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令和 3 年度　学位論文

低分子医薬品の標的分子拡充を志向した
カルボランの創薬研究

東京工業大学大学院生命理工学院

ライフエンジニアリングコース

浅輪泰允

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Abbreviations

Ac	acetyl
AcOH	acetic acid
Å	ångström
ADH	alcohol dehydrogenase
Ala	alanine
AR	androgen receptor
Arg	arginine
ATP	adenosine triphosphate
aq.	Aqueous.
Bak	Bcl-2 homologous antagonist/killer
Bcl-2	B-cell/CLL lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
Bn	benzyl
BNCT	boron neutron capture therapy
Boc	*t*-butoxycarbonyl
BOPP	boronated porphyrin
br	broad
BSA	bovine serum albumin
Bu	butyl
c	centi-, calculated
˚C	degrees Celsius
CA	carbonic anhydrase
CADD	computer-aided drug design
cal	calorie
CBP	cAMP response element binding protein binding protein
CD	circular dichroism
CMC	comprehensive medicinal chemistry
CMV	*Cytomegalovirus*
conc.	Concentration, concentrated
cont.	control
COX	cyclooxygenase
COMU	1-[(1-(Cyano-2-ethoxy-2-oxo-ethylideneaminooxy)dimethyl-aminomorpholino)]-uronium hexafluorophosphate
Ct	threshold cycle
CXCR	CXC chemokine receptor type
d	doublet
δ	chemical shift in parts per million
Δ	heat
DB-LAMS	droplet-beam infrared (IR) laser ablatio
DCM	dichloromethane
DDR1	discoidin domain receptor 1
DDQ	2,3-dichloro-5,6-dicyano-*p*-benzoquinone
DIAD	diisopropyl azodicarboxylate
DIEA	*N*,*N*-diisopropyl ethylamine
DME	dimethoxy ethane
DMF	*N*,*N*-dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DOS	Diversity-Oriented Synthesis
ECFP	extended connectivity fingerprint
EDCI	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide Hydrochloride
EGFP	Enhanced Green Fluorescent Protein
FDA	food and drug administration
FFU	focus forming unit
FITC	Fluoresceinisothiocyanate isomer-I
ER	estrogen receptor
eq.	equivalent
ESI-MS	electrospray ionization mass spectrometry
Et	ethyl
Et_2O	diethylether
FACS	fluorescence activated cell sorting
FITC	fluorescein isothiocyanate
g	gram(s)

IC_{50}	half growth inhibition concentration
Ile	isoleucine
IPA	isopropyl alcohol
IUPAC	international union of pure and applied chemistry
GA	geldanamycin
Gd	gadolinium
GLUT	glucose transporter
GPC	gel permeation chromatography
h	hour(s), hecto-
HA	hemagglutinin
HATU	hexafluorophosphate-azabenzotriazole-tetramethyl Uronium
Her3	human epidermal growth factor receptor 3
HIF	hypoxia inducible factor
His	histidine
HOBt	1-HydroxybenzotriazoleMonohydrate
HRE	hypoxia response element
HSP	heat shock protein
HyT	hydrophobic tag
Hz	hertz
i-	iso
IC_{50}	half inhibiting concentration
ICP-OES	inductivity coupled plasma optical emission spectrometer
IR	infrared
J	coupling constant
k	kilo
K_a	acid dissociation constant
L	liter(s), ligand
LBDD	ligand-based drug design
LC	liquid chromatography
LDA	lithium diisopropylamide
Leu	leucine
LNCaP	lymph node carcinoma of the prostate
m-	meta
M	moles per liter
m	meter(s), milli, multiplet,
μ	micro
max	maximum
Me	methyl
MD	molecular dynamics
MDM2	murine double minute 2
MHz	megahertz
MIC	maleimide conjugated caborane
min	minute(s)
MM	molecular mechanics
MMP	matrix metalloprotease
MMS	matched molecular series
mol	mole(s)
MRI	magnetic resonance imaging
MS	mass spectrometry
MTT	3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	microwave
n	nano
n-	normal
N2a	Neuro 2a
NAD	nicotinamide adenine dinucleotide
NAMPT	nicotinamide phosphoribosyltransferase
NMN	nicotinamide mononucleotide
NMNAT	nicotinamide mononucleotide adenylyltransferase
NMR	nuclear magnetic resonance
NSF	nephrogenic systemic fibrosis
o-	ortho
p-	para
p	pico
P	partition

PAGE	polyacrylamide gel electrophoresis
PARP	poly ADP-ribose polymerase
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PDB	protein data bank
PET	positron emission tomography
PKC	protein kinase C
Pd	palladium
Ph	phenyl
Phe	phenylalanine
Phth	phthalimide
PMB	*p*-methoxybenzyl
PMI	principal moment of inertia
POI	protein of interest
PPI	protein-protein interaction
ppm	parts per million
Pr	propyl
Pro	proline
PROTAC	proteolysis-targeting chimera
PRPP	phosphoribosyl diphosphate
Py	pyridine
q	quartet
QM	quantum mechanics
QSAR	quantitative structure activity relationship
quant.	quantitative
quint.	quintet
R	functional group
RAF	rapidly accelerated fibrosarcoma
RABV	rabies virus
Ref	reference
RNA	ribo nucleic acid
RNN	recurrent neural network
RNP	ribonucleoprotein
RPMI	roswell park memorial institute medium
λ	wavelength
r.t.	room temperature
RT	real time
s	singlet, second(s)
SAR	structure activity relationship
SARD	selective androgen receptor degrader
SBDD	structure-based drug design
SDF	structure data file
SDS	sodium dodecyl sulfate
SMILES	simplified molecular input line entry system
t	triplet
t-	tertiary
TACE	tumor necrosis factor-α-converting enzyme
TBS	*tert*-butyldimethylsilyl
temp.	temperature
TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TEA	triethylamine
Tf	trifluoromethanesulfonyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TIPS	triisopropylsilyl
TLC	thin layer chromatography
TMS	trimethylsilyl
Tyr	tyrosine
Ts	*p*-toluenesulfonyl
µ	micro
Ub	ubiquitin
UV	ultraviolet
VEGF	vascular endothelial growth factor
VHL	von Hippel Lindau
W	watt(s)
WST	water soluble tetrazolium
X	halide, leaving group
XIAP	X linked inhibitor of apoptosis protein

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バイオインタラクション研究会、東京医科歯科大学、**2021 年 11 月**

その他（受賞歴）

1) Gold Student Award, 11th Workshop on Organic Chemistry for Junior Chemists (WOCJC-11)

2) 日本薬学会第 140 年会（京都）講演ハイライト（2 /175 件）

3) 優秀ポスター賞、第 10 回 CSJ 化学フェスタ 2020

第一章

序論

1-1　創薬化学

生体分子は生命システムの維持に重要な役割を果たしている。すなわち、タンパク質や核酸、脂質、糖鎖といった有機分子が相互に作用することで、堅牢な機能をもつ複雑なネットワークやフィードバックシステムが生命維持のために構築されている。このようなシステムの機能不全は疾病につながるが、医薬品はその生物活性を生体内において発現し、システムを正常化させつつ、できる限り少ない副作用で体外に排出される。医薬品の研究は今から 100 年以上前より研究されている。1890 年、フィッシャーエステル化で有名な Emil Fischer 博士はタンパク質と基質の相互作用を「鍵と鍵穴モデル」で提唱した[1]。また 20 世紀初頭、Paul Ehrlich 博士は狙った標的の生体分子のみを攻撃する医薬品を「魔法の弾丸」と呼び、化学療法（Chemotherapy）の概念を初めて提唱した[2]。この概念は、1910 年に秦佐八郎博士とともに梅毒治療のための低分子医薬品としてサルバルサン（ラテン語で世を救うヒ素の意）の開発に成功したことで実証された。一方で、Ehrlich 博士は 1908 年に抗体医薬品の基礎となる側鎖説[a]の提唱も行っており、1908 年ノーベル医学生理学賞を受賞している。このように 100 年以上前から提唱されている、生体分子を医薬品で制御するという創薬化学の概念のもと、今日まで様々な創薬戦略が研究されている（Figure 1-1）[3]。

生体分子の中でもタンパク質はシグナル伝達、輸送、品質管理など多くの機能をもっているため、低分子医薬品の分子標的として最も広く研究されている[4–6]。この機能を制御する低分子を生物活性分子（もしくは医薬品候補化合物）と呼ばれ、その構造変化がタンパク質の機能に与える影響を追究する構造活性相関（Structure Activity Relationship:; SAR）研究が最も一般的な戦略として利用されている。本論文では、標的タンパク質と、様々な創薬戦略に基づいて設計したカルボランを基盤とする分子の SAR を解明し、新たな低分子医薬品創出の可能性を提唱する。

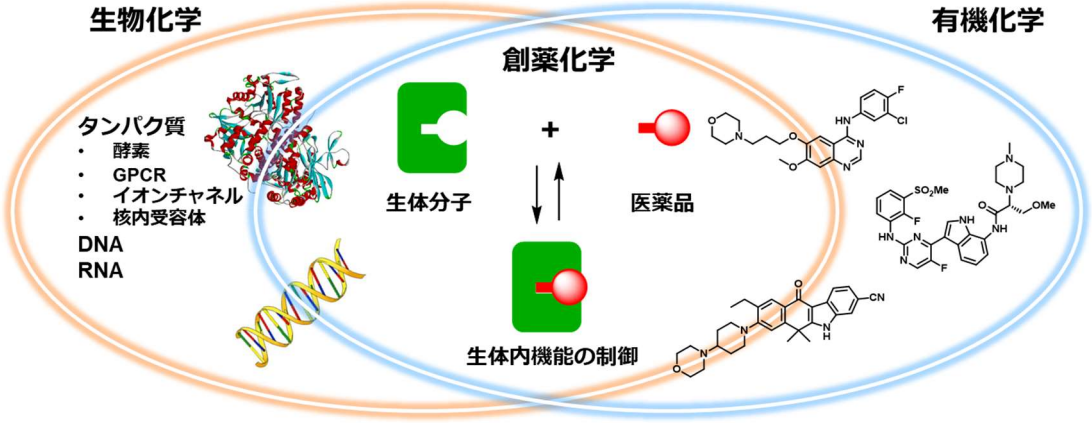


Figure 1-1. 生体分子を制御する医薬品の創出を目指す創薬化学

[a] 生細胞の免疫応答を説明するために提唱した理論。細胞表面には、外来の物質（抗原）と反応する側鎖が存在し、物質と側鎖が反応すると細胞内応答が変化するというもの。この側鎖こそが、現在の抗体である。

1-2　タンパク質-タンパク質相互作用（Protein Protein Interaction; PPI）を標的とした戦略

これまで多くの疾病治療の効果的な標的分子は、リガンドで活性化される転写因子や酵素が主流だった[7]。しかし、こうした標的に対する医薬品開発コストが年々増加していること[8]、未だ治療法のない疾病に対する PPI の普遍的な重要性が明らかになっていることから、PPI が魅力的な創薬標的として注目されている[9–11]。抗体医薬品は PPI を標的にした先駆的なものだが、細胞透過性に乏しいため、細胞内の PPI を標的にすることが困難であった。低分子医薬品は優れた細胞膜透過性や経口投与の可能性を有するが、PPI には酵素などに見られる鍵と鍵穴モデルのような、せまくて深い基質ポケット（~300 Å^2）が存在せず、約 750～1,500 Å^2 の表面積からなる広くて浅い界面で構成されるため、低分子による PPI 制御分子の設計は容易でない[12–14]。しかし、その界面には、「ホットスポット」とよばれるいくつかの疎水性相互作用が点在するため[15]、近年、このホットスポットに標的を絞って設計した低分子化合物が PPI を制御できることが示されている[9,16,17]。実際、2016 年にファーストインクラスの低分子 PPI 阻害剤として、Venetoclax（ABT-199）が FDA に承認された[18,19]。Venetoclax はアポトーシス抑制シグナル Bcl-2/Bak の PPI を阻害するため、抗がん剤として利用される。本項では、このように新たな治療標的である PPI に対する創薬アプローチを紹介する。

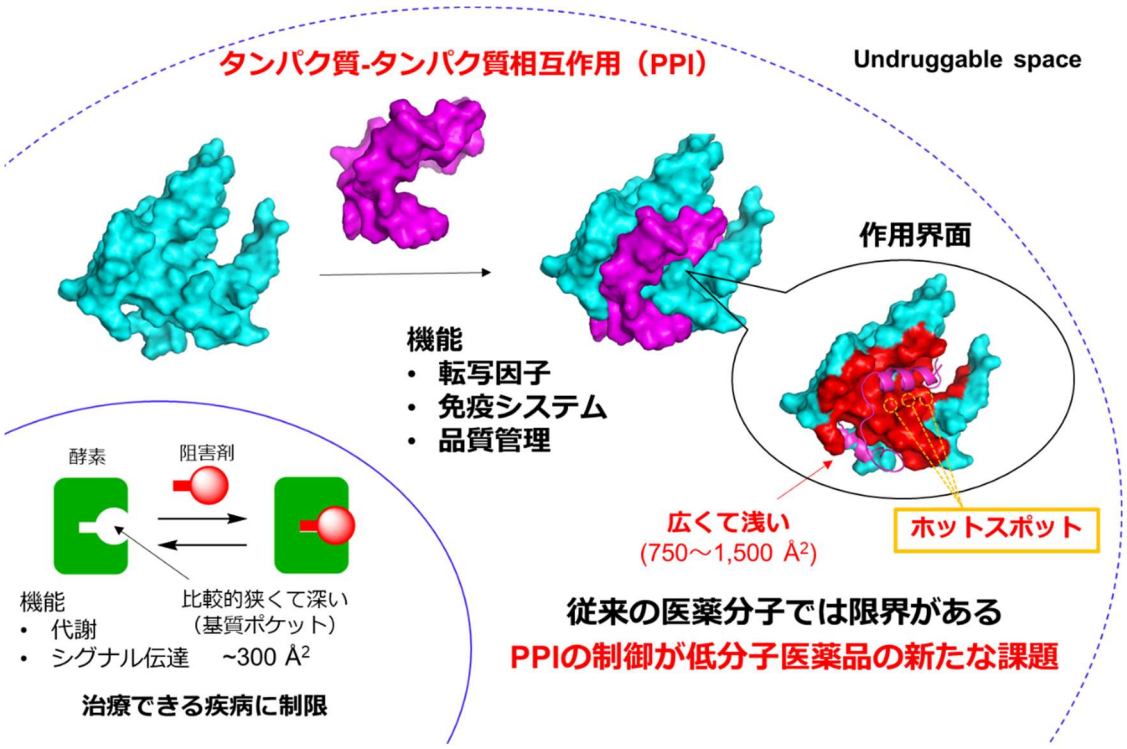


Figure 1-2. 創薬標的のパラダイムシフト

1-2-1　ペプチド模倣分子の設計

タンパク質の2次構造は、主鎖のアミド基間で生じる水素結合により、α-ヘリックス、β-シート、β-ターンといった特定の立体構造をとり、側鎖の方向を規定する[20–22]。そのため、この2次構造を模倣できる低分子骨格を用いることで、ホットスポットに存在するペプチドの側鎖と同じ配置をもつ分子を設計し PPI を制御する研究が盛んに行われている（Figure 1-3）。特に、α-ヘリックスは現在解明されている PPI を構成する2次構造の62%を占めるため[23]、模倣するテンプレートとして最も広く用いられる[24]。1992年、Rees らによって 1,6-disubstited indane を骨格として、初めて低分子による α-ヘリックス模倣が報告された[25]。それ以降、Terphenyl[26,27]、Oligoamide[28]、Benzamide[29]、Oligoozopiperadine[30] と様々な骨格が開発された。さらに近年では、Bicyclo[3.3.1]non-2-ene[31] や(Di)Azatricyclododecene[32] などのかご状骨格も α-ヘリックスの側鎖配置を模倣できることが示唆されている。先に紹介した Venetoclax（ABT-199）も Bcl-xL のペプチドをテンプレートとした分子設計され、承認薬にまで発展した。このようにペプチド模倣に基づく PPI 制御分子設計は、強力な創薬手法として確立されてきている。

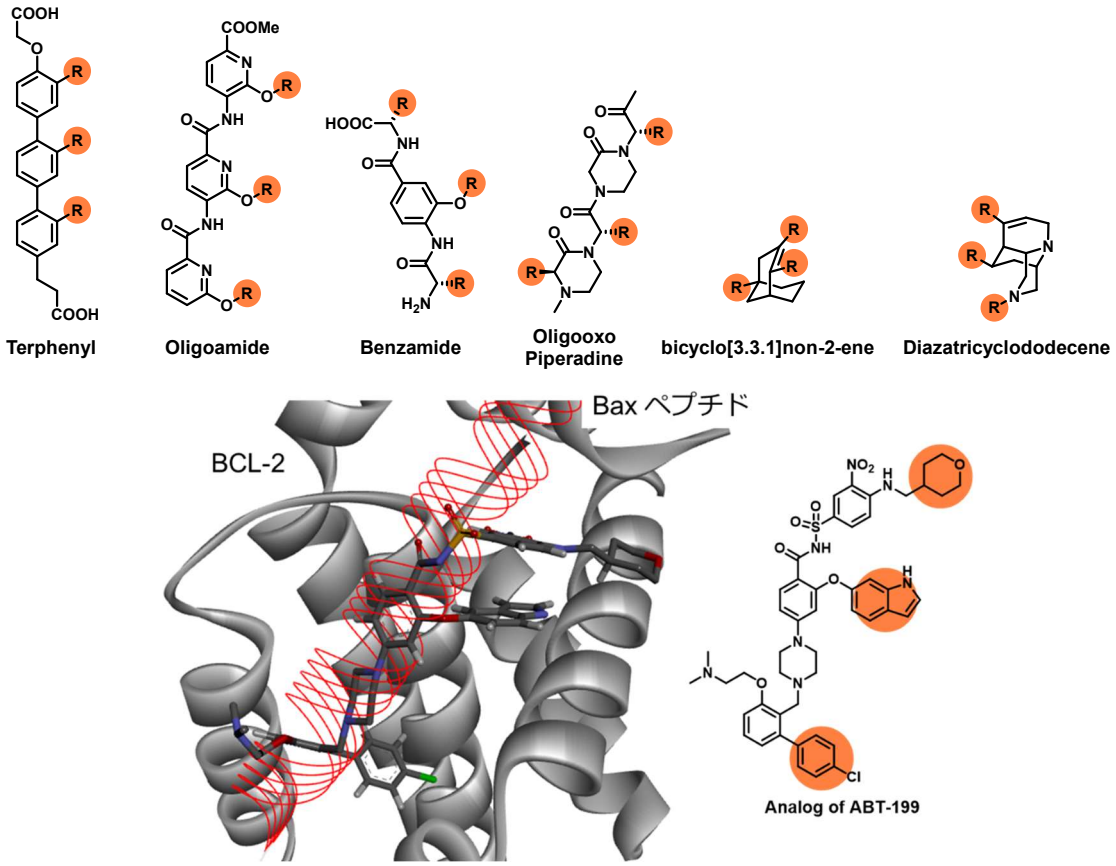


Figure 1-3. これまで開発されてきた α-ヘリックス模倣分子（PDB:4MAN, 2XA0）

1-2-2　三次元分子の設計

これまでの医薬分子はせまくて深い基質ポケット適応するため、直線的もしくは平面的な分子形状をもつことが、Principal Moment of Inertia（PMI）解析[b]から明らかになっている[33]。PMI 解析は、分子の立体構造から主慣性モーメントを算出し、逆三角形のグラフにプロットする。その各頂点が、2-ブチン、ベンゼン、アダマンタンと分布し、右上に行くほど分子の三次元性が高いことが示される。低分子医薬品のデータベース（DrugBank[34]）を PMI 解析した結果を Figure 1-4 に示す。グラフ左側が密、右上が疎であることから、明らかに医薬分子の形状は直線・平面的で、アダマンタンのような球状の分子は非常に限られていることがわかる。さらに、ほとんどの球状構造は、天然物に含まれるような大環状ペプチドやスピロ環、複雑な立体構造をもつ多環性骨格が占め、合成が困難という課題がある一方で、低分子中の sp^3 炭素中心の割合（Fsp^3）が高いほど、その低分子が創薬から臨床試験を経て医薬品になる確率が高いことが指摘されている[35,36]。また、分子構造の剛直性が PPI の制御に重要であることも示唆されている[37]。このように、新たな創薬標的である PPI を標的にした医薬分子を設計するためには、球状の特徴をもち、より三次元的で剛直な骨格を、容易に構築できる合成戦略が必要とされている。

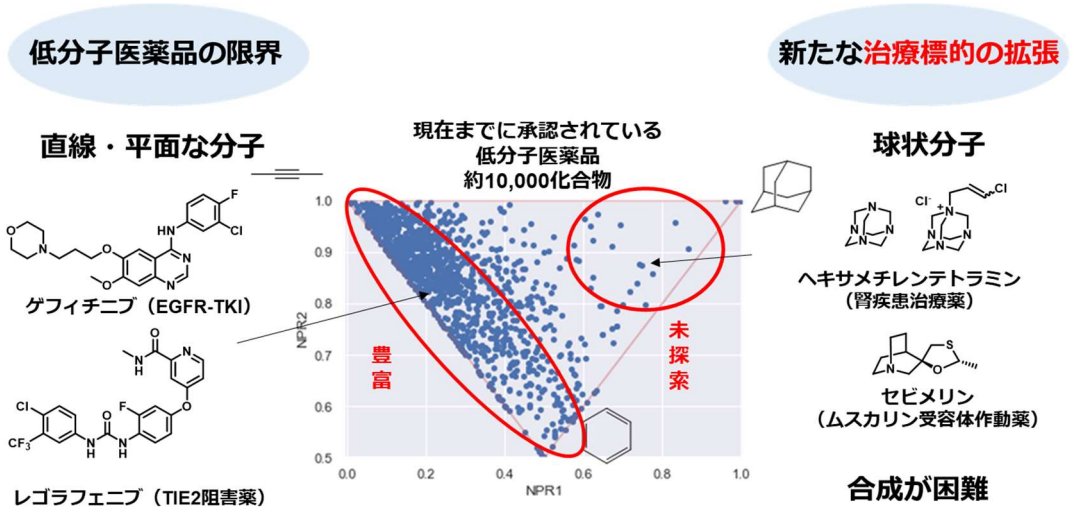


Figure 1-4. PMI 解析による今日の承認医薬品の分子形状分布

[b] PMI 解析：任意の xyz 軸の主慣性モーメントを小さい順に I_1, I_2, I_3 とし、縦軸に（I_1/I_2）、横軸に（I_2/I_3）として標準化した値をプロットする。

1-2-3　分子の多様性を志向した分子設計（Diversity-Oriented Synthesis; DOS）

新たな創薬標的に対する医薬品設計を困難にする理由として、分子構造の多様性の枯渇が指摘されている [38,39]。しかし、理論的に低分子医薬品となりうる分子の数は、10^{60} にも上ると計算されており、この「化学空間（ケミカルスペース）」のすべてを網羅的に合成することは時間と費用の面で不可能とされる [40]。この課題を解決するために、Schreiber らが開発した DOS が利用されている [41,42]。DOS とは、構造的および機能的に高度な多様性をもつ分子を意図的かつ効率的に合成するアプローチであり、未開拓な化学空間を探索できる分子を構築することが目的であるとされる [39]。例えば、Figure 1-5 に示すように、単環のイミニウムカチオンから、多環式の生物活性分子に応用できる骨格の合成が報告されている [43]。近年では、この中心骨格に天然物や金属錯体を用いることで、三次元性の高い DOS ライブラリも構築されている [44,45]。しかし、このように様々な分子構造に基づく DOS の開発が進められているものの、骨格の多様性を重視するあまり、個々の骨格における体系的な置換基の導入法は確立されていないケースが多い。医薬品分子の設計には、側鎖の多様性も重要であるため、体系的な置換基導入法が求められる。

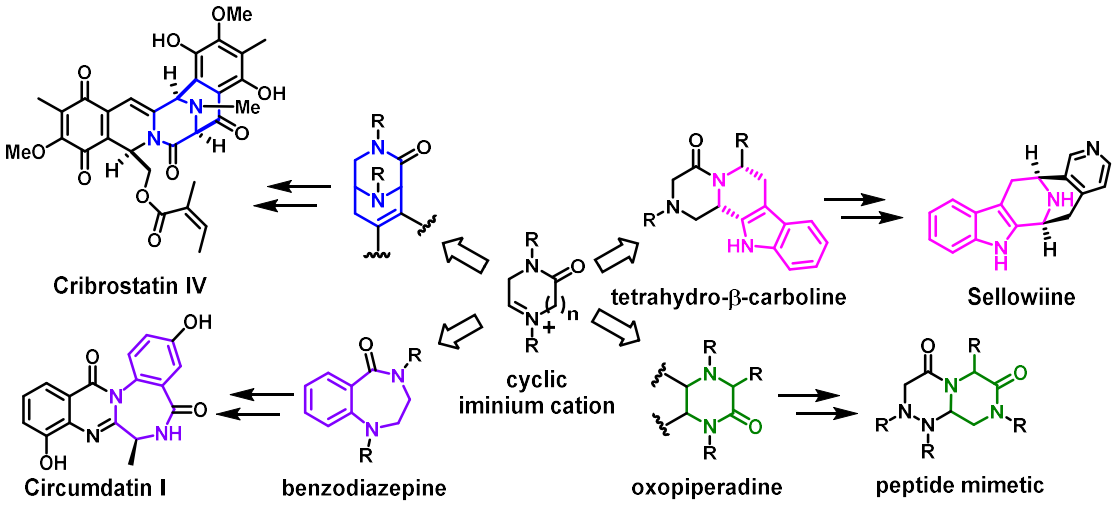


Figure 1-5. PMI 解析による今日の承認医薬品の分子形状分布

1-3　タンパク質の分解を狙った戦略

疾患の原因となるタンパク質の細胞内レベルを低下させることは、その治療に向けた有望な戦略の一つである。アンチセンスオリゴヌクレオチドや RNA 干渉などの核酸を用いた細胞内タンパク質修飾は、分子生物学の分野ではタンパク質ノックダウン技術として広く受け入れられているが、血清中での不安定性や免疫原性の問題から、医薬品開発への応用はまだ困難であるとされている[46,47]。こうした中、より生体内での安定性が高く、細胞膜透過性も良いとされる低分子を使った、細胞内のタンパク質品質管理機構であるユビキチン-プロテアソーム系を意図的に誘導することで、タンパク質の分解を意図的に誘導する薬剤の開発が進められている[48–50]。なお、ユビキチンプロテアソーム系とは、ユビキチン活性化酵素 E1 によって活性化された、ユビキチン結合酵素 E2 と E3 リガーゼが複合体を形成し、E3 リガーゼによって認識されたタンパク質がユビキチン化され、26S プロテアソームによる分解が誘導される分解系である[51]。本項では、近年利用されている 2 つの戦略を紹介する。

1-3-1　Proteolysis-Targeted Chimera（PROTAC）

PROTAC は、2 つのリガンドからなる 2 つの機能性分子を用いる技術であり、1 つは目的のタンパク質（Protein Of Interest; POI）に結合し、もう 1 つは E3 リガーゼをリクルートして POI のユビキチン化を誘導する[48,52–54]（Figure 1-6A）。坂本らが報告した最初の PROTAC は、ユビキチン化のための Skp1-Cullin-F box 複合体のリガンドである IκBα ペプチドと、メチオニンアミノペプチダーゼ-2（MetAP-2）の共有結合体であるオバリシンを組み合わせて、ユビキチン-プロテアソーム依存的に MetAP-2 を分解することを示した[55]。POI を認識する E3 リガーゼは、ヒトゲノム上に 600 種類以上の遺伝子が存在し、その標的特異性を実現している[56]。例えば、nutlin-3a（MDM2 リガンド）[57]、bestatin（XIAP リガンド）[58]、von Hippel Lindau（VHL）リガンド[59,60]、pomalidomide（cereblon リガンド）などの E3 リガーゼを標的にした分子が開発されている[61,62]。2021 年現在、Arvinas 社の開発品 ARV-110 や ARV-471 で第 2 相臨床試験が実施されているなど、PROTAC 分子の実用化が期待されている[63]。

1-3-2　疎水性タグ（HyT）システム

PROTAC が、POI のリガンドと E3 リガーゼのリガンドを複合させた設計であるのに対し、E3 リガーゼリガンドの代わりに「疎水性タグ（Hydrophobic Tag; HyT）」と呼ばれる疎水性の高い部分構造を導入することで、タンパク質分解を誘導する戦略が開発されている（Figure 1-6B）。既に 2002 年、エストロゲンを選択的に分解できる Fulvestrant が転移性乳がん治療薬として FDA (Food and Drug Administration)から承認されている[64]。もともと選択的エストロゲン受容体モジュレーター（SERM）として設計されたが、後

に Fulvestrant の perfluoroethyl 部位の高い疎水性により、エストロゲン受容体 α（ERa）受容体の分解が誘導されることが明らかになった [65]。

一方、2011 年に POI として HaloTag タンパク質を用いて、HyT13（Figure 1-6B）が有意な分解を誘導することを Neklesa らが見出したことを契機に、本格的に HyT システムの研究が始まった [66]。細胞内のアンフォールドやミスフォールドしたタンパク質は、熱ショックタンパク質（HSP）ファミリーであるシャペロン分子によって直ちに認識され、ユビキチン-プロテアソーム系で除去される [67,68]。この品質管理機構を利用し、タンパク質の表面に HyT を導入し、タンパク質の構造を変化させることで、部分的な変性状態が模倣され、シャペロン分子による認識の後、POI の分解が誘導されると考えられている。Selective Androgen Receptor Degrader（SARD）を用いた実験から、シャペロン分子として HSP70 が重要な役割を有することが示唆されているが [69]、これ以上の詳細な機構解明はされていない。HyT システムを誘導する部分構造として、これまでに HyT として HyT13 や SARD のようなアダマンチル部位だけでなく、*tert*-ブチルカルバメートで保護されたアルギニン（Boc3Arg）も利用されている [70]。この 2 つの疎水性タグが一般的に使われており、アンドロゲン受容体やエストロゲン受容体の他に、Her3 や PARP といった様々なタンパク質の分解誘導剤が開発されている [71,72]。しかし、Boc3Arg は mTORC1 を介した翻訳機構を阻害するといったオフターゲット効果も確認されているため [73,74]、新たな HyT の設計も必要とされている。

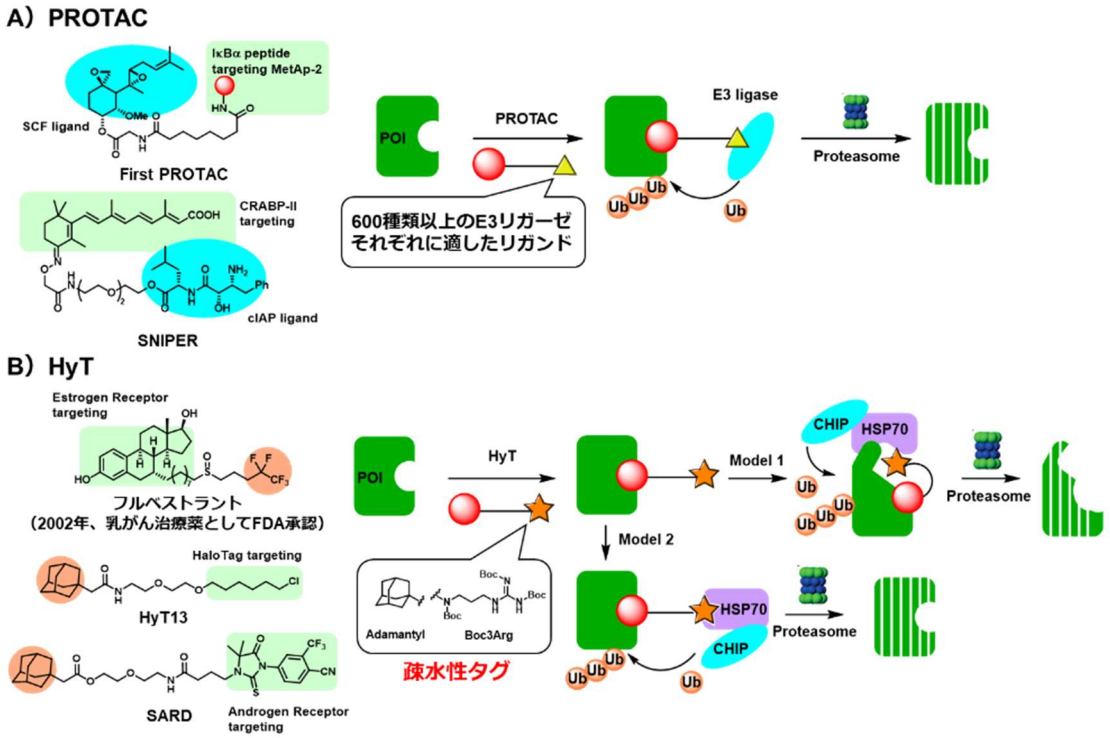


Figure 1-6. 低分子による標的タンパク質の分解誘導戦略；A）PROTAC；B）HyT システム

1-4　Computer-Aided Drug Design（CADD）を利用した戦略

1-2-3 項で述べたような推定 10^{60} 個の分子からなるケミカルスペースから、標的分子に対して活性のある化合物を探索するために開発された計算機的アプローチを Computer-Aided Drug Design （CADD）と呼び [75,76]、タンパク質の結晶構造を基にした Structure-based Drug Design（SBDD）[77,78]、活性化合物の解析から新たな分子を設計する Ligand-based Drug Design（LBDD）に大別される（Table 1-1）。コンビナトリアルケミストリー[79,80] やハイスループットスクリーニング（HTS）による構造活性相関情報 [81,82]、Protein Structure Initiative（PSI）やタンパク 3000 プロジェクトによるタンパク質と低分子の複合体構造情報が、今日までに ChEMBL[c]、ZINC[d]、DrugBank[e]、Protein Data Bank[f]（PDB）といった公共のデータベースに蓄積されている [76]。

SBDD では、主に PDB からタンパク質-低分子複合体構造を取得し、結合部位における低分子とタンパク質の相互作用をエネルギー計算によって求めることが基本となる [77,78] 。またタンパク質、低分子ともに三次元構造を使って立体配座の予測を行うことから、配座の生成方法や入力する分子の立体構造（初期配座）が結果に大きな影響を与える [83]。さらにドッキングシミュレーションでは、クーロン力やファンデルワールス力といった静的な分子間力の計算、Molecular Dynamics（MD）シミュレーションでは運動方程式を解き、動的な結合力を予測するといったようにエネルギーの計算法が異なる [84]。近年では、QM/MM（Quantum Mechanics/Molecular Mechanics）法という量子力学計算を組み合わせた手法も用いられているが、予測精度は高くなる反面、それに比例して計算量も多くなる傾向にある [85,86]。一方で、共結晶構造からファーマコフォアを抽出し、それを満たす分子および立体配座を探索するファーマコフォアフィッティングも比較的計算量の少ない方法として使われる。なお、ファーマコフォアとは、タンパク質と低分子の分子認識に必要とされる官能基特性であり、疎水性、水素結合ドナー、アクセプター、カチオン、アニオン、芳香環といった特徴が利用される。ドッキングシミュレーションが分子の立体配座とタンパク質間の相互作用を逐時計算して最適な配座を探索するのに対し、ファーマコフォアフィッティングは、あらかじめ分子の取りうる立体配座を生成させたデータベースを用意しておき、各ファーマコフォアとの重なる範囲が多い立体配座に高いスコアを付与する。そのため、ファーマコフォアの作成元である結晶構造に含まれる分子と同じ配座をとりうる構造を探索しやすい [87]。

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[c] ChEMBL：生物活性分子のデータベース、現在 210 万化合物収録されている。https://www.ebi.ac.uk/chembl/

[d] ZINC：最も利用されている化合物構造データベース、10 億近い化合物が収録されている。https://zinc.docking.org/

[e] DrugBank：医薬品および医薬品候補化合物のデータベース、https://go.drugbank.com/

[f] PDB：タンパク質の結晶構造データベース、現在 18 万以上の構造が登録されている。https://www.rcsb.org/

Table 1-1. CADD の手法

	SBDD	LBDD
データベース	PDB	ChEMBL、ZINC
評価関数（f(x)）	エネルギー計算	回帰モデル
入力データ（x）	タンパク質と低分子の立体構造	特徴量
計算時間	多い	少ない
主な手法	ドッキングシミュレーション	QSAR
	MD、QM/MM	機械学習
	ファーマコフォアフィッティング	

LBDD では、ChEMBL などから取得した構造活性情報をもとに化合物の構造や化学特徴量（記述子 : Descriptor）を変数として活性値を予測する回帰モデルを構築することが主に行われる[88,89]。SBDD と異なり高度な計算を行わないため、より多くの化合物を取り扱うことができる。さらに、Figure 1-7 に示すように、化合物の構造表現を変換することで計算量を減らすことができる。化合物の構造は２次元構造式の他に、IUPAC 命名法によって一意に表すことができるが、ルールが複雑な上に名称が長くなる。また慣用名もあるため、言葉による表現はコンピュータには適さない。そこで、一次元文字列配列として表現する SMILES が開発された[90]。構造式から結合と水素を省き、原子の配列とし、幾何異性体や立体異性体も「/」や「@」を用いることで表現可能である。しかし、構造の大きさに文字列数が依存することや、表記する始点の原子の違いによって無数の表現方法が生じるという問題点がある。一方で、Fingerprint（FP）と呼ばれる「0,1」のビット表現にすることで分子の特徴を抽出できることが知られる。一度ビット表現に変換してしまうと元の構造を特定できなくなるが、ビット数を規定することで、情報量を統一することができる。特に、Extended Connectivity FingerPrint （ECFP）と呼ばれる原子から特定の距離にある部分構造を数え上げるアルゴリズムがよく使われる[91]。FP 化した化合物の類似性を谷本係数[g]などでスコアリングし、活性を予測する Quantitative Structure Activity Relationship（QSAR）法が使われる。QSAR は、構造的に類似した化合物が類似した活性を示すという前提に基づき、活性化合物との類似度が高くなるように分子を設計する[89]。この方法は、従来の酵素などの標的分子以外にも、PPI に対する分子設計にも利用されている[92]。しかし、必然的に QSAR モデルを作成した時のデータベースに含まれる構造に類似した化合物でしか適用できないという欠点がある。

[g] 谷本係数：化合物 A と B に共通する部分構造の数（c）を化合物 A に含まれる部分構造（a）と B に含まれる部分構造（b）の総数で割った値。すなわち、谷本係数 S = c /(a+b+c) と計算できる。例えば、FP 化した構造 A「0011」、B「1100」、C「1011」とした場合、A との谷本係数は、B = 0/4 = 0、C = 3/4 = 0.75 となる。

また、二次元構造式と立体配座情報を合わせた Structure Data File（sdf）形式も分子の表現方法として使われる。これらは OMEGA[93] や iCon[94] といった様々な配座生成アルゴリズムによって得られる。活性化合物の三次元構造をもとにファーマコフォアを作成し、ファーマコフォアフィッティングにより新たな活性化合物を見出す例も報告されている [95]。この方法は LBDD と SBDD を組み合わせたような手法であり、QSAR 法の構造類似性の課題を克服できる。

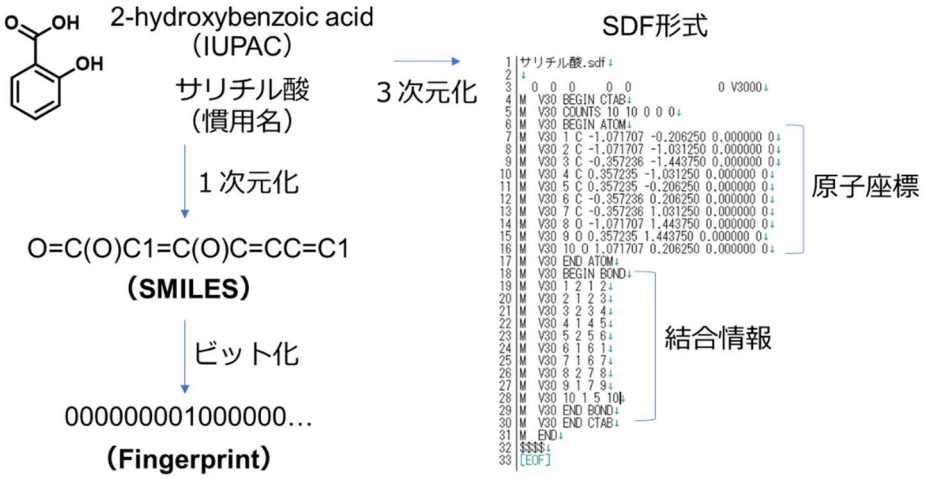


Figure 1-7. LBDD に使われる分子の表現方法

1-5　カルボラン創薬

1-5-1　カルボランの化学的特徴

化学式 $C_2B_{10}H_{12}$ で表される Dicarba-*closo*-dodecaborane（以下、カルボラン）は二十面体の疎水性クラスター分子である。2 個の炭素原子と 10 個のホウ素原子、各頂点に 1 個ずつ計 10 個の水素原子で構成され、*closo* の名前が示す通り「かご状」分子である。骨格を構成するホウ素原子と炭素原子は 3 中心 2 電子結合を有し、それぞれから 2 つ、3 つずつ供給される計 26 個（4n+2 個）の電子が分子の球状表面に非局在化しているため、ヒュッケル則に基づく芳香族性およびウェイド則[h]に基づく三次元的構造安定性をもつ。この電子が分子表面に非局在化し、三次元的に構造が安定化していることを超芳香族性と呼ぶ。さらにカルボランのホウ素原子は、3 中心 2 電子結合により空の 2p 軌道がなく、一般的なホウ素化合物がもつ高いルイス酸性を示さない。これらのことから、カルボランは特徴的な、かご状構造でありながらもカルボランは高い化学的安定性と炭化水素に匹敵する疎水性をもち、「三次元的なベンゼン化合物」と称されている（Figure 1-8A）[96,97]。

カルボランには、2 つの炭素原子の位置により *o*-, *m*-, *p*-の三つの構造異性体が存在する [98]。炭素原子が隣接する *o*-カルボランはアルキンとデカボラン（$B_{10}H_{14}$）から合成され、465-500 ℃で *o*-カルボランから *m*-カルボランに異性化し、615-700 ℃で *m*-カルボランから *p*-カルボランに異性化する [99]。しかし合成の複雑さから数万円/g と非常に高価である。*o*-＞*m*-＞*p*-の順に分極が大きく、*p*-＞*m*-＞*o*-の順に疎水性が高い。これは炭素（X＝2.55）とホウ素（X＝2.05）の電気陰性度の違いから、双極子モーメントが生じているためである。

疎水性の他に、その大きさも特徴的である。X 線結晶構造からファンデルワールス体積として計算されており、*o*-、*m*-、 *p*-カルボランでそれぞれ 148、143、141 Å^3 であり、分極と同様に異性体間で異なる [99]。一方で、アダマンタンのファンデルワールス体積が 136 Å^3、ベンゼンを 360° 回転させたときに占有する体積が 102 Å^3 であることと比較すると、カルボランは疎水性を有する炭化水素であるアダマンタンやベンゼンよりも大きな疎水性分子と言える。

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h ウェイド則：1971 年、Kenneth Wade によって報告されたボランおよびその類縁体の価電子数と安定な立体構造の間の関係を示す法則 [123]。分子式が BmHn のボラン化合物においてイオン価数 x の時、m+n+x の値によって、ボラン化合物の安定な構造（*closo*, *nido*, *arachno*, *hypo*）が推定できる。カルボランのように炭素が含まれるカルバボランの場合、BH を CH_2 に置き換えても同様に成り立つ。例えば m + n +x = 2m + 2 の時、closo の骨格をとり、m + n +x = 2m + 4 の時、*closo* の一個頂点が取れた *nido* の骨格をとると言われている。

1-5-2　カルボランの反応性

カルボランの修飾に用いられる一般的な反応を Figure 1-8B に示す[97,100,101]。先述の通り、*o*-カルボランは、Lewis 塩基存在下、アセチレンとデカボラン（$B_{10}H_{14}$）を加熱し、反応させることで得られる。置換アルキンも同様に反応するため、一（二）置換の *o*-カルボランは容易に調製できる。*m*-, *p*-カルボランは、それぞれ 465-500 ℃、615-700 ℃で熱異性化によって合成される[99]。

カルボランは、①*n*-BuLi（p*K*a = 35）などの強塩基による炭素上の脱プロトン化と、続く求電子剤との反応、②求電子置換反応が進行する。さらに異性体によって、反応点が異なるため、様々な方向に置換基を導入したカルボランを合成できる。*o*-,*m*-, *p*-カルボランは、それぞれ 1,2 位（*o*-）、1,7 位（*m*-）、1,12 位（*p*-）の炭素上で反応①が進行する。この反応性を利用すると、1,2-、1,7-、1,12-置換体はそれぞれ、63.4 度（$\cos^{-1}(1/\sqrt{5})$）、116.5 度（$\cos^{-1}(-1/\sqrt{5})$）、180 度に置換基配置を決定することができる。一方で、9,12 位（*o*-）、9,10 位（*m*-）2 位（*p*-）のホウ素上で反応②が進行する。ただし、条件によって多置換体を与え、4,5,7,8,9,10,11,12-オクタメチル-*o*-カルボラン[102]や 3,4,5,6,7,8,9,10,11,12-ドデカクロロ-*o*-カルボラン[103]なども合成されている。一方で、他の異性体に比べて分極が最も大きい *o*-カルボランは、③塩基性条件下、3 位のホウ素原子に対して求核攻撃が容易に進行し、ホウ素が脱離した *nido*-カルボランを与える[104]。*closo*-カルボランとは異なり、10,11 位のホウ素に水素が架橋したイオン性の分子であり、さらに *n*-BuLi 等で処理すると、この架橋している水素は脱プロトン化される。得られたジアニオンと遷移金属と反応させることで、安定なサンドイッチ型錯体（メタラカルボラン）が合成できる[105]。さらに近年、Xie グループによって金属触媒を用いた *o*-カルボランの位置選択的ホウ素置換反応が精力的に研究されている[106,107]。

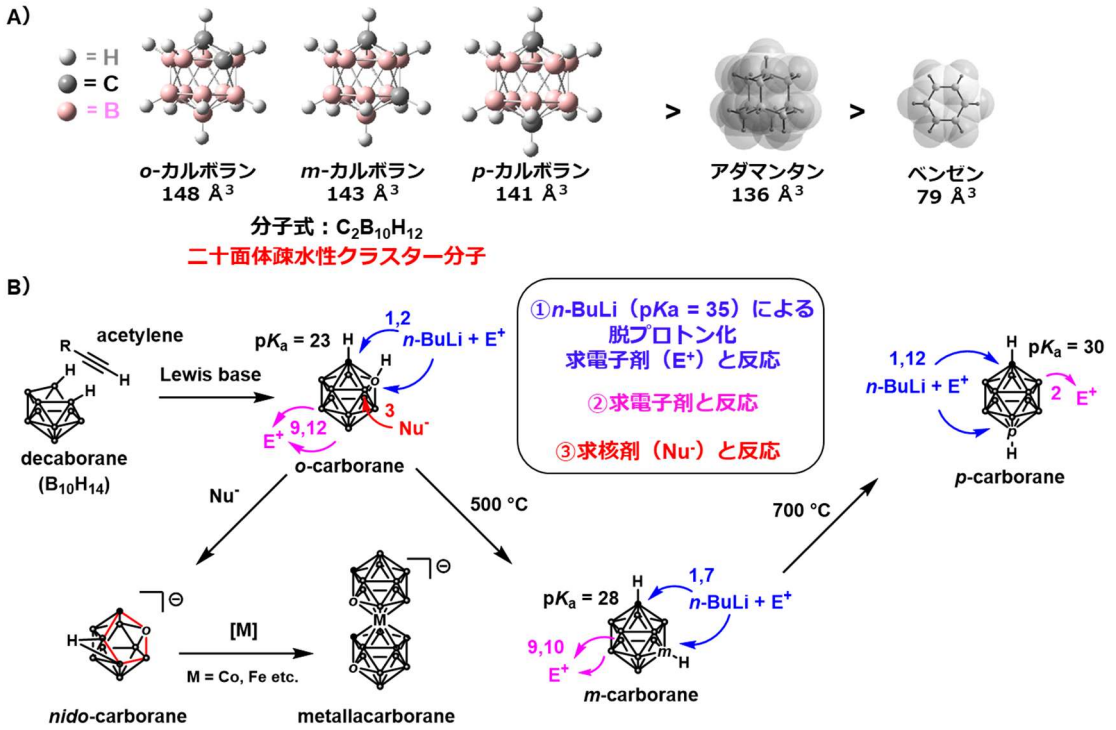


Figure 1-8. A）カルボランの構造と B）反応性

1-5-3　創薬のビルディングブロック

これまで述べたように、特徴的な構造と反応性をもつカルボランは、生物活性分子の設計において疎水性ファーマコフォアとして用いられている[99,108]。その例を Figure 1-9A に示す。1999 年に遠藤らによって BE120 が初のカルボランを疎水性ファーマコフォア利用した分子として報告された[109]。BE120 はエストロゲン受容体作動薬であり、エストロゲン受容体の基質である 17β-エストラジオールに比べて 10 倍以上の親和性を示した。さらにモデルマウスを用いた *in vivo* 試験が実施されており、BE120 の投与によって卵巣摘出マウスにおける骨量の減少に対して効果を示した[110]。2010 年には、当研究室は LW6 のアダマンタンをカルボランに置換した GN26361 が HIF-1α の分解を誘導するだけなく、HSP60 を標的としていることを報告した[111]。一方で LW6 は von Hippel-Lindau（VHL）の活性化を標的にしていることから、カルボランはタンパク質に対して、アダマンタンとは異なる特異的な相互作用を有することが示唆された[112]。他にも、インドメタシンのカルボラン誘導体や天然物であるマナサンチン A の誘導体が報告されている[113,114]。

またカルボランがタンパク質の疎水性環境で結合することは、共結晶構造解析から明らかになっており（Figure 1-9B）[115]、2021 年現在、38 の共結晶構造が Protein Data Bank（PDB）に登録されている。このように、カルボランは疎水性ファーマコフォアとして、創薬のビルディングブロックとして利用されてきている[116]。

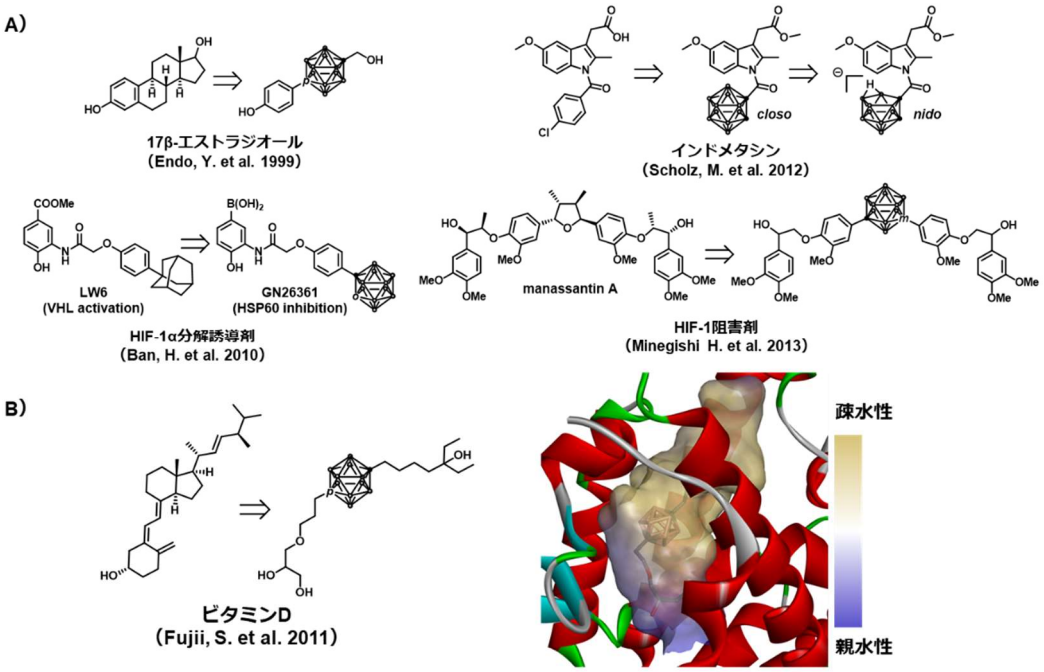


Figure 1-9. A）疎水性ファーマコフォアとしてカルボランを利用した生物活性分子；B）ビタミン D 受容体とカルボラン誘導体の共結晶構造（PDB：3VJS）

さらにイオン性の *nido*-カルボランも創薬に応用されている。2013 年に *o*-、*m*-、*nido*-カルボランを有する炭酸脱水素酵素 II（CA II）の阻害剤が共結晶構造とともに報告された（Figure 1-10A）[119]。*nido*-カルボランが *o*-カルボランや *m*-カルボランと同じ CA II の疎水性部位に配置されることを見出している。*nido*-カルボランはイオン性であり親水性を向上させるにもかかわらず、タンパク質には疎水性置換基として認識されるという知見は非常に興味深い。

加えて、カルボランはその特徴的な B-H 結合により新たな分子間相互作用として二水素結合が注目されている[120,108]。電気陰性度を比較すると水素（X＝2.20）の方がホウ素（X = 2.05）より大きく、B-H の水素原子はヒドリド性と言える。これは従来の水素結合 Donor である O-H や N-H のプロトン性水素と逆の性質を示し、これまでに NH…HB、CH…HB、SH…HB の二水素結合が報告されている。一方で、その結合力は従来の水素結合に比べて弱く、1 つの結合当たり 6.1－7.6 kcal/mol で形成可能距離は、1.7-2.2 Å と言われている[121]。先述の CAII と *nido*-カルボランや *m*-カルボランの共結晶構造を基にした量子力学計算が実施され、アスパラギン残基と *nido*-カルボランに強い二水素結合

（4.2 kcal/mol, 1.7Å）の形成が示唆されている（Figure 1-10B）[122]。一方で *m*-カルボランでは、同じ結合ポケットに配置されているにもかかわらず、この結合はそれほど強くなかったため、カルボランの構造によって二水素結合力が異なることも示されている。

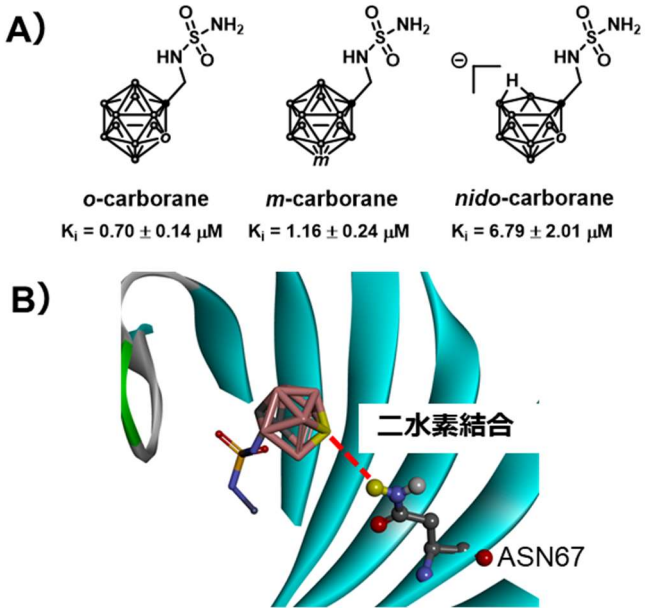


Figure 1-10. A）イオン性の *nido*-カルボラン骨格を有する薬剤；B)二水素結合の形成

しかし、カルボランを含んだ化合物が医薬品となった例はない。脳腫瘍に対する光線力学療法のために開発されたカルボラン含有ポルフィリン誘導体（BOPP）が唯一、第Ⅱ相試験まで進んだが、50%生存期間が初発 5 カ月、再発 11 カ月であり、顕著な有効性を示さなかった [117,118]。カルボラン含有化合物の臨床試験の実施数は非常に限られており、代謝安定性など薬物動態はほとんど解明されていない [3]。

1-6　本論文の概要

本論文は「低分子医薬品の標的分子拡充を志向したカルボランの創薬研究」と題し、以下7章から構成される。Figure 1-11に示すように、カルボランを基盤とした分子設計により、新たな標的分子に対する医薬品候補化合物を創出した。

まず、Autodock Vinaを用いたドッキングシミュレーションに基づいて分子設計を行い、疎水性ファーマコフォアとしてカルボランを利用したNAMPT阻害剤の創出を行った（第二章）。この知見から、カルボランを疎水性タグとしてタンパク質分解誘導剤に活かした新しい分子設計を着想し、HyTシステムの機構解明を行った（第三章）。同時に磁気共鳴イメージング（MRI）志向したGdメタラカルボラン錯体の合成に挑戦した（第四章）。一方で、膨大な構造活性相関情報のこれまでにない網羅的な解析法（SARM法）を用いて高活性化合物の創出を行った（第五章）。

そして、第二章で得られたもう一つの知見である、カルボランの異性体を用いた厳密な置換基配置と、第五章で得られた網羅的なSAR解析法を融合した「カルボランの12頂点から任意の3か所に置換基を導入することで、ケミカルスペースにおけるすべての空間を網羅する」という全く新しい分子設計戦略を提唱し、この概念の実証を行った（第六章）。

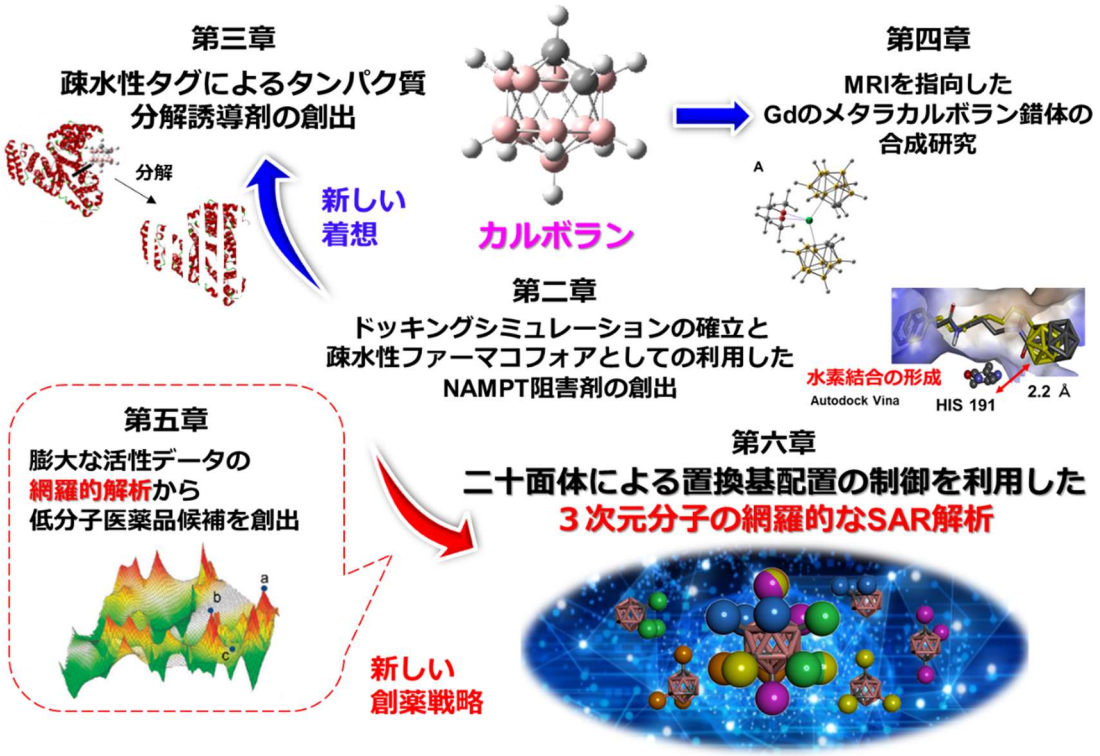


Figure 1-11. 本論文の概要

第 1 章「序論」では、まず低分子医薬品の新たな標的分子として PPI を紹介し、従来の標的であった酵素とは異なった広くて浅い作用界面に適応する分子設計が必要であることを述べた。また、この課題に対する戦略としてペプチド模倣分子、三次元分子の設計、DOS を紹介したが、三次元的で剛直な骨格の簡便な合成と体系的な側鎖の導入が課題であることを指摘した。次に、低分子医薬品によるタンパク質を分解する戦略として PROTAC と HyT システムを挙げ、多くの臨床試験が実施されていることを述べた一方で、HyT システムの分解機構においてシャペロン分子に認識されるタンパク質の構造変化が明らかにされていない点を指摘した。3 つ目に CADD による分子設計法を紹介し、創薬の強力なツールであることを述べた。最後に、カルボランの構造的特徴と反応性について説明し、疎水性ファーマコフォアとして利用されていることを述べた。

第 2 章「カルボランのドッキングシミュレーションの確立と疎水性ファーマコフォアとして利用した NAMPT 阻害剤の創出」では、カルボランのドッキングシミュレーションにより設計した新たな NAMPT 阻害剤の構造活性相関を明らかにするとともに、ジカルボラン化合物の活性結果から、カルボランの異性体を利用することで、厳密な置換基配置の制御であることを見出した。

第 3 章「疎水性タグとしてカルボランを利用したタンパク質分解誘導剤の創成と機能解明」では、まず、カルボランを細胞内でのタンパク質分解誘導のための HyT として利用できることを示した。続いて、MIC-BSA を用いて HyT によるタンパク質構造変化を模倣し、HyT によってリジン残基が露出することを確認し、BSA-MIC が細胞内でユビキチンプロテアソーム系によって分解されることを示した。

第 4 章「磁気共鳴イメージングを指向した Gd メタラカルボラン錯体の合成研究」では、2 つのカルボランをアミド基リンカーでつなげることで安定な錯体の合成を試みたが、予想外の錯体が確認された。この結果を基に新たな Gd メタラカルボラン錯体モデルを提唱し、その量子力学計算による解析から、より安定な錯体モデルの合成可能性を示した。

第 5 章「公共データベースの網羅的な SAR 解析による Activity cliff の予測とその実験的検証」では、大規模な SAR の公共データベースである ChEMBL から得た、SAR 情報の網羅的解析を SAR matrix (SARM)を用いて可能とし、新たな Activity cliff の予測、その実験的検証を行った。

第 6 章「カルボランの置換基配置を駆使した三次元ケミカルスペースの網羅的探索」では、「カルボランの 12 頂点から任意の 3 か所に置換基を導入することで、ケミカルスペースにおけるすべての空間を網羅する」という概念を 5 パターンの 3 置換カルボランを合成し、新たな生物活性化合物を見出すことによって実証した。他の創薬アプローチでは得られなかった、三次元的かつ系統的に置換基を配置できるカルボランを基盤とした分子設計戦略の大きな可能性を示した。

第 7 章「結論」では、本論文を総括している。

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第二章

カルボランのドッキングシミュレーションの確立と疎水性ファーマコフォアとして利用したNAMPT 阻害剤の創出

2-1　はじめに

第一章 **1-5-3** において、カルボランは二十面体の疎水性クラスター分子であり、過去 20 年で疎水性ファーマコフォアとして利用されていることを述べた [1–3]。さらにカルボランは疎水性相互作用に加えて、二水素結合相互作用によって生体分子と強く結合することができる [4]。興味深いことに、この相互作用の強さは、カルボランの異性体によって異なる。Lee らは、カルボランを含むニコチンアミドホスホリボシルトランスフェラーゼ（NAMPT）阻害剤 **1a-c** を合成し、3 つのカルボラン異性体（*o*-、*m*-、*p*-、Figure 2-1）間の活性を比較し、*m*-カルボランを含む化合物 **1b** が最も活性の高い NAMPT 阻害剤であることを報告している（Figure 2-1）[5]。

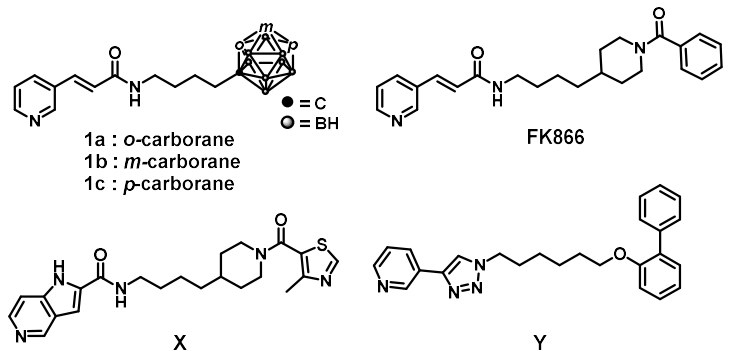


Figure 2-1. これまでに報告されている NAMPT 阻害剤

NAMPT は、哺乳類のニコチンアミド・アデニン・ジヌクレオチド（NAD^+）リサイクル経路における律速酵素で、ニコチンアミドをニコチンアミド・モノヌクレオチド（NMN）に変換する。最近、NAMPT が代謝、細胞増殖、細胞生存、炎症反応などに中心的な役割を果たしていることが明らかになり、この酵素の阻害剤は癌、アルツハイマー病、糖尿病、関節炎など多くの疾患の治療に有用であると知られる [6,7]。例えば、FK866 は、NAMPT の特異的かつ高活性な阻害剤として初めて臨床試験が行われていたが [8]、近年その副作用とし血小板減少症などが挙げられており、新たな NAMPT 阻害剤の開発が望まれる [9]。一方で 1*H*-ピロロ[3,2-*c*]ピリジン部位を有する化合物 **X** は、非常に強力なアミド基含有の NAMPT 阻害剤であり [10]、また 1,2,3-トリアゾール環を有する化合物 **Y** は、マウスを用いた *in vivo* 試験において抗腫瘍剤として作用するだけでなく、大腸炎の予後を改善することを明らかにし、NAMPT 阻害剤の新たな可能性を示した [11]。

タンパク質の結晶構造を利用し、エネルギー計算を行うことで合理的に分子を設計する Structure-based drug design （SBDD）が広く使われている [12]。実際に FK866-NAMPT 複合体（PDB code: 2GVJ）の結晶構造から [13]、NAMPT の疎水性部位に化合物 **1a-c** が重なっていることが示されている。しかし、カルボランの力場が不足しているため、ドッキングシミュレーションによる NAMPT に対する **1a-c** の詳細な結合様式は明らかに

されていない。SBDD で使われる他の方法として QM/MM 法や MD シミュレーションを用いた精力的な研究が行われているが、これらの方法は膨大な計算時間を必要とし、これらの計算方法に従うことができる化合物は少ない[14,15]。

本章では、カルボランのホウ素原子を C3 型原子に置き換えて近似し、ホウ素クラスターを人工的に炭素原子だけのクラスターとして扱うことで[16,17]、Autodock vina 計算プログラムを利用したドッキングシミュレーションを可能とし、新規カルボラン含有 NAMPT 阻害剤の創出を目指した。

2-2　カルボランのドッキングシミュレーションの妥当性評価

2009 年、Tiwari らによってカルボランのホウ素原子を炭素原子に置き換えた炭素クラスター分子として AutoDock（現：AutoDock Vina[a]）によりドッキングシミュレーションが可能であることが報告された[16]。しかし、本手法を用いて SBDD を行った例はないため、まずは本手法の妥当性を評価した。検証は、カルボランを含む化合物とビタミン D 受容体の共結晶構造（PDB：5E7V）を用いたリドッキングによって行った。リドッキングとは、結晶構造に含まれるリガンドを取り出した後、同じリガンドを用いてドッキングシミュレーションを実行し、出力されたリガンドの立体配座と元の X 線結晶構造を比較し、シミュレーションの精度評価を行うことである。一般に、配座のずれを示す Root mean squared deviation（RMSD）が 2.0 Å以下であれば、十分な精度を得られているとされる[18]。

Figure 2-2 にリドッキングの結果を Figure 2-2 に示す。まず、結晶構造を解析すると、カルボランがビタミン D 受容体の疎水性ポケットに配置されており、本手法の妥当性評価に適した結晶構造であるといえる。リドッキングした配座は、結晶構造と相違ない配座を示し（RMSD＜1.9 Å）、本手法が、カルボランの疎水性ファーマコフォアとしての機能を予測するシミュレーションとして妥当であることが示された。

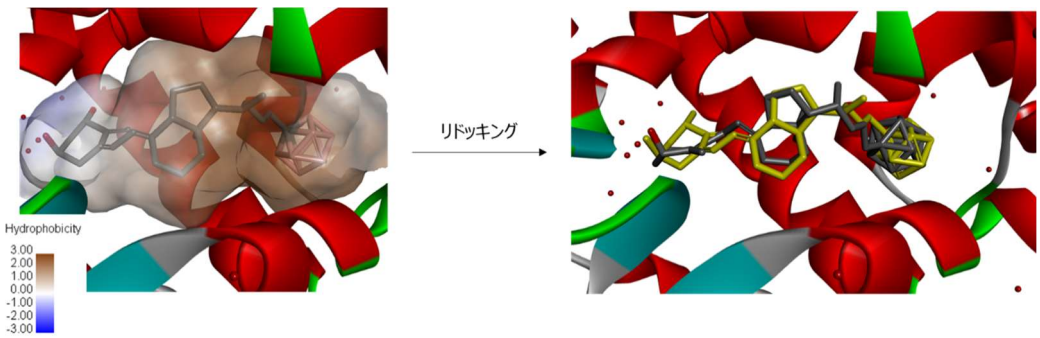


Figure 2-2. ドッキングシミュレーションの妥当性評価；左）X 結晶構造（5E7V）、茶色～青色で疎水性～親水性の環境を表す。右）リドッキングの結果、黄色が結晶構造の立体配座（左図と同じ）、黒色がリドッキングの立体配座（RMSD＜1.9 Å）

[a] http://vina.scripps.edu/index.html

2-3　分子設計

化合物 **1** の構造をもとに，カルボラン含有 NAMPT 阻害剤を設計した。まず、Figure 2 に示すように、化合物 **1** をニコチンアミド模倣部位、リンカー部位、カルボラン部位の 3 つに分けた。Figure 1 の NAMPT 阻害剤 FK866、 **X**、 **Y** の構造に基づき、ニコチンアミド模倣部位として、ピリジニルアクリルアミド骨格 A、ピロロピリジン骨格 B、ピリジニルトリアゾール骨格 C の 3 つの骨格を選択した（Figure 2-3）[10]。リンカー部位は、NAMPT の筒状の疎水性部位に配置される可能性が高いため、ニコチンアミド模倣部位とカルボラン部位の間に 4 つおよび 5 つの炭素鎖を導入した。最後にカルボラン部位には、その調製の容易さから *o*-カルボランを選択した。またカルボラン部位とリンカー部位をメチレン基（CH_2）、ピペリジルカルボニル基（Pip-CO）、アミド基（NHCO）とした。

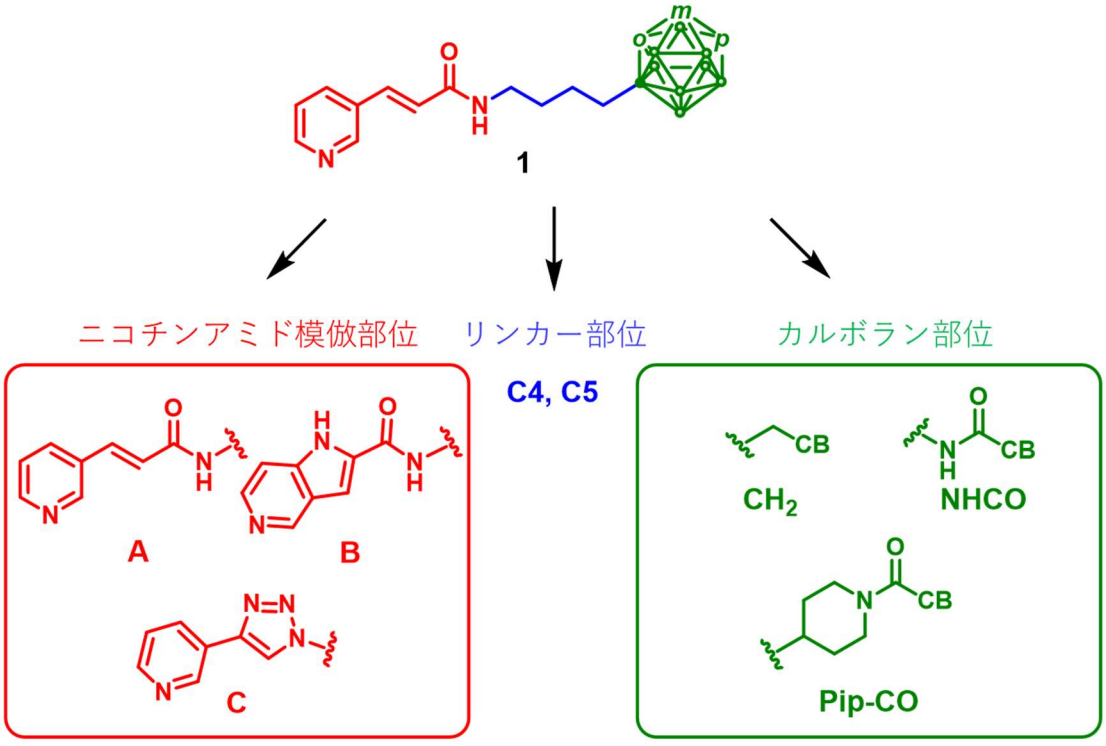


Figure 2-3. 化合物 **1** に基づいた新たなカルボラン含有 NAMPT 阻害剤の設計

化合物 **1** と FK866 において、それぞれメチレン基とピペリジルカルボニル基が使われており、アミド基は、ドッキングシミュレーションに基づいて採用した。Figure 2-4 に示すように、化合物 **2** のアミドカルボニル基と NAMPT の 191 番目のヒスチジン残基（H191）との間に水素結合相互作用が形成されていることが予測された（計算値は約 2.2Å）。H191 残基は、野生型 NAMPT の酵素活性に重要なアミノ酸残基であることが知られており、実際、H191R の変異により FK866 に対する耐性細胞株が得られている [19]。

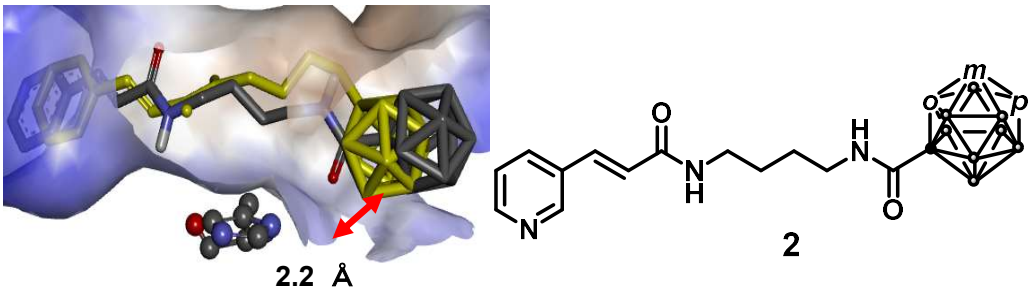
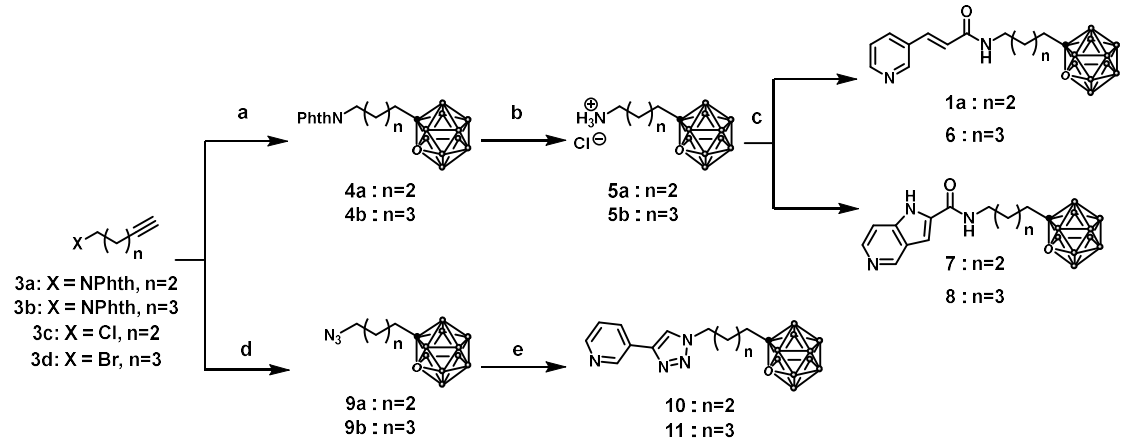


Figure 2-4. NAMPT の酵素活性部位と化合物 **2** のドッキングシミュレーション

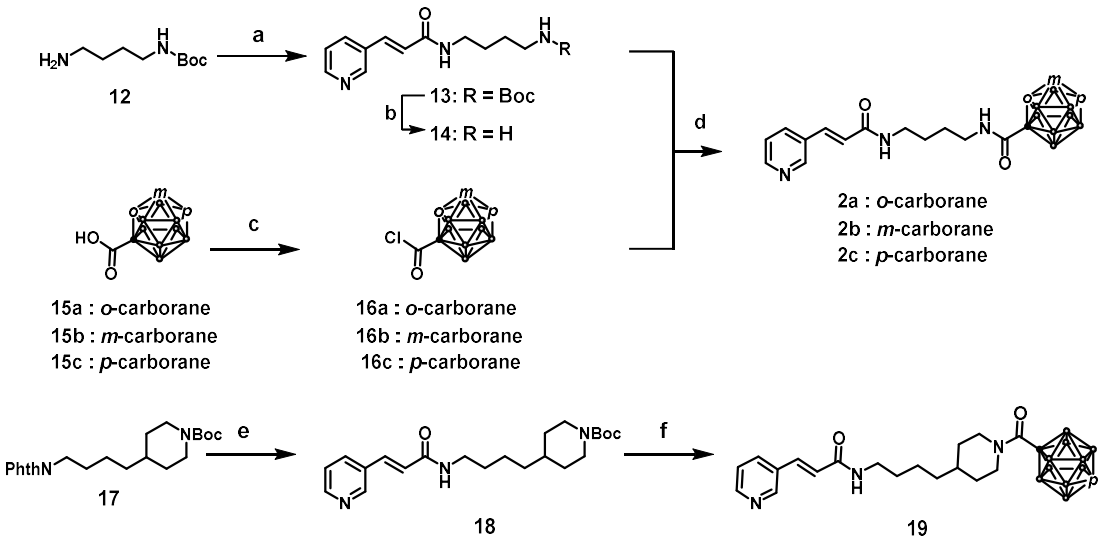
2-4　設計した分子の合成

Scheme 2-1 に示すように、アルキン **3** から化合物 **1a**、**2**、**6-8**、**10**、**11** を合成した。まず、**1a** を報告されている方法に従って合成した [5]。次に、アルキン **3a-b** をクロロベンゼン中、マイクロ波照射条件下でデカボランと反応させて，*o*-カルボラン誘導体 **4a-b** を合成した。$NaBH_4$ で還元した後、塩酸で処理すると、対応するアンモニウム塩 **5a-b** が得られた。アンモニウム塩 **5a-b** を(*E*)-3-(ピリジン-3-イル)アクリル酸または 1*H*-ピロロ[*3,2-c*]ピリジン-2-カルボン酸と縮合させ、**6-8** を得た。アルキン **3c-d** についても同様に *o*-carborane 誘導体を生成した後、NaN_3 で処理すると、対応するアジド **9a-b** が得られた。アジド **9a-b** を CuI 触媒の存在下で 3-ethynyl pyridine を用いて Hüisgen 環化付加反応を行うと、それぞれ対応するピリジニルトリアゾール **10** と **11** が得られた。



Scheme 2-1. 化合物 **1a**、**2**、**6-8**、**10**、**11** の合成 ; 反応条件 : (a) $B_{10}H_{14}$, *N,N*-dimethylaniline, chlorobenzene, MW, 130 ℃, 86%-quant.; (b) (i) $NaBH_4$, IPA/H_2O, r.t.; (ii) HCl, 80 ℃; (c) RCOOH, HATU, DIEA, DMF, r.t. 26-81% 2 steps; (d) (i) $B_{10}H_{14}$, *N,N*-dimethylaniline, chlorobenzene, MW, 130 ℃; (ii) NaN_3, DMF, 80 ℃, 61-79% 2 steps; (e) 3-ethynylpyridine, CuI, Na ascorbate, DMF/H_2O, 34-40%.

続いて、カルボランカルボン酸 **15a-c** から化合物 **2a-c** および **19** の合成を Scheme 2-2 に従って行った。アミン **12** を文献の手順に従って調製した [20]。アミン **12** を、1-（3-ジメチルアミノプロピル）-3-エチルカルボジイミド（EDCI）、1-ヒドロキシベンゾトリアゾール（HOBt）、N,N-ジイソプロピルエチルアミン（DIPEA）の存在下、(*E*)-4-(ピリジン-4-イル)アクリル酸と縮合させ、化合物 **13** を得た。**13** の *tert*-ブトキシカルボニル（Boc）基を脱保護した後、得られた **14** を酸塩化物 **16a-c**（カルボランカルボン酸 **15a-c** から調製）で処理した [21]。対応する *m* および *p*-カルボラン誘導体 **2b** および **2c** は良好な収率（それぞれ 43%および 63%、2 ステップ）で得られたが、*o*-カルボラン誘導体 **2a** は不安定で、おそらく *o*-カルボランが脱ホウ素化したために容易に分解されたと考えられる。*o*-カルボランは他の異性体と比べて分極が大きく、さらに 3 位のホウ素原子が電子吸引性のアミド基によって活性化され、化合物 **2a** の *o*-カルボランの求電子性が高まったと考えられる。このような現象は、近年 Hey Hawkins らのグループからも報告されている [22]。実際、脱ボロン化反応は ^{11}B NMR で経時的にモニターすることができた（Figure 2-5）。なお、*nido*-カルボランの生成は、-37 ppm （1 位の B）、-33 ppm（10 位の B）により確認した [23,24]。化合物 **17** を報告文献に従って調製し [25]、化合物 **13** の調製と同様の方法で(*E*)-4-(ピリジン-4-イル)アクリル酸と縮合させた。得られた **18** を Boc 基の脱保護後，*p*-カルボランカルボン酸 **15c** と反応させ、ピペリジルカルボニル *p*-カルボラン **19** を得た。



Scheme 2-2. 化合物 **2**、**19** の合成；反応条件：(a) (*E*)-3-(pyridin-3-yl)acrylic acid, EDCI, HOBt, DIPEA, THF, r.t., 82%; (b) HCl, dioxane, r.t.; (c) $(COCl)_2$, toluene, reflux; (d) TEA, DCM, r.t., 43-63% 2 steps; (e) (i) NH_2NH_2-H_2O, EtOH, reflux; (ii) (*E*)-3-(pyridin-3-yl)acrylic acid, EDCI, HOBt, THF, r.t. quant. 2 steps; (f) (i) HCl, dioxane, r.t.; (ii) **15c**, HATU, DIPEA, THF, r.t. 3.6%.

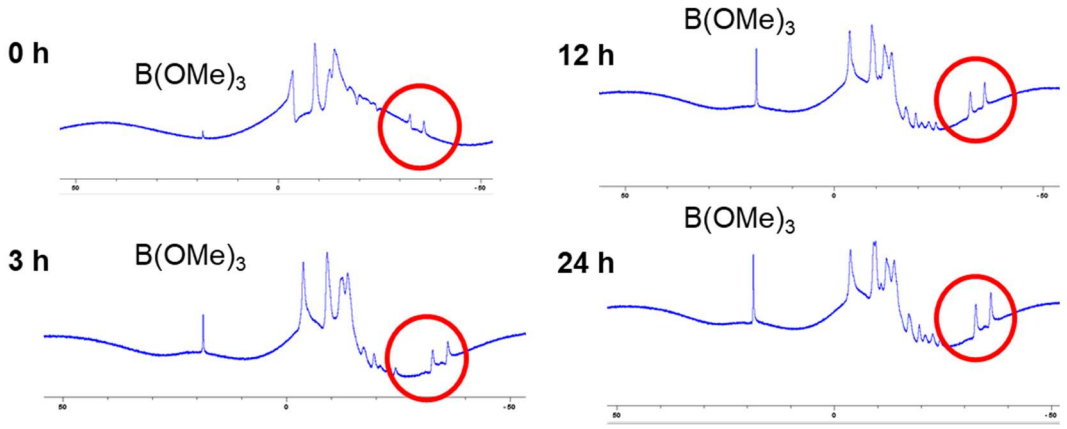
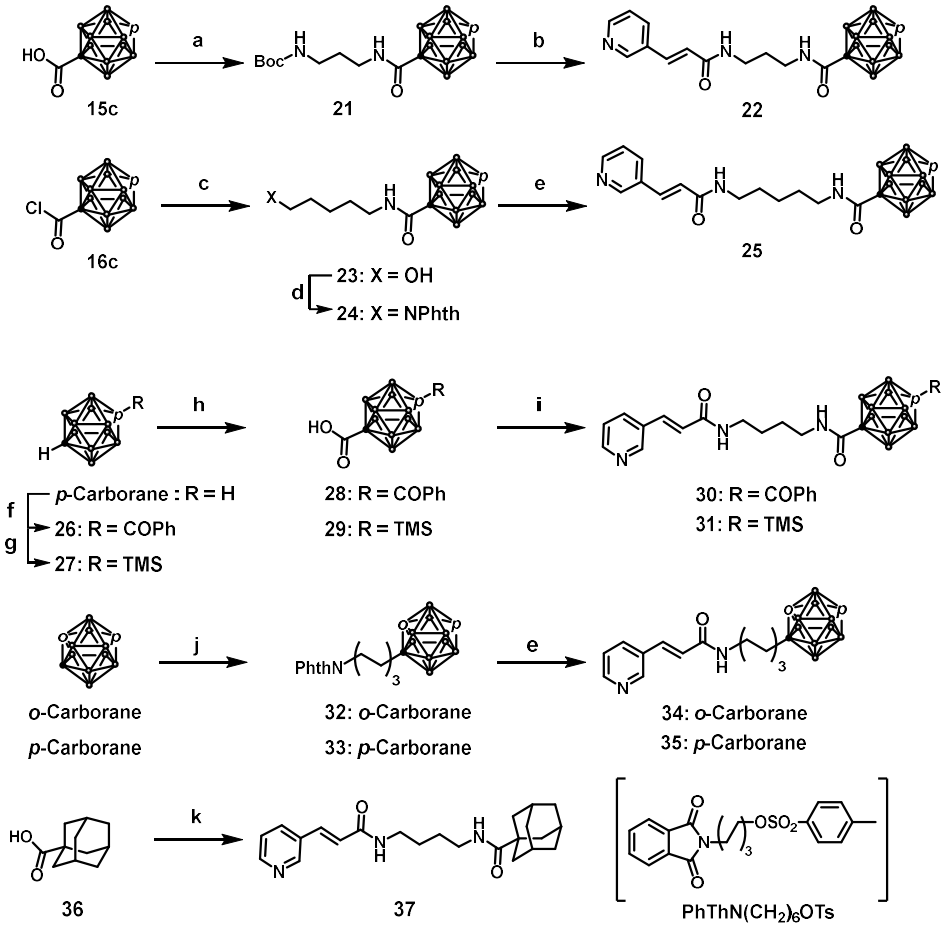


Figure 2-5. メタノール中における化合物 **3-14a** の時間依存的な分解を ^{11}B NMR によって解析；赤丸は *nido*-カルボランの 1 位の B（-37 ppm）、10 位の B（-33 ppm）を示す。

さらなる検討のため、Scheme 2-3 に従って化合物 **2c** の誘導体を設計・合成した。化合物 **22** は **2c** よりも炭素数が 1 つ短いタイプ、化合物 **25** は炭素数が 1 つ長いタイプである。化合物 **30** および **31** は，*p*-カーボランの *p*-位にそれぞれベンゾイルおよびトリメチルシリル（TMS）の置換基を有する。化合物 **34** および **35** は，ブチルアミドリンカー（C4）の代わりにヘキシルリンカー（C5）を有する *m*-および *p*-カルボラン誘導体である。さらに，カルボランとアダマンタンの立体電子的効果を比較するために，化合物 **37** も対応するアダマンチルカルボン酸 **36** から合成した。

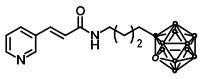
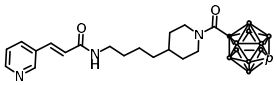
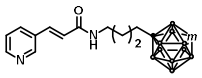
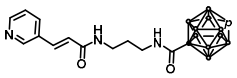
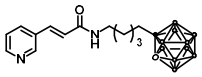
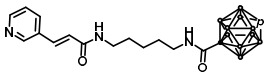
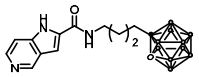
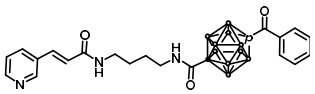
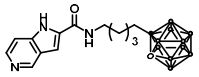
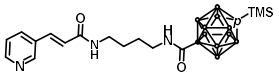
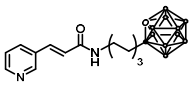
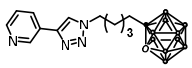
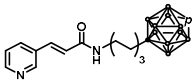
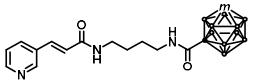
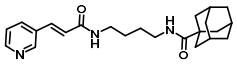
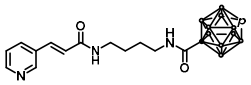
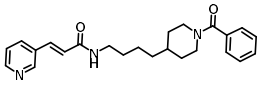


Scheme 2-3. 化合物 **2c** 誘導体の合成；反応条件：(a) **20**, HATU, DIPEA, THF, r.t.; (b) (i) HCl, dioxane, r.t.; (ii) (*E*)-3-(pyridin-3-yl)acrylic acid, HATU, DIPEA, THF, r.t., 41%, 3 steps; (c) 5-aminopentan-1-ol , TEA, DCM, r.t.; (d) Phthalimide, PPh_3, DIAD, THF, r.t.; (e) (i) NH_2NH_2-H_2O, EtOH, reflux; (ii) (*E*)-3-(pyridin-3-yl)acrylic acid, HATU, DIPEA, DMF, r.t. (**25**: 11%, 4 steps, **34**: 10% 3 steps, **35**: 12%, 3 steps); (f) *n*-BuLi, PhCOCl, THF, −78 °C to 0 °C, quant.; (g) *n*-BuLi, TMSCl, Et_2O, −78 °C to 0 °C; (h) diisopropylamine, *n*-BuLi, CO_2, THF, −78 °C to 0 °C; (i) **14**, HATU, DIPEA, DMF, r.t. (**30**: 43% 2 steps and **31**: 25%, 3 steps); (j) *n*-BuLi, PhthN$(CH_2)_6$OTs,[26] THF, −78 °C to 0 °C; (k) **14**, HATU, DIPEA, DMF, r.t., 30%.

2-5　合成した分子の NAMPT 阻害活性評価

NAMPT の酵素活性は、CycLex® NAMPT colorimetric assay kit を用いて評価した。その結果を Table 2-1 に示す。予想に反して、**6-11** は **1a** および **1b** よりも高い NAMPT に対する阻害活性を示さなかった。カルボランを含む NAMPT 阻害剤を設計する際には、ピリジルアクリルアミド（A）（Figure 2-3）をニコチンアミド模倣部位として選択することが最適であることが示された。次に、カルボランアミドを有する **2b**、**2c**、**19** の IC_{50} 値は、**1a**、**1b** や FK866 よりも有意に低かった。特に **2c** は最も優れた NAMPT 阻害活性を示し、FK866 の 10 倍の値を示した。これらの結果は、ドッキングシミュレーションで示唆されたように、His191 とカルボランアミドの間の水素結合相互作用によるものと考えられる（Figure 2-4）。また、これまでの研究とは異なり、*p*-カルボラン基をもつ化合物において *m*-カルボラン誘導体よりも 2 倍高い活性が認められた。これは、カルボランの分極による二重水素結合が、*p*-カルボランの効果にさらに寄与していることを意味している。一方、FK866 のフェニル基を *p*-カルボランで置換した化合物 **19** は、**1b** と同程度のレベル（IC_{50} = 0.32 ± 0.05 μM）で、FK866 の約 2 倍の有意な NAMPT 阻害活性を示した。これらの結果から、カルボラン骨格の疎水性に加えて 3 次元的な空間充填の影響が、NAMPT の疎水性ポケットに適していることが示唆された。次に、**2b** や **2c** のようにカルボランアミド結合を持つ化合物の中で、NAMPT 阻害活性を比較した。まず、NAMPT 阻害活性はリンカーの長さに大きく影響され、短いリンカーを持つ **22** の IC_{50} 値は有意に増加したが、長いリンカーを持つ **25** の IC_{50} 値は増加しなかったためである。これは、NAMPT の結合部位が円筒形をしており、リンカーが短い場合（**22**）、ニコチンアミド模倣部位が活性部位に到達できず、阻害活性がほとんど見られなかったためと考えられる。**30** の場合、長いリンカーは結合部位に収めることができたが、**30** のカルボランアミドと H191 との距離が長すぎて水素結合を作ることができなかった。そのため、**30** の NAMPT に対する親和性は **2c** のよりも低かったと考えられる。2 つ目に、**30** と **31** の NAMPT 阻害活性も、**2c** よりも低かった。この NAMPT との親和性の低下は、カルボラン上に導入した置換基の立体障害に起因すると考えられる。3 つ目に、**2c** と **35** の IC_{50} 値を比較すると、**35** の NAMPT 阻害活性は **2c** のそれよりも有意に低かった。この結果は、**2c** のアミド結合と NAMPT の H191 との間の水素結合が結合親和性を高めることを裏付けた。さらに、**35** の *p*-カルボランを *o*-カルボランに変更した **34** も同様の結果を示した。最後に、**2c** と **37** の比較から、カルボラン部位が NAMPT 阻害活性を高めることを解明した。**2c** は、アダマンタンを含む **37** よりも 100 倍強い阻害活性を持っていた。このように、**2c** は、適切なリンカー長、カルボラン部位を有すること、カルボランアミドと H191 の間に水素結合が存在することから、強力な NAMPT 阻害剤であることがわかった。

Table 2-1. 合成した化合物の NAMPT 阻害活性

Compound		NAMPT IC_{50} [μM]	Compound		NAMPT IC_{50} [μM]
	**1a**	0.61 ± 0.01		**19**	0.32 ± 0.05
	**1b**	0.37 ± 0.03		**22**	> 30
	**6**	± 0.2		**25**	0.17 ± 0.02
	**7**	> 30		**30**	0.63 ± 0.04
	**8**	> 30		**31**	0.18 ± 0.01
	**10**	> 30		**34**	0.37 ± 0.02
	**11**	> 30		**35**	0.25 ± 0.06
	**2b**	0.098 ± 0.008		**37**	8.0 ± 1
	**2c**	0.057 ± 0.001		FK 866	0.62 ± 0.05

各化合物はあらかじめ NAMPT と混合し、CycLex® kit の 1 step assay buffer を加えた。450nm の吸光度を測定し、化合物の NAMPT に対する阻害率 IC_{50} を算出した（n=3）。

2-6　合成した分子の細胞死誘導活性評価

続いて有意な NAMPT 阻害作用を示した化合物 **1a**、**2b**、**2c** の抗がん活性を調べるために、A549（ヒト肺胞基底上皮腺癌）、MDA-MB 468（ヒト乳癌）、HeLa（ヒト子宮頸癌）細胞に対する細胞生存率を MTT アッセイで評価した。その結果を Table 2-2 に示す。残念ながら、合成された化合物は試験したすべての細胞に対しても FK866 よりも高い細胞毒性を示さなかった。これは、**2b** または **2c** の水溶性がカルボランアミドによって損なわれ、その結果、細胞膜の透過性が低下したものと考えられる。一方、**2b** と **2c** の比較から、カルボラン異性体間の細胞毒性の違いは特定の細胞株に依存せず、細胞毒性は NAMPT 阻害活性に比例して増加することが示された。

Table 2-2. 合成した化合物の各細胞に対する抗がん活性評価

	A549 [nM]	HeLa [nM]	MDA-MB 468 [nM]
1a	17.8 ± 8	32.3 ± 7	21.2 ± 3
2b	14.4 ± 5	25.6 ± 7	11.8 ± 3
2c	3.77 ± 0.8	14.4 ± 2	7.19 ± 0.6
FK866	1.74 ± 0.5	3.33 ± 0.3	2.83 ± 0.4

2-7　ジカルボラン化合物の設計

化合物 **19** の NAMPT 阻害活性を FK866 と比較すると、フェニル基をカルボランに置換することで 2 倍程度活性が向上することがわかった（Table 2-1）。カルボランの 3 次元的な疎水性により、活性が向上したと考えられる。さらに、Figure 2-5A に示すように、**19** と NAMPT を用いて行ったドッキングシミュレーションでは、化合物 **1** のカルボランが **19** のピペリジン部位に位置することが示唆された。 また、C4-リンカーを有する **1a** の IC_{50} 値は 0.61±0.01 μM、C6-リンカーを有する **34** の IC_{50} 値は 0.37±0.02 μM であったのに対し、C5-リンカーを有する **6** の IC_{50} 値は 4.9±0.2 μM であった。この活性相関は興味深く、NAMPT のリガンド結合部位には 2 つの潜在的なカルボラン結合ポケットがあるのではないかと考えた。この仮説に基づいて、2 つのカルボランを含むジカルボラン化合物 **38** を設計した（Figure 2-5B）。ドッキングシミュレーションでは、**19** との良好なオーバーラップが確認された（Figure 2-5C）。また、**38** の各カルボランは、**1a** と **34** のカルボラン結合部位に位置していた（Figure 2-5D）。そこで、さらなる研究のために、ジカルボラン化合物を合成することにした。

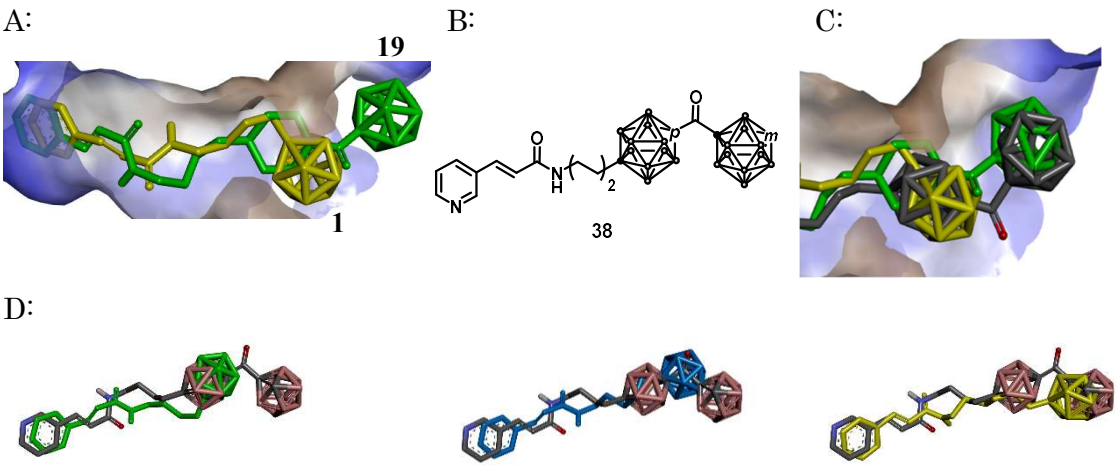


Figure 2-5. ドッキングシミュレーションによるジカルボラン化合物 **38** の設計: (A) 化合物 **1a** (yellow) と **19** (green)のドッキング配座比較 ; (B) 化合物 **38** の構造 ; (C) 化合物 **1a** (yellow)、**19** (green)、**38** (black)のドッキング配座比較 ; (D) 化合物 **38** と **1a** (green)、**6** (blue)、**34** (yellow)との重ね合わせ。

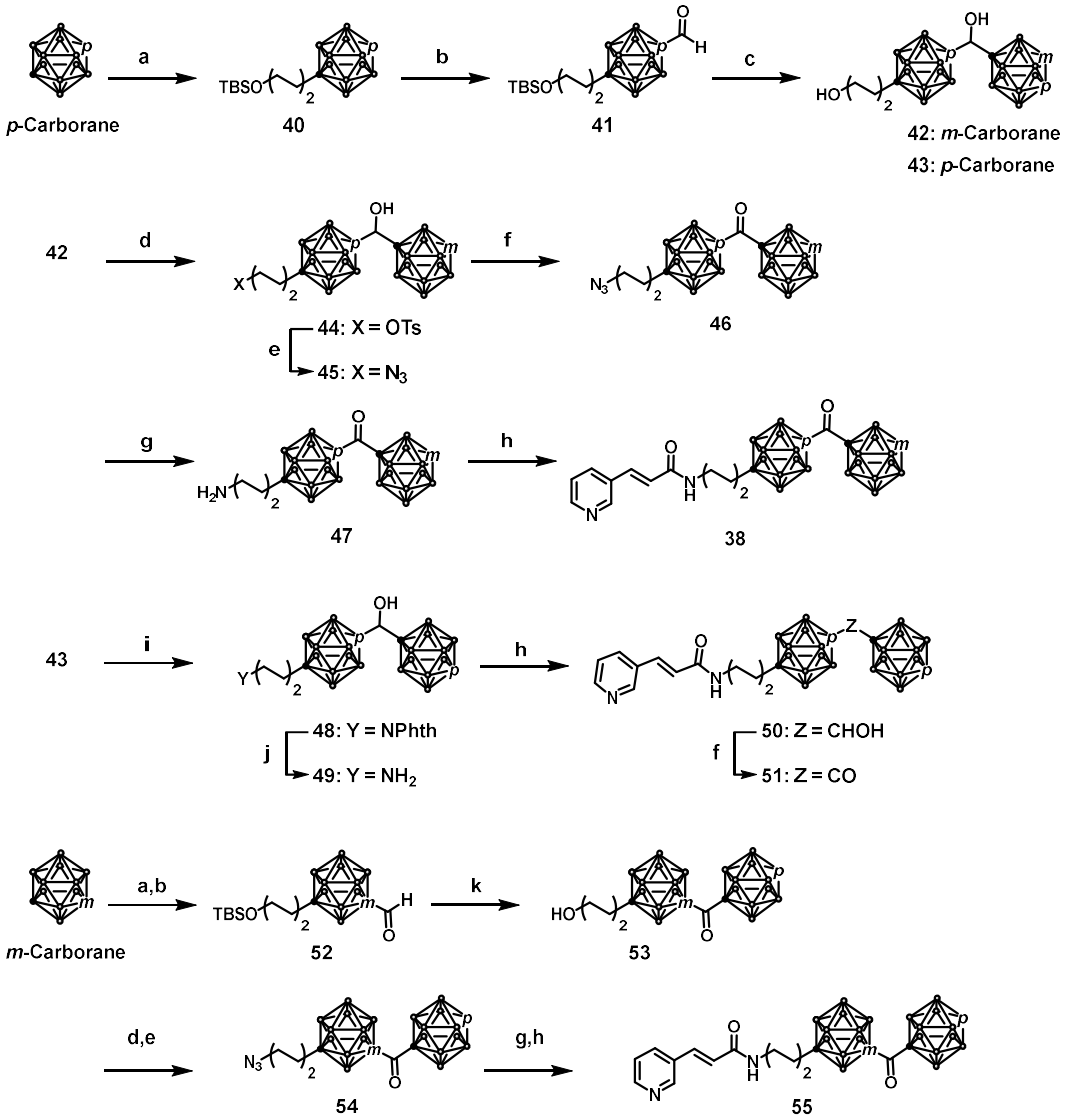
2-8　設計したジカルボラン化合物の合成と NAMPT 阻害活性評価

Scheme 2-4 に従って、化合物 **38**、**50**、**51**、**55** を合成した。まず、*p*-カルボランを THF 中で *n*-BuLi で処理し，リチオ化 *p*-カルボランを生成した。これを tert-butyl(4-iodobutoxy)dimethylsilane (**39**)と反応させ、化合物 **40** を得た。続いて THF 中で **40** を *n*-BuLi でリチオ化した後、ギ酸メチルでホルミル化して **41** を得た。これをリチオ化した *m*-または *p*-カルボランと反応させた後、塩酸で処理すると、TBS 基が脱保護されたジカルボラン誘導体 **42** と **43** がそれぞれ得られた。**42** の一級アルコールをトシル化した後、アジドで置換し、得られたアジド **45** を Dess-Martin Periodinane で酸化して、ジカルボラン **46** を得た。**46** のアジドをアミノ基に Staudinger 還元した後、得られたアミン **47** を(*E*)-4-(ピリジン-4-イル)アクリル酸と縮合させ、ジカルボラニルケトン **38** を得た。一方，*p*-カルボラン誘導体 **43** を光延反応によりフタルイミドに変換して化合物 **48** を得た後、ヒドラジンを用いて対応するアミン **49** に変換した。アミン **49** を(*E*)-4-(ピリジン-4-イル)アクリル酸と縮合させ、ジカルボラニルアルコール **50** を得た。これを酸化してジカルボラニルケトン **51** を得た。同様にして、*p*-カルボランの代わりに *m*-カルボランがリンカーに結合したジカルボラニルケトン **55** も合成した。

次に、合成した化合物の NAMPT 阻害活性を評価した（Table 2-3）。まず、ジカルボラン化合物が μM オーダーの NAMPT 阻害活性を示したことから、2 つの疎水性ポケットが実際に存在していることが明らかになった。しかし、これらの化合物は FK866 や **1a** よりも活性が低く、2 つのカルボランの正しい配置が重要であると考えた。実際、2 つのカルボランを柔軟な第 2 級アルコールリンカーによって結合した **50** の活性は、より剛直なカルボニルリンカーをもつ **51** の活性よりも優れていた。興味深いことに、**38** と **51** を比較すると、末端のカルボランがメタ異性体である **38** の NAMPT 阻害活性は、パラ異性体である **51** の NAMPT 阻害活性よりも高かった。これは、カルボランの異性体がタンパク質と相互作用する方法の違い、特に二重水素結合を介した方法の違いを示唆していると考えられる。最後に、化合物 **55** は最も活性の高いジカルボラン化合物であったのに対して、リンカーに結合したカルボランがパラ異性体である **51** では活性が大幅に低減した。この結果から、NAMPT 阻害活性には末端のカルボランの配置が重要であることだけでなく、カルボランの異性体を駆使することで、カルボラン上の置換基の立体配置を厳密に制御できることが示唆された。

Table 2-3. 合成したジカルボラン化合物の NAMPT 阻害活性

Compound	IC_{50} [μM]
1a	0.61 ± 0.01
38	3.76 ± 0.5
50	4.51 ± 0.9
51	> 10
55	2.92 ± 0.5



Scheme 2-4. 設計したジカルボラン化合物の合成；反応条件：(a) **39**, *n*-BuLi, THF, 0 ℃; (b) *n*-BuLi, HCO_2CH_3, THF, -78 ℃; (c) (i) Carborane, *n*-BuLi, Et_2O, -78 ℃ to 0 ℃; (ii) HCl, dioxane, r.t.; (d) TsCl, TEA, DMAP, DCM, r.t.; (e) NaN_3, DMF, 80 ℃, 11%, 6 steps; (f) Dess-Martin periodinane, DCM, r.t.; 53%; (g) PPh_3, THF/H_2O, r.t.; (h) (*E*)-4-(pyridin-4-yl)acrylic acid, EDCI, HOBt, DIPEA, THF, r.t., (**38**: 43%, 3 steps, **50**: 29%, 3 steps, **55**: 37%, 7 steps; (i) Phthalimide, PPh_3, DIAD, THF, r.t.; (j) NH_2NH_2-H_2O, EtOH, reflux; (k) (i) *p*-Carborane, *n*-BuLi, Et_2O, -78 ℃ to 0 ℃; (ii) Dess-Martin periodinane, DCM, r.t. (iii) HCl, dioxane, r.t.

2-9　まとめ

本章では、NAMPT 阻害剤として一連のカルボラン誘導体を設計・合成した。合成した化合物の中では、**2c** が最も強力な NAMPT 阻害剤であり、FK866 の 10 倍以上の阻害活性をもつことを見出した（Figure 2-6）。ドッキングシミュレーションを行った結果、**2c** のカルボランアミドと NAMPT の H191 との間に水素結合が形成されることで、**2c** の NAMPT 阻害活性が向上すると予測された。実際、カルボランアミドを含まない化合物 **35** は、**2c** に比べて約 4 倍の活性を示したことから、高い NAMPT 阻害活性にはカルボランアミド部分が重要であることがわかった。また、カルボランの 3 次元的な疎水性により、活性が向上したと考えられた（**2c** と **37** の比較）。**2c** を用いて構造活性相関研究をさらに進めると、(1) 炭素鎖 4 本のリンカーが最適であること、(2) 柔軟性とカルボランの位置決めのためにカルボランの周りに少し空間が必要であること、(3) カルボランのアミド骨格が H191 と水素結合を確立するために必要であること、を明らかにした。さらにドッキングシミュレーションによって設計された 2 つの疎水性ポケットを充填するジカルボラン化合物 **38**、**50**、**51**、**55** は、中程度の NAMPT 阻害活性を示したものの、**2c** に比べて効果が低かった。しかし、これらの結果は、NAMPT の基質結合部位に 2 つの疎水性ポケットを有することを実証しただけでなく、カルボランの異性体を利用することで、厳密な置換基配置制御が可能であることを示した。本研究で得られた知見は、NAMPT 阻害剤のさらなる設計に有用なだけでなく、カルボランを用いた医薬品開発にも役立つものである。

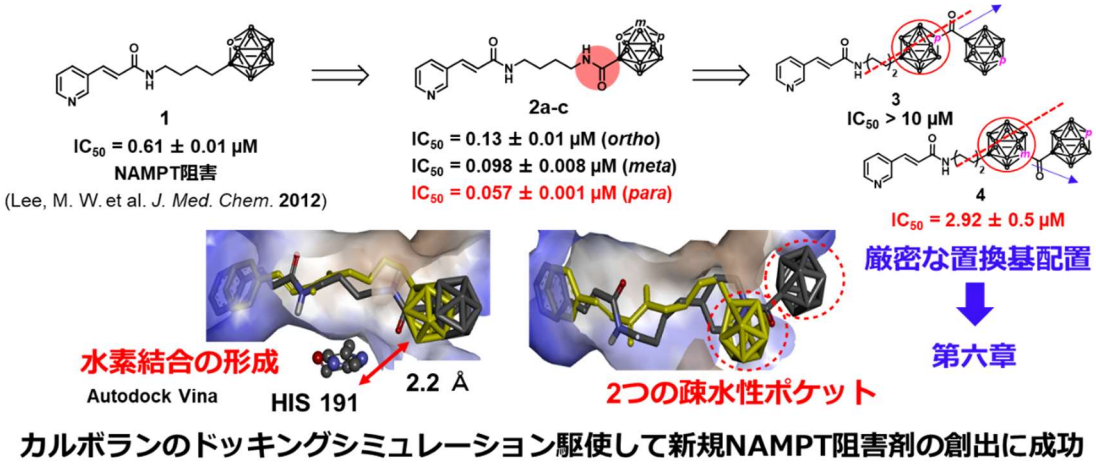


Figure 2-6. 第二章まとめ

Reference

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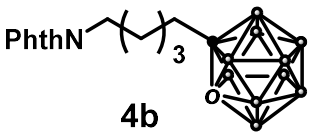
Experimental Section

Synthesis

General Methods

NMR spectra were recorded on a Bruker biospin AVANCE II (400 MHz for 1H, 100 MHz for 13C) or a Bruker biospin AVANCE III (500 MHz for 1H, 125 MHz for 13C) instrument in the indicated solvent. Chemical shifts are reported in units per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane for solutions in $CDCl_3$ (7.26 ppm for 1H, 77.16 ppm for 13C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, q; quartet, m; multiplet, br; broad, J; coupling constants in Hertz. Mass spectra were measured using a JMS-700 Mstation. HRMS (EI, 70 eV) was calibrated as perfluorokerosene. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light (254 nm) and were visualized using an aqueous alkaline $KMnO_4$ solution, ninhydrin AcOH solution and/or *p*-anisaldehyde EtOH solution. Column chromatography was performed on Silica Gel 60 N, purchased from Fuji Silysia Chemical Ltd. Microwave-assisted synthesis were performed on microwave synthesizer (Biotage, Initiator). Compound **1a** was known and synthesized from **3a** according to the literature procedures.

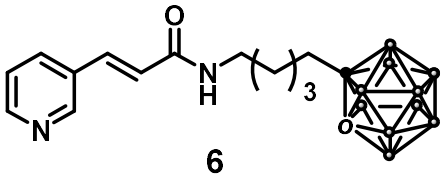
Synthesis of 1-(Phtalimido-*N*-pentyl)-1,2-dicarba-*closo*-dodecaborane (4b)



To a solution of alkyne **3b** (131 mg, 0.541 mmol) and *N,N*-dimethylaniline (103 μL, 0.812 mmol) in chlorobenzene, was added decaborane (79 mg, 0.649 mmol). The resulting mixture was stirred at 130 ℃ under argon atmosphere for 10 min with microwave synthesizer. The resulting mixture was added 1 M HCl and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude materials were purified by column chromatography on silica gel (40% EtOAc in hexane) afforded **4b** as a white solid (184 mg, 0.531 mmol, quant.). ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.85-7.83 (m, 2H), 7.73-7.71 (m, 2H), 3.67 (t, J = 5.8 Hz, 2H), 3.56 (s, 1H), 2.19 (t, J = 6.0 Hz, 2H), 1.67 (q, J = 6.0 Hz, 2H), 1.51 (q, J = 6.0 Hz, 2H),1.31 (q, J = 6.0 Hz, 2H), 2.5-1.6 (m, 10H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 168.5, 134.1, 132.2, 123.4, 75.2, 61.1, 38.0, 37.6, 28.8, 28.2, 26.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.36, -5.824, -9.35, -11.5, -12.3, -13.2; HRMS (ESI, pos) for $C_{15}H_{25}B_{10}NO_2$

(m/z): calcd 382.2788 $(M+Na)^+$, found 303.2796.

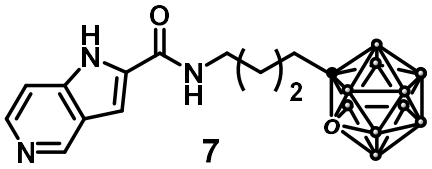
Synthesis of 1-((*E*)-4-(3-(pyridin-3-yl)acrylamido)pentyl)-1,2-dicarba-*closo*-dodecaborane (6)



6

To a solution of phthalimide **4b** (84 mg, 0.234 mmol) in 2-propanol: water (6:1, v/v), was added $NaBH_4$ (44 mg, 1.17 mmol). The resulting mixture was reacted at room temperature until the full conversion of a starting material was observed. After which, 12 M HCl aq. was slowly added and stirred at 80 ℃ for 6 h under argon atmosphere. The reaction mixture was concentrated under vacuum. The resulting ammonium salt **5b** was dissolved in water and washed twice with CH_2Cl_2. The aqueous phase was concentrated under vacuum, and the residue was dissolved in ethanol and filtered through a small cotton plug. After removal of ethanol under vacuum, the crude product was used for the next step without further purification. To a solution of the crude **5b**, (*E*)-3-(pyridin-3-yl)acrylic acid (35 mg, 0.234 mmol), and 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) (89 mg, 0.234 mmol) in THF was slowly added *N,N*-diisopropylethylamine (DIEA) (102 μL, 0.585 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **6** as a white solid (20 mg, 0.0554 mmol, 27% from **4b**). m.p. 167-168 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.75 (s, 1H), 8.58 (d, J = 2.2 Hz, 1H), 7.78 (d, J = 4.0 Hz, 1H), 7.62 (d, J = 8.0 Hz, 1H), 7.33-7.30 (m. 1H), 6.43 (d, J = 8.0 Hz, 1H), 5.67 (s. 1H), 3.57 (s. 1H), 3.38 (ddd, J = 10, 5.0 Hz, 2H), 2.21 (t, J = 7.5 Hz, 2H), 1.61-1.50 (m. 4H), 1.37-1.33 (m. 2H), 2.5-1.9 (br. 10H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 165.3, 150.6, 149.3, 137.8, 134.5, 130.7, 123.8, 122.6, 61.17, 39.6, 38.1, 29.8, 29.5, 29.0, 26.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.29, -5.77, -9.33, -11.5, -12.2, -13.2; HRMS (ESI, pos) for $C_{15}H_{28}B_{10}N_2O$ (m/z): calcd 383.3104 $(M+Na)^+$, found 383.3108.

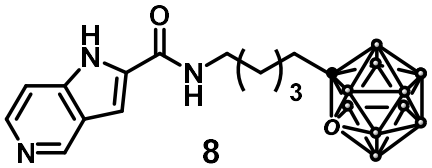
Synthesis of 1-(5-(1*H*-Pyrrolo[3,2-*c*]pyridine-2-carboxamido)butyl)-1,2-dicarba-closo-dodecaborane (7)



7

This compound was prepared from compound **4a** (42 mg, 0.123 mmol) and 1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (20 mg, 0.123 mmol) using the procedure described for **6**. Purification by column chromatography (10% MeOH in CH_2Cl_2) gave **7** in 62% yield as amorphous. ^{1}H NMR (500 MHz; CD_3CN): δ 8.92 (s, 1H), 8.26 (d, *J* = 6.0 Hz, 1H), 7.40 (d, *J* = 5.0 Hz, 1H), 7.06 (s, 1H), 4.12 (s, 1H), 3.34 (t, *J* = 6.0 Hz, 2H), 2.25 (t, *J* = 8.0 Hz, 2H), 1.94-1.91 (m, 2H), 1.53-1.48 (m, 2H), 2.5-1.6 (br. 10H); ^{13}C NMR (125 MHz; CD_3CN): δ161.8, 146.2, 143.0, 140.5, 133.8, 125.9, 108.0, 102.1, 77.4, 63.3, 39.2, 37.7, 29.4, 27.3; ^{11}B NMR (160 MHz; CD_3CN): δ -3.03, -6.31, -9.88, -11.60, -12.02, -13.26; ; HRMS (ESI, pos) for $C_{15}H_{26}B_{10}N_2O_1$ (m/z): calcd 361.3072 $(M+H)^+$, found 361.3070.

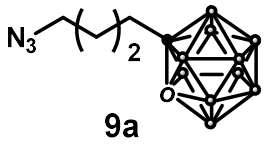
Synthesis of 1-(5-(*1H*-Pyrrolo[3,2-*c*]pyridine-2-carboxamido)pentyl)-1,2-dicarba-*closo*-dodecaborane (8)



8

This compound was prepared from compound **4b** (18 mg, 52.4 μmol) and 1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (10 mg, 52.4 μmol) using the procedure described for **6**. Purification by column chromatography (10% MeOH in CH_2Cl_2) gave **8** in 26 % yield as a white solid. m.p. 148-149 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.83 (bs, 1H), 9.00 (s, 1H), 8.35 (d, *J* = 5.5 Hz, 1H), 7.36 (d, *J* = 6.0 Hz, 1H), 6.91 (s, 2H), 6.36 (s, 1H), 3.57 (s, 1H), 3.52-3.48 (m, 2H), 2.21 (t, *J* = 5.6 Hz, 2H) 1.66 (q, *J* = 7.6 Hz, 2H), 1.53 (q, *J* = 8.0 Hz, 2H), 1.38 (q, *J* = 8.5 Hz, 2H), 2.5-1.9 (br. 10H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 165.4, 150.6, 149.3, 138.0, 134.6, 130.6, 123.9, 122.5, 75.1, 61.4, 39.0, 37.6, 29.2, 26.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.17, -5.60, -9.25, -11.5, -12.2, -13.1; HRMS (ESI, pos) for $C_{15}H_{27}B_{10}N_3O_1$ (m/z): calcd 374.3237 $(M+H)^+$, found 374.3238.

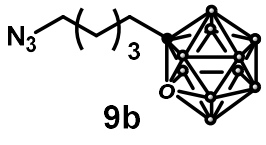
Synthesis of 1-(4-Azidebutyl)-1,2-dicarba-*closo*-dodecaborane (9a)



9a

To a solution of alkyne **3c** (48 µL, 0.400 mmol) and *N,N*-dimethylaniline (76 µL, 0.600 mmol) in chlorobenzene, was added decaborane (57 mg, 0.480 mmol). The resulting mixture was stirred at 130 °C under argon atmosphere for 10 min with microwave synthesizer. The resulting mixture was added 1 M HCl and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The resulting crude material was used for the next step without further purification. To a solution of the crude material in dimethylformamide (DMF), was added sodium azide (78 mg, 1.20 mmol) at room temperature and then the mixture was stirred at 80 °C under argon atmosphere for 12 h. The reaction was quenched with water and the mixture was extracted with Et_2O, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (30% EtOAc in hexane) to afford azide **9a** as color less oil (55 mg, 0.228 mmol, 61%). ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.57 (s, 1H), 3.30 (s, 2H), 2.25-2.21 (m, 2H), 1.56 (m, 4H), 2.80-1.50 (m, 10H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 75.3, 61.7, 51.4, 38.2, 28.8, 27.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.17, -5.60, -9.28, -11.5, -12.2, -13.1; HRMS (ESI, nega) for $C_6H_{19}B_{10}N_3$ (m/z): calcd 278.2241 $(M+Cl)^-$, found 278.2242.

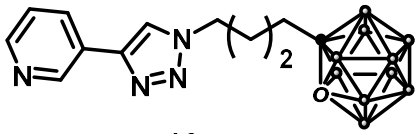
Synthesis of 1-(5-Azidepentyl)-1,2-dicarba-closo-dodecaborane (9b)



9b

This compound was prepared from compound **3d** (100 mg, 0.571 mmol) and decaborane (84 mg, 0.685 mmol) using the procedure described for **9a**. Purification by column chromatography (30% EtOAc in hexane) gave **9b** in 79% yield as a colorless liquid. ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.56 (s, 1H), 3.27 (t, J = 6.6 Hz, 2H), 2.21 (dd, J = 8.5 Hz, 17 Hz, 2H), 1.61-1.56 (m, 2H), 1.53-1.47 (m, 2H), 1.38-1.34 (m, 2H) ; ^{13}C NMR (125 MHz; $CDCl_3$): δ 75.1, 61.2, 51.2, 38.1, 28.9, 28.6, 26.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.17, -5.60, -9.28, -11.5, -12.2, -13.1; HRMS (ESI, nega) for $C_7H_{21}B_{10}N_3$ (m/z): calcd 254.2658 $(M-H)^-$, found 254.2651.

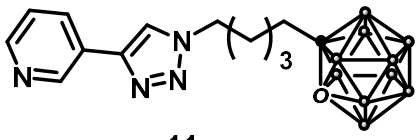
Synthesis of 1-(4-(4-(Pyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)butyl)-1,2-dicarba-*closo*-dodecaborane (10)



10

To a solution of 3-ethynylpyridine (17 mg, 0.164 mmol) in DMF:H_2O (4:1, v/v), were added CuI (3.5 mg, 6.85 μmol), sodium ascorbate (7.2 mg, 0.0137 mmol) and azide **9a** (33mg, 0.137 mmol). The mixture was stirred at room temperature under argon atmosphere for 8 h. The reaction was quenched with water and the mixture was extracted with CH_2Cl_2, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (100% EtOAc) to afford **10** as a white solid (16.1 mg, 0.0467 mmol, 34%). m.p. 150-151 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.99 (d, *J* = 1.8 Hz, 1H), 8.59 (dd, *J* = 1.8 Hz, 6.0 Hz, 1H), 8.20 (td, *J* = 2.4 Hz, 6.0 Hz, 1H), 7.83 (s, 1H), 7.39 (dd, *J* = 6.0 Hz, 9.6 Hz, 1H), 4.43 (t, *J* = 8.7 Hz, 2H), 3.59 (s, 1H), 2.27 (dd, *J* = 11 Hz, 21 Hz, 2H), 2.00-1.93 (m, 2H), 1.61-1.53 (m, 2H), 3.00-1.50 (m, 10H).^{13}C NMR (125 MHz; $CDCl_3$): δ 149.5, 147.2, 145.1, 133.1, 126.7, 123.9, 119.9, 74.5, 61.3, 49.8, 37.4, 29.6, 26.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.24, -5.65, -9.24, -11.6, -12.2, -13.0; HRMS (ESI, pos) for $C_{13}H_{24}B_{10}N_4$ (m/z): calcd 345.3083 $(M+H)^+$, found 345.3091.

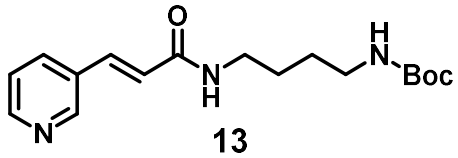
Synthesis of 1-(4-(4-(Pyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)pentyl)-1,2-dicarba-*closo*-dodecaborane (11)



11

This compound was prepared from azide **9b** (58 mg, 0.198 mmol) and 3-ethynylpyridine (25 mg, 0.238 mmol) using the procedure described for **10**. Purification by column chromatography (100% EtOAc) gave **10** in 40% yield as a white solid. m.p. 85-86 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.00 (d, *J* = 1.6 Hz, 1H), 8.60 (dd, *J* = 1.6 Hz, 4.8 Hz, 1H), 8.22 (td, *J* = 1.9 Hz, 6.0 Hz, 1H), 7.85 (s, 1H), 7.40 (dd, *J* = 4.8 Hz, 8.0 Hz, 1H), 4.44 (t, *J* = 7.0 Hz, 2H), 3.58 (s, 1H), 2.20 (dd, *J* = 8.7 Hz, 17 Hz, 2H), 2.00 (tt, *J* = 7.7 Hz, 7.7 Hz, 2H), 1.57-1.50 (m, 2H), 1.38-1.33 (m, 2H), 3.00-1.60 (m, 10H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 149.4, 147.1, 145.0, 133.1, 126.8, 124.0, 119.9, 74.9, 61.3, 50.3, 37.9, 30.0, 28.8, 26.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.24, -5.65, -9.24, -11.6, -12.2, -13.0; HRMS (ESI, pos) for $C_{14}H_{26}B_{10}N_4$ (m/z): calcd 381.3060 $(M+Na)^+$, found 381.3056.

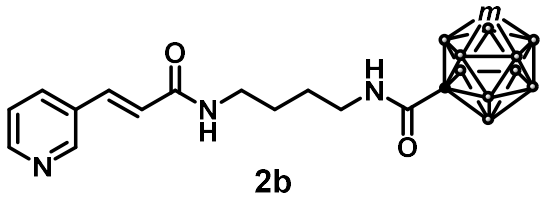
Synthesis of *tert*-Butyl (*E*)-(4-(3-(pyridin-3-yl)acrylamido)butyl)carbamate (13)



13

To a solution of **12** (226 mg, 1.20 mmol), carboxylic acid (149 mg, 1.00 mmol), and HATU (380 mg, 1.00 mmol) in THF (3 mL) was slowly added DIEA (261 μL, 1.50 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **13** (390 mg, quant.) as a white solid. ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.72 (s, 1H), 8.53 (d, J = 2.2 Hz, 1H), 8.07 (d, J = 4.0 Hz, 1H), 7.57-7.49 (m, 2H), 6.74 (d, J = 8.0 Hz, 1H), 3.76-3.70 (m, 2H), 3.42-3.37 (m, 2H), 1.63-1.55 (m, 4H), 1.44 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 165.4, 156.4, 150.4, 149.2, 137.1, 134.4, 130.9, 123.8, 123.2, 79.5, 40.1, 39.6, 28.5, 28.0, 28.0, 26.2; HRMS (ESI, pos) for $C_{17}H_{25}N_3O_3$ (m/z): calcd 342.1788 $(M+Na)^+$, found 342.1787.

Synthesis of (*E*)-*N*-(4-(1,7-Dicarba-closo-dodecaboranyl)amidebutyl)-3-(pyridin-3-yl)acrylamide (2b)



2b

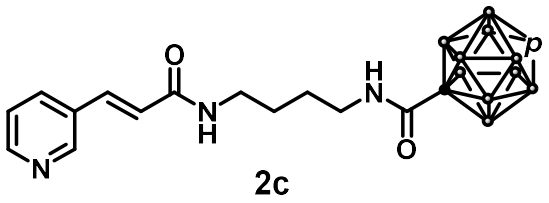
To a solution of the dicarba-*closo*-dodecaborane-1-acetic acid **15b** (43 mg, 0.221 mmol) in toluene, was slowly added excess oxalyl chloride. After the resulting mixture was stirred at 60 ℃ under argon atmosphere for 12 h, the reaction mixture was concentrated under pressure. The crude materials **16b** were used to the next reaction without further purification.

In another flask, to a solution of **13** (47 mg, 0.147 mmol) in CH_2Cl_2, was slowly added TFA in dioxane. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. After evaporation of the solvent, **14** was used to the next reaction without further purification.

To a solution of the unpurified amine **14** and triethylamine (60 μL, 0.441 mmol) in CH_2Cl_2, was slowly added dicarba-*closo*-dodecaborane-1-acetyl chloride (**16b**) in CH_2Cl_2 at 0 ℃. After the

resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, the resulting mixture was added NH_4Cl aq. and CH_2Cl_2 after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. Then, the crude materials were purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **2b** as a white solid (24.4 mg, 0.063 mmol, 43%). m.p. 131-132 ℃; ^{1}H NMR (500 MHz; CD_3OD): δ 8.71 (s, 1H), 8.51 (dd, *J* = 5.0 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 16 Hz, 1H), 7.47 (dd, *J* = 5.0 Hz, 8.0 Hz, 1H), 6.72 (d, *J* = 16 Hz, 1H), 3.65 (s, 1H), 3.27 (t, *J* = 5.5 Hz, 2H), 3.13 (t, *J* = 7.8 Hz, 2H), 1.48-1.45 (m, 4H), 1.53-1.52 (m, 4H), 2.87-1.59 (m, 10H); ^{13}C NMR (125 MHz; CD_3OD): δ 167.7, 162.5, 150.7, 149.7, 137.4, 136.2, 132.9, 125.5, 124.8, 77.4, 56.8, 41.3, 40.1, 27.6, 27.5; ^{11}B NMR (160 MHz; CD_3OD): δ -5.61, -7.45, -11.1, -11.6, -13.4, -15.6; HRMS (ESI, pos) for $C_{15}H_{27}B_{10}N_3O_2$ (m/z): calcd 390.3187 $(M+H)^+$, found 390.3195.

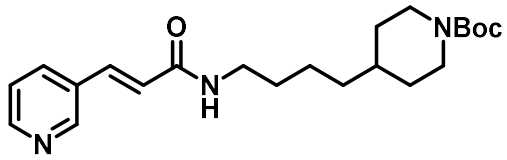
Synthesis of (*E*)-*N*-(4-(1,12-Dicarba-closo-dodecaboranyl)amidebutyl)-3-(pyridin-3-yl)acrylamide (2c)



2c

Preparation and purification of compound **2c** was carried out according to the procedure of compound **2b** (Yield: 63%). m.p. 173-174 ℃; ^{1}H NMR (500 MHz; CD_3OD): δ 8.70 (d, *J* = 2.0 Hz, 1H), 8.51 (dd, *J* = 1.3 Hz, 4.9 Hz, 1H), 8.03 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 16 Hz, 1H), 7.47 (dd, *J* = 1.3 Hz, 8.0 Hz, 1H), 6.70 (d, *J* = 16 Hz, 1H), 3.35 (s, 1H), 3.27-3.25 (m, 2H), 3.27 (t, *J* = 13 Hz, 2H), 3.07 (t, *J* = 13 Hz, 2H), 1.47-1.46 (m, 2H), 2.87-1.59 (m, 10H); ^{13}C NMR (125 MHz; CD_3OD): δ 166.4, 163.5, 149.3, 148.3, 136.0, 134.9, 131.5, 124.2, 123.5, 54.5, 38.9, 38.7, 30.3, 26.2, 26.1; ^{11}B NMR (160 MHz; CD_3OD): δ -13.6, -15.2; HRMS (ESI, pos) for $C_{15}H_{27}B_{10}N_3O_2$ (m/z): calcd 413.2975 $(M+Na)^+$, found 413.2966.

Synthesis of *tert*-Butyl (*E*)-4-(4-(3-(pyridin-3-yl)acrylamido)butyl)piperidine-1-carboxylate (18)

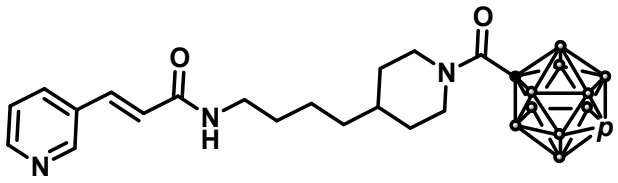


18

To a solution of **17** (204 mg, 0.528 mmol) in EtOH (6.3 mL) was added hydrazine monohydrate (70 μL, 1.32 mmol). The resulting mixture was stirred at reflux temperature under argon atmosphere until the full conversion of a starting material was observed. After evaporation of the solvent, to the reaction mixture were added water and CH_2Cl_2. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of adamantane-1-carboxylic acid (79 mg, 0.528 mmol), HATU (95 mg, 0.634 mmol) in THF (1.5 mL) was slowly added DIEA (138 μL, 0.792 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (5% MeOH in CH_2Cl_2) afforded **18** as a white solid (231 mg, 0.596 mmol, quant.). ^{1}H NMR (400 MHz; $CDCl_3$): δ 8.72 (s, 1H), 8.53 (d, *J* = 2.2 Hz, 1H), 8.07 (d, *J* = 4.0 Hz, 1H), 7.57-7.49 (m. 2H), 6.74 (d, *J* = 8.0 Hz, 1H), 3.76-3.70 (m, 2H), 3.42-3.37 (m, 2H), 1.63-1.55 (m, 4H), 1.44 (s, 9H); HRMS (ESI, pos) for $C_{22}H_{33}N_3O_3$ (m/z): calcd 410.2414 $(M+Na)^+$, found 410.2420.

Synthesis of (*E*)-*N*-(4-(1-(1,12-Dicarba-*closo*-dodecaboranyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (19)

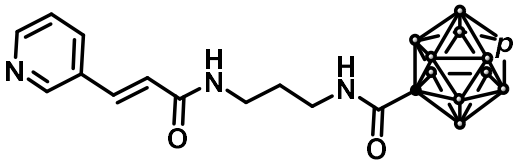


19

To a solution of 4 N HCl in dioxane was added **18** (54.0 mg, 0.139 mmol). The resulting mixture was stirred at room temperature for 12 h under argon atmosphere. The resulting mixture was concentrated under vacuum. It was used to the next step without further purification.

To a solution of the crude material, **15c** (32 mg, 0.167 mmol), and HATU (63 mg, 0.167 mmol) in THF was slowly added DIEA (50 μL, 0.278 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **19** as a solid (2.3 mg, 0.0050 mmol, 3.6% 2 steps). m.p. 138-139 °C; ^{1}H NMR (500 MHz; CD_3OD): δ 8.71 (d, *J* = 2.0 Hz, 1H), 8.51 (dd, *J* = 2.0 Hz, 4.9 Hz, 1H), 8.04 (dt, *J* = 2.0 Hz, 8.0 Hz 1H), 7.53 (d, *J* = 20 Hz, 1H), 7.47 (dd, *J* = 4.9 Hz, 8.0 Hz, 1H), 6.72 (d, *J* = 20 Hz, 1H), 4.35 (d, *J* = 10 Hz, 2H) 3.45 (s, 1H), 3.30-3.29 (m, 3H), 2.77 (t, *J* = 12.5 Hz, 2H), 1.72 (d, *J* = 12.5 Hz, 1H), 1.59-1.51 (m, 3H), 1.42-1.35 (m, 2H), 1.30-1.25 (m, 2H), 1.01-0.93 (m, 2H), 3.10-1.70 (m, 10H).^{13}C NMR (125 MHz; $CDCl_3$): δ 165.3, 159.2, 150.3, 149.1, 137.2, 134.4, 130.9, 123.8, 123.1, 83.8, 64.0, 47.3, 39.8, 35.9, 35.8, 32.6, 29.9, 24.0; ^{11}B NMR (160 MHz; CD_3OD): δ -12.6, -15.3; HRMS (ESI, pos) for $C_{20}H_{35}B_{10}N_3O_2$ (m/z): calcd 493.3401 $(M+Cl)^-$, found 493.3391.

Synthesis of *tert*-Butyl (1,12-dicarba-*closo*-dodecaboranylamido)propyl)carbamate (22)



22

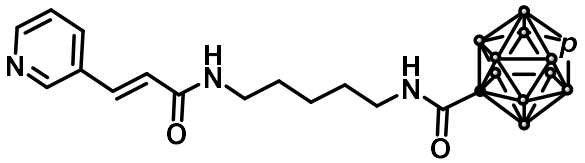
To a solution of **15c** (77 mg, 0.408 mmol), **20** (71 mg, 0.408 mmol) HATU (155 mg, 0.408 mmol) in THF (2 mL) was slowly added DIEA (100 μL, 0.612 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. It was used to the next step without further purification.

To a solution of 4 M HCl (100 μL, 0.408 mmol) in dioxane was added the crude material. The resulting mixture was stirred at room temperature for 12 h under argon atmosphere. The resulting mixture was concentrated under vacuum. It was used to the next step without further purification.

To a solution of the crude material, (*E*)-3-(pyridin-3-yl)acrylic acid (61 mg, 0.408 mmol), and HATU (155 mg, 0.408 mmol) in THF was slowly added DIEA (142 μL, 0.816 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full

conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **22** as amorphous (48.6 mg, 0.129 mmol, 41%, 3 steps). ^{1}H NMR (500 MHz; CD_3OD): δ 8.72 (s, 1H), 8.52 (d, J = 4.8 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 20 Hz, 1H), 7.43 (dd, J = 6.1 Hz, 8.6Hz, 1H), 6.67 (d, J = 20 Hz, 1H), 3.36 (bs, 1H), 3.23 (t, J = 10 Hz, 2H), 3.10 (t, J = 10 Hz, 2H), 1.62 (td, J = 5 Hz, 10 Hz, 2H); ^{11}B NMR (160 MHz; CD_3OD): δ -12.6, -15.3; ^{13}C NMR (125 MHz; CD_3OD): δ 168.0, 163.1, 150.7, 149.7, 137.6, 136.2, 132.8, 125.5, 124.6, 63.3, 55.8, 38.9, 37.7, 30.0; HRMS (ESI, pos) for $C_{14}H_{25}B_{10}N_3O_2$ (m/z): calcd 398.2855 $(M+Na)^+$, found 398.2849.

Synthesis of (*E*)-*N*-(4-(1,12-Dicarba-*closo*-dodecaboranyl)amidepentyl)-3-(pyridin-3-yl)acrylamide (25)

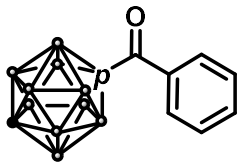


25

To a solution of 5-aminopentan-1-ol (103.2 mg, 1.00 mmol) and triethylamine (340 μL, 2.00 mmol) in CH_2Cl_2, was slowly added carbonic acid chloride in CH_2Cl_2 at 0 ℃. After the resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, the resulting mixture was added NH_4Cl aq. and CH_2Cl_2 after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. It was used to the next step without further purification.
To a solution of PPh_3 (262 mg, 1.00 mmol), phthalimide (220 mg, 1.00 mmol) in THF was slowly added 1.9 M diisopropyl azodicarboylate in Toluene (526 μL, 1.00 mmol) at 0 ℃. The resulting mixture was stirred at room temperature for 1 h under argon atmosphere. To the resulting mixture was added the crude material at 0 ℃. The resulting mixture was stirred at room temperature for 16 h. After evaporation of solvent, it was used to the next step without further purification.
To a solution of the crude material in EtOH was added Hydrazine Monohydrate (125 μL, 2.50 mmol). The resulting mixture was stirred at reflux temperature for 12 h. After evaporation of the solvent, the crude product was resolved in CH_2Cl_2 and filtered. The filtrate was concentrated under vacuum. The crude materials were used as amine to the next reaction without further purification.
To a solution of the crude material, (*E*)-3-(pyridin-3-yl)acrylic acid (149 mg, 1.00 mmol), and

HATU (380 mg, 1.00 mmol) in THF (1 mL) was slowly added DIEA (348 μL, 2.00 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **25** as a white solid (45.5 mg, 0.113 mmol, 11%, 4 steps). m.p. 85-86 °C; ^{1}H NMR (500 MHz; CD_3OD): δ 8.71 (d, *J* = 2.0 Hz, 1H), 8.51 (dd, *J* = 6.1 Hz, 2.0 Hz, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 7.54 (d, *J* = 20 Hz, 1H), 7.46 (dd, *J* = 6.1 Hz, 8.6 Hz, 1H), 6.73 (d, *J* = 20 Hz, 1H), 3.32 (bs, 1H), 3.28 (t, *J* = 7.0 Hz, 2H), 1.54 (quin, *J* = 7.0 Hz, 2H), 1.44 (quin, *J* = 7.0 Hz, 2H), 1.29-1.23 (m, 2H), 2.97-1.85 (m, 10H); ^{11}B NMR (160 MHz; CD_3OD): δ -12.6, -15.3; ^{13}C NMR (125 MHz; CD_3OD): δ 167.6, 163.0, 150.7, 149.7, 137.3, 136.2, 132.9, 125.5, 124.8, 84.4, 63.2, 41.3, 40.4, 29.9, 29.6, 24.9; HRMS (ESI, pos) for $C_{15}H_{29}B_{10}N_3O_2$ (m/z): calcd 426.3163 $(M+Na)^+$, found 426.3169.

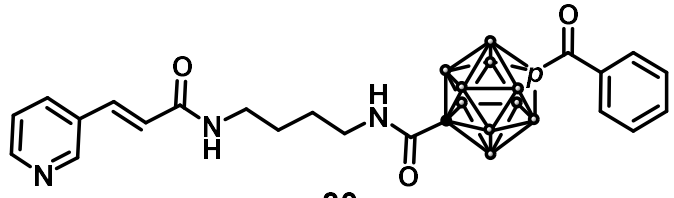
Synthesis of 1-Benzoyl-1,12-dicarba-*closo*-dodecaborane (26)



26

To a solution of *p*-carborane (200 mg, 1.39 mmol) in Et_2O, was slowly added *n*-BuLi 1.6 M solution in hexane (984 μL, 1.53 mmol) at -78 °C. After the resulting mixture was stirred -78 °C under argon atmosphere for 1 h, benzoyl chloride (176 μL, 1.53 mmol) was added. Then, the resulting mixture was stirred at room temperature for 2 h. After that, the reaction mixture was concentrated under pressure. The resulting mixture was added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (10 % Et_2O in hexane) afforded **26** as white solid (356.4 mg, 1.435 mmol, quant.). ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.48-7.44 (m, 3H), 7.36-7.32 (m, 2H), 2.88 (s, 1H), 3.01-1.57 (m, 10H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.6, -15.3; ^{13}C NMR (125 MHz; $CDCl_3$): δ 189.7, 136.4, 131.9, 128.3, 87.3, 63.6; HRMS (ESI, pos) for $C_9H_{16}B_{10}O$ (m/z): calcd 271.2101 $(M+Na)^+$, found 271.2093.

Synthesis of (*E*)-*N*-(4-(1-Benzoyl-1,12-dicarba-*closo*-dodecaboran-12-yl)amidebutyl)-3-(pyridin-3-yl)acrylamide (30)

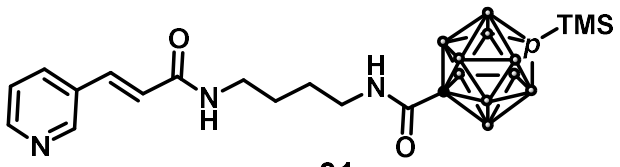


30

To a solution of isopropyl amine (18 μL, 0.127 mmol) in Et_2O, was *n*-BuLi 1.6 M solution in hexane (71 μL, 0.110 mmol) slowly added at 0 ℃. After the resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, was added a solution of **26** (21 mg, 0.0846 mmol). Then, the resulting mixture was stirred at room temperature CO_2 atmosphere for 3 h. After that, the reaction mixture was concentrated under pressure. The resulting mixture was added and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, the aqueous layer was added 6 M HCl and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of the crude material, excess amount of **14** and HATU (48 mg, 0.127 mmol) in THF was slowly added DIEA (44 μL, 0.254 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **30** as a solid (18.0 mg, 0.0365 mmol, 43%, 2 steps). m.p. 64-65 ℃; ^{1}H NMR (500 MHz; CD_3OD): δ 8.71 (d, *J* = 2.0 Hz, 1H), 8.51 (dd, *J* = 6.1 Hz, 2.0 Hz, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 7.56-7.40 (m, 7H), 6.70 (d, *J* = 20 Hz, 1H), 3.29-3.26 (m, 2H), 3.09-3.06 (m, 2H), 1.47-1.45 (m, 4H), 3.20-1.88 (m, 10H); ^{11}B NMR (160 MHz; CD_3OD): δ -12.6, -15.3; ^{13}C NMR (125 MHz; CD_3OD): δ 189.3, 166.3, 161.2, 149.3, 148.3, 136.1, 136.0, 134.9, 131.8, 131.5, 127.8, 127.6, 124.1, 123.4, 85.2, 83.4, 39.8, 38.7, 26.2, 26.1; HRMS (ESI, pos) for $C_{22}H_{31}B_{10}N_3O_3$ (m/z): calcd 517.3242 $(M+Na)^+$, found 517.3233.

Synthesis of (*E*)-*N*-(4-(1-Trimethylsilyl-1,12-dicarba-*closo*-dodecaboran-12-yl)amidebutyl)-3-(pyridin-3-yl)acrylamide (31)



31

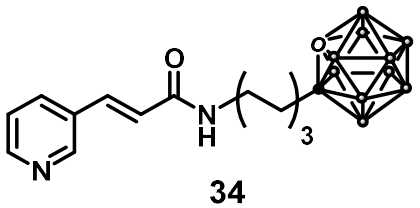
To a solution of *p*-carborane (27 mg, 0.185 mmol) in Et_2O, was slowly added *n*-BuLi 1.6 M solution in hexane (120 μL, 0.185 mmol) at -78 ℃. After the resulting mixture was stirred -78 ℃ under argon atmosphere for 1 h, TMSCl (23 μL, 0.185 mmol) was added. Then, the resulting mixture was stirred at room temperature for 2 h. After that, the reaction mixture was concentrated under pressure. The resulting mixture was added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. It was used to the next step without further purification.

To a solution of the crude material in Et_2O, was slowly added *n*-BuLi 1.6 M solution in hexane (120 μL, 0.185 mmol) at -78 ℃. The resulting mixture was stirred at room temperature under argon atmosphere for 1.5 h. The resulting mixture was stirred at room temperature CO_2 atmosphere for 3 h. After that, the reaction mixture was concentrated under pressure. The resulting mixture was added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, the aqueous layer was added 6 M HCl and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was used to the next reaction without further purification.

To a solution of the crude material, excess amount of **14** and HATU (70 mg, 0.185 mmol) in THF (1 mL) was slowly added DIEA (64 μL, 0.370 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **31** as a solid (21.0 mg, 0.0455 mmol, 25%, 3 steps). m.p. 65-66 ℃; ^{1}H NMR (500 MHz; CD_3OD): δ 8.72 (d, *J* = 2.0 Hz, 1H), 8.53 (dd, *J* = 6.1 Hz, 2.0 Hz, 1H), 8.06 (d, *J* = 8.6 Hz, 1H), 7.52 (d, *J* = 20 Hz, 1H), 7.49 (dd, *J* = 6.1 Hz, 8.6 Hz, 1H), 6.72 (d, *J* = 20 Hz, 1H), 3.33-3.22 (m, 2H), 3.09-3.08 (m, 2H), 1.49-1.47 (m, 4H), 2.95-1.71 (m, 10H),

0.01 (s, 9H) ; ^{11}B NMR (160 MHz; CD_3OD): δ -12.6, -15.3; ^{13}C NMR (125 MHz; CD_3OD): δ 166.3, 161.7, 149.3, 148.3, 148.3, 136.0, 134.9, 131.5, 124.2, 123.4, 85.8, 71.6, 39.7, 38.7, 26.2, 26.1, -2.69; HRMS (ESI, pos) for $C_{18}H_{35}B_{10}N_3O_2Si$ (m/z): calcd 485.3374 $(M+Na)^+$, found 485.3376.

Synthesis of 1-((*E*)-4-(3-(Pyridin-3-yl)acrylamido)hexyl)-1,2-dicarba-*closo*-dodecaborane (34)



34

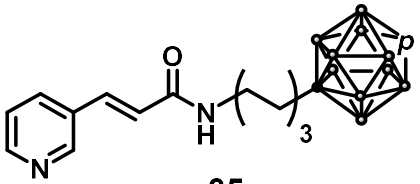
To a solution of *o*-carborane (40 mg, 0.277 mmol) in THF (2 mL) was slowly added *n*-BuLi 1.6 M solution in hexane (179 μL, 0.277 mmol) at 0 ℃. The resulting mixture was stirred at 0 ℃ for over 1 h under argon atmosphere. Then $PhthN(CH_2)_6OTs$ (111 mg, 0.277 mmol) was added at 0 ℃. After the resulting mixture was stirred for 12 h at room temperature, the resulting mixture was added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel afforded products. The crude material was used to the next reaction without further purification.

To a solution of the crude material in 2-propanol:water (2.0 mL, 6:1, v/v), was added $NaBH_4$ (52 mg, 1.39 mmol). The resulting mixture was stirred at room temperature. After 2 h, conc.HCl (200 μL) was added after which was reacted at 80 ℃ for 6 h under argon atmosphere. The reaction mixture was dried by repeated co-concentration with methanol. The product was dissolved in water and washed twice with CH_2Cl_2. The aqueous phase was concentrated in vacuo, dissolved in ethanol and filtered through a small cotton plug. The crude material was used without further purification.

To a solution of the crude material, (*E*)-3-(pyridin-3-yl)acrylic acid (41 mg, 0.277 mmol) and HATU (105 mg, 0.277 mmol) in THF (500 μL) was slowly added DIEA (97 μL, 0.554 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (5% MeOH in CH_2Cl_2) afforded **34** as a solid (10.5 mg,

0.028 mmol, 10%, 3 steps). m.p. 125-126 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.71 (d, *J* = 2.0 Hz, 1H), 8.51 (dd, *J* = 6.1 Hz, 2.0 Hz, 1H), 8.04 (d, *J* = 8.6 Hz, 1H), 7.54 (d, *J* = 20 Hz, 1H), 7.46 (dd, *J* = 6.1 Hz, 8.6 Hz, 1H), 6.73 (d, *J* = 20 Hz, 1H), 4.52 (bs, 1H), 2.27 (m, 2H), 1.58-1.46 (m, 4H), 1.40-1.29 (m, 6H), 2.97-1.85 (m, 10H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.24, -5.65, -9.24, -11.6, -12.2, -13.0; ^{13}C NMR (125 MHz; $CDCl_3$): δ 167.6, 150.7, 149.6, 137.3, 136.2, 132.9, 125.5, 124.8, 86.0, 59.7, 40.4, 40.0, 30.4, 30.2, 29.7, 27.5; HRMS (ESI, pos) for $C_{16}H_{30}B_{10}N_2O$ (m/z): calcd 374.3476 $(M+H)^+$, found 374.3463.

Synthesis of 1-((*E*)-4-(3-(Pyridin-3-yl)acrylamido)hexyl)-1,12-dicarba-*closo*-dodecaborane (35)



35

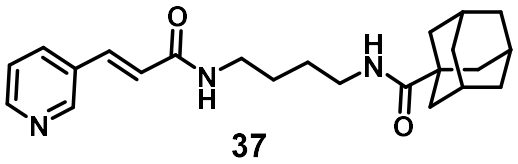
To a solution of *p*-carborane (40 mg, 0.277 mmol) in THF (2 mL) was slowly added *n*-BuLi 1.6 M solution in hexane (179 μL, 0.277 mmol) at 0 °C. The resulting mixture was stirred at 0 °C for over 1 h under argon atmosphere. Then $PhthN(CH_2)_6OTs$ (111 mg, 0.277 mmol) was added at 0 °C. After the resulting mixture was stirred for 12 h at room temperature. The resulting mixture was added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel afforded products. The crude material was used to the next reaction without further purification.

To a solution of the crude material in EtOH (1 mL) was added hydrazine monohydrate (19 μL, 0.277 mmol). The resulting mixture was stirred at reflux temperature for 6.5 h under argon atmosphere. After evaporation of solvent, to the reaction mixture were added water and CH_2Cl_2. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was used without further purification.

To a solution of the crude material, (*E*)-3-(pyridin-3-yl)acrylic acid (41 mg, 0.277 mmol) and HATU (105 mg, 0.277 mmol) in THF (500 μL) was slowly added DIEA (97 μL, 0.554 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine,

dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (5% MeOH in CH_2Cl_2) afforded **35** as a white solid (12 mg, 0.033 mmol, 12%, 3 steps). m.p.147-148 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.71 (s, 1H), 8.51 (s, 1H), 8.04 (s, 1H), 7.57-7.47 (m, 2H), 6.71 (m, 1H), 3.27 (m, 2H), 3.10 (bs. 1H), 1.64 (s. 2H), 1.51 (m, 2H), 1.30 (bs, 2H), 1.18 (m, 4H), 2.5-1.9 (br. 10H); ^{11}B NMR (160 MHz; CD_3OD): δ -13.0, -15.9; ^{13}C NMR (125 MHz; $CDCl_3$): δ 167.6, 150.7, 149.6, 137.3, 136.2, 132.9, 125.5, 124.8, 86.0, 59.7, 40.4, 40.0, 30.4, 30.2, 29.7, 27.5; HRMS (ESI, pos) for $C_{16}H_{30}B_{10}N_2O$ (m/z): calcd 397.3261 $(M+Na)^+$, found 397.3268.

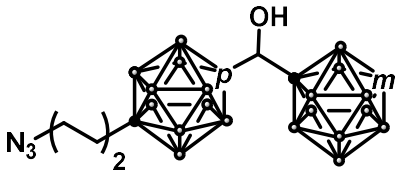
Synthesis of (3*r*,5*r*,7*r*)-*N*-(4-((*E*)-3-(pyridin-3-yl)acrylamido)butyl)adamantane-1-carboxamide (37)



37

To a solution of adamantane-1-carboxylic acid (30 mg, 0.166 mmol), excess amount of **14**, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (34 mg, 0.177 mmol), 1-hydroxybenzotriazole (HOBt) (34 mg, 0.249 mmol) in THF was slowly added DIEA (87 μL, 0.498 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% MeOH in CH_2Cl_2) afforded **37** as a white solid (19.2 mg, 0.0503 mmol, 30%). m.p. 194-195 ℃; ^{1}H NMR (400 MHz; CD_3OD): δ 8.71 (d, *J* = 1.6 Hz, 1H), 8.51 (dd, *J* = 6.0 Hz, 1.6 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 20 Hz, 1H), 7.47 (dd, *J* = 6.0 Hz, 8.0 Hz, 1H), 6.72 (d, *J* = 20 Hz, 1H), 3.34-3.22 (m, 2H), 3.09-3.08 (m, 2H), 2.00 (s, 3H), 1.86-1.71 (m, 12H), 1.56 (t, *J* = 3.2 Hz, 4H); ^{13}C NMR (125 MHz; CD_3OD): δ 181.0, 167.7, 150.7, 149.7, 137.4, 136.2, 132.9, 125.5, 124.9, 41.8, 20.3, 40.3, 40.2, 40.0, 37.6, 29.7, 28.0, 27.7; HRMS (ESI, pos) for $C_{23}H_{31}N_3O_2$ (m/z): calcd 404.2308 $(M+Na)^+$, found 404.2328.

Synthesis of (4-(4-Azidobutyl)-1,12-dicarba-*closo*-dodecaboranyl) -1,7-dicarba-*closo*-dodecaboranyl)methanol (45)



45

To a solution of *p*-carborane (72 mg, 0.50 mmol) in THF (4 mL) was slowly added *n*-BuLi 1.6 M solution in hexane (318 μL, 0.50 mmol) at -78 ℃. The resulting mixture was stirred at 0 ℃ for over 1 h under argon atmosphere. Then **39** (157 mg, 0.50 mmol) was added at -78 ℃. After the resulting mixture was stirred for 12 h at room temperature, the resulting mixture was added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel afforded products. Then, the crude material **40** was used to the next reaction without further purification.

To a solution of the crude material **40** in THF (2 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (318 μL, 0.50 mmol) at 0 ℃. After the resulting mixture was stirred at 0 ℃ under argon atmosphere for 1 h, the resulting mixture was cooled to -78 ℃. Methyl formate (62 μL, 1.00 mmol) was slowly added the resulting mixture was stirred at the same temperature for 2 h. After that, the resulting mixture was quenched by NH_4Cl aq. and concentrated under pressure. The resulting mixture was added water and hexane, after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. Then, the crude material **41** was used to the next reaction without further purification.

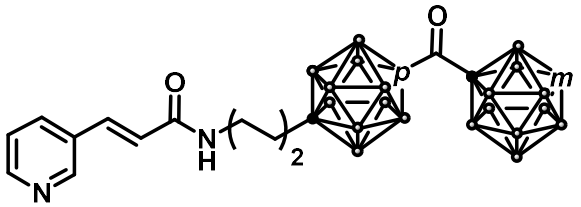
To a solution of *m*-carborane (72 mg, 0.50 mmol) in Et_2O (2 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (318 μL, 0.50 mmol) at 0 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 1 h, the resulting mixture was cooled to -78 ℃. The crude material **41** in Et_2O was slowly added the resulting mixture was stirred at the same temperature for 2 h. After that, the resulting mixture was quenched by NH_4Cl aq., the resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. Next, to a solution of 4 M HCl in Dioxane (500 μL) was added the crude material at room temperature. After the resulting mixture was stirred for 12 h, and concentrated under vacuum. Then, the crude material **42** was used to the next reaction without further purification.

To a solution of the crude material **42**, triethylamine (205 μL, 1.50 mmol), and DMAP (6.1 mg,

0.050 mmol) in dichloromethane, was slowly added *p*-toluenesulfonylchloride (191 mg, 1.00 mmol) at room temperature. After the resulting mixture was stirred at room temperature under argon atmosphere until full conversion of a starting material was observed. The resulting mixture was added water and CH_2Cl_2 after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material **44** was used to the next reaction without further purification.

To a solution of the crude material **44** in dimethylformamide, was added sodium azide (162 mg, 2.50 mmol). After the resulting mixture was stirred at 80 ℃ under argon atmosphere until full conversion of a starting material was observed. The resulting mixture was added water and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel afforded **45** as a colorless oil (23.8 mg, 0.0570 mmol, 11%, 6 steps). ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.84 (d, *J* = 8.0 Hz, 1H), 3.20 (t, *J* = 10.8 Hz, 2H), 3.10-1.60 (m, 20H), 2.85 (s, 1H), 2.23 (d, *J* = 8.0 Hz, 1H), 1.67-1.62 (m, 2H), 1.44-1.37 (m, 2H), 1.26-1.23 (m, 2H); ^{11}B NMR (160 MHz; $CDCl_3$): δ –3.99, -6.94, -11.2, -13.2, -15.6; ^{13}C NMR (125 MHz; $CDCl_3$): δ 84.4, 82.6, 74.5, 66.0, 51.1, 37.5, 28.5, 26.2; HRMS (ESI, pos) for $C_9H_{31}B_{20}N_3O$ (m/z): calcd 436.4367 $(M+Na)^+$, found 436.4367.

Synthesis of (*E*)-*N*-(4-(4-(1,7-Dicarba-*closo*-dodecaboranecarbonyl) -1,12-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (38)



38

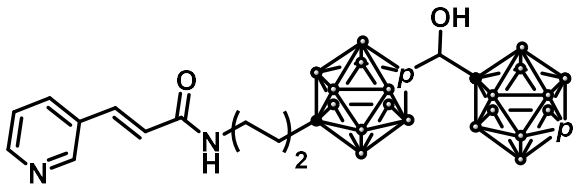
To a solution of **45** (8.3 mg, 0.020 mmol) in CH_2Cl_2 was added Dess–Martin periodinane (20 mg, 0.040 mmol) at room temperature. The resulting mixture was stirred under argon atmosphere until full conversion of a starting material was observed. The resulting mixture was quenched by $NaHCO_3$ aq., after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. Then, the crude material was used to the next step without further purification.

To a solution of the crude material in THF : H_2O (500 μL, 10:1, v/v), was added PPh_3 (10 mg,

0.040 mmol). The resulting mixture was stirred at room temperature for 12 h under argon atmosphere. After evaporation of solvent, the crude product **47** was used to the next reaction without further purification.

To a solution of the crude material **47**, (*E*)-3-(pyridin-3-yl)acrylic acid (3.0 mg, 0.020 mmol), EDCI (4.6 mg, 0.024 mmol), HOBt (4.6 mg, 0.030 mmol) in THF was slowly added DIEA (10 μL, 0.060 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (5% MeOH in CH_2Cl_2) afforded **38** as a white solid (4.4 mg, 8.5 μmol, 43%, 3 steps). m.p. 127-128 °C; ^{1}H NMR (500 MHz; CD_3OD): δ 8.71 (s, 1H), 8.51 (d, *J* = 5.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.54 (d, *J* = 15 Hz, 1H), 7.47 (dd, *J* = 5.0 Hz, 8.0 Hz, 1H), 6.70 (d, *J* = 15 Hz, 1H), 3.72 (s, 1H), 3.22 (t, *J* = 7.0 Hz, 2H), 3.10-1.60 (m, 20H), 1.72 (t, *J* = 8.5 Hz, 1H), 1.41-1.37 (m, 2H), 1.24-1.21 (m, 2H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.56, -10.9, -13.1, -14.9; ^{13}C NMR (125 MHz; CD_3OD): δ 181.7, 167.7, 150.7, 149.7, 137.5, 136.3, 132.9, 125.6, 124.7, 79.5, 57.5, 57.1, 54.8, 39.9, 39.0, 29.7, 27.8; HRMS (ESI, pos) for $C_{17}H_{36}B_{20}N_2O_2$ (m/z): calcd 540.4652 $(M+Na)^+$, found 540.4637.

Synthesis of (*E*)-*N*-(4-(4-(1,12-Dicarba-*closo*-dodecaboranyl(hydroxy)methyl) -1,12-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (50)



50

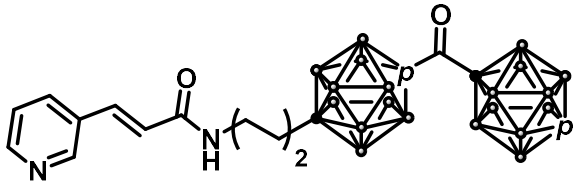
To a solution of *p*-carborane (34 mg, 0.240 mmol) in Et_2O (2 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (151 μL, 0.240 mmol) at 0 °C. After the resulting mixture was stirred at -78 °C under argon atmosphere for 1 h, the resulting mixture was cooled to -78 °C. The crude material **41** (described above) was slowly added the resulting mixture was stirred at the same temperature for 2 h. After that, the resulting mixture was quenched by NH_4Cl aq., the resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. Next, to a solution of 4 M HCl in Dioxane (500 μL) was added the crude material at room temperature. After the resulting mixture was stirred for 12 h, and concentrated under vacuum. Then, the crude product was used to the next reaction without further purification.

To a solution of PPh_3 (63 mg, 0.240 mmol), phthalimide (35 mg, 0.240 mmol) in THF (1.5 mL) was slowly added 1.9 M diisopropyl azodicarboylate in toluene (126 μL, 0.240 mmol) at 0 ℃. The resulting mixture was stirred at room temperature for 1 h under argon atmosphere. To the resulting mixture was added the crude material at 0 ℃. The resulting mixture was stirred at room temperature for 16 h. After evaporation of solvent, the crude product was used to the next reaction without further purification.

To a solution of the crude material in EtOH, was added hydrazine monohydrate (30 μL, 0.600 mmol). The resulting mixture was stirred at reflux temperature under argon atmosphere until full conversion of a starting material was observed. After evaporation of solvent, to the reaction mixture were added water and CH_2Cl_2. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of the crude material, (*E*)-3-(pyridin-3-yl)acrylic acid (36 mg, 0.240 mmol), EDCI (55 mg, 0.029 mmol), HOBt (55 mg, 0.360 mmol) in THF was slowly added DIEA (126 μL, 0.720 mmol). The resulting mixture was stirred at room temperature under argon atmosphere until full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were dried over sodium sulfate and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (5% MeOH in CH_2Cl_2) afforded **50** as a white solid (36 mg, 0.0686 mmol, 29%, from **41**). m.p. 204-205 ℃; ^{1}H NMR (500 MHz; CD_3OD): δ 8.71 (s, 1H), 8.51 (d, *J* = 5.0 Hz, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.53 (d, *J* = 15 Hz, 1H), 7.47 (dd, *J* = 5.0 Hz, 8.0 Hz, 1H), 6.70 (d, *J* = 15 Hz, 1H), 3.44 (s, 1H), 3.25 (s, 1H), 3.21 (t, *J* = 8.0 Hz, 2H), 2.90-1.50 (m, 20H), 1.67 (t, *J* = 8.5 Hz, 1H), 1.40-1.37 (m, 2H), 1.24-1.19 (m, 2H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.9, -15.7; ^{13}C NMR (125 MHz; CD_3OD): δ 167.7, 150.7, 149.7, 137.5, 136.3, 132.9, 125.5, 124.7, 90.6, 85.3, 75.6, 64.4, 54.8, 40.0, 38.5, 29.8, 28.0; HRMS (ESI, pos) for $C_{17}H_{38}B_{20}N_2O_2$ (m/z): calcd 542.4809 $(M+Na)^+$, found 542.4813.

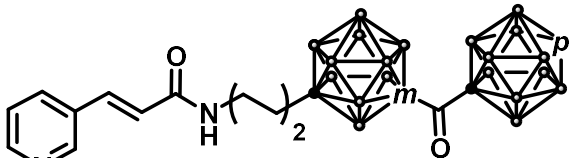
Synthesis of (*E*)-*N*-(4-(4-(1,12-Dicarba-*closo*-dodecaboranecarbonyl) -1,12-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (51)



51

To a solution of **50** (16.3 mg, 0.029 mmol) in CH_2Cl_2 (500 μL) was added Dess–Martin periodinane (24.6 mg, 0.058 mmol) at room temperature. The resulting mixture was stirred under argon atmosphere until full conversion of a starting material was observed. The resulting mixture was quenched by $NaHCO_3$ aq., after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were washed with brine, dried over magnesium sulfate and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (5% MeOH in CH_2Cl_2) afforded **51** as a white solid (8.1 mg, 0.0155 mmol, 53%). m.p. 176-177 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.74 (s, 1H), 8.57 (d, *J* = 4.0 Hz, 1H), 7.77 (d, *J* = 8.0 Hz, 1H), 7.60 (d, *J* = 16 Hz, 1H), 7.30 (dd, *J* = 4.0 Hz, 8.0 Hz, 1H), 6.43 (d, *J* = 16 Hz, 1H), 5.72 (s, 1H), 3.28 (dd, *J* = 7.0 Hz, 14 Hz, 2H), 2.87 (s, 1H), 2.90-1.60 (m, 20H), 1.62 (t, *J* = 8.5 Hz, 1H), 1.41-1.35 (m, 2H), 1.25-1.16 (m, 2H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.0, -15.4; ^{13}C NMR (125 MHz; $CDCl_3$): δ 180.5, 165.2, 150.5, 149.2, 137.7, 134.5, 130.7, 123.8, 122.7, 86.6, 85.9, 70.7, 65.2, 39.4, 37.9, 29.2, 26.8; HRMS (ESI, pos) for $C_{17}H_{36}B_{20}N_2O_2$ (m/z): calcd 540.4652 $(M+Na)^+$, found 540.4653.

Synthesis of (*E*)-*N*-(4-(4-(1,12-Dicarba-*closo*-dodecaboranecarbonyl) -1,7-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (55)



55

Preparation and purification of compound **55** was carried out according to the procedure of compound **38** as a white solid (11.8 mg, 0.023 mmol, 37%, 7 steps). m.p. 209-211 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.74 (d, *J* = 1.5 Hz, 1H), 8.57 (dd, *J* = 1.5 Hz, *J* = 6.0 Hz, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 15 Hz, 1H), 7.31 (dd, *J* = 5.0 Hz, 8.0 Hz, 1H), 6.45 (d, *J* = 15 Hz, 1H), 5.80 (s, 1H), 3.35 (q, *J* = 6.7 Hz, 2H), , 2.91 (s, 1H) 3.10-1.50 (m, 20H), 1.95 (t, *J* = 8.5 Hz, 1H), 1.51-1.42 (m, 2H), 1.41-1.38 (m, 2H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.24, -7.24, -11.0, -12.8, -15.3; ^{13}C NMR (125 MHz; $CDCl_3$): δ 180.2, 165.3, 150.5, 149.3, 137.8, 134.5, 130.7, 123.8,

122.7, 85.8, 77.7, 75.6, 65.4, 39.4, 36.7, 29.3, 27.3; HRMS (ESI, pos) for $C_{17}H_{36}B_{20}N_2O_2$ (m/z): calcd 539.4686 $(M+Na)^+$, found 539.4682.

Biology

Recombinant NAMPT Inhibition Assay

The assay was performed according to the manufacturers protocol (CycLex NAMPT Colorimetric Assay Kit, MBL International Corp., Woburn, MA). The NAMPT assay was performed according manufacturers protocol. Briefly we used the "1-Step Assay Method" for which following reagents were mixed to make assay buffer and kept at ice before starting the assay: 10 µL each of 10X NAMPT assay buffer, nicotinamide, PRPP, ATP and EtOH; 2 µL each of recombinant NMNAT1, WST-1, ADH, diaphorase and dH_2O. The NAMPT inhibition assay was performed by mixing 2 µL of various concentrations (DMSO as vehicle control) with the following: 2 µL recombinant NAMPT and 36 µL dH_2O. The reaction was initiated by adding 60 µL of 1- Step Assay Buffer to each well and mixed thoroughly followed by incubation at 30 ℃ for 20 mins. After this period, the absorbance at 485 nm was measured and compared with the positive control.

MTT assay

Human epithelioid cervical carcinoma HeLa cells were used for the cell viability assay. The cells (5×10^4 cells/mL of 96-well plate) were incubated at 37 °C for 72 h in 100 µL of RPMI-1640 medium containing various concentrations of compounds. After the incubation, 10 µL of 3'-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, TCI) in PBS (5 mg/ml) was added into the each well, and the cells were further incubated at 37 ºC for 2 h. After the removal of the medium, 100 µL of DMSO was added and the absorbance at 595 nm was determined by a microplate S6 reader. The drug concentration required to reduce cell viability by 50% (IC_{50}) was determined from semilogarithmic dose-response plots.

Modeling

Docking study

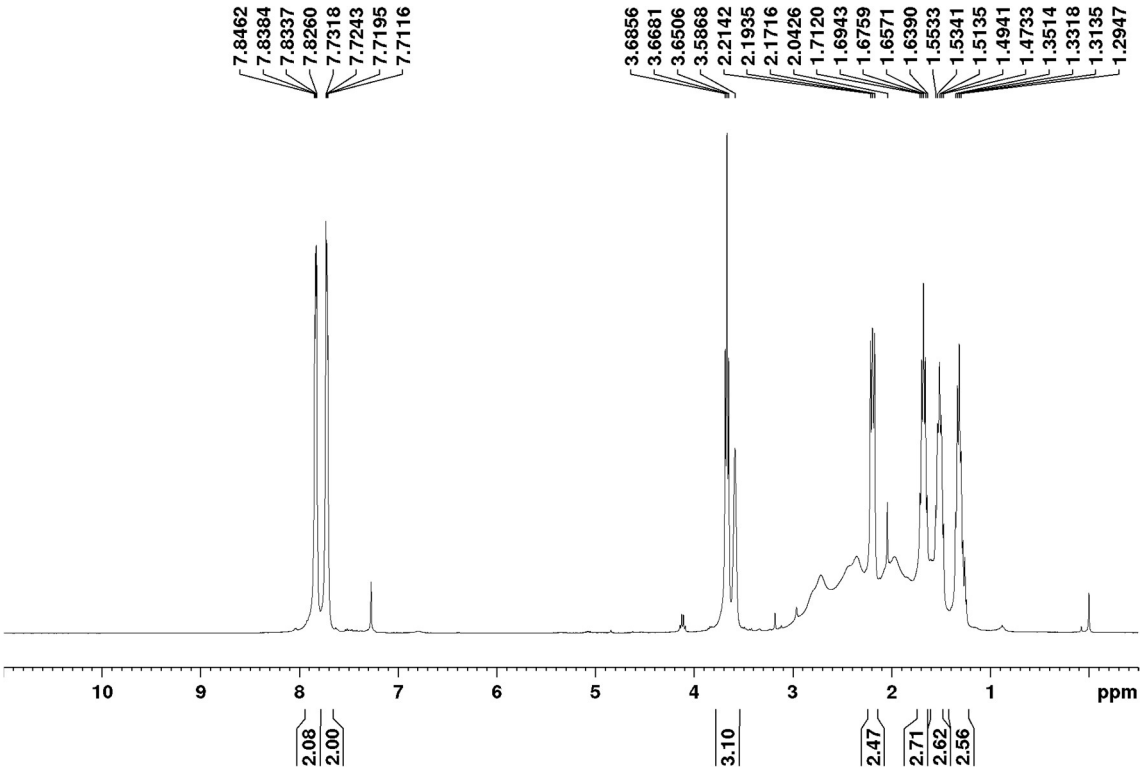
Ligand structures and geometries were optimized with Discovery Studio 4.1 using CHARMm force field. The crystal structures for NAMPT complexed with the NAMPT- selective inhibitor FK866 (PDB ID: 2GVJ) were downloaded from the Protein Data Bank (PDB). All ligands and water molecules were removed. One monomer of NAMPT was prepared for docking with AutoDock Vina. The optimized geometries of the ligands were prepared for docking. Ligand nonpolar hydrogens (including carborane C−H protons) were merged to conform to the AutoDock atom types, and all of the torsion angles within the carborane clusters were set to nonrotatable. A

new atom type (B) was defined for the boron atoms, utilizing the force field parameters reported by Tiwari et al. for docking of carborane-containing ligands. The docking area was defined using AutoGrid, Three-dimensional affinity grid with 1.0 Å grid point spacing was placed around the NAMPT active site. Docking was performed with AutoDock Vina.

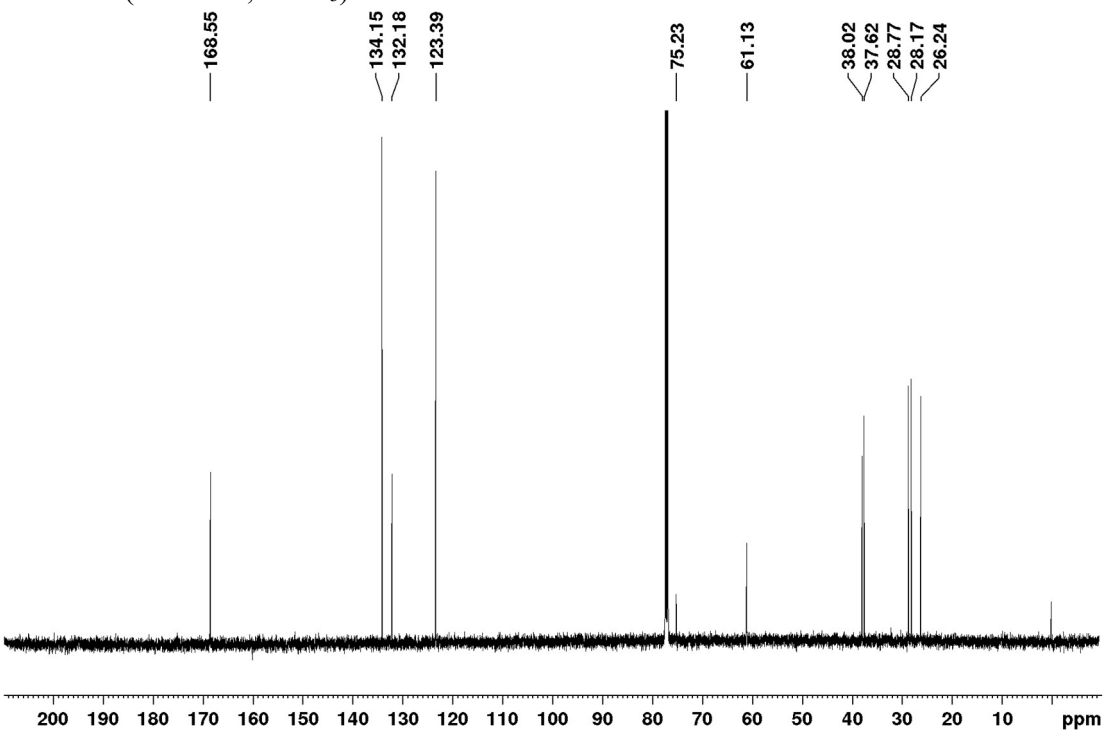
Spectra data

1-(Phtalimido-*N*-pentyl)-1,2-dicarba-*closo*-dodecaborane (4b)

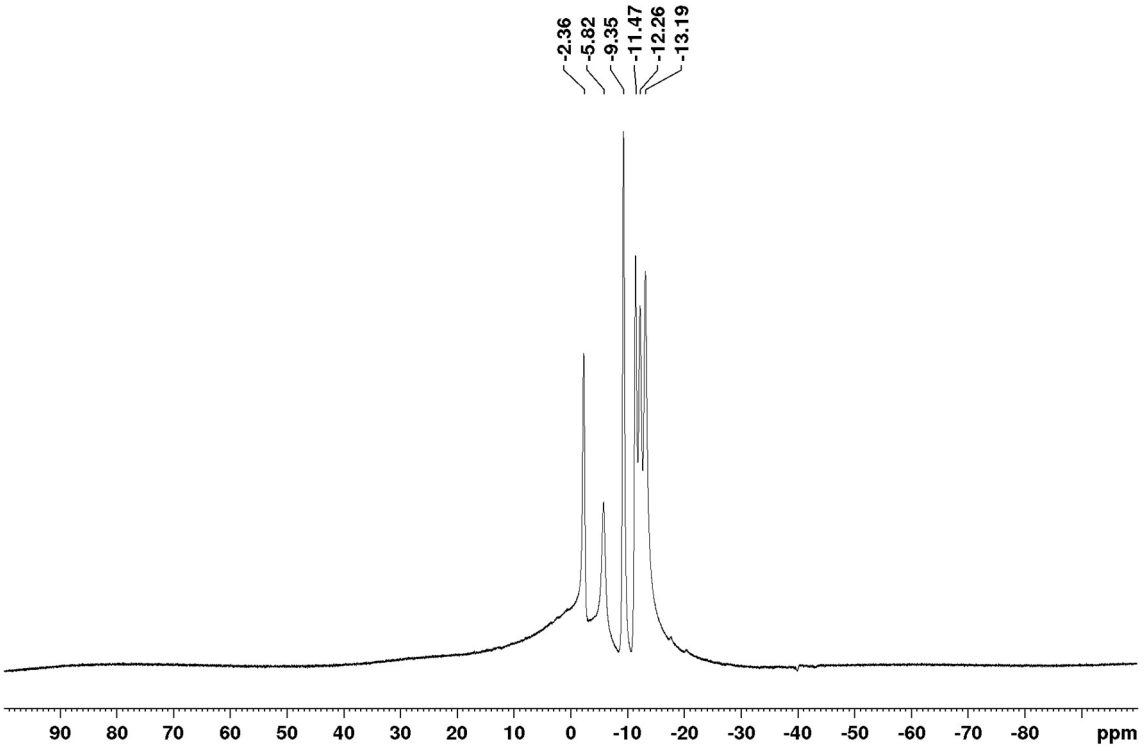
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

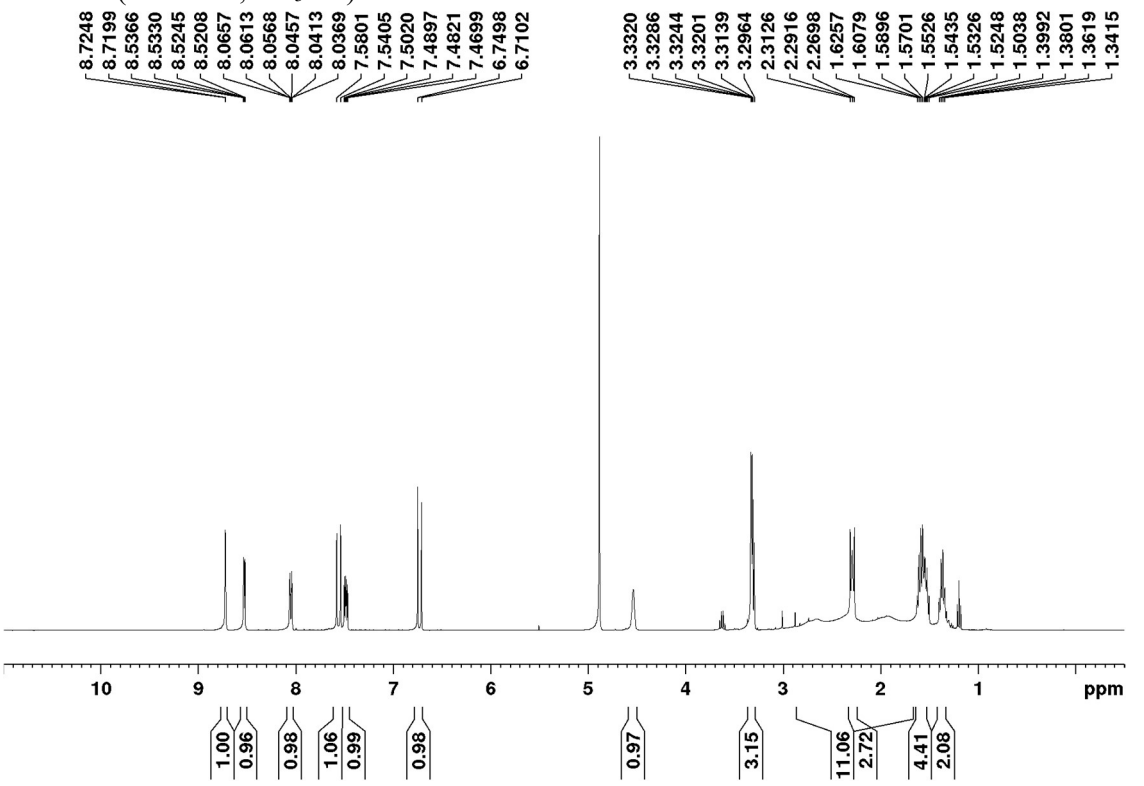


^{11}B NMR (160 MHz; $CDCl_3$)

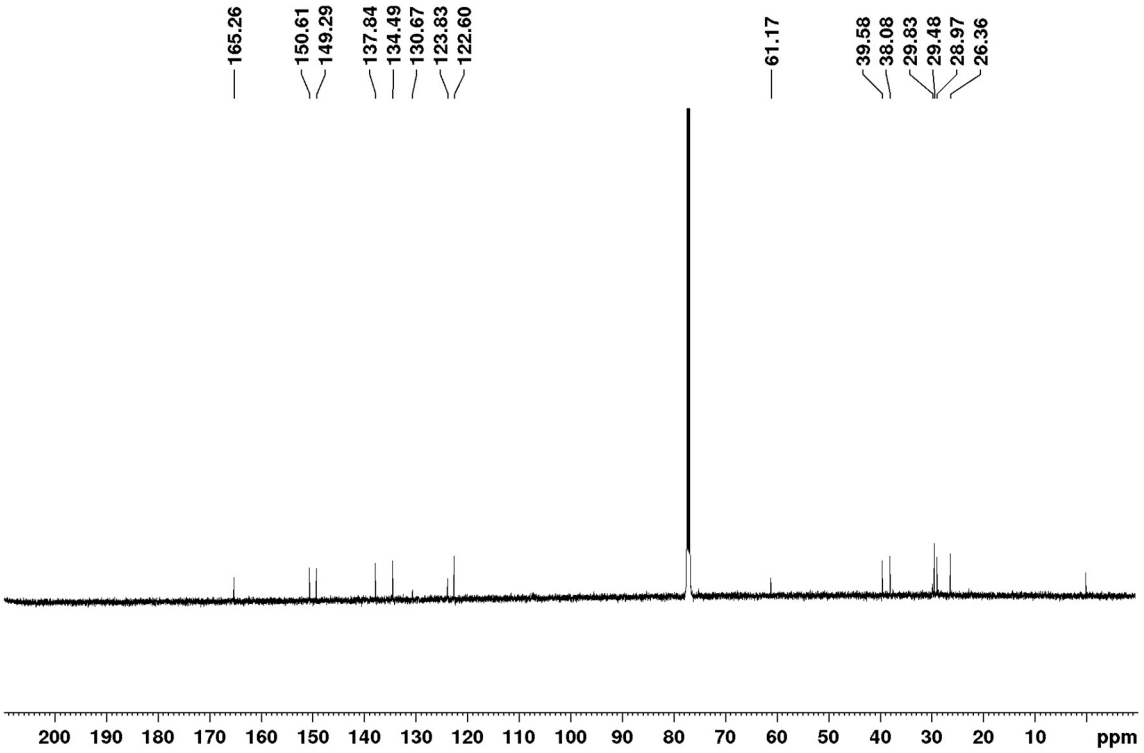


1-((*E*)-4-(3-(pyridin-3-yl)acrylamido)pentyl)-1,2-dicarba-*closo*-dodecaborane (6)

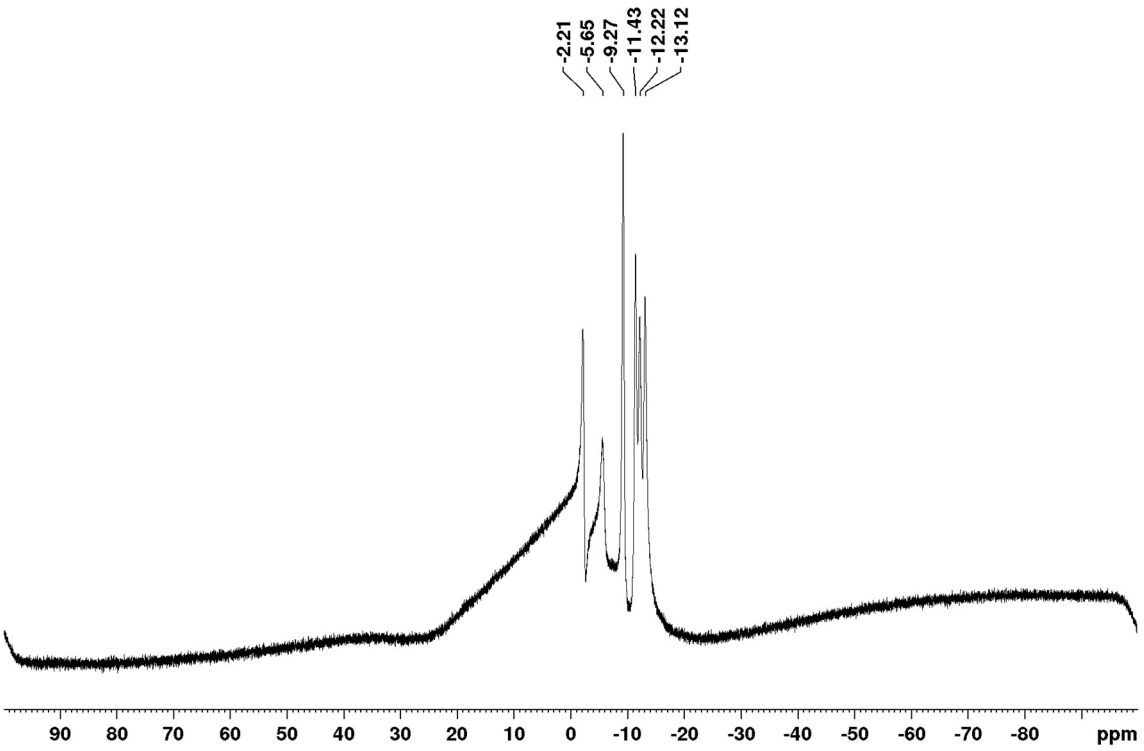
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; $CDCl_3$)

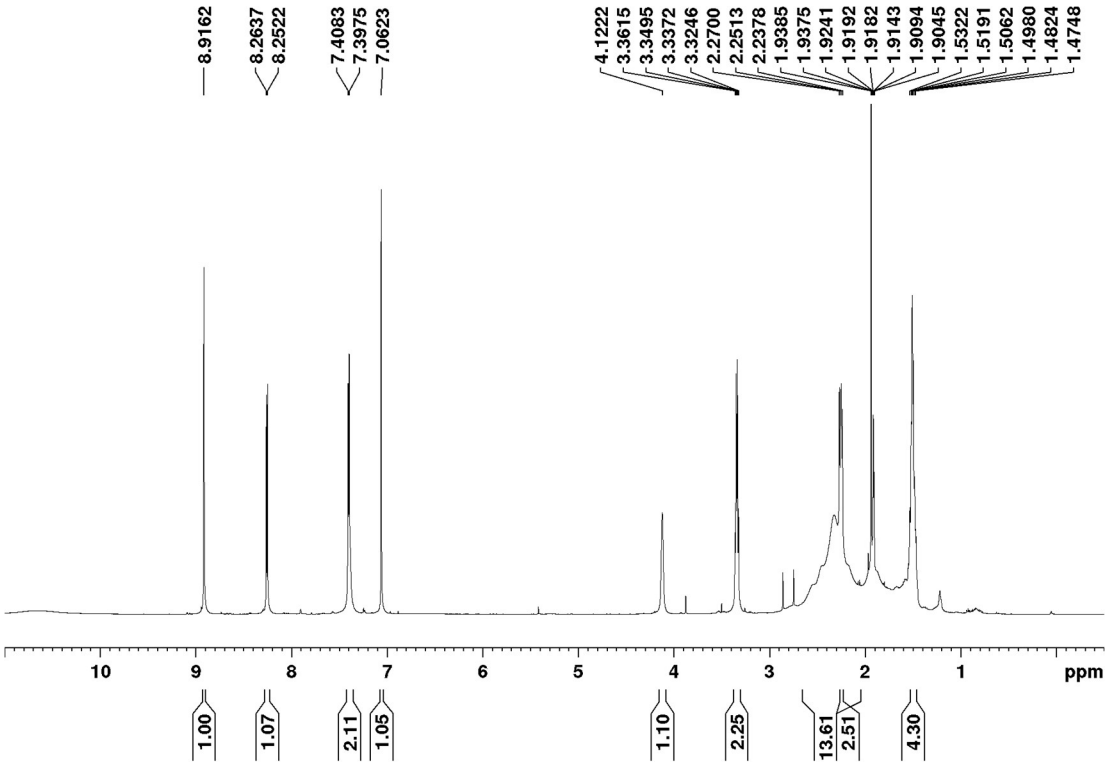


^{11}B NMR (160 MHz; $CDCl_3$)

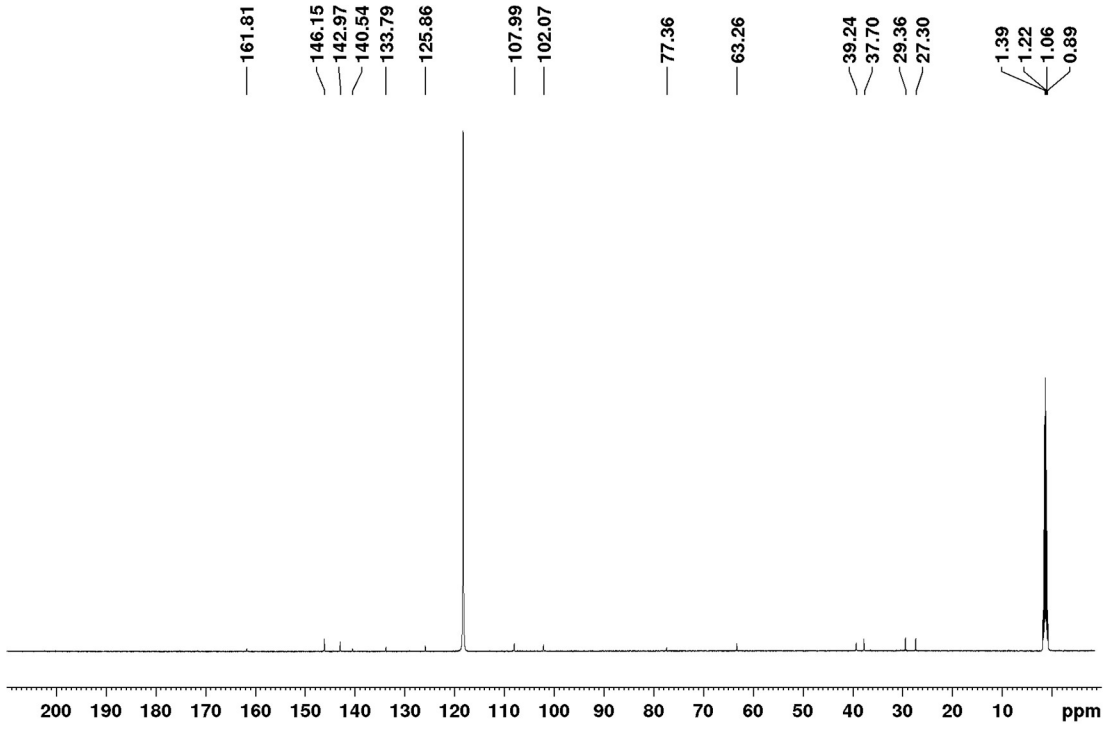


1-(5-(1*H*-Pyrrolo[3,2-*c*]pyridine-2-carboxamido)butyl)-1,2-dicarba-*closo*-dodecaborane (7)

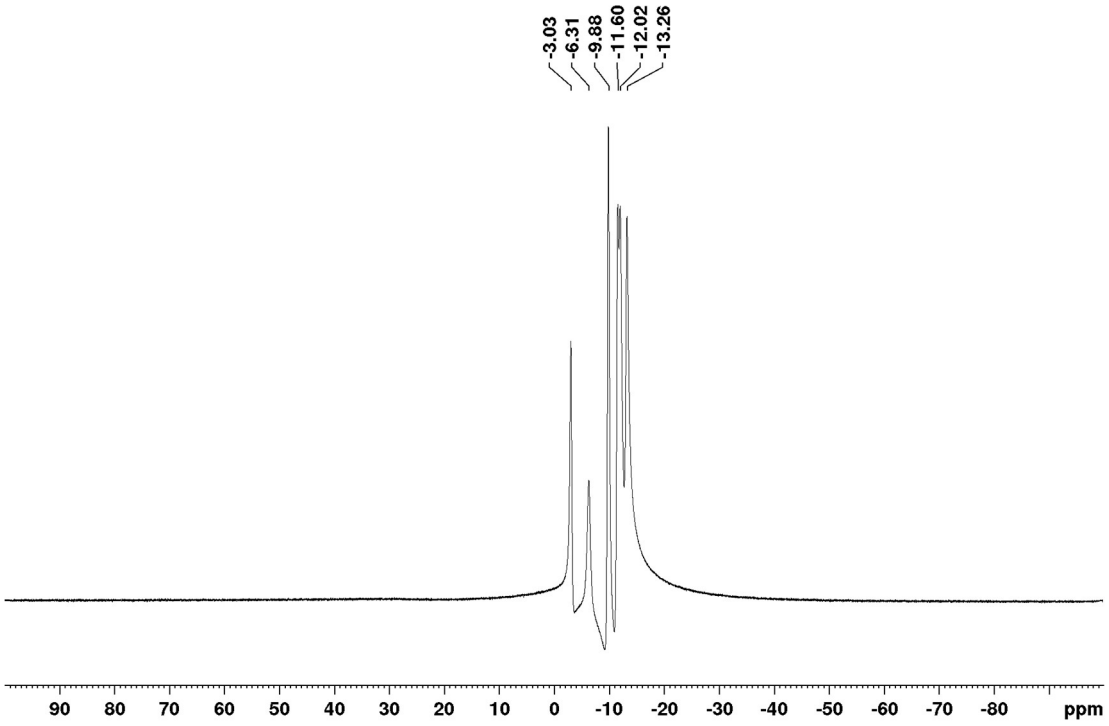
^{1}H NMR (500 MHz; CD_3CN)



^{13}C NMR (125 MHz; CD_3CN)

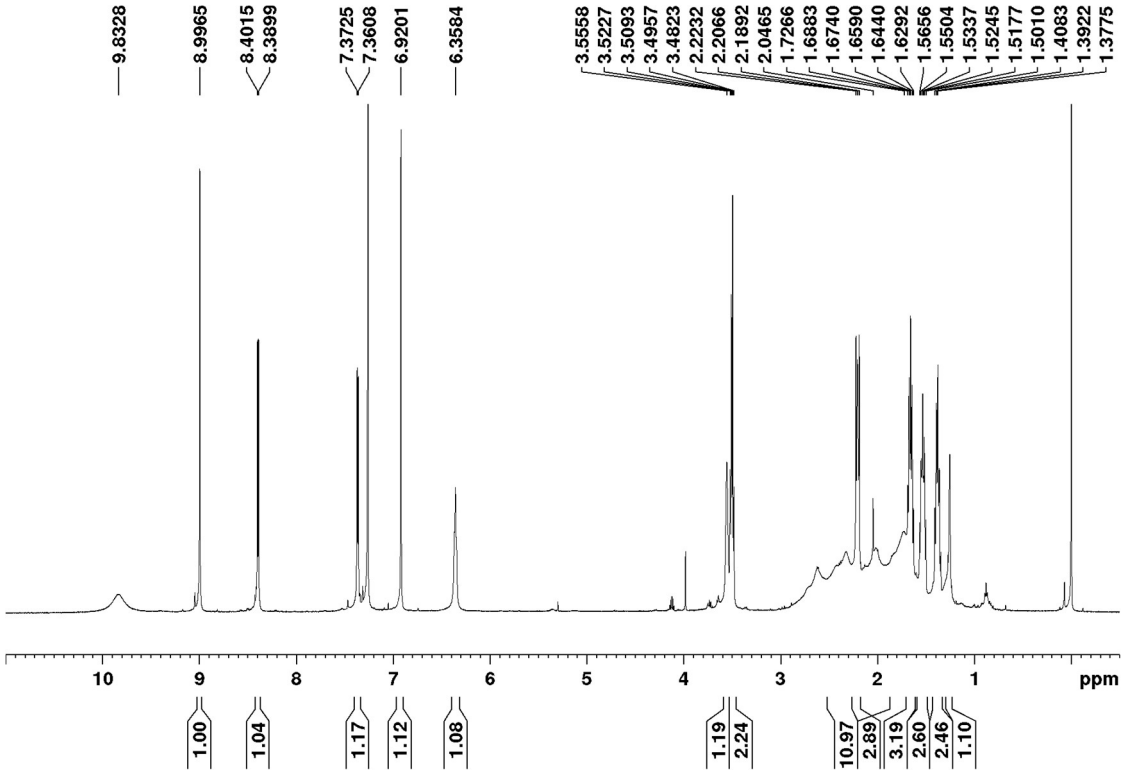


^{11}B NMR (160 MHz; CD_3CN)

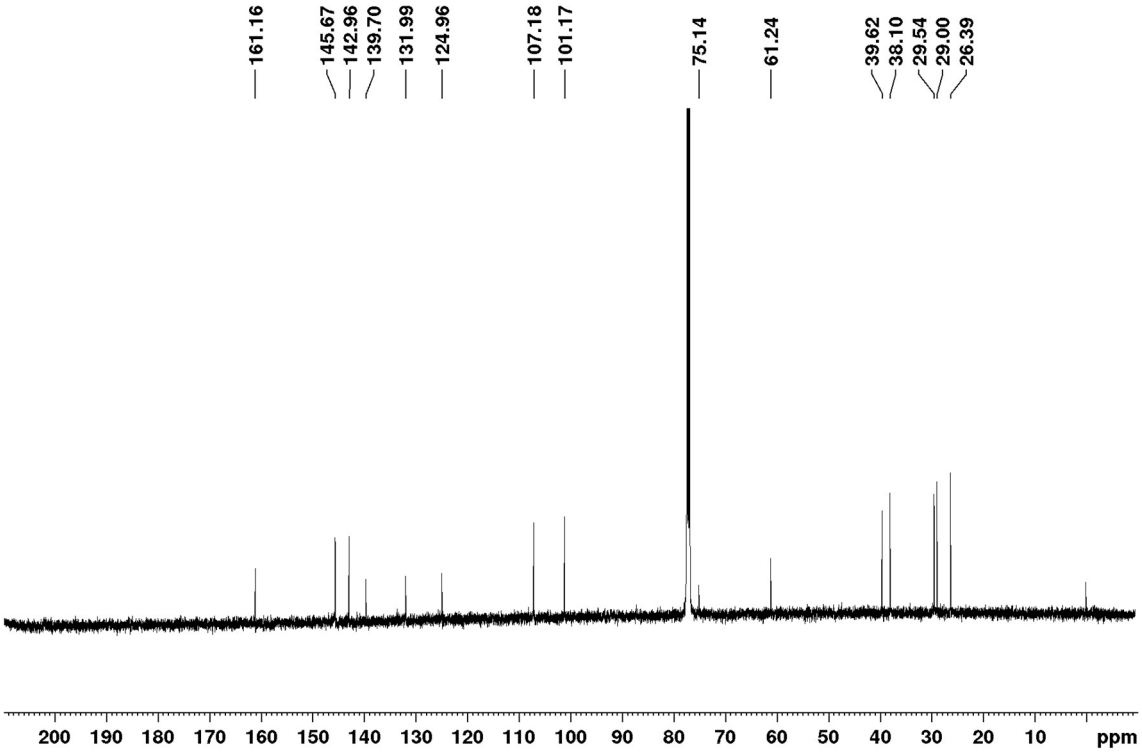


1-(5-(*1H*-Pyrrolo[3,2-*c*]pyridine-2-carboxamido)pentyl)-1,2-dicarba-*closo*-dodecaborane (8)

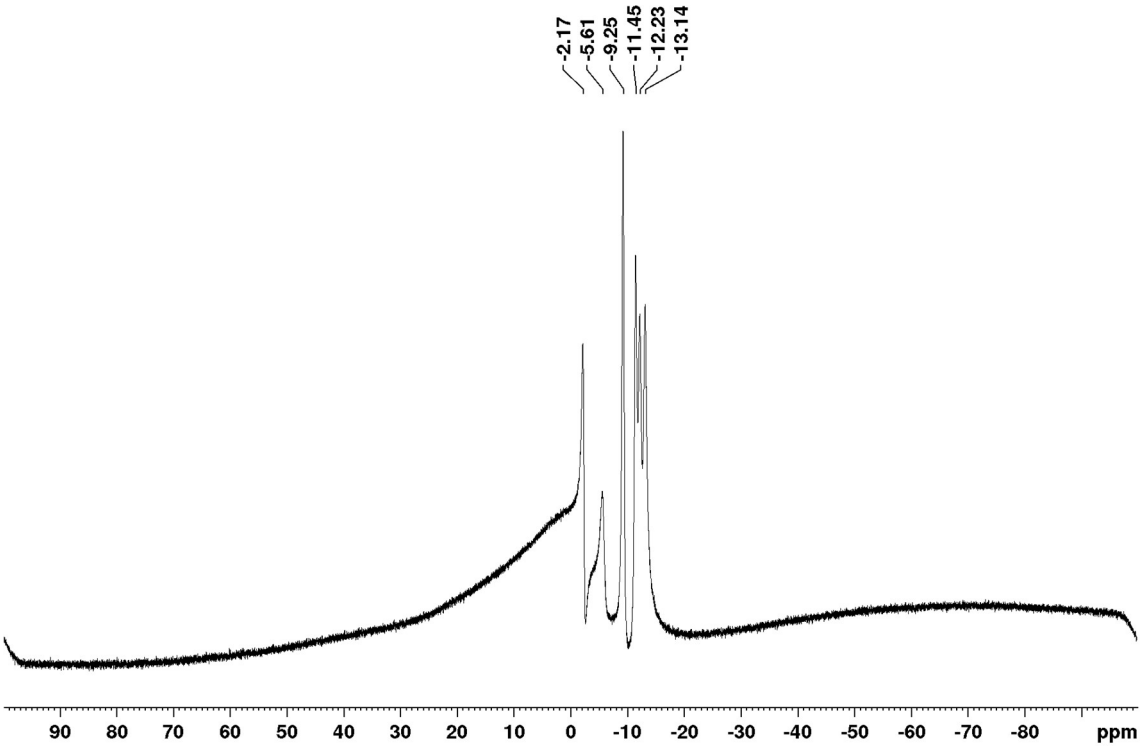
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

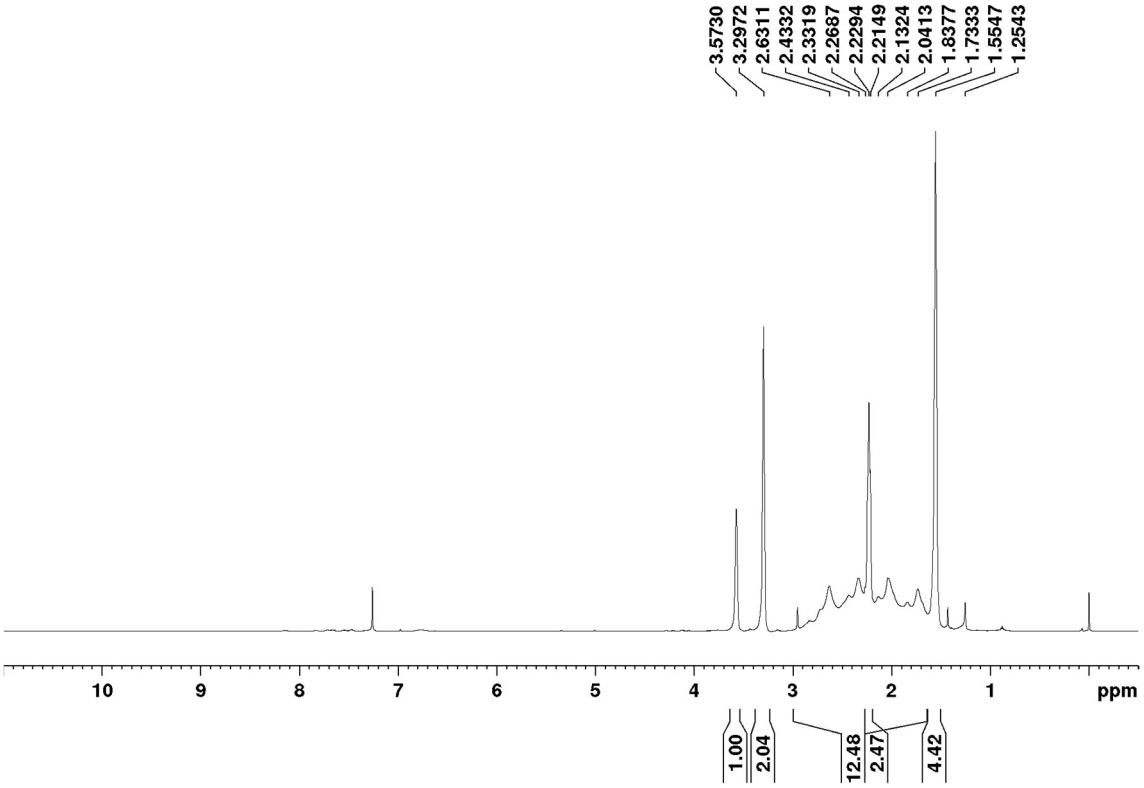


^{11}B NMR (160 MHz; $CDCl_3$)

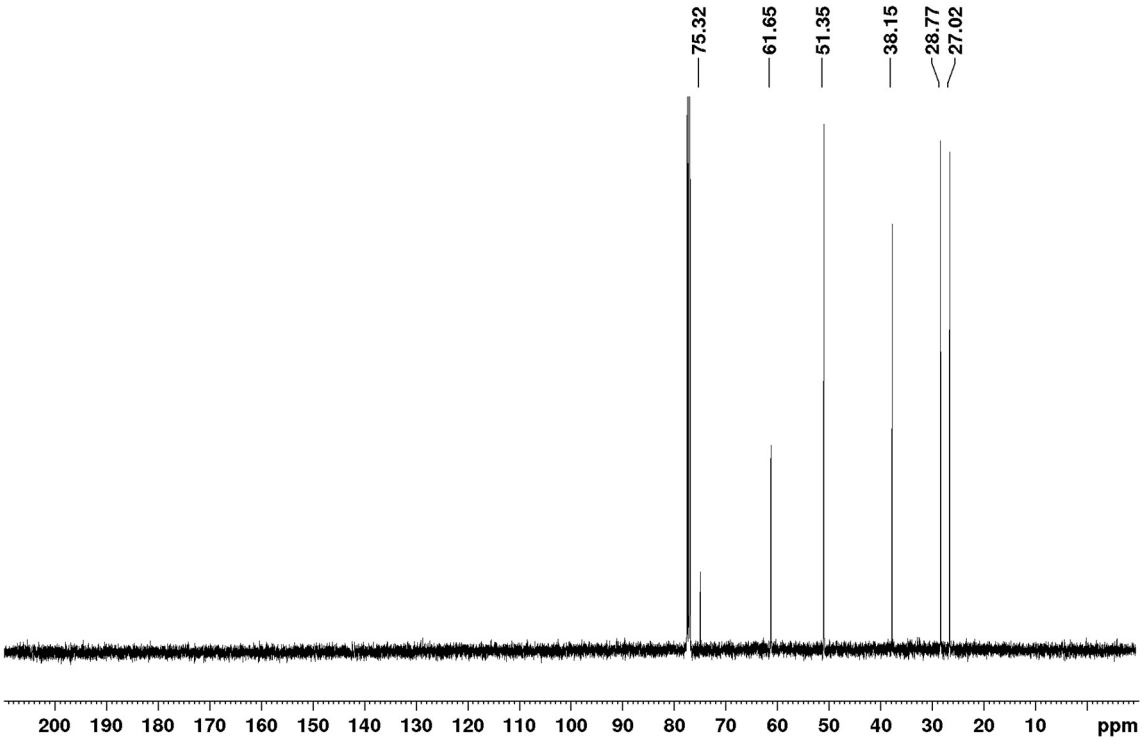


1-(4-Azidebutyl)-1,2-dicarba-*closo*-dodecaborane (9a)

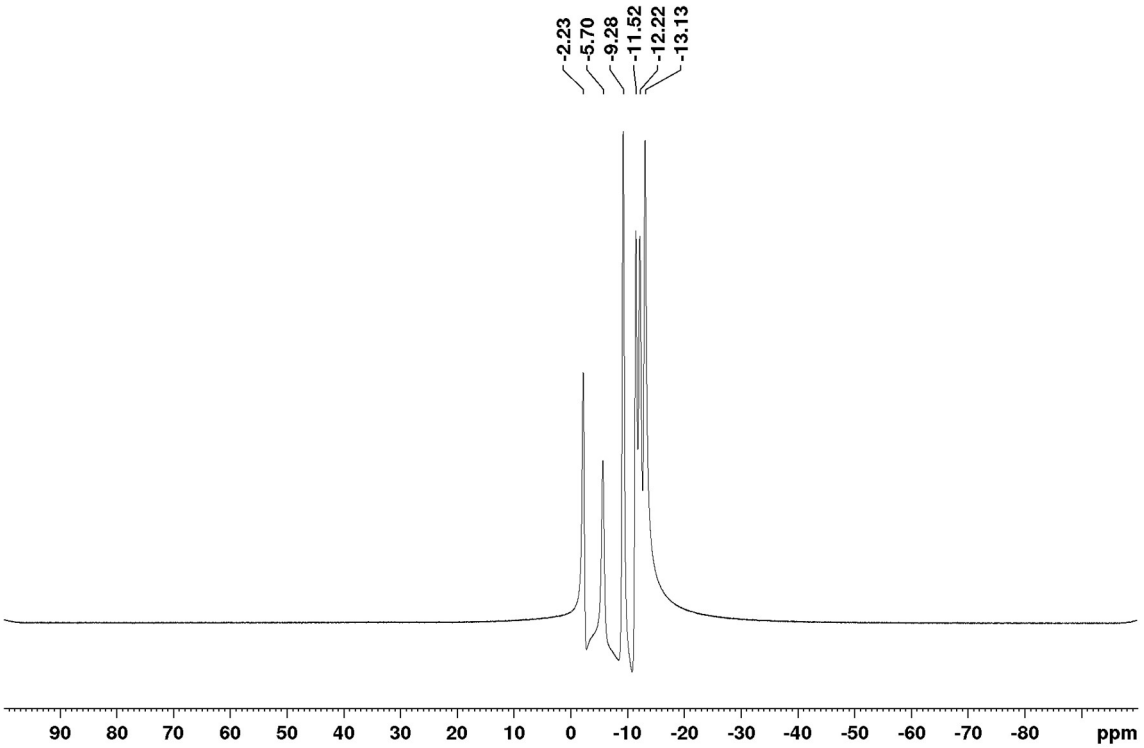
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

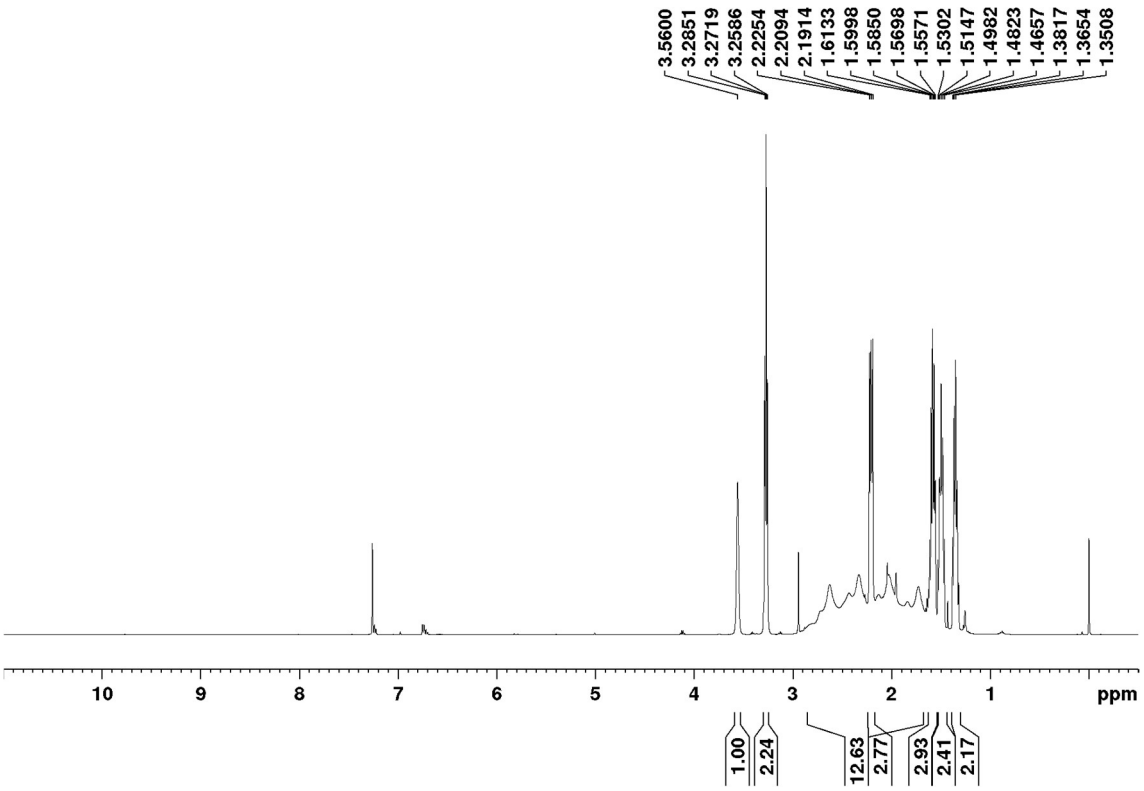


^{11}B NMR (160 MHz; $CDCl_3$)

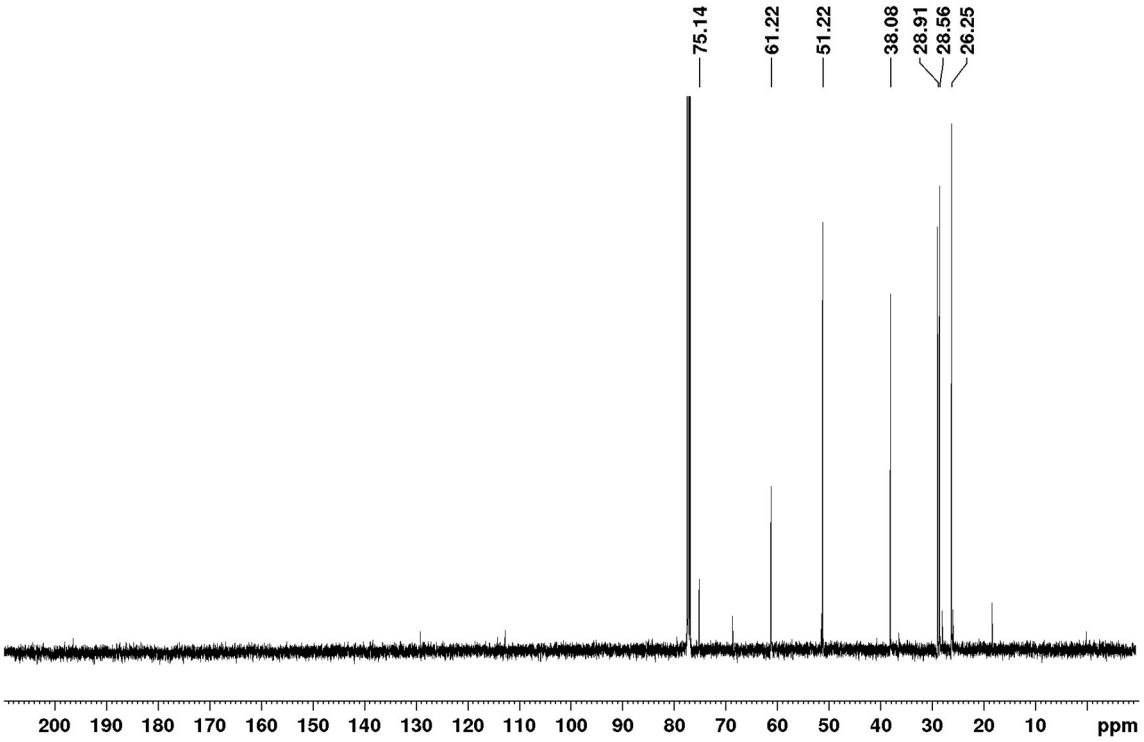


1-(5-Azidepentyl)-1,2-dicarba-closo-dodecaborane (9b)

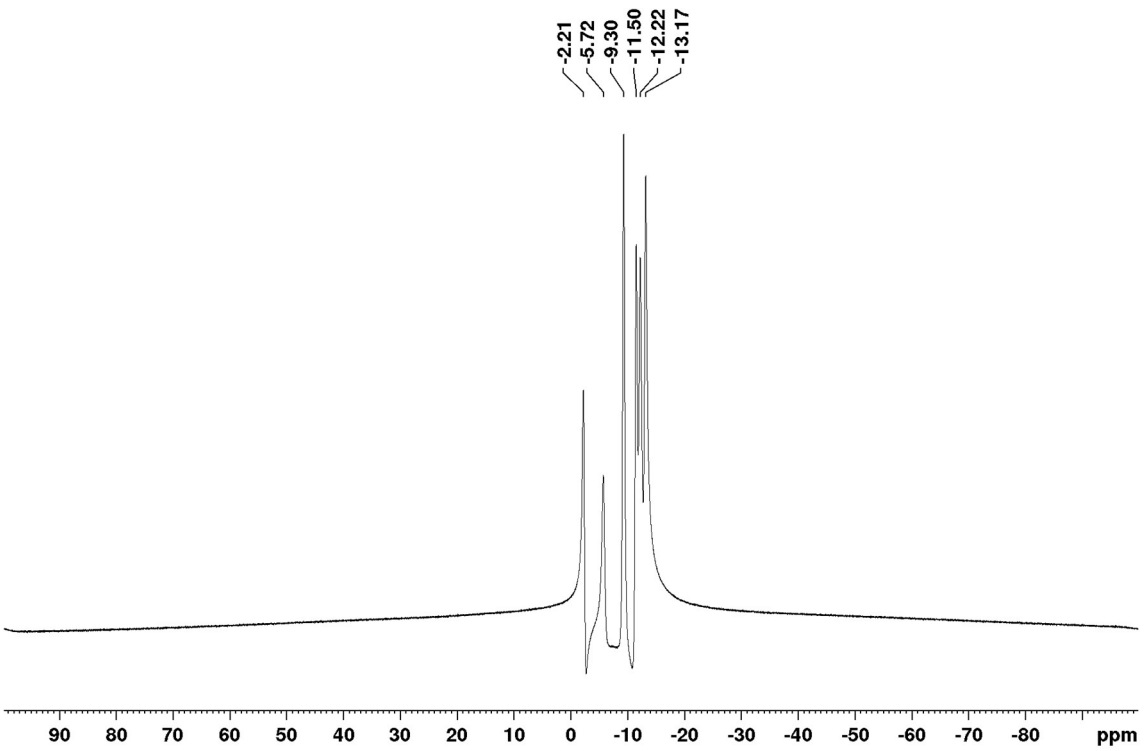
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

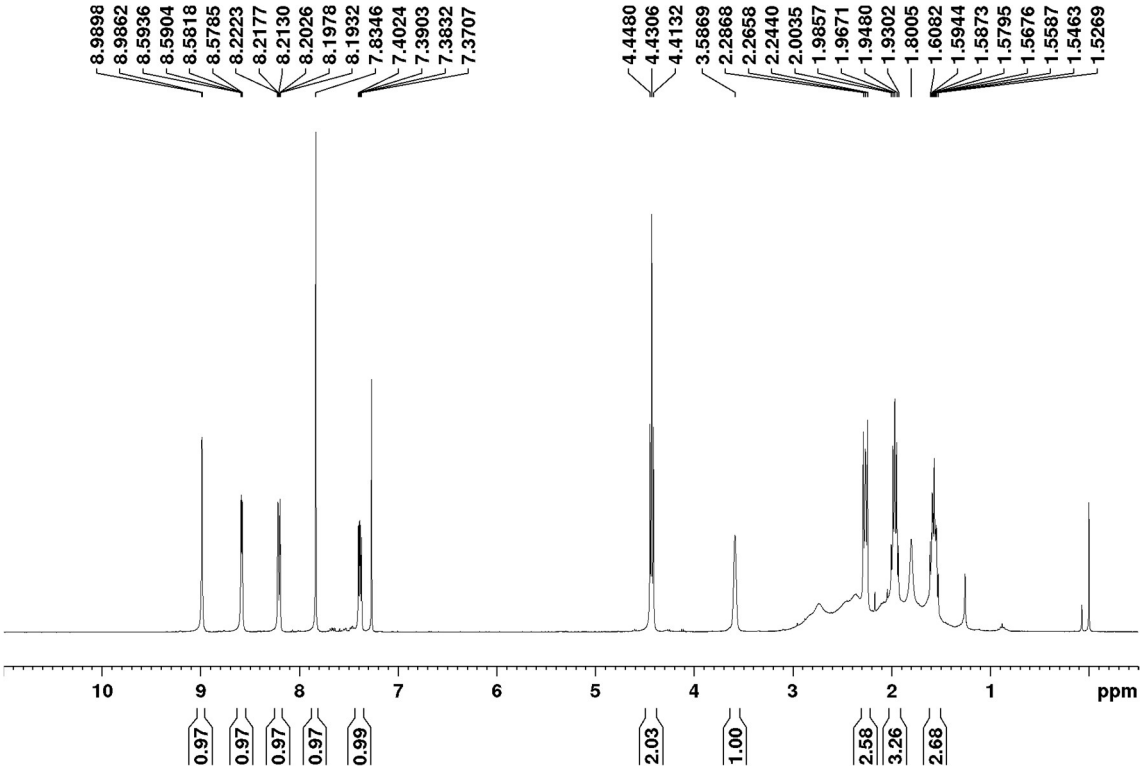


^{11}B NMR (160 MHz; $CDCl_3$)

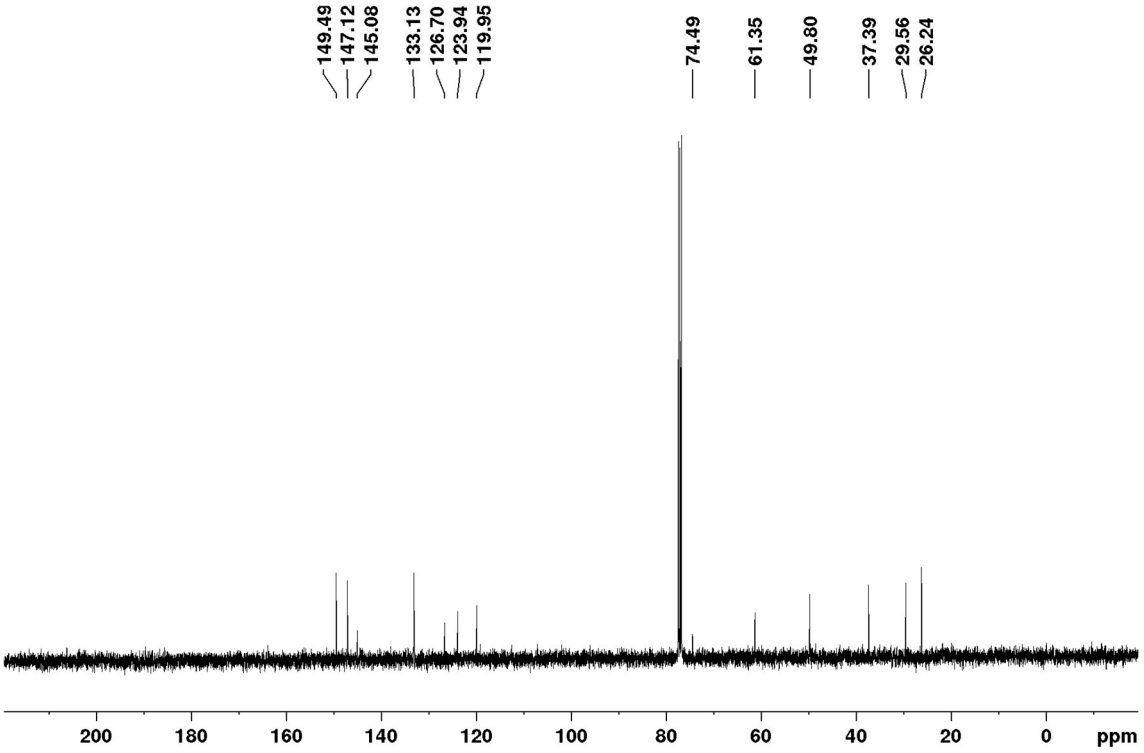


1-(4-(4-(Pyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)butyl)-1,2-dicarba-*closo*-dodecaborane (10)

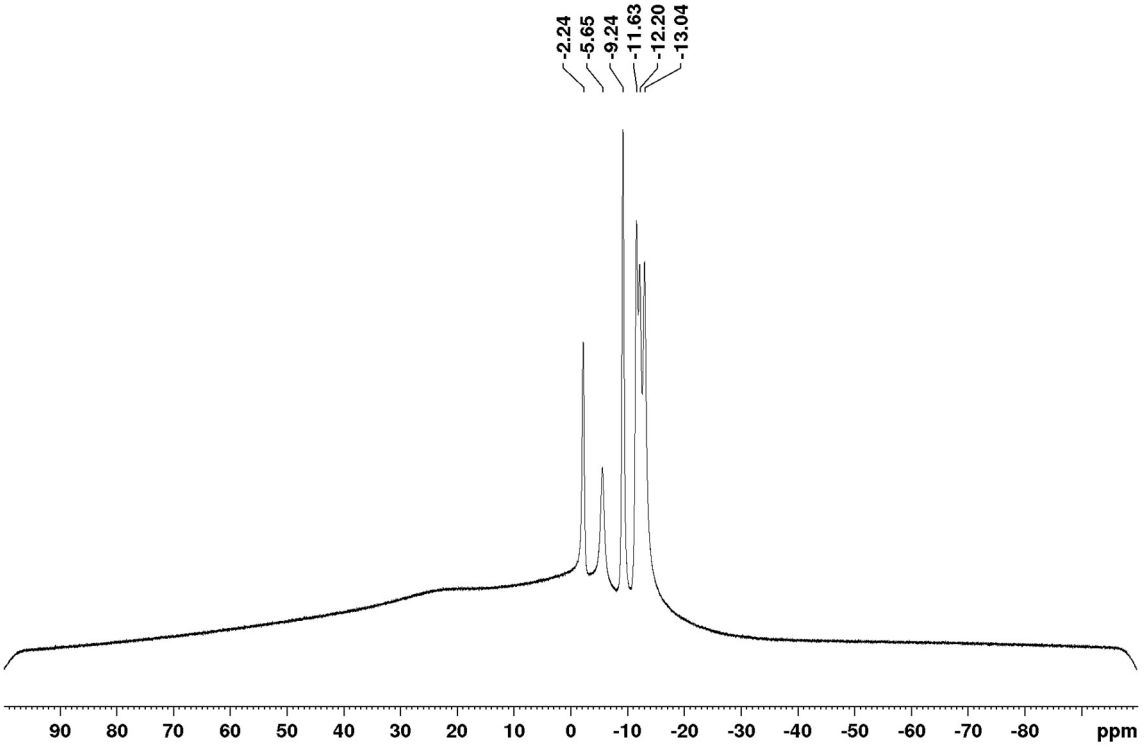
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

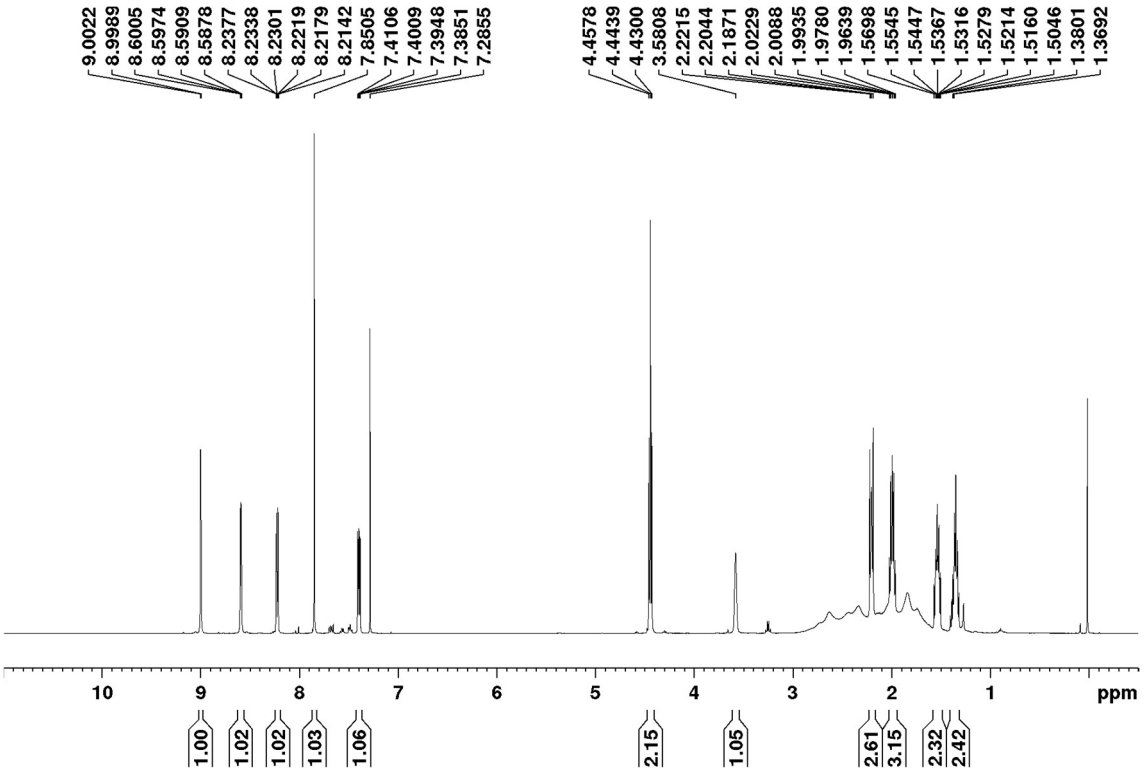


^{11}B NMR (160 MHz; $CDCl_3$)

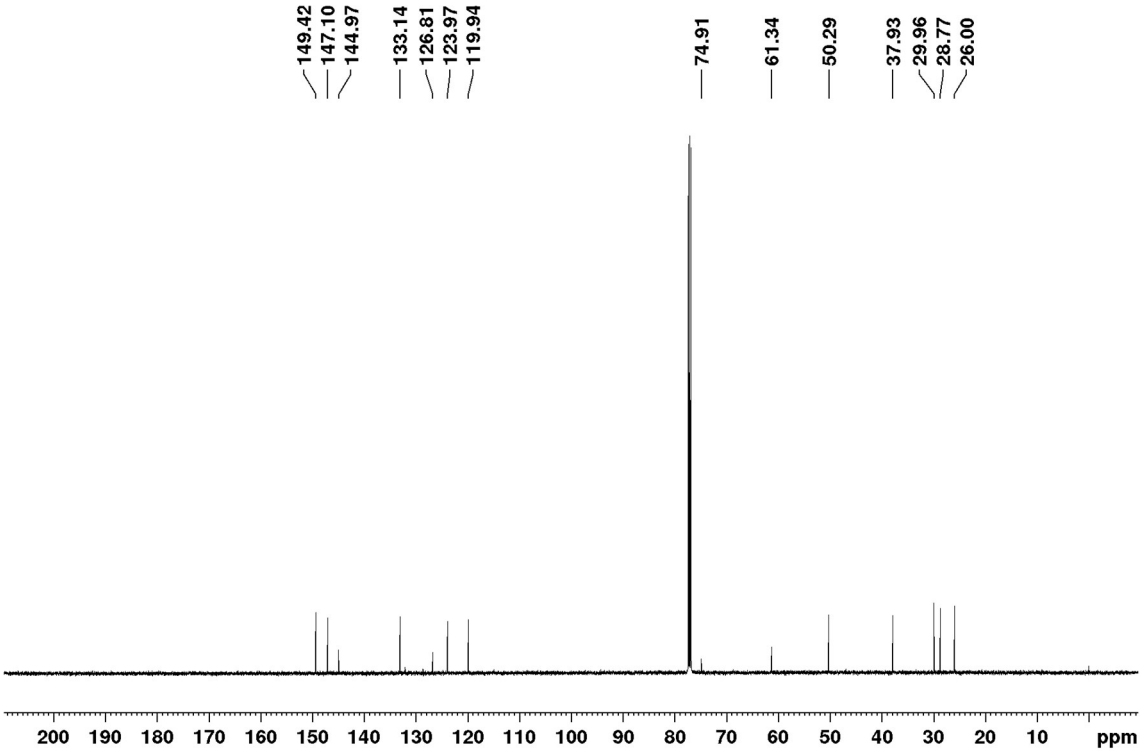


1-(4-(4-(Pyridin-3-yl)-1*H*-1,2,3-triazol-1-yl)pentyl)-1,2-dicarba-*closo*-dodecaborane (11)

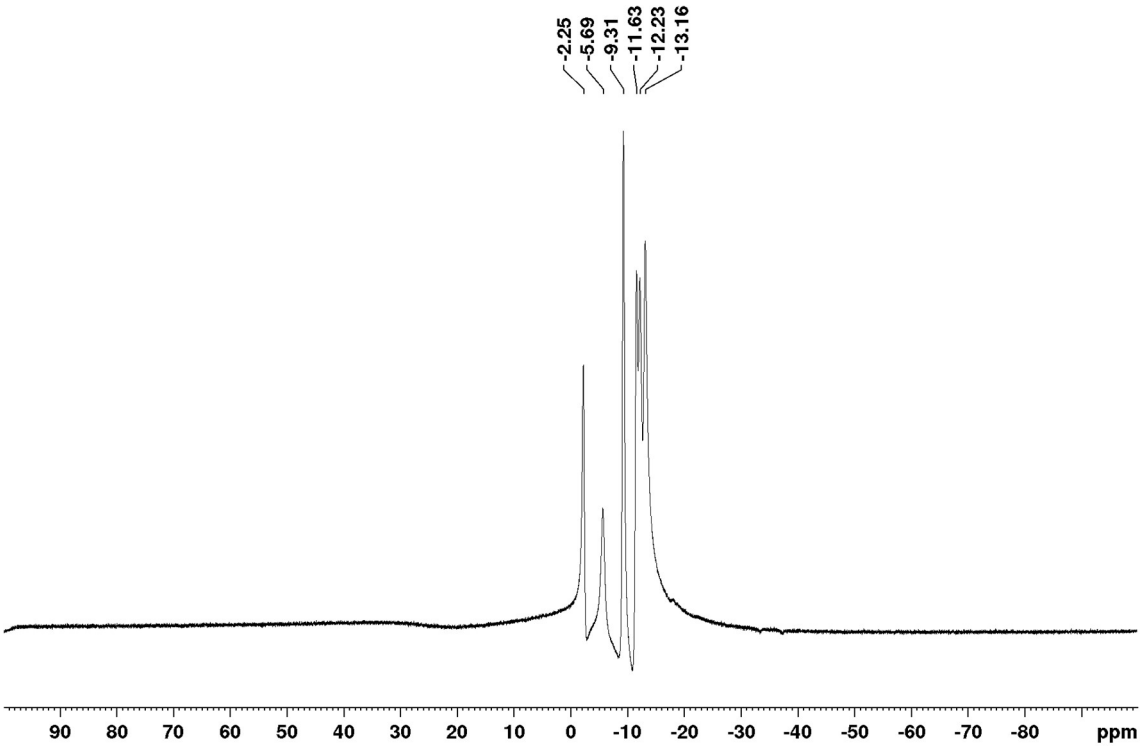
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

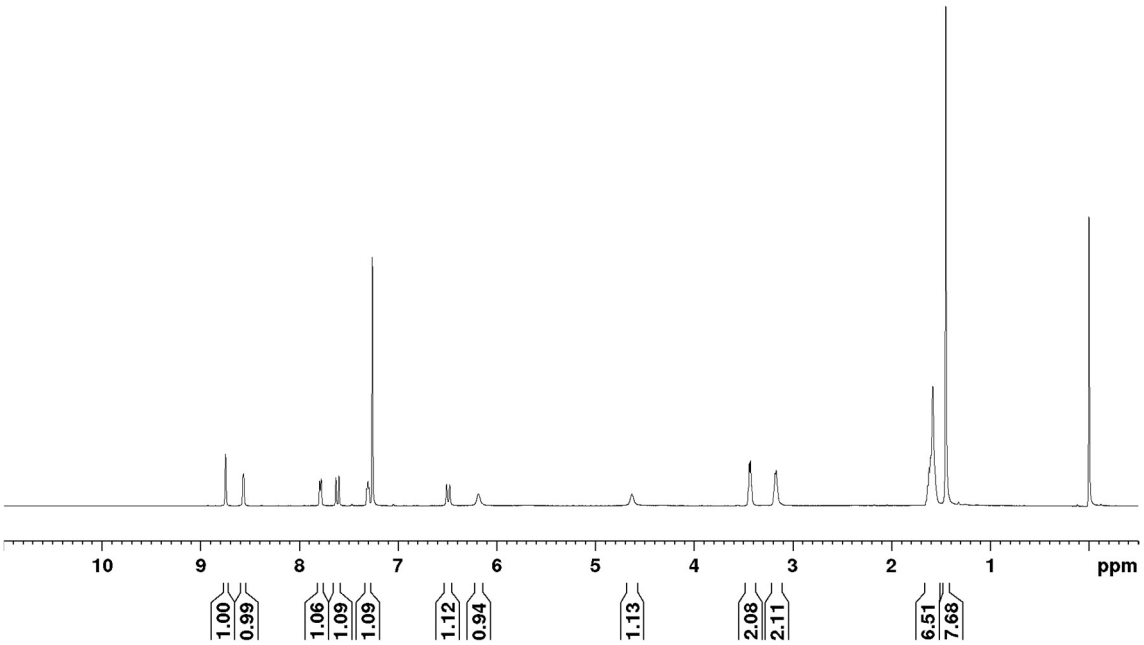


^{11}B NMR (160 MHz; $CDCl_3$)

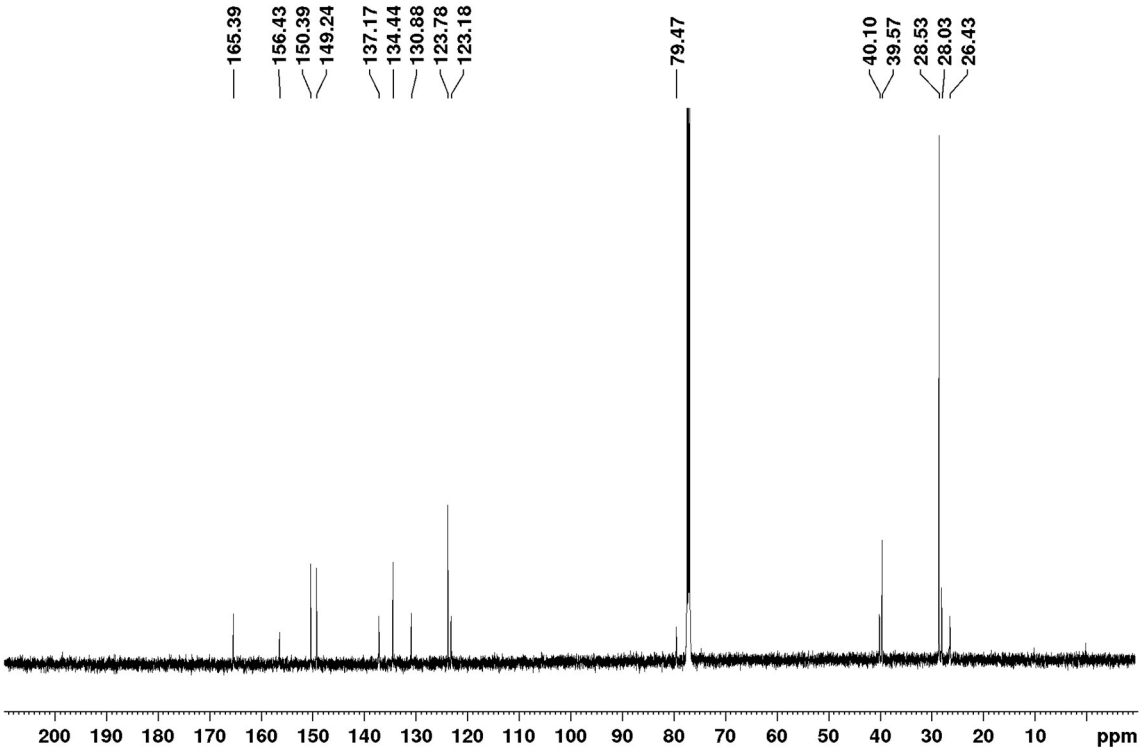


***tert*-Butyl (*E*)-(4-(3-(pyridin-3-yl)acrylamido)butyl)carbamate (13)**

^{1}H NMR (500 MHz; $CDCl_3$)

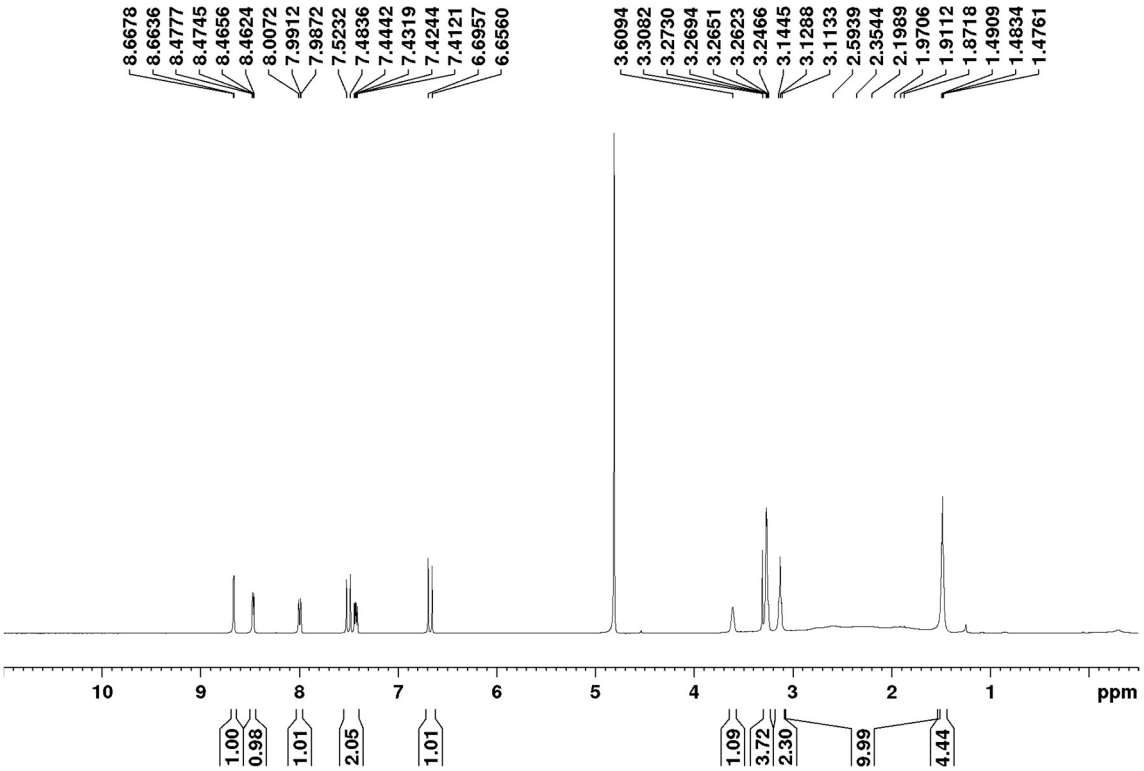


^{13}C NMR (125 MHz; $CDCl_3$)

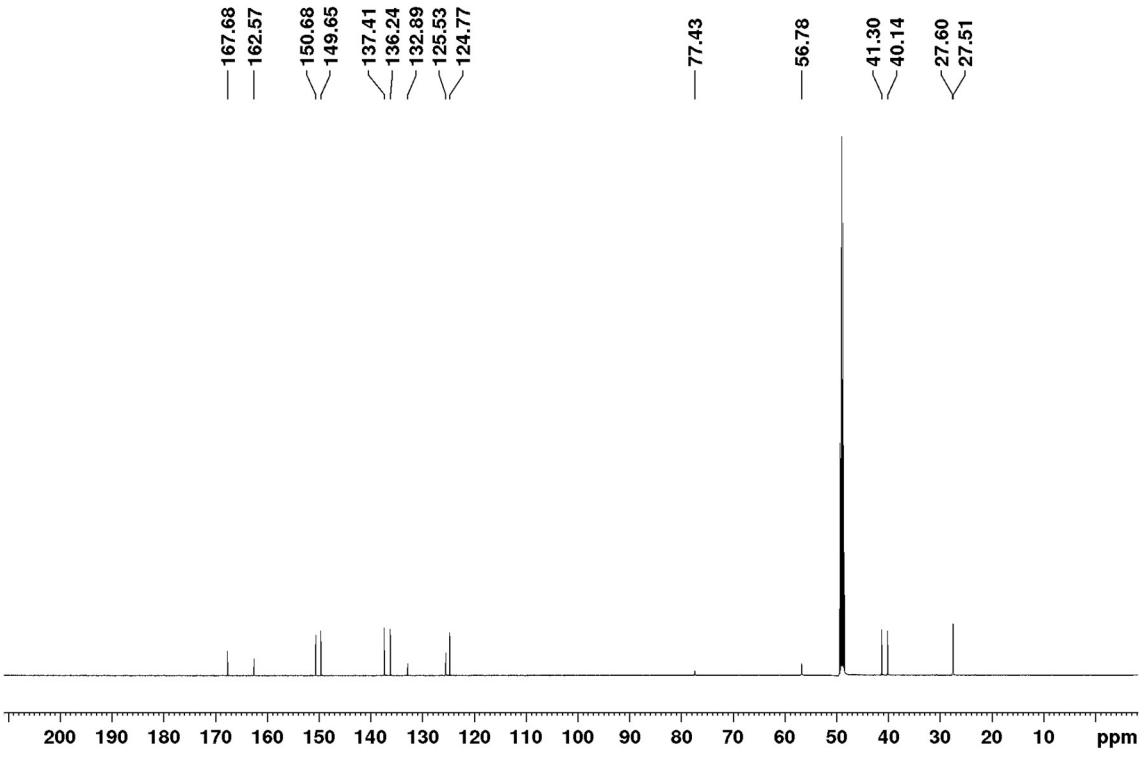


(*E*)-*N*-(4-(1,7-Dicarba-*closo*-dodecaboranyl)amidebutyl)-3-(pyridin-3-yl) acrylamide (2b)

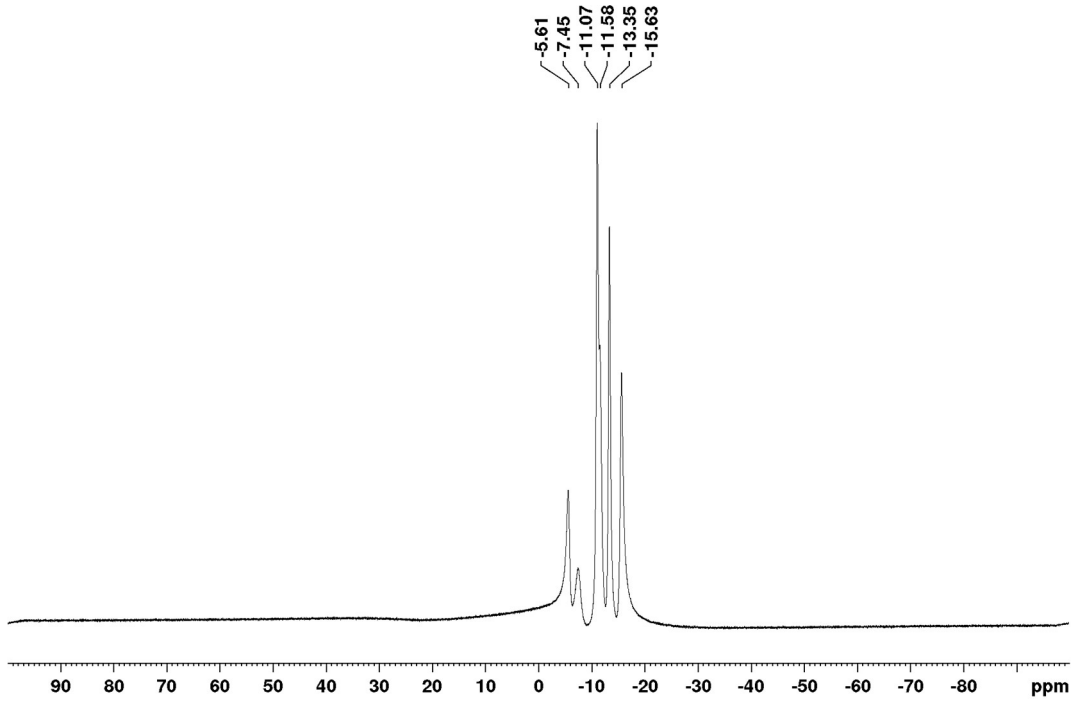
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

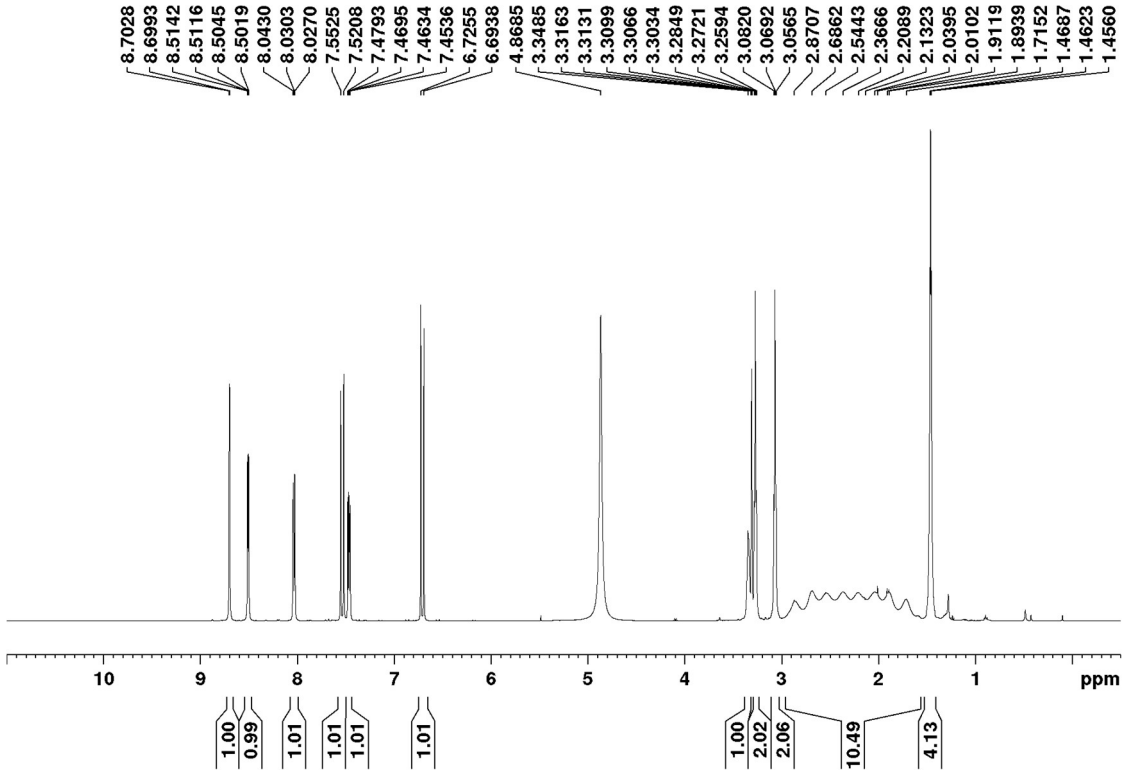


^{11}B NMR (160 MHz; CD_3OD)

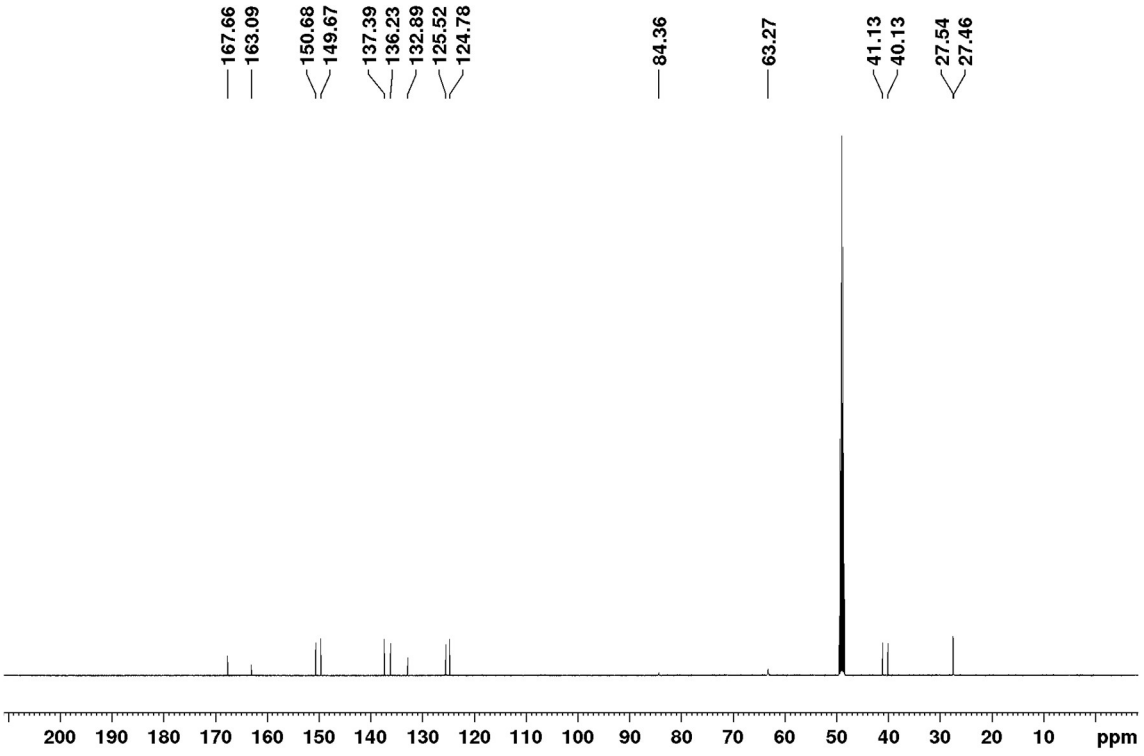


(*E*)-*N*-(4-(1,12-Dicarba-*closo*-dodecaboranyl)amidebutyl)-3-(pyridin-3-yl) acrylamide (2c)

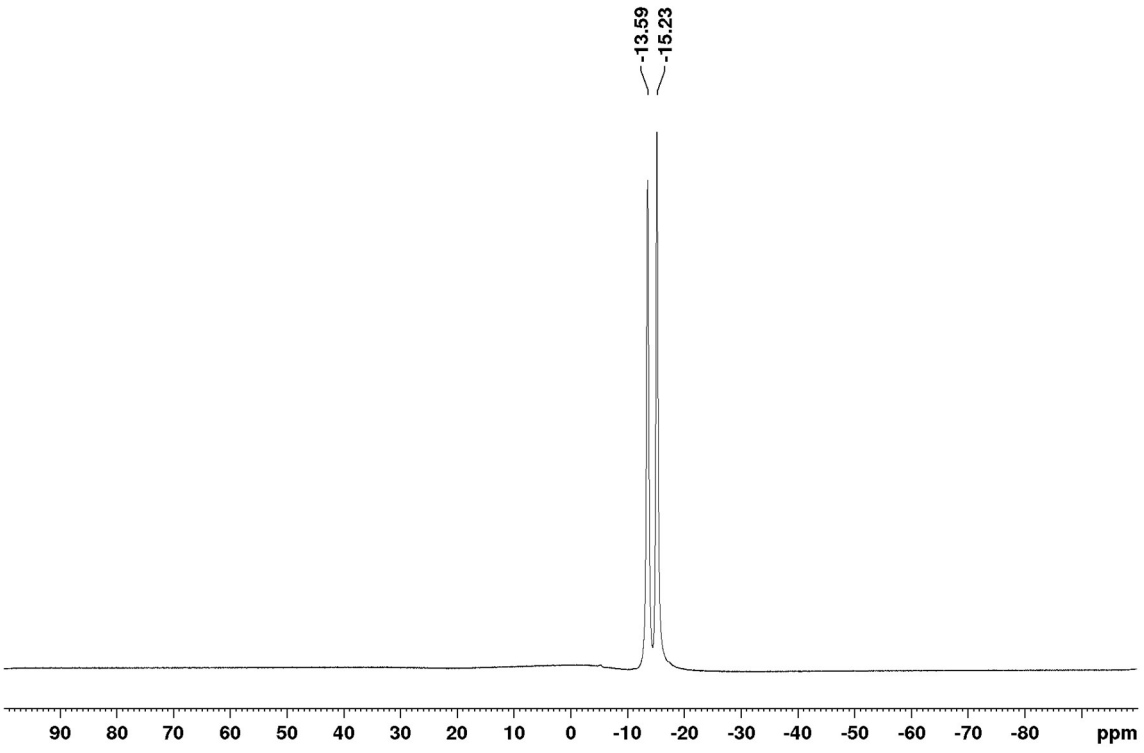
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

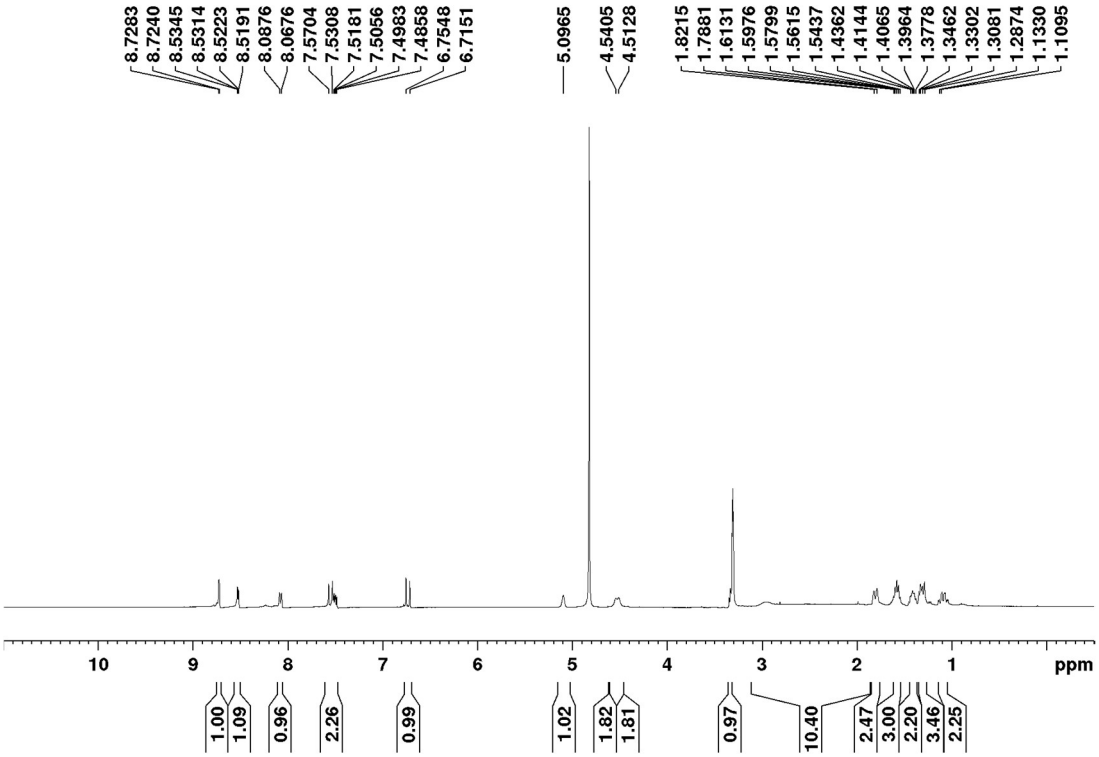


^{11}B NMR (160 MHz; CD_3OD)

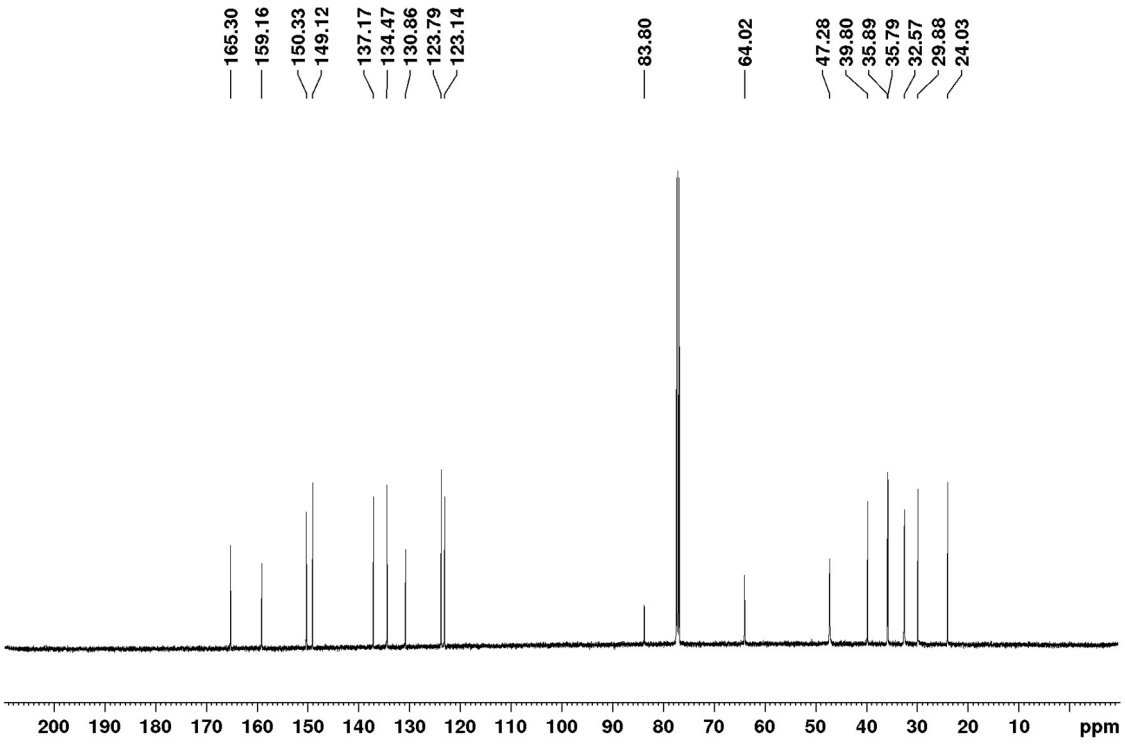


(*E*)-*N*-(4-(1-(1,12-Dicarba-*closo*-dodecaboranyl)piperidin-4-yl)butyl)-3-(pyridin-3-yl)acrylamide (19)

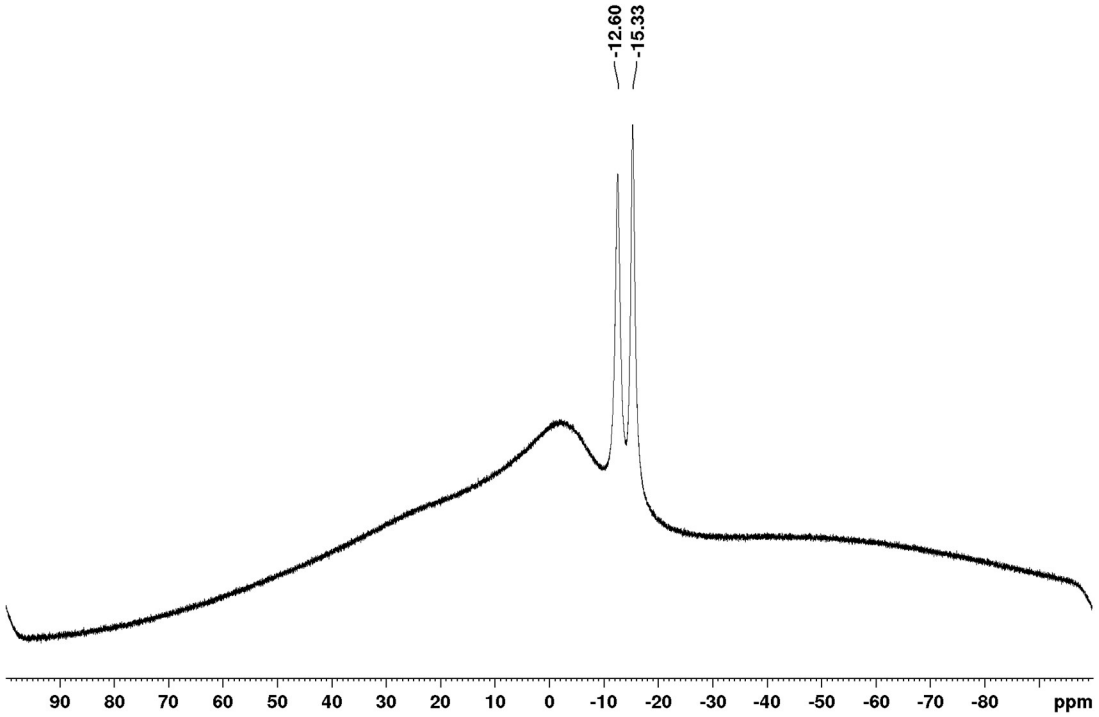
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; $CDCl_3$)

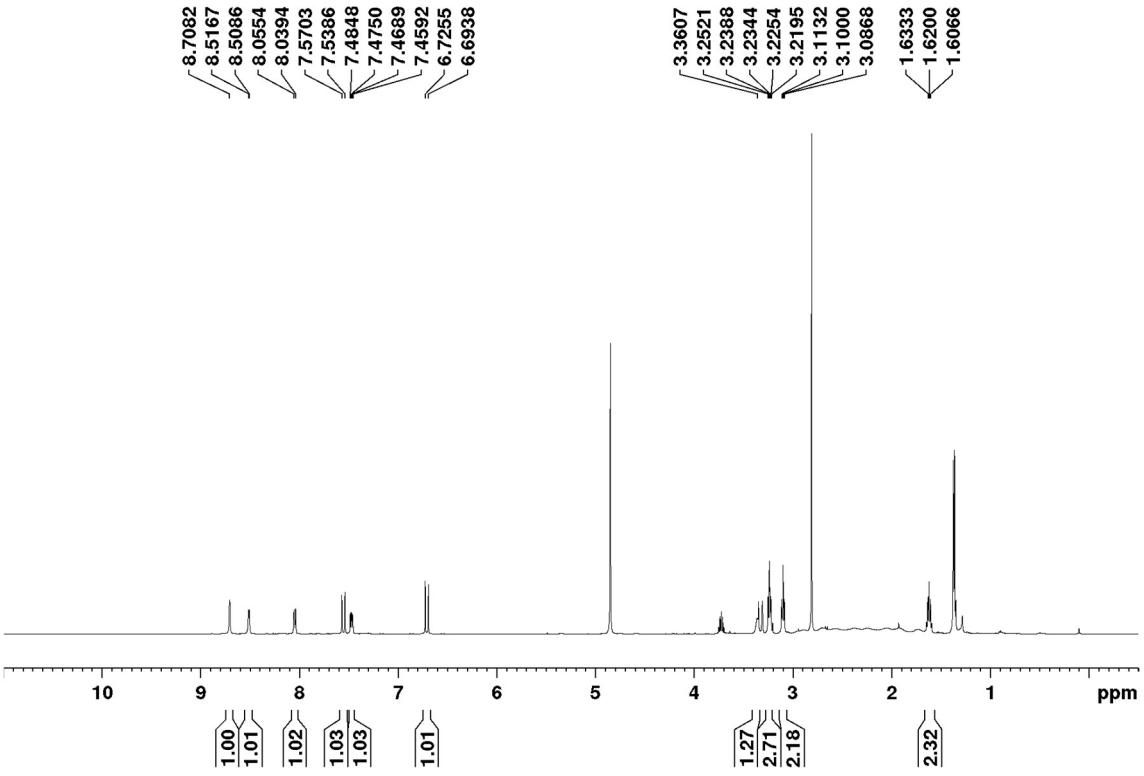


^{11}B NMR (160 MHz; CD_3OD)

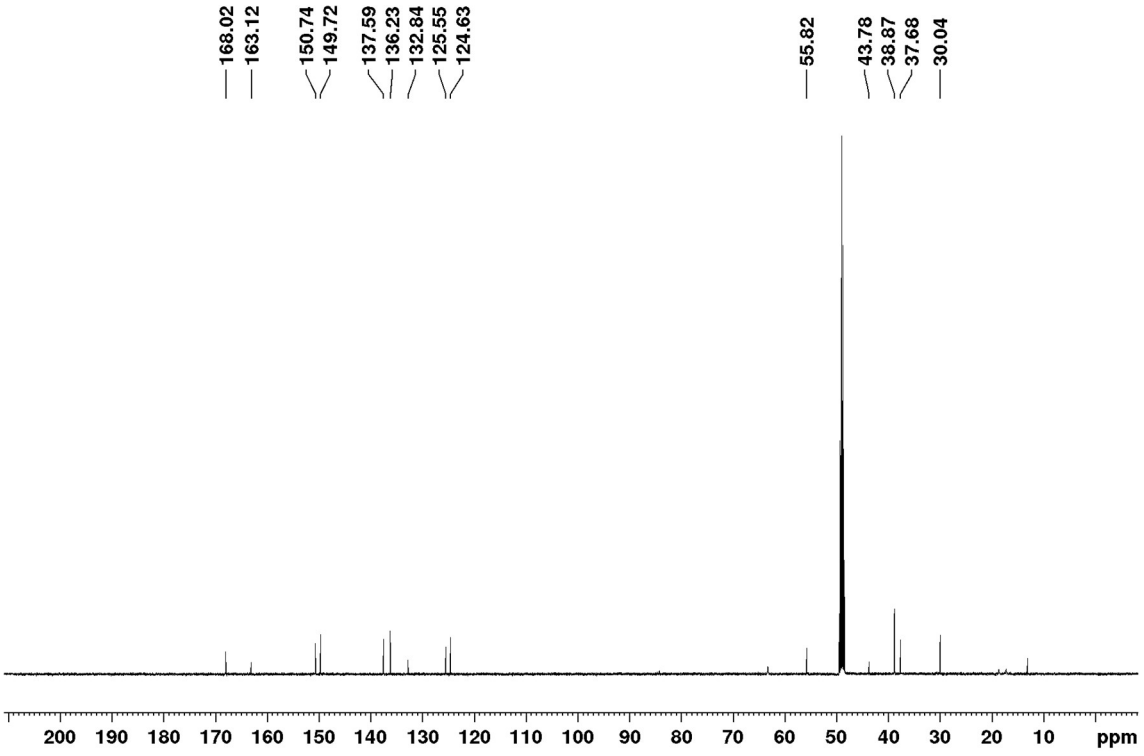


(*E*)-*N*-(4-(1,12-Dicarba-*closo*-dodecaboranyl)amidepropyl)-3-(pyridin-3-yl)acrylamide (22)

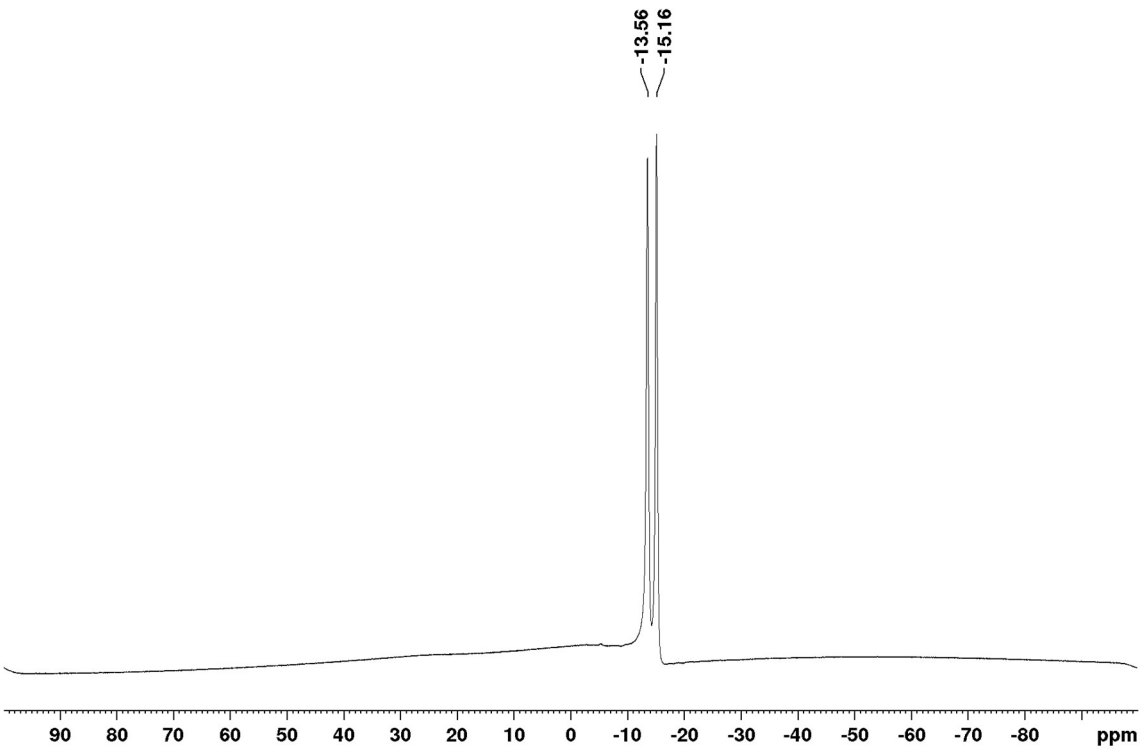
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

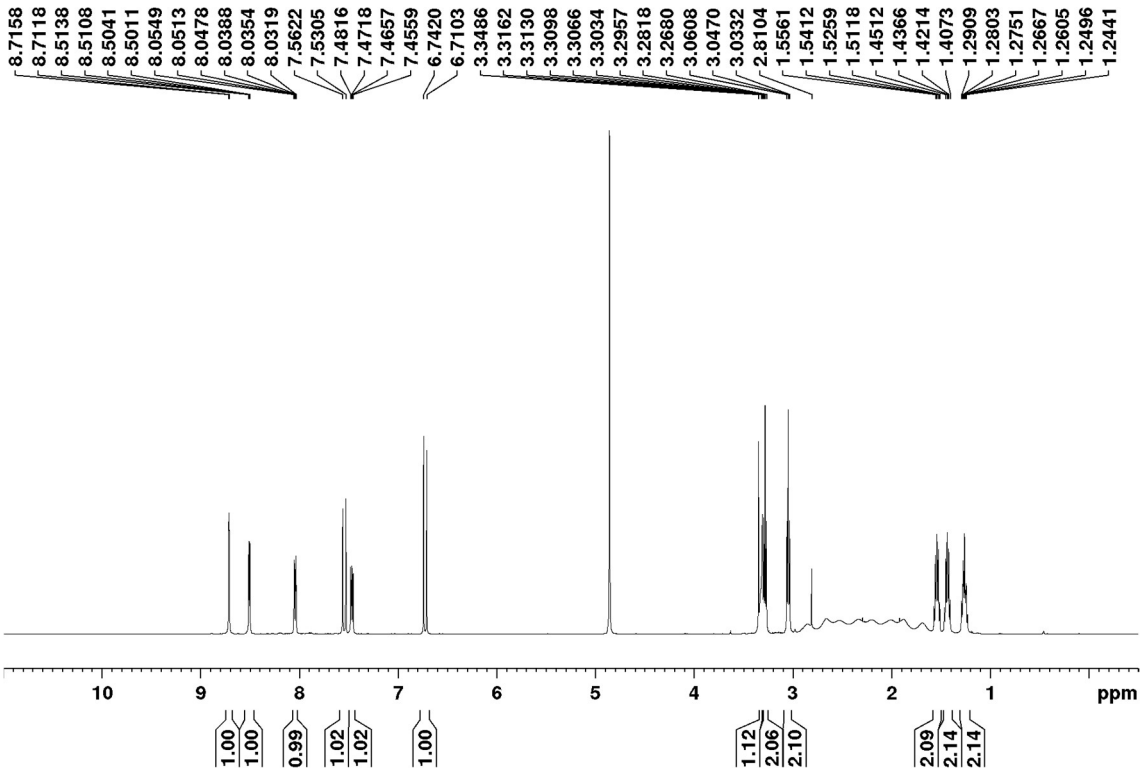


^{11}B NMR (160 MHz; CD_3OD)

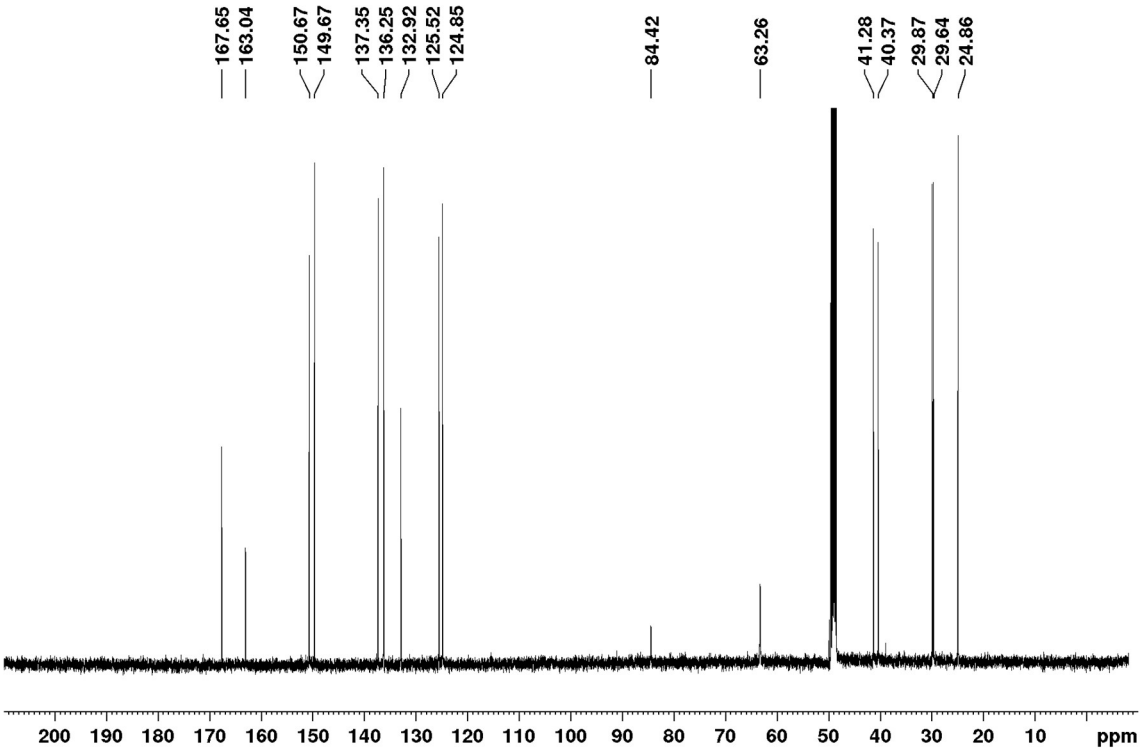


(*E*)-*N*-(4-(1,12-Dicarba-*closo*-dodecaboranyl)amidepentyl)-3-(pyridin-3-yl)acrylamide (25)

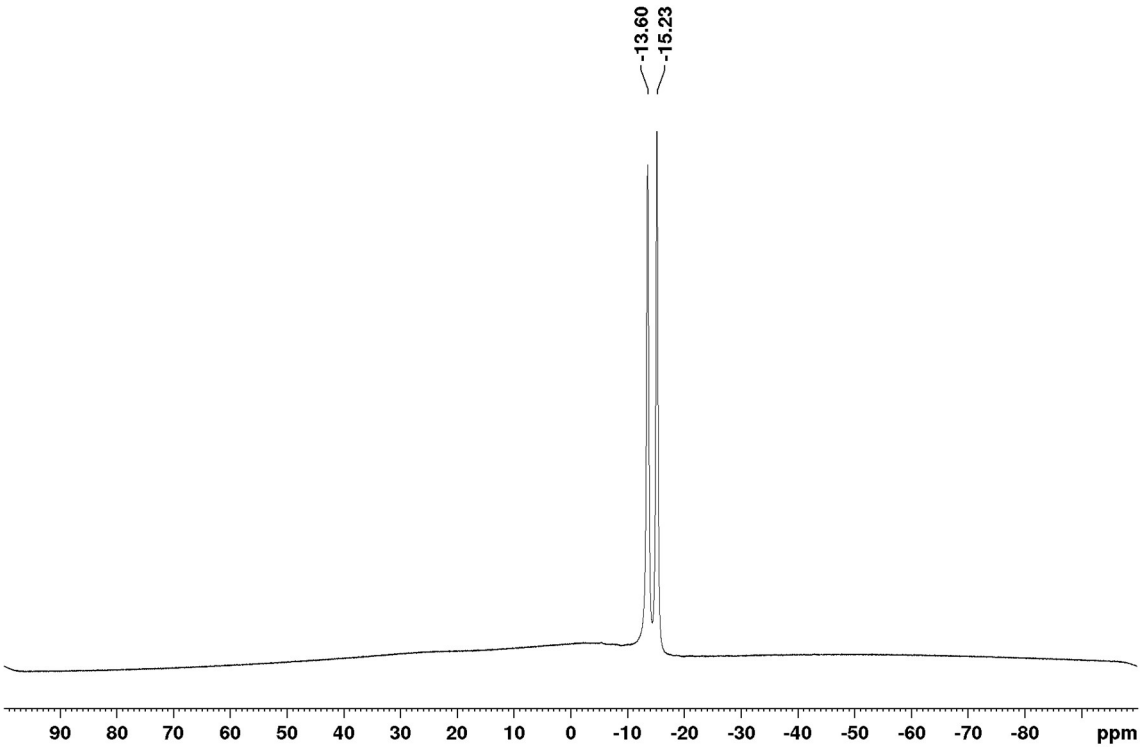
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

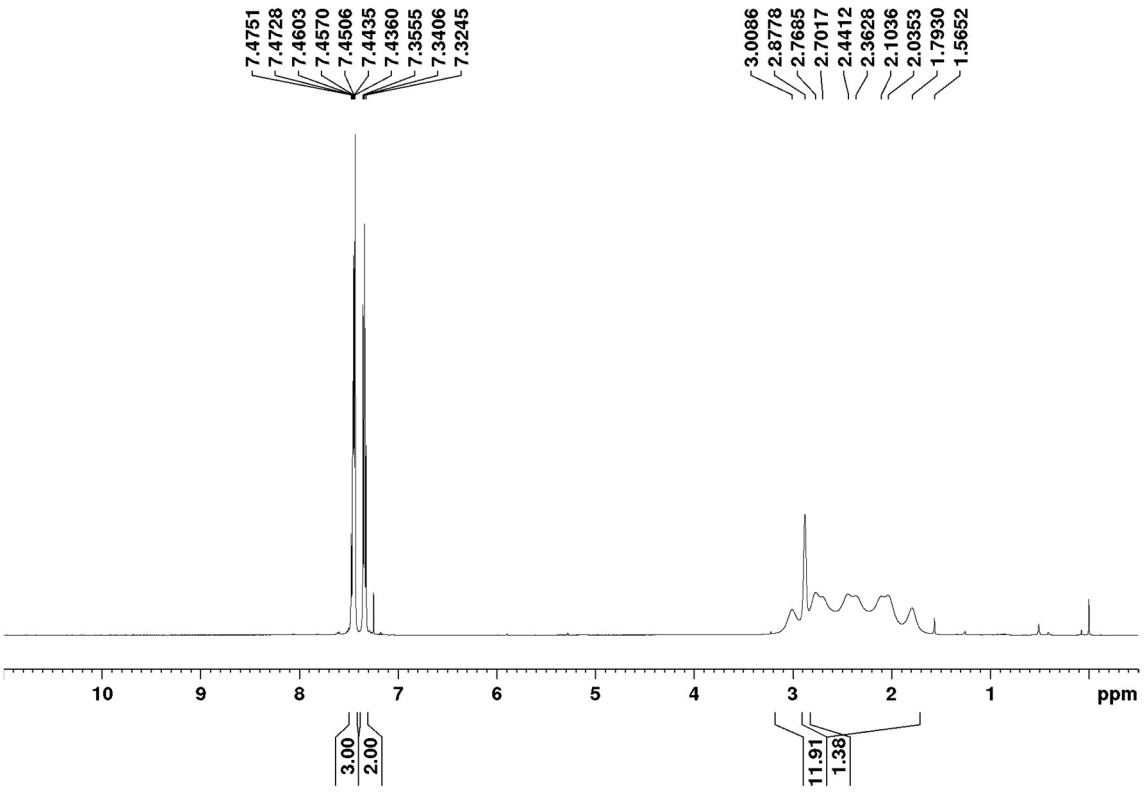


^{11}B NMR (160 MHz; CD_3OD)

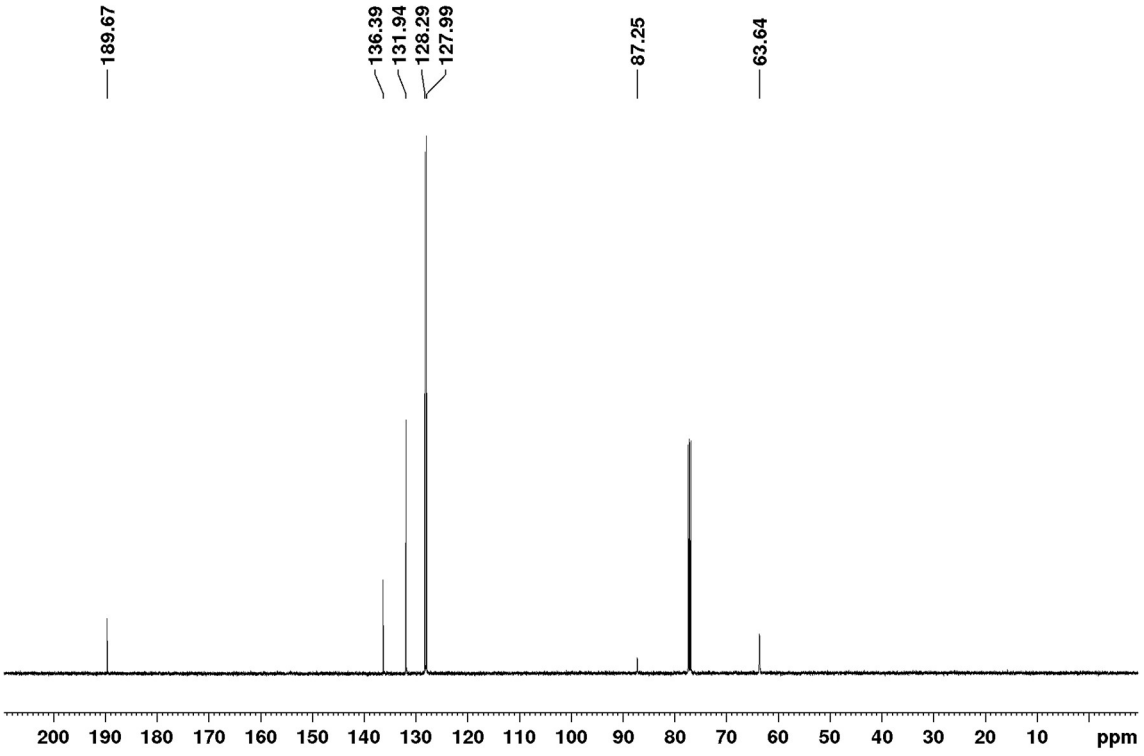


1-Benzoyl-1,12-dicarba-*closo*-dodecaborane (26)

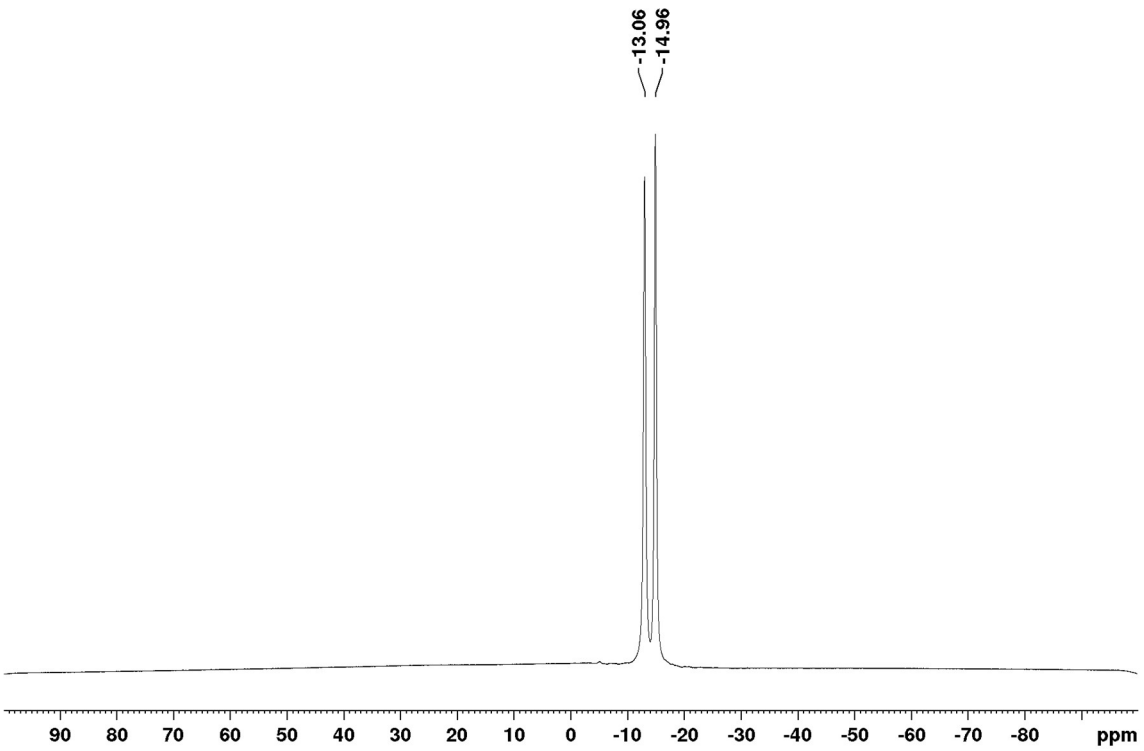
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

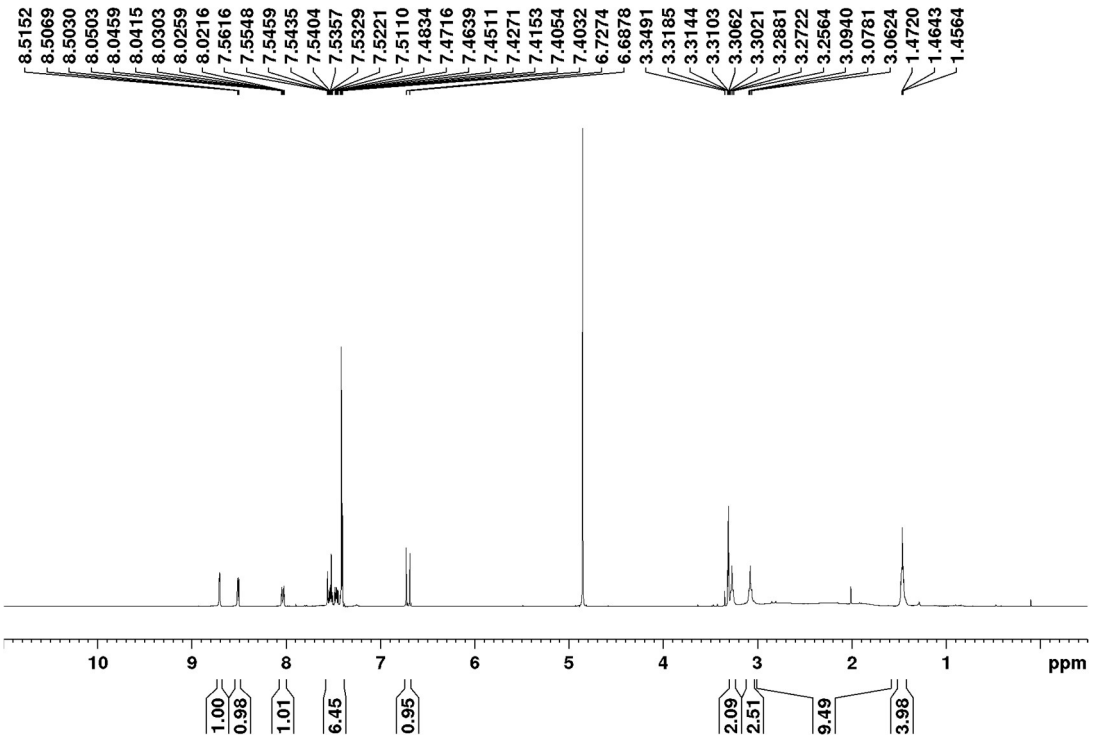


^{11}B NMR (160 MHz; $CDCl_3$)

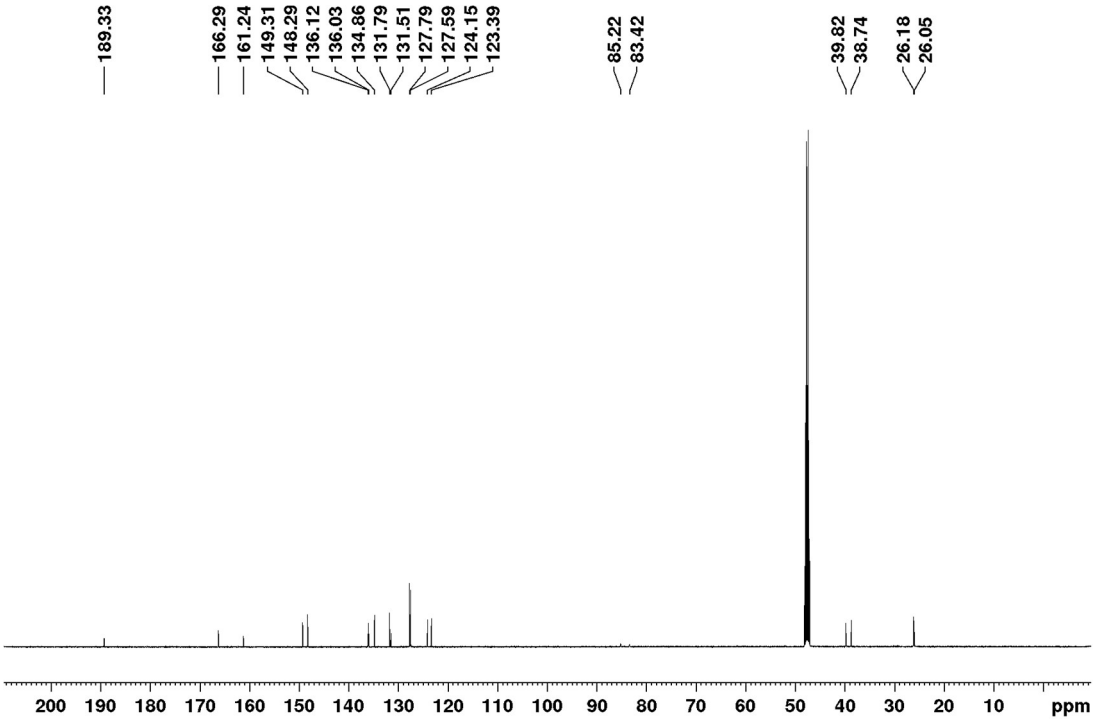


(*E*)-*N*-(4-(1-Benzoyl-1,12-dicarba-*closo*-dodecaboran-12-yl)amidebutyl)-3-(pyridin-3-yl)acrylamide (30)

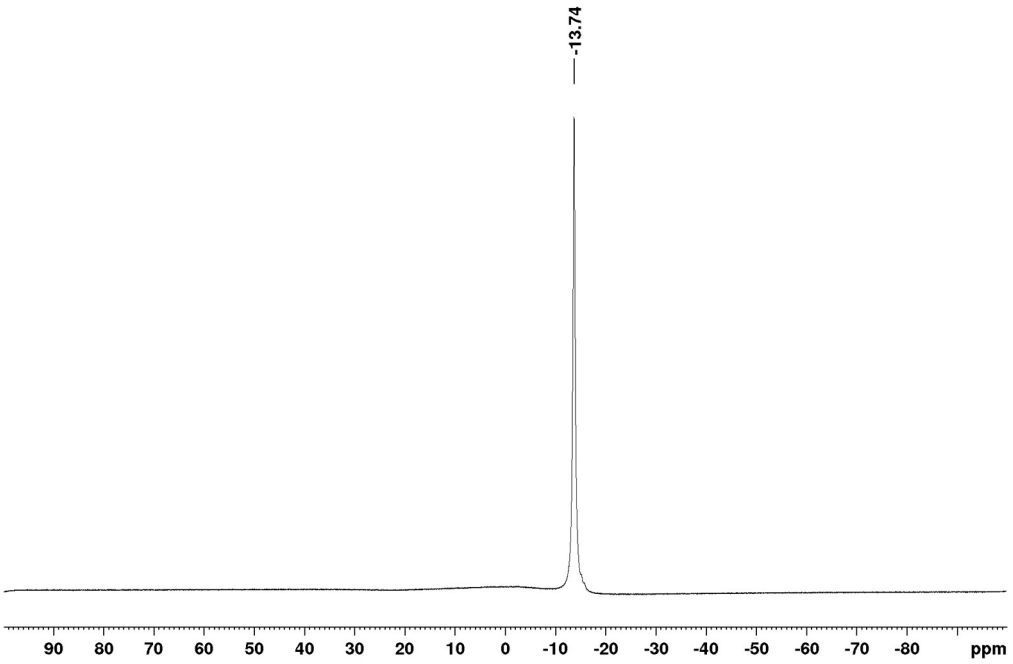
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

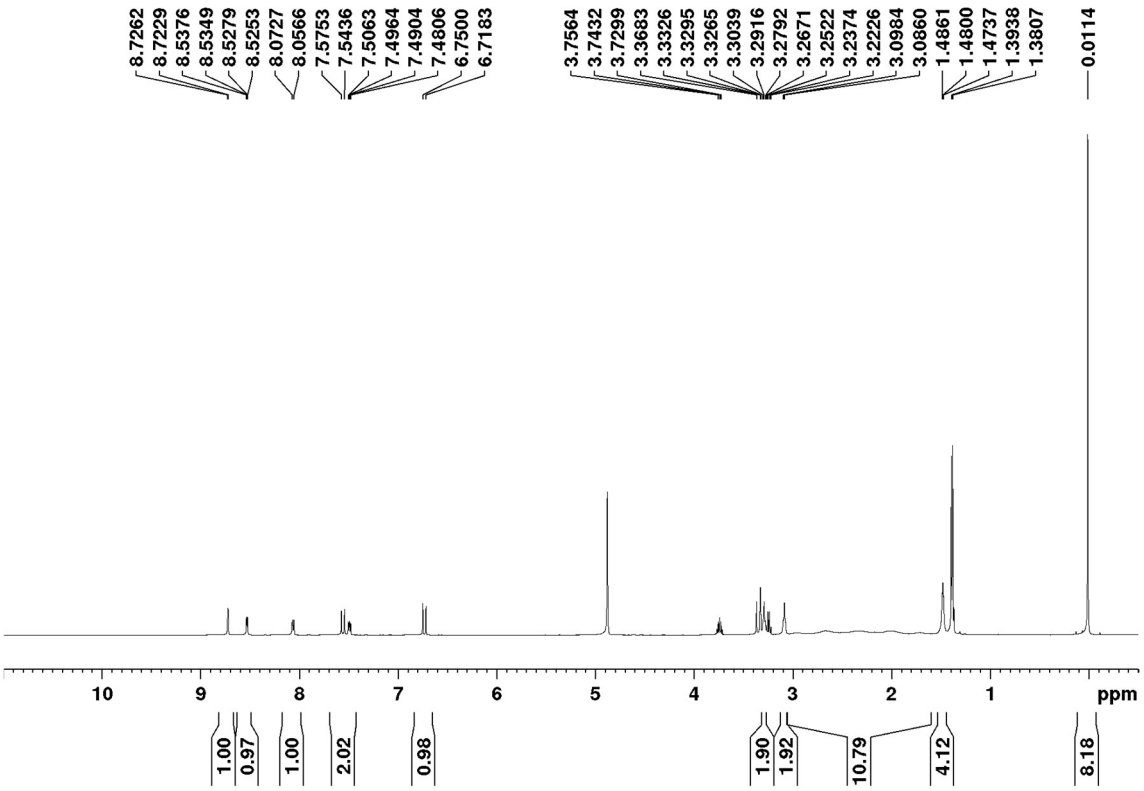


^{11}B NMR (160 MHz; CD_3OD)

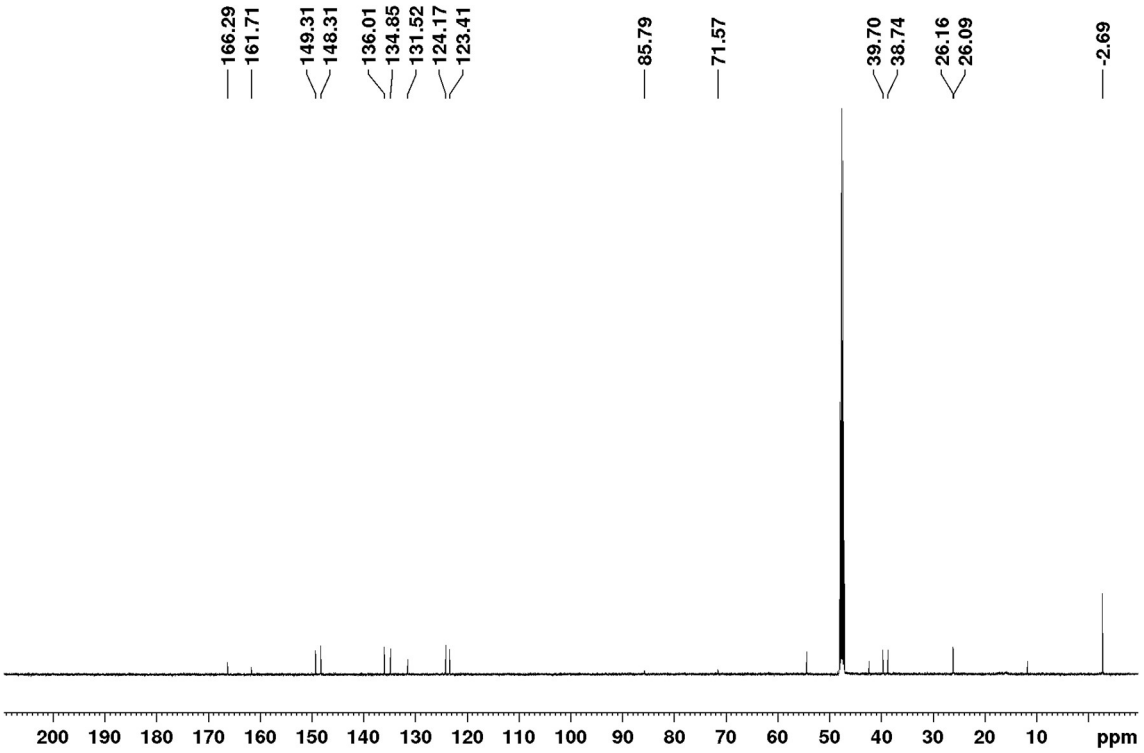


(*E*)-*N*-(4-(1-Trimethylsilyl-1,12-dicarba-*closo*-dodecaboran-12-yl)amidebutyl)-3-(pyridin-3-yl)acrylamide (31)

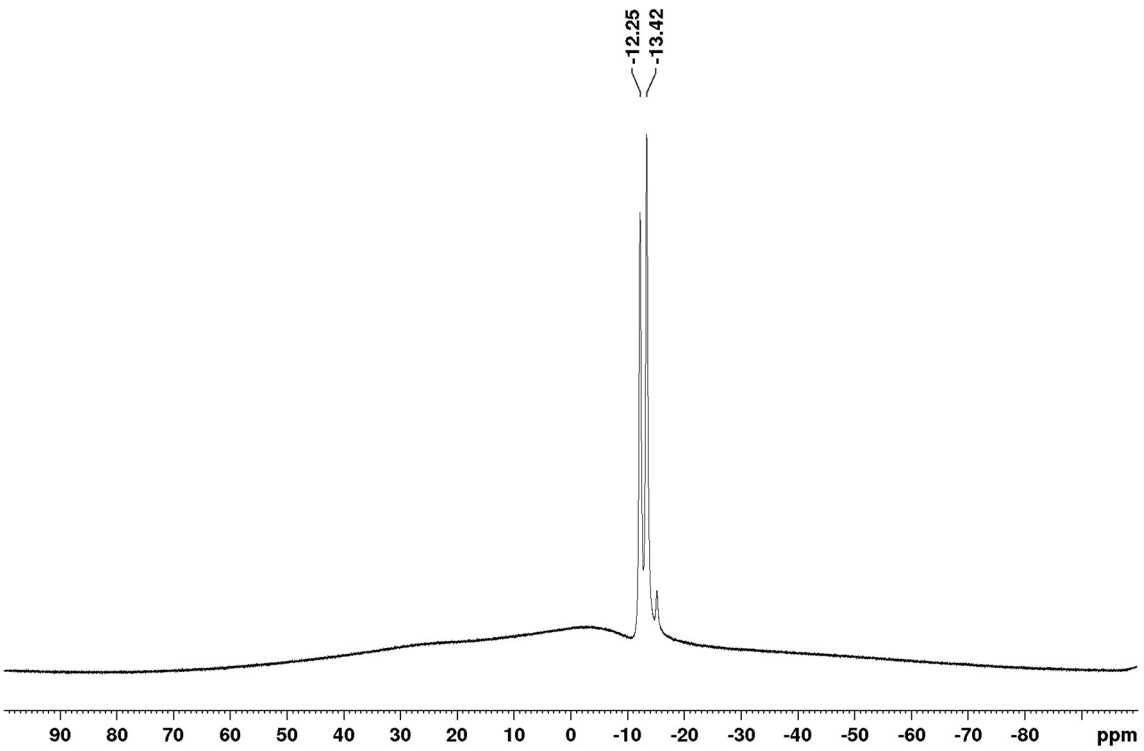
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

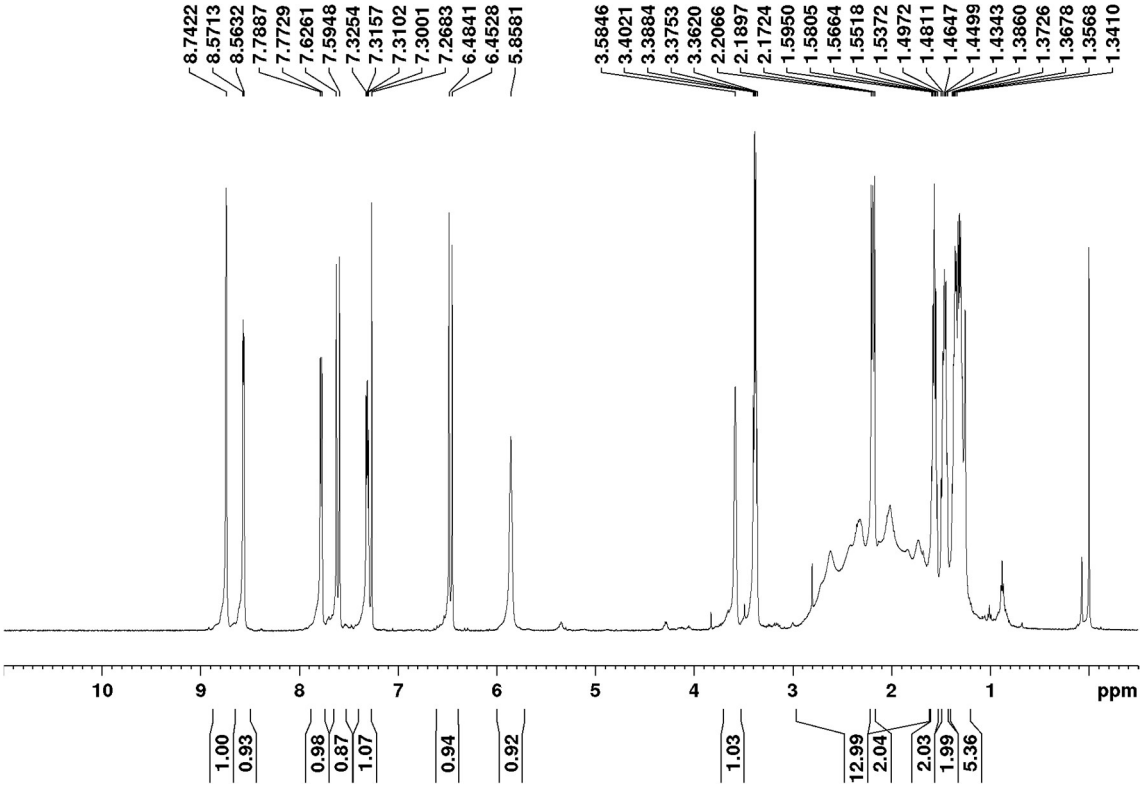


^{11}B NMR (160 MHz; CD_3OD)

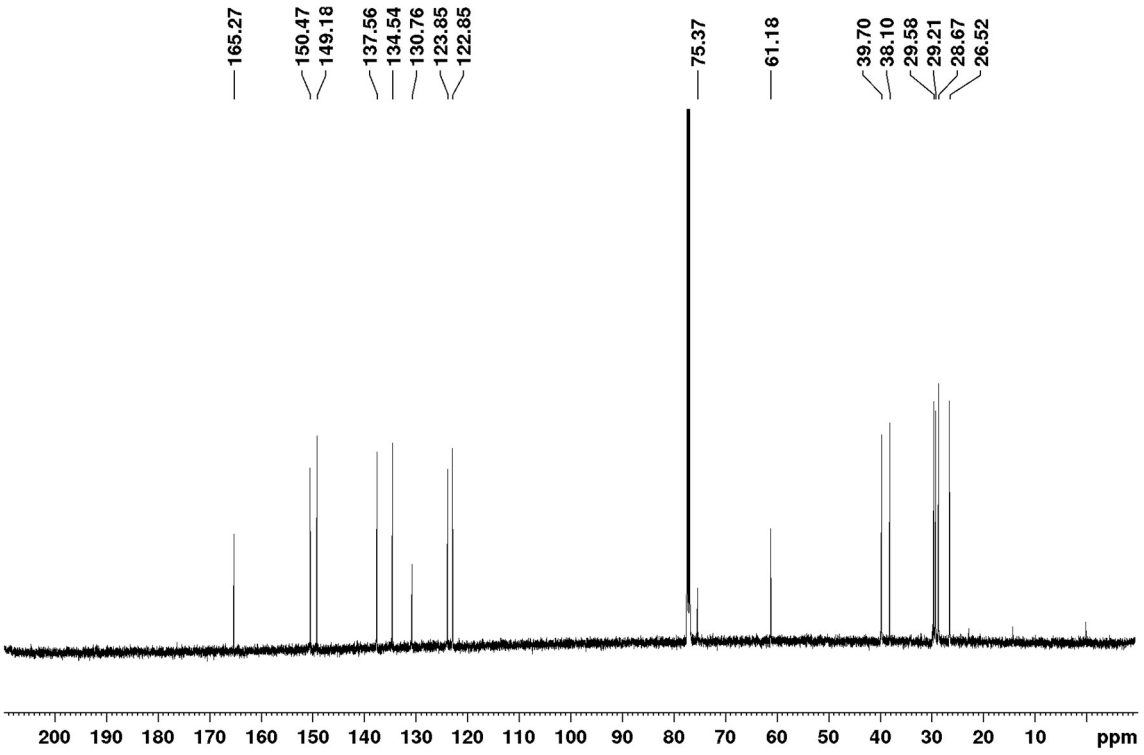


1-((*E*)-4-(3-(Pyridin-3-yl)acrylamido)hexyl)-1,2-dicarba-*closo*-dodecaborane (34)

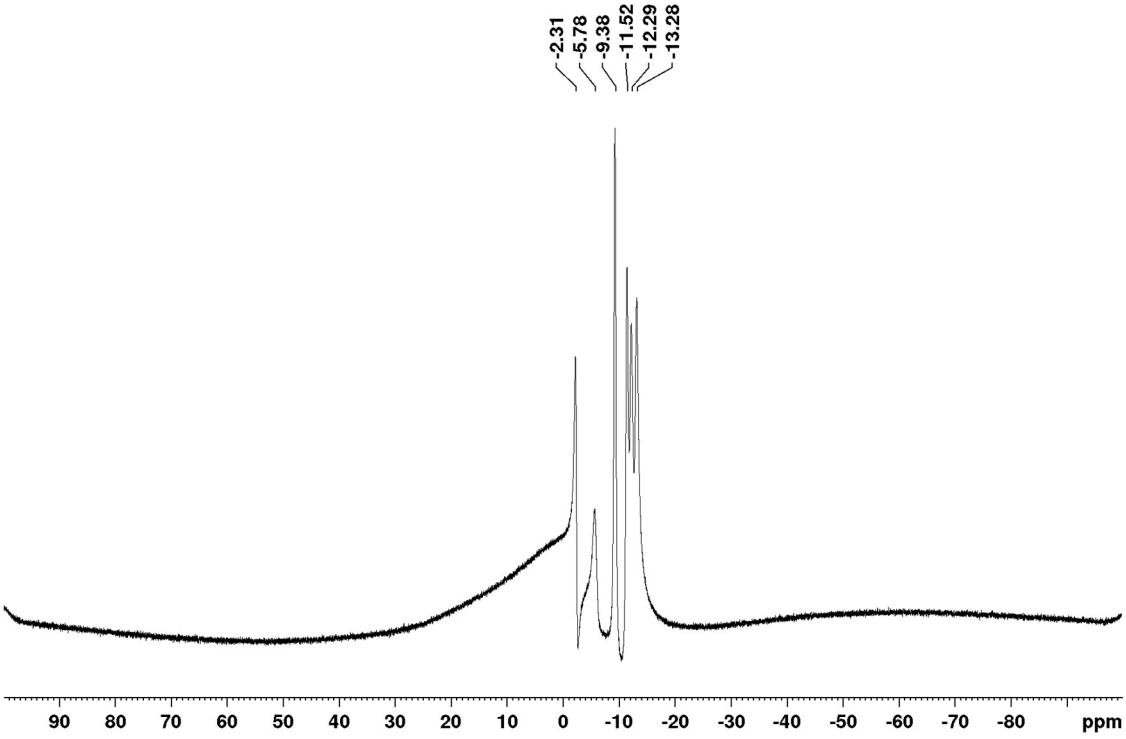
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

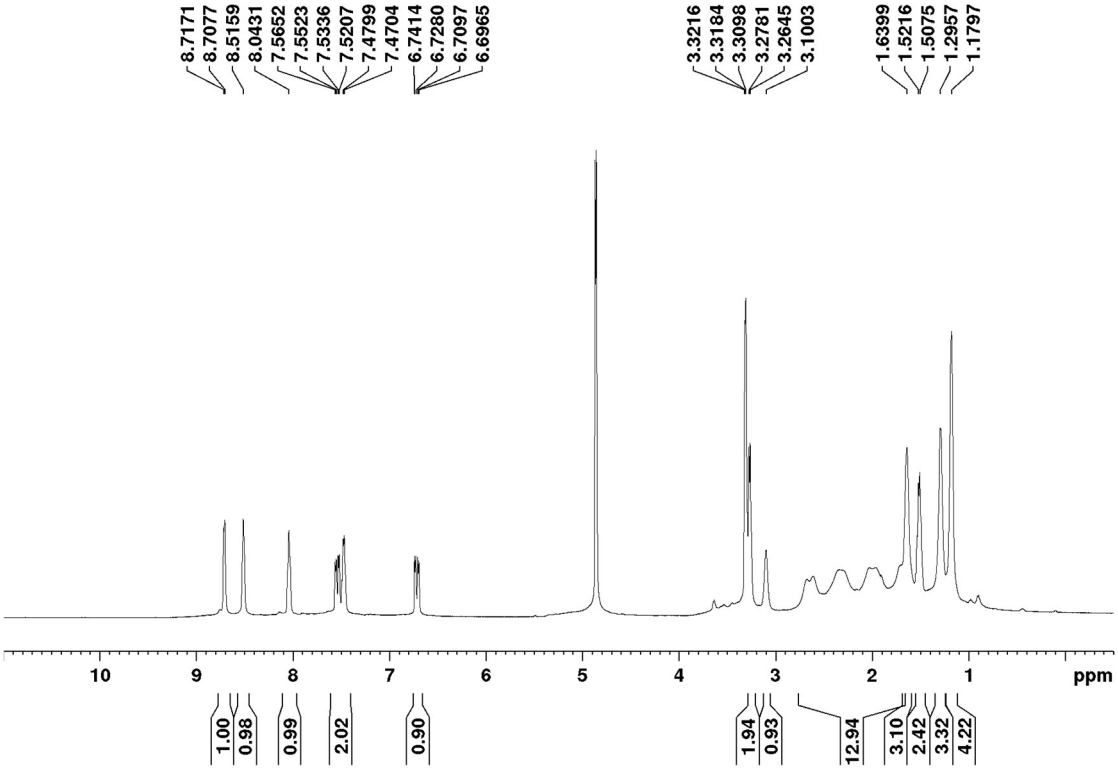


^{11}B NMR (160 MHz; $CDCl_3$)

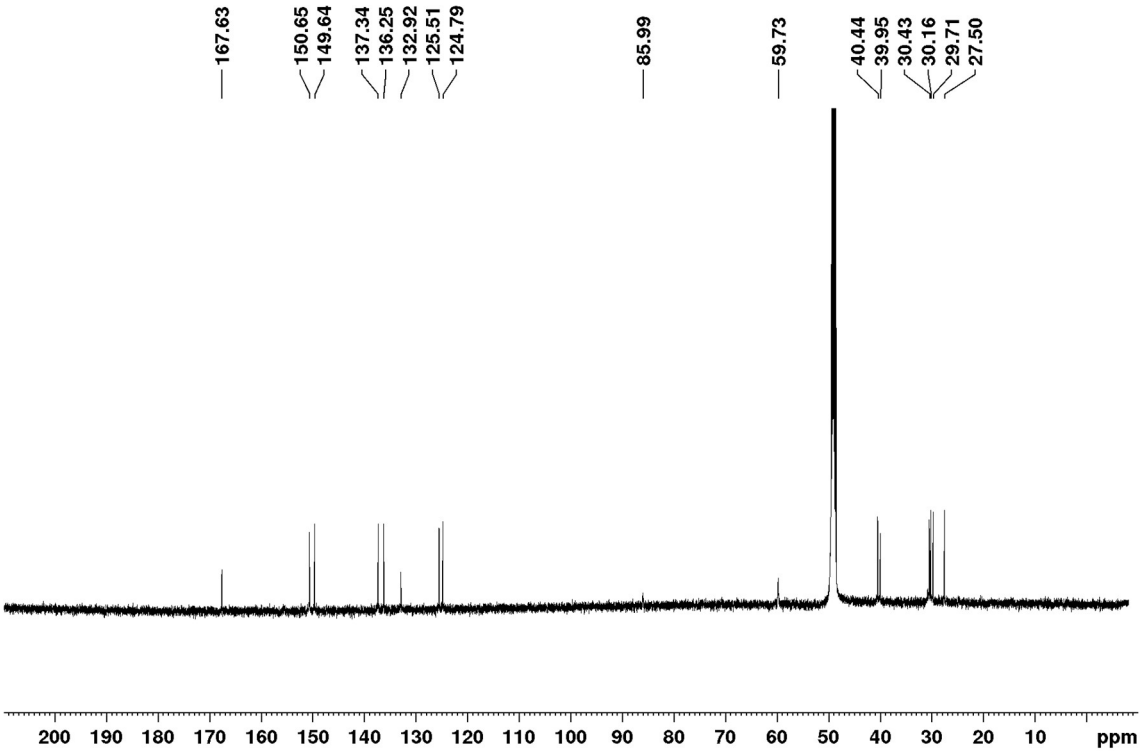


1-((*E*)-4-(3-(Pyridin-3-yl)acrylamido)hexyl)-1,12-dicarba-*closo*-dodecaborane (35)

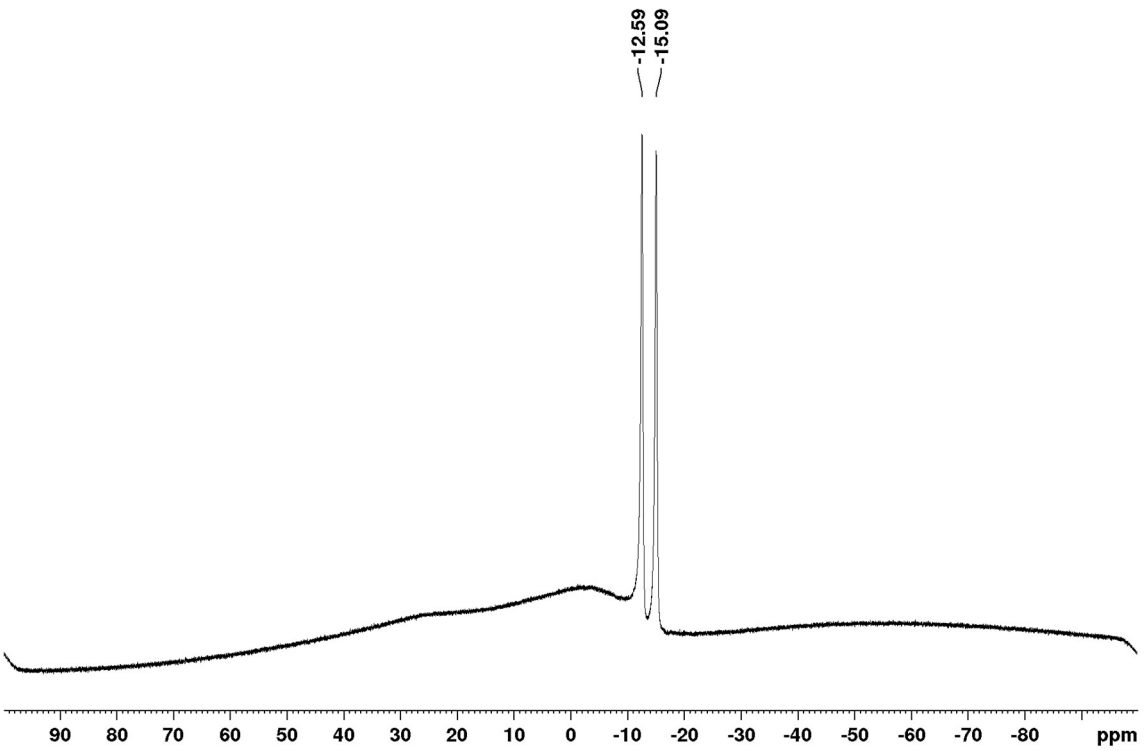
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

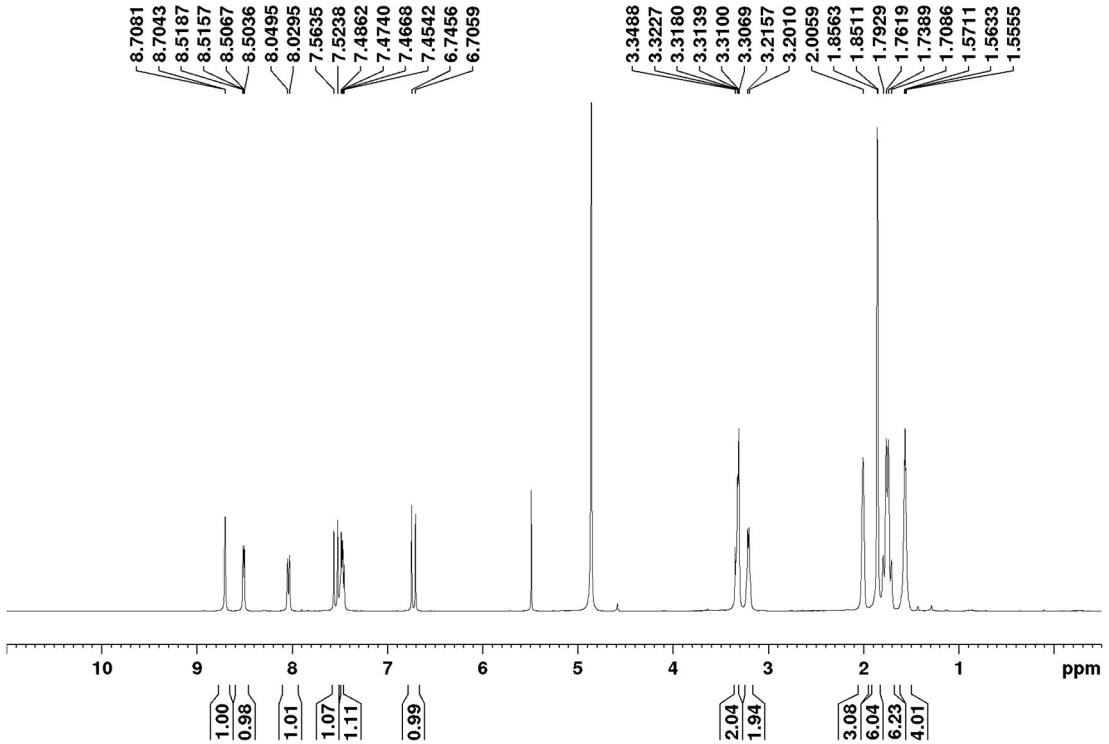


^{11}B NMR (160 MHz; CD_3OD)

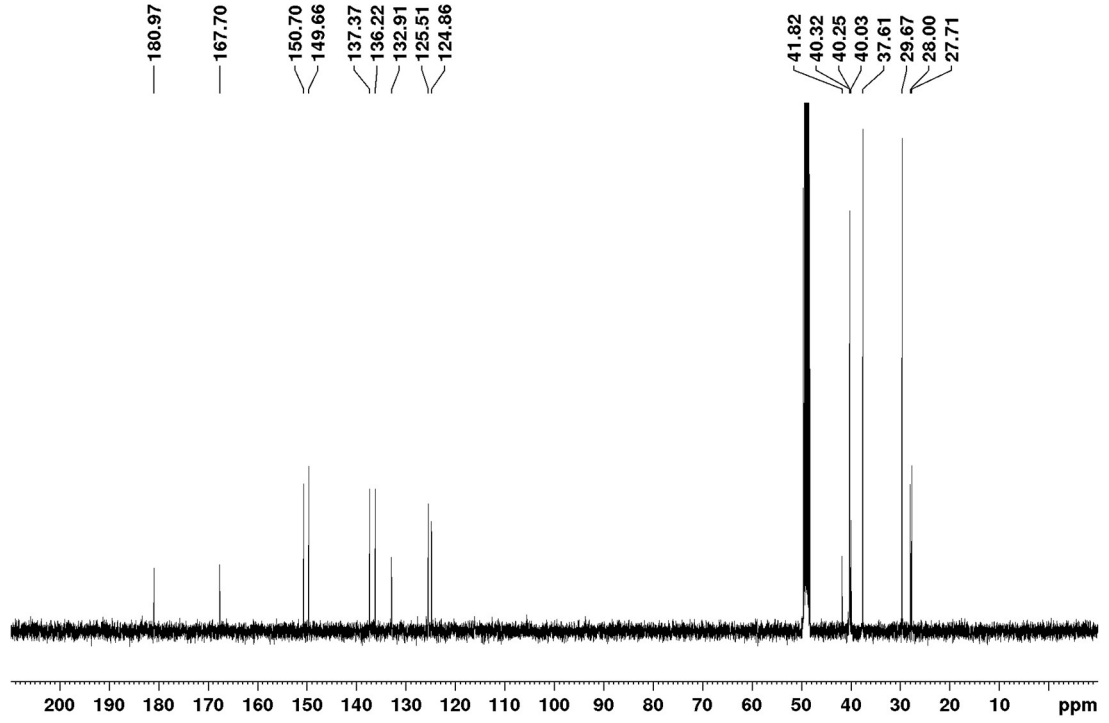


(3*r*,5*r*,7*r*)-*N*-(4-((*E*)-3-(pyridin-3-yl)acrylamido)butyl)adamantane-1-carboxamide (37)

^{1}H NMR (400 MHz; CD_3OD)

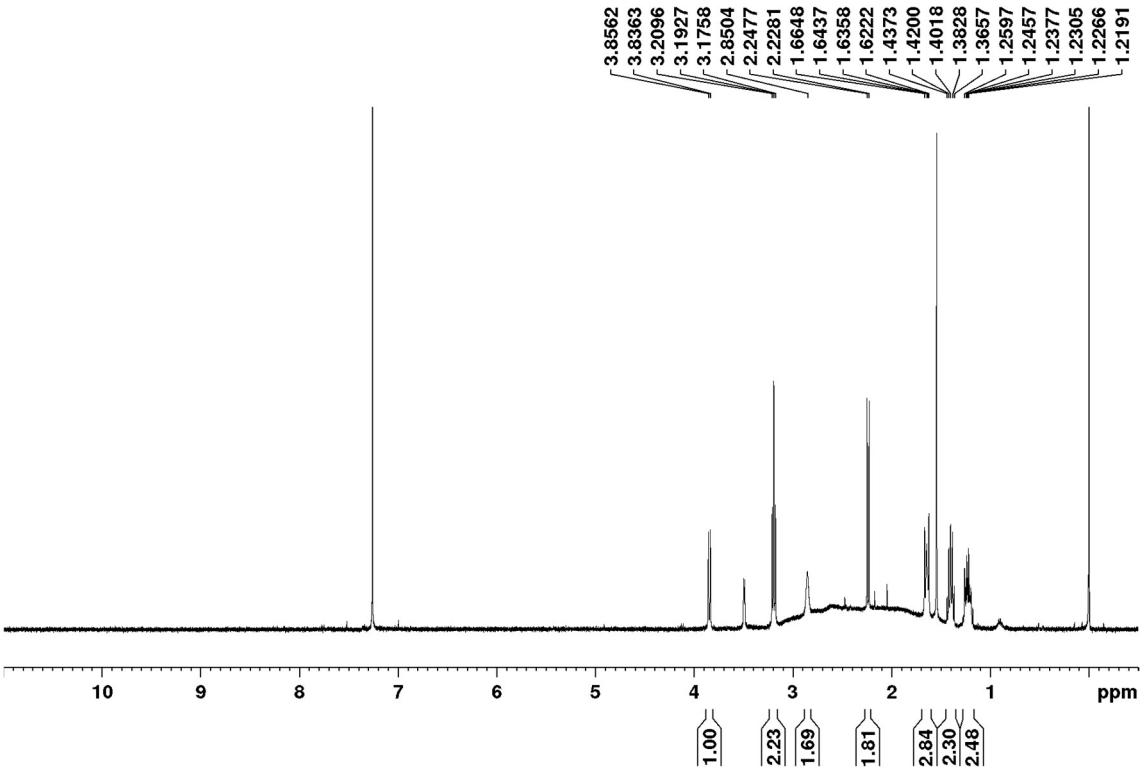


^{13}C NMR (125 MHz; CD_3OD)

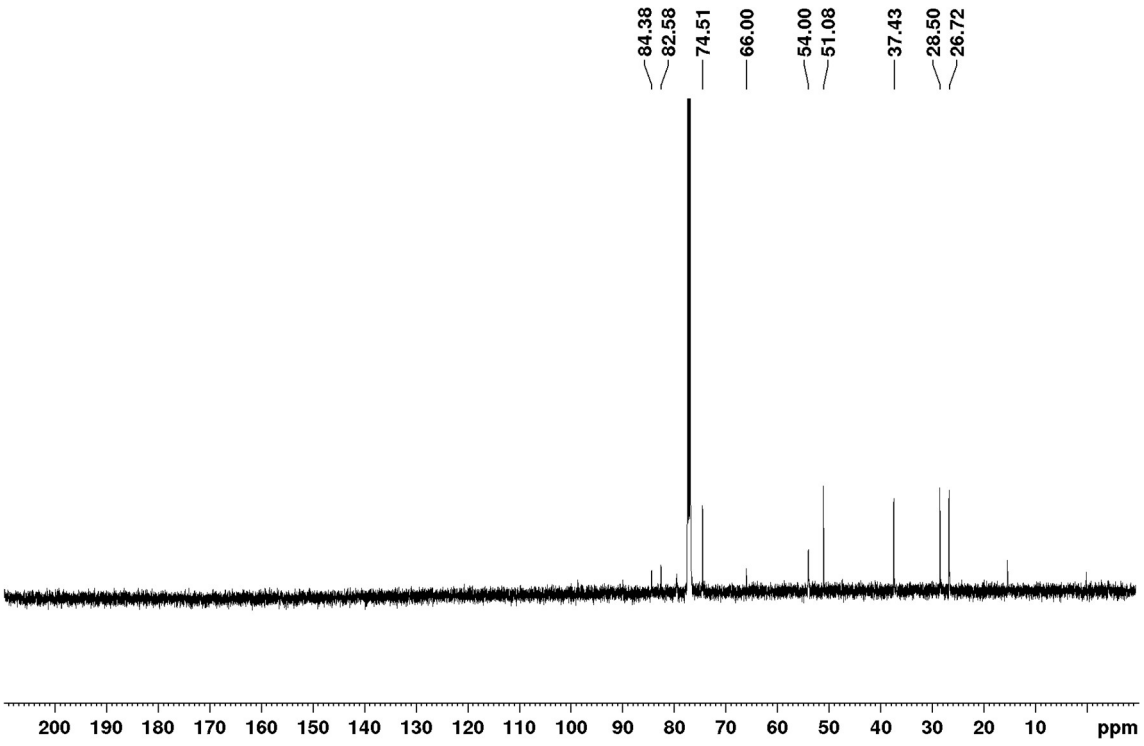


(4-(4-Azidobutyl)-1,12-dicarba-*closo*-dodecaboranyl) (1,7-dicarba-*closo*-dodecaboranyl)methanol (45)

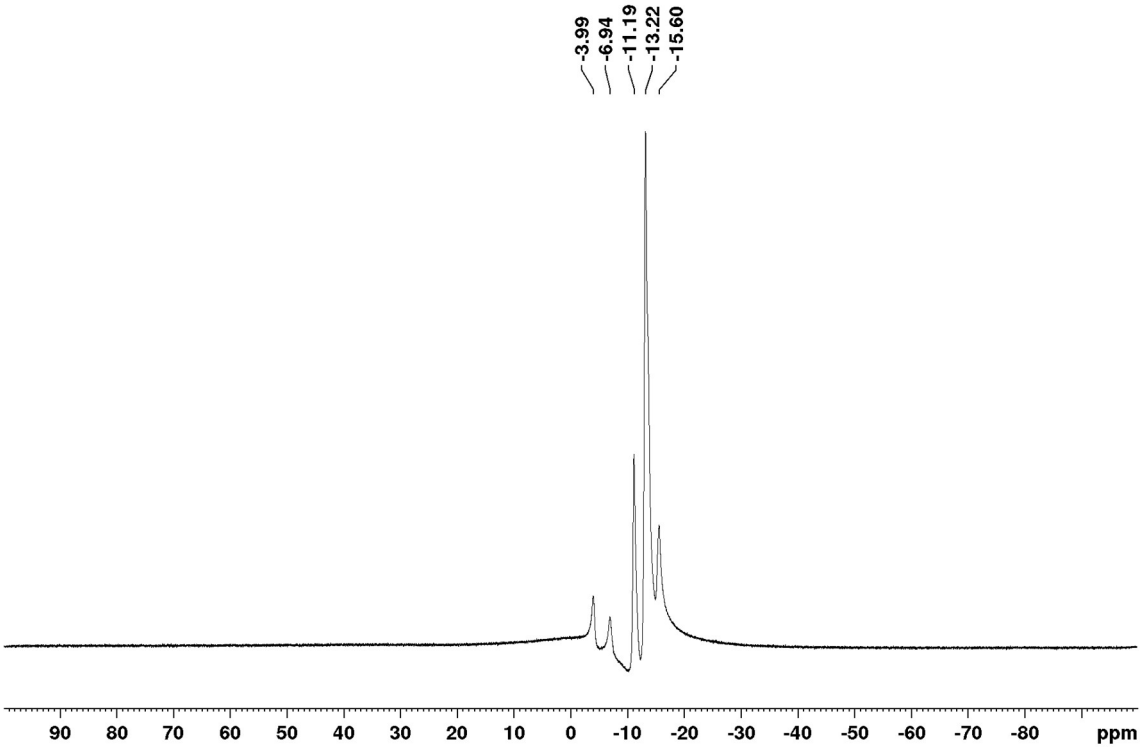
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

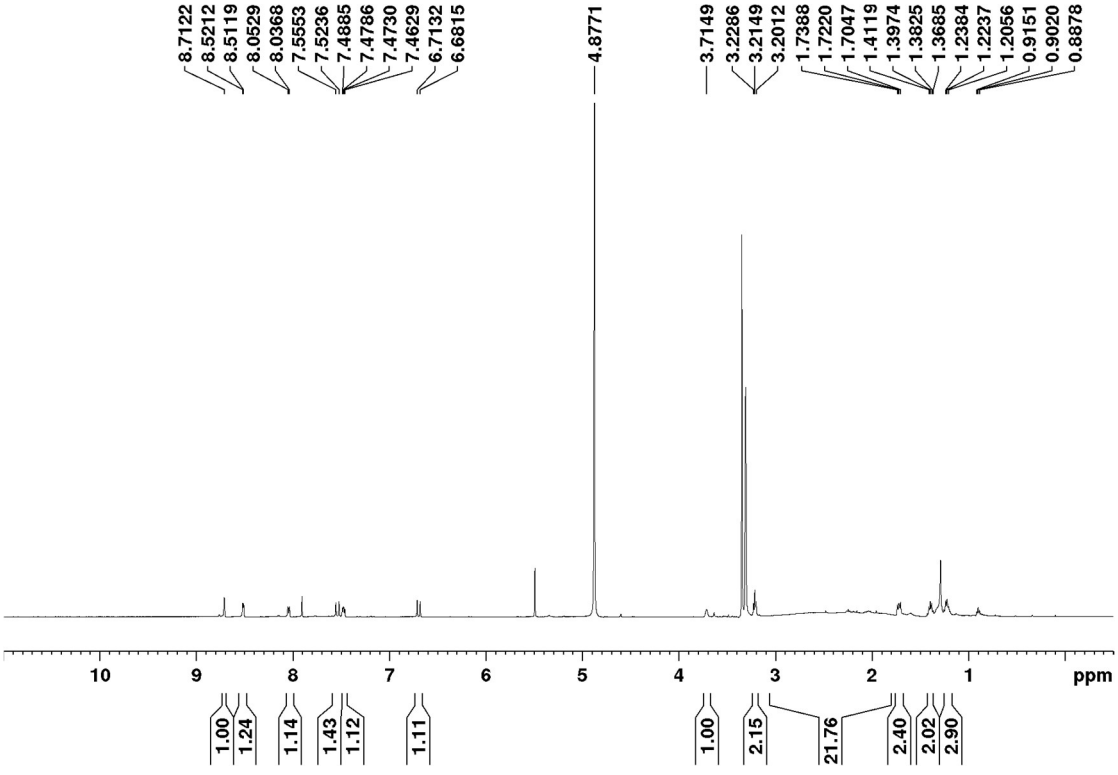


^{11}B NMR (160 MHz; $CDCl_3$)

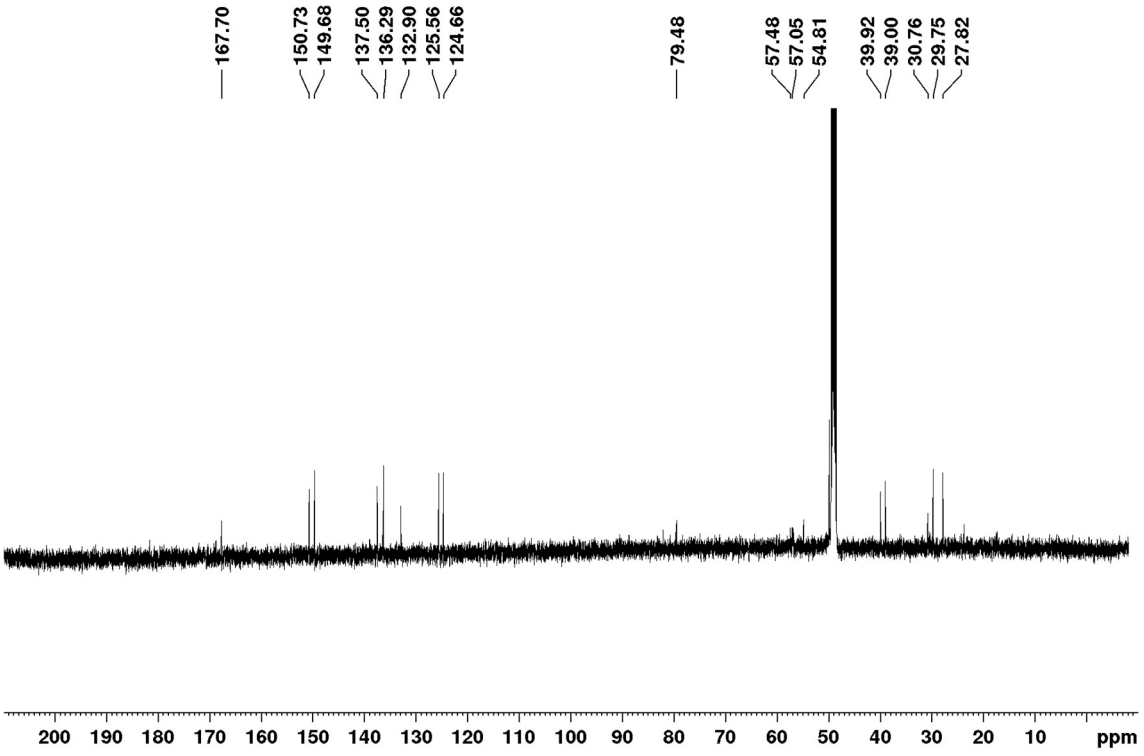


(*E*)-*N*-(4-(4-(1,7-Dicarba-*closo*-dodecaboranecarbonyl)-1,12-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (38)

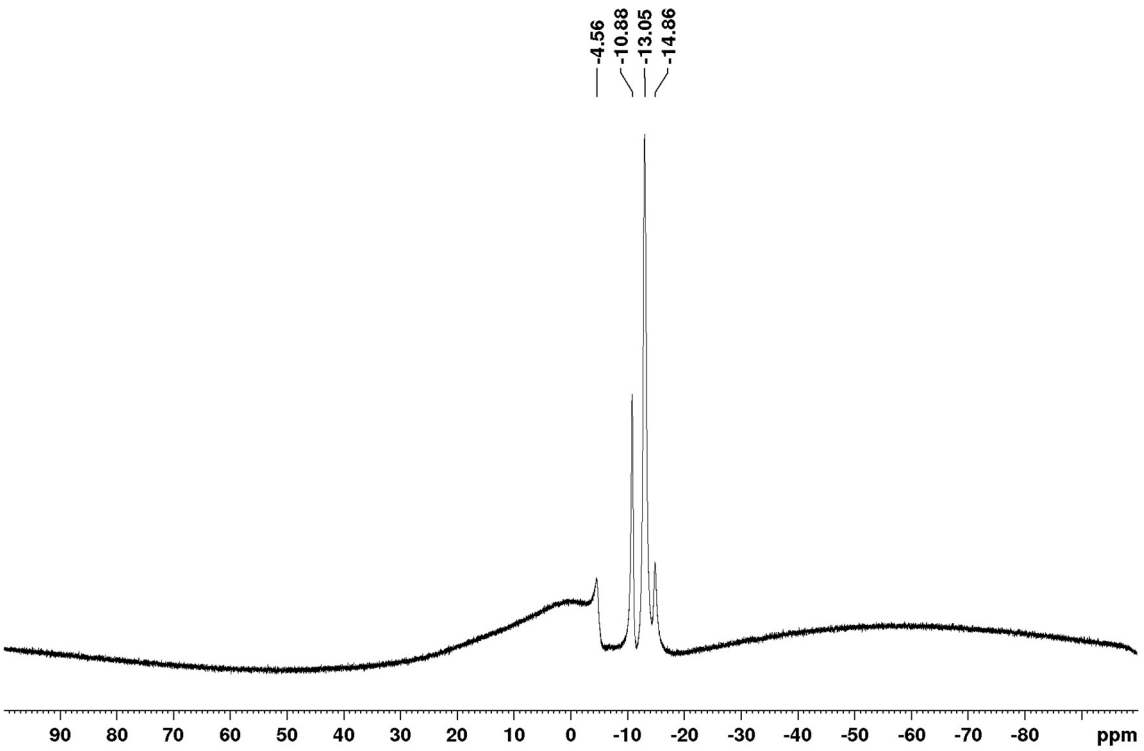
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

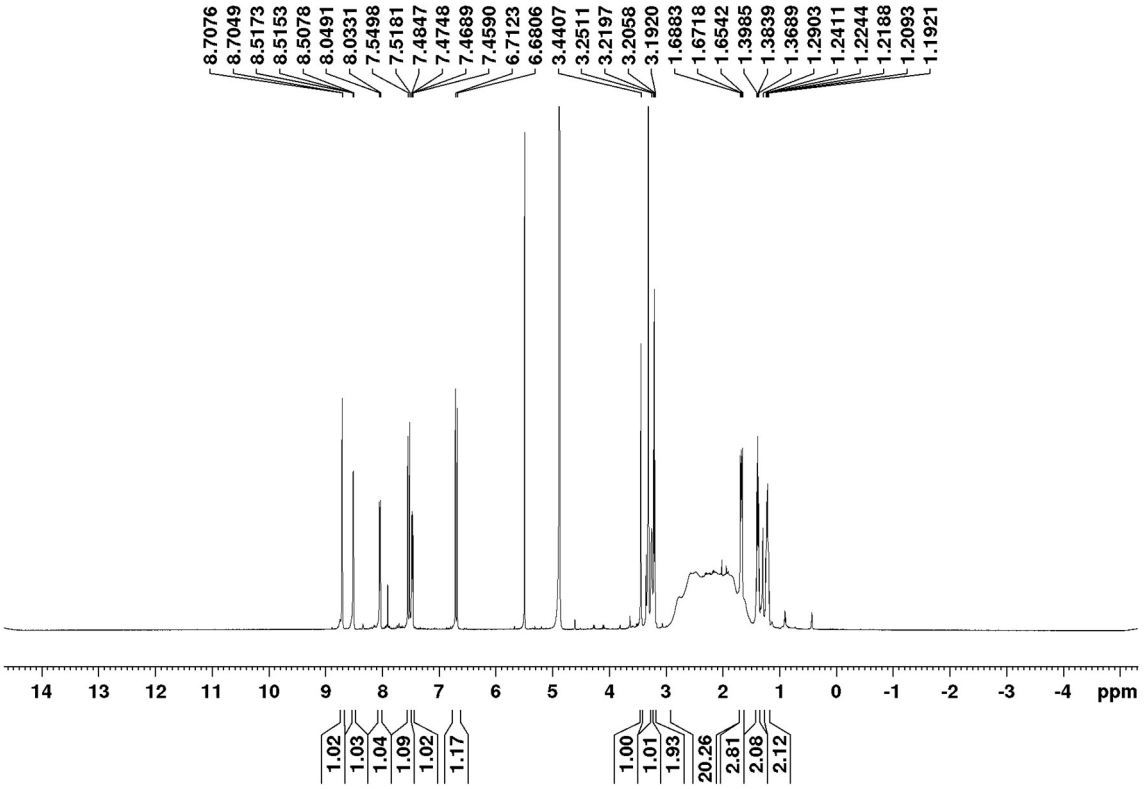


^{11}B NMR (160 MHz; CD_3OD)

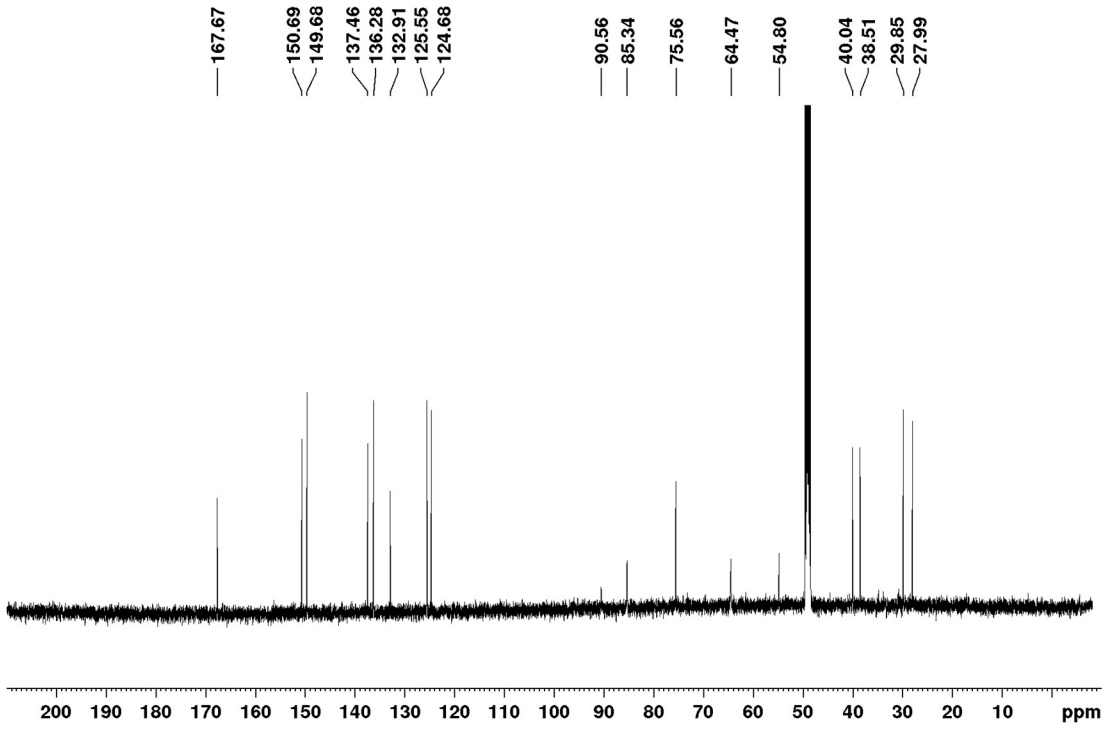


(*E*)-*N*-(4-(4-(1,12-Dicarba-*closo*-dodecaboranyl(hydroxy)methyl)-1,12-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (50)

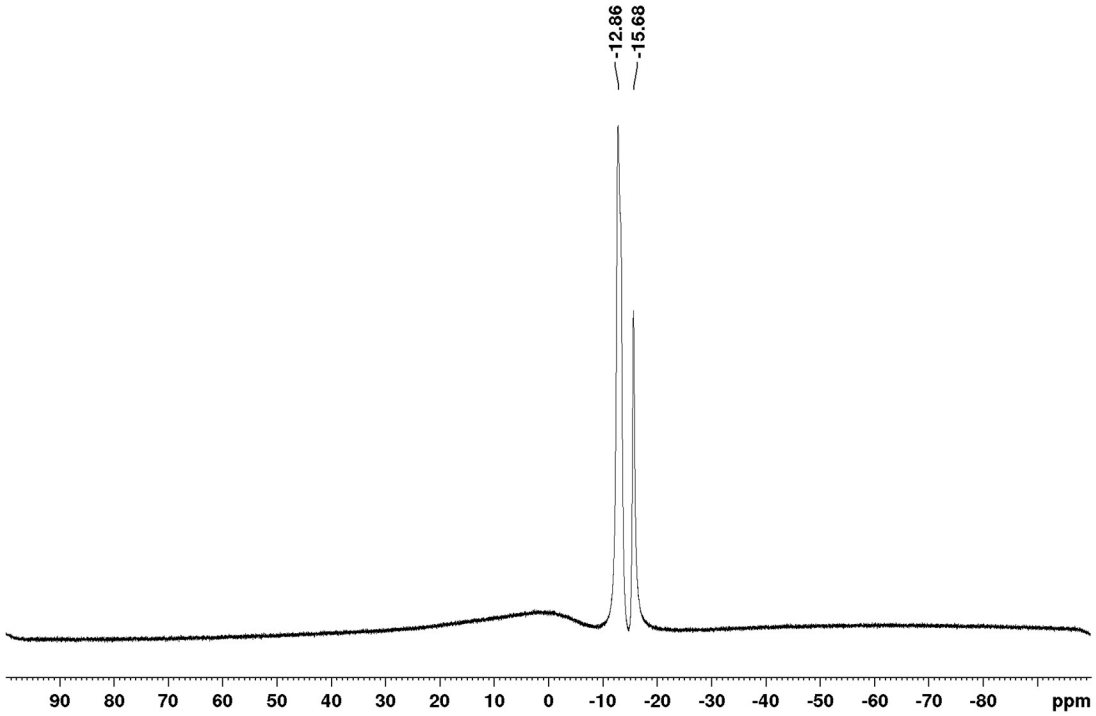
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

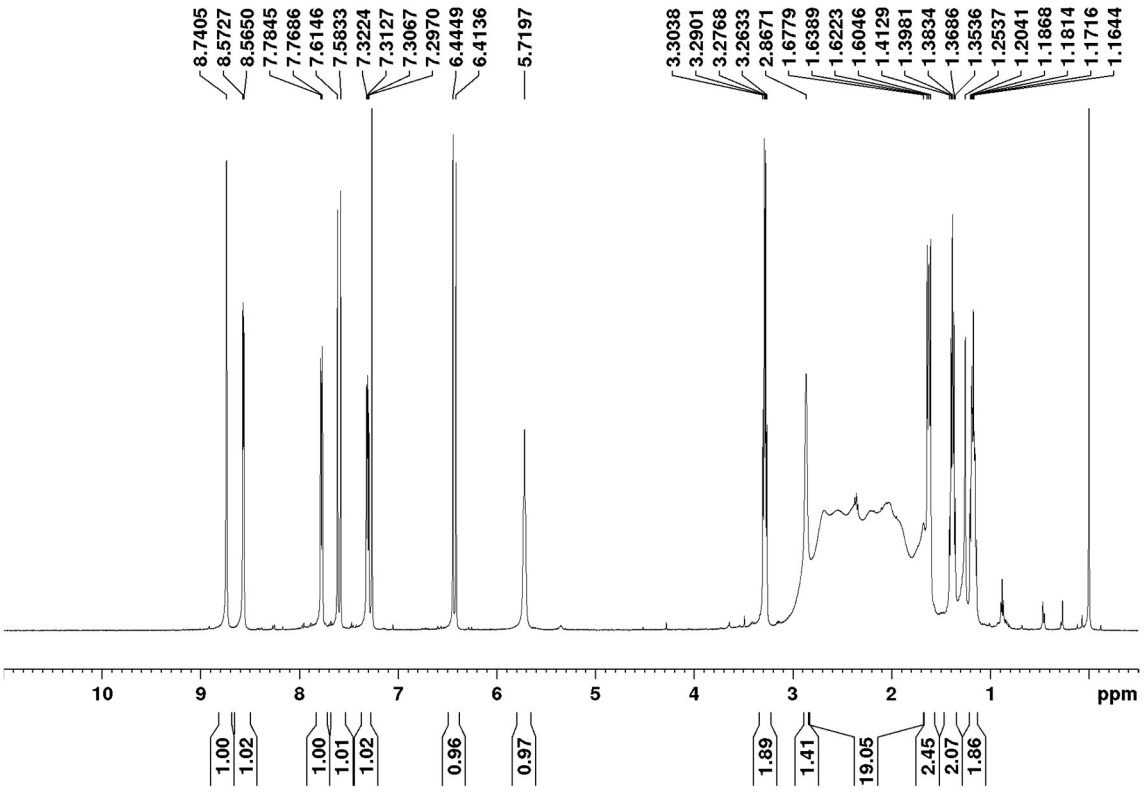


^{11}B NMR (160 MHz; CD_3OD)

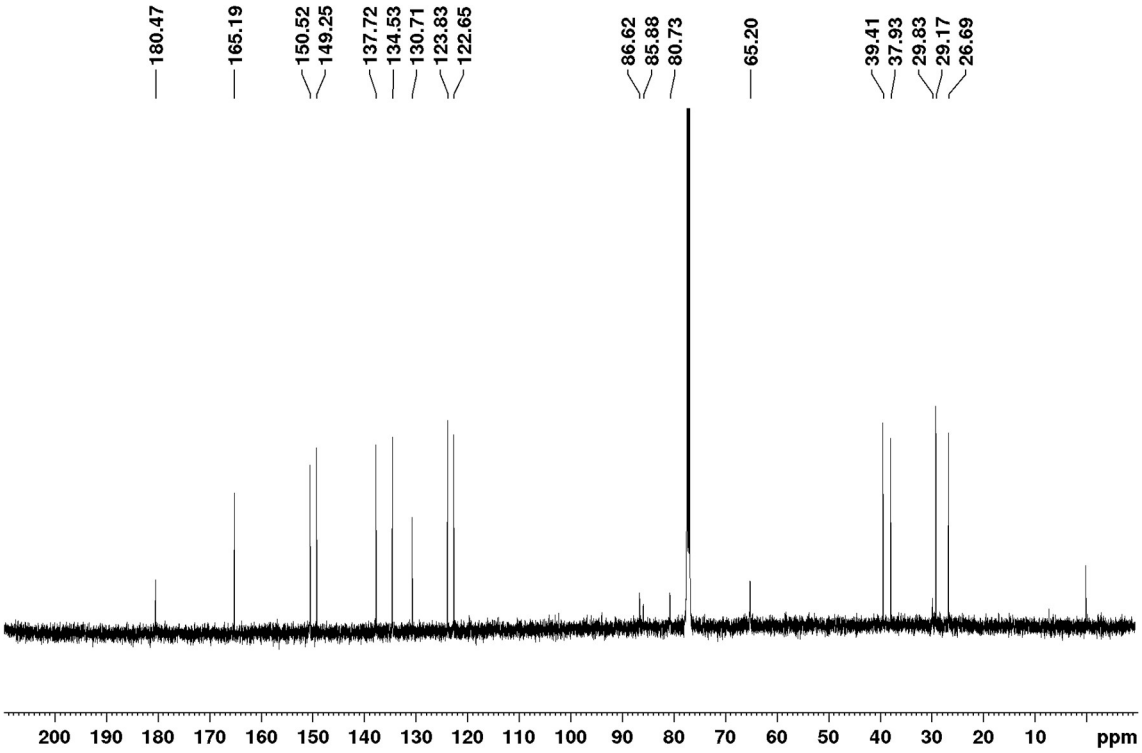


(*E*)-*N*-(4-(4-(1,12-Dicarba-*closo*-dodecaboranecarbonyl)-1,12-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (51)

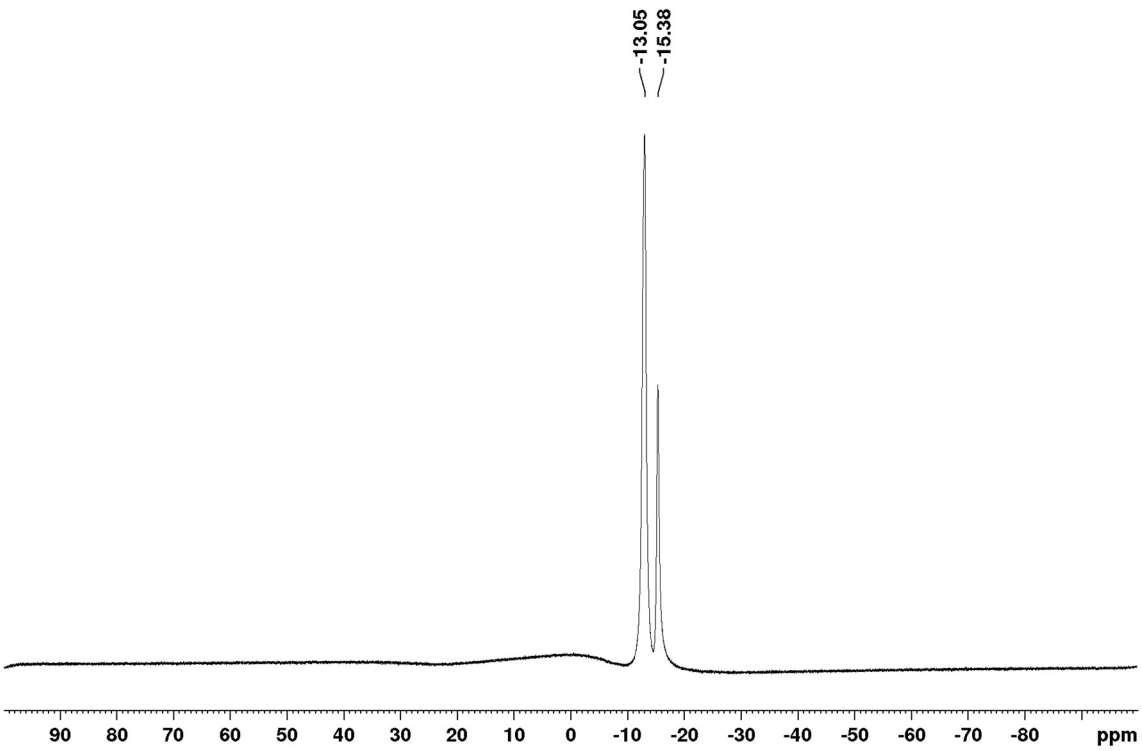
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

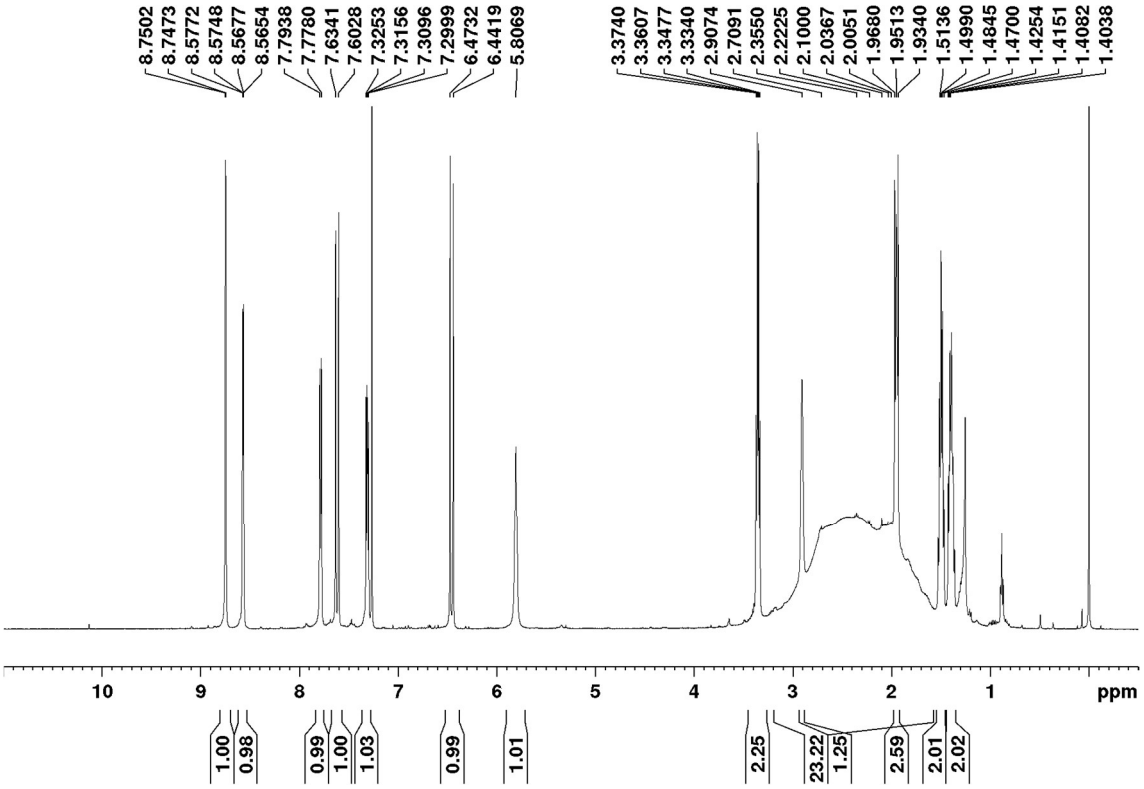


^{11}B NMR (160 MHz; CD_3OD)

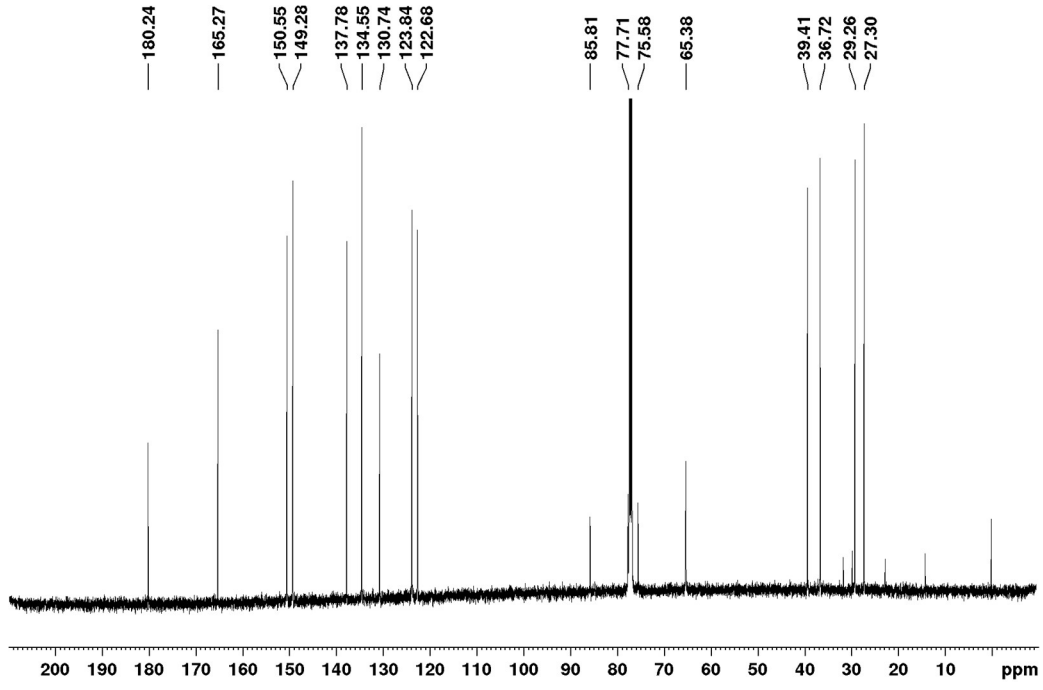


(*E*)-*N*-(4-(4-(1,12-Dicarba-*closo*-dodecaboranecarbonyl)-1,7-dicarba-*closo*-dodecaboranyl)butyl)-3-(pyridin-3-yl)acrylamide (55)

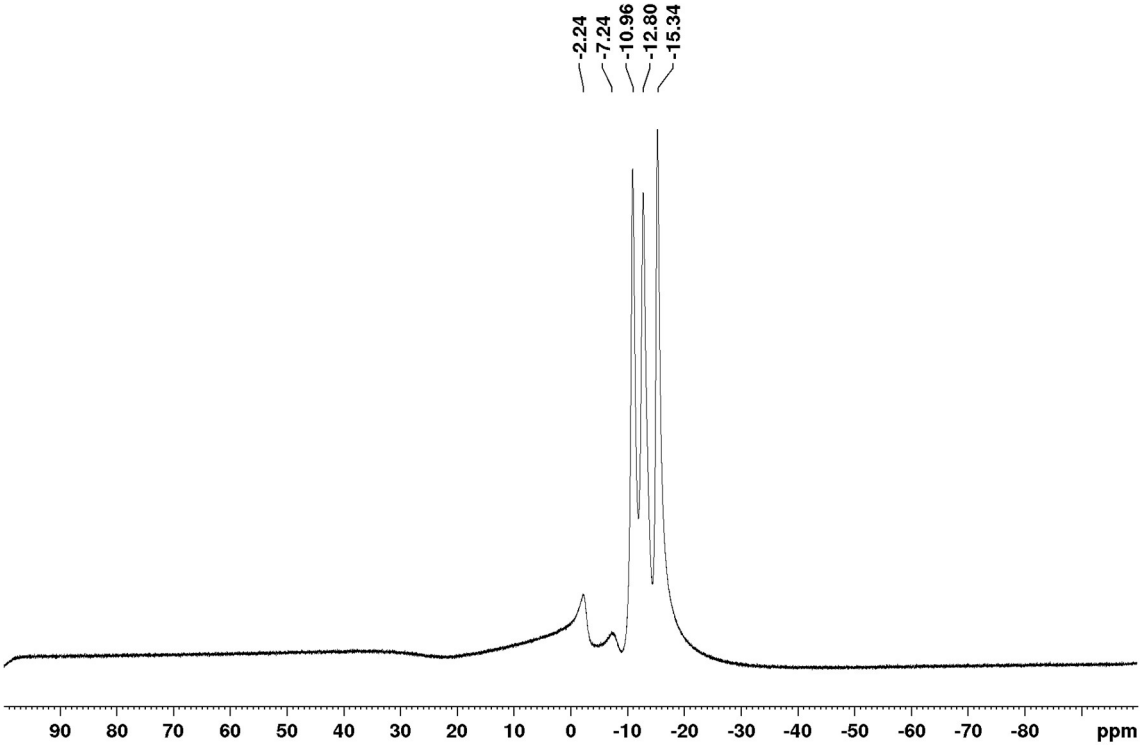
^{1}H NMR (500 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)



^{11}B NMR (160 MHz; CD_3OD)



第三章

疎水性タグとしてカルボランを利用したタンパク質分解誘導剤の創成と機能解明

3-1 はじめに

第一章 **1-3** において、疾患の原因となるタンパク質の細胞内レベルを低下させる戦略として PROTAC（Proteolysis-Targeting Chimera）と疎水性タグ（HyT）システムについて述べた。この２つの作用機序には、低分子によって E3 リガーゼと標的分子（POI）の近接を誘導するか、シャペロン分子の POI に対する認識能を高めるかという違いがある（Figure 1-6）。そのため、POI のリガンドに加えて PROTAC では 600 種類以上あるとされている E3 リガーゼのリガンドを選択する一方で、HyT システムでは POI のミスフォールドを模倣する HyT を導入する。一般的にアダマンタンや *tert*-ブチルカルバメートで保護されたアルギニン（Boc3Arg）が HyT として用いられる（Figure 3-1）。

これまでカルボランは疎水性ファーマコフォアとして創薬のビルディングブロックとして利用されてきた。第二章においても、この特徴的な疎水性を生かした NAMPT 阻害剤の設計について述べた。この知見から、本章ではカルボランが新たな HyT としてタンパク質分解に利用できるのではないかと考え、HaloTag リンカーと直接結合したカルボランを設計し、HyT システムの誘導を評価した。さらに、疎水性タグ付けされたタンパク質のモデルとして、カルボランをウシ血清アルブミン（BSA）に修飾した Maleimide conjugated carborane-BSA（MIC-BSA）を合成し、その物理化学的特性を測定した。最後に、MIC-BSA を生細胞にエレクトロポレーション法によって導入し、HyT システムによる分解を調べた。

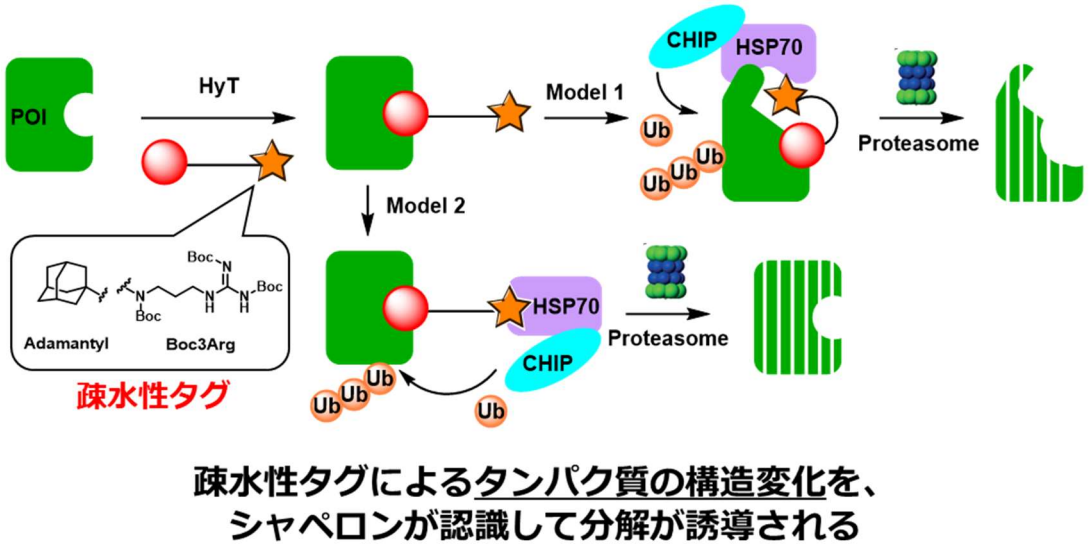


Figure 3-1. HyT システムによるタンパク質の分解

3-2 カルボランを疎水性タグとして導入したタンパク質分解誘導剤の設計とその合成

Crews らはアダマンタンを有する HyT13（**1**）の他に、*o*-carboranylphenoxy 誘導体 HyT55（**2**）を HyT として検討しているが、GFP-HaloTag2 に対する HyT55 の分解効果は HyT13 のようなアダマンチル疎水性タグに比べて十分ではなかった[1]。また、彼らは同様に 22 種類の疎水性構造を HyT として検討していたが、cLogP[a]値と分解効率の間に有意な相関は確認されなかった。しかし、検討された HyT の構造的特徴に着目したところ、HyT に平面構造を含む場合、分解効率が低下する傾向に気づいた。そこで、アダマンタンとカルボランのタンパク質分解効率を直接比較するために、Figure 3-2 に示すように、アミド基と直接結合した *m*-カルボラン（**3**）と、メチレンを介してアミド基と結合した *o*-カルボラン（**4**）の 2 種類のカルボラン含有タンパク質分解誘導剤を設計した[2]。

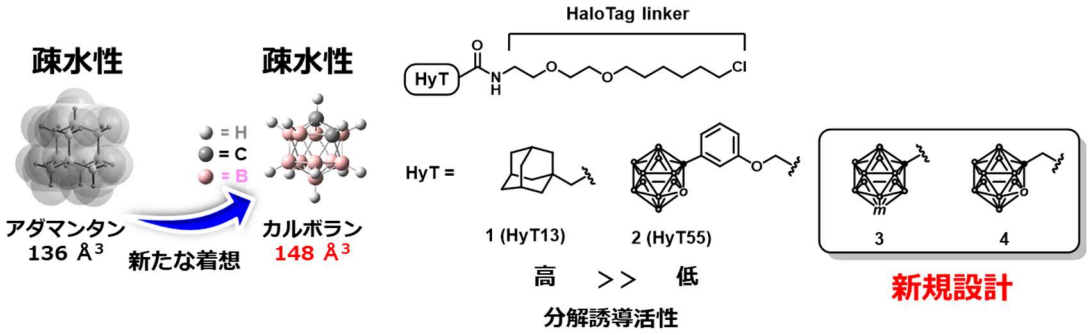
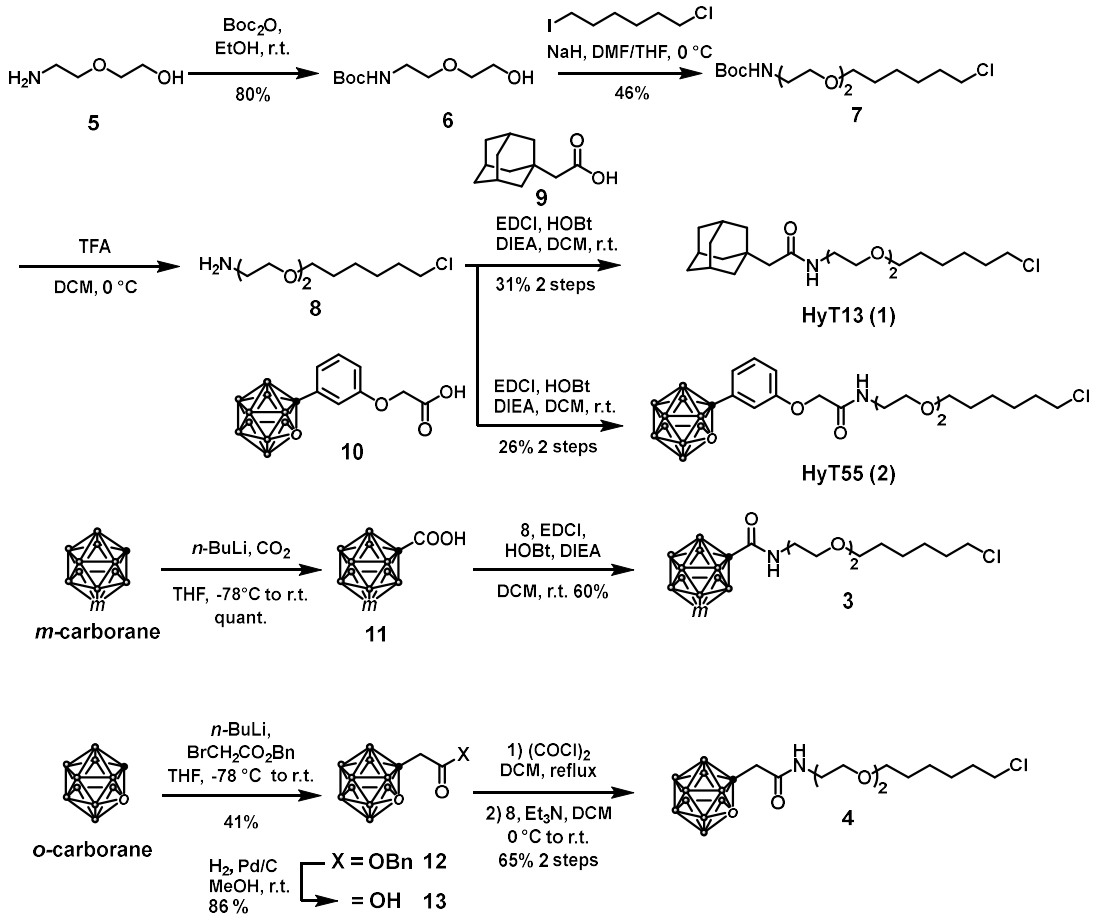


Figure 3-2. アダマンタンに代わる HyT としてカルボランをもつ POI 分解誘導剤の設計

化合物 **1-4** を Scheme 3-1 に従って合成した。まず文献に従って、2-（2-アミノエトキシ）エタン-1-オール（**5**）から HaloTag リンカー**8** を調製し、1-アダマンチル酢酸（**9**）と縮合させることで化合物 **1** を合成した[3]。また化合物 **2** も既知法に従って、HaloTag リンカー**8** とカルボラン含有のカルボン酸 **10** と縮合させることで合成した[4]。*m*-カルボラニルカルボン酸 **11** は、*n*-BuLi で処理して生成した *m*-カルボランアニオンを二酸化炭素と反応させて調製した[5]。HaloTag リンカー**8** を、1-（3-ジメチルアミノプロピル）-3-エチルカルボジイミド（EDCI）、1-ヒドロキシイベンゾトリアゾール（HOBt）、N,N-ジイソプロピルエチルアミン（DIEA）の存在下で **11** と反応させ、化合物 **3** を収率 60%で得た。同様に *n*-BuLi で処理して生成した *o*-カルボランアニオンをブロモ酢酸ベンジルと反応させ、エステル **12** を収率 41%で得た。水素添加反応によりベンジル基を脱保護した後、得られたカルボン酸 **13** を塩化オキサリルで処理し、次いで Et_3N の存在下で HaloTag リンカー**8** と反応させ、化合物 **4** を収率 65%で得た。

[a] cLogP（calculated LogP）：分子の有機層（オクタノール層）と水層中の平衡状態での濃度比率の常用対数値（LogP）の予測値



Scheme 3-1. HyT13（**1**）、HyT55（**2**）、**3**、**4** の合成

3-3　合成したカルボラン化合物のタンパク質分解誘導能評価

細胞内の HaloTag2 融合タンパク質に対する化合物 **3** および **4** の分解効果を評価した。コントロールとして HyT13（**1**）と HyT55（**2**）を用いた。ヘマグルチニン（HA）、Enhanced Green Fluorescent Protein（EGFP）、および HaloTag2 の融合タンパク質（HA-HaloTag2-EGFP）を Flp-In 293 細胞で安定的に発現させた。この細胞を化合物と 24 時間インキュベートした後、細胞内の HA-HaloTag2-EGFP の蓄積量を、HA 抗体を用いたイムノブロッティングで測定し、フローサイトメーターを用いて EGFP の蛍光強度を検出する、2 つの独立した評価法で定量した。その結果を Figure 2 に示す。イムノブロッティングの結果、HA-HaloTag2-EGFP のタンパク質レベルは、1 μM の HyT13、HyT55、および化合物 **3**、**4** によって、化合物を添加しない場合と比較して、それぞれ 49、79、43、65%の発現レベルとなり、有意に減少したことが明らかとなった（Figure 3-3A）。アダマンタンは、HyT として広く使用されているが、カルボランを HyT として含有する化合物 **3** は HyT13 よりも高いタンパク質分解誘導効果を示した。

同様のタンパク分解効果はフローサイトメトリー分析でも観察された。HA-HaloTag2-EGFP を発現する Flp-In 293 細胞の平均細胞内蛍光強度は、1 μM の HyT13 と化合物 **3** および **4** で処理することにより減少した（Figure 3-3B）。

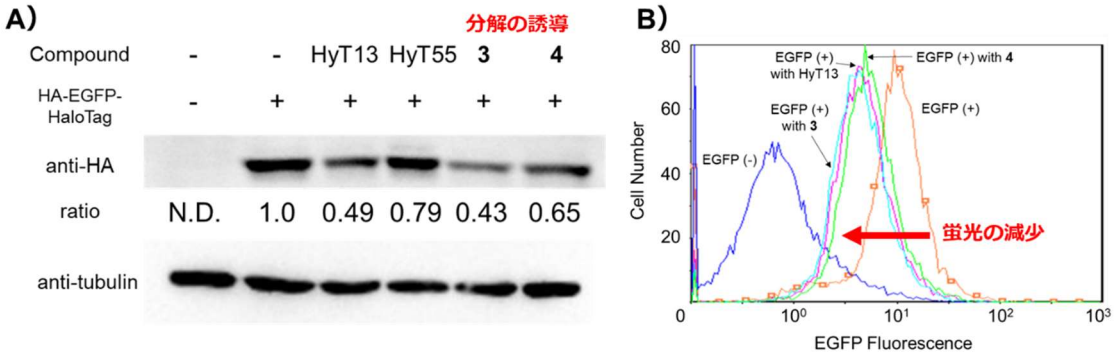


Figure 3-3. 細胞内の HaloTag2 融合タンパク質に対する化合物 **3** および **4** の分解誘導効果（細胞: Flp-In 293 cell（HA-EGFP-HaloTag 恒常発現株）、化合物濃度: 1.0 μM、薬剤暴露時間: 24 h）; A）HA-抗体を用いたイムノブロッティング、tubulin 量で標準化した HA の蓄積量を画像下部に示す。B）フローサイトメトリー分析による EGFP の蛍光強度の測定

続いて、化合物 **3** のタンパク質分解が、ユビキチン-プロテアソーム系に依存するか調べるため、プロテアソーム阻害剤である MG132[6] の添加による分解効率の変化を、先と同様に HA 抗体を用いたイムノブロッティングで解析した（Figure 3-4）。その結果、HyT13 と化合物 **3** のタンパク質分解効果は、化合物添加しない場合とほとんど変わらなかった。このことから化合物 **3** は、定説通りユビキチン-プロテアソーム系を介したタンパク質分解を誘導することが示された。

これらの結果から、カルボランがタンパク質分解のための HyT として使用できることが示された。さらに化合物 **3** が今回検討した化合物の中で最も高い分解誘導効率を示したことから、今後の調査では *m*-カルボランを HyT として使用することにした。

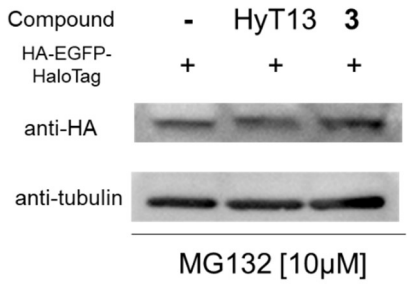


Figure 3-4. プロテアソーム阻害剤 MG132（10 μM）添加による HyT13 および化合物 3 のタンパク質分解誘導活性の抑制（培養条件は、Figure 3-3 に同じ）

3-4　HyT システムにおける熱ショックタンパク質（HSP）の関与

最後に、HyT システムにおいて POI の構造変化を認識する役割をもつと考えられている HSP ファミリーの関与を調べるため、Figure 3-5A に構造を示す HSP90、70、60 阻害剤である geldanamycin（GA）[7]、apoptozole（Az）[8]、GN26361（26）[9] をそれぞれ 1 μM 添加した細胞を使って、HyT13 と化合物 **3** の HA-HaloTag2-EGFP 分解誘導効果を測定した。なお、GA は HSP90 の阻害によって、そのフィードバック機構から HSP70 の発現量を増加させることが知られている[10]。はじめにイムノブロッティングの結果を Figure 3-5B に示す。まず、HyT を添加しない場合（①～④）に比べて、HyT13（⑤～⑧）と化合物 **3**（⑨～⑫）の添加によって HA-HaloTag2-EGFP 分解が、Figure 3-3 に示す結果と同様に確認された。続いて、HyT13 を添加した条件で比較すると、各 HSP 阻害剤は分解誘導効果を高める傾向が観察された（⑤ vs. ⑥～⑧）。また Figure 3-5C に示すフローサイトメトリーの結果からも、HSP 阻害剤の添加によってタンパク質の分解誘導が促進されていることがわかる。同様な傾向は、化合物 **3** を添加した場合においても観察された。GA の添加によって HSP90 が阻害されると同時に、細胞内の HSP70 の発現量が増えるため、HyT による分解誘導活性が促進することは、Crews らの先行研究の結果と一致する[11]。一方で HSP60 阻害剤である GN26361 の添加では、分解誘導の抑制がみられなかったため、HSP60 の関与の可能性は低いと考えられる。しかし、Az は HSP70 の ATP 拮抗阻害剤として HSP70 のシャペロン活性を阻害することが知られており、HyT によるタンパク質の分解誘導を抑制するはずであったが、⑦、⑪に示すようにむしろ分解誘導が促進した。これらの結果から、さらなる検討の余地はあるものの、HSP70 以外にも HyT システムに関与するシャペロンタンパク質の存在が示唆された。

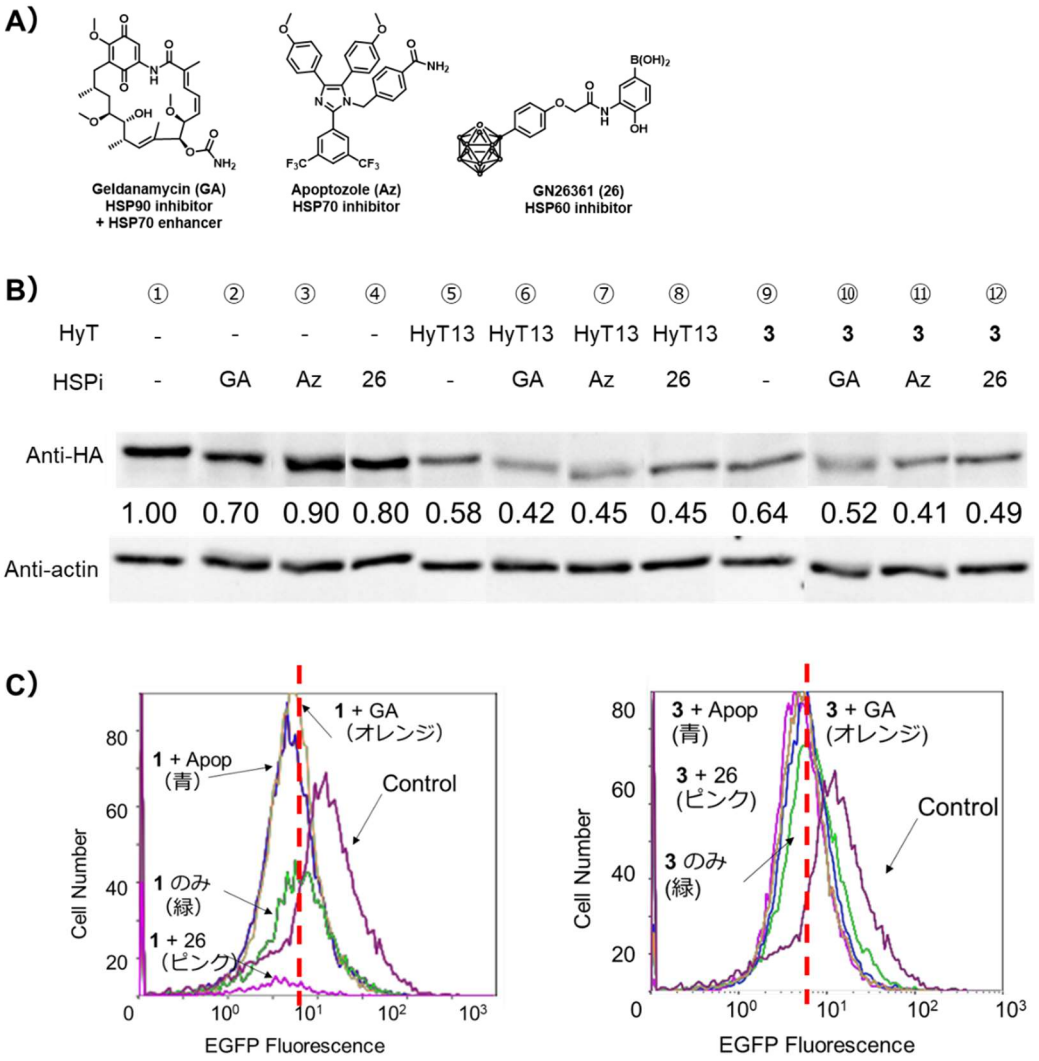
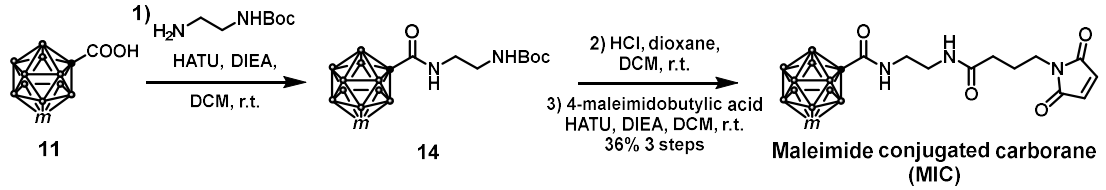


Figure 3-5. HSP 阻害剤添加時における HyT13 および化合物 **3** のタンパク質分解誘導効果 ; A）HSP 阻害剤の構造 ; B）HA-抗体を用いたイムノブロッティング、tubulin 量で標準化した HA の蓄積量を画像下部に示す。B）フローサイトメトリー分析による EGFP の蛍光強度の測定

3-5 MIC-BSA の合成

続いて HyT によるタンパク質の構造変化を明らかにするために、**3-3** で最も高い分解誘導効率を示した *m*-カルボランを疎水性タグとして修飾したタンパク質を合成し、その特性評価を行った。まず、細胞外タンパク質として BSA を選択し、*m*-カルボランがアミドリンカーを介してマレイミドと結合した Maleimide-conjugated carborane（MIC: Scheme 3-2）と酸緩衝生理食塩水（PBS）緩衝液中で反応させ、MIC-BSA を合成した（Figure 3-6A）。なお、生理的条件下では、マレイミド基がタンパク質のシステイン残基に選択的に結合することは広く知られているが、ホウ素クラスターを結合させたマレイミド基は、システイン残基とリジン残基にも結合しうる知見を得ている[12]。

次に 1 分子の BSA に対して何分子の MIC が結合するか明らかにするべく、液滴ビーム赤外レーザーアブレーション（DB-LA）質量分析法[13]を用いて MIC-BSA の分子量を測定した[b]。一般的にタンパク質のイオン化には、エレクトロスプレー法（ESI）やマトリックス支援レーザーイオン法（MALDI）が利用されるが、DB-LA は、水溶液サンプルの液滴に水分子の OH 伸縮振動に対応する IR レーザーを照射することで、水溶液中の特性を維持したまま、フラグメンテーションを起こすことなくタンパク質のイオン化を行うことができる[14]。未修飾の BSA と、10 当量の MIC と BSA を 37 ℃の PBS 中で 6 時間反応させた MIC-BSA（BSA/MIC＝10/1）の DB-LA 質量分析の結果を Figure 3-6B に示す。未修飾の BSA では、その分子量に相当する 66 kDa のピークが観察された一方で、MIC-BSA(10/1）では BSA に加えて平均 6.2 分子の MIC に相当する分子量が確認された。MIC を 10 当量加えたにもかかわらず、6.2 分子の結合が見られたことから、今回 MIC-BSA(10/1）を調整した条件で飽和状態になることが分かった。そこで、反応させる MIC の量を 3 当量として、異なる MIC 担持量の MIC-BSA(3/1)の合成を行った。そして未修飾の BSA、MIC-BSA(10/1)、MIC-BSA(3/1)のカルボラン担持量を、抗カルボラン抗体を用いたイムノブロッティングで定量した結果、Figure 3-6C に示すように、期待通り、異なる MIC 担持量をもつ MIC-BSA の調製に成功した。



Scheme 3-2. MIC の合成

[b] 河野淳也 教授、浅見祐也博士（学習院大学 理学部）との共同研究によって実施された。

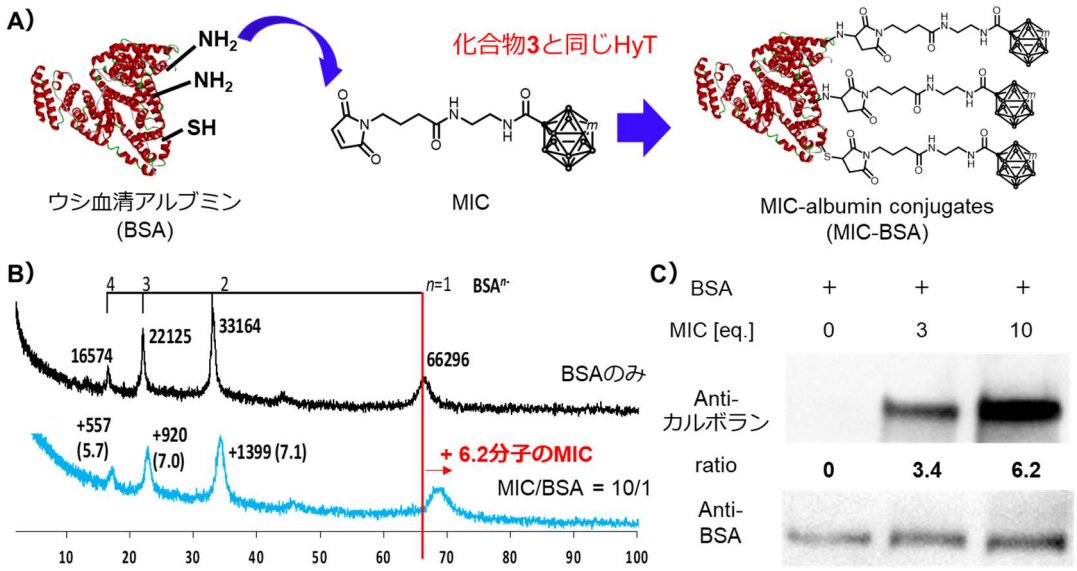


Figure 3-6. MIC-BSA の合成；A）スキーム；B）DB-LA 質量分析法で得られた BSA および MIC-BSA の質量スペクトル。50 μM のサンプルを含む水溶液に 3591cm^{-1}、1 パルスあたり 5.5 mJ の赤外レーザーを照射して測定；C）抗カルボラン抗体を用いたイムノブロッティング、BSA 量で標準化したカルボランの担持量比を画像下部に示す。

3-6 MIC-BSA の特性評価

まず、MIC-BSA の二次構造を、円偏光二色性（CD）スペクトルの測定によって確認した（Figure 3-7）。BSA および MIC-BSA の CD スペクトルは、30 ℃で 208～222 nm の間に負のバンドを示し，BSA の α-ヘリックス構造に起因していることがわかった [15]。加えて、MIC-BSA (10/1)は、未修飾の BSA や MIC-BSA (3/1)に比べて、熱による構造変化が抑制されていた。これらの結果から、MIC-BSA の二次構造は未修飾 BSA と同等であり、BSA の熱安定性にわずかな変化をもたらすことが示唆された。

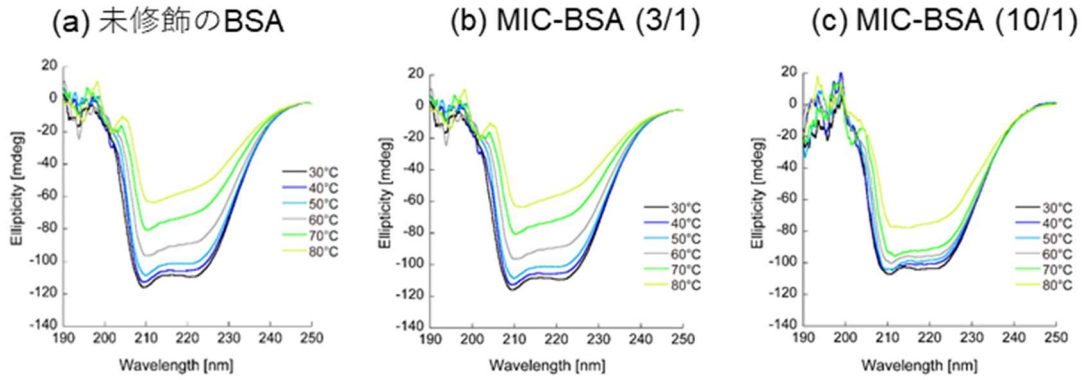


Figure 3-7. CD スペクトルによる MIC-BSA の二次構造解析と熱安定性評価（30-80℃間の 10℃刻みで測定）

続いて MIC-BSA の流体力学的な粒子径（DLC）とゼータ電位を Table 3-1 に示す。粒子径は MIC/BSA の混合比とは相関しないが、これらの混合比はゼータ電位に大きく影響した。すなわち、MIC の混合比の増加は BSA の表面上の負電荷を増加させ、HyT の付加は BSA の構造変化を誘発する可能性を示唆している。おそらく HyT がタンパク質内部の疎水性領域に導かれることによって表面上に多くのリジン残基が出現したためと考えた。

Table 3-1. MIC-BSA の DLS とゼータ電位

	BSA	MIC-BSA (3/1)	MIC-BSA (10/1)
粒子径 [nm]	8.32 ± 0.67	7.19 ± 0.59	6.85 ± 0.59
ゼータ電位 [mV]	**-4.91 ± 1.20**	-7.52 ± 0.66	**-8.43 ± 2.55**

測定条件：Zetasizer Nano ZS (Malvern Panalytical) at 25 °C. The concentration of each sample was prepared at 0.1 mg/mL. All measurements were performed in triplicate.

3-7 露出したリジン残基の定量による HyT 結合タンパク質の構造変化解析

この露出したリジン残基の検証を行うべく、フルオレセイン・イソチオシアネート・アイソマーI（FITC）を用いた標識により定量した（Figure 3-8A）。FITC は、イソチオシアネート部位が第一級アミン基と選択的に反応するため、タンパク質のリジン残基の蛍光標識によく用いられる。そこで、BSA および MIC-BSA (10/1)、MIC-BSA (3/1)を PBS 中の FITC で処理した。ドデシル硫酸ナトリウム-ポリアクリルアミドゲル電気泳動（SDS-PAGE）の後、各バンドの FITC 蛍光強度を定量した。Figure 3-8B に示すように、FITC 蛍光強度は、MIC/BSA 比に依存して増加した。なお、各サンプルの BSA の量は、Coomassie Brilliant Blue（CBB）染色によって同一であることを確認している。実際、MIC-BSA (10/1)の蛍光強度は、未修飾 BSA の強度の 1.6 倍であり、疎水性のカルボラン修飾によって、BSA 上のリジン残基の露出が誘導されていることが示唆された（Figure 3-8C）。Figure 3-7 の CD スペクトルや Table 3-1 の粒子径の結果を合わせて考えると、タンパク質に結合した HyT は、二次構造や全体の大きさにほとんど影響を与えないものの、リジン残基の露出を伴う構造変化を濃度依存的に誘導することが明らかとなった。

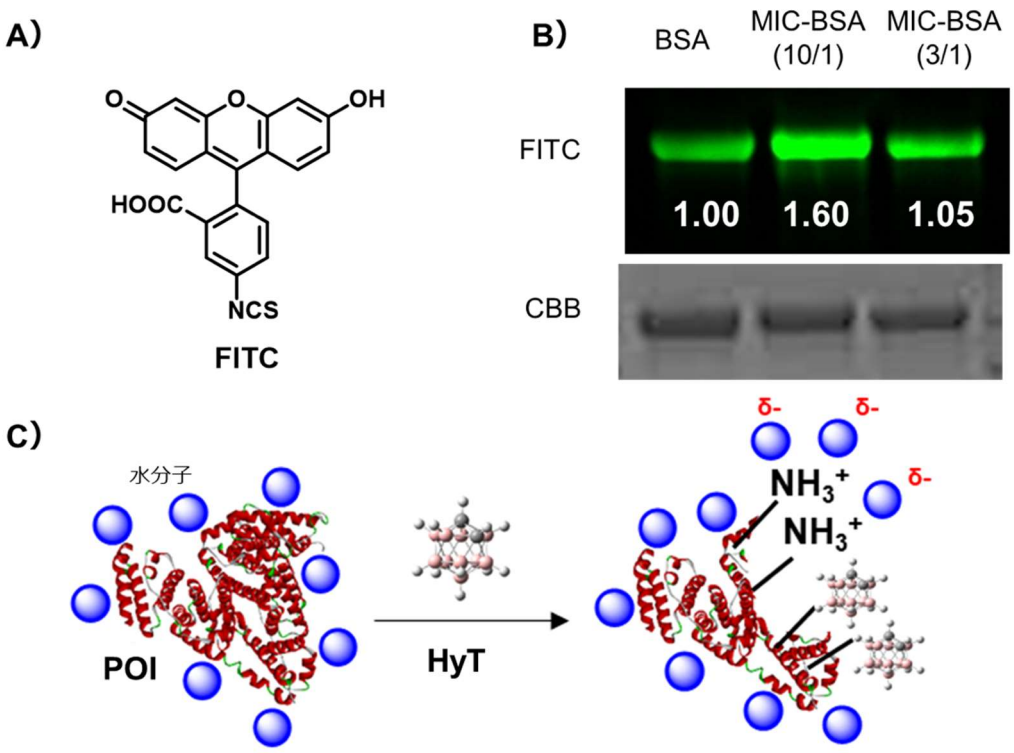


Figure 3-8. カルボラン修飾による BSA の構造変化；A）FITC の構造、B）MIC-BSA への FITC 標識。反応条件：BSA および MIC-BSA を 1 mM FITC を 4 ℃で 8 時間、暗所で反応させた。30 kDa の限外ろ過後、濃縮液を PBS で希釈し、50 mM Tris, pH 7.4, 4%ドデシル硫酸ナトリウム[SDS]、10%グリセロール、4% 2-チオエタノール、50 μg/mL ブロモフェノールブルーを含むバッファーで変性させた。その後、SDS-PAGE によって泳動し、FITC の蛍光（λ_{ex}= 498 nm, λ_{em}= 522 nm）を測定した（n＝3）；C）今回明らかにした HyT の結合によるタンパク質の構造変化の概要図

3-8 細胞外タンパク質の生細胞における分解[c]

最後に MIC-BSA の分解が細胞内で誘導されているかどうかを明らかにするため、細胞に導入した MIC-BSA の分解をイムノブロッティングで観察した。MIC による分解は、細胞質内のタンパク質分解システムを介して行われると考えられるため、リソソームを介さずに細胞質内に送り込む必要がある。そのため、LNCaP 細胞にエレクトロポレーション法で MIC-BSA を導入した。フローサイトメトリーによる解析では、LNCaP 細胞の生存率を低下させることなく、FITC 結合 BSA のトランスフェクション効率が高くなるように、MIC-BSA のエレクトロポレーションの条件を検討した（Table 3-2、Figure 3-9)。その結果、FITC-BSA の導入量が多く、かつ細胞毒性に比較的影響の少ない Condition 2 を最適条件とした。

Table 3-2. FITC-BSA を用いたエレクトロポレーションの条件検討

Condition[a]	Voltage [V]	Pulse length [ms]	Interval [ms]	Number	Decay rate [%]	Polarity
1	150	10	50	5	20	+
2	150	20	50	5	20	+
3	150	10	50	1	20	+
4	150	30	50	5	20	+
5	150	10	50	5	10	+
6	150	20	50	5	10	+

[a] Condition for poring pulse. Condition for transfer pulse was constant as follows: transfer pulse; pulse voltage, 20 V; pulse length, 50 ms; pulse interval, 50 ms; pulse number, 20; decay rate, 30; polarity, +/-.

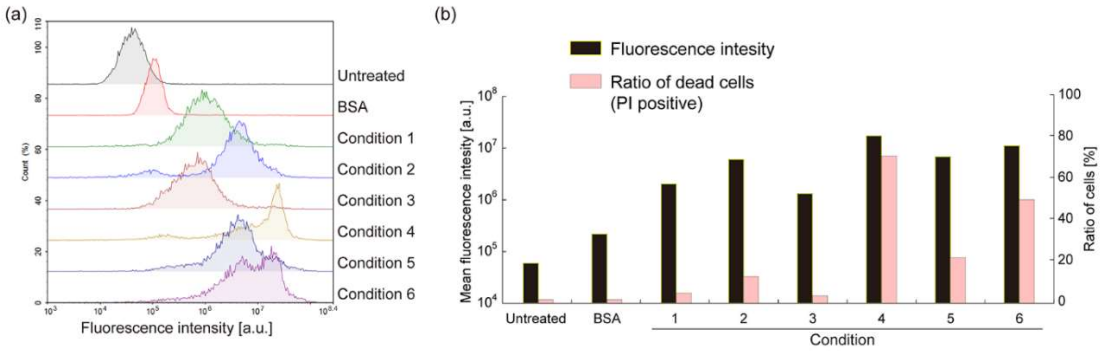


Figure 3-9. FITC-BSA を用いたエレクトロポレーションの条件検討の結果

[c] 田中賢 教授、西田慶 博士（九州大学　先導物質化学研究所）との共同研究によって実施された。

続いて、LNCaP 細胞に導入した HyT コンジュゲート BSA の量をウエスタンブロットによって定量した（Figure 3-10A）。なお比較のため、MIC のカルボランをアダマンタンに置換した Maleimide conjugated adamantane （MIA）を用いて、MIC-BSA と同様の手順で MIA-BSA を調製した。MIC-BSA、MIA-BSA の時間依存的な分解効果を比較するために、0, 6, 9 時間における LNCaP 細胞の BSA 量を測定した。また、Figure 3-10B にアクチン量で標準化した MIC-BSA の蓄積量をまとめた（n＝3）。その結果、未修飾の BSA の値は 6-9 時間のインキュベーションではほとんど変化が見られなかったが、MIC-BSA では時間依存的な分解が確認され、MIC-BSA(10/1)は MIC-BSA(3/1)に比べて BSA の減少が早かった。同じ傾向が、MIA-BSA を用いた実験においても観測された（Figure 3-10A 下）。このことから、BSA に修飾された HyT の数が、細胞内の BSA の分解を誘導することに関係していると考えられた。

最後に HyT システムはユビキチン E3 リガーゼが関与する 26S プロテアソーム経路を介してタンパク質の分解を誘導することが報告されているため[16,17]、カルボランによる BSA の分解のメカニズムをより深く調べるべく、MIC-BSA を導入した LNCaP 細胞を 26S プロテアソーム阻害剤である MG-132[6] と HDM2 ユビキチン E3 リガーゼ阻害剤である HLI-373[18] で処理した（Figure 3-10C）。MG-132 または HLI-373 で処理した LNCaP 細胞では、MIC-BSA（3/1）および MIC-BSA（10/1）の分解が抑制されたが、LNCaP 細胞に導入した未修飾の BSA の値はほとんど変化しなかった。これらの結果は、タンパク質にカルボランを修飾したことによって、ユビキチンプロテアソーム系の経路を介して、そのタンパク質の分解を誘導する可能性を示している。さらに、タンパク質のリジン残基は、E3 リガーゼを介したユビキチン化に必須の役割を果たしていることが報告されている[19]。Figure 3-8B に示した結果から、BSA のリジン残基の露出度は、カルボランの結合数が多いほど高くなった。このことから、カルボランがタンパク質に結合すると、微視的な構造変化によってタンパク質のリジン残基が露出し、ユビキチン E3 リガーゼ関連のプロテアソームを介して分解されることが示唆される。

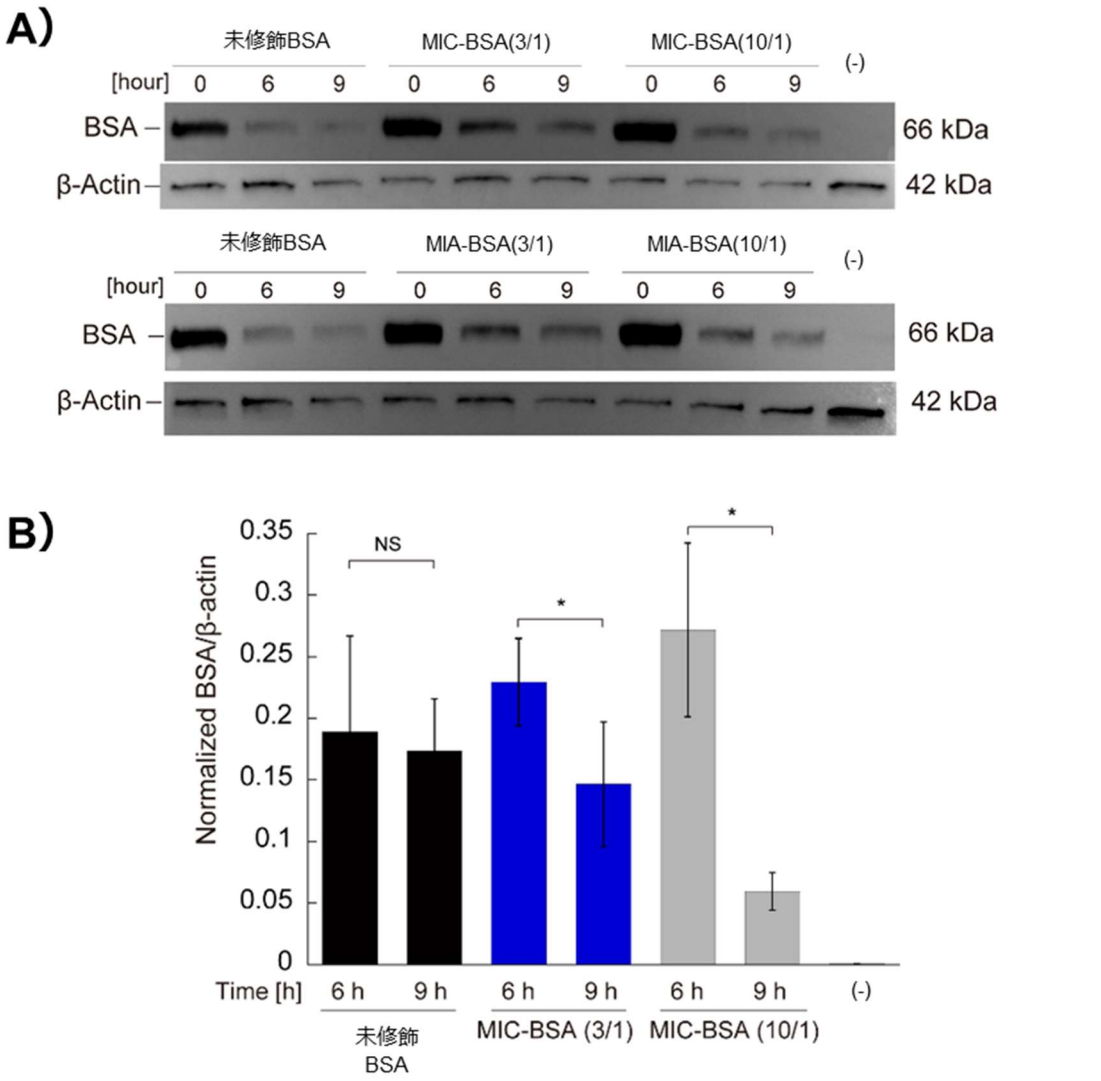


Figure 3-10. LNCaP 細胞における MIC-BSA の分解；A）抗 BSA 抗体を用いたイムノブロッティング；BSA、MIC−BSA (3/1)、MIC−BSA (10/1)、MIA−BSA (3/1)をトランスフェクションして 0-9 時間培養後、細胞破砕し、イムノブロッティングを行った。 B）β-actin 量で標準化した MIC-BSA の量（n = 3）。mean ± SD (n = 3, *p < 0.05, **p < 0.01, ***p < 0.001, NS: not significant).

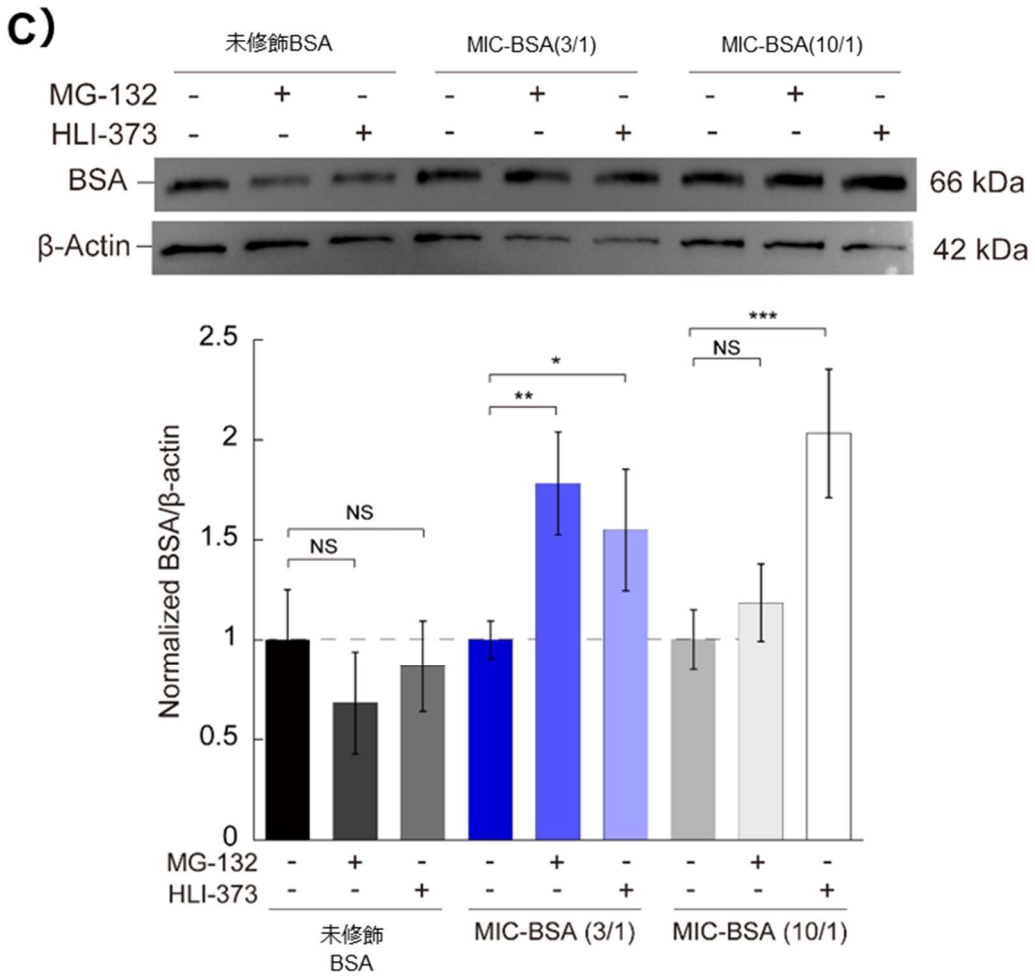


Figure 3-10.（続き）C）MG-132（26S プロテアソーム阻害剤）、HLI-373（ユビキチン E3 リガーゼ阻害剤）が MIC-BSA の分解に与える影響。mean ± SD (n = 3, *p < 0.05, **p < 0.01, ***p < 0.001, NS: not significant).

3-9 まとめ

本章では、アダマンタンに代わる新たな HyT としてカルボランを利用したタンパク質分解誘導剤の創出を行った（Figure 3-11）。興味深いことに、カルボランをもつ化合物 **3** は、これまで一般的に用いられてきた HyT13（**1**）に比べて高いタンパク質分解効率を示した（43% vs. 49%）。HyT の分解誘導活性は、疎水性だけでなく、3 次元的で、より大きなファンデルワールス体積をもつことが重要であることが示唆された。一方で、HyT システムに重要な役割をもつ熱ショックタンパク質（HSP）の阻害剤を用いた実験から、これまで提唱されている HSP70 の寄与だけなく、他のシャペロンタンパク質による影響が示唆された。

また、カルボランを結合させた細胞内タンパク質（HA-HaloTag2-EGFP）だけでなく、細胞外のタンパク質（MIC-BSA）も細胞内のユビキチンプロテアソーム系で分解を受けることを明らかにした。HyT システムを用いて細胞外タンパク質を分解した初の例である。さらに、MIC-BSA のゼータ電位の測定から、カルボランが BSA の構造変化を誘発することが示唆された。これは、カルボランがタンパク質内部の疎水性領域に誘導されることにより、表面に多くのリジン残基が出現したためと考えられる。実際、FITC で標識した MIC-BSA の蛍光強度は MIC/BSA 比に依存して増加した。カルボランによって引き起こされる詳細なタンパク質分解機構はまだ明らかにされていないが、カルボランが結合することにより、BSA のわずかな構造変化がリジン残基の露出を誘導し、これがユビキチン E3 リガーゼおよびプロテアソームに影響を与え、タンパク質分解に導かれると考えられる。以上のことから、本研究で得られた知見は、新たな HyT の設計に役立つだけでなく、HyT システムの完全な機構解明に向けた重要な手がかりであるといえる。

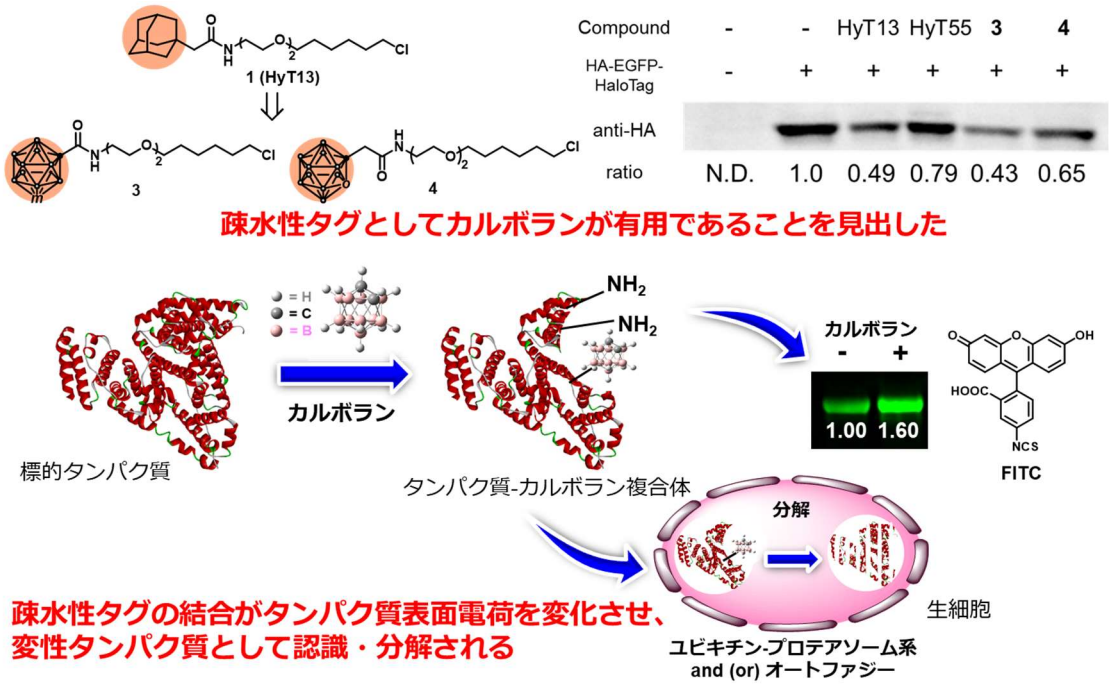


Figure 3-11. 第三章まとめ

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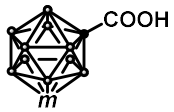
Experimental Section

Synthesis

General

NMR spectra were recorded on a Bruker biospin AVANCE II (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker biospin AVANCE III (500 MHz for ^{1}H, 125 MHz for ^{13}C, 470 MHz for ^{11}B) instrument in the indicated solvent. Chemical shifts are reported in units per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane solutions in $CDCl_3$ (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, q; quartet, m; multiplet, br; broad, J; coupling constants in Hertz. Mass spectra were measured using a JMS-700 Mstation. IR spectra were recorded on a JASCO FT/IR-4100 spectrometer. IR spectrum was recorded on a JASCO Corporation FT/IR-4100 FT-IR Spectrometer. ATR PRO ONE was attached to the FT/IR-4100 in measuring solid IR spectroscopy by single reflection attenuated total reflection. Only the strongest and/or structurally important peaks were reported as the IR data given in cm^{-1}. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-TOF-MS (micrOTOF II). All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light (254 nm) and were visualized using Hanessian's stain solution. Column chromatography was performed on Silica Gel 60 N, purchased from Fuji Silysia Chemical Ltd. HyT13 (**1**), HyT55 (**2**) and 2-(2-((6-chlorohexyl)oxy)ethoxy)-ethanamine (HaloTag linker **8**) were known and synthesized according to the literature procedures.[3,4]

Synthesis of 1-carboxyl-1,7-dicarba-*closo*-dodecaborane (11)

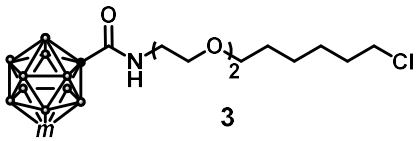


11

To a solution of *m*-carborane (488 mg, 3.38 mmol) in THF (15 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (2 mL, 3.21 mmol) at -78 ℃. After the resulting mixture was stirred room temperature under argon atmosphere for 1 h, the CO_2 balloon was attached. Then, the resulting mixture was stirred at room temperature CO_2 atmosphere for 3 h. After that, the reaction mixture was concentrated under pressure. The resulting mixture was added and Hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Hexane. Then, the aqueous layer was added 6 M HCl and Hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was

extracted with Hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum to afford **11** (444 mg, 2.36 mmol, 74%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.03 (brs, 1H), 4.10-1.60 (m, 10H). This spectrum is consistent with the reported values.[20]

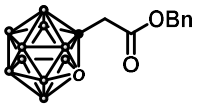
Synthesis of *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1,7-dicarba-*closo*-dodecaborane-1-carboxamide (3)



3

To a solution of **3** (6.1 mg, 0.031 mmol.) and HaloTag linker **6** (40 mg, 1.78 mmol) in DCM (1.2 mL) were added HOBt (15 mg, 0.11 mmol) and EDCI (17.9 mg, 0.093 mmol). After the reaction mixture was cooled to 0 ℃, was slowly added DIEA (40 μL, 3.09 mmol). The resulting mixture was stirred at room temperature for 8.5 h and quenched with H_2O. The mixture was extracted twice with ethyl acetate and the combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (70% EtOAc in Hexane) afforded **3** as slight yellow oil (7.5 mg, 0.019 mmol, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.32 (bs, 1H), 3.61-1.59 (m, 2H), 3.57-3.51 (m, 6H), 3.47 (t, J = 6.7 Hz, 2H), 3.39 (q, J = 5.2 Hz, 2H), 3.02 (s, 1H), 2.80-1.80 (m, 10H), 1.82-1.76 (m, 2H), 1.65-1.59 (m, 2H), 1.48-1.44 (m, 2H), 1.41-1.36 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.6, 71.7, 70.8, 70.4, 69.3, 55.2, 45.4, 40.8, 32.9, 30.0, 29.8, 27.0, 25.8. ^{11}B NMR (160 MHz, $CDCl_3$): δ -5.68, -7.46, -10.9, -11.6, -13.3, -15.7; IR (NaCl disc): 2926, 2856, 2604, 1686, 1519, 1276, 1216, 1114, 760 cm^{-1}; HRMS (ESI, positive) for $C_{13}H_{32}B_{10}ClNO_3$ (m/z): calculated 418.2927 $(M+Na)^+$, found 418.2918.

Synthesis of 1-(2-(benzyloxy)-2-oxoethyl)-1,2-dicarba-*closo*-dodecaborane (12)

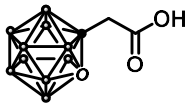


12

To a solution of *o*-carborane (757 mg, 5.25 mmol) in THF (20 mL), was added *n*-BuLi 1.6 M solution in hexane (3.28 mL, 5.25 mmol) at -78 ℃. After the resulting mixture was stirred at 0 ℃ under argon atmosphere for 1 h, benzyl bromoacetate (784 μL, 5.0 mmol) was added. Then, the resulting mixture was stirred at room temperature for 3 h. After that, the reaction mixture was quenched with H_2O. The mixture was extracted twice with ethyl acetate and the combined extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (10% EtOAc in Hexane) afforded **12** as colorless oil (691 mg, 2.36 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40-7.33 (m, 5H), 5.14 (s, 2H), 4.35

(s, 1H), 3.27 (s, 2H), 3.00-1.60 (m, 10H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 167.3, 134.6, 129.1, 128.1, 128.8, 67.9, 58.7, 41.7; ^{11}B NMR (160 MHz, $CDCl_3$): δ -2.05, -4.98, -9.23, -10.5, -12.0, -12.8; IR (NaCl disc): 2592, 1739, 1178 cm^{-1}; HRMS (ESI, positive) for $C_{11}H_{20}B_{10}O_2$ (m/z): calculated 317.2305 $(M+Na)^+$, found 317.2307.

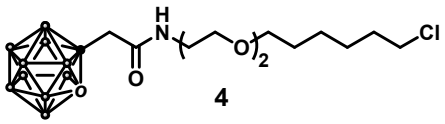
Synthesis of 1-(carboxymethyl)-1,2-dicarba-*closo*-dodecaborane (13)



13

To a solution of **12** (207 mg, 0.71 mmol) in MeOH (10 mL), was added Pd/C wetted with water (34 mg). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. The reaction mixture was filtered on celite pad and concentrated. The resulting mixture was dissolved with aq. $NaHCO_3$ and washed with hexane three times. After the water layer was acidified with aq. HCl, extracted with EtOAc, dried over Na_2SO_4, filtered, and concentrated afforded **13** (123 mg, 0.61 mmol, 86%). ^{1}H NMR (400 MHz, CD_3CN): δ 8.93 (br, 1H), 4.64 (s, 1H), 3.28 (s, 2H), 3.10-1.40 (m, 10H). This spectrum is consistent with the reported values.[21]

Synthesis of *N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1,7-dicarba-*closo*-dodecaborane-1-acetamide (4)



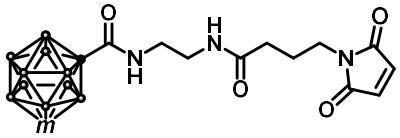
4

To a solution of **13** (40 mg, 0.20 mmol) in CH_2Cl_2 (1 mL), was slowly added excess amount of oxalyl chloride. After the resulting mixture was stirred at 50 ℃ under argon atmosphere for 12 h, the reaction mixture was concentrated under pressure. The crude materials were used to the next reaction without further purification.

To a solution of HaloTag linker **8** (44 mg, 0.20 mmol) and triethylamine (400 μL) in CH_2Cl_2 (1 mL) at 0 ℃ was slowly added the crude material in CH_2Cl_2 (500 μL). After the resulting mixture was stirred at 0 ℃ for 30 min, it was warmed up to room temperature and stirred for 1 h. The reaction mixture was quenched with 1M aq. HCl. The mixture was extracted three times with CH_2Cl_2 and the combined extracts were dried over Na_2SO_4, filtered, and concentrated. Then, the crude materials were purified by column chromatography on silica gel (70% EtOAc in Hexane) afforded **4** as a white solid (50 mg, 0.12 mmol, 62%). m.p. 38-40 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.36 (bs, 1H), 4.60 (s, 1H), 3.64-3.62 (m, 2H), 3.59-3.52 (m, 6H), 3.48 (t, J = 6.7 Hz, 2H), 3.43 (q, J = 5.2 Hz, 2H), 3.04 (s, 2H), 2.80-1.60 (m, 10H), 1.81-1.75 (m, 2H), 1.64-1.59 (m, 2H), 1.50-

1.44 (m, 2H), 1.40-1.36 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 166.3, 71.4, 70.5, 70.1, 69.3, 69.2, 58.8, 45.1, 43.5, 39.7, 32.6, 29.6, 26.8, 25.5. ^{11}B NMR (160 MHz, $CDCl_3$): δ -2.15, -5.17, -9.53, -10.7, -11.8, -12.7; IR (NaCl disc): 2937, 2865, 2590, 1659, 1556, 1098 cm^{-1}; HRMS (ESI, positive) for $C_{14}H_{34}B_{10}ClNO_3$ (m/z): calculated 432.3084 $(M+Na)^+$, found 432.3073.

Synthesis of 1-((2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)ethyl)carbamoyl)-1,7-dicarba-*closo*-dodecaborane (MIC)



Maleimide conjugated carborane (MIC)

To a solution of **3** (270 mg, 1.43 mmol), HATU (815 mg, 214 mmol), and DIEA (369 mg, 2.86 mmol) in CH_2Cl_2, was added *N*-Boc-ethylenediamine (400 mg, 2.50 mmol) and stirred for 3 h at room temperature. The mixture was washed with 1 M HCl aq., saturated $NaHCO_3$ aq., and Brine. The organic layer was dried over $MgSO_4$, and dried up under the reduced pressure. To a solution of the resulting crude materials in CH_2Cl_2 (1.0 mL), was added 4 M HCl in dioxane (4.0 mL) at 0 ℃ and the mixture was stirred at room temperature for 10 h. After removal of the solvent under the reduced pressure, the residue was suspended in EtOAc and washed with saturated $NaHCO_3$ aq. and brine. The organic layer was dried over $MgSO_4$, the solvent was removed. The crude materials were used to the next reaction without further purification.

To a solution of 4-maleimidobutyric acid (256 mg, 1.40 mmol), HATU (570 mg, 1.50 mmol), and DIEA (387 mg, 3.00 mmol) in CH_2Cl_2 was added the crude material in CH_2Cl_2, and stirred at room temperature for 11 h. The resulting mixture was washed with 1 M HCl aq., saturated $NaHCO_3$ aq., and Brine. The organic layer was dried over $MgSO_4$, and dried up under the reduced pressure. The crude materials were purified by column chromatography on silica gel (20% EtOAc in Hexane) afforded **MIC** as a white solid (204 mg, 0.516 mmol, 36% 3 steps). m.p. 48-50 ℃;^{1}H NMR (500 MHz; $CDCl_3$) δ 7.09 (s, 1H), 6.73 (s, 2H), 6.39 (s, 1H), 3.58 (t, J = 6.5 Hz, 2H), 3.33-3.38 (m, 4H), 3.05 (s, 1H,), 2.17 (t, J = 7.0 Hz, 2H), 1.94 (quin, J = 6.5 Hz, 2H). ^{13}C NMR (125 MHz; $CDCl_3$) δ 173.2, 171.0, 161.18, 134.2, 75.45, 42.10, 38.82, 36.93, 33.08, 24.60. ^{11}B NMR (160 MHz; $CDCl_3$) δ -5.63, -7.46, -10.87, -11.57, -13.27, -15.73; IR (NaCl disc): 3343, 3062, 2939, 2604, 1706. HRMS (ESI, positive) for $C_{13}H_{25}B_{10}N_3O_4$ (m/z): calculated 420.2690 $(M+Na)^+$; found 420.2688.

Biology

Cell culture and materials.

Flp-In™ 293 cell line purchased from Invitrogen (California, USA) was incubated with DMEM (high glucose) medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Gibco; ThermoFisher Scientific, Waltham, MA). Cells were incubated in a cell incubator with 5% CO_2 at 37 ℃. HA-EGFP-HaloTag2 was a gift from Craig Crews (Addgene plasmid # 41742; http://n2t.net/addgene:41742; RRID: Addgene_41742) from pHT2 vector (Promega). pOG44 Flp-Recombinase Expression Vector was purchased from Invitrogen. Stable Flp-In™ 293 cells were raised by co-transfection of HA-EGFP–HaloTag2 plasmid and pOG44 according to the manufacturer's procedure. A clonal population of cells expressing HA-EGFP–HaloTag2 was isolated using hygromycin. These cells were used for Immunoblotting and Flow cytometry analysis study. LNCaP cell line, a human cell line derived from prostate adenocarcinoma, was obtained by RIKEN BRC (Tsukuba, Japan), and cultivated under a humidified 5% CO_2 atmosphere at 37 °C in RPMI1640 (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% (v/v) fetal bovine serum (FBS), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}).

Immunoblotting for HA-EGFP-HaloTag2 degradation using HyTs.

The HA-EGFP–HaloTag2 expressing cells (2×10^5 cells per well of a 12-well plate) were incubated for 24 h with or without drugs. The indicated cells were washed twice with cold PBS, and the cells were lysed in 100 μL of lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100) with protease inhibitor cocktail (Promega, Wisconsin, USA). The lysates were cleared by centrifugation at 16,100 g for 5 min. The total protein concentration was determined by BCA assay using Pierce™ BCA Protein Assay Kit (Thermo Scientific, Massachusetts, USA). 50 μg of protein was lysed with a sample buffer (50 mM Tris, pH 7.4, 4% sodium dodecyl sulfate [SDS], 10% glycerol, 4% 2-thioethanol, and 50 μg/mL of bromophenol blue) and boiled for 5 min. The cell lysates were then subjected to SDS–polyacrylamide gel electrophoresis (PAGE), transferred to a polyvinylidene difluoride (PVDF) membrane (GE Healthcare Buckinghamshire, UK), and immunoblotted with anti-HA antibody (BioLegend, California, USA), anti-tubulin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-β-actin antibody (Sigma-Aldrich, Missouri, USA), anti-BSA antibody (Bioss, Massachusetts, USA), and anti-MIC antibody (prepared from SCRUM Inc. Japan). Following overnight incubation at 4 °C with the primary antibodies, the membranes were further incubated with a horseradish peroxidase (HRP)-conjugated secondary antibody at 4 °C for 1 h. The membranes were then treated with ImmunoStar LD (Wako Chemical, Japan), and protein expression was visualized with iBright™ CL1500 Imaging System (Invitrogen, California, USA).

Preparation of BSA-MICs.[38]

To a solution of BSA in PBS (pH = 7.4) solution (6.6 mg/mL) was added various concentration of maleimide-conjugated MIC in DMSO (10 eq.) and incubated in an Eppendorf tube at 37 °C for 6 h. The reaction mixture was diluted with PBS (400 μL), ultrafiltered, and rediluted 3 times with PBS, and then the mixture was diluted with PBS (200 μL). BSA-MIC solution was finally obtained.

Flow cytometry analysis.

The HA-EGFP–HaloTag2 expressing cells (2×10^5 cells per well of a 12-well plate) were incubated for 24 h with or without drugs, washed with PBS, trypsinized. These cells were centrifugated and removed of the supernatant. The resulting cell precipitates were resuspended in PBS and the intracellular EGFP level was measured by Cytomics FC500 (Beckman Coulter, California)

DLS and zeta-potential.

The dynamic light scattering (DLS) and zeta potential analysis of BSA, MIC-BSA (3/1), and MIC-BSA (10/1) was performed using Zetasizer Nano ZS (Malvern Panalytical) at 25 °C. The concentration of each sample was prepared at 0.1 mg/mL. All measurements were performed in triplicate.

FITC capping assay.[39]

To a solution of 0.1 mM BSA or BSA-MIC in PBS (20 μL) was added 0.2 μL of 100 mM FITC in DMSO and incubated in an Eppendorf tube at 4 °C for 8 h in the dark. The reaction mixture was diluted with PBS (100 μL), ultrafiltered, and rediluted 3 times with PBS, and then the mixture was diluted with PBS (30 μL). After that, FITC modified BSA or BSA-MIC solution was lysed with a sample buffer (50 mM Tris, pH 7.4, 4% sodium dodecyl sulfate [SDS], 10% glycerol, 4% 2-thioethanol, and 50 μg/mL of bromophenol blue) and boiled for 5 min. The resulting mixture were then subjected to SDS-PAGE. The fluorescence of gels was detected with iBright™ CL1500 Imaging System (Invitrogen, California, USA).

Circular dichroism (CD) spectra.

The solutions of BSA, MIC-BSA (3/1), and MIC-BSA (10/1) dissolved in PBS (0.1 mg/mL) were prepared, and their CD spectra were recorded on a JASCO spectropolarimeter, model PS-450J (JASCO, Tokyo, Japan). The spectra were scanned between 190 and 250 nm with 0.2-nm resolution at a heating rate of 1.0°C/min.

Electroporation for a transduction of MIC-BSA.

LNCaP cells were incubated for 24 h under serum-reduced Opti-MEM (Gibco, Grand Island, USA) to reduce the value of albumin in cells. Unmodified BSA, 3MIC-BSA, and 10MIC-BSA (80 μg) were electroporated into the albumin-reduced LNCaP cells (2×10^5 cells/800 μL in Opti-MEM) using the Super Electroporator NEPA 21 (NEPA GENE Co. Ltd., Chiba, Japan). The electroporation was conducted under following conditions (condition 2, Table 3-2): (1) poring pulse; pulse voltage, 150 V; pulse length, 10 ms; pulse interval, 50 ms; pulse number, 50; decay rate, 20%; polarity, +; and (2) transfer pulse; pulse voltage, 20 V; pulse length, 50 ms; pulse interval, 50 ms; pulse number, 20; decay rate, 30; polarity, +/-. The electroporated LNCaP cells were washed with PBS twice. The cells were seeded on 6 well-plate dish (Iwaki, Japan) coated with fibronectin (Sigma-Aldrich) and incubated for 6 and 9 h. For the inhibition measurement for protease system, MG132 (Sigma-Aldrich) (20 μM) and HLI-373 (10 μM) were pretreated to LNCaP cells for 30 min before the electroporation. After the electroporation in the same manner, the electroporated LNCaP cells was incubated with MG132 and HLI-373 for 6 h.

To determine the electroporation condition, FITC-labeled BSA was electroporated into LNCaP cells with following condition (Table 3-2). The electroporated LNCaP cells was collected by trypsin treatment, stained with propidium iodide (PI) (3 μM). The fluorescence intensity of cells was measured with flow cytometry (EC-800; Sony, Tokyo, Japan). On condition 2, the electroporated LNCaP cells showed a higher fluorescence intensity for FITC-BSA and a lower fluorescence intensity for PI. Hence, the electroporation for MIC-BSA into LNCaP cells was performed by the condition 2.

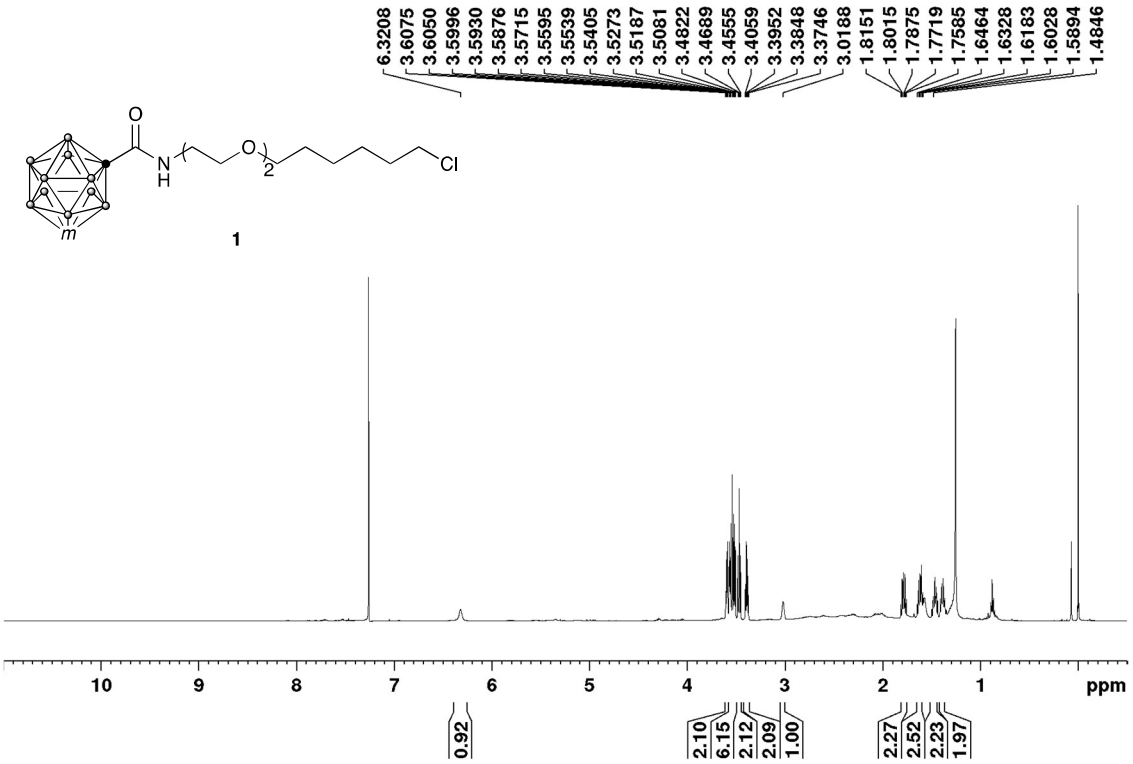
Immunoblotting for MIC-BSA intracellular degradation.

The electroporated LNCaP cells cultured on 6-well plate dish for prescribed time was washed twice with PBS and lysed in RIPA buffer (Wako Chemical) containing protease inhibitors (Nacalai Tesque) and phosphatase inhibitors (Nacalai Tesque). The lysate was clarified by centrifugation at 12,000 rpm for 10 min at 4 °C and the supernatant was collected. The supernatant was mixed with Laemmli buffer (BioRad) containing 10% 2-mercaptoethanol. SDS-PAGE was performed on a 12% poly(acrylamide) gel for 50 min at 150 V. The separated proteins were then transferred to a PVDF membrane (Bio-Rad, Hercules, CA, USA) using a Trans-Blot Turbo transfer system (Bio-Rad). The membrane was blocked with TBS-T (20 mM Tris-HCl, 500 mM NaCl, 0.05% Tween 20, pH 7.5) containing 5% skim milk for 60 min at room temperature. The membrane was treated overnight with the following primary antibodies at 4 °C: mouse polyclonal anti-BSA (1:1,000 dilution) (BSA-7G10; Abcam) and mouse polyclonal anti-β-actin (6D1; MBL) (1:1,000 dilution). Then, the membrane was treated with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (MBL) (1:10,000 dilution) for 60 min at room temperature, and was treated with ImmunoStar Zeta (Wako Chemical) for 5 min. The chemiluminescence images were acquired on an LuminoGraph I (WSE-6100; ATTO). The band intensity of the captured images was measured using ImageJ software.

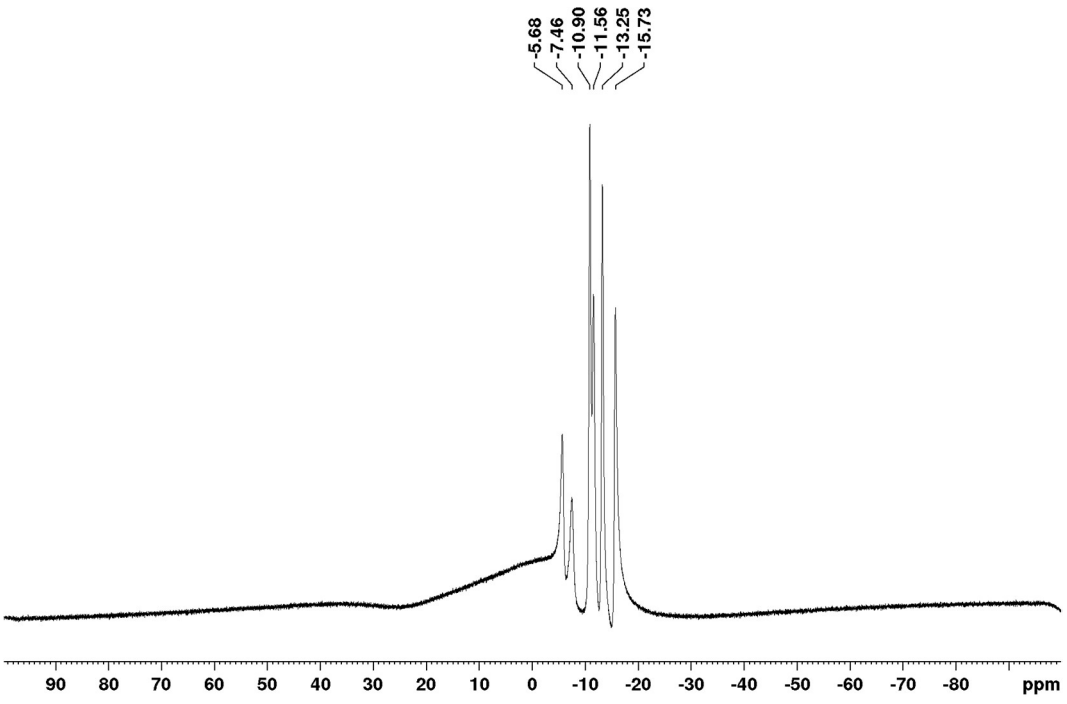
Spectra data

***N*-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1,7-dicarba-*closo*-dodecaborane-1-carboxamide (3)**

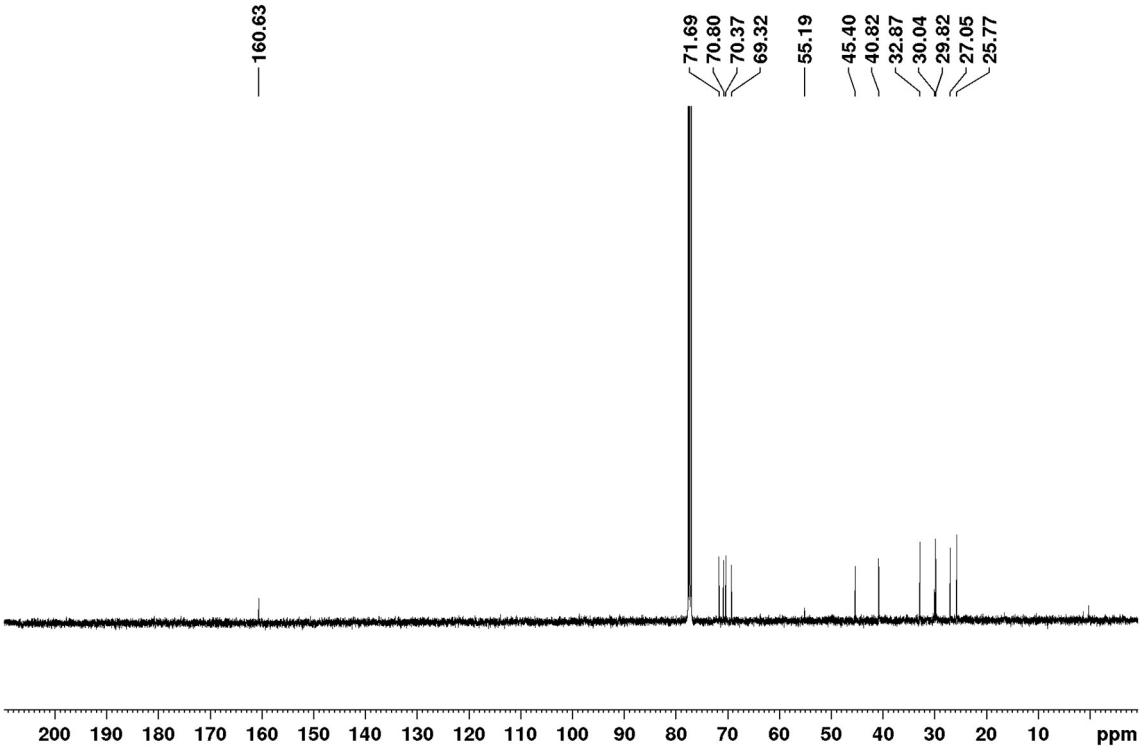
^{1}H NMR (400 MHz, $CDCl_3$) (**1**)



^{11}B NMR (160 MHz, $CDCl_3$)

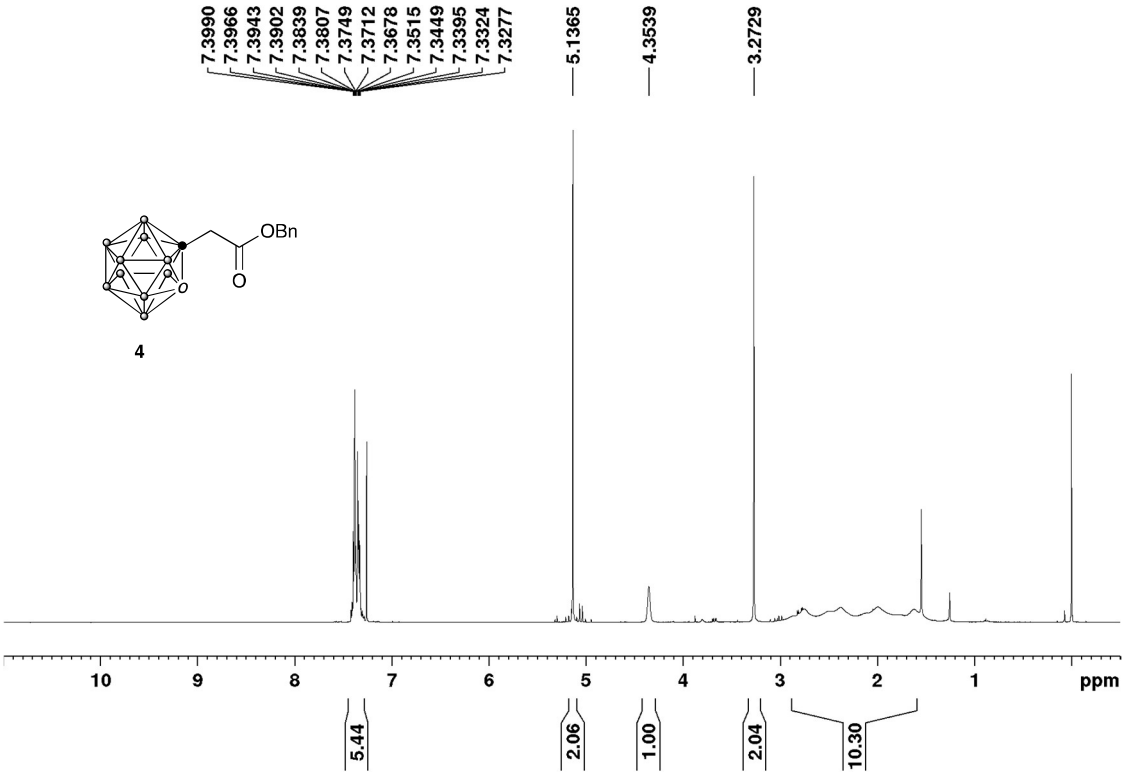


^{13}C NMR (125 MHz, $CDCl_3$) (**1**)

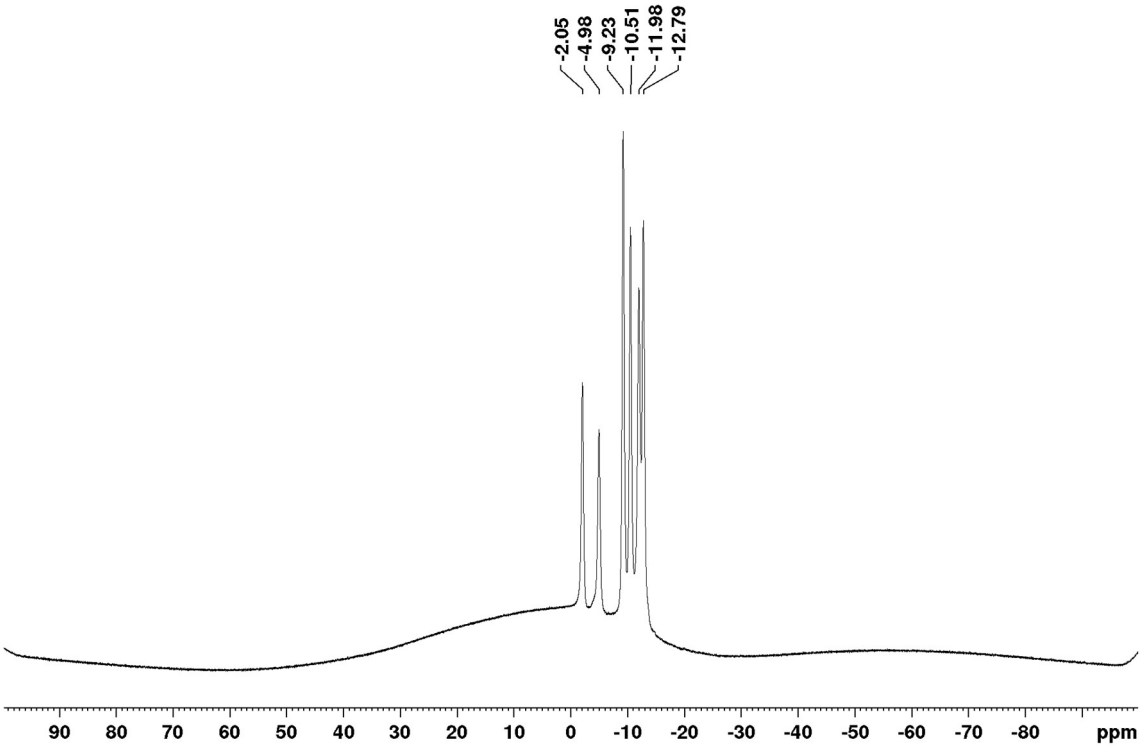


1-(2-(benzyloxy)-2-oxoethyl)-1,2-dicarba-*closo*-dodecaborane (4)

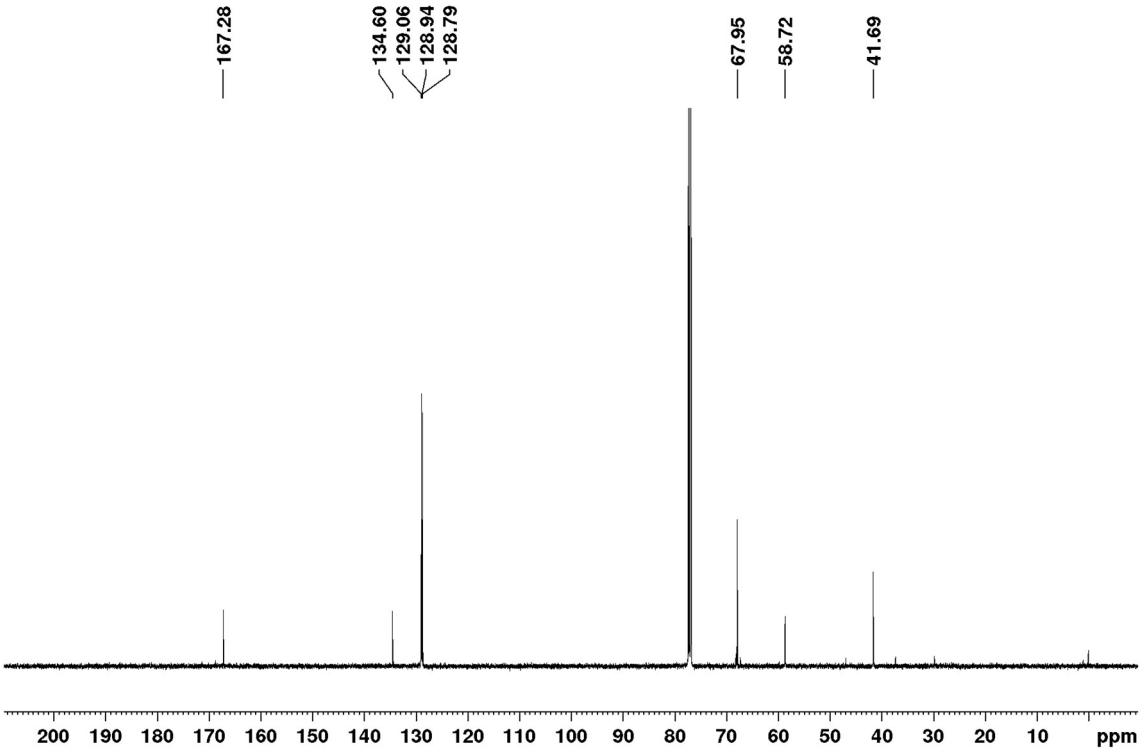
^{1}H NMR (400 MHz, $CDCl_3$) (**4**)



^{11}B NMR (160 MHz, $CDCl_3$) (**4**)

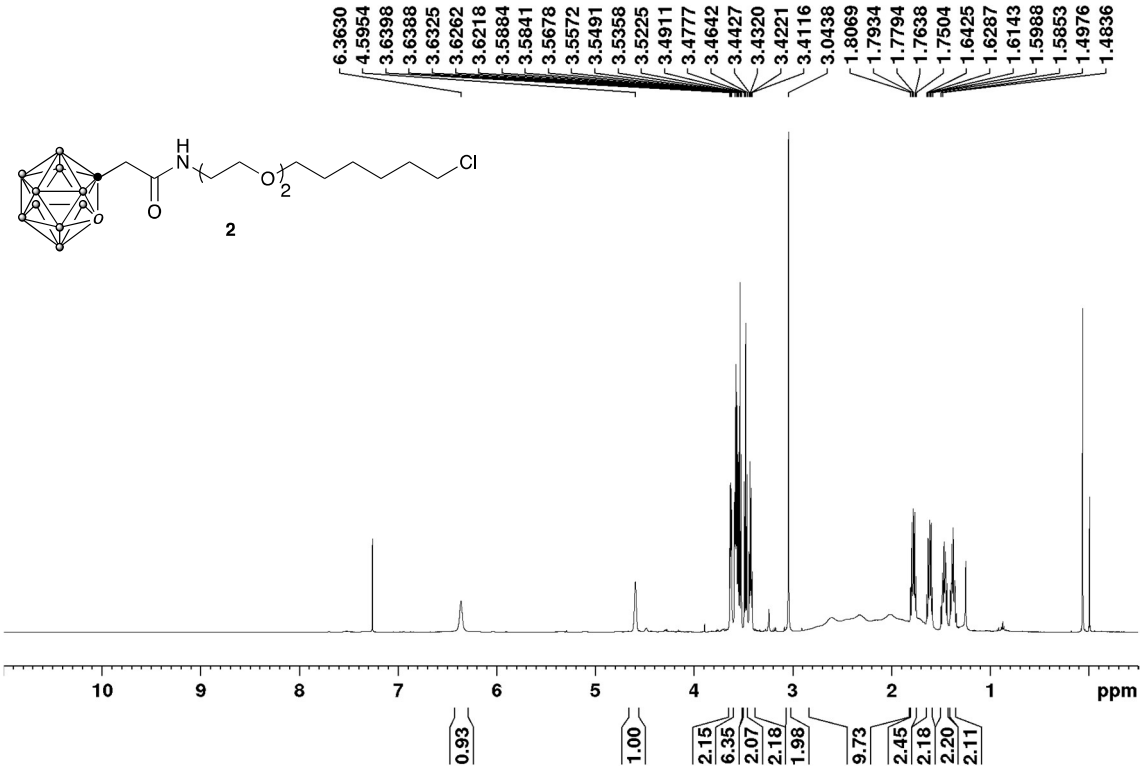


^{13}C NMR (125 MHz, $CDCl_3$) (**4**)

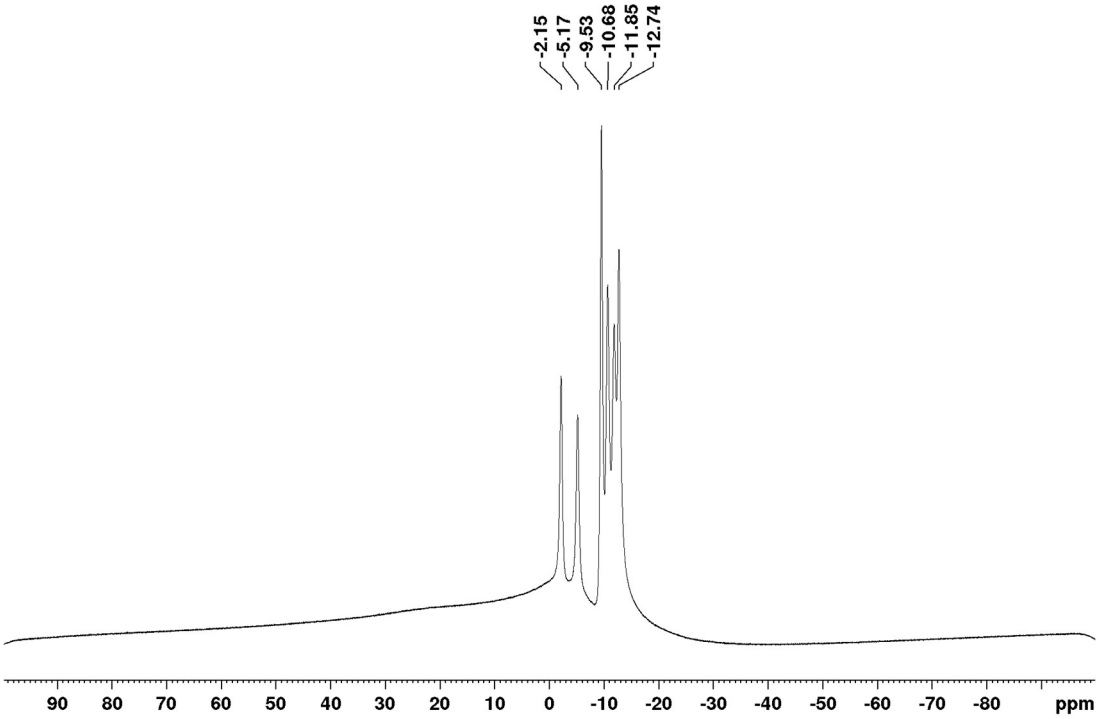


_N_-(2-(2-((6-chlorohexyl)oxy)ethoxy)ethyl)-1,7-dicarba-_closo_-dodecaborane-1- acetamide (2)

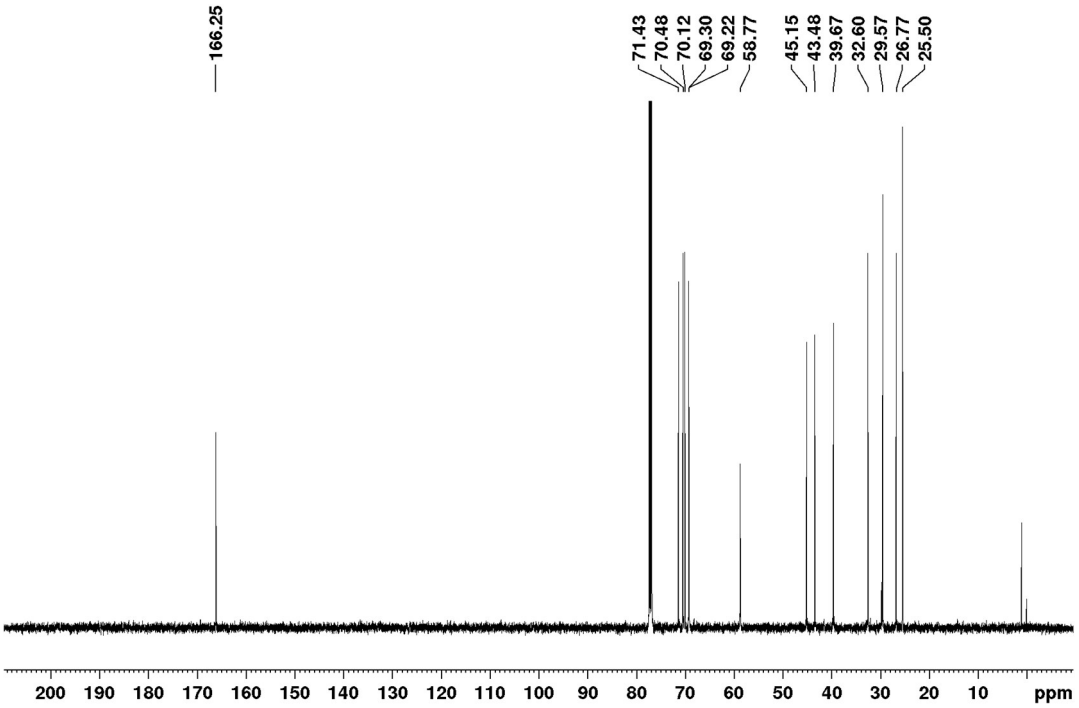
^{1}H NMR (400 MHz, $CDCl_3$) (**2**)



^{11}B NMR (160 MHz, $CDCl_3$) (**2**)

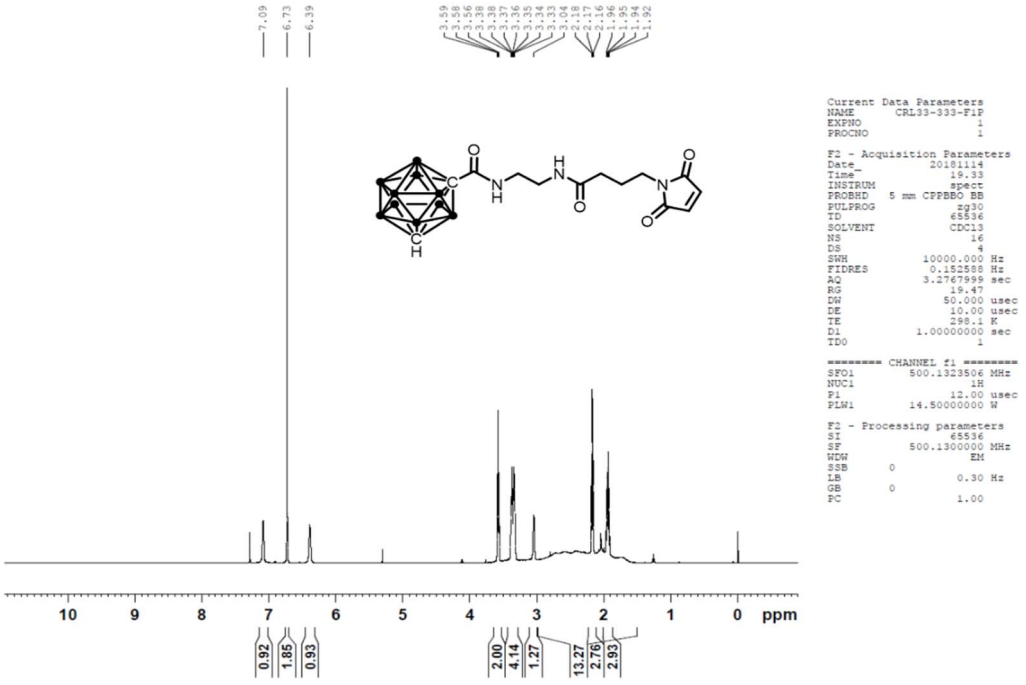


^{13}C NMR (125 MHz, $CDCl_3$) (**2**)

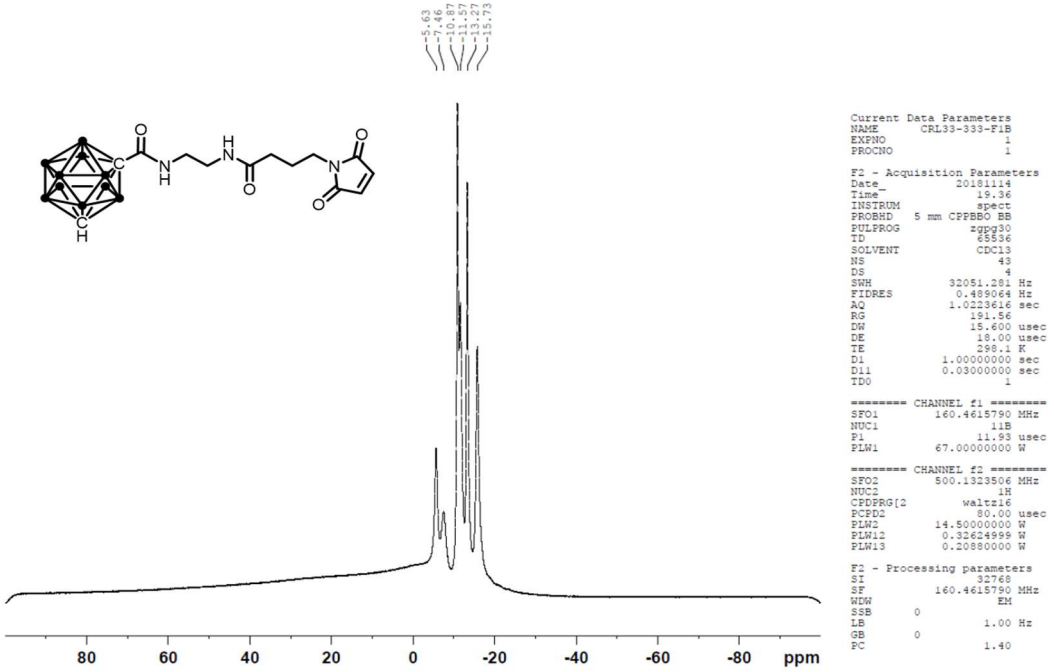


1-((2-(4-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)butanamido)ethyl)carbamoyl)-1,7-dicarba-*closo*-dodecaborane (MIC)

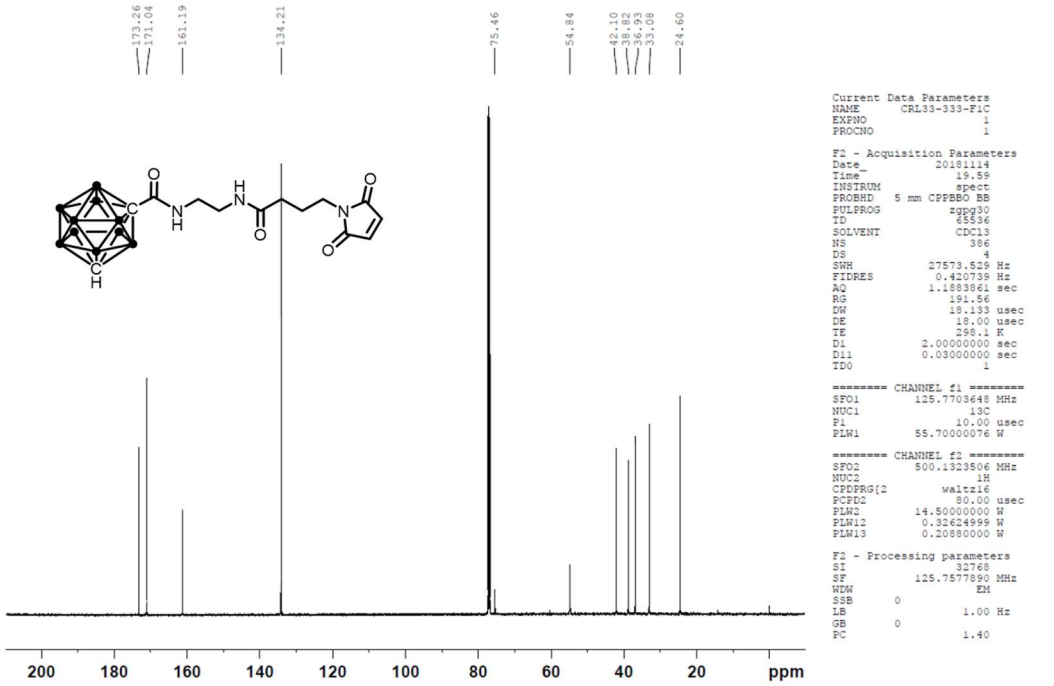
^{1}H NMR (400 MHz, $CDCl_3$)



^{11}B NMR (160 MHz, $CDCl_3$) (**MIC**)



^{13}C NMR (125 MHz, $CDCl_3$) (**MIC**)



第四章

磁気共鳴イメージングを指向した Gd メタラカルボラン錯体の合成研究

4-1 はじめに

ホウ素中性子捕捉療法（BNCT）は、ホウ素（^{10}B）と熱中性子との核反応で生じるα粒子（He^{+}）を用いてがん細胞を破壊する放射線治療であり、2020年3月に初めてステボロニンが薬事承認を受けたことで第 5 のがん治療法として益々期待が高まっている[1]。この核反応で発生するα粒子の飛程が約 10〜14 μm であり、がん細胞 1 個分の直径に匹敵することから、がん細胞特異的にホウ素化合物を集積することで、低侵襲で選択的ながん治療が可能となる。したがって本治療を効果的に行うためは、ホウ素を含む薬剤の体内分布と腫瘍への蓄積をコントロールすることが重要である。BNCT 薬剤に使われる ^{10}B 同位体は非放射性であるため、その体内分布を非侵襲的に測定することはかなり困難であり[2]、通常、ポジトロン・エミッション・トモグラフィー（PET）用の放射性標識を導入するか[3]、即発γ線放射化分析（PGAA）を用いて ^{10}B 原子核が中性子を捕獲して生じる二次放射線を測定するかのいずれかが必要となる[4]。しかし、これらの方法はいずれも特殊な装置を必要とし、通常の医療機関では利用できない。そこで、医療診断法として最も広く用いられている Gd をイメージング剤として利用した磁気共鳴イメージング（MRI）に着目した[5–7]。MRI は磁気と電波を使い、生体内の水や脂肪の分布を断層画像として、非侵襲的に観察する技術であり、被爆の危険性もないため比較的簡便に利用できる。さらに、^{157}Gd も ^{10}B と同様に中性子捕捉が可能であり、その断面積は ^{10}B の約 60 倍であるとされる。^{10}B の核反応でα線（飛程 9 nm≒腫瘍細胞）が生じるのに対し、^{157}Gd の核反応では、γ線（飛程 100 μm≒腫瘍組織）が生じるため、薬剤が蓄積したがん細胞だけでなく、がん組織周辺の浸潤細胞に対しても有効であり、同時に発生するオージェ電子による局所的な腫瘍殺傷も期待できるといった利点が考えられる。これまでにジエチレントリアミン五酢酸（DTPA）や 1,4,7,10-テトラアザシクロドデカン-1,4,7,10-テトラ酢酸（DOTA）キレート剤に Gd が配位した化合物とカルボランが連結した分子の合成が報告されている[8–10]（Figure 4-1A）。しかし、このような Gd 錯体は、生理的な pH7.4 の条件で尿とともに完全な排泄が行われると推定されるが[11]、しばしば体内での Gd^{3+}の漏出や滞留が指摘される。Gd^{3+}は腎性全身性線維症（NSF）とよばれる四肢の皮膚の硬化を引き起こし、特に腎機能が低下している場合、死に至るリスクの高い副作用になることが分かっている[12,13]。一方で、*nido*-カルボランは多くの遷移金属と安定なサンドイッチ型錯体（メタラカルボラン）を形成することが知られている（Figure 4-1B）[14]。そこで本章では、ホウ素送達と Gd によるイメージングを同時に実現するため、Gd-メタラカルボランの合成を目指した。

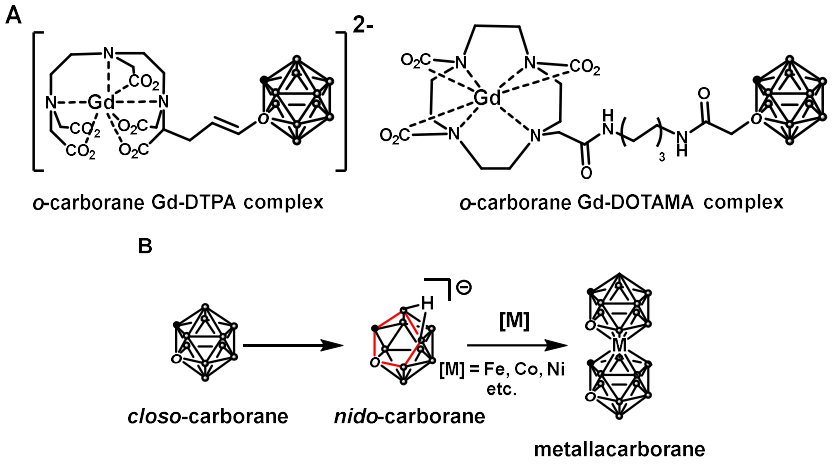


Figure 4-1. A）これまでに研究されてきたホウ素とガドリニウムを連結した分子；B）メタラカルボランの合成法

4-2　安定な Gd-メタラカルボランのためのリガンド設計と合成

これまでに、Gd-メタラカルボランとして[3,3-(THF)$_2$-3,3’ -Gd(1,2-$C_2B_9H_{11}$)$_2$]$^-$の合成に関する文献は 1 例のみあるが、その特性は信頼できるものとしては不十分であった[15]。また Gd が属するランタノイドのメタラカルボランは、一般的に溶媒分子の損失により不安定であるが、近年ペンダント型のドナー基を含む置換基をもつカルボラン配位子を用いることで、その安定性を高めることができることがわかった[16]。この場合、ドナー基のキレート効果が排除される溶媒和よりも優ることによって錯体の安定性が向上することになる。さらに、これらのドナー基を閉じた鎖状構造の中に含めることで、より安定性が向上すると考えられる。そこで、メタラカルボランにクラウンエーテルやチオクラウンを導入した合成知見に基づき[17,18]、2 つのニドカルボランが 2 つのアミド基を含むリンカーで連結されたリガンド **1**、**2** を設計した（Figure 4-2）。

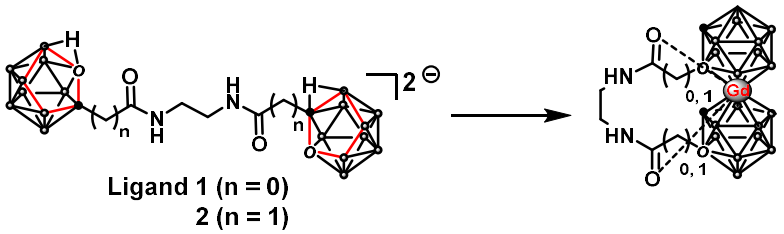
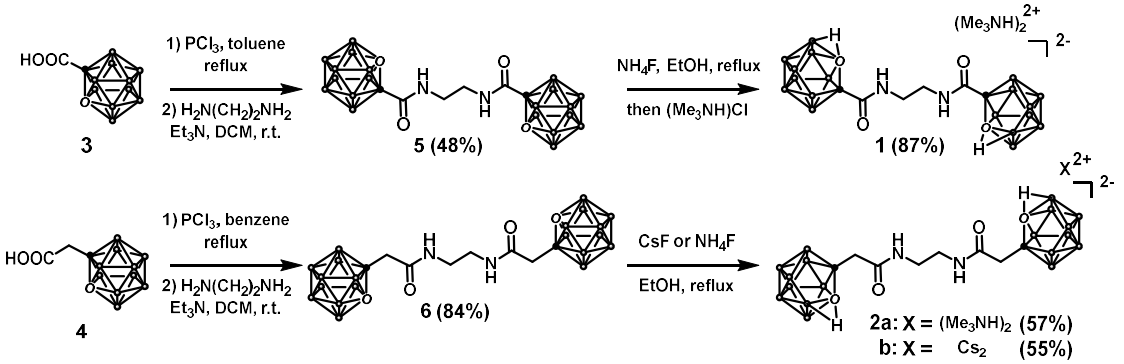


Figure 4-2. ドナー基を導入したカルボラン配位子の設計

続いてリガンド **1**、**2** を Scheme 4-1 に従って合成した。まず、カルボラニルカルボン酸 1-HOOC-1,2-$C_2B_{10}H_{11}$（**3**）とカルボラニル酢酸 1-$HOOCCH_2$-1,2-$C_2B_{10}H_{11}$（**4**）を文献に従い調整し[19,20]、これらを対応する酸クロリドに変換した後、トリエチルアミン存在下エチレンジアミンと反応させ、1,1’-μ-($CH_2NH(O)C$-1,2-$C_2B_{10}H_{11})_2$（**5**）、1,1’-μ-($CH_2NH(O)CCH_2$-1,2-$C_2B_{10}H_{11})_2$（**6**）をそれぞれ収率 48%、84%で得た。最後に、化合物 **5**、**6** をエタノール中でフッ化アンモニウムまたはフッ化セシウムで処理すると、*o*-カルボランの脱ホウ素化反応が進行し、*nido*-カルボランを含むリガンド **1**、**2** を 55-87%の収率で得た。



Scheme 4-1. 設計したリガンド **1**、**2** の合成

なお、化合物 **5** の構造は、単結晶 X 線構造解析によって決定した（Figure 4-3A）。このとき、化合物 **5** は、1 分子のヘキサンとの溶媒和物の形（1:1）で結晶化が確認された。アミド基の結合長（C_{carbon}-C(O) 1.527 および 1.516 Å；C=O 1.231 および 1.23Å；C(O)-N 1.332 および 1.337Å）は, 既知の *o*-カルボラニルアミドの結合長と同程度であった[21,22]。観測された分子のコンフォメーションでは、結晶パッキングから 1 分子あたり 2 つの分子間水素結合が確認される一方で（Figure 4-3C）、分子内水素結合は観察されないことがわかる（Figure 4-3A）。そこで分子内水素結合が形成される可能性を調べるため、量子化学計算を用いてその構造を最適化した（Figure 4-3B）。その結果、予想通り、分子内水素結合（-4.4kcal/mol）を有し、結晶構造とは少し異なるコンフォメーションが予測された。また最適化した構造は、カルボラン上の H3 と H4 の間の弱い引力相互作用（-0.3kcal/mol）によっても安定している。このような相互作用は、結晶構造中でも確認できた。

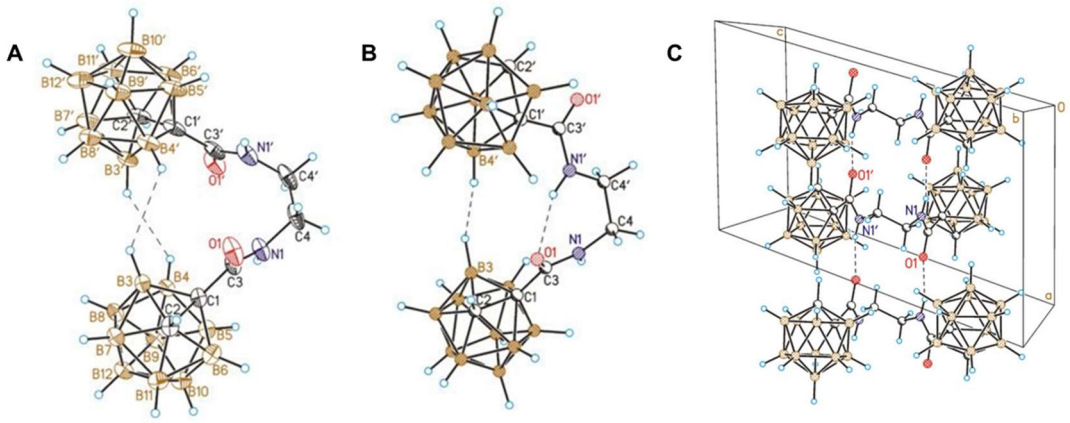
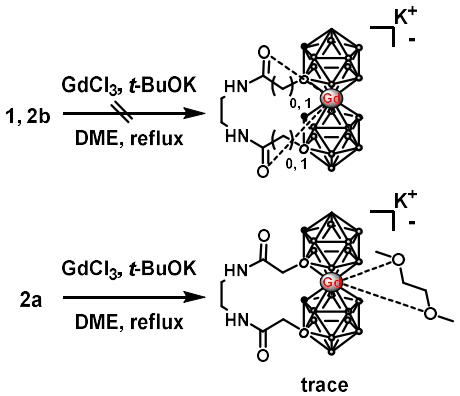


Figure 4-3. A) 化合物 **5** の単結晶 X 線構造；B) 量子化学計算による **5** の最安定構造；C) **5** の結晶パッキング

4-3 Gd-メタラカルボランの合成検討と$[Gd(C_2B_9H_{11})_2(DME)]^-$のモデリング

Scheme 4-2 に示すように、*t*-BuOK 存在下、1,2-ジメトキシエタン（DME）中で，無水塩化ガドリニウム（$GdCl_3$）とリガンド **1**、**2b** をそれぞれ反応させたところ、期待する錯体は得られなかった一方で、リガンド **2a** を反応させたところ、反応混合物の質量分析（ESI-MS）スペクトルには、$[GdL_2(DME)]^-$ 錯体に対応する 650.8 のマイナーシグナルが観測された。しかし、Gd の配位圏に DME 分子が存在するということは、当初の設計ではカルボラン配位子のアミド基が占めるはずだった場所を DME が占めていることを意味する。この場合、カルボラン配位子は予想より回転していると考えられ、両者をつなぐリンカーは、錯形成の安定化ではなく、逆に不安定化させる効果をもつことになる。



Scheme 4-2. Gd-メタラカルボランの合成検討

そこで $[Gd(C_2B_9H_{11})_2(DME)]^-$錯体モデルの量子化学計算を行い、Gd の配位圏の 2 つのサイトが DME で占められている場合の、カルボラン配位子の好ましい配向を調べた（Figure 4-4）[a]。既知の d-金属のメタラカルボランでは、無置換および対称的に置換されたカルボラン配位子の場合、3 つの非等価な安定ロータマーが存在し[23–25]、非対称的に置換された配位子の場合、5 つの非等価な異性体が存在する[24–27]。一方でカルボランが曲がって配位する錯体では、可能なロータマーの数がはるかに多い。これは、２つのカルボラン配位子の回転により生じる互いの相対的な位置関係だけでなく、キレート化された DME 配位子との相対的な位置関係も変化するためであると考えられる。2 つのカルボラン配位子を DME 配位子に対して相対的に回転させることで、$[Gd(C_2B_9H_{11})_2(DME)]^-$の取りうるロータマーを列挙し、最も安定な配座（Figure 4-4A）と不安定な配座（Figure 4B）を示した。DME に対するカルボラン配位子の回転評価には、二面角 cent(*OO*)-Gd-B_{10}-cent(*CC*) を用いた（cent は対応する原子対の中心を意味し、cent(*OO*)は DME の二つの酸素原子の中心、cent(*CC*)はカルボランの二つの炭素の中心を表す)。それぞれのカルボランに対して、この値を D1 と D2 と定義した。

最も好ましいと計算されたロータマーでは、カルボラン配位子の CH 基が DME 配位子に対して *anti*-の位置にあり（D_1 = 165°, D_2 = 163° ；Figure 4A)、一方、最も好ましくないロータマーでは、両方のカルボランの CH 基が DME 配位子に対して *syn*-の位置にある（D_1 = 27°, D_2 = 9° ；Figure 4-4B)。他の予測されうるロータマーでも、D1、D2 のいずれかが 125° に近い位置にある（Table 4-1)。またロータマーのエネルギーが D1 と D2 の値に一貫して依存している（D を大きくすると安定化する）ことから、これらの複合体では、カルボラン配位子が DME 配位子と *anti*-の位置を好むことが明らかとなった。

[a] Dr. Sergey A. Anufriev、Dr. Kyrill Yu. Suponitsky、Dr. Oleg A. Filippov、Prof. Igor B. Sivaev（A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences）との共同研究により、実施された。

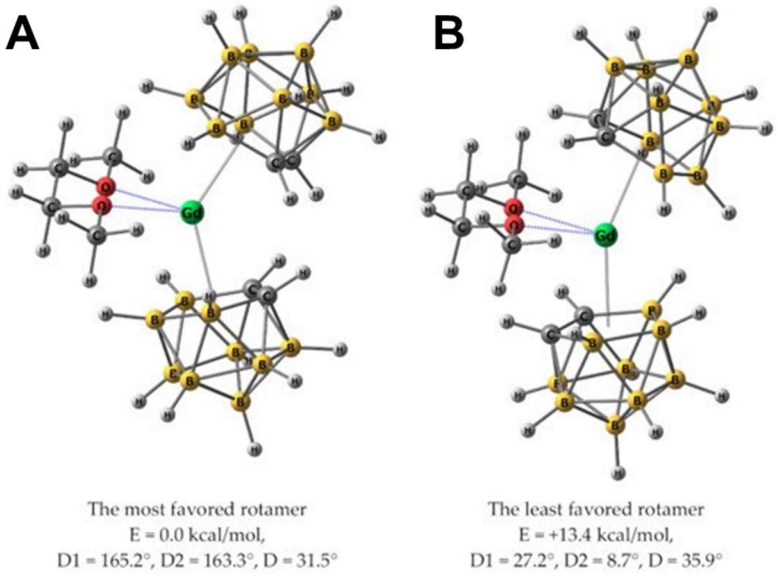


Figure 4-4. 量子化学計算によって予測された$[Gd(C_2B_9H_{11})_2(DME)]^-$のロータマー；A) 最安定構造、B) 不安定構造

Table 4-1. $[Gd(C_2B_9H_{11})_2(DME)]^-$のロータマーにおけるエネルギーと二面角

rotamer	1	2	3	4	5	6	7	8	9
ΔE	13.4	5.8	3.0	6.4	5.3	0.0	3.1	7.4	3.1
D1	27.24	-14.25	-15.39	13.21	30.30	165.22	179.28	87.22	165.93
D2	8.69	125.28	-164.85	-127.32	152.45	163.3	-145.98	125.03	-125.14

4-4　まとめ

本章では、ホウ素の送達と MRI イメージングを同時に可能にする BNCT 薬剤の開発を目指して、ドナー基を導入したカルボラン配位子を設計、合成した後、Gd メタラカルボランの合成に挑戦した（Figure 4-5）。残念ながら、安定な錯体を得ることはできなかったが、反応混合物から $[GdL_2(DME)]^-$ 錯体に対応する 650.8 のマイナーシグナルが質量分析（ESI-MS）で観測された。この結果から、当初の Gd が配位すると予測していたカルボラン配位子のアミド基の空間を、DME の酸素原子が占有していることが示唆された。そこで、新たな錯体モデル$[Gd(C_2B_9H_{11})_2(DME)]^-$を提唱し、量子化学計算により，Gd 含有メタラカルボランのさまざまなコンフォメーションの安定性を評価した。その結果、最も安定なコンフォメーションでは、カルボラン配位子の CH 基が DME 配位子に対して *anti*-に位置しており、カルボラン配位子を回転させると、系の安定性が低下することがわかった。これらの結果より、カルボラン配位子の設計を合理化し、それをベースにしたガドリニウムメタラカルボランの合成が可能になった。

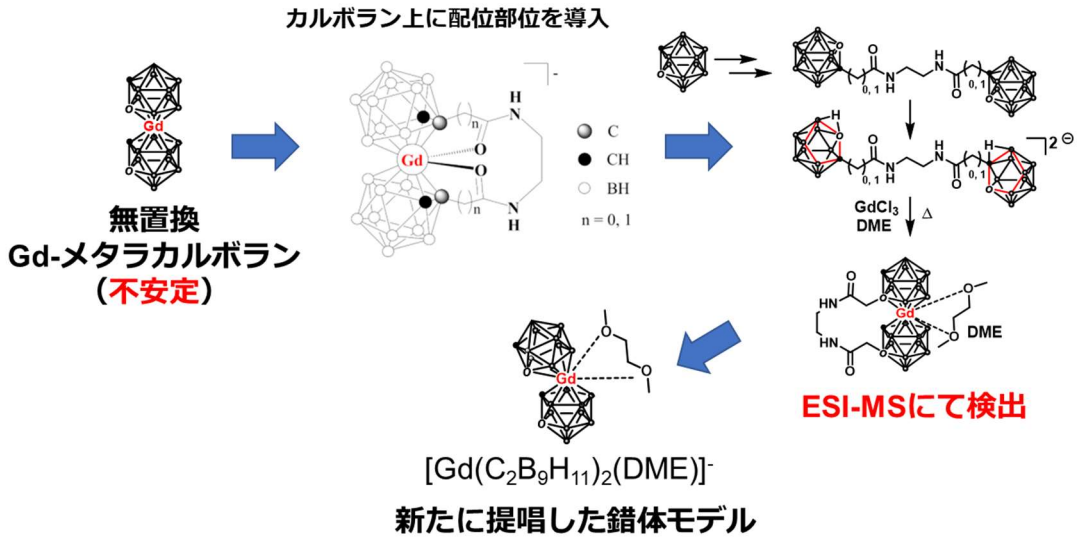


Figure 4-5. Gd-メタラカルボランの合成検討

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Experimental Section

Synthesis

General Method

1-HOOC-1,2-$C_2B_{10}H_{11}$ (**1**) and 1-HOOCCH_2-1,2-$C_2B_{10}H_{11}$ (**2**) were prepared according to the literature procedures.[19,20] Diethyl ether, tetrahydrofuran, 1,2-dimethoxyethane, dichloromethane, benzene and toluene were dried using standard procedures. All other chemical reagents were purchased from Sigma Aldrich, Acros Organics and ABCR and used without purification. The reaction progress was monitored by thin layer chromatography (Merck F254 silica gel on aluminum plates) and visualized using 0.5% $PdCl_2$ in 1% HCl in aq. MeOH (1:10). Acros Organics silica gel (0.060–0.200 mm) was used for column chromatography. The NMR spectra at 400 MHz (1H), 128 MHz (11B) and 100 MHz (^{13}C) were recorded with an Inova 400 spectrometer (Varian). The residual signal of the NMR solvent relative to Me_4Si was taken as the internal reference for ^{1}H- and ^{13}C-NMR spectra. ^{11}B-NMR spectra were referenced using $BF_3{\cdot}Et_2O$ as external standard. High resolution mass spectra (HRMS) were measured on a micrOTOF II instrument (Bruker) using electrospray ionization (ESI). The measurements were done in a negative ion mode, mass range from m/z 50 to m/z 3000. Low resolution mass spectra (MS) were measured on a 2010 EV Liquid Chromatograph-Mass Spectrometer (LC-MS) instrument (Shimadzu) using electrospray ionization (ESI). The measurements were done in a negative mode, mass range from m/z 200 to m/z 1000.

Synthesis of 1-Cl(O)C-1,2-$C_2B_{10}H_{11}$ (3)

Phosphorus pentachloride (13.50 g, 64.9 mmol) was slowly added to 1-HOOC-1,2-$C_2B_{10}H_{11}$ (**1**) (11.70 g, 62.2 mmol) in 50 mL of toluene and the reaction mixture was stirred for 30 min. Then, the toluene and phosphoryl chloride were removed by distillation at 110 °C. The crude product was purified by vacuum distillation (5 mbar) to collect 1-Cl(O)C- 1,2-$C_2B_{10}H_{11}$ (**3**) (8.70 g, yield 68%, bp = 40 °C). 1-Cl(O)C-1,2-$C_2B_{10}H_{11}$ (**3**): ^{1}H-NMR (400 MHz, $CDCl_3$): δ 4.13 (1H, br s, CH_{Carb}), 3.4–1.4 (10H, br m, BH_{Carb}) ppm. ^{11}B-NMR (128 MHz, $CDCl_3$): δ −1.4 (1B, d, J = 108 Hz), −2.1 (1B, d, J = 133 Hz), −8.5 (2B, d, J = 153 Hz), −11.5 (4B, d, J = 187 Hz), −13.2 (2B, d, J = 191 Hz) ppm. IR (film): υmax 3074 (CH_{Carb}), 2588 (BH_{Carb}), 1763 (CO) cm^{-1}. The spectral data match those described in the literature.[19]

Synthesis of 1,1'-($CH_2NH(O)C$-1,2-$C_2B_{10}H_{11}$)$_2$ (5)

Under an argon atmosphere a mixture of ethylenediamine (0.10 mL, 1.5 mmol) and triethylamine (0.42 mL, 3.0 mmol) in 10 mL of dichloromethane was added dropwise to 1-$Cl(O)C$-1,2-$C_2B_{10}H_{11}$ (**3**) (0.62 g, 3.0 mmol) in 10 mL of dichloromethane and reaction mixture was stirred for 60 min. Thereafter, volatiles were removed under reduced pressure. The column chromatography on silica using ethyl acetate as eluent gave pure pale-yellow solid of **5** (0.29 g, yield 48%). ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.84 (2H, br s, NH), 4.88 (2H, br s, CH_{Carb}), 3.45 (4H, t (1:1:1), J^2 = 2.7 Hz, CH_2), 3.2–1.4 (br m, BH_{Carb}) ppm. ^{11}B-NMR (128 MHz, acetone-d_6): δ −3.9 (4B, d, J = 146 Hz), −9.2 (4B, d, J = 150 Hz), −12.7 (8B, m), −13.6 (4B, d, J = 154 Hz) ppm. ^{13}C-NMR (100 MHz, acetone-d_6): δ 160.3 (CO), 73.7 (C_{Carb}CO), 59.2 (C_{Carb}H), 41.0 (CH_2) ppm. IR (film): υmax 3360 (NH), 3074 (CH_{Carb}), 2597 (BH_{Carb}), 1684 (CO) cm^{-1}. HRMS (ESI): found m/z 423.4043 [M +Na]$^+$, $C_8H_{28}B_{18}N_2NaO_2$, calculated for C $C_8H_{28}B_{18}N_2NaO_2$ [M +Na]$^+$ = 423.4054.

Synthesis of 1-$Cl(O)CCH_2$-1,2-$C_2B_{10}H_{11}$ (4) and 1,1'-($CH_2NH(O)CCH_2$-1,2-$C_2B_{10}H_{11}$)$_2$ (6)

Under an argon atmosphere phosphorus pentachloride (0.21 g, 1.0 mmol) was slowly added to 1-$HOOCCH_2$-1,2-$C_2B_{10}H_{11}$ (0.20 g, 1.0 mmol) in 5 mL of toluene and reaction mixture was stirred for 10 min. Then, the reaction mixture was heated under reflux for 1 h. Thereafter, the dichloromethane and phosphoryl chloride were removed by distillation at 110 °C. The obtained **4** was used in the next step without further purification.

Under argon atmosphere mixture of ethylenediamine (0.03 mL, 0.5 mmol) and triethylamine (0.14 mL, 1.0 mmol) in 10 mL of dichloromethane was added dropwise to **4** in 10 mL of dichloromethane and reaction mixture was stirred for 60 min. Thereafter, volatiles were removed under reduced pressure. The column chromatography on silica using a mixture of ethyl acetate and hexane (1:1, v/v) as eluent gave pure white solid of **6** (0.18 g, yield 43%). ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.75 (2H, br s, NH), 4.98 (2H, br s, CH_{Carb}), 3.32 (4H, t (1:1:1), J^2 = 2.7 Hz, NCH_2), 3.23 (4H, s, CH2), 3.1–1.3 (br, m, BH_{Carb}) ppm. 11B-NMR (128 MHz, acetone-d_6): δ −2.8 (2B, d, J = 147 Hz), −5.6 (2B, d, J = 145 Hz), −9.7 (4B, d, J = 130 Hz), −10.6 (4B, d, J = 137 Hz), −11.7 (4B, d, J = 152 Hz), −13.0 (4B, d, J = 158 Hz) ppm. ^{13}C-NMR (100 MHz, acetone-d_6): δ 167.4 (CO), 71.6 (C_{Carb}CH2), 61.0 (C_{Carb}H), 43.3 (NCH_2), 39.7 (CH_2) ppm. HRMS (ESI): found m/z 429.4545 [M+H]$^+$, $C_{10}H_{33}B_{20}N_2O_2$, calculated for $C_{10}H_{33}B_{20}N_2O_2$ [M+H]$^+$ = 429.4549.

Synthesis of $(Me_3NH)_2$[7,7'(8')-$(CH_2NH(O)C$-7,8-$C_2B_9H_{11})_2$] (1)

Ammoniumfluoride (0.86 g, 23.3mmol) was added to 1,1'-$(CH_2NH(O)C$-1,2- $C_2B_{10}H_{11})_2$ (**4**) (0.47 g, 1.2 mmol) in 30 mL of ethanol and the reaction mixture was heated under reflux until the disappearance of starting material on TLC. Thereafter, volatiles were removed under reduced pressure and to the residue water (10 mL) was added. The aqueous solution was filtered and added to solution of $(Me_3NH)Cl$ (0.33 g, 3.5 mmol) in 5 mL of water to form precipitate. White solid was filtered and dried over P_2O_5 to give $(Me_3NH)_2$[7,7'(8')-$(CH_2NH(O)C$-7,8-$C_2B_9H11)_2$] (**1**) (0.51 g, yield 87%). ^{1}H-NMR (400 MHz, acetone-d_6): δ 6.66 (2H, br s, NH), 3.18 (4H, s, CH^2), 3.07 (18H, s, Me_3NH^+), 2.39 (2H, br s, CH_{Carb}), 2.8–0.5 (br m, BH_{Carb}), −2.76 (2H, br q (1:1:1:1), J = 44 Hz, BHB) ppm. ^{11}B- NMR (128 MHz, acetone-d_6): δ −9.5 (2B, d, J = 102 Hz), −10.2 (2B, d, J = 119 Hz), −14.4 (2B, d, J = 154 Hz), −17.3 (4B, d, J = 125 Hz), −20.9 (2B, d, J = 145 Hz), −23.0 (2B, d, J = 150 Hz), −32.6 (2B, dd, J^l = 132 Hz, J^2 = 44 Hz), −35.8 (2B, d, J = 139 Hz) ppm. ^{13}C-NMR (100 MHz, acetone-d_6): δ 173.6 (CO), 59.7 ($C_{Carb}CO$), 45.7 (Me_3NH^+), 41.5 ($C_{Carb}H$), 40.3 (CH_2) ppm. IR (film): υmax 3410 (NH), 3037 (CH_{Carb}), 2528 (BH_{Carb}), 1617 (CO) cm^{-1}. HRMS (ESI): found m/z 380.4036 $[M+H]^-$, $C_8H_{29}B_{18}N_2O_2$, calculated for $C_8H_{29}B_{18}N_2O_2$ $[M+H]^-$ = 380.4024.

Synthesis of $(Me_3NH)_2$[7,7'(8')-$(CH_2NH(O)CCH_2$-7,8-$C_2B_9H_{11})_2$] (2a) and Cs_2[7,7'(8')-$(CH_2NH(O)CCH_2$-7,8-$C_2B_9H_{11})_2$] (2b)

(a) Ammonium fluoride (0.11 g, 3.0 mmol) was added to 1,1'-$(CH_2NH(O)C$-1,2-$C_2B_{10}H_{11})_2$ (**7**) (0.09 g, 0.2 mmol) in 30 mL of ethanol and reaction mixture was heated under reflux until the disappearance of starting material on TLC. Thereafter, volatiles were removed under reduced pressure and to the residue water (5 mL) was added. The aquatic solution was filtered and added to trimethylamine hydrochloride (0.10 g, 1.0 mmol) in 5 mL of water to form precipitate. White solid was filtered and dried over P_2O_5 to give $(Me_3NH)_2$[7,7'(8')-$(CH_2NH(O)C$-7,8-$C_2B_9H_{11})_2$] (**2a**) (0.06 g, yield 57%). ^{1}H-NMR (400 MHz, acetone-d_6): δ 7.15 (br s, NH), 3.36 (4H, m, NCH_2), 3.20 (18H, s, Me_3NH^+), 2.55 (2H, d, J = 15.0 Hz, CHH), 2.24 (2H, d, J = 15.0 Hz, CHH), 1.81 (2H, br s, CH_{Carb}), −2.71 (2H, br q (1:1:1:1), J = 42 Hz, BHB) ppm. ^{11}B-NMR (128 MHz, acetone-d_6): δ −10.1 (2B, d, J = 133 Hz), −11.2 (2B, d, J = 138 Hz), −13.7 (2B, d, J = 170 Hz), −15.0 (2B, d, J = 135 Hz), −19.4 (4B, d, J = 130 Hz), −21.0 (2B, d, J = 156 Hz), −32.7 (2B, dd, J^l = 128 Hz, J^2 = 42 Hz), −36.6 (2B, d, J = 135 Hz) ppm.

(b) Cesiumfluoride (3.12 g, 20.5 mmol) was added to 1,1'-$(CH_2NH(O)CCH_2$-1,2-$C_2B_{10}H_{11})_2$ (**7**) (1.47 g, 3.4 mmol) in 30 mL of ethanol and reaction mixture was heated under reflux for 48 h. Thereafter, the reaction mixture was filtered, volatiles were removed under reduced pressure. The column chromatography on silica using ethanol as eluent gave pure white solid of Cs_2[7,7'(8')-$(CH_2NH(O)CCH_2$-7,8-$C_2B_9H_{11})_2$] (**2b**) (1.24 g, yield 55%). ^{1}H-NMR (400 MHz, acetone-d_6): δ

7.40 (2H, br s, NH), 3.40 (4H, s, NCH_2), 2.57 (2H, d, J = 14.9 Hz, CHH), 2.25 (2H, d, J = 14.9 Hz, CHH), 1.80 (2H, br s, CH_{Carb}), −2.79 (2H, br q (1:1:1:1), J = 54 Hz, BHB) ppm. ^{11}B-NMR (128 MHz, acetone-d_6): δ −9.9 (2B, d, J = 137 Hz), −11.6 (2B, d, J = 151 Hz), −13.8 (4B, d, J = 133 Hz), −20.4 (6B, d, J = 130 Hz), −32.5 (2B, d, J = 118 Hz), −36.5 (2B, d, J = 138 Hz) ppm. HRMS (ESI): found m/z 203.7113 $[M]^{2-}$, $C_{10}H_{32}B_{18}N_2O_2$, calculated for $C_{10}H_{32}B_{18}N_2O_2$ $[M]^{2-}$ = 203.7133.

Single Crystal X-ray Diffraction Study

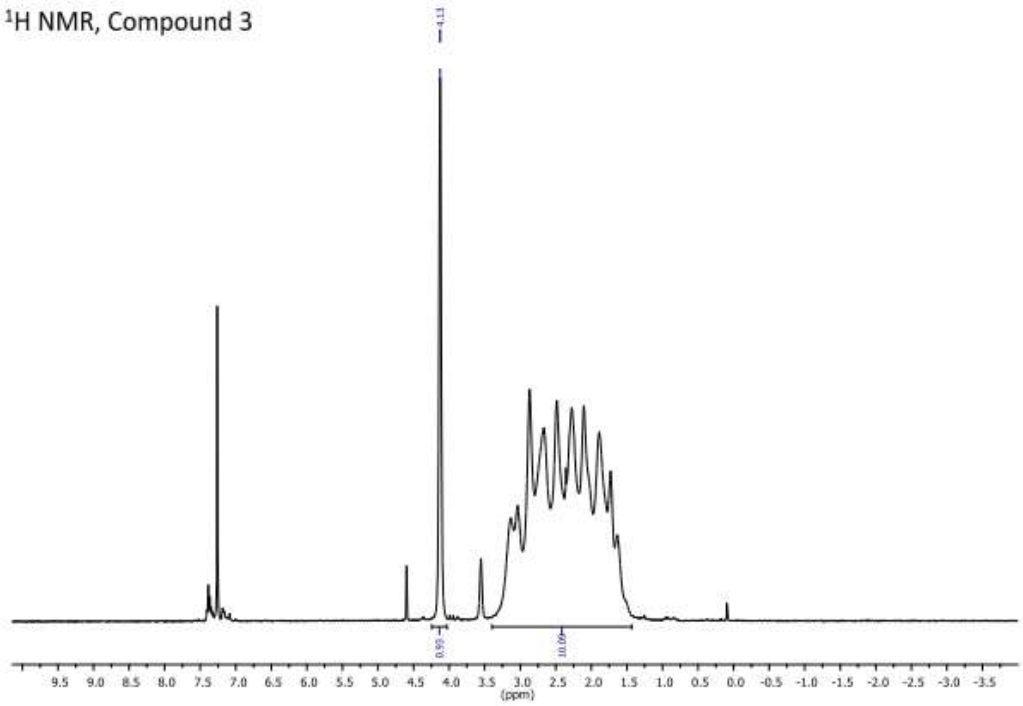
X-ray diffraction experiment for compound 4 was carried out using a SMART APEX2 CCD diffractometer (Bruker) (λ(Mo-Kα) = 0.71073 Å, graphite monochromator, ω-scans) at 120 K. Collected data were processed by the SAINT and SADABS programs incorporated into the APEX2 program package. The structure was solved by the direct methods and re-fined by the full-matrix least-squares procedure against F^2 in anisotropic approximation. The refinement was carried out with the SHELXTL program. Both solvate hexane molecules occupy special position and are disordered over two positions. Occupancy ratios are 0.728(7):0.272(7) and 0.869(9):0.131(9). The CCDC number 2061631 contains crystallographic data for **4**: $C_8H_{28}B_{20}N_2O_2 \cdot C_6H_{14}$ are triclinic, space group P-1: a = 9.6759(11) Å, b = 10.3862(12) Å, c = 16.0979(17) Å, α = 105.859(3)◦, α = 104.585(2)◦, γ = 90.158(3)◦, V = 1501.6(3) Å^3, Z = 1, M = 486.69, d_{cryst} = 1.076 g·cm^{-3}. wR2 = 0.2388 calculated on F^2_{hkl} for all 7214 independent reflections with 2θ < 56.0◦, (GOF = 1.049, R = 0.0763 calculated on F_{hkl} for 4938 reflections with I > 2σ(I))

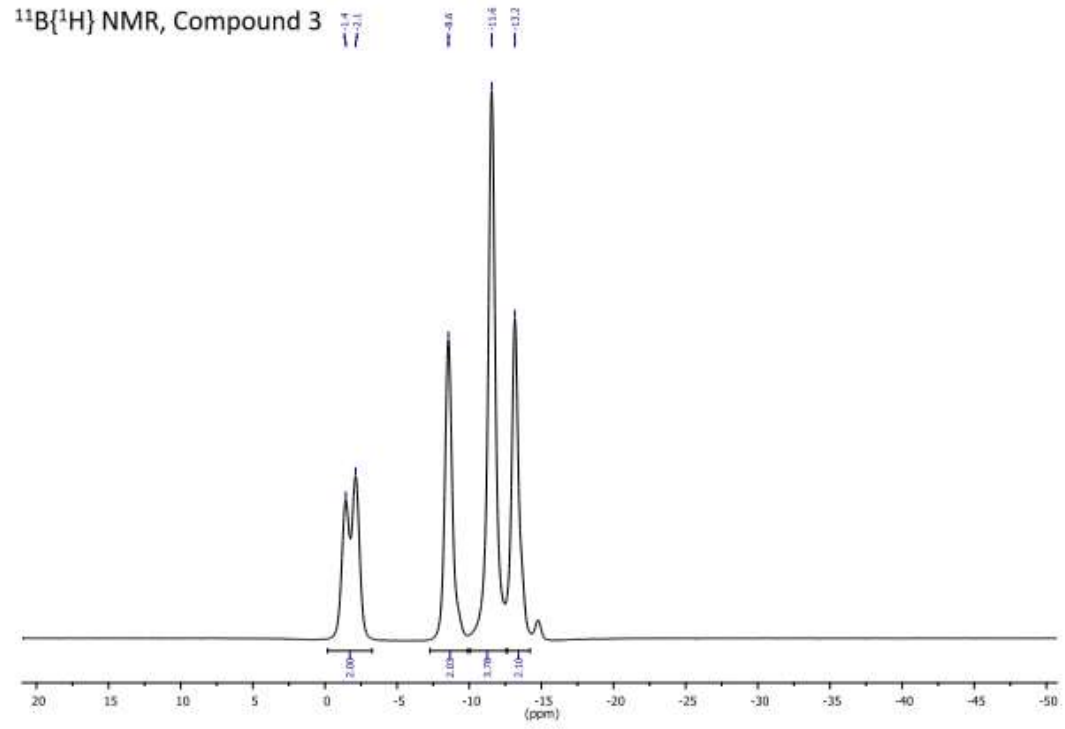
Quantum-Chemical Calculations

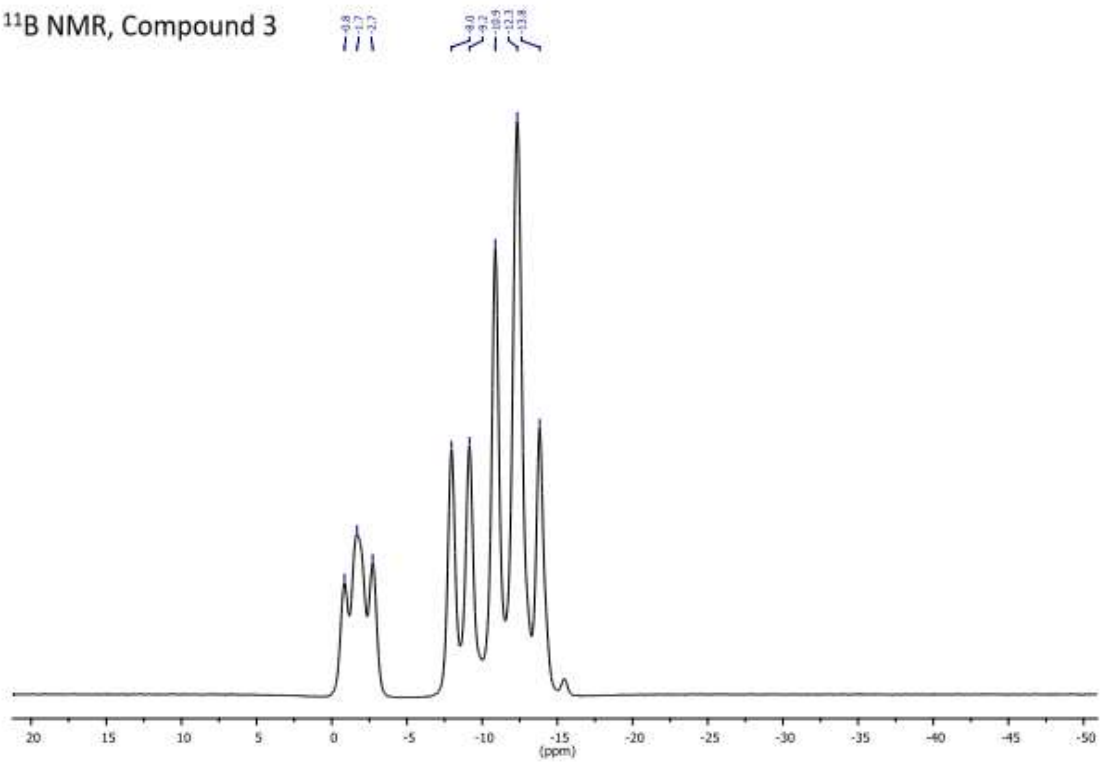
For geometry optimization of the $[Gd(C_2B_9H_{11})_2(DME)]^-$ complex ORCA v4.2.0 was used.[28] ZORA Hamiltonian[29] was used for treating of relativistic effects, PBE0 functional[30] with Grimme's DFT-D3 correction[31] and Becke–Johnson damping (D3BJ)[32] was applied. Use of PBE0 functional was proved to be reliable for estimation of geometry and energy of different classes of compounds.[33–35] The def2-TZVP for light (H, B, C, O) atoms, and scalar all-electron relativistic TZVP basis set (SARC-ZORA- TZVP)[36] for Gd atom were used with the general-purpose auxiliary basis set SARC/J. The RIJCOSX was used to improve the calculations efficiency. The calculations were performed by using an unrestricted formalism (S = 7/2). Such computational approach was shown to lead to a free energy difference of ca. 2–5 kcal/mol in comparison with experiments[37] for lanthanide complexes. Calculated electron density of compound 4 was analyzed with AIM theory using AIMAll program. Energies of intramolecular noncovalent interactions were estimated using correlation between energy of a contact and potential energy density at bond crit- ical point (E = 1/2V(r))[38] that is widely used for energetic analysis of noncovalent interactions.[39–41]

Spectra data

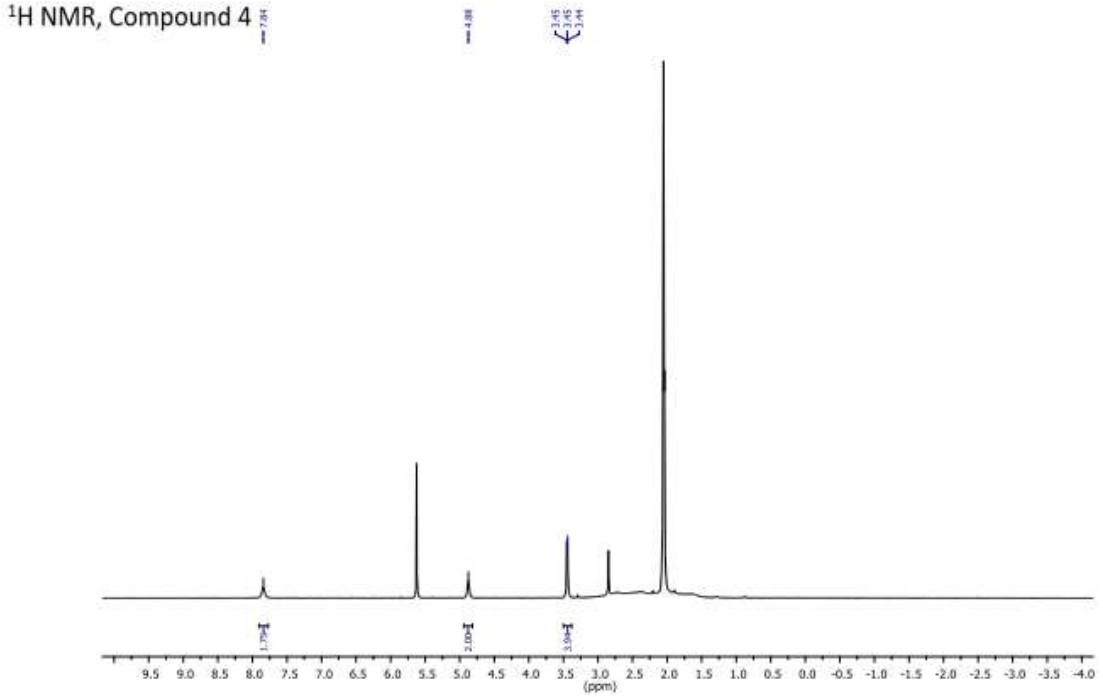
1-Cl(O)C-1,2-$C_2B_{10}H_{11}$ (3)



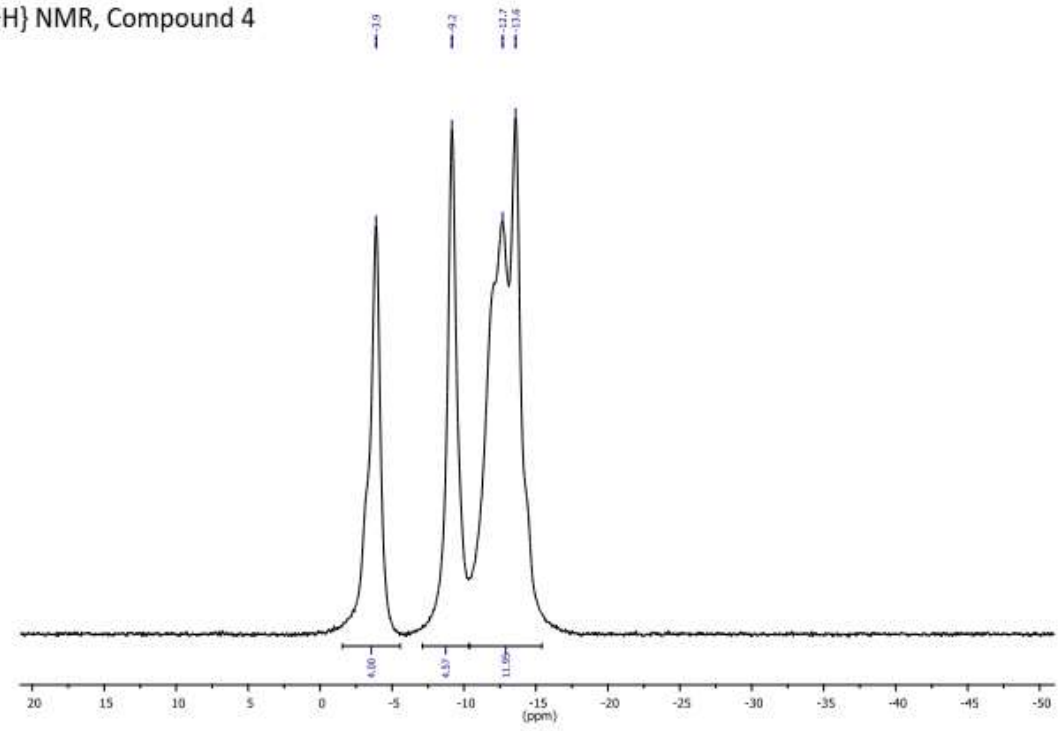




1,1'-($CH_2NH(O)C$-1,2-$C_2B_{10}H_{11}$)$_2$ (5)

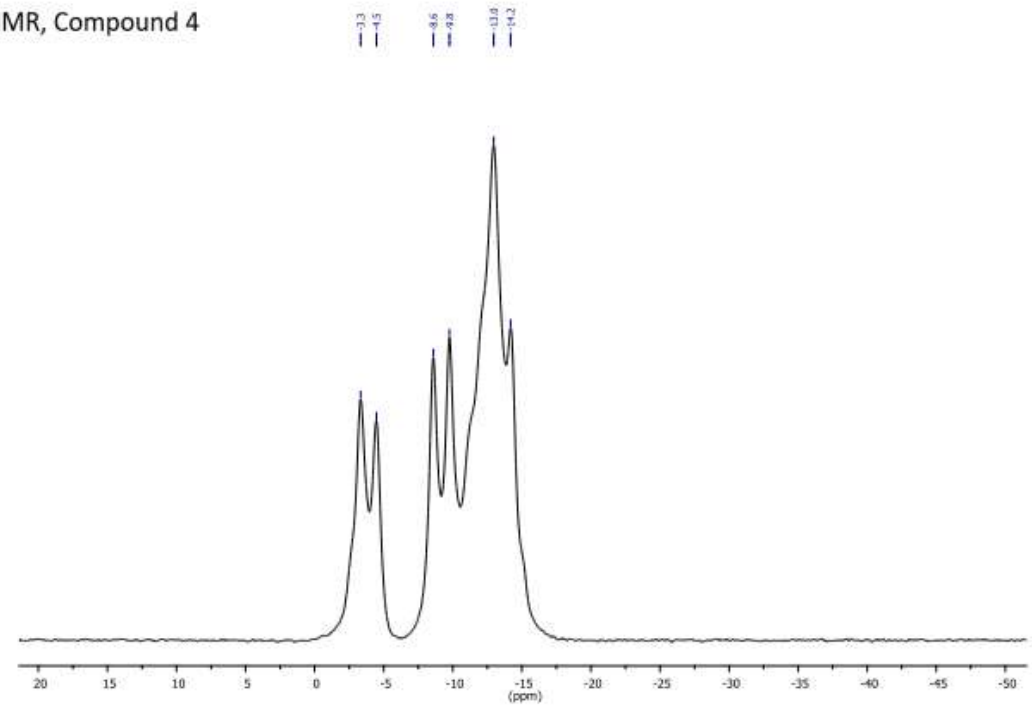


$^{11}B\{^{1}H\}$ NMR, Compound 4

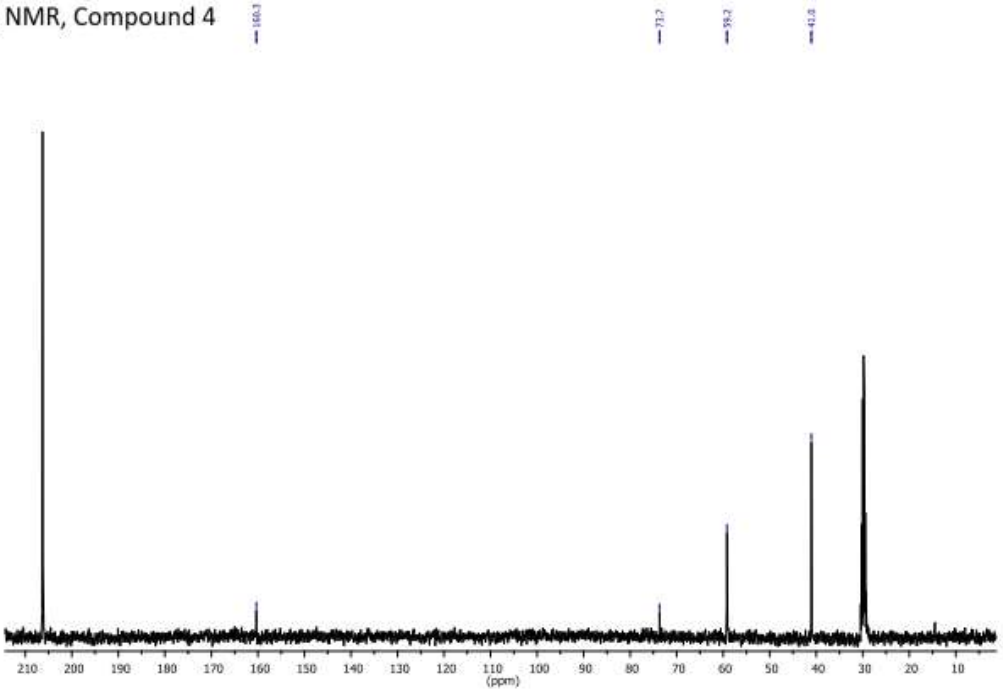


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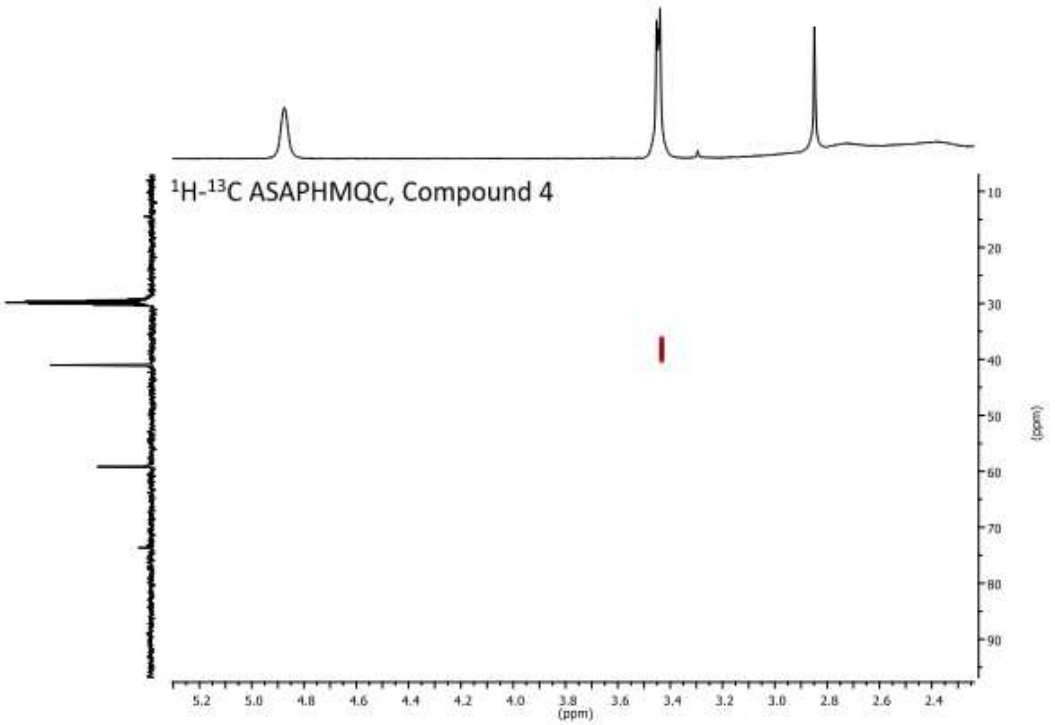
^{11}B NMR, Compound 4



$^{13}C\{^{1}H\}$ NMR, Compound 4

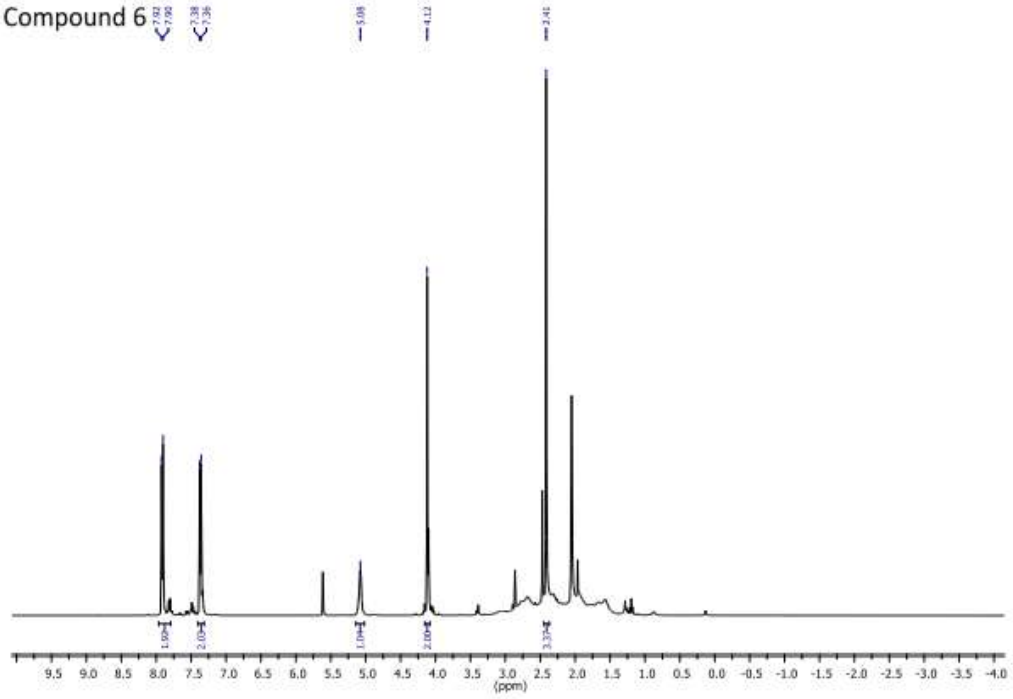


^{1}H-^{13}C ASAPHMQC, Compound 4

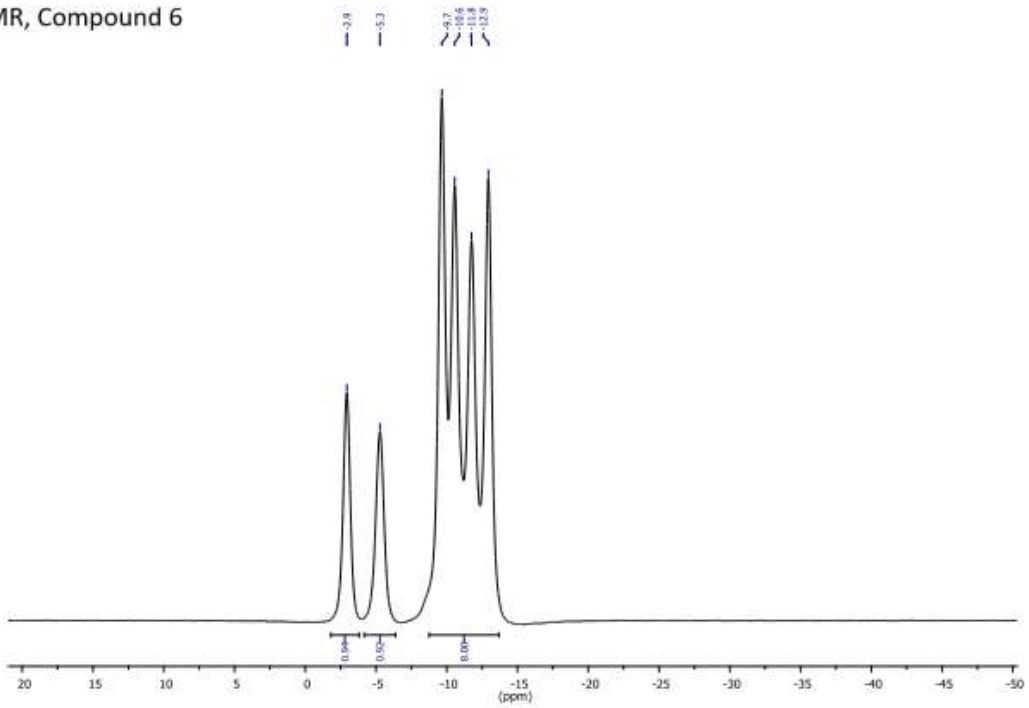


1-Cl(O)CCH$_2$-1,2-C$_2$B$_{10}$H$_{11}$ (4) and 1,1'-(CH$_2$NH(O)CCH$_2$-1,2-C$_2$B$_{10}$H$_{11}$)$_2$ (6)

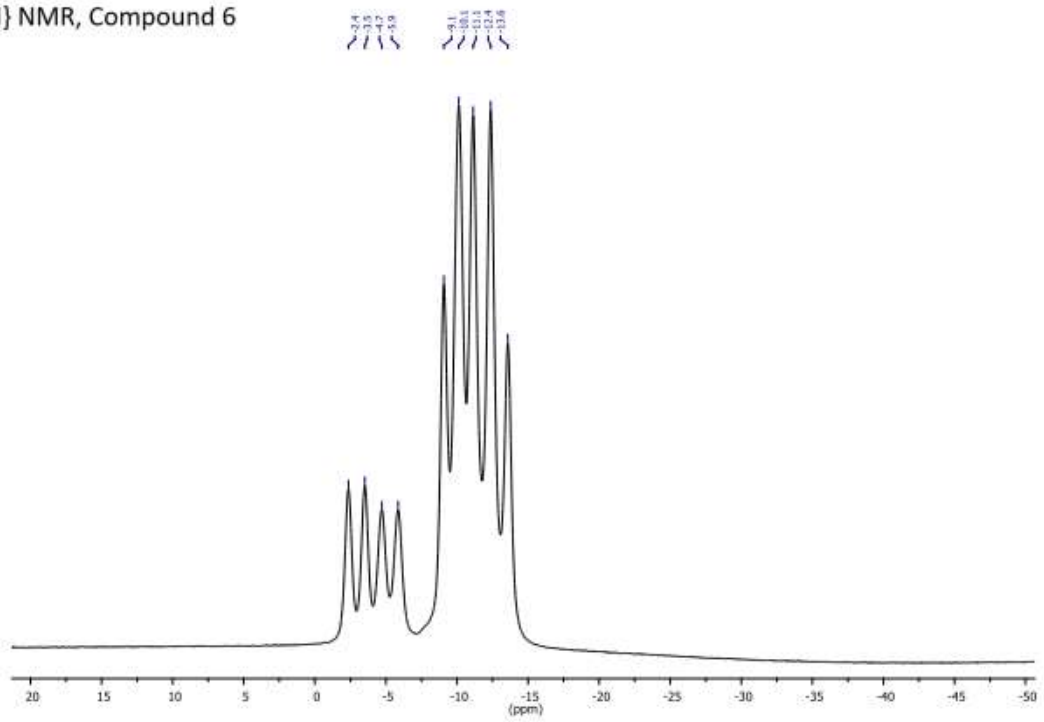
^{1}H NMR, Compound 6



^{11}B NMR, Compound 6

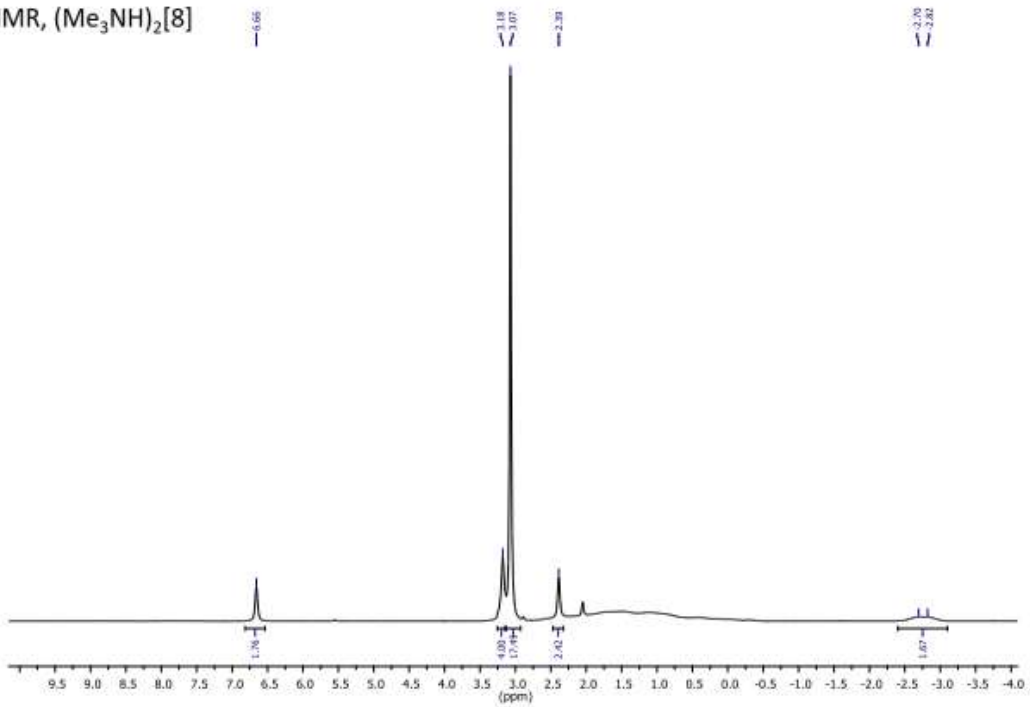


$^{11}B\{^{1}H\}$ NMR, Compound 6

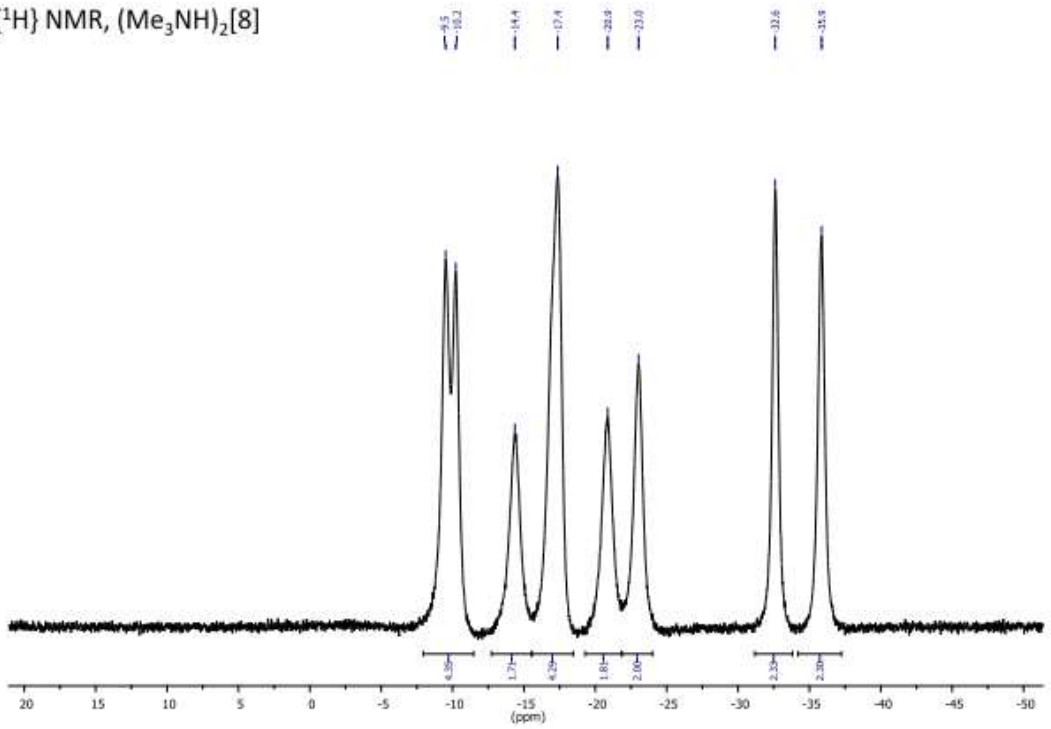


$(Me_3NH)_2[7,7'(8')-(CH_2NH(O)C)-7,8-C_2B_9H_{11})_2]$ (1)

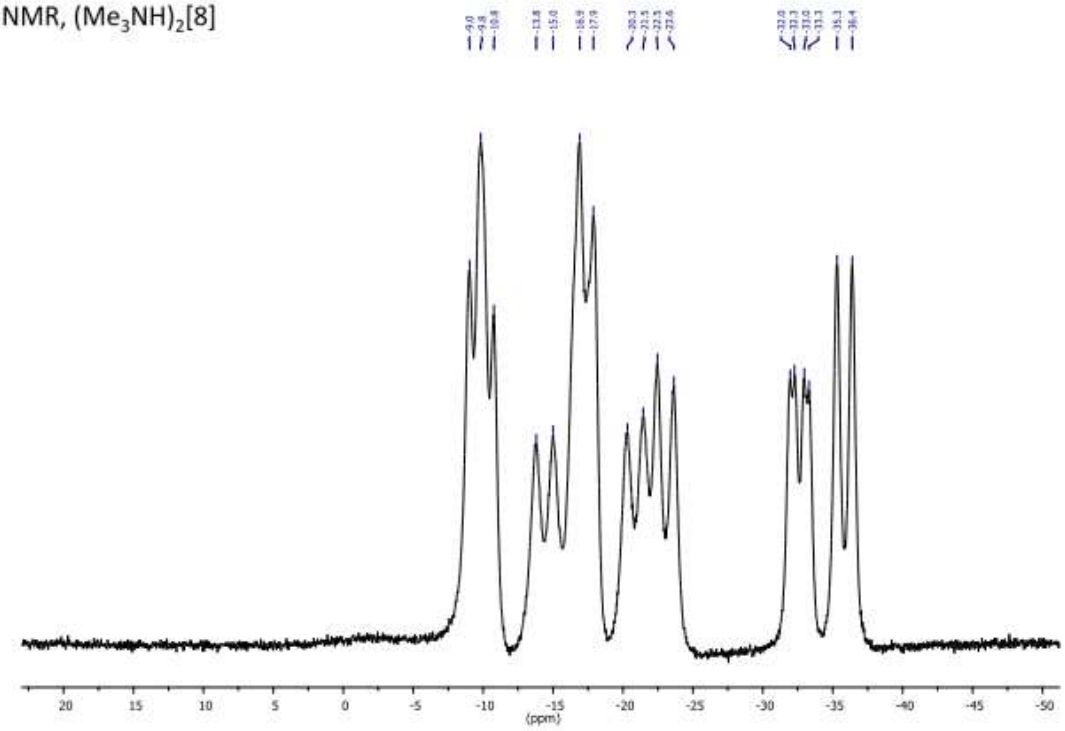
^{1}H NMR, $(Me_3NH)_2[8]$



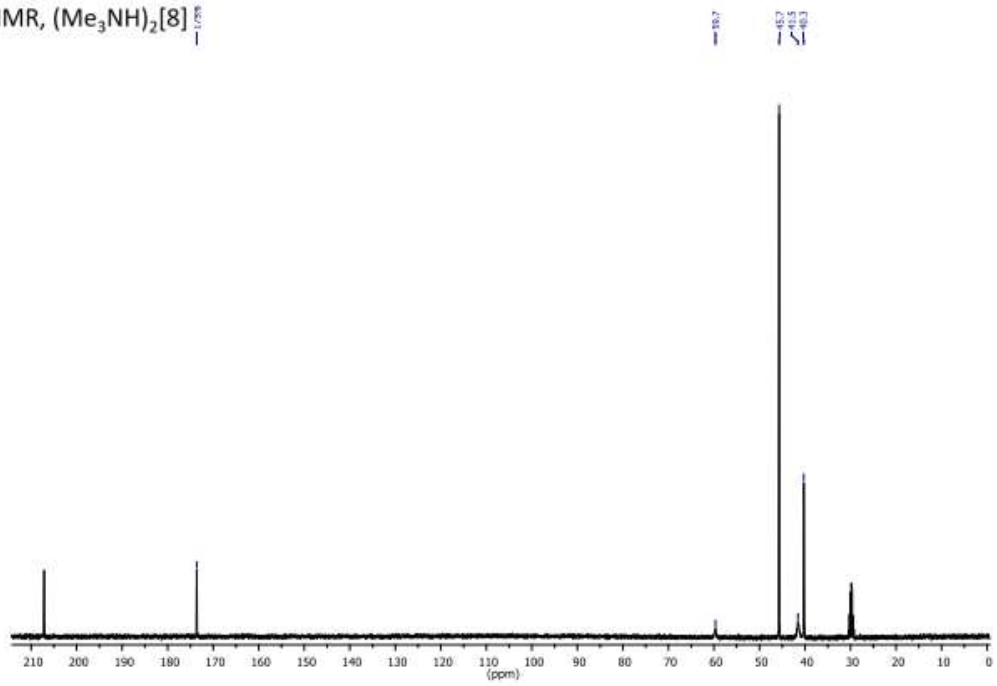
$^{11}B\{^{1}H\}$ NMR, $(Me_3NH)_2[8]$



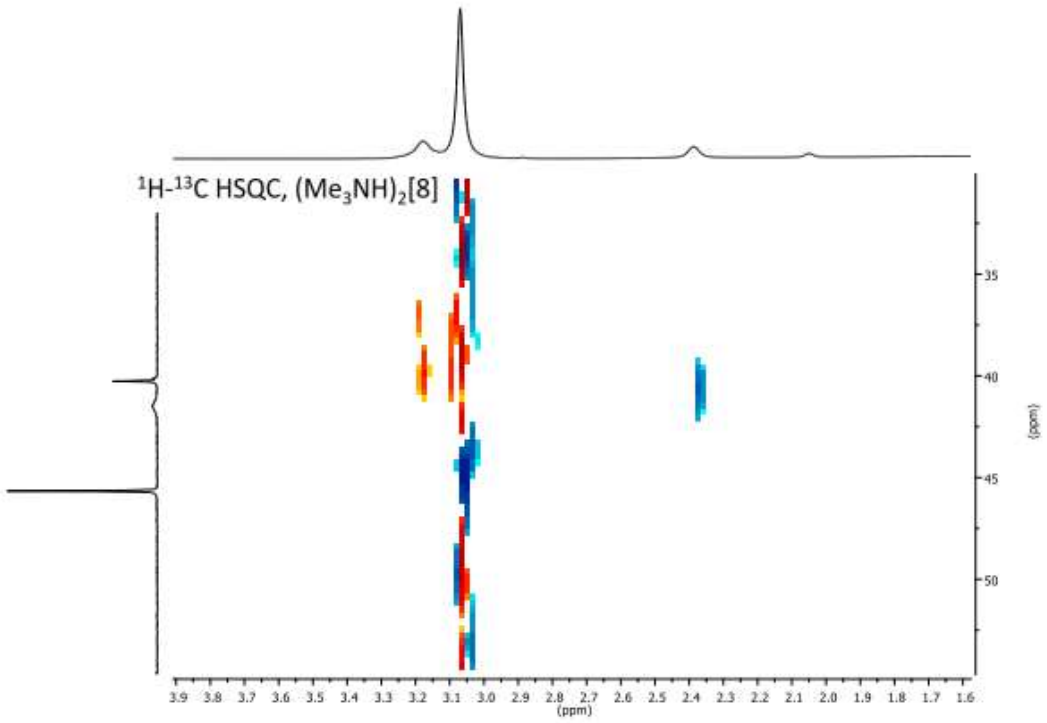
^{11}B NMR, $(Me_3NH)_2[8]$



$^{13}C\{^{1}H\}$ NMR, $(Me_3NH)_2[8]$

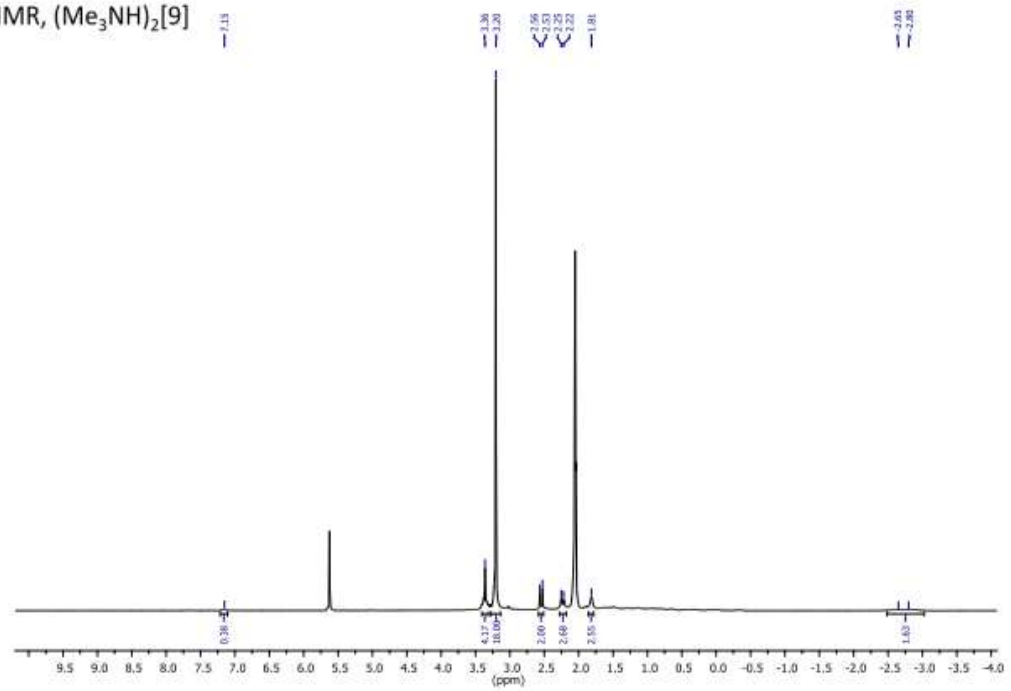


^{1}H-^{13}C HSQC, $(Me_3NH)_2[8]$

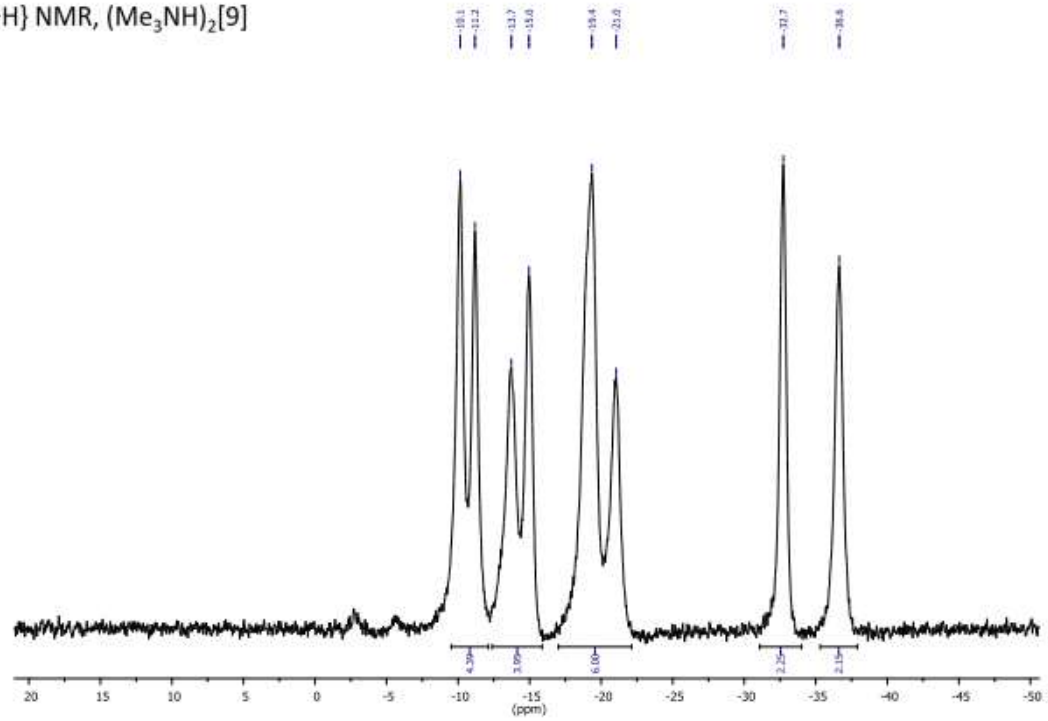


$(Me_3NH)_2$[7,7'(8')-$(CH_2NH(O)CCH_2$-7,8-$C_2B_9H_{11})_2$] (2a)

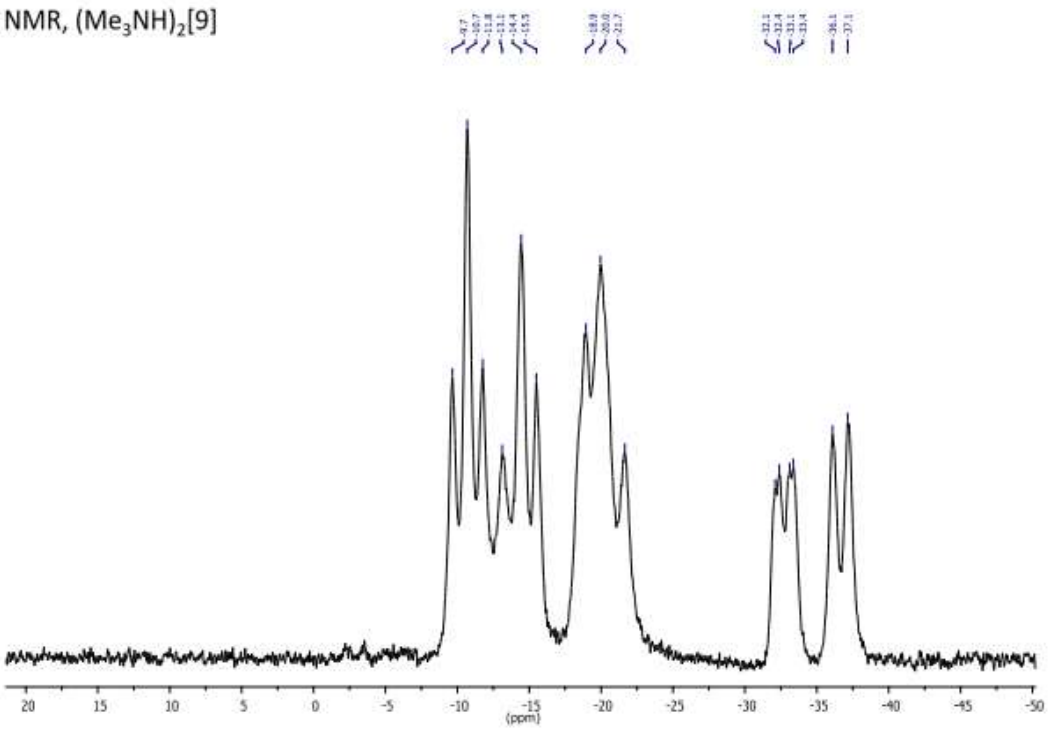
^{1}H NMR, $(Me_3NH)_2$[9]



^{11}B{^{1}H} NMR, $(Me_3NH)_2$[9]

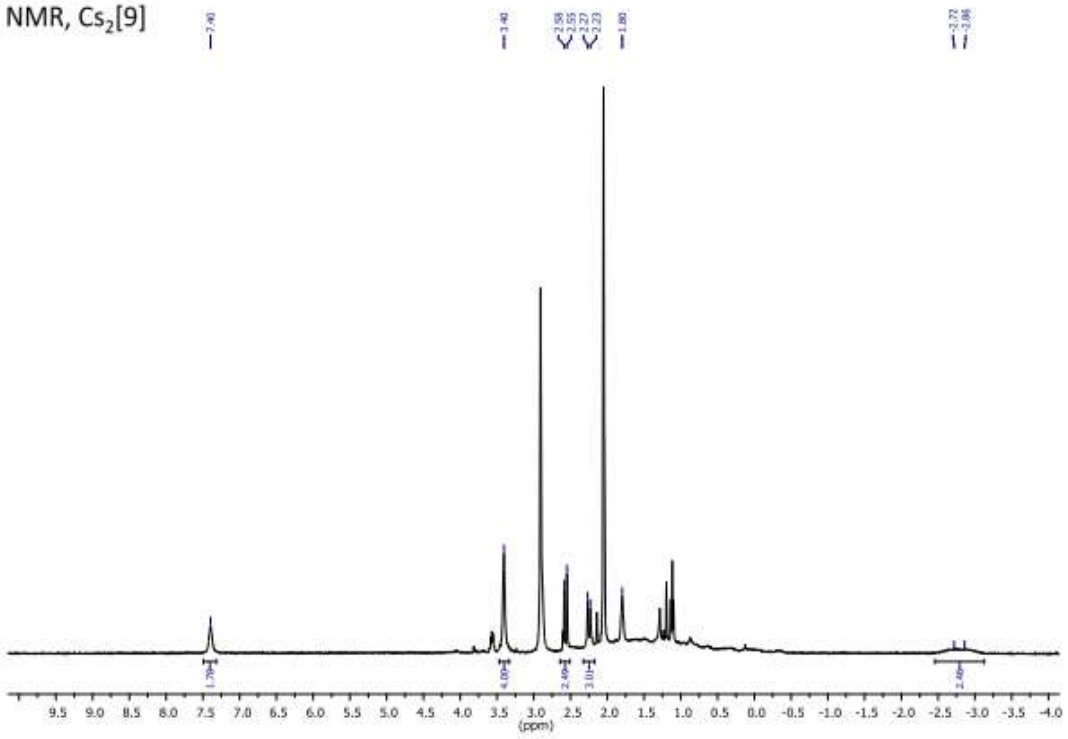


^{11}B NMR, $(Me_3NH)_2$[9]

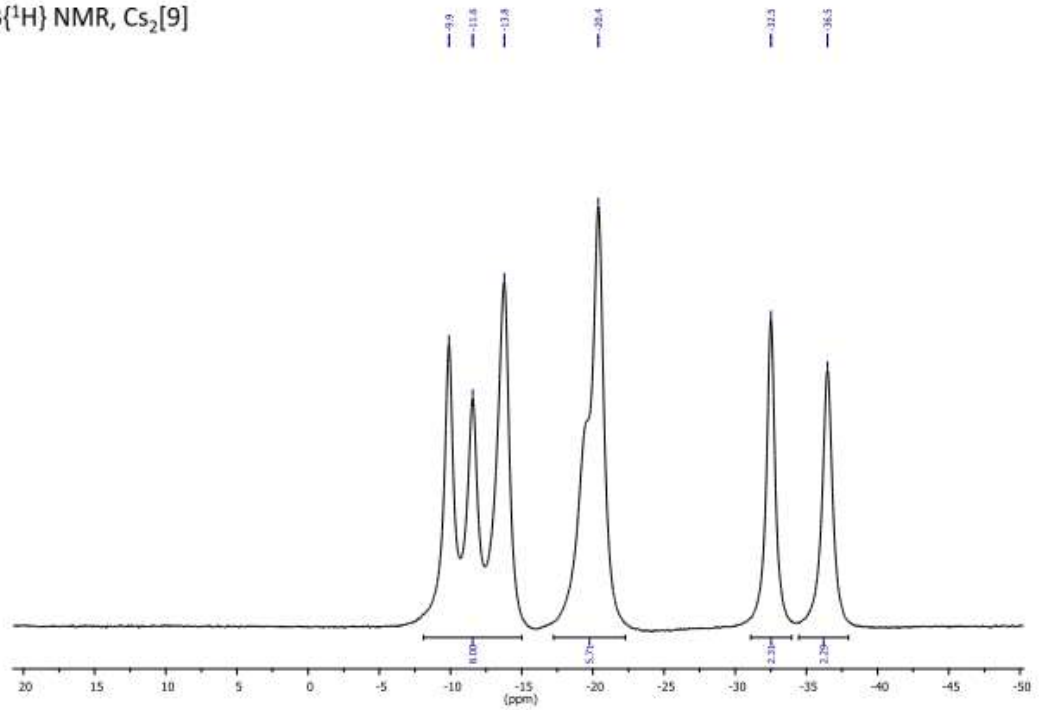


Cs_2[7,7'(8')-($CH_2NH(O)CCH_2$-7,8-$C_2B_9H_{11}$)$_2$] (2b)

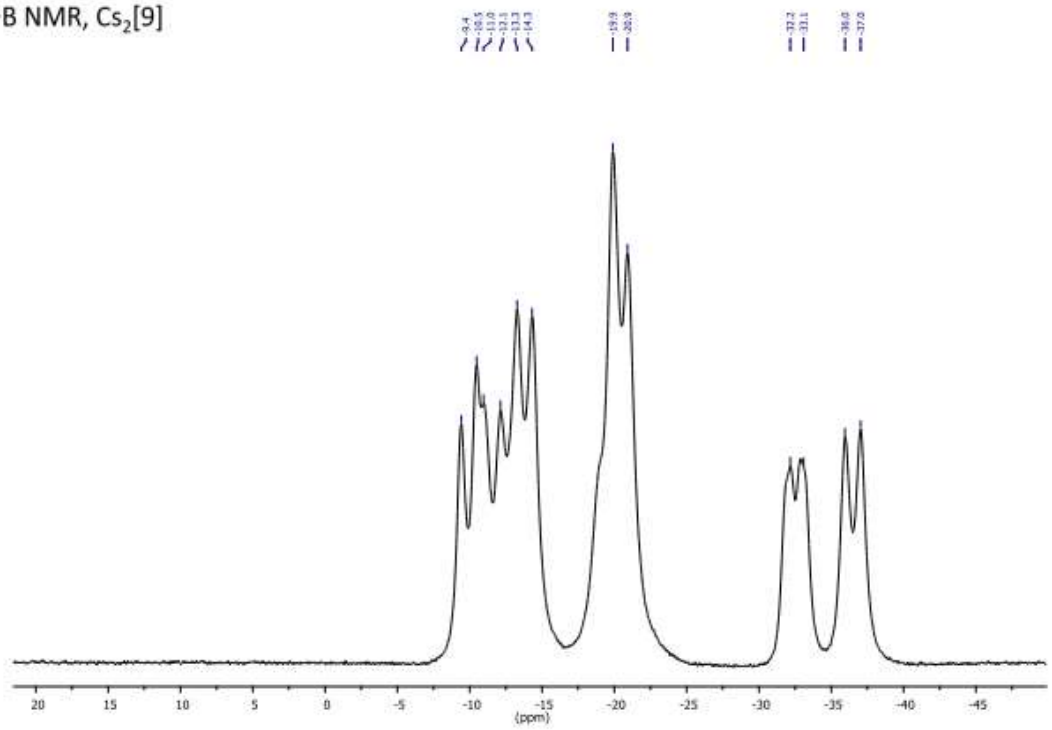
^{1}H NMR, Cs_2[9]



$^{11}B\{^{1}H\}$ NMR, $Cs_2[9]$



^{11}B NMR, $Cs_2[9]$



第五章

公共データベースの網羅的 SAR 解析による Activity cliff の予測とその実験的検証

5-1　はじめに

第一章 **1-4** で述べた通り、コンピュータを使った構造活性相関（Structure Activity Relationship; SAR）解析は、より強力な活性を有する化合物の予測に重要な役割を果たしている。一般的に、SAR の連続性がある場合、化合物の部分構造の変換によって活性が徐々に変化する[1]。このような場合には、定量的構造活性相関（Quantitative SAR; QSAR）法を用いて類縁体の活性を予測することができる[2]。一方、活性化合物の小さな化学修飾によって活性が大きく変化する SAR の不連続性が確認される場合があり、これを”Activity cliff”と呼ぶ[3,4]。このような場合、QSAR の手法は適用できず、化合物の活性を予測することは困難である[5,6]。しかし、既存の SAR データを網羅的に解析することで、新規の活性化合物を予測できる可能性がある。

SAR Matrix (SARM) は、SAR データセットを系統的に分析するとともに、既知の活性化合物からバーチャルの構造類似体を提案するために開発された。SARM は、類縁体構造と関連する SAR 情報を、データセット内の構造的関係に基づいてマトリックスに整理し、Activity cliff を探索できる[7,8]。その生成アルゴリズムを Figure 5-1 に示す。大まかに三つの段階から構成される。

１．入力構造のフラグメント化による Key と Value の生成

データベース上のそれぞれの化合物に対して、環状構造上にある単結合を１つ切断する。すると、二つのフラグメントになり、原子数が大きい方を Key、小さい方を Value 1 と定義する。

２．Key のフラグメント化による Key MMS の生成

１で生成した Key に対して、先と同様の手順で単結合を２つ切断する。ただし、環上の結合だけなく、非環状構造の結合も１つのみ切断可能とする。すると、３つのフラグメントが生成され、改めて原子数により Key と Value 2 に分類する。そこで、Value 2 内の２つのフラグメントが一致する場合の Key を集めると、系統的に構造が類似した母骨格を抽出することができる。これを Key MMS（Matched Molecular Series）と呼ぶ。なお、MMS は構造が類似する化合物群を意味する。

３．Key MMS と Values のグリッドマップ化

２で得た Key MMS の構造が含まれるフラグメントを、１で生成した Key の中から抽出し、縦軸に並べる。また、それらの Key がもつ Value 1 をすべて横軸に並べてグリッドマップを作成する。最後に、構造がデータベース内に含まれているマスに活性データを色分けして埋め込む。

このように生成された SARM 内の白抜けマスは、バーチャルな構造類似体であり、データベースに含まれていない（活性が確認されていない）化合物である。さらに SARM をベースにした Free-Wilson モデル[a,9] を用いて、その活性値を予測することができる。実際、このような予測により、in-house なデータベースから新規の活性化合物が見出されている [10] 。

本章では、SAR の大規模な公共データベースである ChEMBL[11] から SARM を生成し、SAR を網羅的に解析することで、新たな Activity cliff の予測にも応用できると考え、マトリックスメタロプロテアーゼ（MMP）-1 阻害剤の Activity cliff を予測し、実験的に検証した。

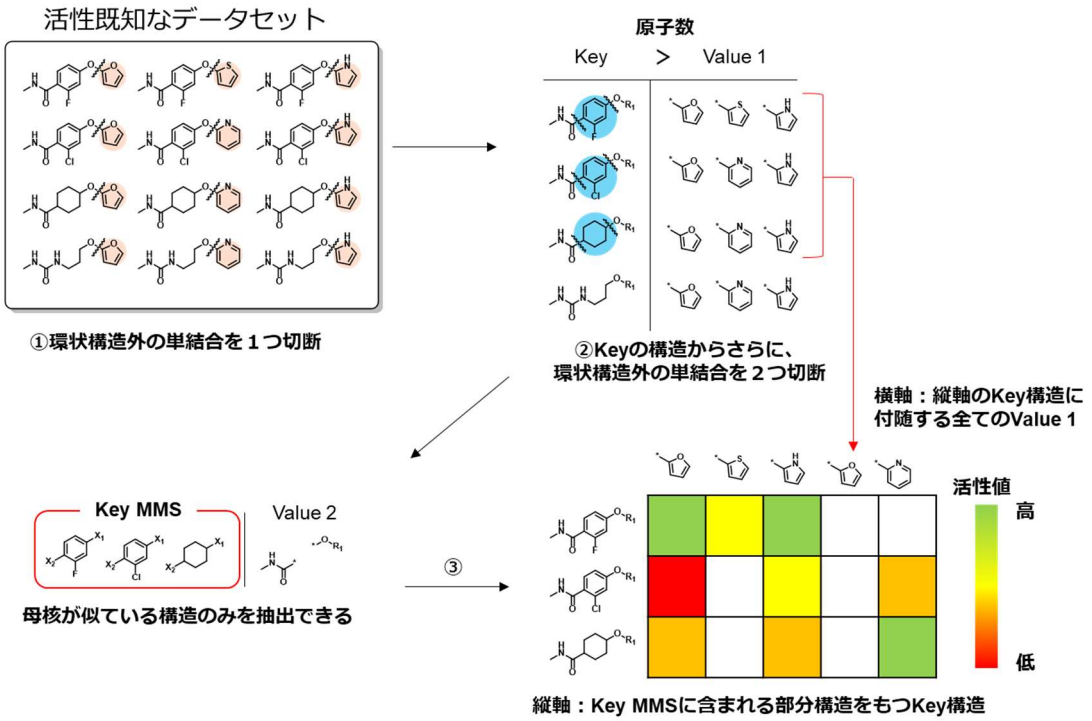


Figure 5-1. SARM の生成アルゴリズム

[a]特定の化学構造の有無により、活性を予測する手法。1964 年に Free と Wilson によって提唱された。

5-2　ChEMBL データベースに基づく SARM の生成と Activity cliff の予測

MMP は、コラゲナーゼファミリーの一つであり、腫瘍進行の主要段階において中心的な役割を果たしている [12,13]。これまで多くの MMP 阻害剤が抗がん剤として研究されてきたが、歯周炎の予防という異なる治療用途で米国食品医薬品局（FDA）に承認されているのは、ドキシサイクリン 1 剤のみである [14]。一方で、別の化合物がカポジ肉腫や脳腫瘍を対象とした第 2 相試験に入っている [15]。こうしたことから現在、様々な MMP-1 阻害剤とその SAR データが ChEMBL データベースに登録されており（ChEMBL332）、K_i 値が入手可能な 644 化合物を利用して SARM を生成した。

Figure 5-2 に示すように、SARM を生成し Activity cliff の予測を行った。この MMP-1 阻害剤セットからは、2,697 個の SARM が得られた。このうち、同じ行の中で pKi の値が 2.0 以上変化する化合物ペア、すなわち同一の母骨格をもちながら側鎖が 1 つ異なるだけで活性が大きく変化する Activity cliff を抽出した。今回の場合、化合物 **1**[16] と **2**[17] の関係が、既知の MMP-1 阻害剤の Activity cliff であり、フェニル基をトリフルオロメチル基に変換させることで活性が大幅に向上していることが分かる。一方で、同じ傾向が異なる行で、バーチャル化合物を一つ含む新たなペアを探索した。なお、前述の通り、バーチャル化合物の活性値は Free-willson モデルによって予測した値を利用した。その結果、化合物 **3**[18] と **4** のペアが、新たな Activity cliff であると予測し、また Figure 2B の式に従って計算し、約 100 倍の活性向上が見込まれた。

バーチャル化合物 **4** の活性値は、異なる母骨格と置換基をもつ化合物 **1** や異なる母骨格をもつ化合物 **2** に基づいて予測されており、従来の QSAR 法では予測できなかったものである。さらに、置換基が部分的に共通する化合物 **1** と **3** の活性値は、異なる SAR 研究により報告されているため、SARM による大規模なデータベースの解析が必要不可欠だったといえる。特に、化合物 **3** は、TACE （tumor necrosis factor-α-converting enzyme）阻害剤として報告されており、MMP-1 に対する活性値は選択性評価の一部として掲載されていた [18]。このように、大規模なデータベースの解析は埋もれたデータ資源を活用できる。

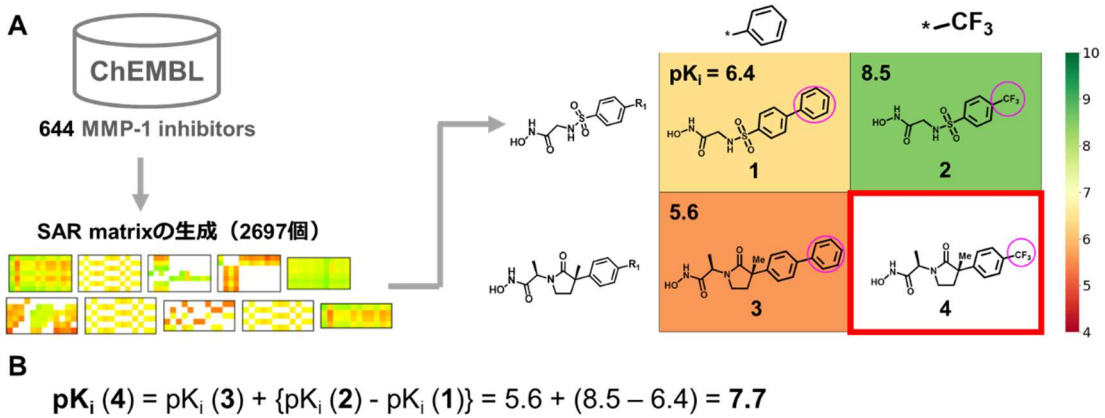
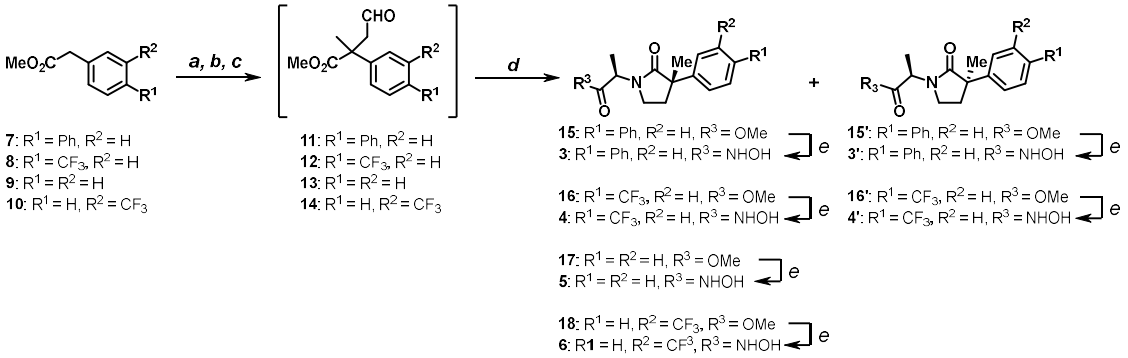


Figure 5-2. MMP-1 阻害剤の SARM から Activity cliff を予測

5-3　予測した Activity cliff 化合物の合成と MMP-1 阻害活性評価

新たな Activity cliff を与えると予測した化合物 **3** と **4** を合成した。さらに、立体化学的な違いが活性に及ぼす影響を調べるために、**3** と **4** のジアステレオマーである化合物 **3'**と **4'**も合成した。また置換基の影響を比較するため、**3** のフェニル基を水素原子に置換した化合物 **5**[18] と、**4** のトリフルオロメチル基をメタ位に置換した化合物 **6** を対照化合物として合成した（Scheme 5-1）。

エステル **7-10** を出発物質とし、THF 中のリチウムジイソプロピルアミド（LDA）で処理して、各エステルの α 位に段階的にメチル基とアリル基を導入し、得られたアリルエステルをオゾン分解によって処理することで、対応するアルデヒド **11-14** を合成した。次にアルデヒド **11-14** の *D*-アラニンメチルエステルによる還元的アミノ化とそれに続くラクトン化を、酢酸中、亜鉛粉末の存在下、ワンポットで行い、対応する γ-ラクタム **15-18** を 29-49%の収率で、1:1 のジアステレオマー比で得た。シリカゲルカラムクロマトグラフィーでジアステレオマーを分離した後、NH_2OH と KOH を用いて γ-ラクタム **15-18** から *N*-ヒドロキシアミド **3-6** および **3'-4'**を 43-98%の収率で得た。

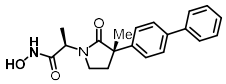
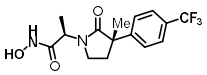
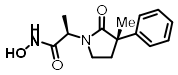
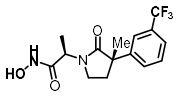
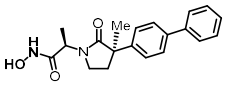
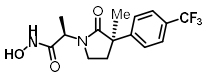


Scheme 5-1. 化合物 **3-6** の合成；反応条件：(a) LDA, MeI, THF, -78 °C; (b) LDA, allyl bromide, THF, -78 °C; (c) O_3, CH_2Cl_2, PPh_3 -78 °C to r.t.; (d) *D*-alanine methyl ester, Zn, acetic acid, reflux (**15**: 29%; **16**: 47%; **17**: 49%; **18**: 29% yield for 4 steps; d.r. = 1:1); (e) NH_2OH, MeOH (**3**: 72%; **3'**: 98%; **4**: 90%; **4'**: 72%; **5**: 49%; **6**: 43% yield).

続いて、合成した化合物 **3-6** の MMP-1 に対する阻害活性を比色定量法によって測定した。このアッセイは、MMP-1 Inhibitor Screening Assay Kit（ab139443）を用いて実施した。MMP-1 の切断部位がチオエステルで置換されているチオペプチド（Ac-PLG-[2-mercapto-4-methyl-pentanoyl]-LG-OC_2H_5）を発色基質として、MMP-1 のプロテアーゼ活性を測定する。チオペプチドが MMP-1 によって加水分解を受けることで生成するスルフヒドリル基（SH 基）が、Ellman 試薬（5-(3-Carboxy-4-nitrophenyl)disulfanyl-2-nitrobenzoic acid: DTNB）のジスルフィド結合を分離し、生成した 2-ニトロ-5-メルカプト安息香酸（TNB）の吸光度（λ_{max}= 412 nm）を定量化する。

評価結果を Table 5-1 に示す。化合物 **4** の IC_{50} 値（0.18 ± 0.03 μM）は、化合物 **3** の IC_{50} 値（11.5 ± 1.3 μM）よりも 60 倍低く、予測した Activity cliff が形成されていることが確認された。一方、ジアステレオマーである **3'**および **4'**は，100 μM でも MMP-1 に対して阻害活性を示さなかった。一方、化合物 **3** のフェニル基を水素に置換した化合物 **5** の阻害活性は中程度（1.54 ± 0.08 μM）であり、立体的もしくは疎水性の向上から化合物 **3** のフェニル基よりもトリフルオロメチル置換基の方が好ましいことが示された。また、メタ位にトリフルオロメチル基を有する化合物 **6** は、化合物 **3** と同程度の活性（11.1 ± 0.5 μM）を示した。

Table 5-1. 合成した化合物の MMP-1 阻害活性

化合物		IC_{50}[a] [μM]
	**3**	11.5 ± 1.3
	**4**	0.18 ± 0.03
	**5**	1.54 ± 0.08
	**6**	11.1 ± 0.5
	**3'**	> 100
	**4'**	> 100

[a] The compound concentration required for 50% inhibition (IC_{50}) was determined from semi-logarithmic dose–response plots, and the results represent the mean ± standard deviation of triplicated samples.

5-4　ファーマコフォアフィッティングによる Activity cliff の解析

最後に化合物 **4** と MMP-1 間の結合相互作用を評価するために、化合物 SC44463 と MMP-1 の複合体の結晶構造（PDB: 1FBL）からファーマコフォアモデルを構築した[19]。SC44463 は、ヒドロキサム酸部位をもつペプチドベースの阻害剤であり、MMP-1 の活性部位の亜鉛イオンとキレート結合する（Figure 5-3A）。この結晶構造から水素結合アクセプター（HA）、疎水性部位（Hy）、亜鉛結合位置特徴（ZL）の 3 つのファーマコフォアを用いた（Figure 5-2B）。MMP ファミリー内で配列が異なる S1'ポケット内の疎水性相互作用と亜鉛キレート相互作用は、MMP 阻害剤の活性と選択性に大きく関係する[20]。実際に、このファーマコフォアモデルに化合物 **4** を重ね合わせた結果、**4** のトリフルオロメチルフェニル基が、SC44463 のイソブチル基が位置する疎水性の S1'ポケットに配置された（Figure 5-2B）。このモデルを考慮すると、化合物 **3** のビフェニル基は嵩高すぎて S1'ポケットに適さないと考えられた。また Figure 5-3C に示すように、化合物 **4** は、トリフルオロメチル基と MMP-1 の ARG214 残基との間で、ハロゲン結合による相互作用を形成する可能性がある。一方、ジアステレオマー**3'**および **4'**は、いずれも **3** および **4** よりも有意に低い MMP-1 に対する阻害活性（IC_{50} > 100 μM）を示した。この結果は、γ-ラクタムのカルボニル基が、LEU164 残基および ALA165 残基との水素結合形成を通じて、MMP-1 阻害活性に重要な役割を果たしていることから説明できる[18]。今回モデルでは、化合物 **4** の γ-ラクタムと MMP-1 の LEU181 および ALA182 との間に水素結合が形成されていることが示唆されたが、異性体 **4'**ではこの水素結合形成が観察されなかった（Figure 5-3C）。

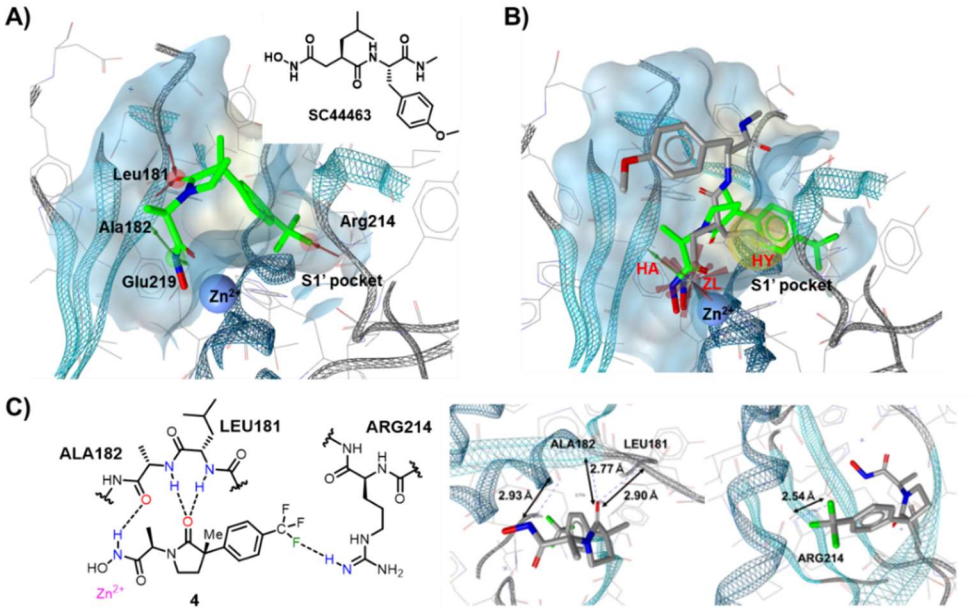


Figure 5-3.（A）SC44463 と MMP-1 の結晶構造（PDB: 1FBL）;（B）化合物 **4** と MMP-1 のファーマコフォアフィッティングモデルと（C）その相互作用解析

5-5　まとめ

本章では、大規模な SAR の公共データベースである ChEMBL から得た、SAR 情報を網羅的に解析し、新たな Activity cliff の予測およびその実験的検証を行った（Figure 5-4）。実際に既知の 644 の MMP-1 阻害剤から SARM を用いて、予測した Activity cliff である化合物 **3** とバーチャル化合物 **4** とその誘導体を合成し、MMP-1 阻害活性を調べた。新たに合成した化合物 **4** は、テンプレート化合物の **3** よりも 60 倍も高い活性を示すことがわかり、予測された Activity cliff の実証に成功した。さらにファーマコフォア解析では、化合物 **4** のパラ位のトリフルオロメチル基と MMP-1 の ARG214 との間に顕著な相互作用があることが示され、モデリングと実験結果が一致した。

今回の実証結果は従来の QSAR では不可能な SARM の有効性を示すものであり、SARM を用いた SAR データの網羅的解析によって設計したバーチャル化合物を、主観的介入なく、体系的にスクリーニングして、予測された Activity cliff を見つける方法に役立つ。しかし、本手法を適用するには、データベース内に多様な母骨格の SAR が含まれている必要がある。すなわち、SARM の行に対応するフラグメントが少なければ、Activity cliff の予測が困難である。これは、タンパク質間相互作用など新たな創薬標的に対する阻害剤を設計する際に課題となると考えられる。実際に、ChEMBL に収録されている HDM2/p53 の PPI 阻害剤から、同様のアプローチで SARM を生成した結果、Figure 5-4 下に示すように、MMP-1 の SARM と比較して行数の少ないマトリックスが確認された。このような場合、Activity cliff の予測ができる可能性は低くなる。そのため、より多様な母骨格を有する化合物を用いた SAR 研究する必要があると言える。

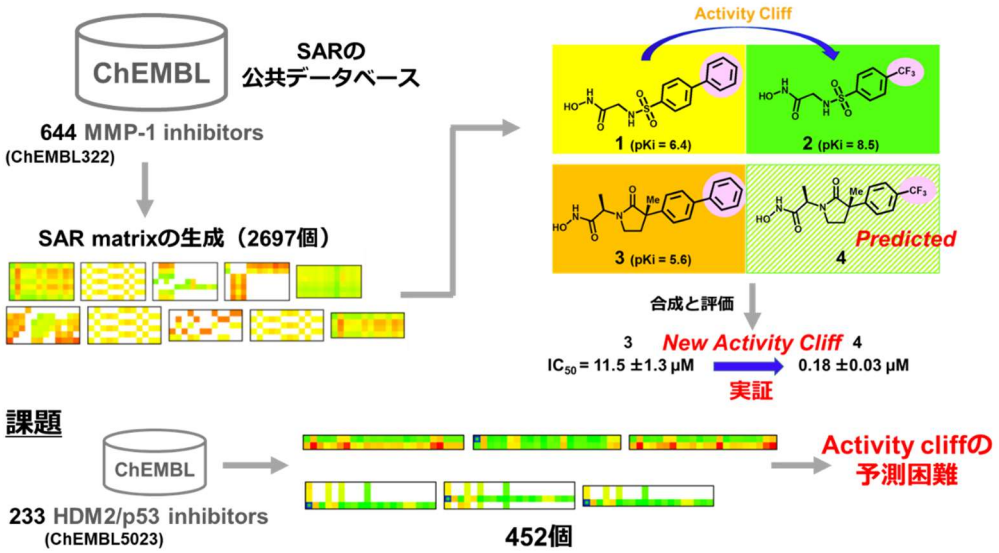


Figure 5-4. 本章のまとめと課題

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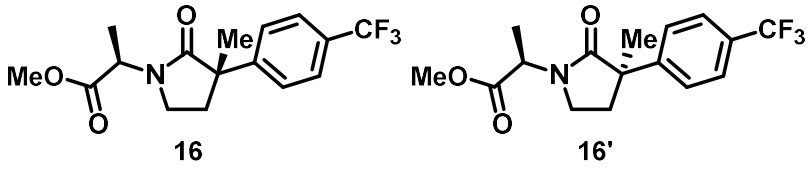
Experimental Section

Synthesis

General Method

Compounds **3** and **5** were known and synthesized according to the literature procedures.[18] Purity of the compounds tested for MMP-1 inhibitory assay was determined by HPLC analysis using Inertsil ODS-3 5 μm (4.6×75 mm; GL Science) with a linear gradient of 0.1% formic acid in water/0.1% formic acid in MeCN (100/0 to 0/100 for 10 min).

Synthesis of methyl (*R*)-2-((*S*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanoate (16) and methyl (*R*)-2-((*R*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)-pyrrolidin-1-yl)propanoate (16')



To a solution of methyl 2-(4-(trifluoromethyl)phenyl)acetate (**8**) (579 mg, 2.56 mmol) in THF (7.5 mL), was slowly added LDA 1.0 M solution in hexane/THF (1 : 2) (3.30 mL, 3.30 mmol) at -78 °C. After the resulting mixture was stirred at 0 °C under argon atmosphere for 1 h, MeI (319 μL, 5.12 mmol) was added. Then, the resulting mixture was stirred at room temperature for 1 h. After that, the reaction mixture was concentrated under pressure. Brine was added to the mixture and the product was extracted with ethyl acetate, dried over sodium sulfate, and concentrated under vacuum. The crude product was used in the next step without further purification.

To a solution of the crude material in THF (7.5 mL), LDA 1.0 M solution in hexane/THF (1 : 2) (3.30 mL, 3.30 mmol) at -78 °C was slowly added. After the mixture was stirred at 0 °C under argon atmosphere for 1 h, allyl bromide (433 μL, 5.12 mmol) was added. The resulting mixture was stirred at room temperature for 1 h and the reaction mixture was concentrated under the reduced pressure. The reaction was quenched with brine and the mixture was extracted with EtOAc, washed with hexane, dried over sodium sulfate, and concentrated under vacuum. The crude product was used in the next step without further purification.

Ozone was pumped into a –78 °C solution of the above crude material in CH_2Cl_2 (5 mL) until the starting material disappeared, as monitored by TLC analysis. The mixture was purged with argon. Triphenylphosphine (806 mg, 3.07 mmol) was added. After 1 h at room temperature, the mixture was concentrated under vacuum. Purification by short column chromatography on silica gel (20% EtOAc in Hexane) gave the crude product 12 which was used to the next step without further

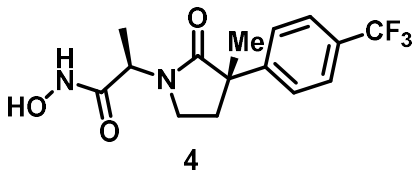
purification.

To a solution of the above product (**12**) and D-alanine methyl ester hydrochloride (275 mg, 1.97 mmol) in acetic acid (10.7 mL) was added portion-wise zinc powder (1.17 g, 17.9 mmol). The mixture was heated to reflux for 12 h, and then cooled to room temperature. Following addition of CH_2Cl_2, the mixture was filtered and the filter cake washed with methanol/ CH_2Cl_2. The filtrate was concentrated at 45 °C in vacuo to remove acetic acid. The residue was treated with ethyl acetate and filtered to remove insoluble materials. The filtrate was concentrated and purified by column chromatography on silica gel (40% EtOAc in hexane) to afford 179 mg of fast eluting isomer (**16'**), 135 mg of slow eluting isomer (**16**), and 80 mg of mixture containing a mixture of both isomers (total 394 mg, 1.20 mmol, 47% yield for 4 steps) as a colorless oil.

16: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.60 (d, *J* = 8.8 Hz, 2H), 7.56 (*J* = 8.8 Hz, 2H), 4.97 (q, *J* = 7.5 Hz, 1H), 3.69 (s, 3H), 3.41 (q, *J* = 4.5 Hz, 2H), 2.48-2.41 (m, 1H), 2.23-2.16 (m, 1H), 1.58 (s, 3H), 1.48 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 177.3, 171.9, 147.9, 129.1 (q, *J* = 32.3 Hz), 126.7, 125.6 (q, *J* = 180.1 Hz), 125.5 (q, *J* = 3.6 Hz), 52.5, 49.8, 48.9, 40.6, 35.3, 24.5, 15.0; ^{19}F NMR (470 MHz, $CDCl_3$): δ 62.5; HRMS (ESI, positive) for $C_{16}H_{18}F_3NO_3$ (m/z): calculated 352.1131 $(M+Na)^+$, found 352.1128.

16': ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.59 (d, *J* = 8.5 Hz, 2H), 7.56 (d, *J* = 8.5 Hz, 2H), 4.93 (q, *J* = 7.5 Hz, 1H), 3.75 (s, 3H), 3.53-3.49 (m, 1H), 3.36-3.30 (m, 1H), 2.46-2.39 (m, 1H), 2.30-2.23 (m, 1H), 1.58 (s, 3H), 1.45 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 177.3, 171.9, 147.8, 129.0 (q, *J* = 14.9 Hz), 126.7, 125.5 (q, *J* = 3.6 Hz), 124.2 (q, *J* = 270.2 Hz), 52.4, 49.8, 48.9, 40.6, 35.3, 24.5, 14.9; ^{19}F NMR (470 MHz, $CDCl_3$): δ 62.5; HRMS (ESI, positive) for $C_{16}H_{18}F_3NO_3$ (m/z): calculated 352.1131 $(M+Na)^+$, found 352.1145.

Synthesis of (*R*)-*N*-hydroxy-2-((*S*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (4)

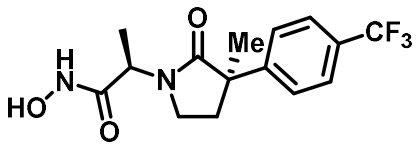


4

Hydroxylamine hydrochloride (234 mg, 34 mmol) in hot methanol (1.2 mL) was treated with a solution of KOH (281 mg, 50 mmol) in methanol (700 μL). The mixture was cooled to room temperature and the insoluble KCl was removed by filtration to yield a clear solution (approximately 1.76 M of NH_2OH). The freshly prepared hydroxylamine solution (371 μL, 0.655 mmol) was added to a solution of 16 (43 mg, 0.131 mmol) in methanol (500 μL). The reaction mixture was stirred at room temperature for 25 min and then adjusted to pH 5-6 by addition of 1N HCl while the flask was cooled on an ice-water bath. The precipitate was collected by filtration,

rinsed with methanol/water (2:1, 500 μL), water (500 μL), and dried under vacuum to give amorphous compound **4** (39 mg, 90%). ^{1}H NMR (500 MHz; CD_3OD): δ 7.63 (d, *J* = 8.5 Hz, 2H), 7.59 (d, *J* = 8.5 Hz, 2H), 4.65 (q, *J* = 7.2 Hz, 1H), 3.61-3.52 (m, 2H), 2.43-2.39 (m, 1H), 2.25-2.20 (m, 1H), 1.55 (s, 3H), 1.43 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz; CD_3OD): δ 179.3, 170.1, 149.6, 130.0 (q, *J* = 32.1 Hz), 128.2 (t, *J* = 14.4 Hz), 126.3 (q, *J* = 3.7 Hz), 125.7 (q, *J* = 269.4 Hz), 50.6, 50.0, 42.3, 36.8, 24.2, 15.2; ^{19}F NMR (470 MHz, CD_3OD): δ 64.0; HRMS (ESI, negative) for $C_{15}H_{17}F_3N_2O_3$ (m/z): calculated 329.1108 $(M-H)^-$, found 329.1109; HPLC purity 99.6%, retention time 7.36 min (MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0:100 to 100:0).

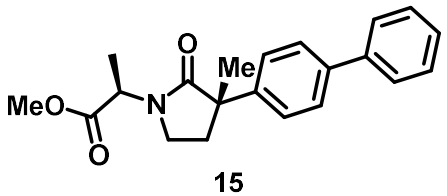
Synthesis of (*R*)-*N*-hydroxy-2-((*R*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (4')



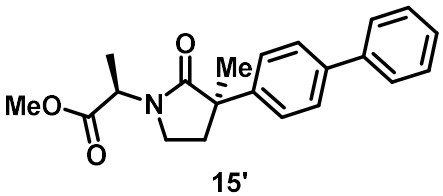
4'

This compound was prepared from ester **16'** (47 mg, 0.413 mmol) using the procedure described for **4** in 72% yield as a white solid. m.p. 105-107 °C; ^{1}H NMR (500 MHz; CD_3OD): δ 7.64 (d, *J* = 8.5 Hz, 2H), 7.58 (d, *J* = 8.5 Hz, 2H), 4.63 (q, *J* = 7.2 Hz, 1H), 3.68-3.63 (m, 2H), 3.48-3.43 (m, 1H), 2.45-2.40 (m, 1H), 2.29-2.25 (m, 1H), 1.55 (s, 3H), 1.41 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz; CD_3OD): δ 179.3, 170.1, 149.5, 130.0 (q, *J* = 32.3 Hz), 128.1, 126.4 (d, *J* = 3.8 Hz), 125.7 (q, *J* = 269.5 Hz), 50.6, 49.9, 42.3, 36.4, 24.2, 15.3; ^{19}F NMR (470 MHz, CD_3OD): δ 64.0; HRMS (ESI, negative) for $C_{15}H_{17}F_3N_2O_3$ (m/z): calculated 329.1108 $(M-H)^-$, found 329.1090; HPLC purity 96.01%, retention time 7.52 min (MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0:100 to 100:0).

Synthesis of methyl (*R*)-2-((*S*)-3-([1,1'-biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-propanoate (15) and methyl (*R*)-2-((*R*)-3-([1,1'-biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-propanoate (15')



15



15'

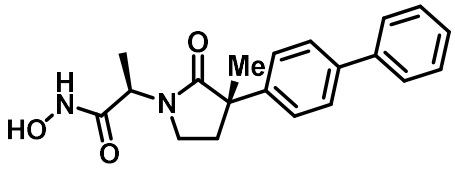
This compound was prepared from ester methyl 2-([1,1'-biphenyl]-4-yl)acetate (**7**) (601 mg, 2.76 mmol) using the procedure described above for **16** and afforded 156 mg of fast eluting isomer

(**15'**), 118 mg of slow eluting desired isomer (**15**) (total yield 274 mg, 0.812 mmol, 29% for 4 steps) as a colorless oil.

15: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.58-7.55 (m, 4H), 7.49 (d, *J* = 6.8 Hz, 2H), 7.41 (d, *J* = 6.1 Hz, 2H), 7.31 (d, *J* = 6.1 Hz, 2H), 4.98 (q, *J* = 5.9 Hz, 1H), 3.67 (s, 3H), 3.42-3.35 (m, 2H), 2.50-2.45 (m, 1H), 2.19-2.13 (m, 1H), 1.59 (s, 3H), 1.46 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 177.8, 172.0, 142.7, 140.8, 139.5, 128.8, 127.2, 127.1, 127.0, 126.8, 52.2, 49.6, 48.6, 40.5, 35.9, 24.6, 14.8; HRMS (ESI, positive) for $C_{21}H_{23}NO_3$ (m/z): calculated 360.1570 $(M+Na)^+$, found 360.1572.

15': ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.58-7.55 (m, 4H), 7.47 (d, *J* = 6.8 Hz, 2H), 7.42 (d, *J* = 6.1 Hz, 2H), 7.33 (d, *J* = 6.1 Hz, 2H), 4.97 (q, *J* = 6.0 Hz, 1H), 3.74 (s, 3H), 3.50-3.46 (m, 1H), 3.36-3.31 (m, 1H), 2.49-2.44 (m, 1H), 2.27-2.21 (m, 1H), 1.60 (s, 3H), 1.45 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 177.9, 172.1, 142.8, 140.9, 139.7, 128.8, 127.3, 127.1, 126.6, 52.4, 49.7, 48.6, 40.6, 35.5, 24.7, 15.0; HRMS (ESI, positive) for $C_{21}H_{23}NO_3$ (m/z): calculated 360.1570 $(M+Na)^+$, found 360.1573.

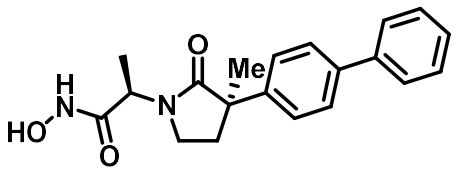
Synthesis of (*R*)-2-((*S*)-3-([1,1'-biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-*N*-hydroxy-propanamide (3)



3

This compound was prepared from ester **15** (58 mg, 0.172 mmol) using the procedure described above for compound **4** in 72% yield as a white solid: m.p. 160-162 °C; ^{1}H NMR was the same as reported[18]; HRMS (ESI, negative) for $C_{20}H_{22}N_2O_3$ (m/z): calculated 337.1547 $(M-H)^-$, found 337.1547; HPLC purity 99.4%, retention time 7.96 min (MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0:100 to 100:0).).

Synthesis of (*R*)-2-((*R*)-3-([1,1'-biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-*N*-hydroxy-propanamide (3')

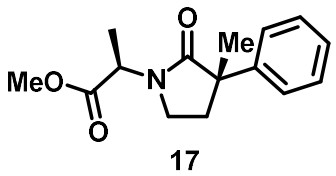


3'

This compound was prepared from ester **15'** (46 mg, 0.136 mmol) using the procedure described

above for compound **4** in 98% yield as a white solid: m.p. 137-138 °C; ^{1}H NMR (500 MHz; CD_3OD): δ 7.59-7.57 (m, 4H), 7.43-7.39 (m, 4H), 7.31 (t, *J* = 7.4 Hz, 1H), 4.66 (q, *J* = 7.2 Hz, 1H), 3.65-3.60 (m, 1H), 3.44-3.35 (m, 1H), 2.45-2.40 (m, 1H), 2.22-2.17 (m, 1H), 1.54 (s, 3H), 1.39 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz; CD_3OD): δ 180.0, 170.1, 143.9, 141.8, 141.0, 129.9, 128.4, 128.1, 127.8, 127.7, 50.3, 49.8, 42.3, 36.7, 24.4, 15.3; HRMS (ESI, negative) for $C_{20}H_{22}N_2O_3$ (m/z): calculated 337.1547 $(M-H)^-$, found 337.1541; HPLC purity 99.20%, retention time 7.91 min (MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0:100 to 100:0)..

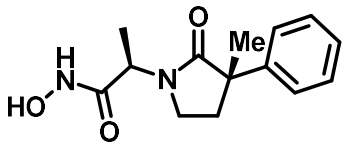
Synthesis of methyl (*R*)-2-((*S*)-3-methyl-2-oxo-3-phenylpyrrolidin-1-yl)propanoate (17)



17

This compound was prepared from ester methyl 2-phenylacetate (**9**) (561 μL, 4.00 mmol) using the procedure described above for **16** and afforded 224 mg of fast eluting isomer, 98 mg of slow eluting desired isomer (**17**), and 190 mg of mixture containing a mixture of both isomers (total yield 512 mg, 1.96 mmol, 49% for 4 steps) as a colorless oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.42 (d, *J* = 7.2 Hz, 2H), 7.33 (d, *J* = 7.6 Hz, 2H), 7.23 (t, *J* = 7.2 Hz, 1H), 4.98 (q, *J* = 7.4 Hz, 1H), 3.68 (s, 3H), 3.37 (t, *J* = 6.7 Hz, 2H), 2.49-2.43 (m, 1H), 2.18-2.11 (m, 1H), 1.56 (s, 3H), 1.46 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 177.9, 171.9, 143.7, 128.5, 126.8, 126.4, 52.3, 49.7, 48.9, 40.5, 36.1, 24.8, 14.9; HRMS (ESI, positive) for $C_{15}H_{19}NO_3$ (m/z): calculated 284.1257 $(M+Na)^+$, found 284.1259.

Synthesis of (*R*)-*N*-hydroxy-2-((*S*)-3-methyl-2-oxo-3-phenylpyrrolidin-1-yl)propanamide (5)

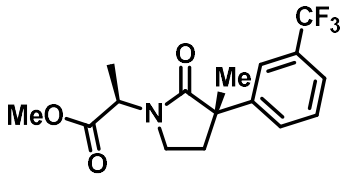


5

This compound was prepared from ester **17** (65 mg, 0.249 mmol) using the procedure described above for compound **4** in 49% yield as amorphous material. The structure was determined by comparison with authentic samples prepared by the literature procedure;[18] HPLC purity 96.1%, retention time 6.17 min (MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0:100 to 100:0).

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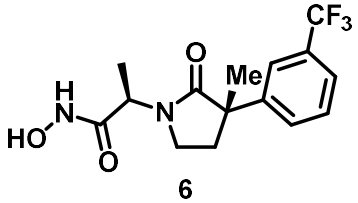
Synthesis of methyl (*R*)-2-((*S*)-3-methyl-2-oxo-3-(3-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanoate (18)



18

This compound was prepared from methyl 2-(3-(trifluoromethyl)phenyl)acetate (**10**) (898 mg, 4.12 mmol) using the procedure described above for **16** afforded 21 mg of fast eluting isomer, 96 mg of slow eluting desired isomer (**18**), and 278 mg of mixture containing a mixture of both isomers (total yield 396 mg, 1.12 mmol, 29% for 4 steps) as a colorless oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.67-7.64 (m, 2H), 7.50-7.46 (m, 2H), 4.98 (q, *J* = 7.4 Hz, 1H), 3.67 (s, 3H), 3.40-3.35 (m, 2H), 2.48-2.42 (m, 1H), 2.24-2.17 (m, 1H), 1.58 (s, 3H), 1.48 (d, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 177.1, 171.7, 144.7, 130.7 (q, *J* = 31.8 Hz), 130.0, 128.9, 123.7 (q, *J* = 3.8 Hz), 123.6 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 270.8 Hz), 52.3, 49.6, 48.8, 40.4, 35.7, 24.7, 14.7; ^{19}F NMR (470 MHz, $CDCl_3$): δ 62.4; HRMS (ESI, positive) for $C_{16}H_{18}F_3NO_3$ (m/z): calculated 352.1131 $(M+Na)^+$, found 352.1135.

Synthesis of (*R*)-*N*-hydroxy-2-((*S*)-3-methyl-2-oxo-3-(3-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (6)



6

This compound was prepared from ester **18** (67 mg, 0.204 mmol) using the procedure described above for compound **4** in 43% yield as amorphous material. ^{1}H NMR (500 MHz; CD_3OD): δ 7.74 (s, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.56-7.51 (m, 2H), 4.64 (q, *J* = 7.2 Hz, 1H), 3.62-3.53 (m, 2H), 2.44-2.39 (m, 1H), 2.27-2.12 (m, 1H), 1.55 (s, 3H), 1.43 (d, *J* = 7.2 Hz, 3H); ^{13}C NMR (125 MHz; CD_3OD): δ 179.3, 170.0, 146.5, 131.7 (q, *J* = 31.0 Hz), 131.4, 130.3, 124.6 (q, *J* = 3.8 Hz), 124.1 (q, *J* = 3.8 Hz), 124.2 (q, *J* = 272.6 Hz), 50.3, 50.0, 42.3, 40.4, 36.6, 24.3, 15.2; ^{19}F NMR (470 MHz, CD_3OD): δ 64.0; HRMS (ESI, negative) for $C_{15}H_{17}F_3N_2O_3$ (m/z): calculated 329.1108 $(M-H)^-$, found 329.1117 ; HPLC purity 95.5%, retention time 7.91 min (MeCN+0.1% formic acid/H_2O+0.1% formic acid, 0-10 min: 0:100 to 100:0).

Biology

Evaluation of MMP-1 inhibitory activity

MMP-1 inhibitory assay was performed using the assay kit according to the manufacturer's protocol. A 96-well clear microplate (1/2 volume), 30.6 U/μL of MMP-1 enzyme, MMP-1 inhibitor (1.3 mM NNGH in DMSO), MMP-1 substrate (25 mM in DMSO), and colorimetric assay buffer were contained in MMP1 Inhibitor Screening Assay Kit (ab139443, abcam). The MMP-1 substrate and an MMP-1 inhibitor were thawed at room temperature. The MMP-1 inhibitor was diluted at 1/200 in assay buffer and brought to 37 °C. Also, the MMP-1 substrate was diluted at 1/200 in assay buffer and brought to 37 °C. The MMP-1 enzyme was diluted at 1/25 in assay buffer and warmed up to 37 °C as soon as the assay was started. After the assay buffer was pipetted appropriately into each well, 20 μL of the prepared MMP-1 enzyme solution was added to the control, MMP-1 inhibitor, and test compound wells. While 20 μL of the prepared MMP-1 inhibitor solution was only added to the MMP-1 inhibitor wells, the desired volume of test inhibitor was added to test compound wells. The microplate was incubated for 30 min at 37 °C. The 10 μL of the prepared MMP-1 substrate solution was added into each well to allow the reaction start. The absorbance of the wells was measured at A_{412nm} using a microplate reader every 1 minute, and data analysis was performed.

Modeling

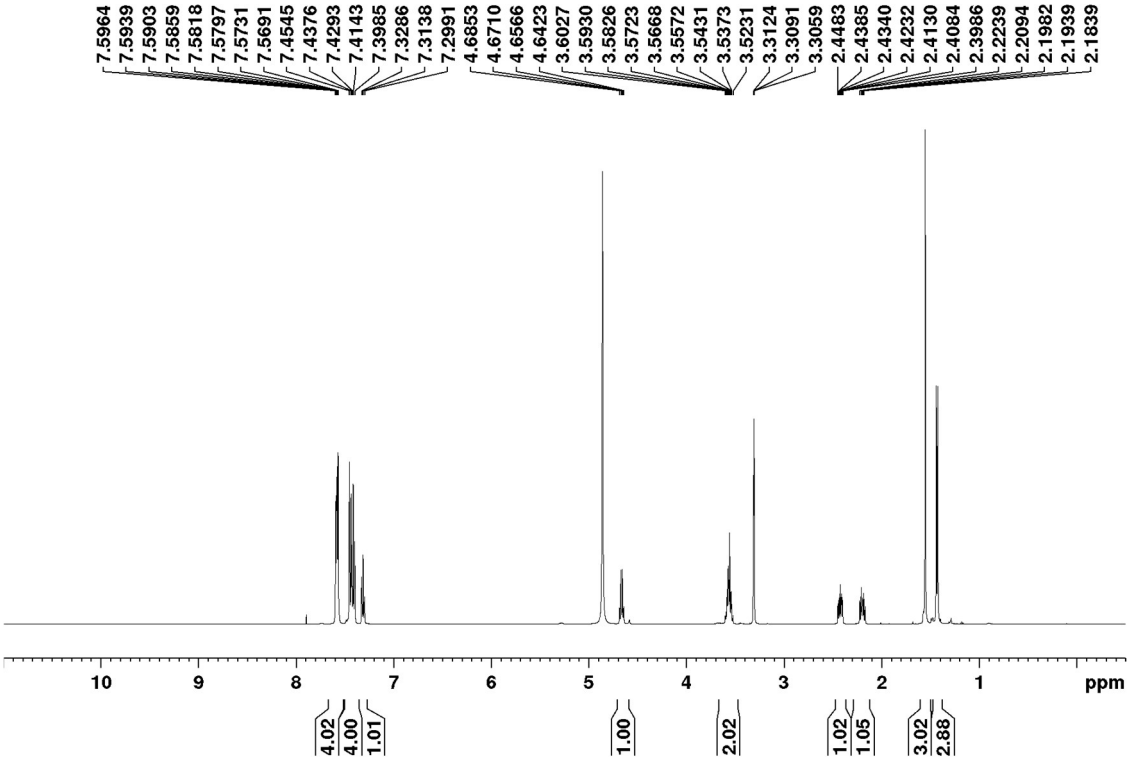
Pharmacophore fitting

To predict binding interaction between compounds and MMP-1, a pharmacophore model was constructed from the crystal structure of SC44463/MMP-1 (PDB: 1FBL) using LigandScout 4.4 (InteLigand GmbH). Then, three pharmacophore features of SC44463 were used including a hydrogen bond acceptor (HA), hydrophobic (Hy) moity, and zinc binding location feature (ZL). For pharmacophore evaluation, the scoring function was set to 'Relative Pharmacophore-Fit'. For alll other parameters, default values were used. Compounds were fitted to the SC44463 pharmacophore model followed by interaction energy minimization with MMP-1.

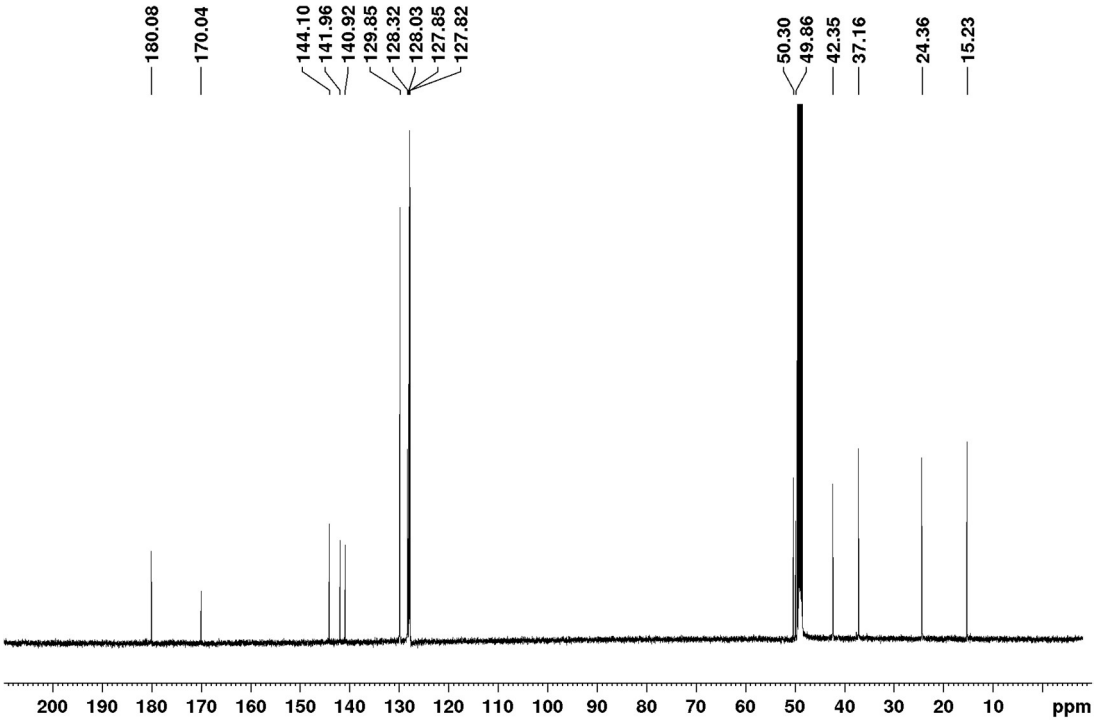
Spectra data

(*R*)-2-((*S*)-3-([1,1'-Biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-*N*-hydroxypropanamide (3)

^{1}H NMR (CD_3OD, 500 MHz)

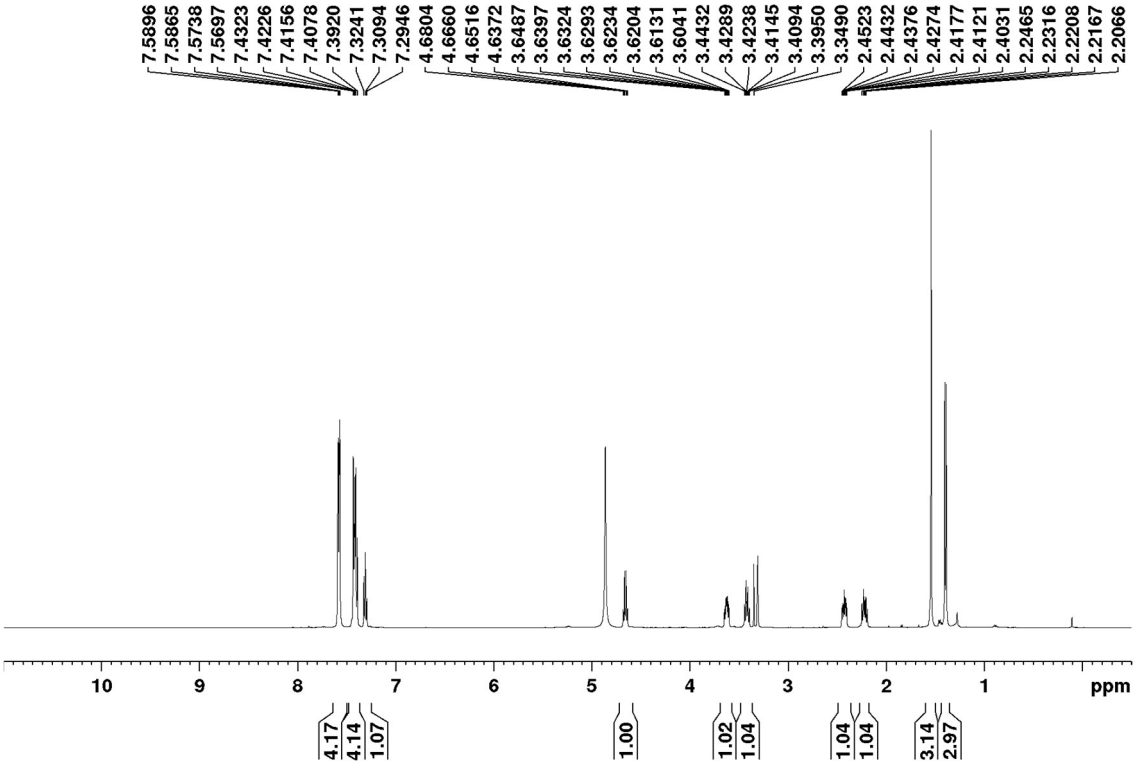


^{13}C NMR (CD_3OD, 125 MHz)

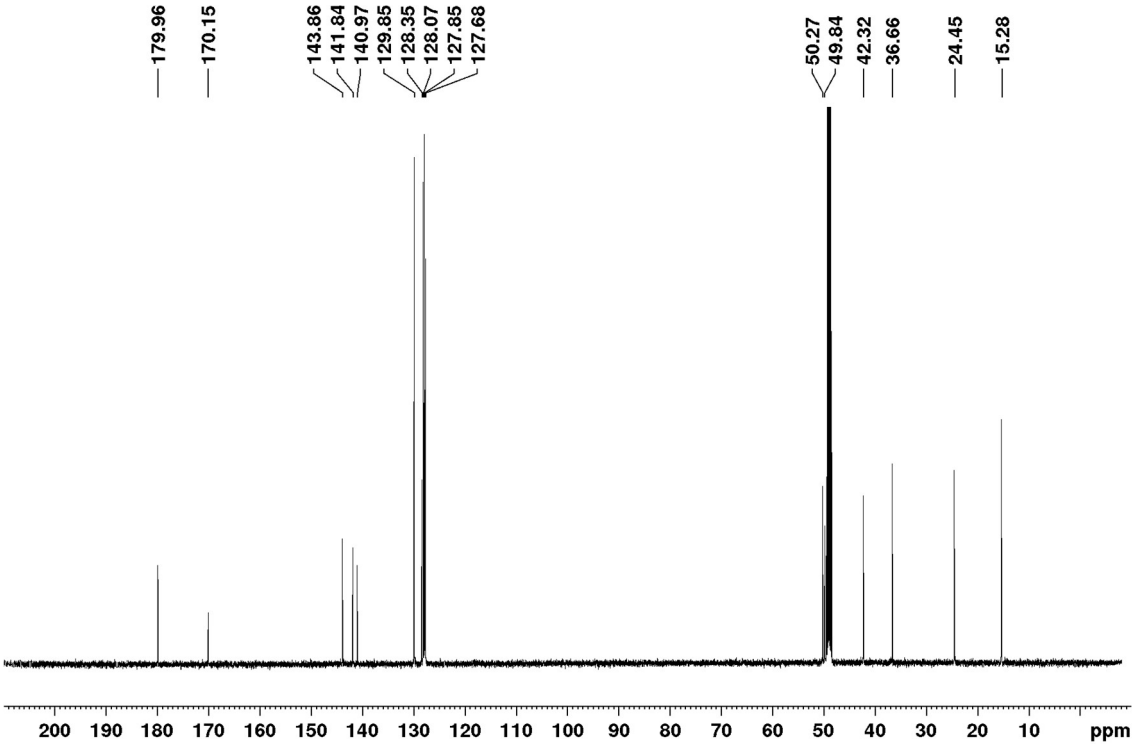


(*R*)-2-((*R*)-3-([1,1'-Biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-*N*-hydroxypropanamide (3')

^{1}H NMR (CD_3OD, 500 MHz)

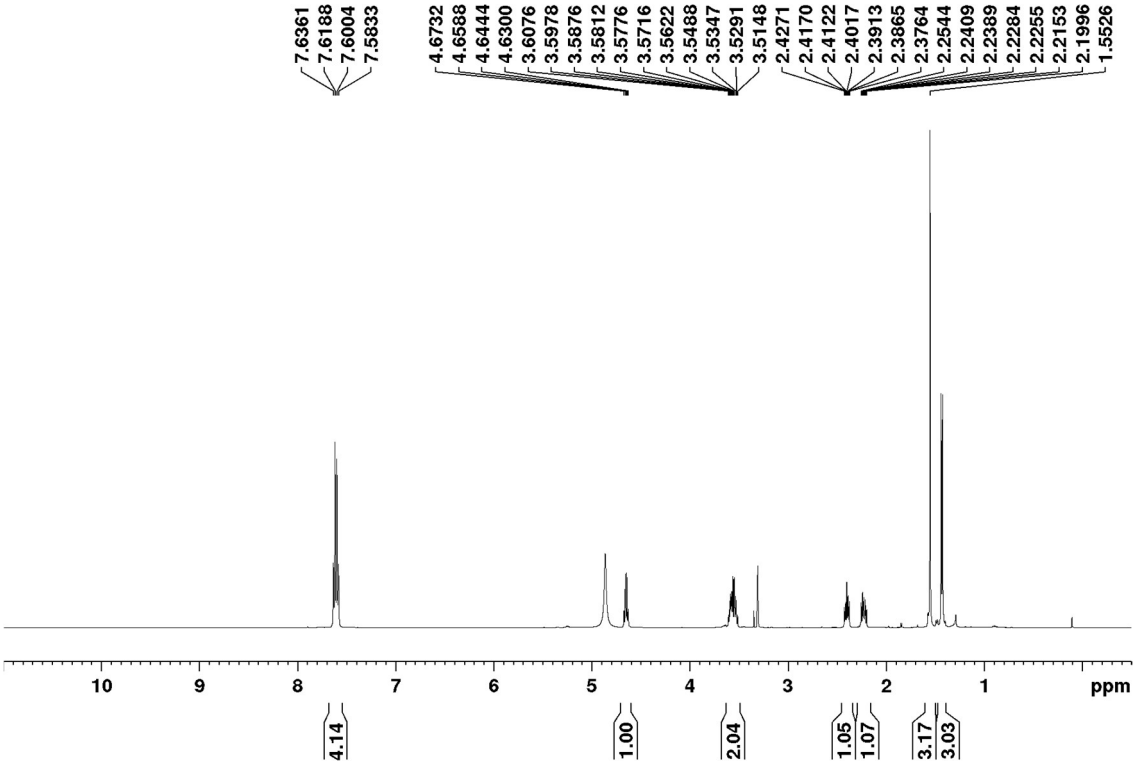


^{13}C NMR (CD_3OD, 125 MHz)

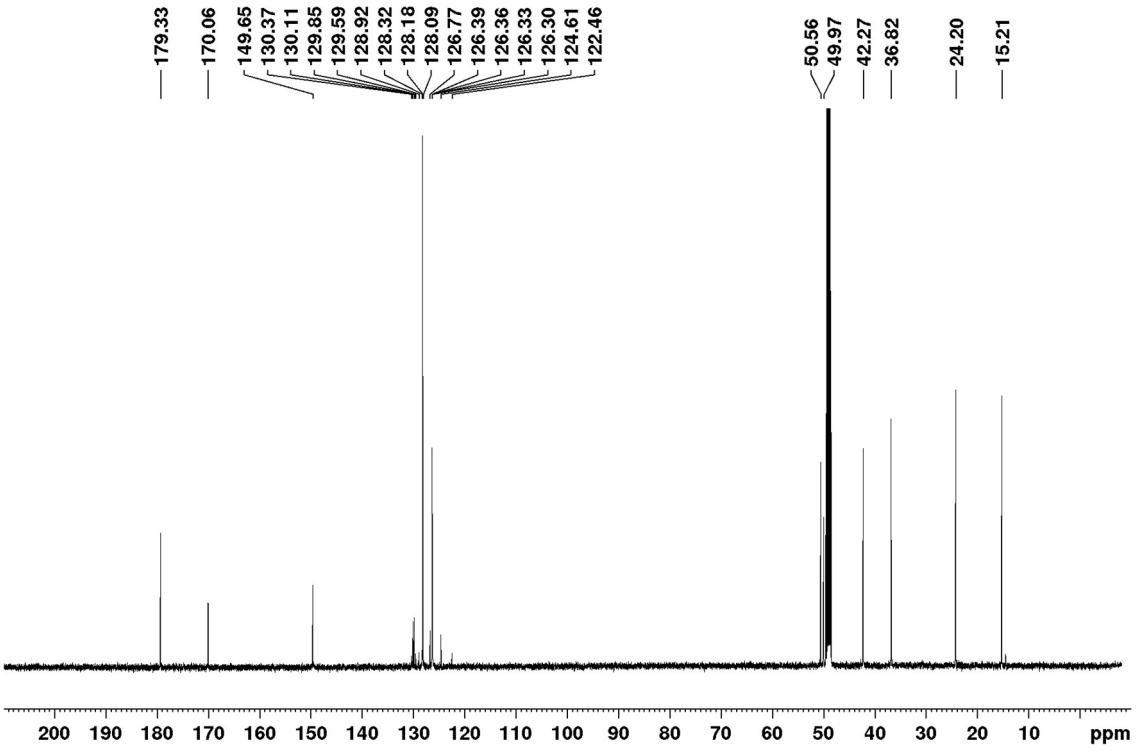


(*R*)-*N*-Hydroxy-2-((*S*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (4)

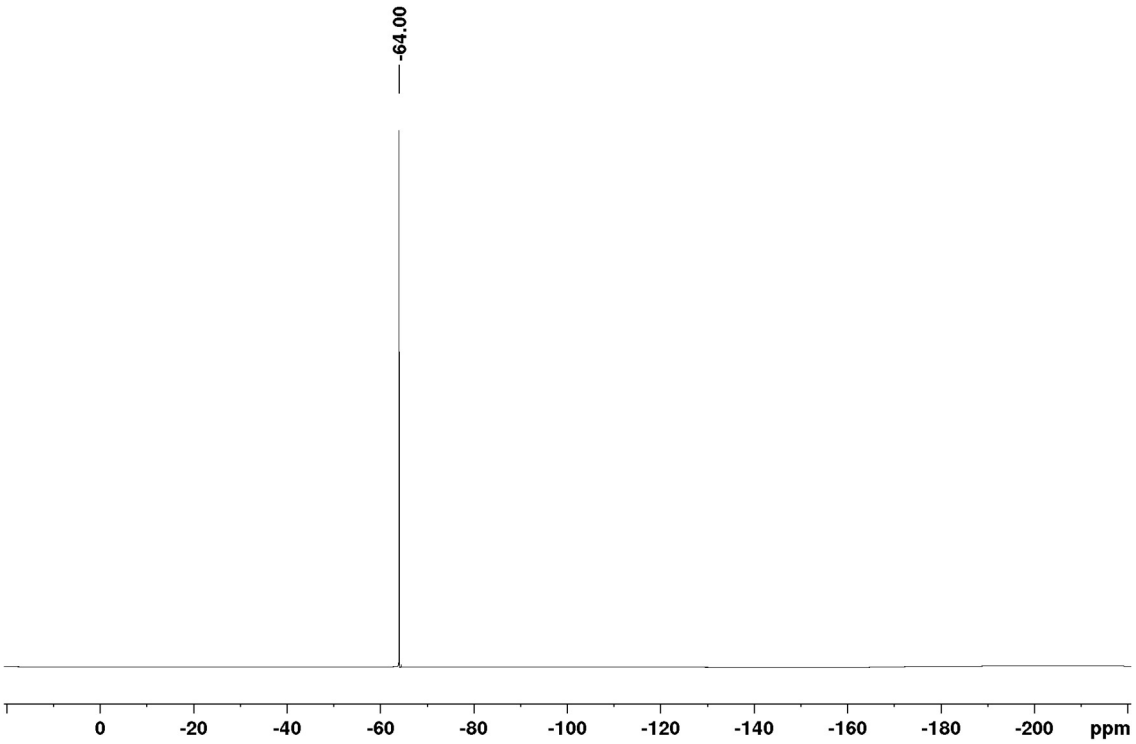
^{1}H NMR (CD_3OD, 500 MHz)



^{13}C NMR (CD_3OD, 125 MHz)

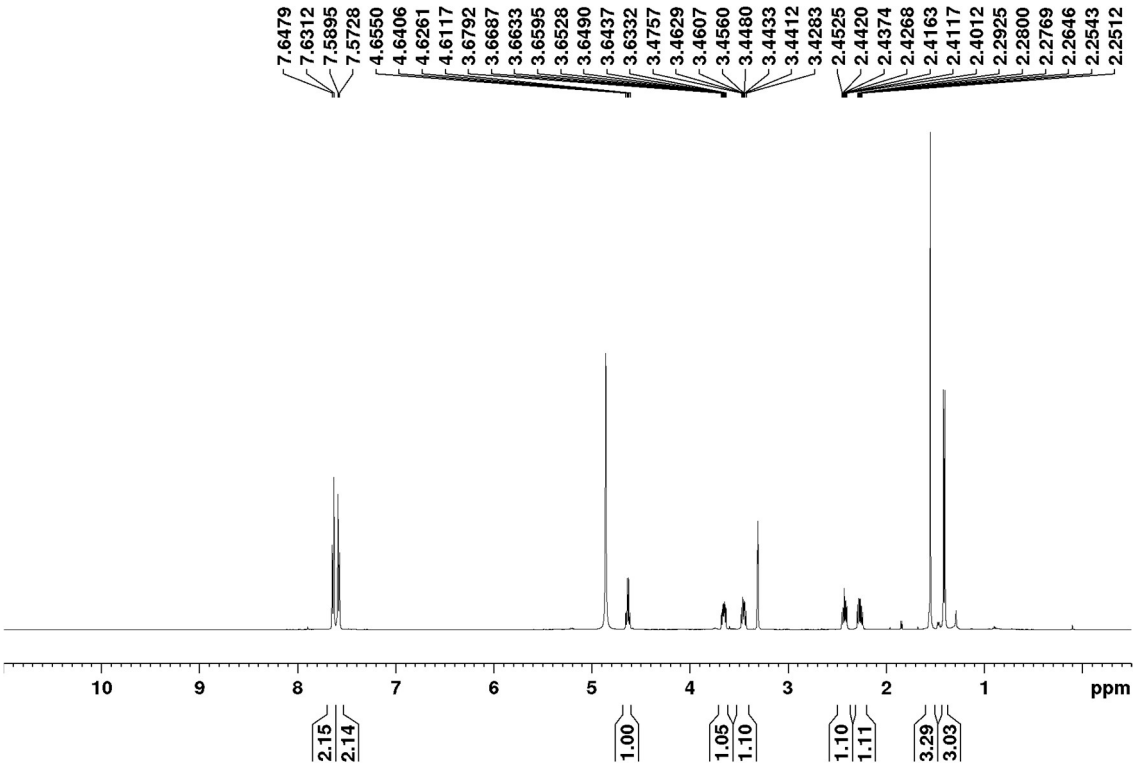


^{19}F NMR ($CDCl_3$, 470 MHz)

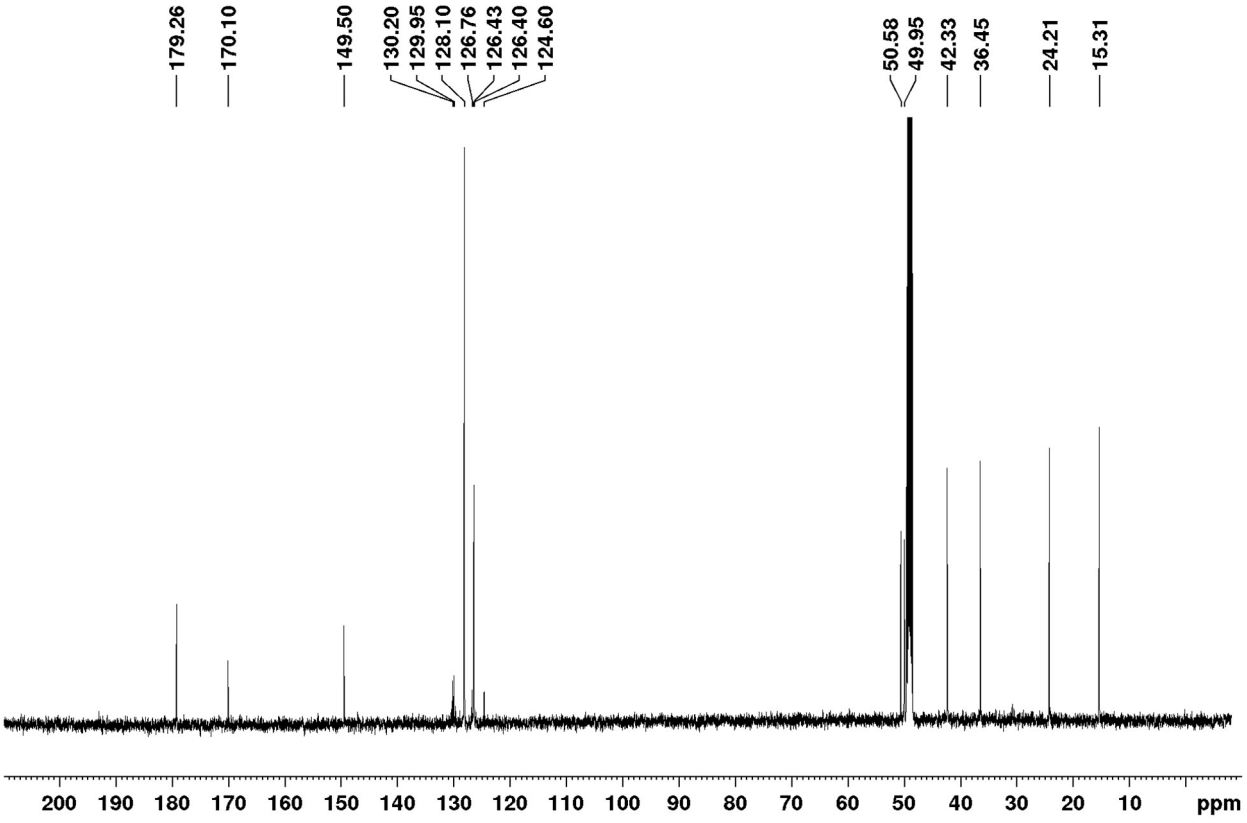


(*R*)-*N*-Hydroxy-2-((*R*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (4')

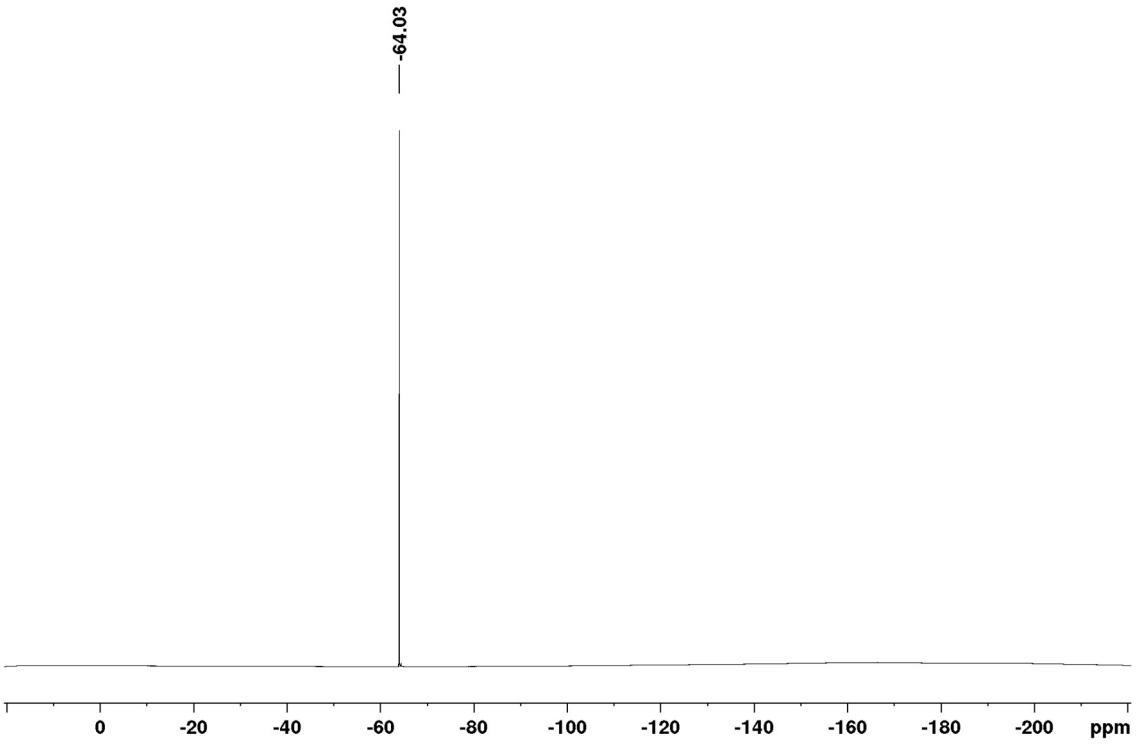
^{1}H NMR (CD_3OD, 500 MHz)



^{13}C NMR (CD_3OD, 125 MHz)

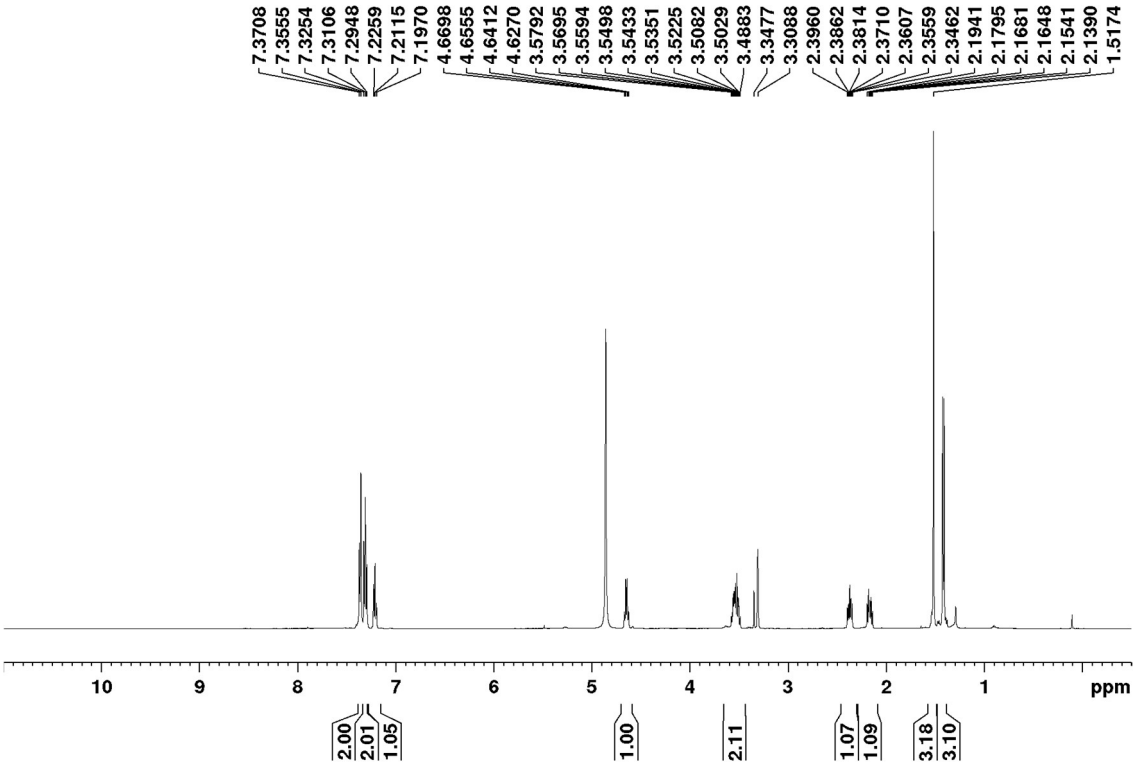


^{19}F NMR (CD_3OD, 470 MHz)

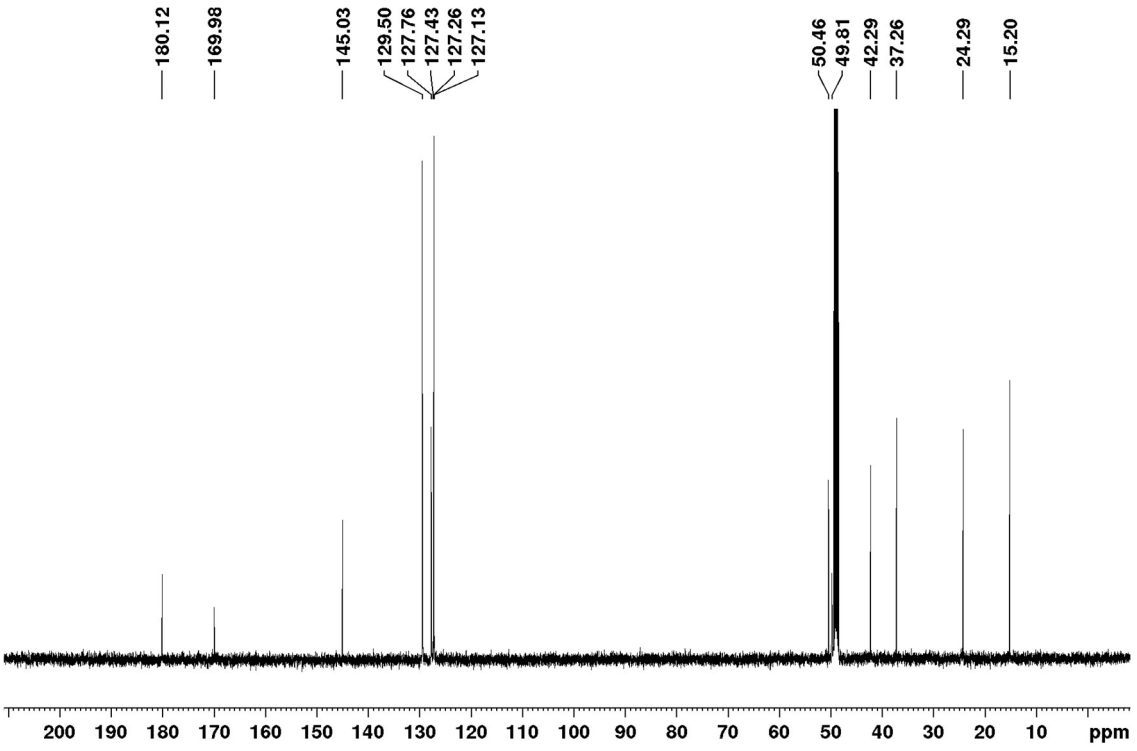


(*R*)-*N*-Hydroxy-2-((*S*)-3-methyl-2-oxo-3-phenylpyrrolidin-1-yl)propanamide (5)

^{1}H NMR (CD_3OD, 500 MHz)

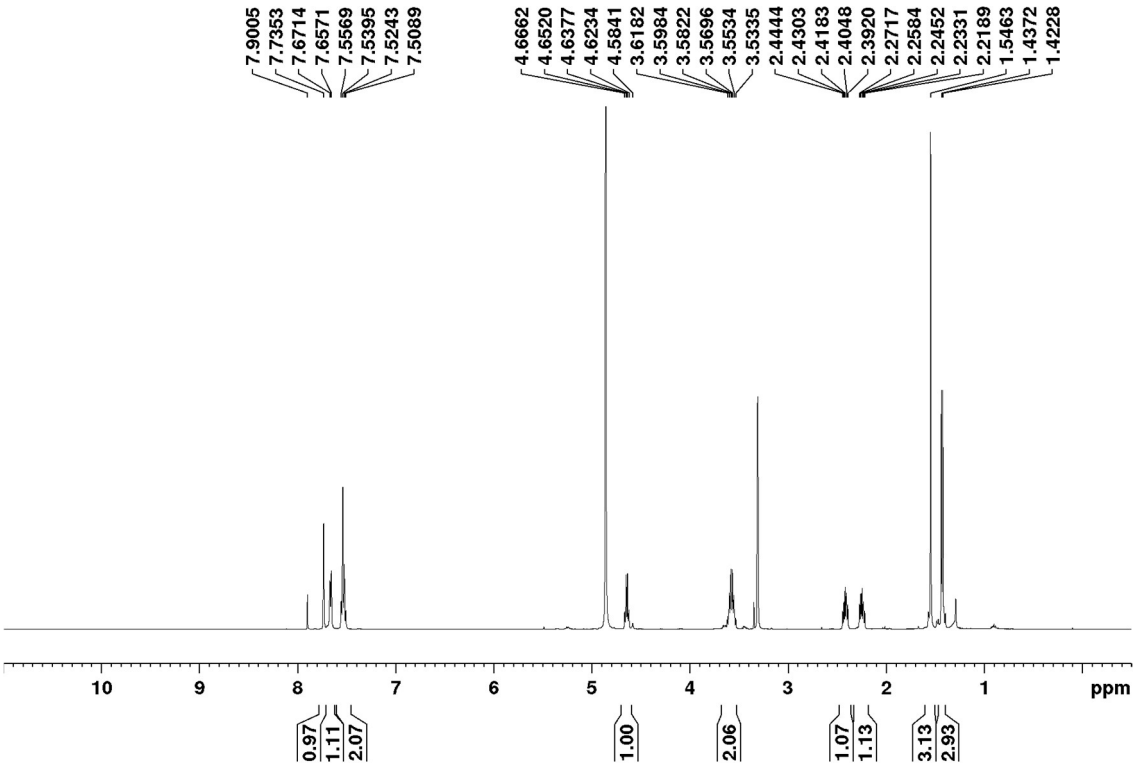


^{13}C NMR (CD_3OD, 125 MHz)

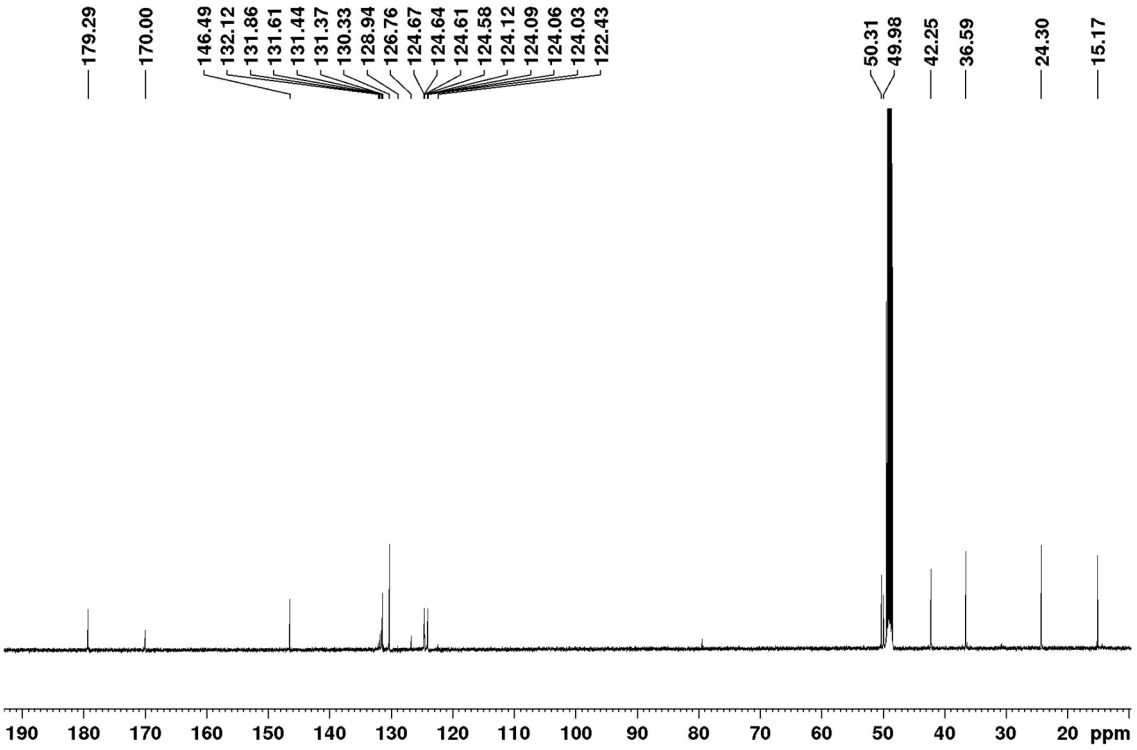


(*R*)-*N*-Hydroxy-2-((*S*)-3-methyl-2-oxo-3-(3-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (6)

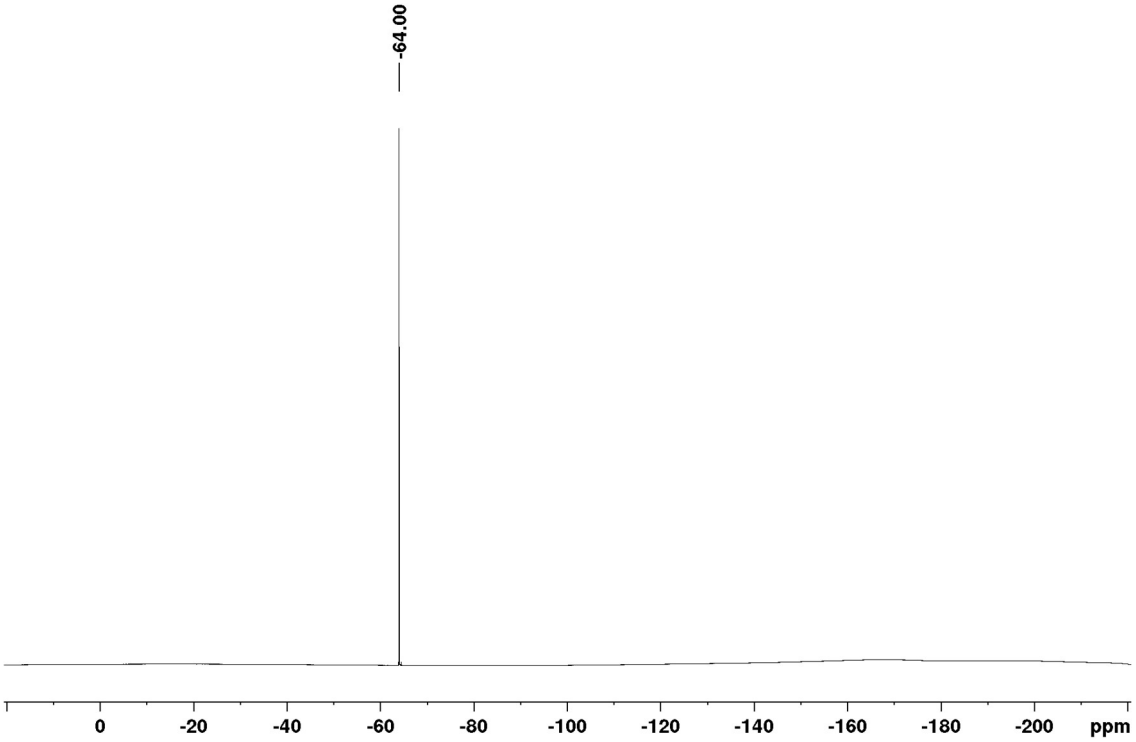
^{1}H NMR (CD_3OD, 500 MHz)



^{13}C NMR (CD_3OD, 125 MHz)

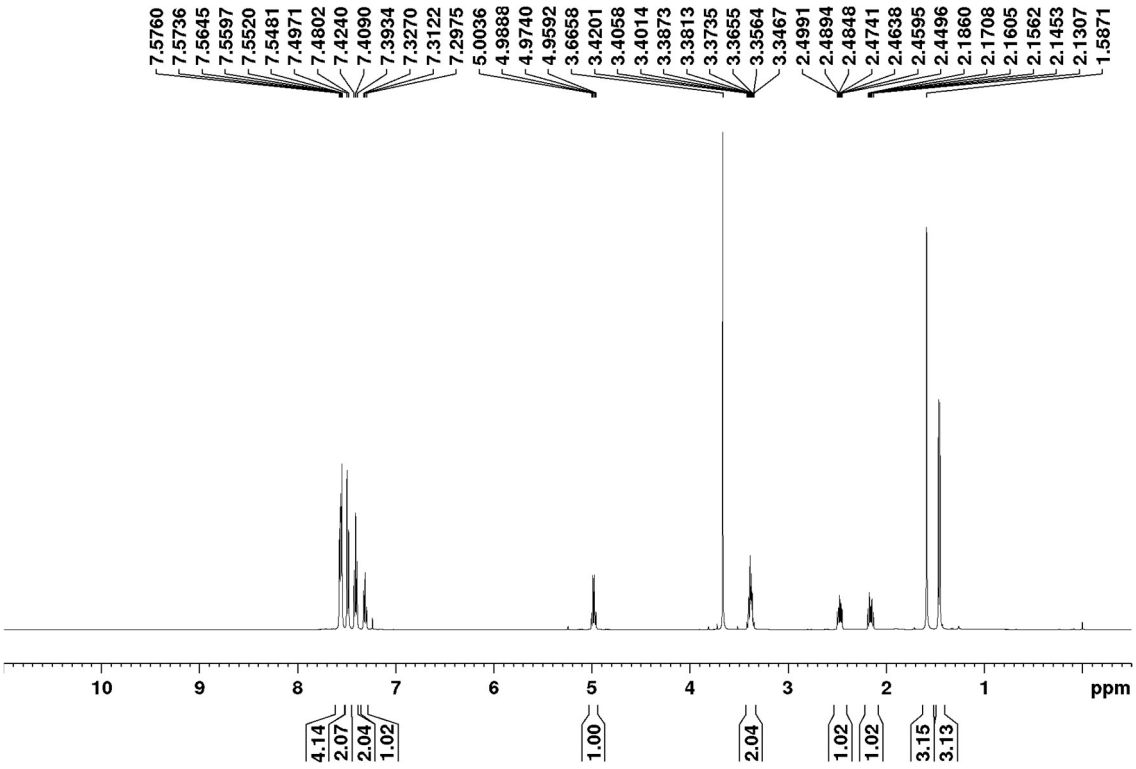


^{19}F NMR (CD_3OD, 470 MHz)

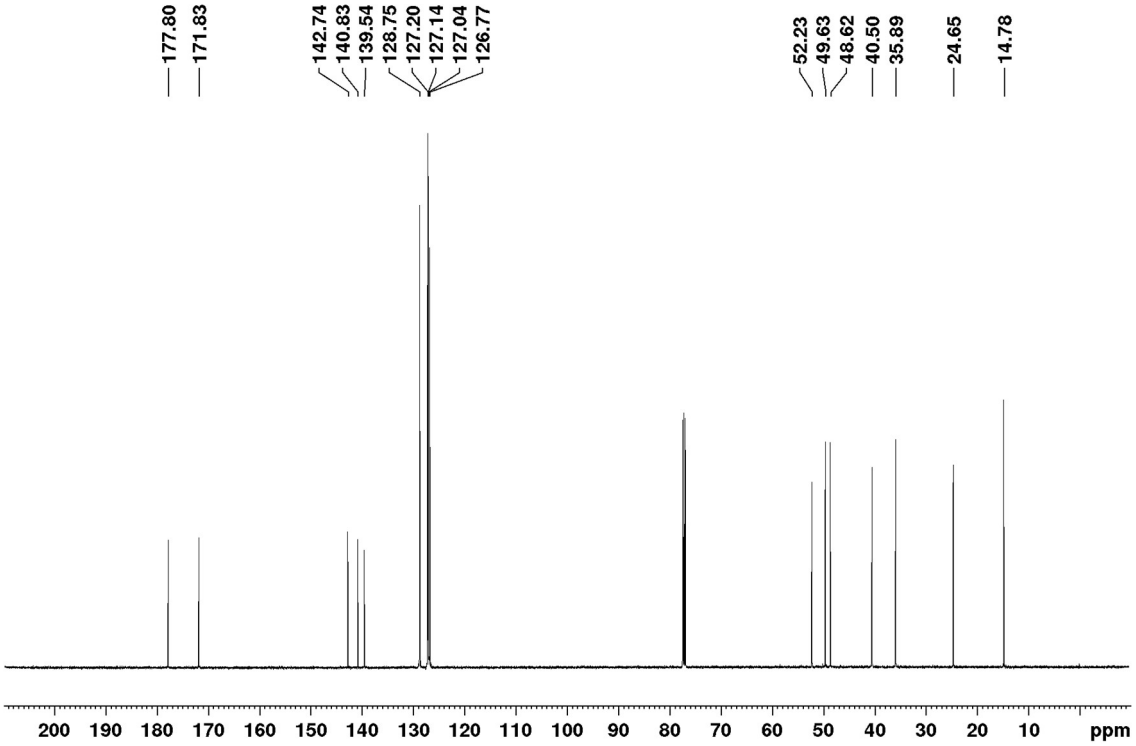


Methyl (*R*)-2-((*S*)-3-([1,1'-biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)propanoate (15)

^{1}H NMR ($CDCl_3$, 400 MHz)

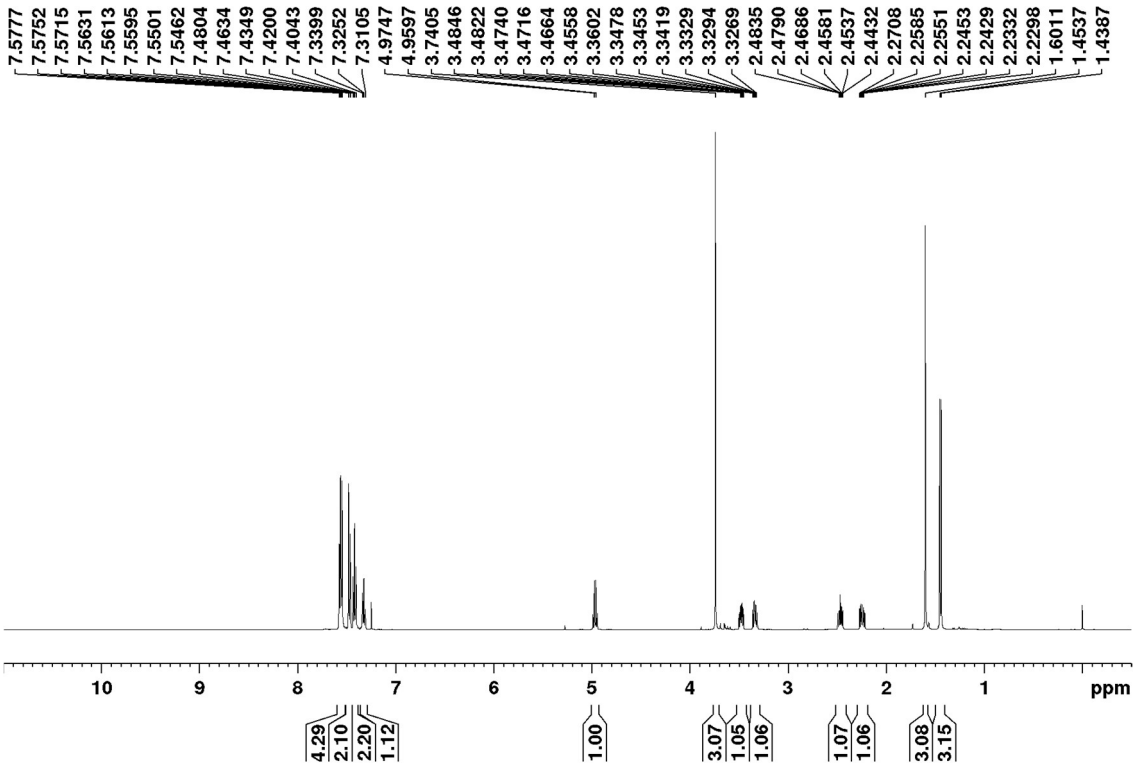


^{13}C NMR ($CDCl_3$, 125 MHz)

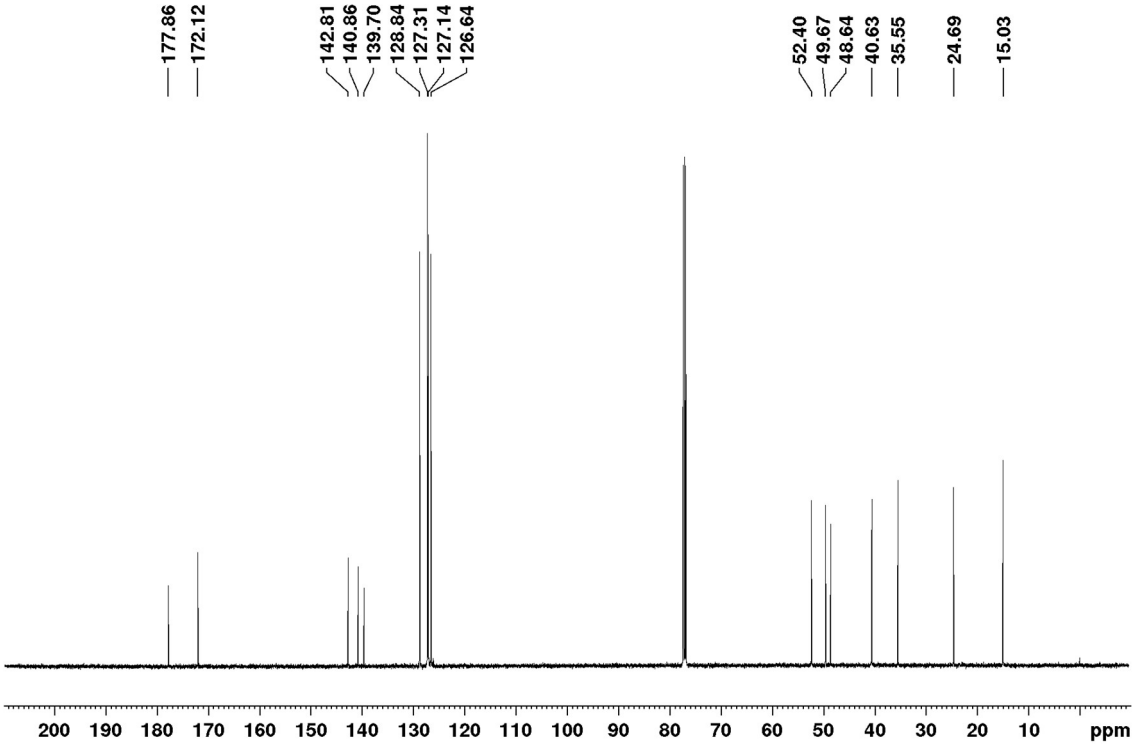


Methyl (*R*)-2-((*R*)-3-([1,1'-biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)propanoate (15')

^{1}H NMR ($CDCl_3$, 400 MHz)

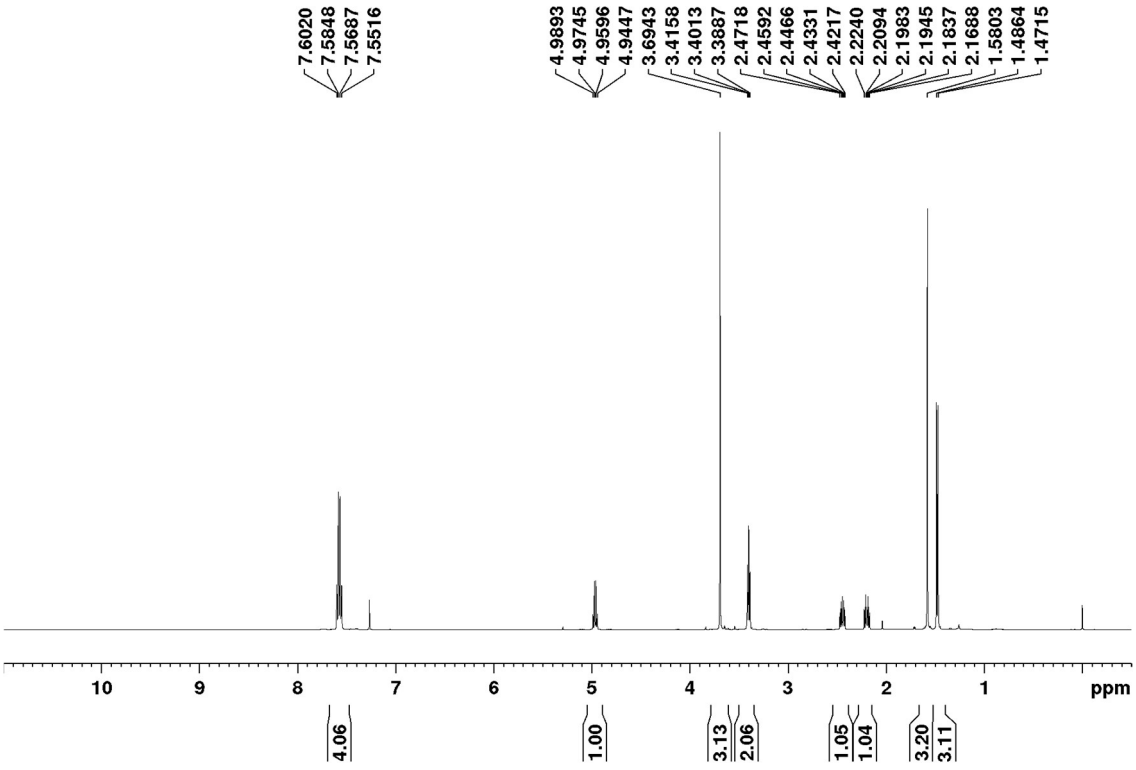


^{13}C NMR ($CDCl_3$, 125 MHz)

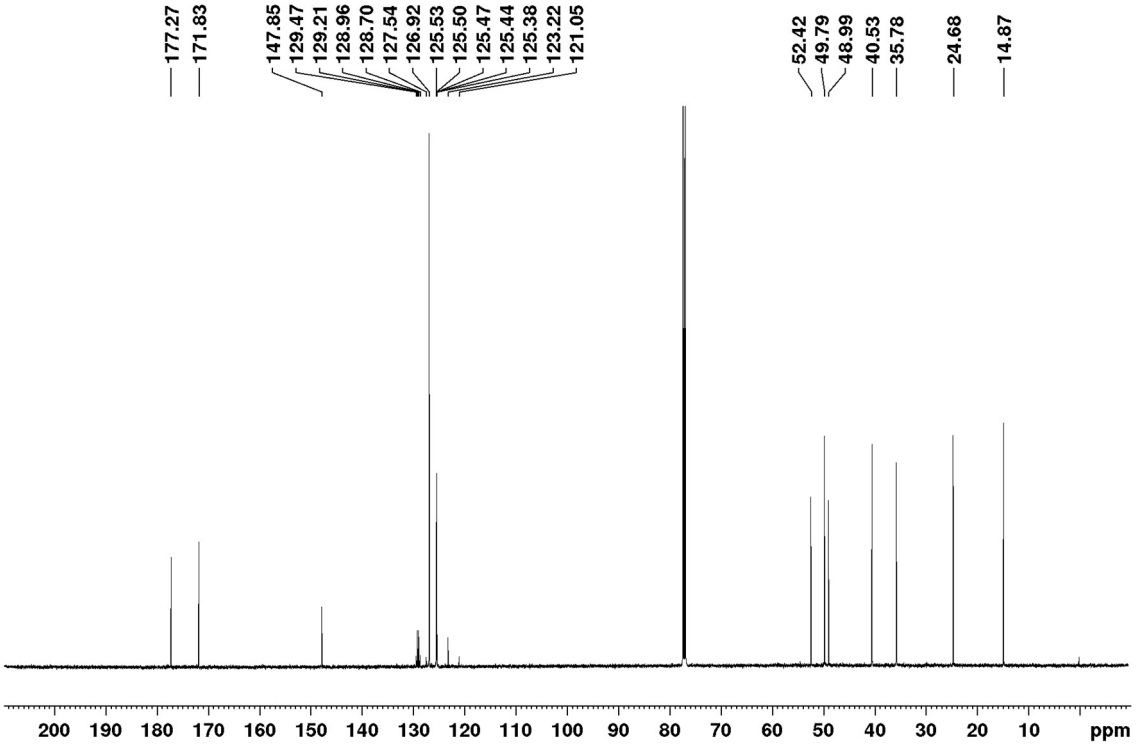


Methyl (*R*)-2-((*S*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanoate (16)

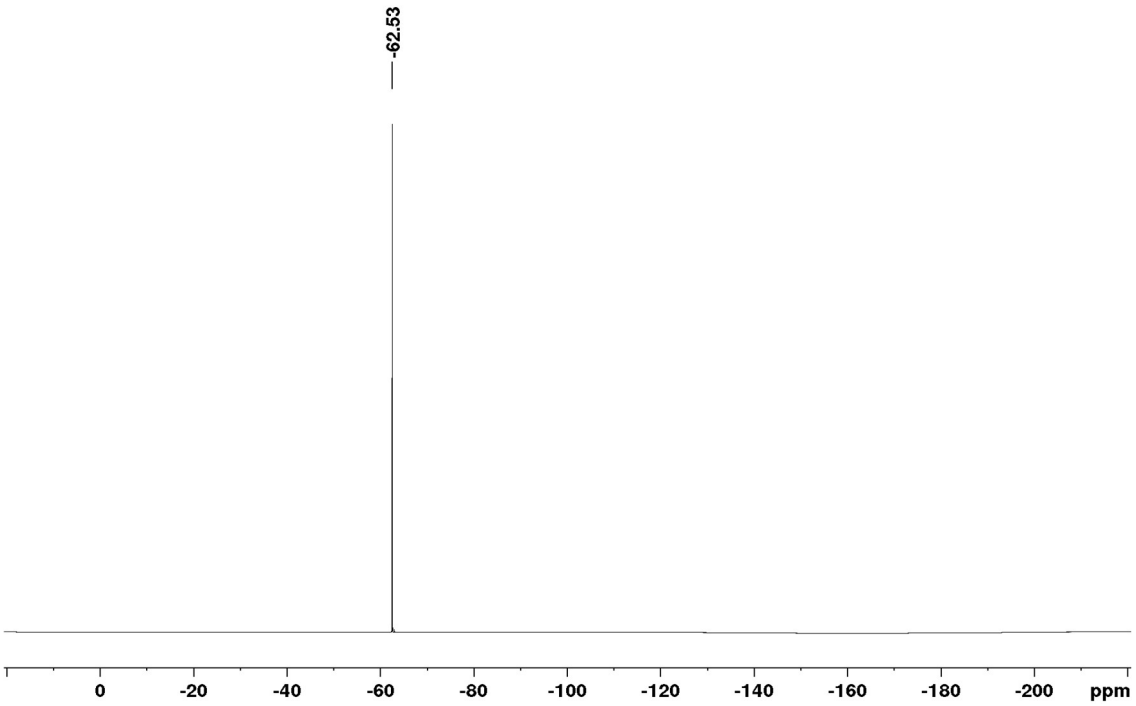
^{1}H NMR ($CDCl_3$, 400 MHz)



^{13}C NMR ($CDCl_3$, 125 MHz)

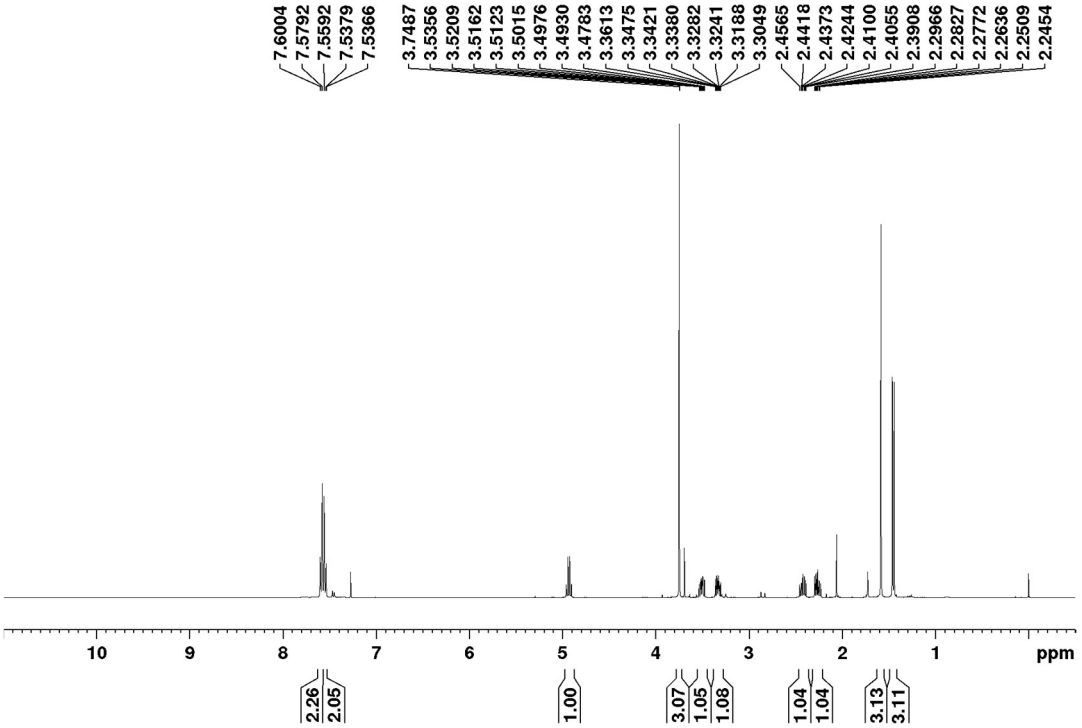


^{19}F NMR ($CDCl_3$, 470 MHz)

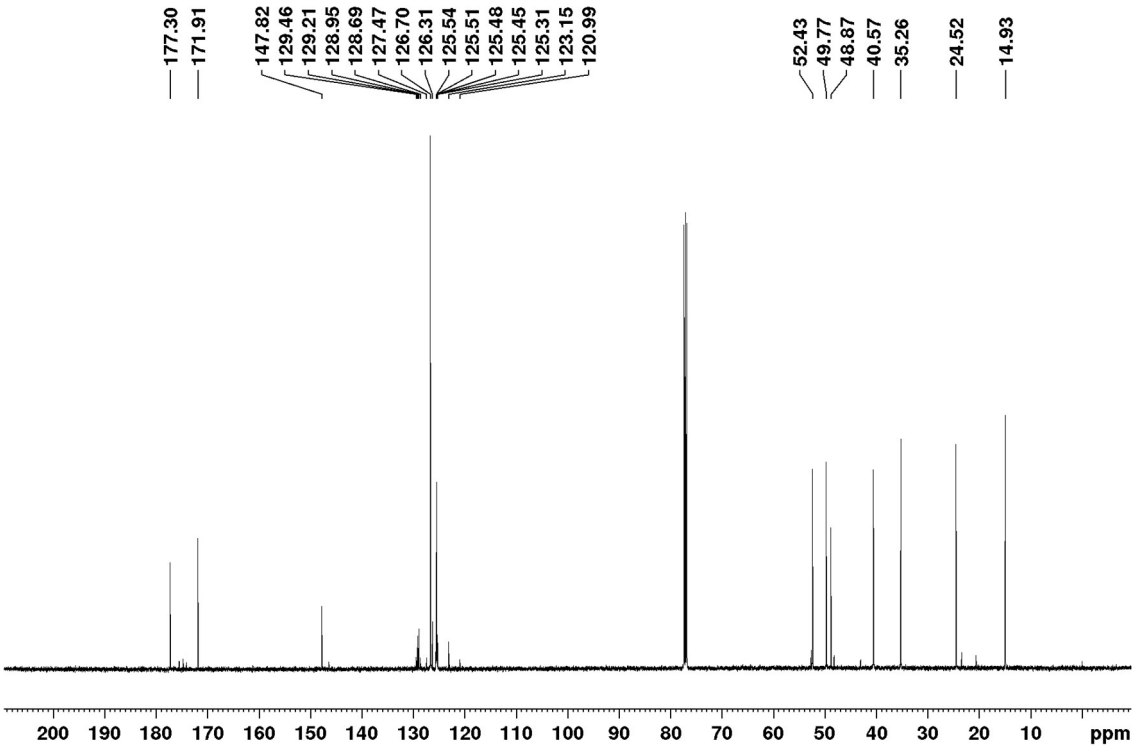


Methyl (*R*)-2-((*R*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanoate (16')

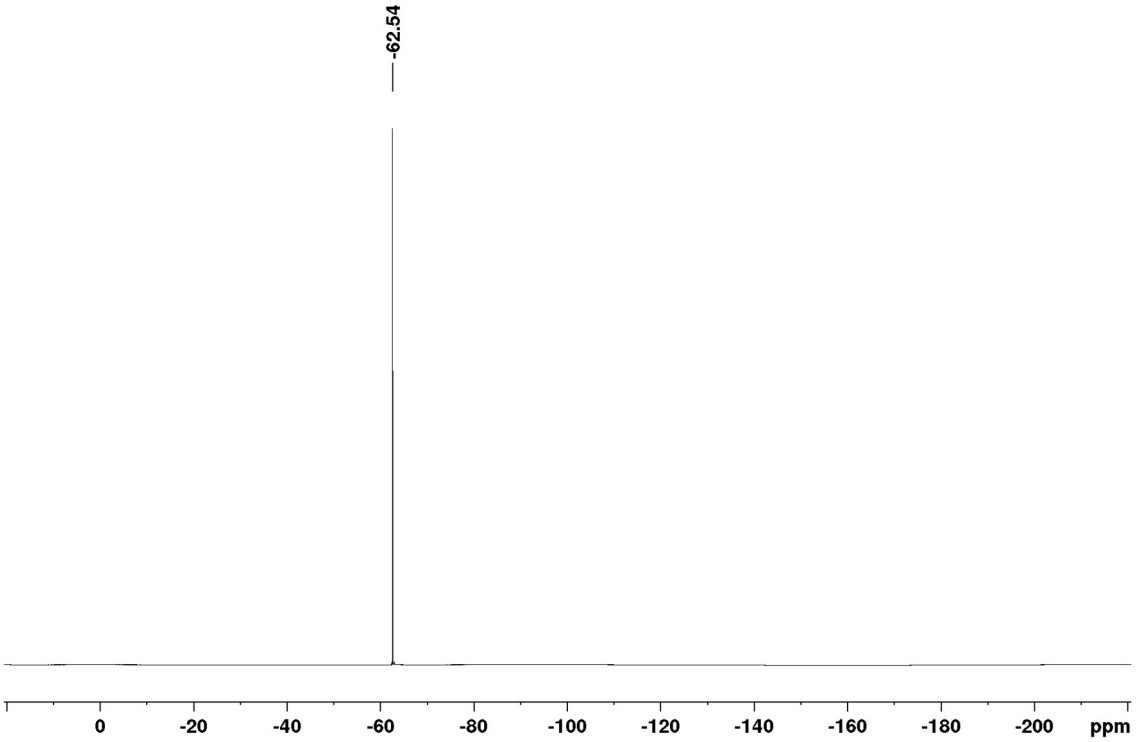
^{1}H NMR ($CDCl_3$, 400 MHz)



^{13}C NMR ($CDCl_3$, 125 MHz)

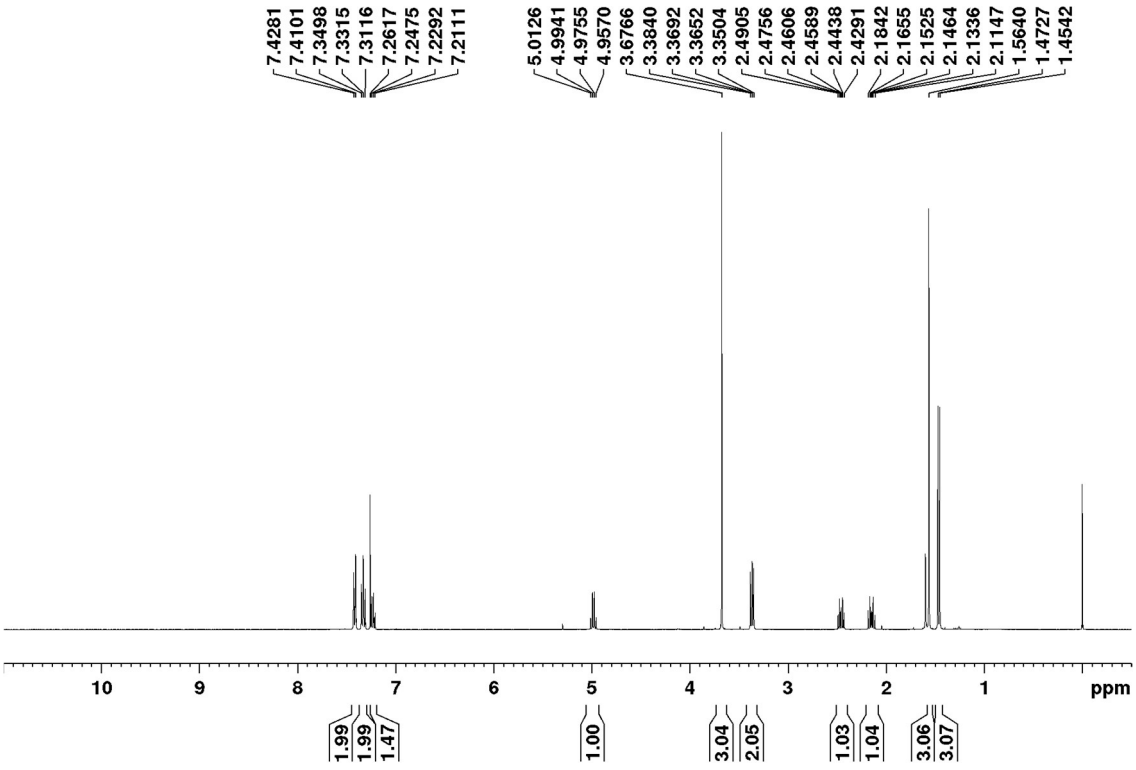


^{19}F NMR ($CDCl_3$, 470 MHz)

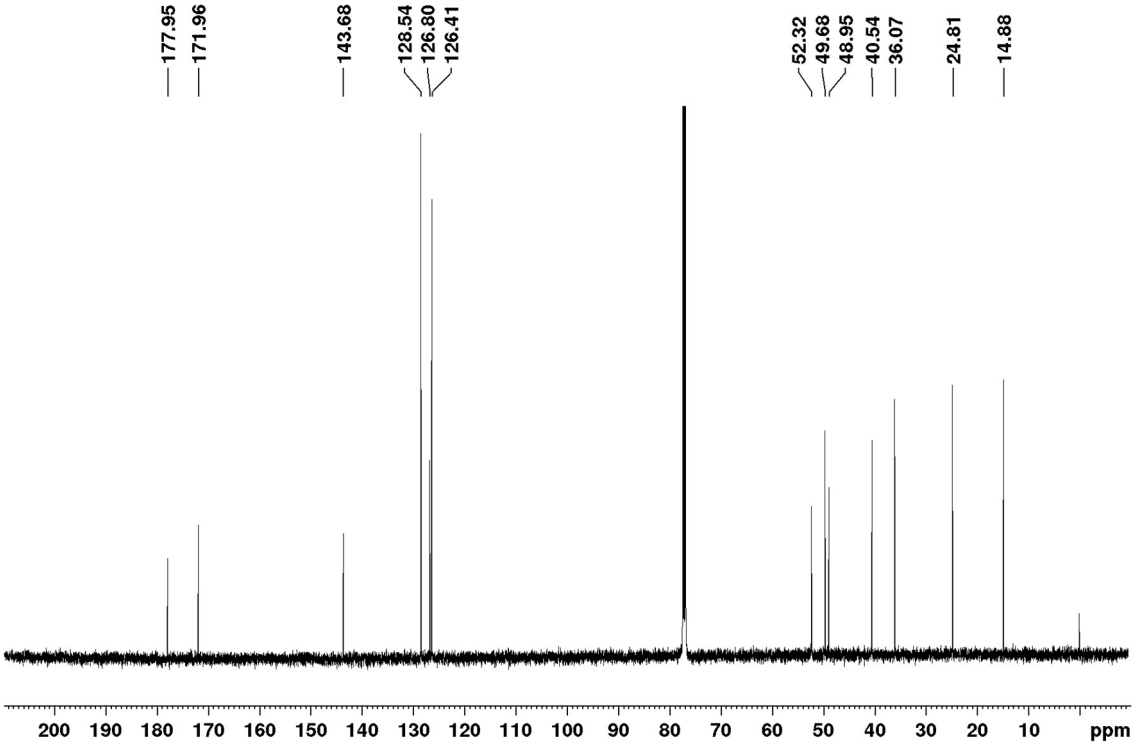


Methyl (*R*)-2-((*S*)-3-methyl-2-oxo-3-phenylpyrrolidin-1-yl)propanoate (17)

^{1}H NMR ($CDCl_3$, 400 MHz)

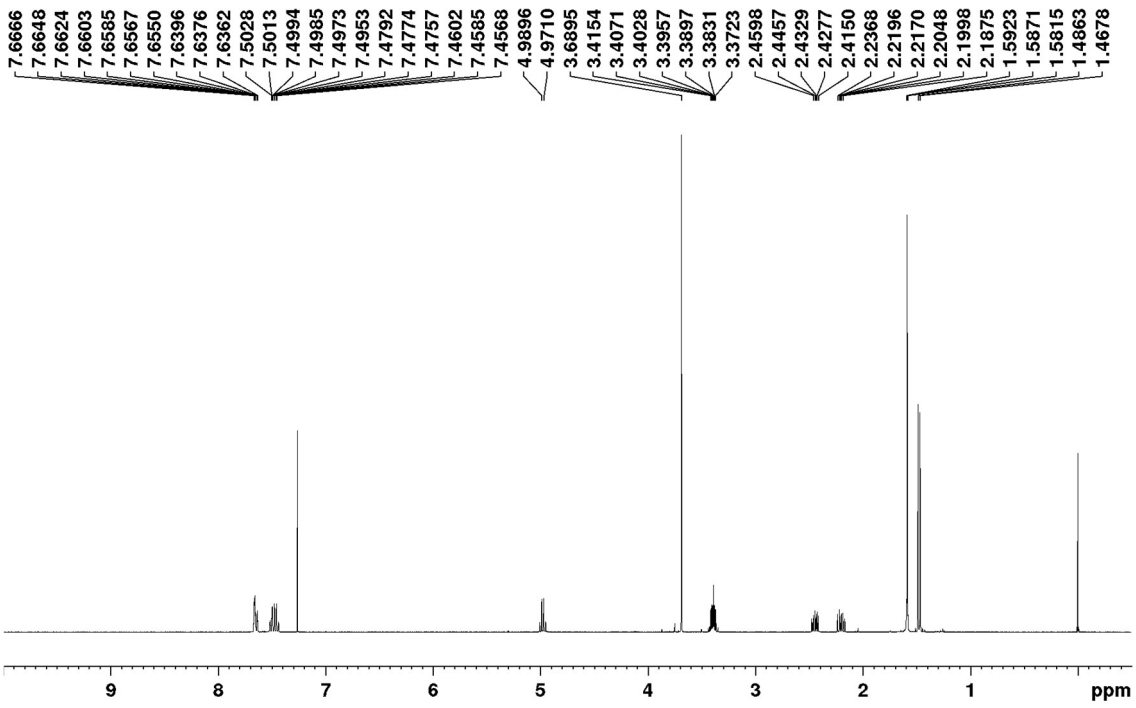


^{13}C NMR ($CDCl_3$, 125 MHz)

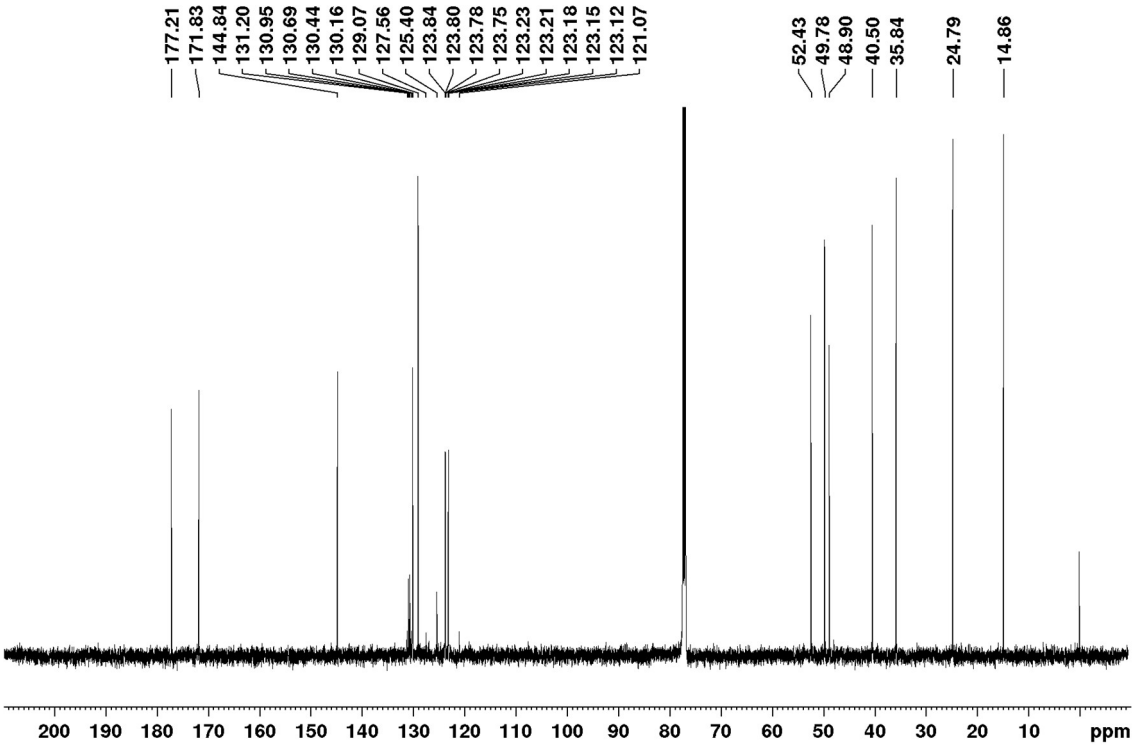


Methyl (*R*)-2-((*S*)-3-methyl-2-oxo-3-(3-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanoate (18)

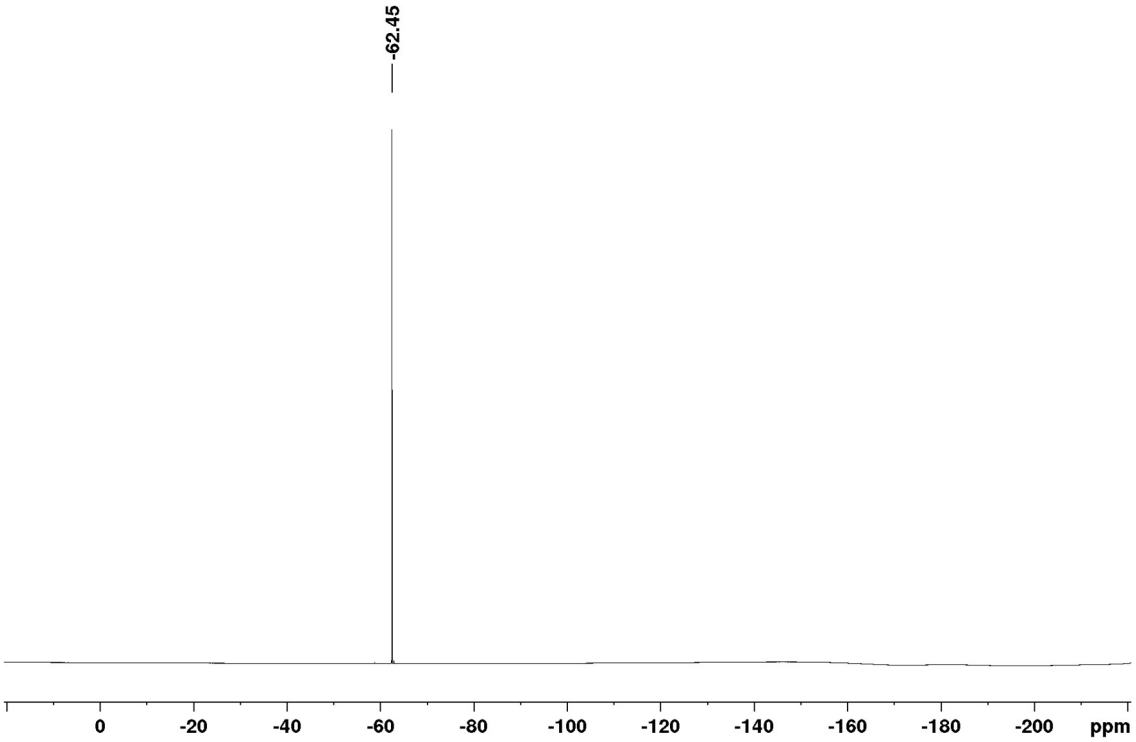
^{1}H NMR ($CDCl_3$, 400 MHz)



^{13}C NMR ($CDCl_3$, 125 MHz)

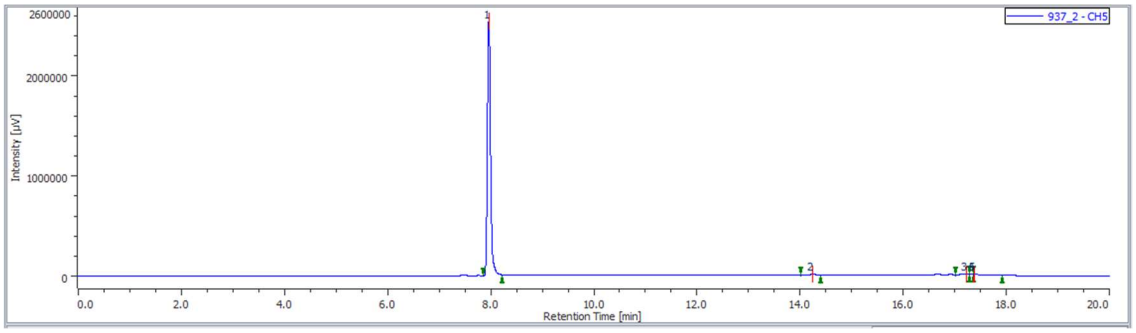


^{19}F NMR ($CDCl_3$, 470 MHz)

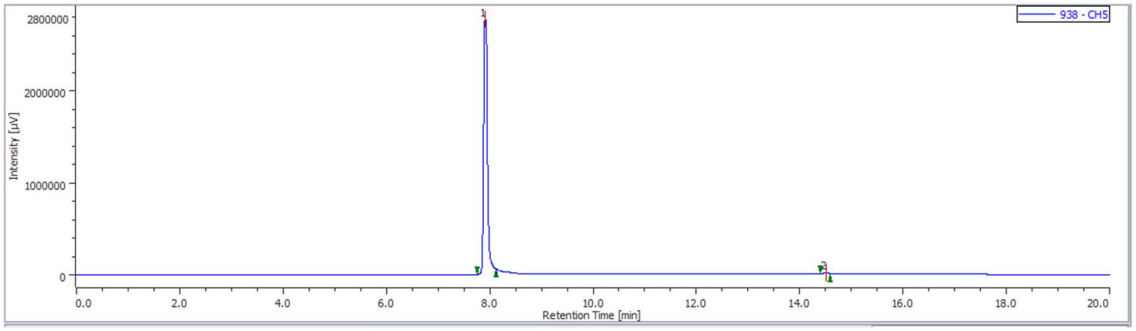


Purity analysis of tested compounds by HPLC

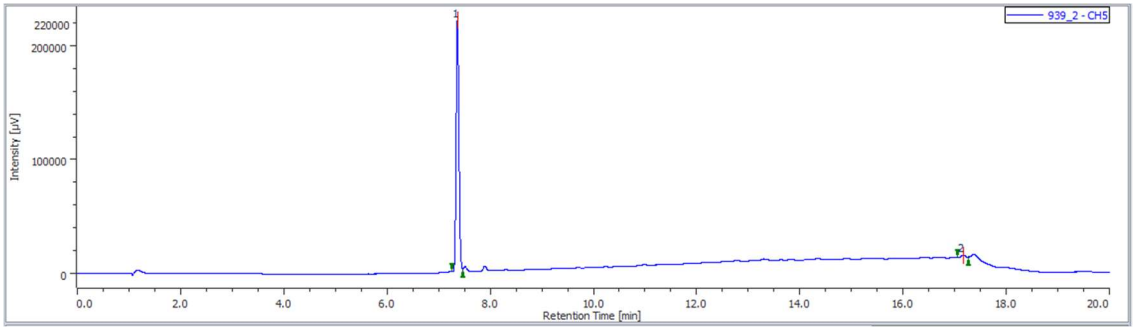
(*R*)-2-((*S*)-3-([1,1'-Biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-*N*-hydroxypropanamide (3)



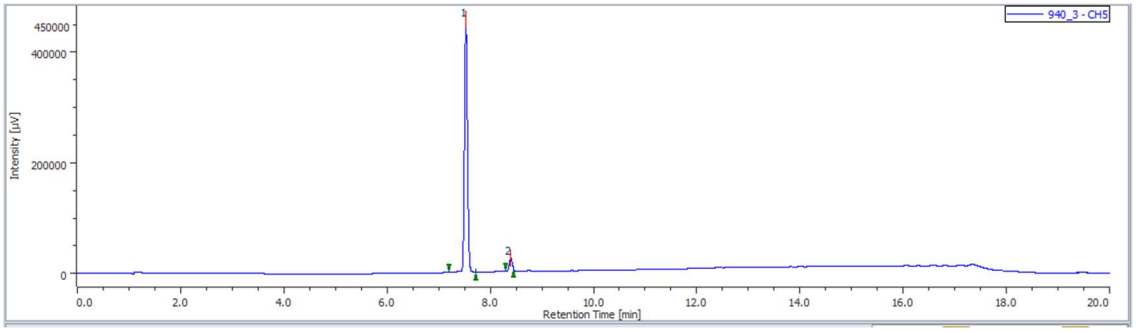
(*R*)-2-((*R*)-3-([1,1'-Biphenyl]-4-yl)-3-methyl-2-oxopyrrolidin-1-yl)-*N*-hydroxypropanamide (3')



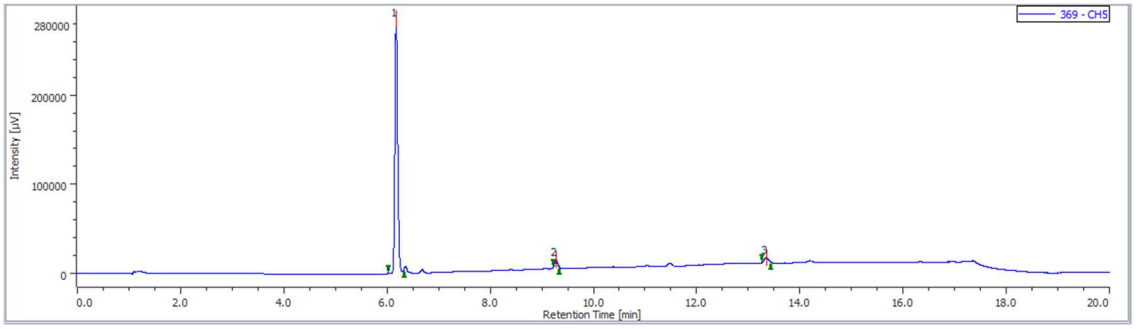
(*R*)-*N*-Hydroxy-2-((*S*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (4)



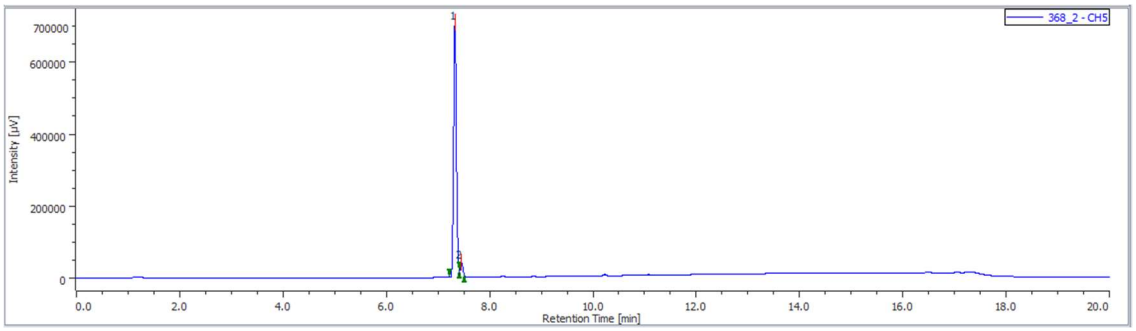
(*R*)-*N*-Hydroxy-2-((*R*)-3-methyl-2-oxo-3-(4-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (4')



(*R*)-*N*-Hydroxy-2-((*S*)-3-methyl-2-oxo-3-phenylpyrrolidin-1-yl)propanamide (5)



(*R*)-*N*-Hydroxy-2-((*S*)-3-methyl-2-oxo-3-(3-(trifluoromethyl)phenyl)pyrrolidin-1-yl)propanamide (6)



第六章

カルボランの置換基配置を駆使した

3 次元ケミカルスペースの網羅的探索

6-1　はじめに

第一章 **1-2-2** で述べたように、現在承認されている医薬品の分子形状は直線または平面的なものが大多数を占めている [1]。さらに第五章で指摘したように、PPI のような新規創薬標的に対する阻害剤を設計するためには、網羅的な SAR 解析を可能とする三次元性の高い母骨格の開発が望まれる。これまでに平面分子の脱却を図った戦略として、天然物の由来の立体的な母骨格を利用する設計や [2,3]、第一章 **1-2-3** で説明した単一の合成中間体から多様な母骨格を合成する Diversity-Oriented Synthesis（DOS）[4–6]、分子の立体性を示すパラメータ値の高い化合物で構成されるバーチャルライブラリの構築 [7] などが報告されている。しかし、これらの戦略で設計される構造は合成が困難でる、置換基が導入できる箇所の制限により網羅的な SAR 解析を妨げるといった課題がある。合成の容易さと網羅性のバランスが取れた母骨格を用いた戦略は、今までに報告がない。

上記の課題を解決できる母骨格として、二十面体構造を有するカルボランに着目した。これまでカルボランは、第二章、第三章の研究のように、特にその疎水性の高さに着目した研究が行われてきた。しかしながら、第二章での研究を通して、カルボランの異性体を使い分けることで置換基配置の厳密な制御が可能であることにも気づいた。実際、この置換基制御は、アンドロゲン受容体リガンド誘導体や manassantin A 誘導体においても確認できる [8,9]。このことから、Figure 6-1 に示すように、「カルボランを母骨格として、その 12 頂点から任意の位置に 3 つの置換基を導入すれば、全方向を空間的に網羅できる」と考えた。またカルボランの 12 頂点を構成する炭素原子とホウ素原子は相互に異なる反応性をもつため、様々な 3 置換カルボランを合成することができる [10]。しかし、実際にこれまで報告されている 3 置換カルボランの数は限られ [11,12]、いずれも医薬品候補化合物の開発を目的としていない。本章では、*o*-カルボランから骨格 I と II、*m*-カルボランから骨格 III と IV、*p*-カルボランから骨格 V の 5 パターンの 3 置換カルボランを合成し、その化合物の生物活性評価を行うことで、網羅的な SAR 解析を実現する。

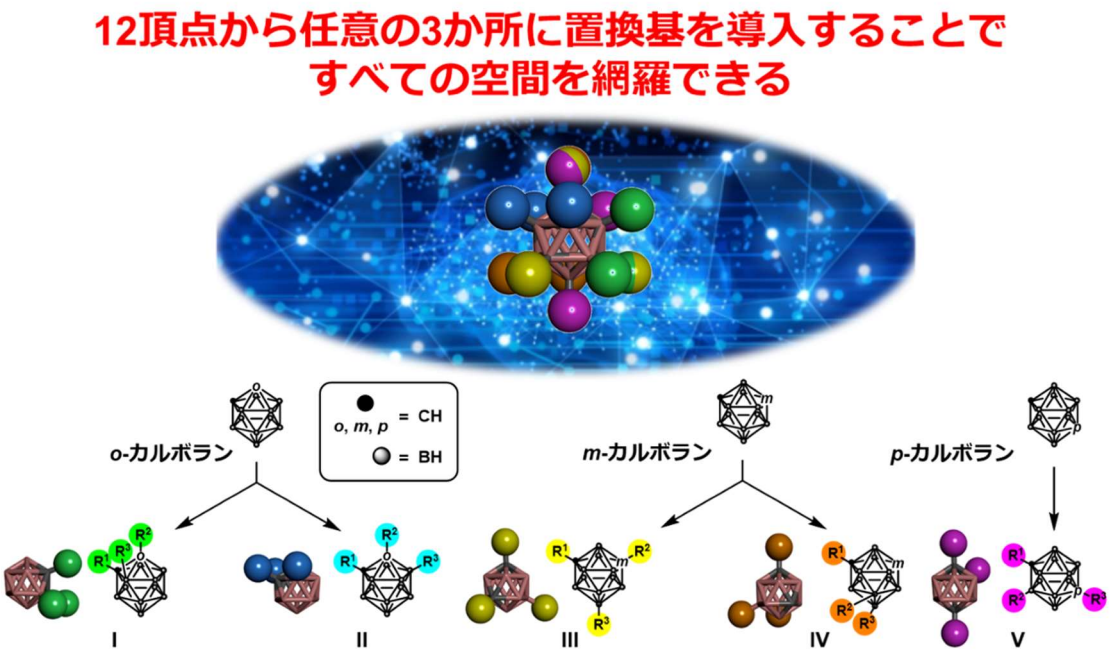


Figure 6-1. すべての空間を網羅する 3 置換カルボランの 5 つのパターン

6-2　3 置換カルボランの合成

3 置換カルボランの網羅的な SAR 解析の標的として、HIF-1α/p300 と狂犬病ウイルス（RABV）の N-タンパク質の PPI を設定した。いずれの PPI 界面にも、α-helix が確認されており、これは他の PPI にも多く見られる構造である [13]。この α-helix を構成するアミノ酸のうち、PPI のホットスポットに位置する疎水性アミノ酸残基が、よくペプチド模倣分子のテンプレートとして利用される [14–17]。疎水性アミノ酸残基の中でもフェニルアラニンとロイシンが、ホットスポットとして頻出するため [18]、カルボラン骨格に対して、それぞれのアミノ酸残基を模倣したベンジル（Bn）基とイソブチル（^{i}Bu）基を置換基として導入することとした。

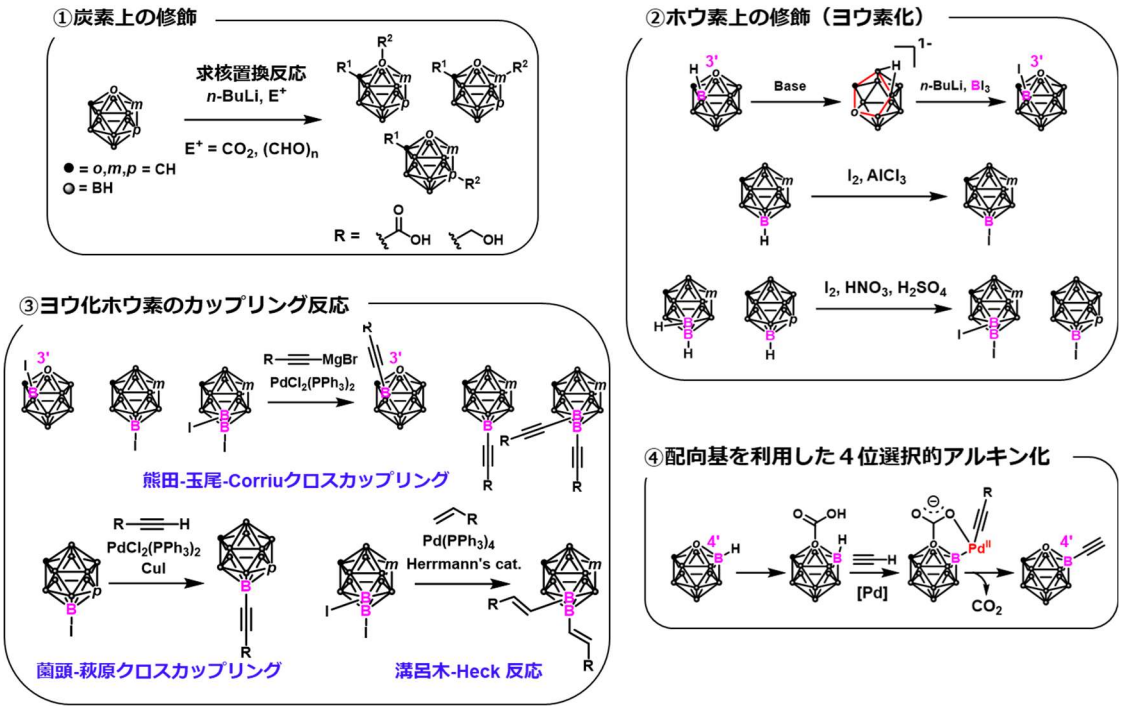
次に、5 パターンの骨格合成に利用する反応として、報告されているカルボランの反応から、より堅牢性の高いものを選択した（Scheme 6-1）。

① カルボランアニオンを経由した求核置換反応；カルボランの反応として最も一般的に使われる反応である。カルボランの C-H の酸解離定数 pKa（*o*-体：23、*m*-体：28、*p*-体：30）は、*n*-BuLi の pKa（35）に比べて低いため、*n*-BuLi 存在下で容易に脱プロトン化し、二酸化炭素やパラホルムアルデヒドなどの求電子剤と反応させることで、C 置換カルボランが得られる [19]。

② ホウ素の位置選択的なヨウ素化反応；カルボラン骨格の各ホウ素原子の電子密度が異なることを利用して、位置選択的なヨウ素化が行える。炭素原子から最も離れたホウ素原子の電子密度が高く、*o*-体であれば 9,12 位、*m*-体であれば 9,10 位、*p*-体であれば 2 位のホウ素原子がヨウ素化される [20]。またヨウ素の当量や酸触媒を変えることで、一置換体と二置換体をそれぞれ選択的に得ることができる [21]。*o*-体の 3 位のホウ素原子に関しては、*nido*-カルボランを経由したヨウ素化が可能である。3 位のホウ素原子は、最も電子密度が低いため、フッ化物イオンや水酸化物イオンにより選択的に脱ホウ素化反応が進行する。続く、*n*-BuLi による脱プロトン化により、ジアニオンを生成させ、トリヨウ化ボラン（BI_3）を反応させると、3 位にヨウ素が導入された *o*-カルボランの合成が可能である [22]。

③ ヨウ化カルボランに対するカップリング反応；ヨウ化カルボランは、ヨウ化アリール基のような反応性を示すことが知られており、グリニャール試薬を用いた熊田-玉尾-Corriu クロスカップリング [21]、末端アルキンを使った薗頭-萩原クロスカップリング [23]、ビニル基との溝呂木-Heck 反応 [24] により、B-C 結合を形成することができる。

④ 配向基を利用した 4 位選択的アルキン反応；①～③で述べた反応は、2010 年以前から知られていたが、2016 年に Xie らによって、はじめて 4 位選択的な B-N[25]、 B-C[26] 結合の形成反応が報告された [11]。これらは、1 位の炭素原子に対してカルボン酸を配向基として導入することで Pd 触媒を 4 位のホウ素原子に近接させ、位置選択的な B-H 活性化を実現し、それぞれバックワルド-ハートウィグのアミノ化や薗頭カップリングを *in situ* で行うことで達成される。



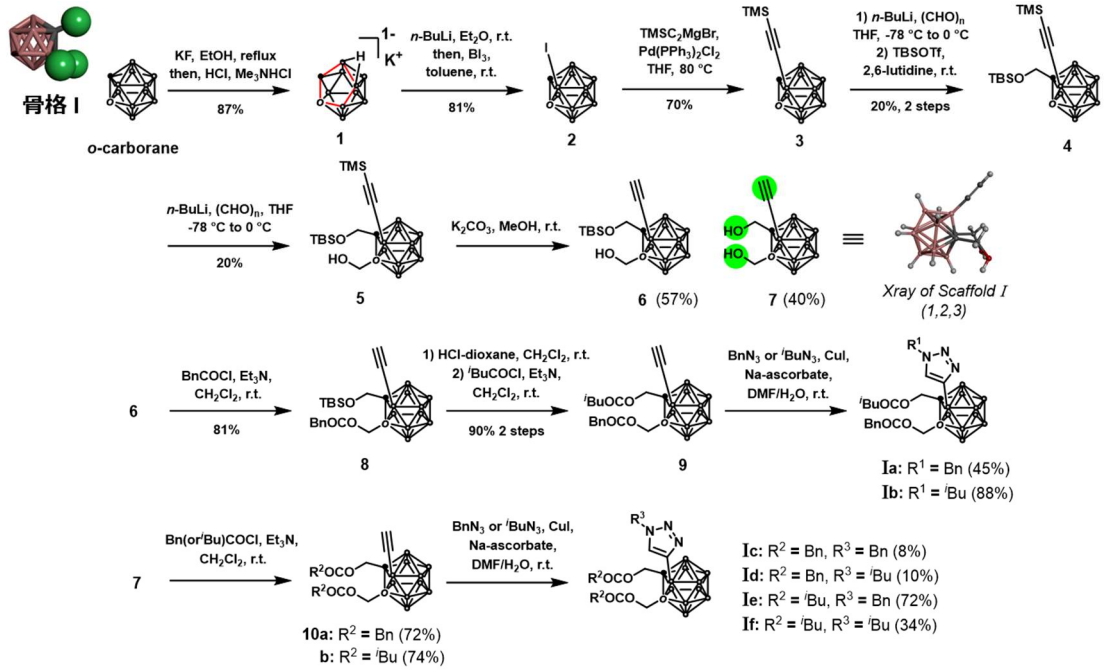
Scheme 6-1. 今回利用したカルボランの骨格構築反応

カルボランの母骨格と SAR 解析のための置換基を介するリンカーとしては、アミド基、エステル基、1,2,3-トリアゾール基を選択した。いずれも医薬品分子に頻出する官能基であり、アミド基やエステル基は、水素結合を形成することでタンパク質との相互作用を強めるだけでなく、化合物の親水性の向上にも寄与する。1,2,3-トリアゾール基は、窒素原子の双極子モーメントが大きいことから、アミド基と同じように水素結合を形成する官能基として用いられる [28,29]。ただし、*o*-カルボラン骨格に直接アミド基を導入すると、その電子吸引性の影響による脱ホウ素化反応が進行するため、化合物の安定性の観点から *o*-カルボランへのアミド基の導入は避けた [30,31]。

以上の合成指針の下、5 パターンの 3 置換カルボランを合成した。

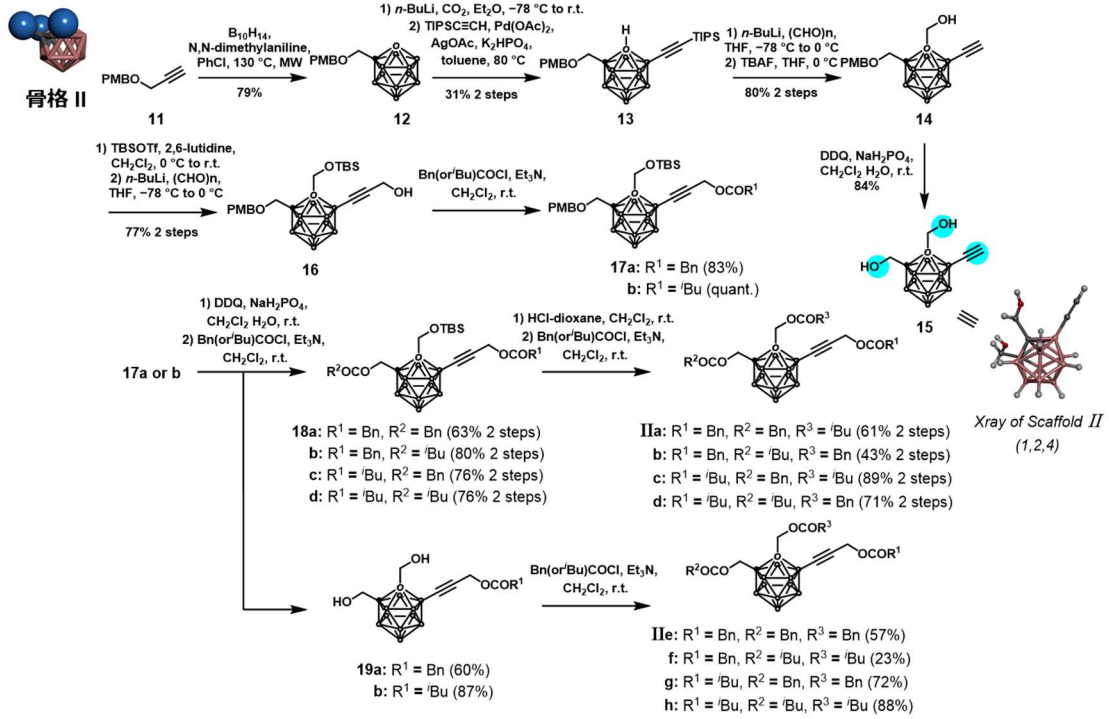
6-2-1　*o*-カルボランを用いた骨格 I と II の合成

骨格 I（1,2,3-trisubstituted-*o*-カルボラン）の合成を Scheme 6-2 に示す。まず、*o*-カルボランの 3 位の選択的ヨウ素化し [22]、得られた B3-ヨウ化カルボランと TMS アセチレンを熊田-玉尾-Corriu クロスカップリングにより反応させると、収率 70%でアルキン **3** が得られた [21]。アルキン **3** の C1 を *n*-BuLi でリチオ化した後、パラホルムアルデヒドを加え、得られたヒドロキシ基を *tert*-butyldimethylsilyl (TBS)基で保護した。C2 位についても同様にヒドロキシメチル基を導入することで、アルコール **5** が得られた。炭酸カリウムを用いてアルコール **5** のトリメチルシリル（TMS）基を除去すると、モノアルコール **6** とジアルコール **7** の混合物がそれぞれ 57%と 40%の収率で得られた。特に、ジアルコール **7** の構造は、X 線構造解析によって決定された。モノアルコール **6** をフェニルアセチルクロリドでエステル化すると、エステル **8** が 81%の収率で得られた。その後、TBS 基を除去し、得られたアルコールをイソバレリルクロリドでエステル化すると、90%の 2 段階収率でアルキン **9** が得られた。最後に、アルキン **9** を CuI とアスコルビン酸ナトリウムの存在下でベンジルアジドまたはイソブチルアジドと Hüisgen 環化付加反応を行い、3 置換カルボラン **Ia** と **Ib** をそれぞれ 45%と 88%の収率で得た。一方，ジアルコール **7** の 2 つのヒドロキシ基を同一の置換基でエステル化すると、アルキン **10a** と **10b** がそれぞれ 72%と 74%の収率で得られた。先と同様に、アルキン **10** に対してベンジルアジドまたはイソブチルアジドとの Hüisgen 環化付加反応を行うと、8～72%の収率でカルボラン **Ic-f** が得られた。このように、骨格 I の 3 置換カルボランの合成を達成した。



Scheme 6-2. 1,2,3-trisubstituted *o*-carborane（骨格 I）の合成

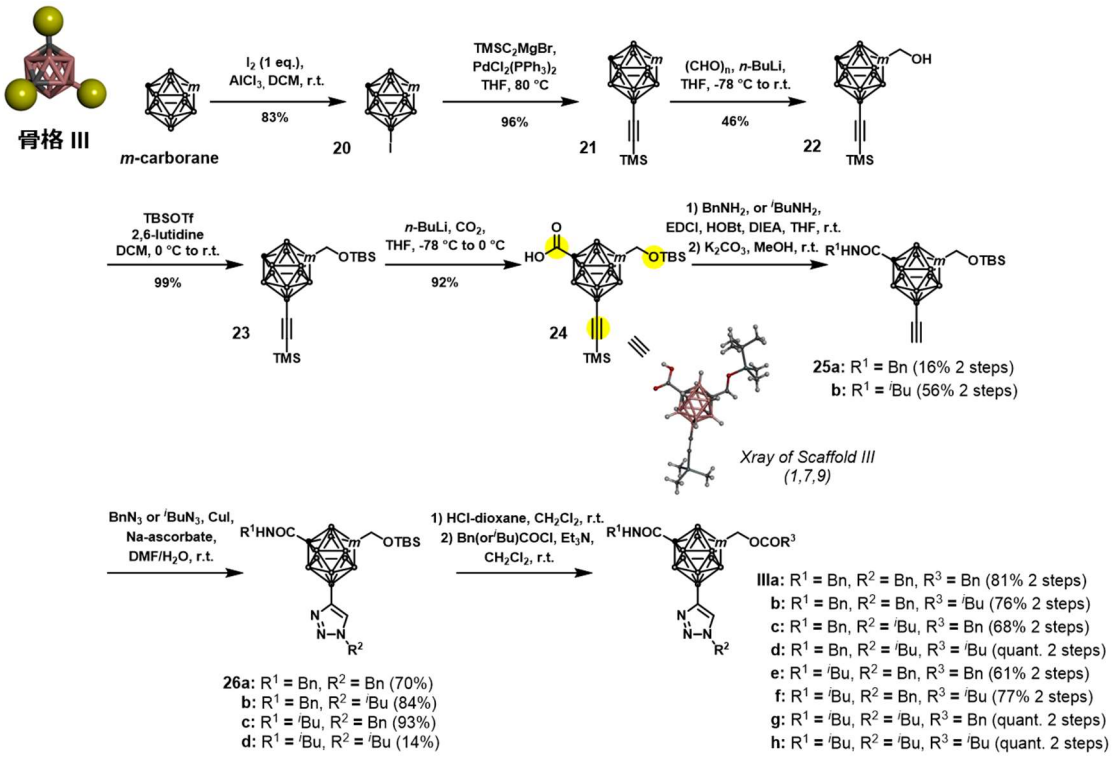
続いて、Scheme 6-3 に示すように、骨格 II（1,2,4-trisubstituted-*o*-カルボラン）の合成を行った。まず、プロパルギル *p*-メトキシベンジル（PMB）エーテル（**11**）とデカボランに対して、クロロベンゼン中、*N*,*N*-ジメチルアニリンの存在下に、マイクロ波（MW）を照射することで、1 置換 *o*-カルボラン **12** を収率 79%で得た [32]。次に、Xie らの報告に従い、*n*-BuLi と二酸化炭素を用いてカルボラン **12** の C2 位にカルボン酸部位を導入し、このカルボン酸を配向基として利用し、B4 位を選択的にアルキニル化し、化合物 **13** を 2 段階で 31%の収率で得た [26]。Scheme 6-1 で示した同様の工程で化合物 **13** の C2 位にヒドロキシメチル基を導入した後、アルキン部位のトリイソプロピルシリル（TIPS）基を除去し、化合物 **14** を 2 段階で 80%の収率で得た。化合物 **14** の PMB 基を除去すると、ジオール **15** が得られ、X 線構造解析により構造が決定された。また、化合物 **14** のヒドロキシ基を TBS で保護した後、末端のアルキンにヒドロキシメチル基を導入することで化合物 **16** が得られた。化合物 **16** のヒドロキシ基には、エステル結合を介してによって Bn 基（**17a**、収率 83%）または ^{t}Bu 基（**17b**、quant.）を導入した。続いて化合物 **17** の PMB 基を 2,3-ジクロロ-5,6-ジシアノ-*p*-ベンゾキノン（DDQ）を用いて選択的に除去し、フェニルアセチルクロリドまたはイソバレリルクロリドでエステル化すると、化合物 **18a-d** が得られた。最後に TBS 基を除去した後、エステル **18** の C2 位に Bn 基と ^{t}Bu 基を導入して、3 置換カルボラン **IIa-d** を 43-89%の収率で得た。さらに、化合物 **17** をジオキサン中、塩酸で処理し、得られたジオール **19** に Bn 基および ^{t}Bu 基を導入して、化合物 **IIe-h** を収率 23-88%で得た。



Scheme 6-3. 1,2,4-Trisubstituted *o*-carborane（骨格 II）の合成

6-2-2　*m*-カルボランを用いた骨格 III と IV の合成

まず、*m*-カルボランを出発原料として、骨格 III（1,7,9-trisubstituted *m*-カルボラン）を合成した（Scheme 6-4）。*m*-カルボランの B9 位を選択的にヨウ素化し、ヨウ化 *o*-カルボランの場合と同様の手順でアルキンを導入した。さらに Scheme 6-1 に示した化合物 **4** の合成方法と同じように、得られた 9-（トリメチルシリルエチニル）*m*-カルボランの C1 位にヒドロキシメチル基を導入し、続く TBS 保護を行うと、4 工程で 42%の収率で 1,9-2 置換 *m*-カルボラン **23** を得た。その後、*n*-BuLi と二酸化炭素を用いて、化合物 **23** の C7 位にカルボン酸を導入した。得られたカルボン酸 **24** を、1-（3-ジメチルアミノプロピル）-3-エチルカルボジイミド塩酸塩（EDCI）、1-ヒドロキシベンゾトリアゾール一水和物（HOBt）、*N,N*-ジイソプロピルエチルアミン（DIEA）の存在下、ベンジルアミンまたはイソブチルアミンを用いてアミド化した。続いて TMS 基を除去し、化合物 **25a** と **25b** をそれぞれ 16%と 55%の収率で得た。次に、この 2 つの化合物を、Scheme 6-1 に示した方法でベンジルアジドまたはイソブチルアジドとの Hüisgen 環化付加反応を行うと、化合物 **26a-d** を 14-93%の収率で得た。最後に、TBS 基を除去した後にエステル化を行うことで、3 置換カルボラン **IIIa-h** を 2 段階で 61%から定量的な収率で得た。

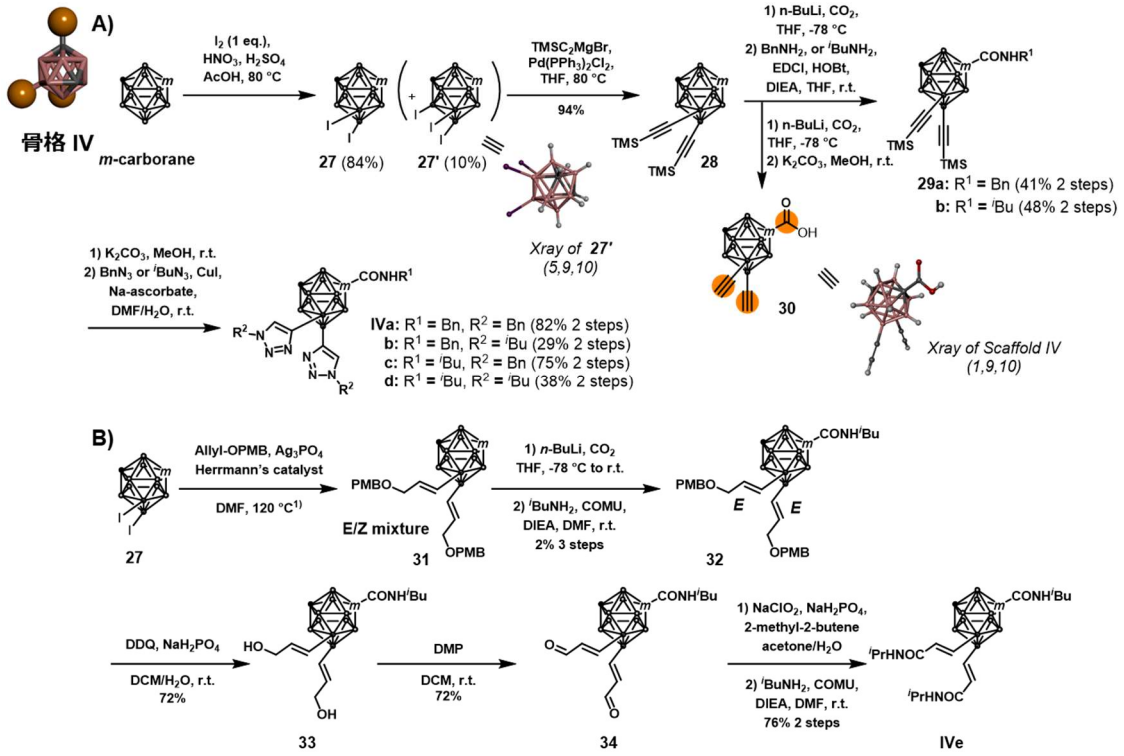


Scheme 6-4. 1,7,9-Trisubstituted *m*-carboranes （骨格 III）

続いて骨格 IV の合成（1,9,10-trisubstituted *m*-カルボラン）を Scheme 6-5 に示す。MW 照射下で *m*-カルボランの 9,10 位を選択的にジヨウ素化し、9,10-ジヨード-*m*-カルボラン **27** を 84%の収率で得た。驚くべきことに、これまで報告されていない 5,9,10-トリヨード-*m*-カルボラン **27'**が副生成物として収率 10%で得られた。**27** と TMS アセチレンとの熊田-玉尾-Corriu クロスカップリングを、Scheme 6-2 と同様の反応条件で行い、9,10-ビス（TMS-エチニル）-*m*-カルボラン **28** を収率 94%で得た。カルボラン **28** の C1 位にカルボン酸を導入した後、得られたカルボン酸に COMU を用いてベンジルアミンまたはイソブチルアミンを加えてアミド化反応を行い [33]、化合物 **29a** および **29b** をそれぞれ 2 段階で 41%および 48%の収率で得た。この時得られたカルボン酸の TMS 基を炭酸カリウム存在下で脱保護すると、化合物 **30** が 2 段階で 30%の収率で得られた。一方で、アミド **29** の TMS 基を除去した後、Hüisgen 環化付加反応を行い、化合物 **IVa-d** を 2 段階で 29-82%の収率で得た。なお、アミド **29** の TMS 基の脱保護から得られる 2 つのアルキンの反応性が同等であるため、1,2,3-トリアゾール基を介して異なる置換基を導入することは、困難であった。

更なる検討として、1,2,3-トリアゾール基の代わりにビニル基をリンカーとした **IVe** の合成を行った（Scheme 6-5B）。ジヨード-*m*-カルボラン **27** に対して、報告されている条件で 1-((allyloxy)methyl)-4-methoxybenzene を反応させると化合物 **31** を位置異性体の混合物で得た。そして、化合物 **29** を合成した手順で、C1 位にカルボン酸の導入、アミド化を行い、シリカゲルカラムクロマトグラフィーにより精製すると、*E* 体の単一化合物 **32** を 3 工程で 2%の収率で得た。PMB 基を除去した後、得られたアルコールを、Dess-Martin 試薬による酸化、続く Pinnick-Kraus 酸化と二段階の酸化条件に伏すことで、カルボン酸へと変換した。得られたカルボン酸とイソブチルアミンと縮合することで、**IVe** を 4 工程で 39%の収率で得た。

最後に、骨格 I と II を X 線結晶構造で明らかにしたように、**18**（骨格 III）、5,9,10-トリヨード-*m*-カルボラン **27'**、および化合物 **30**（骨格 IV）の構造も X 線構造解析によって決定した。

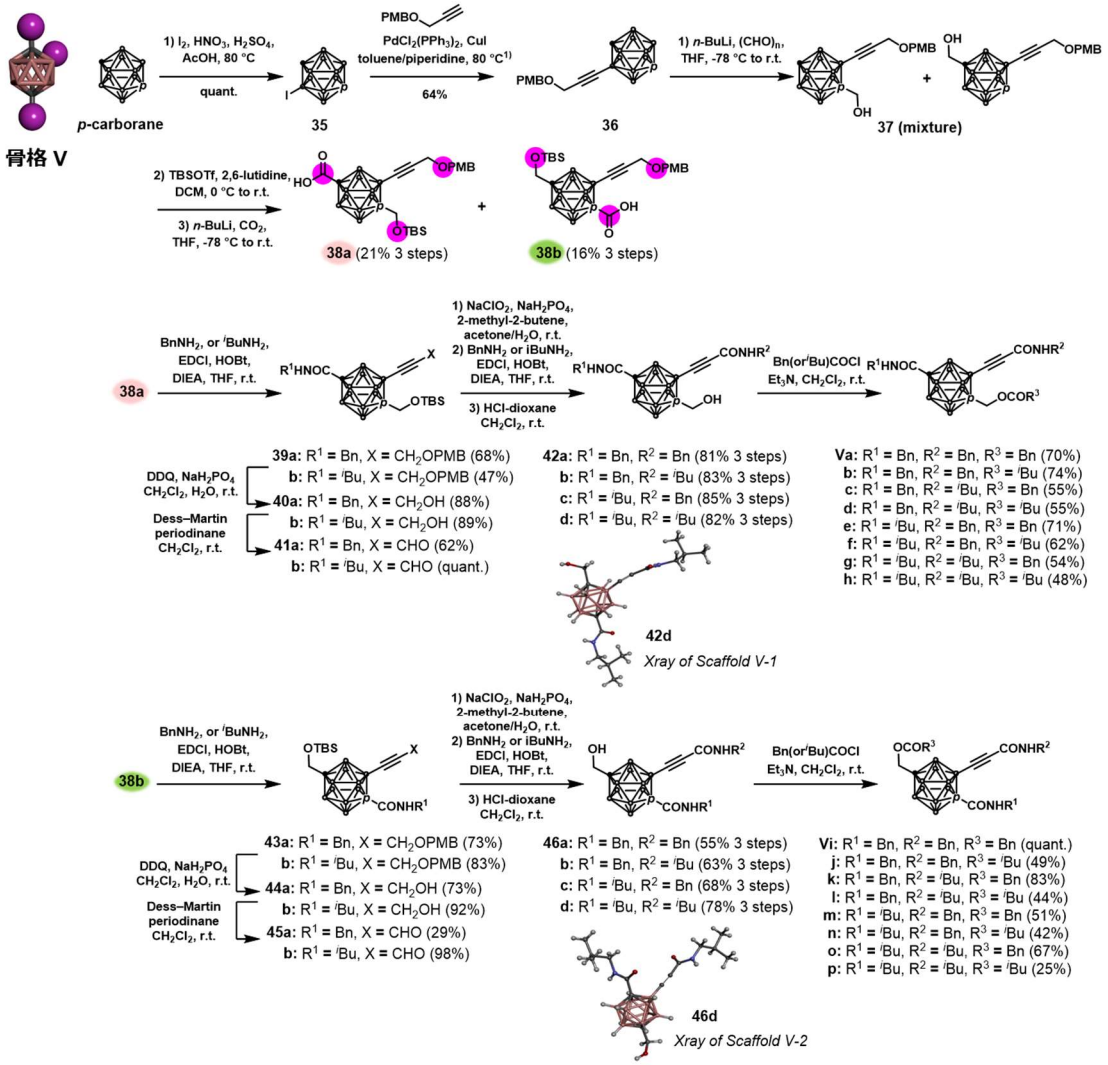


Scheme 6-5. (A) 1,9,10-trisubstituted *m*-carboranes （骨格 IV）の合成、(B) ビニル基をリンカーとして用いた化合物 **IVe** の合成

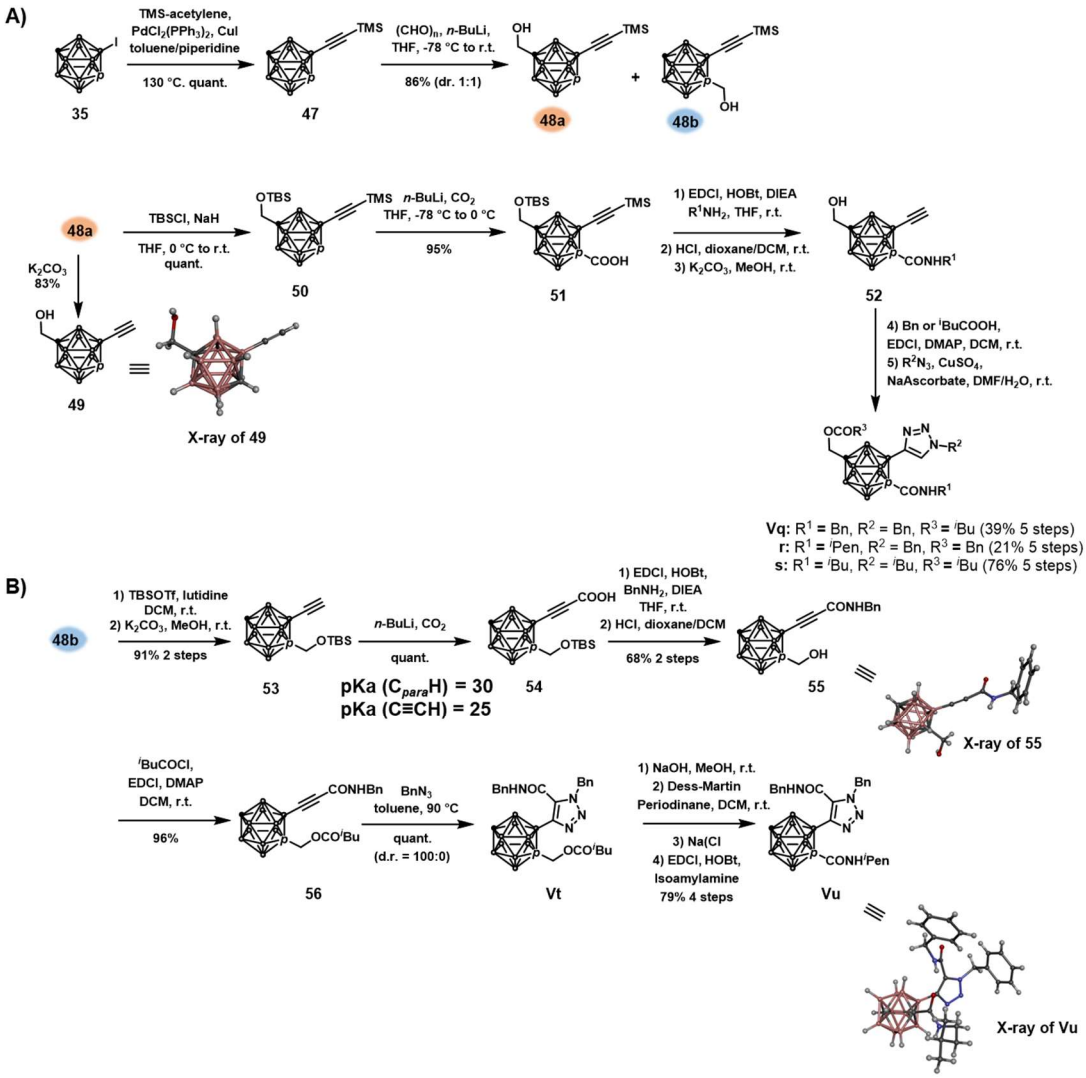
6-2-3　*p*-カルボランを用いた骨格 V とその誘導体の合成

最後に、*p*-カルボランを用いて, 1,2,12-置換カルボランの合成を行った（Scheme 6-6）。*p*-カルボランの B2 位を求電子的にヨウ素化した後、プロパルギル PMB エーテルを用いて薗頭-萩原クロスカップリングを行うと、2 段階で 49%の収率で B2 位置換カルボラン **36** が得られた。*n*-BuLi によるリチオ化とパラホルムアルデヒドへの付加反応により、**36** にヒドロキシメチル基を導入すると、1,2-二置換カルボランと 1,7-二置換カルボランがジアステレオマー混合物 **37** として得られた。ヒドロキシ基を保護するために TBS 基を導入した後、もう一方の炭素原子にカルボキシ基を導入して得られた混合物を、分取 HPLC を用いた分離操作を行うことで、3 工程で 1,7,12-三置換カルボラン **38a** と 1,2,12-三置換カルボラン **38b** をそれぞれ 21%と 16%の収率で得た。このカルボン酸 **38a** と **38b** を、Scheme 6-4 と同様の方法で、ベンジルアミンまたはイソブチルアミンを用いたアミド化反応を行った。続いて、PMB 基を除去した後、Dess-Martin 酸化を行うと、3 段階で 15-75%の収率でアルデヒド **31a**,**b** と **35a**,**b** が得られた。これらのアルデヒドを Pinnick-Kraus 酸化し、得られたカルボン酸を再びベンジルアミンまたはイソブチルアミンでアミド化した後、TBS 基を除去すると、**32a-d** および **36a-d** が 3 段階で 55-85%の収率で得られた。最後に、Scheme 6-1 と同様の手順でフェニルアセチルクロリドまたはイソバレリルクロリドでエステル化すると、化合物 **Va-p** が 25%-定量的な収率で得られた。この 2 つの異性体の絶対構造は、X 線結晶構造解析により **32d** と **36d** であることが確認された。

続く合成検討として、骨格 V の誘導体合成を行った（Scheme 6-7）。まず、骨格 **Vj,m,p** のエチニルアミド基を 1,2,3-トリアゾール基に変換した骨格 **Vq-s** の合成を Scheme 6-7A に示す。ヨウ化 p-カルボラン **35** に対して、Scheme 6-6 と同様の手順で、TMS アセチレンを用いた薗頭-萩原カップリングにより導入し、続く *n*-BuLi によるリチオ化とパラホルムアルデヒドへの付加反応により、得られたジアステレオマー混合物をシリカゲルカラムクロマトグラフィーで分離することで、1,7-二置換カルボラン **48a** と 1,2-二置換カルボラン **48b** を 2 段階で 86%の収率で得た（d.r. = 1:1）。カルボラン **48a** を炭酸カリウム存在下、TMS 基の脱保護を行うとアルコール **49** が定量的な収率で得られ、X 線結晶構造解析により、その構造を決定した。一方で、48a のヒドロキシ基を TBS 基で保護した後、*n*-BuLi によるリチオ化と二酸化炭素の付加反応により、カルボン酸 **51** を 2 段階で 95%の収率で得た。最後に、Scheme 6-4 と同じ方法で、ベンジルアミン、イソブチルアミンまたはイソペンチルアミンを用いたアミド化、TBS 基および TMS 基の除去、フェニル酢酸またはイソ吉草酸を用いたエステル化、ベンジルアジドまたはイソブチルアジドを用いた Hüisgen 環化付加反応を行うことで、骨格 **Vq-s** を 5 段階で 21～76%の収率で得た。



Scheme 6-6. 1,2,12-trisubstituted *p*-carboranes （骨格 V）の合成



Scheme 6-7. 骨格 V の誘導体合成；A) 1,2,3-トリアゾールをリンカーとして用いた 3 置換カルボラン **Vq-s** の合成、B) 3 置換トリアゾールを有する 3 置換カルボラン **Vt**、**Vu** の合成

続いて、**48b** を出発原料として、1,2,3-トリアゾール基を介して 2 つの置換基を有する 3 置換カルボラン **Vt, Vu** の合成を行った（Scheme 6-7B）。まず、**48b** のヒドロキシ基を TBS 基により保護した後、炭酸カリウム存在下、TMS 基を選択的に除去することで **53** を 2 段階で 91%の収率で得た。続いて、*p*-カルボラン 12 位の炭素原子にある水素（pKa = 30）よりも、末端アセチレン上の水素（pKa = 25）の方が脱プロトン化しやすいことを利用して、1 当量の *n*-BuLi によるリチオ化と二酸化炭素の付加反応により、アセチレン末端にカルボキシ基が導入された化合物 **54** を定量的な収率で得た。続いて、ベンジルアミンを用いたアミド化をした後、塩酸酸性条件下、TBS 基を除去することで、**55** を 2 段階で 68%の収率で得た。なお、化合物 **55** は X 線結晶構造解析にて構造を同定した。

55 のヒドロキシ基を、フェニルアセチルクロリドを用いてエステル化し、**56** を 96%の収率で得た。続いて、**56** とベンジルアジドをトルエン溶媒中、100 ℃で反応させると 1,3-双極子環化付加反応が進行し、驚くべきことに 1,2,3-トリアゾール基の 1,4,5 位が修飾された化合物 **Vt** のみが位置選択的に定量的な収率で得られた。一般に、2 置換アルキンに対して 1,3-双極子環化付加反応を行う場合、生成物の位置選択性制御が困難であるが、その生じる位置異性体は ^{1}NMR で異なる化学シフトを有するため、生成率を判断することができる（Figure 6-2）[34–36]。しかしながら、本反応においては、カルボランの立体障害を避けたためか、骨格 **Vt** の化学シフトのみが観測された。最後に、**Vt** のエステル基を水酸化ナトリウム存在下、加水分解によってヒドロキシ基に変換し、Scheme 6-5 と同様の手順で二段階酸化、続くイソアミルアミンとのアミド化により、**Vu** を 4 段階で 79%の収率で得た。そして、**Vu** の絶対構造を X 線結晶構造解析によって同定した。**Vt**, **Vu** の置換基配置が明らかになっただけでなく、興味深いことに **Vu** の 2 つのアミド基が 2.0 Å の近傍に位置しており、水素結合を形成する可能性があることが分かった（Figure 6-3）。

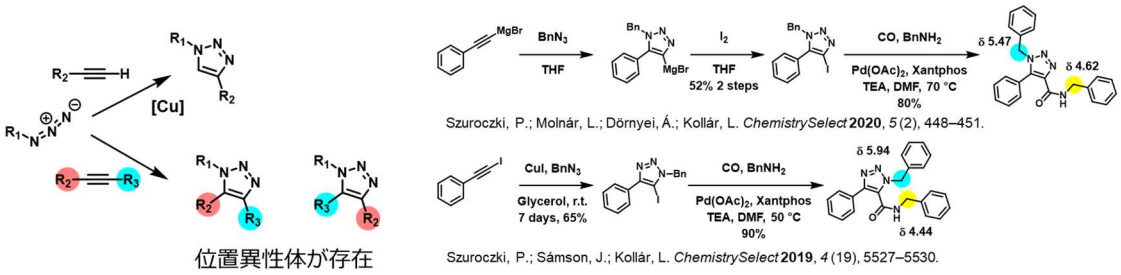


Figure 6-2. 3 置換トリアゾールの位置選択性制御

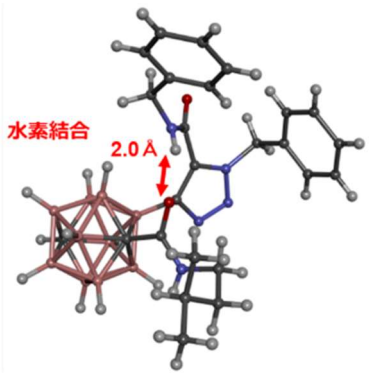


Figure 6-3. Vu の結晶構造で確認された水素結合

6-3　合成した 3 置換カルボランの PMI 解析

合成した化合物の分子形状を調べるために、PMI 解析を行った。序論で述べたように、この解析は、2-ブチン（棒状）、ベンゼン（円盤）、アダマンタン（球状）と比較して化合物の分子形状を評価するために、一般的に用いられる手法である [1]。予め、3 置換カルボランの立体配座を、LigandScout 4.4 の「iCon fast」オプションを用いて生成した [37]。各化合物生成した立体配座から RDKit[a]を用いて PMI 値を計算し、プロットした（Figure 6-4）。球状を表す右上に骨格 I および II の化合物（緑、青の点）が分布し、他の骨格よりも多く含まれていることが明らかとなった。一方、骨格 V（マゼンタの点）を用いた化合物は、グラフの棒状および円盤状の部分を占める傾向があった。これは、2 つの置換基が互いにパラ位で直線的に並んでいるためと考えらえる。*m*-カルボランに由来する骨格 III および IV の化合物（黄、橙の点）は、グラフ中央部を占めており、球状および円盤状の特徴をもつことが分かる。このように今回合成した、骨格 I～V でパターン分けされた 3 置換カルボラン化合物は、PMI 解析によって表される 3 次元の分子形状空間をすべての領域でカバーできることが示された。

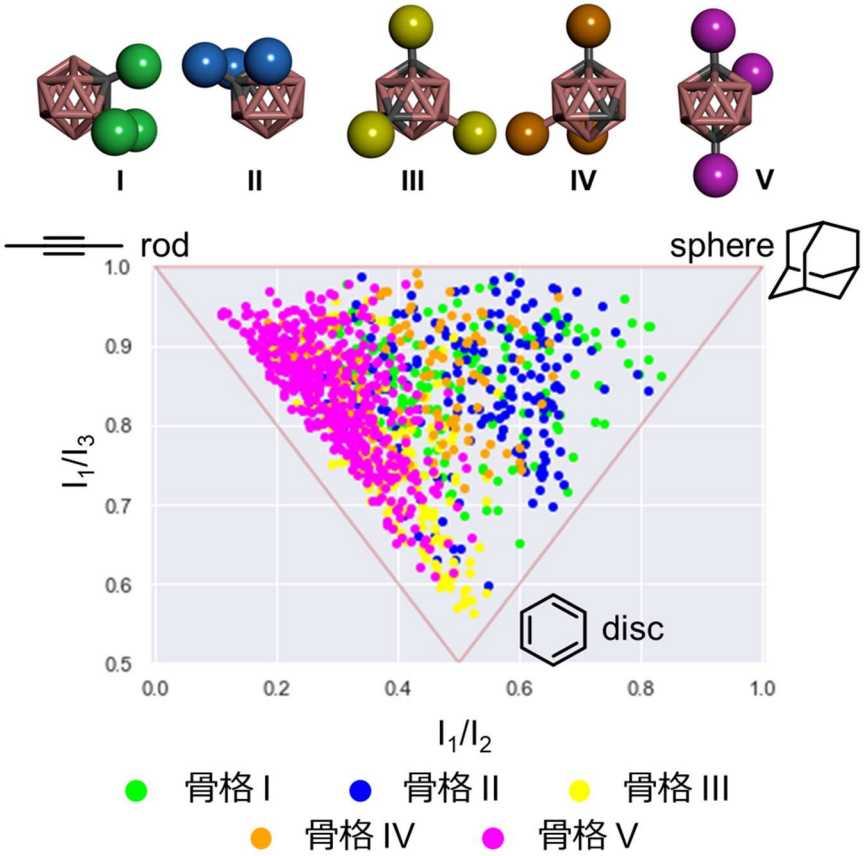


Figure 6-4. 合成した 3 置換カルボランの PMI 解析

[a] RDKit: Open-source cheminformatics tool; http://www.rdkit.org

6-4　合成した 3 置換カルボランの生物活性評価

3 置換カルボランライブラリーの生物学的活性を評価するため、細胞ベースでの低酸素誘導因子（HIF）-1 転写活性阻害、抗狂犬病ウイルス（RABV）活性、および細胞毒性を測定した。HIF-1 転写活性は、低酸素応答エレメント（HRE）依存性のルシフェラーゼレポーター遺伝子を安定的に導入した HeLa 細胞を用いて行った。化合物を添加し、低酸素条件下で 12 時間培養した後、ルシフェリンを添加し、得られた発光強度をから化合物の IC_{50} 値を算出した。なお、前述の通り HIF-1α の C 末端 helix（helix3）の Leu818、Leu819、Leu822 の 3 つのロイシン残基は、転写活性を誘導する HIF-1α と p300 の PPI に関与している [38]。一方で、抗 RABV 活性は N2a 細胞をベースに Gaussia ルシフェラーゼレポーターを融合させた狂犬病ウイルス RNA を用いてウイルス感染細胞を作成した後、化合物存在下で培養した。細胞外に分泌された細胞上清中のルシフェラーゼ活性を測定し、IC_{50} 値を決定した。RABV は、N-タンパク質の重合により増殖されることが知られており、その界面には、C 末端ヘリックスの Phe438、Phe441、Leu442 の疎水性残基がホットスポットとして存在する [39]。また、いずれのレポーター遺伝子を用いた測定は化合物の細胞毒性の影響を受けるため、MTT（3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide）法を用いて、HeLa 細胞および N2a 細胞に対する細胞生存率を測定した。

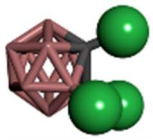
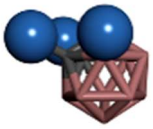
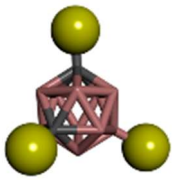
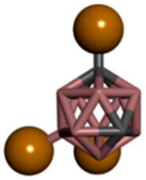
一連の結果を Table 6-1 に示す。まず合成した化合物のうち、骨格 V に属する **Vi-m** および **Vo** は、HeLa 細胞に対する毒性が確認されたものの（IC_{50} = 15.0-30 μM）、それよりも低濃度で HIF-1 の転写活性を有意に阻害することがわかった（IC_{50} = 3.24-23.2 μM）。また、**Vj**, **Vm**, **Vp** のリンカーであるエチニルアミド基を 1,2,3-トリアゾール基に変換した **Vq-s** も IC_{50} = 3.24-5.59 μM で HIF-1 の転写阻害活性を示した。一方で他の骨格 I～IV や V の異性体（**Va-Vh**）を骨格としてもつ化合物では、HIF-1 転写活性にほとんど影響を与えないことから、タンパク質の分子認識がカルボランの置換基導入パターンに強く依存することがが示唆される。また 3 置換トリアゾールを有する **Vt, Vu** も顕著な HIF 阻害活性を示した（IC_{50} = 1.34, 1.46 μM）が、興味深いことに **Vt** は **Vu** よりも HeLa 細胞に対して 10 倍程度強い細胞毒性も確認された（1.88 μM vs. 16.2 μM）。この違いは、**Vu** の X 線結晶構造解析（Figure 6-3）からわかるようにアミド基があることによって分子内の水素結合が形成されるが、**Vt** ではエステル基になっているため、この水素結合は形成せず、**Vt** と **Vu** の立体配座の安定性が異なることに起因すると考えられる。

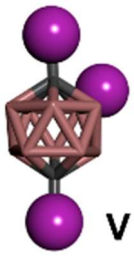
次に、骨格 II に属する **IIb-g** および骨格 V の **Va-h** は，RABV に対する抗ウイルス活性（IC_{50} = 2.17-8.37 μM）および宿主細胞に対する低い細胞毒性（IC_{50} > 30 μM）を示した。また、HIF-1 転写阻害活性の時と同様に、骨格 V の異性体を含めた他の骨格ではほとんど影響を与えないことが分かった。一方で、骨格 IV を有する **IVb** や 3 置換トリアゾールを有する **Vt** は、高い抗ウイルス活性（IC_{50} = 5.96 μM, 2.51 μM）と HIF-1 転写阻

害活性（IC_{50} = 17.4 μM, 1.34 μM）の両方の活性を示すため、作用機序を明らかにすれば 1 分子で複数の標的に作用するデュアル阻害剤としての利用が期待される[40]。

さらに、驚くべきことに **Vi-l**、**Vo**、**Vp** は、RABV の増殖を亢進することがわかった。これまでに、RABV の増殖を活性化する化合物の報告はなく、ここで初めて見いだされた化合物である。興味深いことに、同じ骨格 V のカルボランである化合物 **Va** が有意な抗ウイルス活性（IC_{50} = 3.87 μM）を示したのに対し、**Vi** は狂犬病ウイルスの増殖を活性化した。同じ骨格 V で、化合物 **Va** と **Vi** が同じ置換基で構成されている（R^1=R^2=R^3=Bn）にもかかわらず、こうした活性の違いが表れたことは、選択するリンカーも活性に影響を与えることが示唆された。同様に、化合物 **Va-h** と化合物 **Vi-p**、**Vq-s** の間にも活性の乖離が見られた。

Table 6-1. 合成した 3 置換カルボラン化合物の生物活性評価結果

Scaffold	Compound	HIF-1[a]	RABV[b]	HeLa cells	N2a cells
 I	**Ia**	> 30	> 9	> 30	> 30
	Ib	> 30	> 9	> 30	> 30
	Ic	> 30	> 9	> 30	> 30
	Id	> 30	> 9	> 30	> 30
	Ie	> 30	> 9	> 30	> 30
	If	> 30	> 9	> 30	> 30
 II	**IIa**	> 30	> 9	> 30	> 30
	IIb	> 30	5.74	> 30	> 30
	IIc	> 30	6.87	> 30	> 30
	IId	> 30	3.30	> 30	> 30
	IIe	> 30	8.14	> 30	> 30
	IIf	> 30	5.78	> 30	> 30
	IIg	> 30	8.37	> 30	> 30
	IIh	> 30	> 9	> 30	> 30
 III	**IIIa**	> 30	> 9	> 30	> 30
	IIIb	26.3	> 9	> 30	> 30
	IIIc	> 30	> 9	> 30	> 30
	IIId	23.0	> 9	> 30	> 30
	IIIe	> 30	> 9	> 30	> 30
	IIIf	> 30	> 9	> 30	> 30
	IIIg	> 30	> 9	> 30	> 30
	IIIh	> 30	> 9	> 30	> 30
 IV	**IVa**	> 30	> 9	> 30	> 30
	IVb	17.4	5.96	> 30	> 30
	IVc	> 30	> 9	> 30	> 30
	IVd	> 30	> 9	> 30	> 30
	IVe	> 30	> 9	> 30	> 30

	Va	> 30	3.87	> 30	> 30
	Vb	> 30	2.17	> 30	> 30
	Vc	> 30	4.44	> 30	> 30
	Vd	> 30	3.95	> 30	> 30
	Ve	> 30	3.46	> 30	> 30
	Vf	> 30	2.56	> 30	> 30
	Vg	> 30	5.08	> 30	> 30
	Vh	> 30	4.00	> 30	> 30
 V	**Vi**	5.46	Activation	16.7	> 30
	Vj	3.24	Activation	> 30	22.6
	Vk	3.64	Activation	15.0	21.2
	Vl	18.1	Activation	21.7	25.2
	Vm	23.2	> 9	> 30	> 30
	Vn	> 30	> 9	> 30	> 30
	Vo	9.21	Activation	20.9	7.90
	Vp	> 30	Activation	> 30	8.00
	Vq	5.59[c]	> 9	> 30[c]	> 30
	Vr	3.48[c]	> 9	> 30[c]	> 30
	Vs	3.24[c]	> 9	> 30[c]	> 30
	Vt	1.34	2.51	16.2	5.69
	Vu	1.46	-	1.88	-

The compound concentration required to inhibit the relative light units by 50% (IC_{50}) was determined based on semi-logarithmic dose–response plots. All the samples were tested in triplicate. [a] YC-1 was used as positive control with IC_{50} of 0.17 μM. [b] Favipiravir was used as a control with IC_{50} of 38 μM. [c] HIF and GI_{50} data were measured by Shao Y. and Li G. at Dalian University of Technology.

続いて、HIF-1 転写阻害活性を示した化合物 **Vo**, **Vi-Vk** を添加した HeLa 細胞における、HIF-1 の下流である Carbonic anhydrase (CA) 9 の発現量をウエスタンブロットによって確かめた（Figure 6-5B）[41]。なお、ポジティブコントロールとして HIF-1 阻害剤である GN44028 を用いた [42]。その結果、いずれの化合物においても HIF-1α の減少を伴わず、CA9 の発現量が減少していることが明らかとなった。さらに **Vk** に関して、RT-PCR により、CA9 の mRNA 量も調べたところ、**Vk** の添加による有意な減少が確認された（Figure 6-5C）。これらの結果は、化合物の HIF-1 転写阻害活性を裏付けるものとなった。

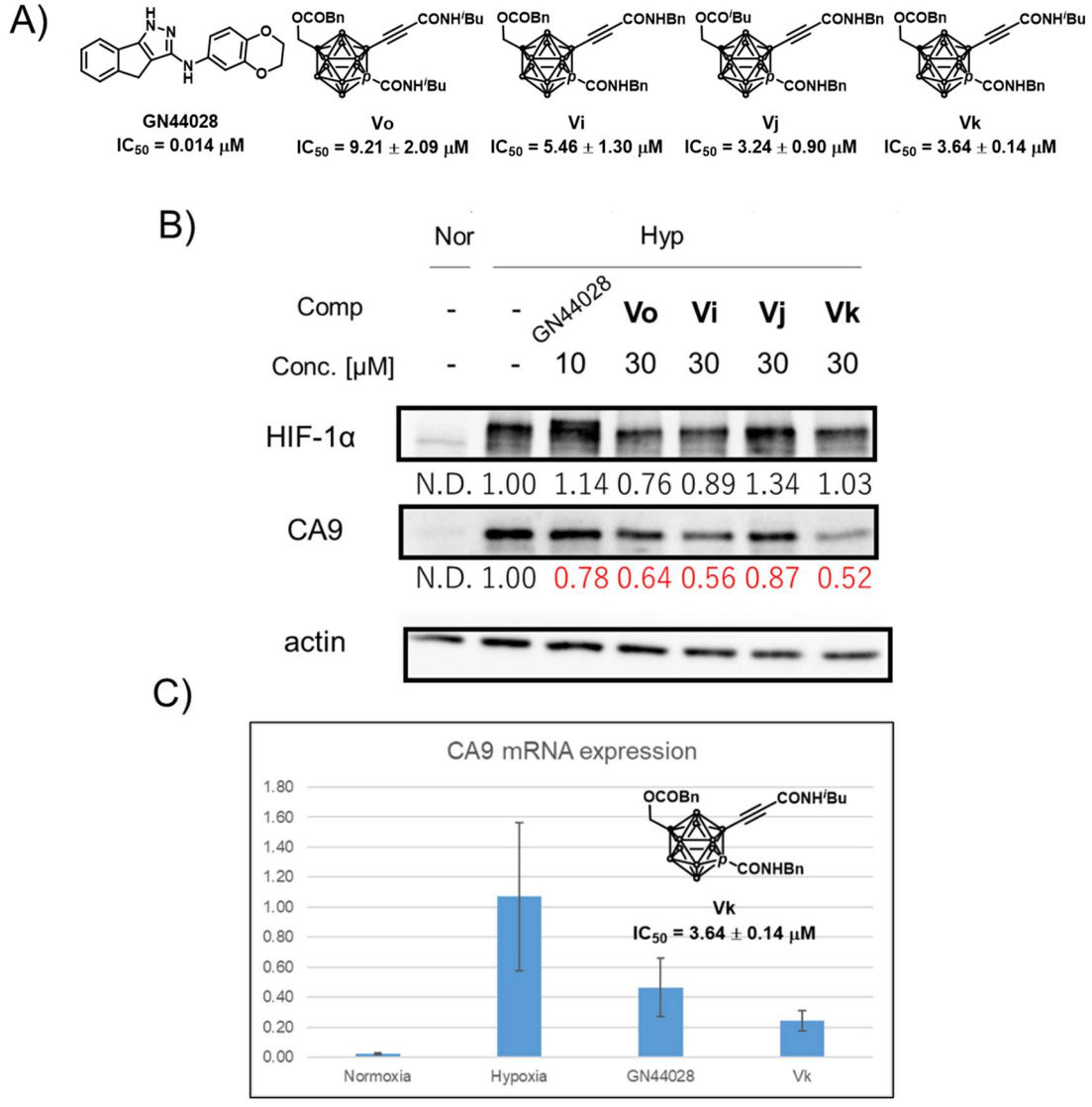


Figure 6-5. 3 置換カルボランの HIF-1 転写阻害活性評価；A）用いた化合物の構造と IC_{50}；B）ウエスタンブロットによるタンパク質定量、アクチンの蓄積量で標準化したそれぞれのタンパク質量を画像下部に示す。C）RT-PCR

最後に、ルシフェラーゼアッセイにて RABV の増殖亢進が示唆された **Vp** と同じ骨格をもつが活性が確認されなかった **Vn** において（Figure 6-6A）、RABV を感染させた N2a 細胞のウイルスタンパク質の蓄積をウエスタンブロットで確認した（Figure 6-6B）[b]。その結果、Vp の添加により、顕著なウイルスタンパク質の増加が確認された。一方で、放出されたウイルス力価の定量結果では、２つの化合物間で差は見られず、化合物を添加していない場合と同じだった（Figure 6-6C）[b]。RABV は、複製されたゲノム RNA と N-タンパク質がリボヌクレオタンパク質（RNP）を形成し、RNP が細胞外に放出されることを考慮し [43]、**Vp** は細胞外のウイルス力価を上げずに、細胞内のウイルスタンパク質の蓄積だけを亢進させていることから、**Vp** は、ゲノム RNA と N-タンパク質との結合を阻害し、RNP 形成を阻害していることが考えられる。ただし、今回の結果では完全な機構解明には不十分であり、**Vp** の効果が細胞に依存しないか、ウイルスゲノム量に影響を与えるかなどを調べることで、更なる機構解明につながると考えている。

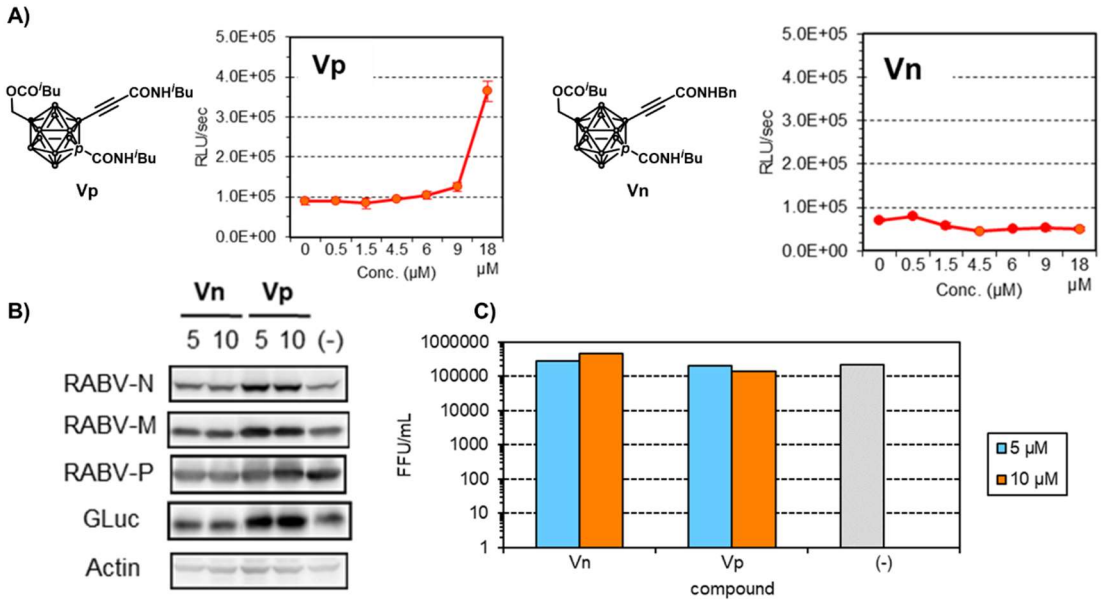


Figure 6-6. RABV 増殖亢進作用を有する化合物 **Vp** とネガティブコントロール **Vn**；A) ウイルスタンパク質の蓄積量、B) ウイルス力価の測定（forcus forming unit（FFU）／mL とは培養液 1 mL 中にウイルスによって変性した細胞がどのくらいいるかを表す。）

[b]山田健太郎博士（宮崎大学）との共同研究によって測定された。

6-5　側鎖の配置に基づく SAR 解析

前項で、3 置換カルボランの骨格に対する標的タンパク質の分子認識が明瞭であることを述べた。続いて、骨格ごとの側鎖に対する SAR matrix（SARM）による解析を行った。第五章で説明したように、SARM は縦軸に構造が類似した母骨格、横軸に側鎖を並べて、SAR をグリッドマップ化する。この方法に従って作成した RABV の阻害化合物の SARM を Figure 6-7 に示す。まず、側鎖に応じた活性変化が確認できた。今回用いた側鎖はフェニルアラニン、ロイシンの模倣であるベンジル基、イソブチル基であり、いずれも疎水性を有するが、ベンジル基は芳香族、イソブチルは脂肪族であることや、大きさの違い（V_{vdw} [Å^3][44]：ベンジル=118 vs.イソブチル=79）があるため、活性に対する違いが表れていると考えられる。次に、骨格ごとの SARM を見ていくと、骨格 V においては側鎖に対して非常に寛容であることが分かる。このことから、これらの化合物が標的タンパク質と結合しているホットスポット（もしくは活性ポケット）は、ある程度の大きめの空間が確保されていることが示唆される。一方で、骨格 II や IV では、顕著に活性が低下する側鎖の組み合わせが見られる。骨格 II の SARM を見ると、左下から右下にかけて活性が高くなる傾向が確認できる。これは、側鎖の組み合わせがベンジルリッチからイソブチルリッチになるパターンと一致している。このことから、骨格 II の化合物が結合するホットスポットでは、芳香族性のπスタッキングよりは、むしろ側鎖の大きさが活性に影響を与えることが示唆される。また骨格 IV では、**IVc** のみが活性をもち、かなり厳密な分子認識が確認できる。さらに他の骨格とは異なり、1,2,3-トリアゾール基がリンカーとして含まれていることに加えて、活性発現にはベンジルを側鎖に 2 つ有する必要がある。このことから、**IVc** と標的タンパク質のホットスポットには芳香族アミノ酸が含まれ、πスタッキングによる相互作用が重要であることが示唆される。このように、3 置換カルボランの SAR 解析を SARM によって行うと、母骨格が共通しているため、側鎖に対する活性の影響を系統的に調べることができる。

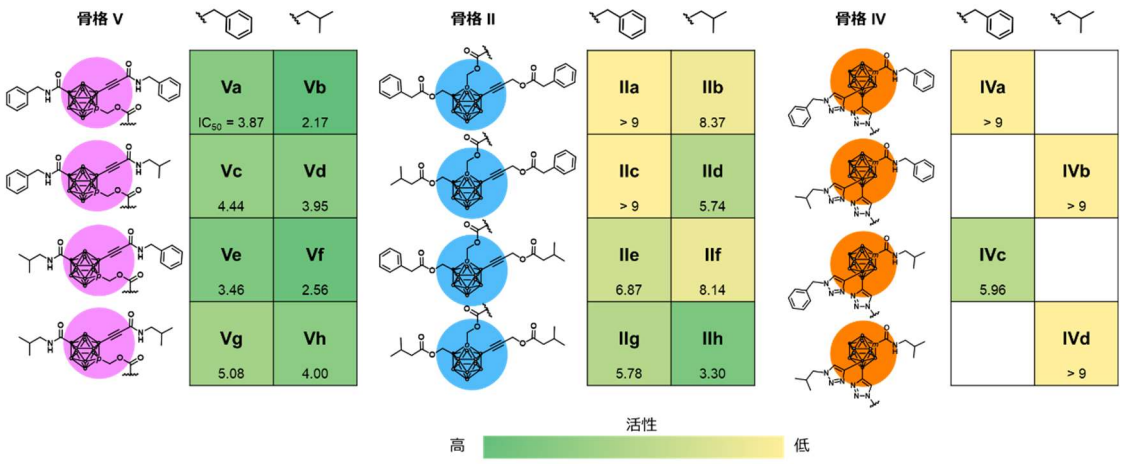


Figure 6-7. RABV 阻害化合物の SAR matrix

6-6　骨格 II および骨格 V の化合物構造を利用した相互作用界面の予測

前項の解析から骨格 II と V の化合物は、疎水性アミノ酸残基から構成される、ある程度の大きさのホットスポットで標的タンパク質と相互作用することが示唆されたため、共通の結合部位をもつのではないかと考えた。そこで、RABV 阻害活性を有する骨格 V の 8 化合物からファーマコフォアを作成し、そのファーマコフォアに対して骨格 II の化合物をフィッティングすることで検証を試みた（Figure 6-8）。まず、LigandScout 4.4 の「Ligand-based Pharmacophore Creation」オプションにより、**Va-Vh** からファーマコフォアを作成した。その結果、水素結合ドナー（HD）、水素結合アクセプター（HA）、疎水性部位（Hy）、排除体積（Ex）からなるモデルが生成された。続いてこのモデルに対して、骨格 II や骨格 III の化合物をフィッティングしたところ、RABV 阻害活性を有する **IId** や **IIb** で良好なフィッティングモデルが得られた一方で、活性のない **IIIa** はファーマコフォアを一部満たさないことが分かった。この結果から、骨格 V の側鎖配置を **IId** や **IIb** などの骨格 II も取りうることが示唆された。また、RABV 阻害化合物に関して PMI 解析も実施した結果、共通する分布が確認され、似たような分子形状をとりうることも分かった。以上の結果から、今回の骨格 II と V から得られた RABV の阻害化合物は、共通の標的タンパク質およびホットスポットを有していると考えられる。

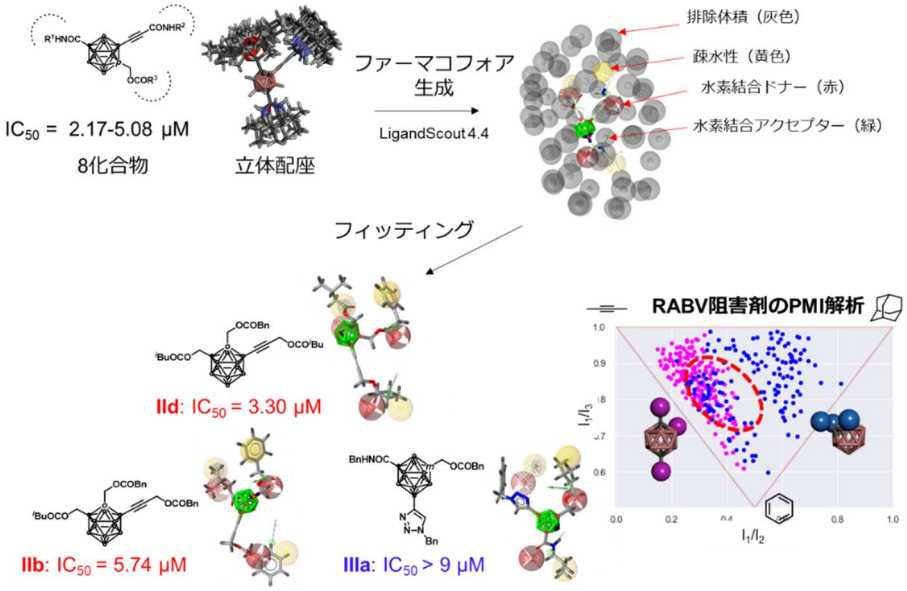


Figure 6-8. 骨格 V のファーマコフォア生成と、そのフィッティング結果

6-7　標的タンパク質とその阻害化合物のファーマコフォアフィッティング

続いて、阻害活性化合物と標的タンパク質の結晶構造に基づく結合様式の予測をファーマコフォアフィッティングにより行った。すなわち、**6-4** 項で述べたように今回の検証標的とした PPI は HIF-1α/p300（PDB: 1L3E）と RABV N-タンパク質の重合（PDB: 2GTT）であり、それぞれの結晶構造を用いて、HIF-1 転写阻害活性をもちつつ下流の CA9 の蓄積量を減少させた **Vk**（IC_{50}=3.64 μM）および RABV 阻害活性を有する **Vb**（IC_{50}=2.17 μM）との結合様式を予測した。まず、タンパク質の結晶構造から各々のホットスポットである HIF-1α の C 末端 helix（helix3）の Leu818、Leu819、Leu822 および RABV N-タンパク質の C 末端ヘリックスの Phe438、Phe441、Leu442 を疎水性部位（Hy）として選択した。このファーマコフォアを満たす化合物の立体配座を LigandScout 4.4 の「Virtual Screening」オプションを利用して探索した結果、Figure 6-9 に示す立体配座をとることで、**Vk** の 2 つのベンジル基、1 つのイソブチル基が HIF-1α のロイシン残基が位置したファーマコフォアにフィッティングすることが分かった。同様に、RABV の N-タンパク質に対しても **Vb** の２つのベンジル基、一つのイソブチル基が、他方の N-タンパク質のフェニルアラニン残基、ロイシン残基と同じ場所に位置した。以上のモデリングから、これらの化合物は、ヘリックス構造を模倣することで、HIF-1α/p300 および RABV N-タンパク質の PPI のホットスポットに結合し、阻害活性を有することが示唆された。

一方で、**Vb** と同じ側鎖パターンをもち、RABV 阻害活性を有する **IIb**（IC_{50}=5.74 μM）も同様にモデリングしたところ、興味深いことに **Vb** と全く同じ側鎖配置で疎水性部位のファーマコフォアを満たすことが分かった。

カルボラン骨格自体の位置は若干違うものの、タンパク質構造ベースのモデリングでも、**6-6** で述べた骨格 II と V が共通の標的タンパク質およびホットスポットを有することが示唆された。

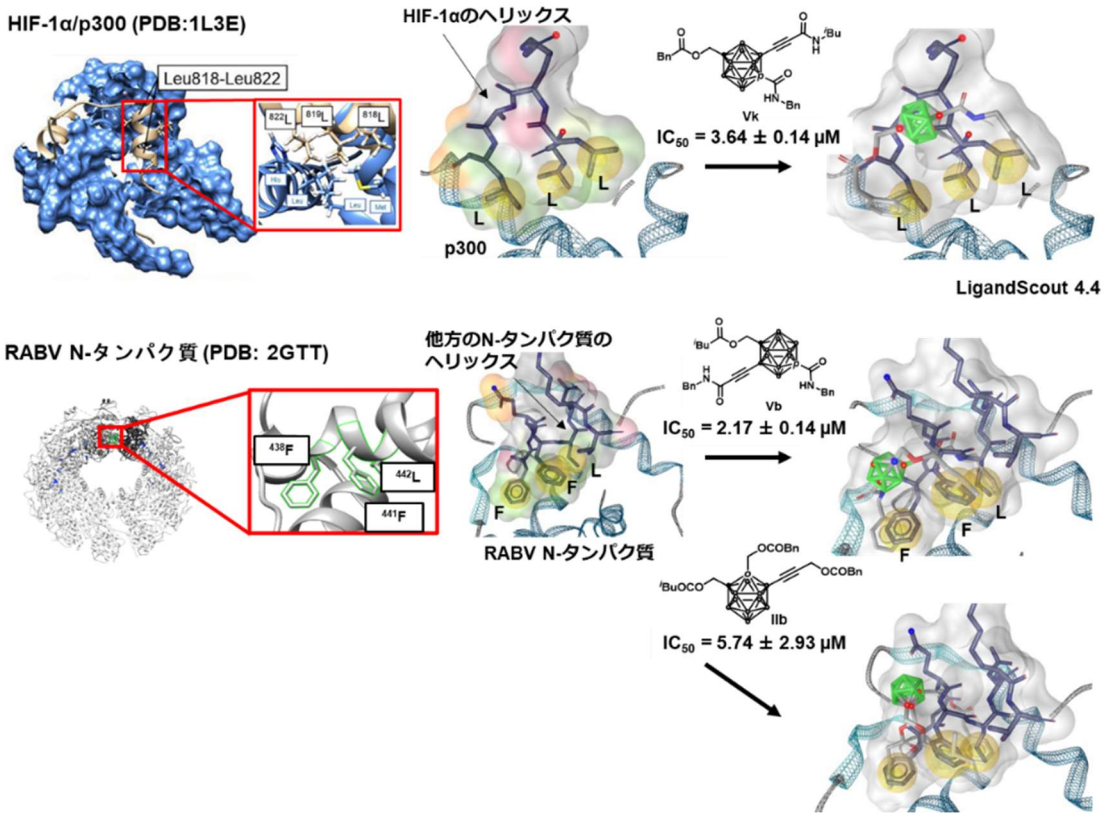


Figure 6-9. タンパク質構造ベースのファーマコフォア生成と、そのフィッティング結果

6-8　化合物 Vt の HIF-1α 分解誘導効果

6-4 項で **Vt** が HeLa 細胞や N2a 細胞に対して細胞死誘導が確認される一方で（IC_{50} = 16.2, 5.69 μM）、HIF-1 転写阻害活性（IC_{50} = 1.34 μM）や RABV 阻害活性（IC_{50} = 2.51 μM）を有することを述べた。そこで、まず Figure 6-5 と同様に、**Vt** を添加した HeLa 細胞内における HIF-1α とその下流のタンパク質である CA9 の蓄積量をウエスタンブロットによって調べた。なお、ポジティブコントロールとして HSP90 阻害剤である Geldanamycin（GA）を用いた。HSP90 は HIF-1α の安定化に重要な役割をもつタンパク質であり、GA の添加により、HIF-1α の細胞内蓄積量が低下することが知られている[45]。なお、細胞死誘導される濃度より高い濃度でウエスタンブロットを行っているが、培養時間が異なるため（実験項参照）、細胞死誘導が行われる前に HIF-1α への影響を調べることができる[46]。その結果を Figure 6-10A に示す。驚くべきことに、30 μM の **Vt** 添加によって顕著な HIF-1α タンパク質の減少、それに伴う CA9 の減少が確認された。さらに、Figure 6-5 と同じ方法で RT-PCR によって、HIF-1α の mRNA と、HIF-1 転写活性に依存するタンパク質である CA9 と血管内皮細胞増殖因子（VEGF）の mRNA を定量したところ、Figure 6-10B に示すように **Vt** の添加により、HIF-1α の mRNA を減少させずに、VEGF の mRNA

を減少させることが分かった。これらの結果から、これまで想定していた HIF-1α/p300 の PPI 阻害ではなく、HIF-1α の分解を伴う異なる作用機構を意味する。

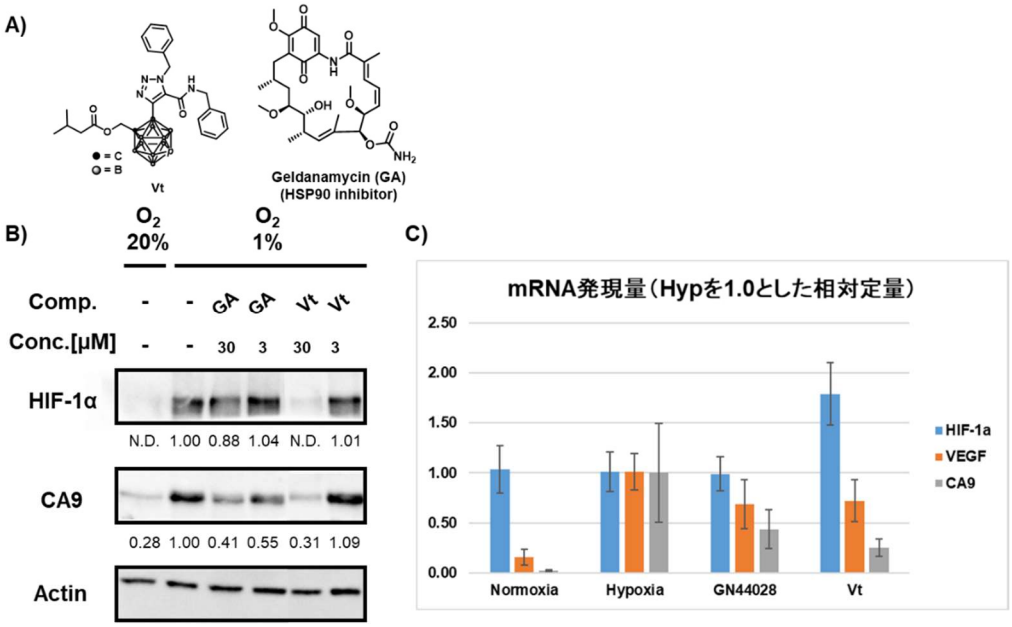


Figure 6-10. Vt による HIF-1α 分解作用の発見；A）**Vt** の構造；B）ウエスタンブロットによる HIF-1 関連タンパク質の定量、アクチンの蓄積量で標準化したそれぞれのタンパク質量を画像下部に示す；B）RT-PCR による HIF-1 関連タンパク質の mRNA の定量

HIF-1 の転写活性は様々な経路で抑制されることが知られる [47]。中でも熱ショックタンパク質（HSP）ファミリーが HIF-1α の安定化に寄与することが明らかになっており、これまでにそれぞれ HSP90、70、60 を阻害し、HIF-1α の蓄積を抑制する化合物が報告されている（Figure 6-11）[45,48,49]。特に、HSP70 阻害剤である IDF-11774 は、アポトーシスを有意に誘導するため、新規抗がん剤として臨床第 1 相試験まで進められている [46]。さらに、HSP70 阻害剤は狂犬病ウイルス（RABV）やデングウイルス（DENV）、ジカウイルス（ZIKV）の阻害剤としても有望であることが明らかになっている [50–53]。以上のことから、HIF-1α 分解作用（Figure 6-10、IC_{50} = 1.34 μM）、細胞死誘導活性（IC_{50} (HeLa) =16.2 μM、IC_{50} (N2a) = 5.69 μM）、RABV 阻害活性（IC_{50} = 2.51 μM）を同時に有する **Vt** が HSP70 阻害剤ではないかと考えられる。

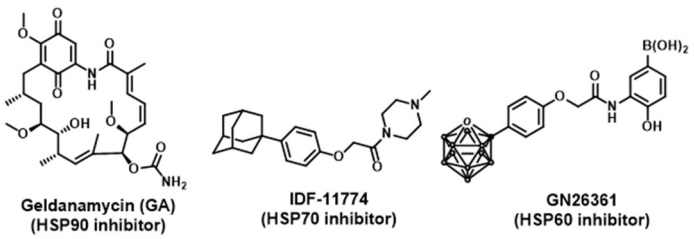


Figure 6-11. HSPs 阻害による HIF-1α 分解誘導活性を有する化合物

6-9　3 置換カルボランの位置異性体と光学異性体

3 置換カルボランの置換基配置は、今回合成した 5 パターンに加えて光学異性体を含めると Figure 6-12 示すパターンに拡張することができる。位置異性体は、**6-2** で述べた方法で合成可能だが、骨格 IV の 9,10 位のホウ素上の置換基が同一であるため、課題が残る。一方で光学異性体は、*o*-カルボラン、*m*-カルボランを出発原料とした骨格 I、II と III、IV に存在し、*p*-カルボランは対称軸をもつため、骨格 V には存在しない（Figure 6-12B）。これらの光学異性体を選択的に合成することは困難であったが、Xie グループから配向基と不斉配位子を用いて、Pd や Ir 触媒下 B-H 活性化を経由した不斉アリール化、アルケニル化反応がごく最近報告された [54,55]。この画期的な反応を利用すれば、骨格 II の光学異性体を考慮した置換基配置が可能になる。また、まだ報告はないものの、骨格 I も同様の戦略で不斉合成が可能ではないかと考えられる。一方で、*m*-カルボランを原料とする骨格 III や IV は、配向基との距離が *o*-カルボランよりも遠くなるため、反応制御が難しいことが予想される。

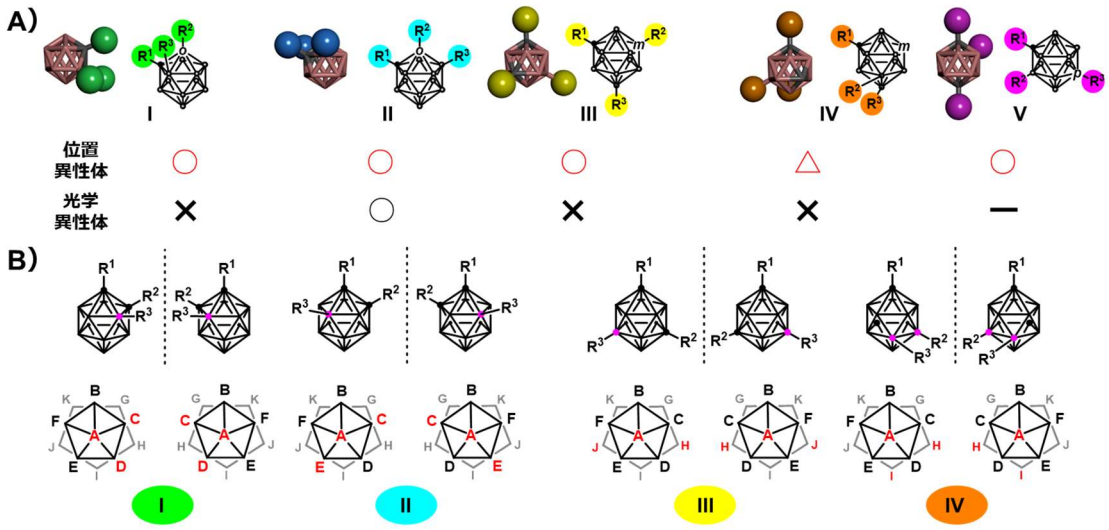
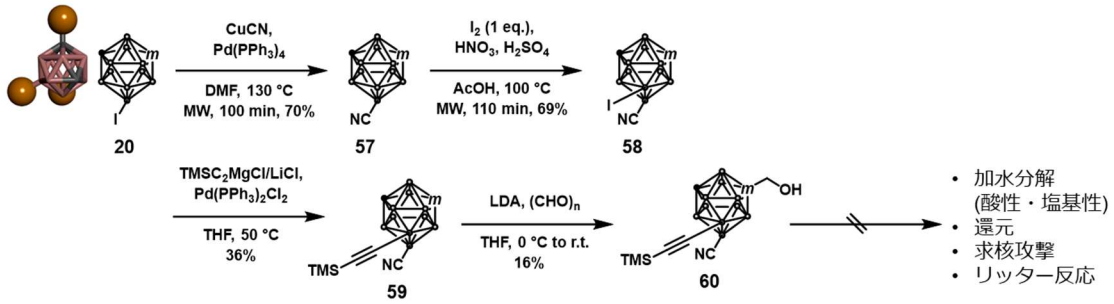


Figure 6-12. 3 置換カルボランに存在しうる位置異性体と光学異性体；A）合成の達成度、○は本研究において達成された合成、〇は本研究において検討していないが反応は開発されている（ref 54）。△は一部未達成、×は反応が未開発、ーは存在しないことを表す。B）骨格 I～IV の存在しうる光学異性体の置換基配置

特に骨格 IV は、位置選択性を実現しなければラセミ体も得られないため、Scheme 6-8 に示すように置換基を入れ分ける検討を鋭意行っている。ヨウ化カルボラン **20** を原料として、9 位の CuCN を用いたシアノ化 [56]、10 位を Scheme 6-4 と同様な方法でエチニル化することで、9,10 位に異なる置換基を導入した化合物 **59** を合成し、続く lithium diisopropylamide（LDA）によるリチオ化とパラホルムアルデヒドへの付加反応により、3 つの異なる置換基を有する化合物 **60** の合成を達成している。しかし、この

シアノ基に対して、酸性及び塩基性条件の加水分解、Diisobutylaluminium hydride（DIBAL）や $LiAlH_4$ を用いた還元、グリニャール試薬の求核攻撃、リッター反応を検討したが、いずれも目的物を与えなかった。このように、真の「任意置換可能なカルボラン化合物の合成」には、達成すべき課題が残されている。



Scheme 6-8. 置換基を入れ分けられる骨格 IV の合成検討

6-10　まとめ

本章では、カルボランの二十面体構造に着目し、12 頂点から任意の 3 か所に置換基を導入 5 パターンの 3 置換カルボランによって、三次元空間を網羅的に探索できると構想し、実証を行った（Figure 6-13）。

第一の実証として、前例のない DOS を志向した 3 置換カルボランの合成を、*o*-、*m*-、*p*-カルボランの炭素、ホウ素からなる 12 頂点の反応性の違いを駆使して達成した。さらに、すべての骨格パターンにおいて、その絶対的な置換基配置を単結晶 X 線構造解析により決定した。今回、確立した合成法に基づけば、側鎖にあたる置換基を簡便かつ自由に変換できるため、SAR 研究に大いに役立つ。また、系統的に合成した 42 化合物の PMI 解析により、5 パターンの骨格は、それぞれで異なる多様な分子形状をもつことが明らかとなった。

第二の実証として、新たな創薬標的である PPI を狙った生物活性評価を行い、新発見の活性を有する化合物を含めて、27 の活性化合物を見出すことに成功した。

この二十面体のすべての空間を探索できる 3 置換カルボラン化合物を用いたスクリーニングによる網羅的な SAR 解析は、医薬品開発の初期段階においてを行うことで、早期に有望な分子を発見することに役立つと期待される。

また興味深いことに、HIF-1α/p300 や RABV N-タンパク質の PPI 阻害活性を有する化合物（骨格 II, IV, V）を見出すだけなく、これまでに報告されたことのない RABV の増殖を亢進する化合物（骨格 V の一部）を見出すことに成功した。対して、その他の骨格（骨格 I や III）では、有意な阻害活性をもたないことから、非特異的な相互作用ではなく、骨格を利用した置換基配置によって、標的タンパク質の分子認識を厳密に制御できることが示唆された。これらの結果から、3 置換カルボランが標的選択的な PPI 制御化合物の設計に有望であることが示された。

さらに骨格ごとに異なる標的に対して活性を示したことから、3 置換カルボランの標的タンパク質による厳密な分子認識が働いていることが明らかとなった。この分子認識から得られた SAR を、骨格ごとに SAR matrix を使って解析したところ、標的タンパク質の構造情報を必要とせずに、その結合部位の相互作用を推察することができた。

一方で、タンパク質の結晶構造に基づくファーマコフォアフィッティングにより骨格 V や II がヘリックス構造を模倣することで、その阻害活性を発現していることを予測した。さらに RABV 阻害活性を有する骨格 V と骨格 II の化合物は、同じ側鎖配置取りうることが示唆され、PMI 解析でも共通する分布が確認されたことから、異なる骨格でも類似の側鎖配置をとることによって同様な活性を発現する可能性があることを示した。この仮説は、タンパク質の構造を用いたファーマコフォアフィッティングによるモデリングからも支持された。本章で合成した 5 パターンの 3 置換カルボランのように、置換基配置を三次元的に制御できる分子を系統的に設計する戦略は、他の創薬アプローチでは得られなかった新たな生物活性分子を創出するという大きな可能性が秘めていると確信している。

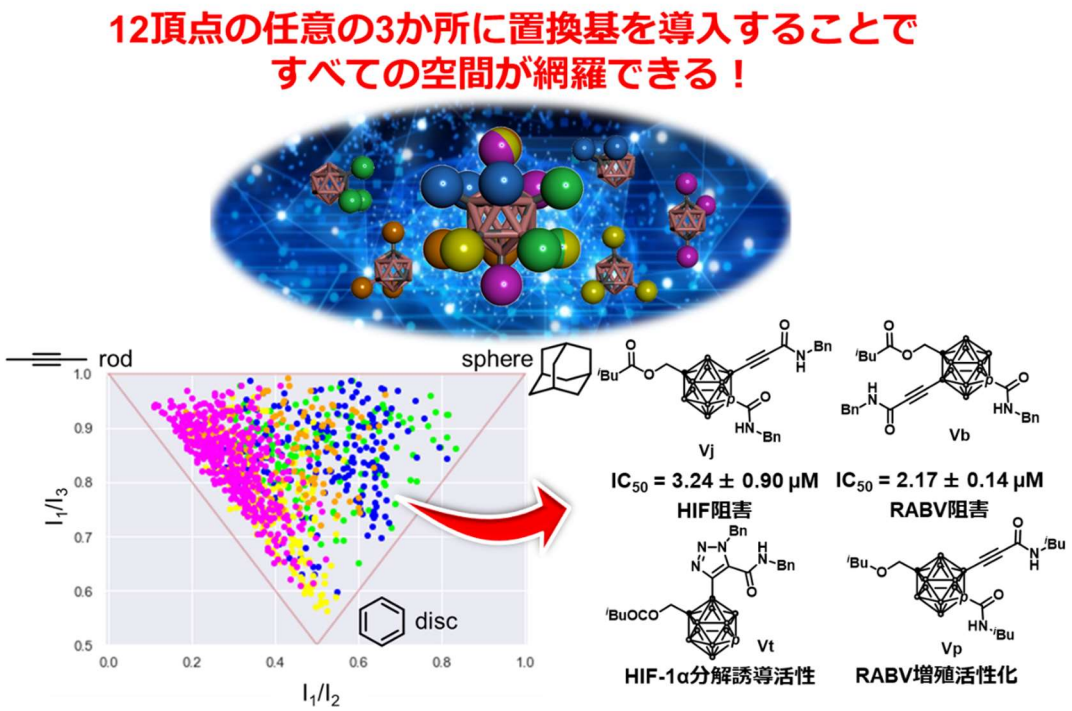


Figure 6-13. 本章のまとめ

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Experimental Section

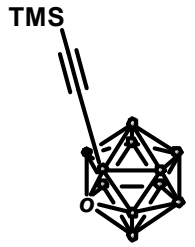
Synthesis

General

NMR spectra were recorded on a Bruker biospin AVANCE II (400 MHz for ^{1}H, 100 MHz for ^{13}C) or a Bruker biospin AVANCE III (500 MHz for ^{1}H, 125 MHz for ^{13}C, 470 MHz for ^{11}B) instrument in the indicated solvent. Chemical shifts are reported in units per million (ppm) relative to the signal (0.00 ppm) for internal tetramethylsilane solutions in $CDCl_3$ (7.26 ppm for ^{1}H, 77.16 ppm for ^{13}C). Multiplicities are reported using the following abbreviations: s; singlet, d; doublet, dd; doublet of doublets, t; triplet, q; quartet, m; multiplet, br; broad, J; coupling constants in Hertz. Mass spectra were measured using a JMS-700 Mstation. High-resolution mass spectra (HRMS) were recorded on a Bruker ESI-TOF-MS (micrOTOF II). All reactions were monitored by thin-layer chromatography carried out on 0.2 mm E. Merck silica gel plates (60F-254) with UV light (254 nm) and were visualized using Hanessian's stain solution. Column chromatography was performed on Silica Gel 60 N, purchased from Fuji Silysia Chemical Ltd.
Compound **1**, **2**[22], **11**[57], **20**, **21**, **35**, **47**[23], **57**[56] were synthesized synthesized according to the literature procedures.

- **Synthesis of scaffold I-type compounds**

Synthesis of 3-(2-(trimethylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (3)

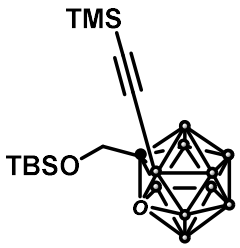


3

To a solution of 3-iodo-1,2-dicarba-*closo*-dodecaborane (**2**) (909 mg, 3.36 mmol) prepared according to the reported literature[58] and dichlorobis(triphenylphosphine)palladium (236 mg, 0.336 mmol) in THF (3 mL), was added trimethylsilylethynylmagnesium bromide (0.9 M, 9.3 mL, 8.41 mmol) freshly prepared from trimethyl acetylene and ethynylmagnesium bromide. The resulting mixture was stirred at 50 °C for 24 h. Then, the resulting mixture was cooled to room temperature and quenched with water, which was filtered on celite pad. The filtrate was wash with brine and dried over sodium sulfate and concentrated under vacuum. The crude material was

purified by column chromatography on silica gel (5% to 10% EtOAc in Hexane) afforded **3** (573 mg, 2.38 mmol, 71%) as a white solid; m.p. 76-77 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.68 (s, 2H), 3.00-1.50 (m, 9H), 0.19 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 105.3, 57.5, -0.22; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.6, -8.7, -11.4, -12.7, -13.7, -14.5; HRMS (ESI, negative) for $C_7H_{20}B_{10}Si$ (m/z): calculated 277.1977 $(M+Cl)^-$, found 277.1966.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-3-((trimethylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (4)

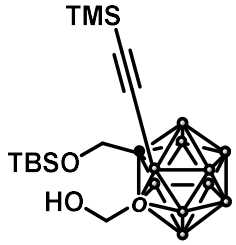


4

To a solution of **3** (572 mg, 2.38 mmol) in THF (9.5 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (1.45 mL, 2.38 mmol) at -78 ℃. After the resulting mixture was stirred at 0 ℃ under argon atmosphere for 2 h, paraformaldehyde (79 mg, 2.62 mmol) was added at 0 ℃. Then, the resulting mixture was warmed up to room temperature and stirred for 3 h under argon atmosphere. The reaction mixture was quenched with aq. 1 M HCl. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc, and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The residue was used in the next step without further purification.

To a solution of the above material and 2,6-lutidine (141 μL, 1.21 mmol) in DCM (3.2 mL) was slowly added TBSOTf (222 μL, 0.967 mmol) at 0 ℃ under argon atmosphere. The resulting mixture was stirred at 0 ℃ until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (0% to 10% EtOAc in Hexane) to afford **4** (187 mg, 0.486 mmol, 20% 2 steps) as white solid. ^{1}H NMR (500 MHz; $CDCl_3$): δ 4.26 (d, J = 10.5 Hz, 1H), 4.03 (d, J = 10.5 Hz, 1H), 3.00-1.50 (m,9H), 0.90 (s, 9H), 0.19 (s, 9H), 0.10 (J = 1.5 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 74.9, 63.8, 58.9, 25.8, 18.4, -0.13, -5.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.9, -5.0, -8.2, -10.5, -11.3, -13.7; HRMS (ESI, positive) for $C_{14}H_{36}B_{10}OSi_2$ (m/z): calculated 409.3156 $(M+Na)^+$, found 409.3157.

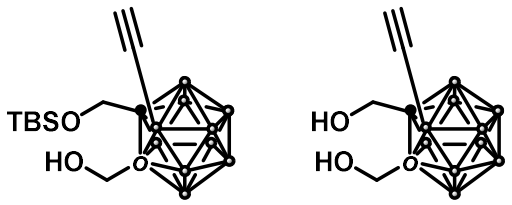
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-hydroxymethyl-3-((trimethylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (5)



5

To a solution of **4** (127 mg, 0.329 mmol) in THF (2.4 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (221 μL, 0.362 mmol) at -78 ℃. After the resulting mixture was stirred at 0 ℃ under argon atmosphere for 2 h, paraformaldehyde (12 mg, 0.395 mmol) was added at 0 ℃. Then, the resulting mixture was warmed up to room temperature and stirred for 3 h under argon atmosphere. The reaction mixture was quenched with aq. 1N HCl. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc, and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford **5** (27.4 mg, 0.0661 mmol, 20%) as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$): δ 4.50 (d, *J* = 12.3 Hz, 1H), 4.24 (dd, *J* = 5.2 Hz, *J* = 13.7 Hz, 1H), 4.11 (d, *J* = 12.3 Hz, 1H), 4.03 (dd, *J* = 5.2 Hz, *J* = 13.7 Hz, 1H), 2.90-2.87 (m, 2H), 2.90-1.40 (m, 9H), 0.92 (s, 9H), 0.22 (s, 9H), 0.14 (*J* = 6.8 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 110.0, 78.3, 77.7, 64.1, 63.5, 25.9, 18.5, -0.2, -5.3; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.94, -10.8, -11.6; HRMS (ESI, positive) for $C_{15}H_{38}B_{10}O_2Si_2$ (m/z): calculated 439.3263 $(M+Na)^+$, found 439.3270.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-hydroxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (6); 1,2-bis(hydroxymethyl)-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (7)



6 **7**

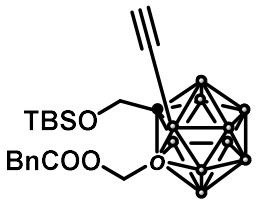
To a solution of **5** (96 mg, 0.232 mmol) in MeOH (1 mL), potassium carbonate (160 mg, 1.16 mmol) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under

pressure. To the resulting mixture was added water and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% to 50% EtOAc in Hexane) to afford faster eluted product **6** (45 mg, 0.132 mmol, 57%) as colorless oil and later eluted product **7** (21 mg, 0.0924 mmol, 40%) as white solid in total 97% yield.

6: ^{1}H NMR (400 MHz; $CDCl_3$): δ 4.49 (d, *J* = 12.4 Hz, 1H), 4.29 (d, *J* = 13.7 Hz, 1H), 4.13 (d, *J* = 12.4 Hz, 1H), 4.07 (d, *J* = 13.7 Hz, 1H), 2.60-1.50 (m, 9H), 2.48 (s, 1H), 0.92 (s, 9H), 0.15 (d, *J* = 7.4 Hz, 6H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 89.3, 78.4, 77.8, 63.9, 63.3, 25.7, 18.3, -5.5, -5.7; ^{11}B NMR (128 MHz; $CDCl_3$): δ -3.8, -9.7, -10.5, -11.5, -12.7; HRMS (ESI, positive) for $C_{12}H_{30}B_{10}O_2Si$ (m/z): calculated 367.2862 $(M+Na)^+$, found 367.2861.

7: ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.46 (s, 2H), 2.98 (s, 1H), 1.77 (s, 1H), 2.90-1.60 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 89.8, 78.3, 63.7; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.6, -9.9, -11.4, -12.8; HRMS (ESI, positive) for $C_6H_{16}B_{10}O_2$ (m/z): calculated 253.1985 $(M+Na)^+$, found 253.1978; m.p. 130-131℃.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-phenylacetoxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (8)



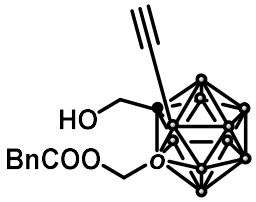
8

To a solution of **6** (35.3 mg, 0.103 mmol) and triethylamine (21 μL, 0.155 mmol) in CH_2Cl_2 (500 μL), was slowly added phenylacetyl chloride (16 μL, 0.124 mmol) in CH_2Cl_2 at 0 ℃. After the resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford **8** (38.3 mg, 0.0831 mmol, 81%) as colorless oil.

^{1}H NMR (400 MHz; $CDCl_3$): δ 7.39-7.27 (m, 5H), 4.74 (d, *J* = 13.1 Hz, 1H), 4.59 (d, *J* = 13.1 Hz, 1H), 4.32 (d, *J* = 12.4 Hz, 1H), 4.11 (d, *J* = 12.4 Hz, 1H), 3.68 (s, 2H), 3.00-1.50 (m, 9H), 0.90 (s, 9H), 0.09 (d, *J* = 8.2 Hz, 1H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 170.1, 133.0, 129.6, 128.8, 127.6, 89.3, 79.2, 74.1, 63.6, 62.3, 41.1, 25.8, 18.3, -5.4, -5.5; ^{11}B NMR (128 MHz; $CDCl_3$): δ -3.4, -4.4, -9.9, -11.0, -11.8; HRMS (ESI, positive) for $C_{20}H_{36}B_{10}O_3Si$ (m/z): calculated 485.3289

$(M+Na)^+$, found 485.3287.

Synthesis of 1-hydroxymethyl-2-phenylacetoxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (S1)

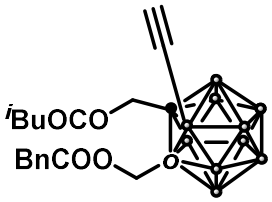


S1

To a solution of **8** (38.3 mg, 83.1 μmol) in CH_2Cl_2 (500 μL), was added 4N HCl in Dioxane (500 μL). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. The crude material was purified by column chromatography on silica gel (25% EtOAc in Hexane) to afford **S1** (26.0 mg, 0.0751 mmol, 90%) as colorless oil.

^1H NMR (400 MHz; $CDCl_3$): δ 7.41-7.29 (m, 5H), 4.89 (d, J = 13.6 Hz, 1H), 4.64 (d, J = 13.6 Hz, 1H), 4.26 (d, J = 14.0 Hz, 1H), 4.14 (d, J = 14.0 Hz, 1H), 3.73 (s, 2H), 3.00-1.50 (m, 9H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 170.8, 132.7, 129.5, 129.0, 127.8, 79.8, 74.9, 63.3, 62.4, 41.2; ^{11}B NMR (128 MHz; $CDCl_3$): δ -3.4, -3.9, -9.5, -10.5, -12.3; HRMS (ESI, positive) for $C_{14}H_{22}B_{10}O_3$ (m/z): calculated 367.2862 $(M+Na)^+$, found 367.2861.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-2-phenylacetoxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (9)

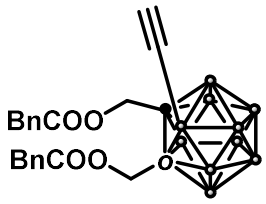


9

To a solution of **S1** (18.9 mg, 43.9 μmol) and triethylamine (18 μL, 0.132 mmol) in CH_2Cl_2 (1 mL), was slowly added isovaleryl chloride (8.0 μL, 65.8 μmol) in CH_2Cl_2 at 0 ℃. After the resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% Et_2O in Hexane) to afford **9** (20.9 mg, 48.5 μmol, quant.) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36-7.26 (m, 5H), 4.77-4.67 (m, 3H), 4.61 (d, *J* = 13.5 Hz, 1H), 3.68 (s, 2H), 3.00-1.50 (m, 9H), 2.44 (s, 1H), 2.20 (d, *J* = 6.5 Hz, 2H), 2.13-2.07 (m, 1H), 0.96 (*J* = 1.2 Hz, *J* = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.4, 169.9, 132.8, 129.5, 128.9, 127.7, 89.8, 75.5, 75.2, 62.2, 61.7, 42.8, 41.0, 25.6, 22.50 22.48; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.5, -9.4, -10.5, -12.0; HRMS (ESI, positive) for $C_{19}H_{30}B_{10}O_4$ (m/z): calculated 455.2997 $(M+Na)^+$, found 455.2997.

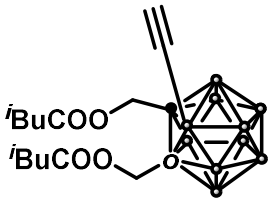
Synthesis of 1,2-bis(phenylacetoxymethyl)-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (10a)



10a

This compound was prepared from **7** (19.5 mg, 85.4 μmol) and 2.0 equivalent of phenylacetyl chloride using the procedure described before for **5** in 72% yield as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34-7.24 (m, 10H), 4.71 (d, *J* = 13.5 Hz, 2H), 4.63 (d, *J* = 13.5 Hz, 2H), 3.00-1.50 (m, 9H), 3.61 (s, 4H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 169.9, 132.8, 129.5, 128.9, 127.7, 89.9, 75.3, 62.2, 40.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.4, -9.4, -10.6, -12.1; HRMS (ESI, positive) for $C_{22}H_{28}B_{10}O_4$ (m/z): calculated 489.2844 $(M+Na)^+$, found 489.2842.

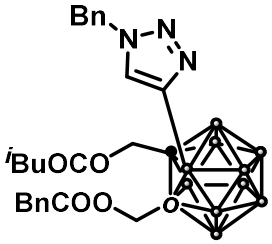
Synthesis of 1,2-bis((3-methylbutanoyl)oxymethyl)-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (10b)



10b

This compound was prepared from **7** (15.3 mg, 67.0 μmol) and 2.0 equivalent of isovaleryl chloride using the procedure described before for **10a** in 74% yield as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$): δ 4.78 (d, *J* = 13.5 Hz, 2H), 4.66 (d, *J* = 13.5 Hz, 2H), 3.00-1.50 (m, 9H), 2.48 (s, 1H), 2.26 (d, *J* = 6.5 Hz, 4H), 2.15-2.10 (m, 2H), 0.98 (d, *J* = 6.7 Hz, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.4, 89.8, 75.4, 61.8, 42.9, 25.6, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.5, -9.4, -10.6, -12.0; HRMS (ESI, positive) for $C_{16}H_{32}B_{10}O_4$ (m/z): calculated 421.3149 $(M+Na)^+$, found 421.3148.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-2-phenylacetoxymethyl-3-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,2-dicarba-*closo*-dodecaborane (Ia)

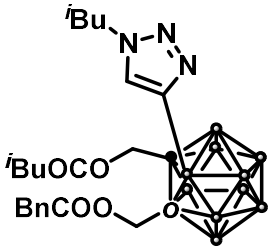


Ia

To a solution of **9** (9.8 mg, 22.8 μmol) in DMF:H_2O (4:1, v/v, 500 μL), were added CuI (0.43 mg, 2.28 μmol), sodium ascorbate (0.90 mg, 4.46 μmol), and benzylazide (6.0 μL, 45.5 μmol). The mixture was stirred vigorously at room temperature for 12 h. The reaction was quenched with water and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **Ia** (11 mg, 20.0 μmol, 88%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.72 (s, 1H), 7.40-7.24 (m, 10H), 5.55 (s, 2H), 4.99 (d, *J* = 13.7 Hz, 2H), 4.97-4.81 (m, 3H), 3.61 (s, 2H), 2.90-1.60 (m, 9H), 2.15 (d, *J* = 6.5 Hz, 2H), 2.09-2.03 (m, 1H), 0.94 (dd, *J* = 1.8 Hz, *J* = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.3, 169.9, 134.3, 133.0, 131.6, 129.5, 129.1, 128.8, 128.4, 127.6, 76.1, 75.7, 62.0, 61.4, 54.1, 42.9, 41.0, 25.5, 22.52, 22.51, ; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.6, -7.2, -9.5, -10.9, -11.8; HRMS (ESI, positive) for $C_{26}H_{37}B_{10}N_3O_4$ (m/z): calculated 588.3644 $(M+Na)^+$, found 588.3645.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-2-phenylacetoxymethyl-3-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,2-dicarba-*closo*-dodecaborane (Ib)



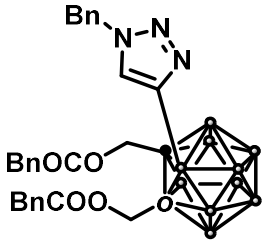
Ib

This compound was prepared from **9** (11.1 mg, 25.8 μmol) and 2.0 equivalent of isovalerylazide using the procedure described before for **Ia** in 45% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.76 (s, 1H), 7.36-7.26 (m, 5H), 5.00 (d, *J* = 13.7 Hz, 2H), 4.91-4.83 (m, 3H), 4.19 (d, *J* = 7.3 Hz, 2H), 3.64 (s, 2H), 2.27-2.21 (m, 1H), 2.16 (d, *J* = 6.5 Hz, 2H), 2.10-2.04 (m, 1H), 0.97-0.94 (m, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.3, 169.9, 133.0, 132.1,

129.5, 128.8, 127.6, 76.0, 75.7, 62.0, 61.4, 57.5, 42.9, 41.1, 29.8, 25.6, 22.5, 20.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.6, -7.2, -9.6, -12.0; HRMS (ESI, positive) for $C_{23}H_{39}B_{10}N_3O_4$ (m/z): calculated 554.3798 $(M+Na)^+$, found 554.3796.

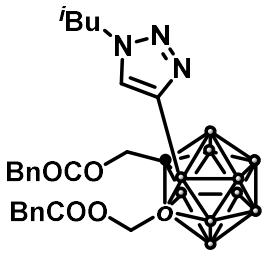
Synthesis of 1,2-bis(phenylacetoxymethyl)-3-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,2-dicarba-*closo*-dodecaborane (Ic)



Ic

This compound was prepared from **10a** (27.6 mg, 59.4 μmol) using the procedure described before for **Ia** in 8% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.68 (s, 1H), 7.38-7.21 (m, 15H), 5.53 (s, 2H), 4.93 (d, *J* = 13.7 Hz, 2H), 4.80 (d, *J* = 13.7 Hz, 2H), 3.55 (s, 4H), 2.80-1.60 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 169.8, 134.3, 133.0, 131.6, 129.5, 129.4, 129.1, 128.8, 128.4, 127.6, 75.8, 62.0, 54.1, 40.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.5, -7.5, -9.5, -11.8; HRMS (ESI, positive) for $C_{29}H_{35}B_{10}N_3O_4$ (m/z): calculated 622.3491 $(M+Na)^+$, found 622.3491.

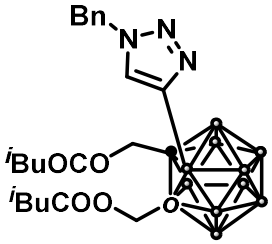
Synthesis of 1,2-bis(phenylacetoxymethyl)-3-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,2-dicarba-*closo*-dodecaborane (Id)



Id

This compound was prepared from **10a** (26.5 mg, 57.0 μmol) and 2.0 equivalent of isovalerylazide using the procedure described before for **Ia** in 10% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.71 (s, 1H), 7.34-7.23 (m, 10H), 4.92 (d, *J* = 13.7 Hz, 2H), 4.81 (d, *J* = 13.7 Hz, 2H), 4.16 (d, *J* = 7.2 Hz, 2H), 3.58 (s, 4H), 2.90-1.80 (m, 9H), 2.24-2.19 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 169.9, 133.0, 132.1, 129.5, 128.8, 127.6, 75.7, 62.0, 57.5, 41.0, 29.8, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ –3.4, -7.3, -9.4, -10.8; HRMS (ESI, positive) for $C_{25}H_{37}B_{10}N_3O_4$ (m/z): calculated 588.3644 $(M+Na)^+$, found 588.3642.

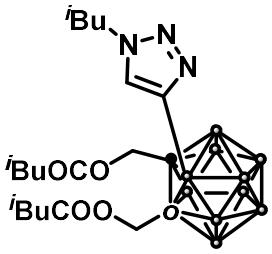
Synthesis of 1,2-bis((3-methylbutanoyl)oxymethyl)-3-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,2-dicarba-*closo*-dodecaborane (Ie)



Ie

This compound was prepared from **10b** (18.4 mg, 46.4 μmol) using the procedure described before for **Ia** in 72% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.76 (s, 1H), 7.41-7.39 (m, 3H), 7.32-7.31 (m, 2H), 5.57 (s, 2H), 4.95 (d, *J* = 13.6 Hz, 2H), 4.87 (d, *J* = 13.6 Hz, 2H), 3.00-1.70 (m, 9H), 2.20 (d, *J* = 6.6 Hz, 2H), 2.13-2.06 (m, 1H), 0.95 (dd, *J* = 1.2 Hz, *J* = 6.6 Hz, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.3, 134.3, 131.7, 129.4, 129.1, 128.4, 76.0, 61.5, 54.2, 43.0, 28.5, 25.6, 22.53, 22.51; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.5, -7.4, -9.6, -10.8, -11.8; HRMS (ESI, positive) for $C_{23}H_{39}B_{10}N_3O_4$ (m/z): calculated 554.3798 $(M+Na)^+$, found 554.3812.

Synthesis of 1,2-bis((3-methylbutanoyl)oxymethyl)-3-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,2-dicarba-*closo*-dodecaborane (If)

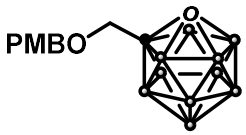


If

This compound was prepared from **10b** (23.7 mg, 59.8 μmol) and 2.0 equivalent of isovalerylazide using the procedure described before for **Ia** in 34% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.82 (s, 1H), 4.95 (d, *J* = 13.7 Hz, 2H), 4.89 (d, *J* = 13.7 Hz, 2H), 4.21 (d, *J* = 7.2 Hz, 2H), 2.29-2.23 (m, 1H), 2.21 (d, *J* = 6.7 Hz, 2H), 2.14-2.07 (m, 2H), 0.98-0.95 (m, 18H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.3, 132.2, 76.0, 61.5, 57.6, 43.0, 29.8, 25.6, 22.53, 22.52, 20.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.5, -7.2, -9.5, -10.9, -11.8; HRMS (ESI, positive) for $C_{20}H_{41}B_{10}N_3O_4$ (m/z): calculated 520.3951 $(M+Na)^+$, found 520.3934.

- **Synthesis of scaffold II-type compounds**

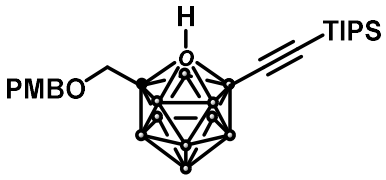
Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-1,2-dicarba-*closo*-dodecaborane (12)



12

To a solution of 1-methoxy-4-((prop-2-yn-1-yloxy)methyl)benzene (**11**) (370 mg, 2.10 mmol) prepared according to the reported literature[59] and *N,N*-dimethylaniline (399 μL, 3.15 mmol) in chlorobenzene, was added decaborane (308 mg, 2.52 mmol). The resulting mixture was stirred at 130 °C under argon atmosphere for 10 min with microwave synthesizer. To the resulting mixture was added 1M HCl and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under vacuum. Then, the crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford **12** (489 mg, 1.66 mmol, 79%) as a white solid. m.p. 65-67 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.19 (d, *J* = 8.8 Hz, 2H), 6.89 (d, *J* = 8.8 Hz, 2H), 4.46 (s, 2H), 3.95 (s, 1H), 3.83 (s, 2H), 3.82 (s, 3H), 3.00-1.60 (m, 10H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.8, 129.6, 128.4, 114.2, 73.5, 72.7, 70.6, 57.8, 55.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.13, -4.95, -9.17, -11.71, -13.37; HRMS (ESI, positive) for $C_{11}H_{22}B_{10}O_2$ (m/z): calculated 319.2461 $(M+Na)^+$, found 319.2460.

Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-4-((triisopropylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (13)



13

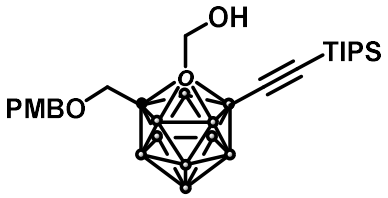
To a solution of **12** (1.56 g, 5.30 mmol) in Et_2O (22 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (3.69 mL, 5.83 mmol) at -78 °C. After the resulting mixture was stirred at -78 °C under argon atmosphere for 2 h, CO_2 balloon was attached. Then, the resulting mixture was warmed up to room temperature and stirred for 12 h under CO_2 atmosphere. After that, the reaction mixture was concentrated under pressure. Hexane was added after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, to the aqueous layer, were added 6 M HCl and Et_2O after which the product was partitioned between

the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was used in the next step without further purification.

To a suspension of the crude material, K_2HPO_4 (1.85 g, 10.6 mmol), $Pd(OAc)_2$ (119 mg, 0.530 mmol), and AgOAc (2.65 g, 15.9 mmol) in toluene (50 mL), was slowly added (triisopropylsilyl)acetylene (3.5 mL, 15.9 mmol) in toluene (3 mL). The resulting mixture was stirred at 80 °C for 16 h. Then, the resulting mixture was cooled to room temperature and quenched with water, which was filtered on celite pad. The filtrate was extracted with Et_2O, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (5% to 10% EtOAc in Hexane) afforded **13** (776 mg, 1.63 mmol, 31%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.20 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.48 (s, 2H), 4.10 (s, 1H), 3.84-3.81 (m, 5H), 2.80-1.60 (m, 9H), 1.09-1.05 (m, 21H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.9, 129.6, 128.4, 114.2, 101.7, 73.6, 72.5, 70.5, 59.2, 55.5, 18.7, 11.3; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.63, -4.12, -8.40, -9.76, -12.10, -12.66; HRMS (ESI, positive) for $C_{22}H_{42}B_{10}O_2Si$ (m/z): calculated 499.3823 $(M+Na)^+$, found 499.3822.

Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-(hydroxymethyl)-4-((triisopropylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (S2)

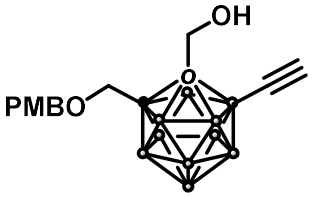


S2

To a solution of **13** (776 mg, 1.63 mmol) in THF (13 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (1.14 mL, 1.80 μmol) at 0 °C. After the resulting mixture was stirred at room temperature under argon atmosphere for 2 h, paraformaldehyde (59 mg, 1.96 mmol) was added at 0 °C. Then, the resulting mixture was warmed up to room temperature and stirred for 3 h under argon atmosphere. The reaction mixture was quenched with aq. 1 M HCl. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc, and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (5% to 10% EtOAc in Hexane) afforded **S2** (647 mg, 1.30 mmol, 80%) as a colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.23 (d, *J* = 8.7 Hz, 2H), 6.90 (d, *J* = 8.7 Hz, 2H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.33-4.23 (m, 2H), 4.04 (d, *J* = 12.1 Hz, 1H), 3.97 (d,

J = 12.1 Hz, 1H), 3.82 (s, 3H), 3.10-3.07 (m, 1H), 3.00-1.70 (m, 9H), 1.07-1.03 (m, 21H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.8, 129.6, 127.9, 114.2, 103.9, 79.0, 76.0, 73.6, 69.9, 63.4, 55.3, 18.6, 11.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.70, -3.67, -9.51, -10.66, -12.07; HRMS (ESI, positive) for $C_{23}H_{44}B_{10}O_3Si$ (m/z): calculated 529.3919 $(M+Na)^+$, found 529.3919.

Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-(hydroxymethyl)-4-ethynyl-1,2-dicarba-*closo*-dodecaborane (14)

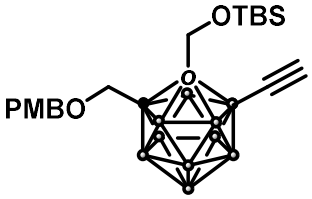


14

To a solution of **S2** (21 mg, 41.6 μmol) in THF (500 μL), was slowly added TBAF 1 M solution in THF (42 μL, 41.6 μmol) at 0 °C. After the resulting mixture was stirred at 0 °C under argon atmosphere for 30 min, the reaction mixture was quenched with wa*ter*he resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was extracted with Et_2O, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) afforded **14** (16 mg, quant.) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.23 (d, *J* = 8.6 Hz, 2H), 6.90 (d, *J* = 8.6 Hz, 2H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.50 (d, *J* = 11.5 Hz, 1H), 4.33-4.29 (m, 1H), 4.21 (m, 1H), 4.05 (d, *J* = 12.0 Hz, 1H), 4.00 (d, *J* = 12.0 Hz, 1H), 3.82 (s, 3H), 3.00 (t, *J* = 7.8 Hz, 1H), 3.00-1.70 (m, 9H), 2.34 (s, 1H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.0, 129.8, 127.8, 114.3, 87.3, 79.2, 76.1, 73.8, 70.1, 63.3, 55.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.56, -3.37, -9.60, -10.71, -11.3, -11.9; HRMS (ESI, positive) for $C_{14}H_{24}B_{10}O_3$ (m/z): calculated 373.2571 $(M+Na)^+$, found 373.2561.

Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-ethynyl-1,2-dicarba-*closo*-dodecaborane (S3)

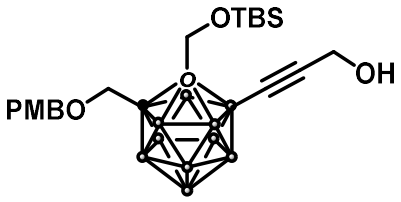


S3

To a solution of **14** (343 mg, 1.00 mmol) and 2,6-lutidine (233 μL, 2.00 mmol) in DCM (4 mL)

was slowly added TBSOTf (276 μL, 1.20 mmol) at 0 ℃ under argon atmosphere. The resulting mixture was stirred at 0 ℃ until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (0% to 10% EtOAc in Hexane) to afford **S3** (451 mg, 0.98 mmol, 98%) as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.22 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.47 (d, *J* = 11.7 Hz, 1H), 4.41 (d, *J* = 12.3 Hz, 1H), 4.20 (d, *J* = 12.3 Hz, 1H), 3.99 (d, *J* = 11.9 Hz, 1H), 3.96 (d, *J* = 11.9 Hz, 1H), 3.82 (s, 3H), 3.00-1.50 (m, 9H), 2.29 (s, 1H), 0.90 (s, 9H), 0.19 (s, 9H), 0.08 (d, *J* = 3.3 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.7, 129.4, 128.8, 114.1, 86.8, 78.9, 76.6, 73.4, 69.6, 63.0, 55.4, 25.8, 18.3, -5.46, -5.47; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.68, -3.70, -10.9, -11.8; HRMS (ESI, positive) for $C_{20}H_{38}B_{10}O_3Si$ (m/z): calculated 487.3446 $(M+Na)^+$, found 487.3453.

Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(3-oxoprop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (16)

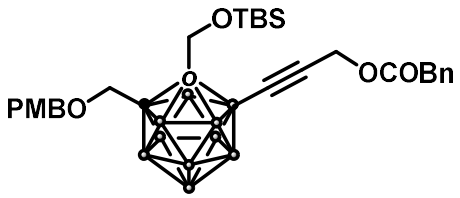


16

To a solution of **S3** (154 mg, 0.333 mmol) in THF (2.7 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (232 μL, 0.366 mmol) at -78 ℃. After the resulting mixture was stirred at 0 ℃ under argon atmosphere for 2 h, paraformaldehyde (12 mg, 0.399 mmol) was added at 0 ℃. Then, the resulting mixture was warmed up to room temperature and stirred for 3 h under argon atmosphere. The reaction mixture was quenched with aq. 1N HCl. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc, and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% to 40% Et_2O in Hexane) to afford **16** (130 mg, 0.263 mmol, 79%) as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$): δ 7.21 (d, *J* = 8.7 Hz, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 4.52 (d, *J* = 11.7 Hz, 1H), 4.46 (d, *J* = 11.7 Hz, 1H), 4.38 (d, *J* = 12.3 Hz, 1H), 4.27 (s, 2H), 4.17 (d, *J* = 12.3 Hz, 1H), 3.98 (d, *J* = 11.9 Hz, 1H), 3.94 (d, *J* = 11.9 Hz, 1H), 3.81 (s, 3H), 3.00-1.50 (m, 9H), 0.89 (s, 9H), 0.19 (s, 9H), 0.07 (d, *J* = 1.3 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.7, 129.4, 128.8, 114.1, 97.2, 78.6, 78.5, 73.4, 69.7, 63.0, 55.4, 51.8, 18.3, -5.46, -5.48; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.77, -3.72, -10.6, -11.9; HRMS (ESI, positive) for $C_{21}H_{40}B_{10}O_4Si$ (m/z): calculated

517.3553 $(M+Na)^+$, found 517.3556.

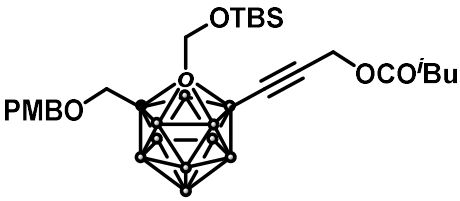
Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (17a)



17a

To a solution of **16** (70 mg, 0.121 mmol) and triethylamine (100 μL, 0.723 mmol) in CH_2Cl_2 (2 mL), was slowly added phenylacetyl chloride (48 μL, 0.361 mmol) in CH_2Cl_2 at 0 °C. After the resulting mixture was stirred 0 °C under argon atmosphere for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% Et_2O in Hexane) to afford **17a** (122 mg, 0.200 mmol, 83%) as colorless oil.
^1H NMR (500 MHz; $CDCl_3$): δ 7.33-7.26 (m, 5H), 7.22 (d, J = 8.7 Hz, 2H), 6.88 (d, J = 8.7 Hz, 2H), 4.71 (s, 2H), 4.52 (d, J = 11.7 Hz, 1H), 4.46 (d, J = 11.7 Hz, 1H), 4.35 (d, J = 12.4 Hz, 1H), 4.14 (d, J = 12.4 Hz, 1H), 3.97 (d, J = 11.9 Hz, 1H), 3.94 (d, J = 11.9 Hz, 1H), 3.81 (s, 3H), 3.66 (s, 2H), 3.00-1.50 (m, 9H), 0.88 (s, 9H), 0.05 (d, J = 3.7 Hz, 1H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.9, 159.7, 133.7, 129.4, 128.82, 128.78, 127.4, 114.1, 73.4, 69.7, 63.0, 55.4, 53.3, 41.1, 25.8, 18.3, -5.50, -5.51; ^{11}B NMR (160 MHz; $CDCl_3$): δ –2.5, -3.6, -10.8, -11.8; HRMS (ESI, positive) for $C_{29}H_{46}B_{10}O_5Si$ (m/z): calculated 635.3980 $(M+Na)^+$, found 635.3992.

Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(3-((3-methylbutanoyl)oxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (17b)

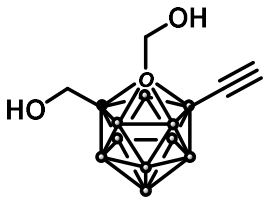


17b

To a solution of **16** (186 mg, 0.377 mmol) and triethylamine (157 μL, 1.13 mmol) in CH_2Cl_2 (2 mL), was slowly added isovaleryl chloride (69 μL, 0.566 mmol) in CH_2Cl_2 at 0 °C. After the

resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **17b** (225 mg, quant.) as colorless oil.
^{1}H NMR (400 MHz; $CDCl_3$): δ 7.21 (d, *J* = 8.7 Hz, 2H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.68 (s, 2H), 4.51 (d, *J* = 11.7 Hz, 1H), 4.45 (d, *J* = 11.7 Hz, 1H), 4.38 (d, *J* = 12.4 Hz, 1H), 4.16 (d, *J* = 12.4 Hz, 1H), 3.98 (d, *J* = 11.9 Hz, 1H), 3.94 (d, *J* = 11.9 Hz, 1H), 3.81 (s, 3H), 3.00-1.50 (m, 9H), 2.21 (d, *J* = 6.8 Hz, 2H), 2.15-2.10 (m, 1H), 0.96 (d, *J* = 6.6 Hz, 6H), 0.89 (s, 9H), 0.08 (d, *J* = 1.7 Hz, 1H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 172.2, 159.7, 129.3, 128.8, 114.1, 92.8, 78.8, 77.3, 76.6, 73.4, 49.7, 63.0, 55.4, 52.5, 43.2, 25.9, 25.8, 22.5, 18.3, -5.53, -5.55; ^{11}B NMR (128 MHz; $CDCl_3$): δ -3.8, -11.0, -11.8; HRMS (ESI, positive) for $C_{26}H_{48}B_{10}O_5Si$ (m/z): calculated 601.4134 $(M+Na)^+$, found 601.4136.

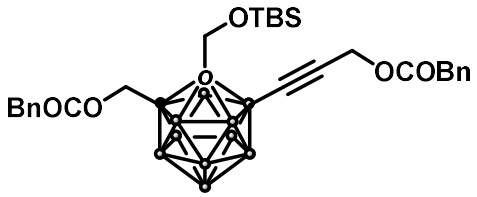
Synthesis of 1,2-bis(hydroxymethyl)-4-ethynyl-1,2-dicarba-*closo*-dodecaborane (15)



15

To a solution of **14** (18.1 mg, 51.9 μmol) and NaH_2PO_4 (12.5 mg, 0.104 mmol) in DCM/H_2O (1 mL, 10:1, v/v) was added DDQ (24.0 mg, 0.104 mmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% EtOAc in Hexane) to afford **15** (10 mg, 43.8 μmol, 84%) as white solid. m.p. 167-168 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 4.47 (d, *J* = 4.8 Hz, 1H), 4.44 (d, *J* = 4.7 Hz, 1H), δ 4.39 (d, *J* = 7.5 Hz, 1H), 4.37 (d, *J* = 7.6 Hz, 2H), 4.22 (d, *J* = 6.0 Hz, 1H), 3.20-1.60 (m, 9H), 2.94 (t, *J* = 6.9 Hz, 1H), 2.85 (d, *J* = 6.9 Hz, 1H), 2.36 (s, 1H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 87.4, 78.6, 64.4, 63.6; ^{11}B NMR (128 MHz; $CDCl_3$): δ -2.2, -2.9, -3.7, -9.1, -10.1, -11.4, -12.3; HRMS (ESI, positive) for $C_6H_{16}B_{10}O_2$ (m/z): calculated 253.1985 $(M+Na)^+$, found 253.1982.

Synthesis of 1-(2-phenylacetoxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18a)



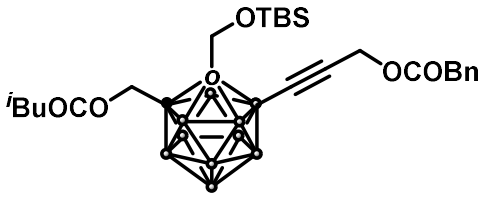
18a

To a solution of **17a** (62.9 mg, 0.103 mmol) and NaH_2PO_4 (24.7 mg, 0.206 mmol) in DCM/H_2O (1 mL, 10:1, v/v) was added DDQ (46.7 mg, 0.206 mmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was shortly purified by column chromatography on silica gel (20% to 40% EtOAc in Hexane) to use in the next step without further purification.

To a solution of crude material and triethylamine (43 μL, 0.309 mmol) in CH_2Cl_2 (1 mL), was slowly added phenylacetyl chloride (20 μL, 0.155 mmol) in CH_2Cl_2 at 0 ℃. After the resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% Et_2O in Hexane) to afford **18a** (39.7 mg, 65.2 μmol, 63%, 2 steps) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.37-7.26 (m, 10H), 4.73-4.71 (m, 3H), 6.88 (d, *J* = 8.7 Hz, 2H), 4.64 (d, *J* = 13.1 Hz, 1H), 4.32 (d, *J* = 12.6 Hz, 1H), 4.20 (d, *J* = 12.6 Hz, 1H), 3.67 (d, *J* = 3.2 Hz, 1H), 3.00-1.50 (m, 9H), 0.89 (s, 9H), 0.08 (d, *J* = 1.1 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.8, 169.9, 133.6, 132.9, 129.5, 129.4, 128.9, 128.8, 127.7, 127.4, 92.8, 79.4, 74.3, 63.5, 62.8, 53.1, 41.10, 41.08, 25.7, 18.2, -5.53, -5.56; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.1, -4.0, -11.4; HRMS (ESI, positive) for $C_{29}H_{44}B_{10}O_3Si$ (m/z): calculated 633.3823 $(M+Na)^+$, found 633.3837.

Synthesis of 1-((3-methylbutanoyl)oxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18b)

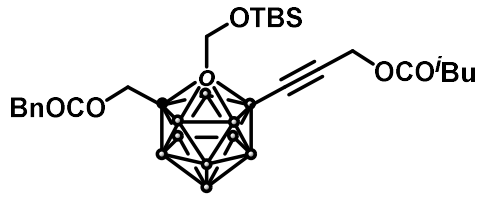


18b

This compound was prepared from **17a** (53.1 mg, 86.9 μmol) and 2.0 equivalent of isovaleryl chloride using the procedure described before for **18a** in 80% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.33-7.28 (m, 5H), 4.72 (m, 3H), 4.63 (d, *J* = 13.1 Hz, 1H), 4.40 (d, *J* = 12.5 Hz, 1H), 4.25 (d, *J* = 12.5 Hz, 1H), 3.66 (s, 2H), 2.25 (d, *J* = 7.1 Hz, 2H), 2.90-1.60 (m, 9H), 2.16-2.09 (m, 1H), 0.98 (d, *J* = 6.6 Hz, 6H), 0.90 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.5, 170.8, 133.6, 129.4, 128.8, 127.4, 79.5, 74.6, 63.5, 62.4, 53.2, 43.0, 41.1, 25.7, 25.6, 22.5, 18.3, -5.51, -5.54; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.3, -4.1, -11.4; HRMS (ESI, positive) for $C_{26}H_{46}B_{10}O_3Si$ (m/z): calculated 599.3977 $(M+Na)^+$, found 599.3971.

Synthesis of 1-(2-phenylacetoxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18c)

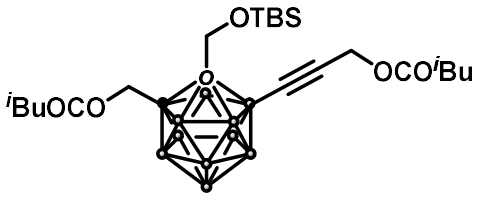


18c

This compound was prepared from **17b** (66.0 mg, 144.5 μmol) and benzyl chloride using the procedure described before for **18a** in 76% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.37-7.26 (m, 5H), 4.71 (d, *J* = 13.1 Hz, 1H), 4.68 (s, 2H), 4.64 (d, *J* = 13.1 Hz, 1H), 4.34 (d, *J* = 12.5 Hz, 1H), 4.21 (d, *J* = 12.5 Hz, 1H), 3.67 (s, 2H), 2.23 (d, *J* = 7.0 Hz, 2H), 2.90-1.60 (m, 9H), 2.15-2.10 (m, 1H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.90 (s, 9H), 0.10 (d, *J* = 3.0 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.3, 169.9, 132.9, 129.5, 128.9, 127.7, 93.1, 79.4, 74.3, 63.5, 62.8, 52.5, 43.2, 41.1, 25.9, 25.7, 22.5, 18.3, -5.5, -5.6; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.1, -4.1, -11.4; HRMS (ESI, positive) for $C_{26}H_{46}B_{10}O_3Si$ (m/z): calculated 599.3977 $(M+Na)^+$, found 599.3972.

Synthesis of 1-(3-methylbutanoyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18d)

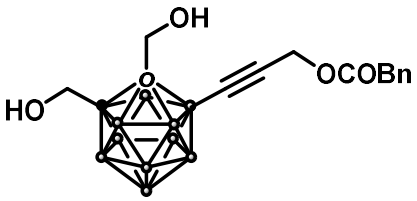


18d

This compound was prepared from **17b** (66.0 mg, 144.5 μmol) and isovaleryl chloride using the procedure described before for **18a** in 76% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 4.70 (d, *J* = 13.1 Hz, 1H), 4.68 (s, 2H), 4.63 (d, *J* = 13.1 Hz, 1H), 4.41 (d, *J* = 12.5 Hz, 1H), 4.27 (d, *J* = 12.5 Hz, 1H), 2.24 (d, *J* = 7.1 Hz, 2H), 2.22 (d, *J* = 7.0 Hz, 2H), 2.90-1.60 (m, 9H), 2.14-2.09 (m, 2H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.96 (d, *J* = 6.6 Hz, 6H), 0.91 (s, 9H), 0.12 (d, *J* = 1.3 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.3, 171.5, 93.2, 79.5, 74.6, 63.5, 62.3, 52.5, 43.2, 43.0, 25.9, 25.7, 25.6, 22.53, 22.48, 18.3, -5.5, -5.6; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.2, -4.1, -11.4; HRMS (ESI, positive) for $C_{23}H_{48}B_{10}O_3Si$ (m/z): calculated 565.4131 $(M+Na)^+$, found 565.4128.

Synthesis of 1,2-bis(hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (19a)

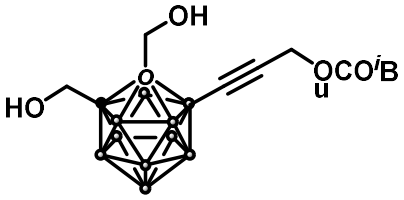


19a

To a solution of **17a** (66 mg, 0.109 mmol) in CH_2Cl_2 (1 mL), was added 4 M HCl in Dioxane (1 mL). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. The crude material was purified by column chromatography on silica gel (25% to 50% EtOAc in Hexane) to afford **19a** (24.7 mg, 0.0656 mmol, 60%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.29 (m, 5H), 4.69 (d, *J* = 15.7 Hz, 1H), 4.64 (d, *J* = 15.7 Hz, 1H), 4.27 (d, *J* = 13.9 Hz, 1H), 4.16-4.13 (m, 3H), 3.68 (s, 2H), 3.00-1.50 (m, 9H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 172.3, 133.4, 129.3, 129.0, 127.6, 92.3, 79.1, 78.8, 64.2, 63.1, 53.7, 41.3; ^{11}B NMR (128 MHz; $CDCl_3$): δ -3.24, -9.97, -10.9, 11.6; HRMS (ESI, positive) for $C_{15}H_{24}B_{10}O_4$ (m/z): calculated 401.2522 $(M+Na)^+$, found 401.2519.

Synthesis of 1,2-bis(hydroxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (19b)

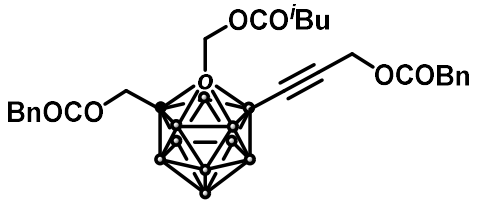


19b

To a solution of **17b** (70 mg, 0.121 mmol) in CH_2Cl_2 (1 mL), was added 4 M HCl in Dioxane (1 mL). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. The crude material was purified by column chromatography on silica gel (25% to 50% EtOAc in Hexane) to afford **19b** (36.7 mg, 0.106 mmol, 87%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 4.67 (d, *J* = 15.7 Hz, 1H), 4.60 (d, *J* = 15.7 Hz, 1H), 4.44 (d, *J* = 13.8 Hz, 1H), 4.29 (d, *J* = 13.8 Hz, 1H), 4.18 (d, *J* = 2.7 Hz, 2H), 3.00-1.50 (m, 9H), 2.24 (t, *J* = 3.9 Hz, 2H) 2.14-2.08 (m, 1H), 0.97 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 173.9, 92.9, 79.1, 78.8, 64.2, 63.3, 53.0, 43.3, 26.0, 22.4; ^{11}B NMR (128 MHz; $CDCl_3$): δ -3.24, -9.97, -10.9, 11.6; HRMS (ESI, positive) for $C_{12}H_{26}B_{10}O_4$ (m/z): calculated 367.2675 $(M+Na)^+$, found 367.2683.

Synthesis of 1-((3-methylbutanoyl)oxymethyl)-2-((3-methylbutanoyl)oxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIa)



IIa

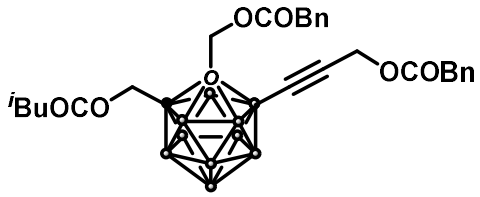
To a solution of **18a** (27 mg, 44.3 μmol) in CH_2Cl_2 (500 μL), was added 4 M HCl in Dioxane (500 μL). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure to use in the next reaction without further purification.

To a solution of the crude material and triethylamine (18 μL, 133 μmol) in CH_2Cl_2 (500 μL), was slowly added isovaleryl chloride (8.1 μL, 66.5 μmol) in CH_2Cl_2 at 0 °C. After the resulting mixture was stirred at room temperature for 9 h, the resulting mixture was concentrated under vacuum. The crude materials were filtered through basic Al_2O_3 pad and purified by column

chromatography on silica gel (25% Et_2O in hexane) afforded **IIa** (15.6 mg, 27.0 μmol, 61% 2 steps) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.33-7.26 (m, 10H), 4.75-4.67 (m, 6H), 3.68 (s, 2H), 3.67 (s, 2H), 2.20 (d, J = 7.1 Hz, 1H), 2.11-2.06 (m, 1H), 3.00-1.50 (m, 9H), 0.96 (d, J = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.2, 170.8, 169.8, 133.7, 132.7, 129.5, 129.4, 129.0, 128.8, 127.8, 127.4, 75.8, 75.3, 63.1, 61.4, 53.0, 42.9, 41.1, 41.0, 25.6, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.1, -11.0; HRMS (ESI, positive) for $C_{28}H_{38}B_{10}O_6$ (m/z): calculated 603.3532 $(M+Na)^+$, found 603.3532.

Synthesis of 1-((3-methylbutanoyl)oxymethyl)-2-(2-phenylacetoxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIb)

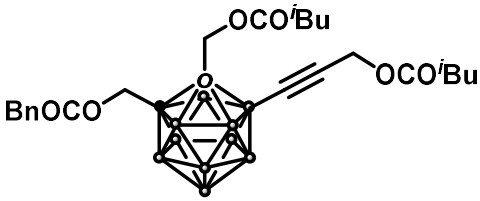


IIb

This compound was prepared from **18b** (39.7 mg, 69.1 μmol) and benzyl chloride using the procedure described before for **IIa** in 43% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.27 (m, 10H), 4.85 (m, 6H), 3.68 (d, J = 13.7 Hz, 1H), 4.80 (d, J = 13.7 Hz, 1H), 4.68 (s, 2H), 4.51 (d, J = 1.0 Hz, 1H), 3.67 (s, 2H), 3.66 (s, 2H), 3.00-1.50 (m, 9H), 2.20 (d, J = 7.0 Hz, 2H), 2.11-2.07 (m, 1H), 0.96 (d, J = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.2, 170.9, 169.8, 133.7, 132.9, 129.5, 129.4, 128.9, 128.8, 127.7, 127.4, 75.61, 75.57, 62.4, 62.0, 53.0, 42.8, 41.1, 41.0, 25.6, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.1, -11.1; HRMS (ESI, positive) for $C_{28}H_{38}B_{10}O_6$ (m/z): calculated 603.3532 $(M+Na)^+$, found 603.3529.

Synthesis of 1-(2-phenylacetoxymethyl)-2-((3-methylbutanoyl)oxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIc)

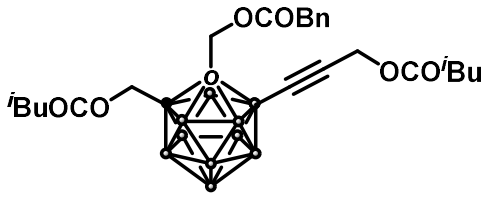


IIc

This compound was prepared from **18c** (29.8 mg, 51.8 μmol) and isovaleryl chloride using the procedure described before for **IIa** in 89% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.37-7.25 (m, 5H), 4.73 (d, *J* = 3.8 Hz, 2H), 4.68 (m, 4H), 3.67 (s, 2H), 2.90-1.60 (m, 9H), 2.24-2.20 (m, 4H), 2.14-2.07 (m, 2H), 0.98-0.96 (m, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.3, 171.2, 169.8, 132.8, 129.5, 129.0, 127.8, 94.0, 75.9, 75.4, 63.1, 61.5, 52.3, 43.2, 42.9, 41.1, 25.9, 25.6, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.1, -11.0; HRMS (ESI, positive) for $C_{25}H_{40}B_{10}O_6$ (m/z): calculated 569.3685 $(M+Na)^+$, found 569.3680.

Synthesis of 1-(3-methylbutanoyl)-2-(2-phenylacetoxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IId)

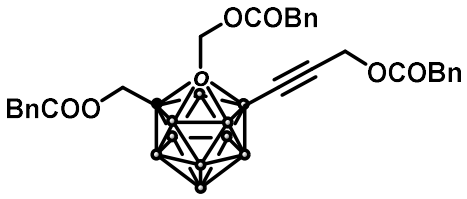


IId

This compound was prepared from **18d** (32.3 mg, 59.7 μmol) and benzyl chloride using the procedure described before for **IIa** in 71% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.36-7.27 (m, 5H), 4.85 (d, *J* = 3.1 Hz, 1H), 4.66 (s, 2H), 4.52 (d, *J* = 0.9 Hz, 1H), 3.69 (s, 2H), 2.90-1.60 (m, 9H), 2.29-2.18 (m, 4H), 2.15-2.05 (m, 2H), 0.96 (d, *J* = 6.6 Hz, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.4, 171.2, 169.8, 132.9, 129.5, 128.9, 127.8, 75.6, 75.5, 62.4, 62.1, 52.4, 43.2, 42.8, 41.0, 25.9, 25.6, 22.49, 22.48; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.9, -11.1; HRMS (ESI, positive) for $C_{25}H_{40}B_{10}O_6$ (m/z): calculated 569.3685 $(M+Na)^+$, found 569.3681.

Synthesis of 1,2-bis(phenylacetoxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIe)



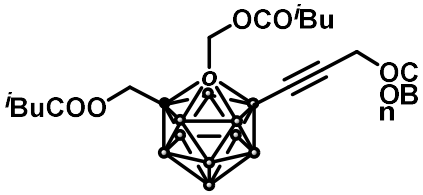
IIe

This compound was prepared from **19a** (11.9 mg, 31.6 μmol) and 2.0 equivalent of phenylacetyl chloride using the procedure described before for **13a** in 57% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.33-7.21 (m, 15H), 4.68 (d, *J* = 4.3 Hz, 4H), 4.49 (s, 2H), 3.66 (s, 2H), 3.61 (s, 4H), 2.90-1.40 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.8, 169.7, 169.7, 133.7, 132.9, 132.7, 129.51, 129.49, 129.41, 129.0, 128.9, 128.8, 127.7, 127.4, 93.5, 75.4, 75.1, 63.0,

62.1, 53.0, 41.1, 41.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.10, -11.4; HRMS (ESI, positive) for $C_{31}H_{36}B_{10}O_6$ (m/z): calculated 637.3378 $(M+Na)^+$, found 637.3386.

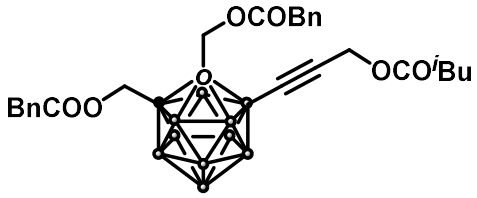
Synthesis of 1,2-bis((3-methylbutanoyl)oxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIf)



IIf

This compound was prepared from **19a** (12.6 mg, 33.5 μmol) and 2.0 equivalent of isovaleryl chloride using the procedure described before for **13b** in 23% yield as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$):δ 7.35-7.27 (m, 5H), 4.87 (d, *J* = 13.8 Hz, 1H), 4.79 (d, *J* = 13.8 Hz, 1H), 4.71 (s, 2H), 4.70-4.65 (m, 2H), 3.68 (s, 2H), 2.90-1.60 (m, 9H), 2.25 (d, *J* = 7.1 Hz, 4H) 2.16-2.07 (m, 2H), 0.99-0.97 (m, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.3, 171.3, 170.8, 133.7, 129.4, 128.8, 127.4, 75.9, 75.7, 62.5, 61.4, 53.0, 42.92, 42.89, 41.1, 25.62, 25.60, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.15, -11.1; HRMS (ESI, positive) for $C_{25}H_{40}B_{10}O_6$ (m/z): calculated 569.3685 $(M+Na)^+$, found 569.3684.

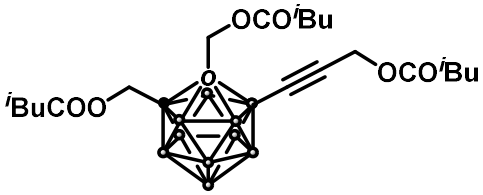
Synthesis of 1,2-bis(2-phenylacetoxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIg)



IIg

This compound was prepared from **19b** (18.6 mg, 54.3 μmol) and 2.0 equivalent of phenylacetyl chloride using the procedure described before for **13a** in 72% yield as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$):δ 7.36-7.28 (m, 6H), 7.25-7.22 (m, 4H), 4.71 (s, 2H), 4.66 (s, 2H), 4.50 (s, 2H), 3.63 (s, 2H), 3.61 (s, 2H), 2.90-1.40 (m, 9H), 2.23 (d, *J* = 7.1 Hz, 2H), 2.16-2.09 (m, 1H), 0.97 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.3, 169.70, 169.69, 132.9, 129.50, 125.49, 128.93, 128.89, 127.8, 127.7, 93.9, 75.4, 75.2, 63.0, 62.1, 52.3, 43.1, 41.0, 25.9, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.9, -10.3, -11.0; HRMS (ESI, positive) for $C_{28}H_{38}B_{10}O_6$ (m/z): calculated 603.3532 $(M+Na)^+$, found 603.3521.

Synthesis of 1,2-bis((3-methylbutanoyl)oxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIh)

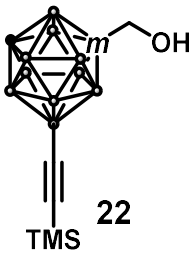


IIh

This compound was prepared from **19b** (19.4 mg, 56.7 μmol) and 2.0 equivalent of isovaleryl chloride using the procedure described before for **13b** in 88% yield as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$):δ 4.88 (d, *J* = 13.7 Hz, 1H), 4.82 (d, *J* = 13.7 Hz, 1H), 4.68-4.67 (m, 4H), 2.90-1.60 (m, 9H), 2.26-2.22 (m, 6H) 2.16-2.08 (m, 3H), 0.98-0.95 (m, 18H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.3, 171.3, 94.0, 75.8, 75.7, 62.5, 43.1, 42.92, 42.88, 25.9, 25.61, 25.60, 22.51, 22.50, 22.47; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.0, -10.3, -11.0; HRMS (ESI, positive) for $C_{25}H_{40}B_{10}O_6$ (m/z): calculated 535.3839 $(M+Na)^+$, found 535.3839.

- **Synthesis of scaffold III-type compounds**

Synthesis of 1-hydroxymethyl-9-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (22)

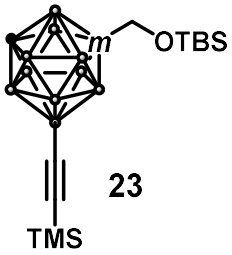


22

To a solution of 9-(2-(trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (**21**) (238 mg, 0.989 mmol) prepared according to the reported literature[21] in THF (4 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (622 μL, 0.989 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 2 h, paraformaldehyde (44 mg, 1.48 mmol) was added at -78 ℃. Then, the resulting mixture was warmed up to room temperature and stirred for 12 h under argon atmosphere. The reaction mixture was quenched with aq. 1 M HCl. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc, and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **22** (122 mg, 0.452 mmol, 46%) as colorless oil.

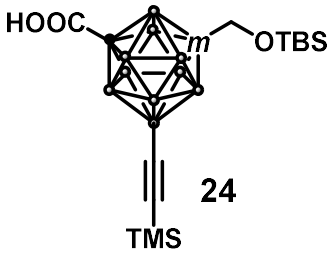
^{1}H NMR (500 MHz; $CDCl_3$): δ 3.81 (s, 2H), 2.93 (s, 1H), 3.40-1.60 (m, 9H), 0.15 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 105.1, 76.2, 65.3, 53.6, 21.2, 0.21; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.3, -8.6, -10.0, -11.5, -12.8, -14.1, -16.7, -18.3; HRMS (ESI, positive) for $C_8H_{22}B_{10}OSi$ (m/z): calculated 295.2281 $(M+Na)^+$, found 295.2273.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl-9-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (23)



To a solution of **22** (132 mg, 0.487 mmol) and 2,6-lutidine (85 μL, 0.730 mmol) in DCM (2 mL) was slowly added TBSOTf (132 μL, 0.584 mmol) at 0 ℃ under argon atmosphere. The resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford **23** (185 mg, 0.480 mmol, 99%) as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.81 (s, 2H), 2.93 (s, 1H), 3.40-1.60 (m, 9H), 0.15 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 105.1, 76.2, 65.3, 53.6, 21.2, 0.21; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.3, -8.6, -10.0, -11.5, -12.8, -14.1, -16.7, -18.3; HRMS (ESI, positive) for $C_{14}H_{36}B_{10}OSi_2$ (m/z): calculated 409.3156 $(M+Na)^+$, found 409.3153.

Synthesis of 7-((*tert*-butyl)dimethylsilyl)hydroxymethyl-9-(trimethylsilyl)ethynyl-1,7-dicarba-*closo*-dodecaboranylcarboxylic acid (24)

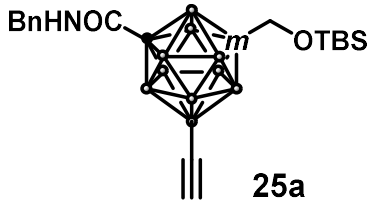


To a solution of **23** (184 mg, 0.478 mmol) in THF (1.8 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (316 μL, 0.502 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 2 h, CO_2 balloon was attached. Then, the resulting mixture was warmed up to room temperature and stirred for 12 h under CO_2 atmosphere. After that, the reaction

mixture was concentrated under pressure. Hexane was added after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, to the aqueous layer, were added 6 M HCl and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum to afford **24** (188 mg, 92%) as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ 3.75 (s, 2H), 3.10-1.80 (m, 9H), 0.89 (s, 9H), 0.16 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 165.7, 105.9, 79.3, 69.2, 65.5, 25.8, 18.4, 0.18, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.9, -6.8, -10.4, -12.7, -17.1; HRMS (ESI, positive) for $C_{15}H_{36}B_{10}O_3Si_2$ (m/z): calculated 453.3056 $(M+Na)^+$, found 453.3051; m.p. 119-120 °C.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-benzylaminocarboxyl-9-ethynyl-1,7-dicarba-*closo*-dodecaborane (25a)



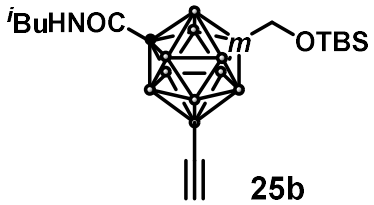
25a

To a solution of **24** (87.8 mg, 0.281 mmol) in THF (1 mL), benzylamine (37 μL, 0.337 mmol), EDCI (65 mg, 0.337 mmol), HOBt (65 mg, 0.422 mmol) and DIEA (143 μL, 0.843 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was used to the next reaction without further purification.

To a solution of the crude material in MeOH (500 μL), potassium carbonate (40 mg, 0.444 mmol) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. To the resulting mixture was added water and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford **25a** (20.5 mg, 0.0460 mmol, 16% 2 steps) as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36-7.17 (m, 4H), 7.18 (d, J = 7.0 Hz, 2H), 6.09 (s, 1H), 4.38 (d, J = 5.5 Hz, 2H), 3.78 (s, 2H), 3.40-1.60 (m, 9H), 2.27 (s, 1H), 0.89 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125

MHz; $CDCl_3$): δ 160.0, 136.8, 129.1, 128.1, 127.6, 86.4, 73.6, 65.5, 45.0, 43.9, 25.8, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.6, -7.9, -10.9, -12.5, -15.3, -16.6; HRMS (ESI, positive) for $C_{19}H_{35}B_{10}O_2NSi$ (m/z): calculated 470.3291 $(M+Na)^+$, found 470.3285.

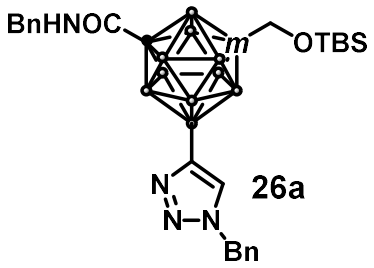
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl-7-isobutylcarbamoyl-9-ethynyl-1,7-dicarba-*closo*-dodecaborane (25b)



25b

This compound was prepared from **24** (102 mg, 0.327 mmol) using the procedure described for **25a** in 56% yield as a white solid. ^1H NMR (500 MHz; $CDCl_3$): δ 5.83 (s, 1H), 3.76 (s, 2H), 3.02 (t, J = 6.4 Hz, 2H), 3.40-1.80 (m, 9H), 2.27 (s, 1H), 1.79-1.70 (m, 1H), 0.88 (s, 9H), 0.87 (d, J = 6.8 Hz, 6H), 0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.9, 86.4, 76.5, 74.0, 65.5, 48.1, 28.5, 25.8, 19.9, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.9, -7.9, -11.0, -12.5, -15.5, -16.6; HRMS (ESI, positive) for $C_{16}H_{37}B_{10}NO_2Si$ (m/z): calculated 436.3444 $(M+Na)^+$, found 436.3423; m.p. 69-70 °C.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-benzylaminocarboxyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (26a)

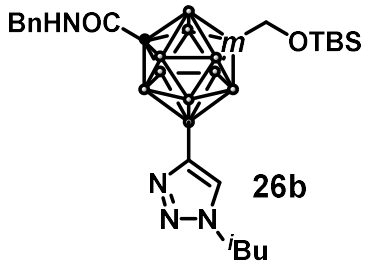


26a

To a solution of **25a** (17 mg, 38.4 μmol) in DMF:H_2O (1:1, v/v, 500 μL), were added CuI (0.7 mg, 3.84 μmol), sodium ascorbate (1.5 mg, 7.67 μmol), and benzylazide (5.1 μL, 38.4 μmol). The mixture was stirred vigorously at room temperature for 12 h. The reaction was quenched with water and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (40% EtOAc in Hexane) to afford **26a** (15.5 mg, 26.8 μmol, 70%) as a white solid.
^1H NMR (500 MHz; $CDCl_3$):δ 7.37-7.26 (m, 10H), 7.18 (d, J = 7.0 Hz, 2H), 6.25 (s, 1H), 5.50 (s, 2H), 4.38 (d, J = 5.5 Hz, 2H), 3.81 (s, 2H), 3.50-1.80 (m, 9H), 0.88 (s, 9H), 0.04 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 136.9, 134.9, 129.2, 129.0, 128.7, 128.3, 128.0, 127.6, 74.1,

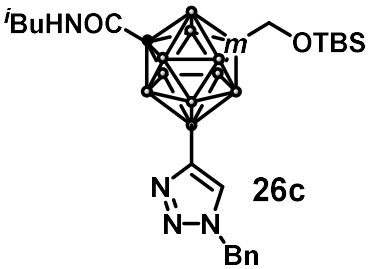
65.5, 53.8, 44.9, 29.8, 25.8, 18.3, -5.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.2, -8.1, -10.6, -12.2, -16.6; HRMS (ESI, positive) for $C_{26}H_{42}B_{10}N_4O_2Si$ (m/z): calculated 603.3938 $(M+Na)^+$, found 603.3939; m.p. 66-68 °C.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (26b)



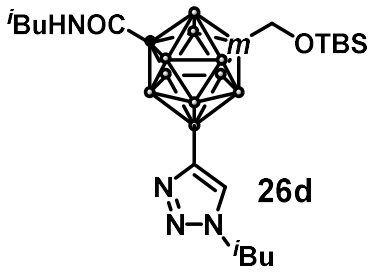
This compound was prepared from **25a** (38.0 mg, 85.3 μmol) and isovalerylazide using the procedure described before for **26a** in 84% yield as a white solid; m.p. 49-51 °C; ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.40 (s, 1H), 7.33-7.26 (m, 3H), 7.18 (d, J = 7.0 Hz, 2H), 6.24 (s, 1H), 4.39 (s, 2H), 4.12 (d, J = 5.7 Hz, 2H), 3.83 (s, 2H), 3.20-2.00 (m, 9H), 2.19 (m, 1H), 0.92 (d, J = 6.4 Hz, 6H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 136.9, 129.0, 128.0, 127.6, 74.0, 65.5, 57.2, 44.9, 29.8, 25.8, 20.1, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.1, -8.1, -12.9, -15.3; HRMS (ESI, positive) for $C_{23}H_{44}B_{10}N_4O_2Si$ (m/z): calculated 569.4092 $(M+Na)^+$, found 569.4075.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-isobutylcarbamoyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (26c)



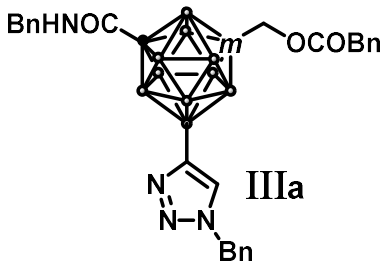
This compound was prepared from **25b** (34.6 mg, 84.1 μmol) using the procedure described before for **26a** in 93% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.37-7.23 (m, 5H), 5.94 (s, 1H), 5.50 (s, 2H), 3.80 (s, 2H), 3.02 (m, 2H), 3.00-1.80 (m, 9H), 0.87 (m, 15H), 0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.2, 135.0, 129.1, 128.7, 128.2, 74.4, 65.5, 48.2, 25.8, 20.0, 18.3, -5.5 ^{11}B NMR (160 MHz; $CDCl_3$): δ –5.1, -8.1, -12.2, -15.2; HRMS (ESI, positive) for $C_{23}H_{44}B_{10}N_4O_2Si$ (m/z): calculated 569.4092 $(M+Na)^+$, found 569.4078.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-isobutylcarbamoyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (26d)



This compound was prepared from **25b** (35.8 mg, 87.0 μmol) and isovalerylazide using the procedure described before for **26a** in 14% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.43 (s, 1H), 6.04 (s, 1H), 4.14 (d, *J* = 7.1 Hz, 2H), 3.82 (s, 2H), 3.04 (m, 2H), 2.24-2.19 (m, 1H), 1.79-1.77 (m, 1H), 0.94 (d, *J* = 6.6 Hz, 6H), 0.89 (s, 9H), 0.87 (d, *J* = 6.7 Hz, 6H), 0.05 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 74.6, 65.6, 57.5, 48.2, 29.9, 28.5, 20.1, 20.0, 18.4, -5.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.4, -8.2, -10.6, -12.5, 16.4; HRMS (ESI, positive) for $C_{20}H_{46}B_{10}N_4O_2Si$ (m/z): calculated 535.4245 $(M+Na)^+$, found 535.4230.

Synthesis of 1-phenylacetoxymethyl-7-benzylaminocarboxyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIa)



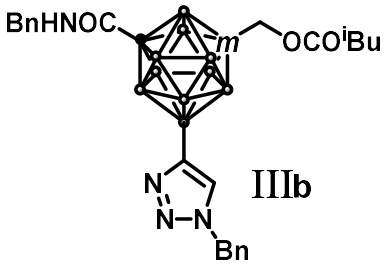
To a solution of **26a** (11.9 mg, 26.8 μmol) in CH_2Cl_2 (500 μL), was added 4N HCl in Dioxane (500 μL). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure to use in the next reaction without further purification.

To a solution of the crude material and triethylamine (16.7 μL, 0.121 mmol) in CH_2Cl_2 (500 μL), was slowly added phenylacetyl chloride (5.3 μL, 40.2 μmol) at 0 ℃. After the resulting mixture was stirred at room temperature for 9 h, the resulting mixture was concentrated under vacuum. The crude materials were filtered through basic Al_2O_3 pad and purified by column chromatography on silica gel (20% to 40% EtOAc in hexane) to afford **IIIa** (12.6 mg, 21.6 μmol, 81% 2 steps) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.40-7.17 (m, 15H), 6.15 (s, 1H), 5.51 (s, 2H), 4.38 (d, *J* = 5.8 Hz, 2H), 4.34 (s, 2H),3.65 (s, 2H), 3.40-1.80 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.1, 159.8,

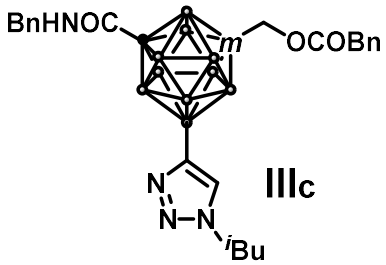
136.7, 135.0, 133.1, 129.5, 129.2, 129.1, 128.8, 128.7, 128.3, 128.1, 127.6, 127.5, 127.0, 74.5, 71.8, 64.6, 53.8, 45.0, 41.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ –4.7, -7.5, -10.3, -12.0, -15.0; HRMS (ESI, positive) for $C_{28}H_{34}B_{10}N_4O_3$ (m/z): calculated 619.3301 $(M+Na)^+$, found 619.3329.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-7-benzylaminocarboxyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIb)



This compound was prepared from **26a** (7.1 mg, 12.3 μmol) and isovaleryl chloride using the procedure described before for **IIIa** in 76% yield as colorless oil.
^{1}H NMR (500 MHz; $CDCl_3$):δ 7.33-7.26 (m, 9H), 7.18 (d, *J* = 8.2 Hz, 2H), 6.15 (s, 1H), 5.51 (s, 2H), 4.38 (d, *J* = 5.7 Hz, 2H), 4.33 (s, 2H), 3.40-1.80 (m, 9H), 2.23 (d, *J* = 7.2 Hz, 2H), 2.14-2.07 (m, 1H), 0.96 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.6, 159.9, 136.7, 135.0, 129.2, 129.1, 128.7, 128.3, 128.1, 127.6, 127.0, 74.5, 72.1, 64.2, 53.8, 45.0, 43.0, 25.6, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ –4.7, -7.5, -10.2, -11.8, -15.3; HRMS (ESI, positive) for $C_{25}H_{36}B_{10}N_4O_3$ (m/z): calculated 585.3455 $(M+Na)^+$, found 585.3460.

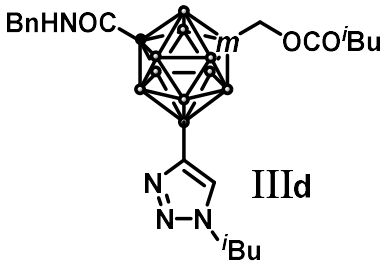
Synthesis of 1-phenylacetoxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIIc)



This compound was prepared from **26b** (17.6 mg, 32.3 μmol) using the procedure described before for **IIIa** in 68% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.39 (s, 1H), 7.36-7.27 (m, 8H), 7.19 (d, *J* = 6.8 Hz, 2H), 6.13 (s, 1H), 4.39 (d, *J* = 5.7 Hz, 2H), 4.36 (s, 2H), 4.13 (d, *J* = 7.2 Hz, 2H), 3.67 (s, 2H), 3.40-1.80 (m, 9H), 2.23-2.17 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.1, 159.8, 136.7, 133.1, 129.5, 129.1, 128.8, 128.1, 127.6, 127.5, 127.5, 74.5, 71.8, 64.6, 57.2, 45.0, 41.0, 29.8, 20.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.5, -7.1, -10.0, -11.6, -15.3; HRMS (ESI, positive) for $C_{25}H_{36}B_{10}N_4O_3$ (m/z): calculated 585.3455

$(M+Na)^+$, found 585.3470.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIId)

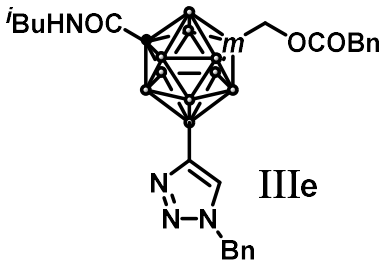


IIId

This compound was prepared from **26b** (20.2 mg, 37.1 μmol) and isovaleryl chloride using the procedure described before for **IIIa** in quantitative yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.41 (s, 1H), 7.35-7.28 (m, 3H), 7.19-7.18 (m, 2H), 6.20 (s, 1H), 4.39 (d, *J* = 5.7 Hz, 2H), 4.35 (s, 2H), 4.13 (d, *J* = 7.2 Hz, 2H), 3.20-1.80 (m, 9H), 2.24 (d, *J* = 7.1 Hz, 2H), 2.22-2.17 (m, 1H), 2.15-2.09 (m, 1H), 0.97 (d, *J* = 6.6 Hz, 6H), 0.93 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.6, 159.9, 136.7, 129.0, 128.1, 127.6, 127.5, 74.5, 72.0, 64.2, 57.2, 45.0, 43.0, 29.8, 25.6, 22.5, 20.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.8, -7.63, -10.4, -12.1, -15.0; HRMS (ESI, positive) for $C_{22}H_{38}B_{10}N_4O_3$ (m/z): calculated 551.3609 $(M+Na)^+$, found 551.3639.

Synthesis of 1-phenylacetoxymethyl-7-isobutylcarbamoyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIe)

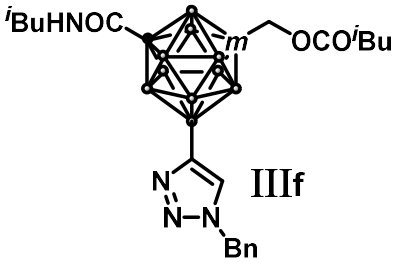


IIIe

This compound was prepared from **26c** (20.8 mg, 38.1 μmol) using the procedure described before for **IIIa** in 61% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.40-7.23 (m, 10H), 5.89 (s, 1H), 5.52 (s, 2H), 4.34 (s, 2H), 3.65 (s, 2H), 3.02 (d, *J* = 6.0 Hz, 2H), 1.78-1.71 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.1, 159.8, 135.1, 133.1, 129.5, 129.2, 128.8, 128.7, 128.3, 127.5, 127.0, 74.9, 71.8, 64.6, 53.8, 48.2, 41.0, 28.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.0, -7.5, -10.4, -12.2, -15.3, -16.7; HRMS (ESI, positive) for $C_{25}H_{36}B_{10}N_4O_3$ (m/z): calculated 585.3455 $(M+Na)^+$, found 585.3456.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-7-isobutylcarbamoyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIf)

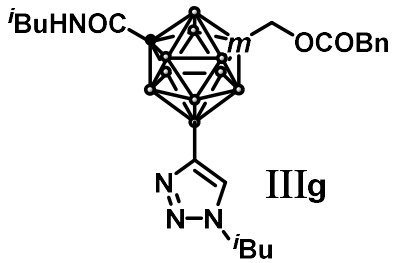


IIIf

This compound was prepared from **26c** (22.0 mg, 40.3 μmol) and isovaleryl chloride using the procedure described before for **IIIa** in 77% yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.40-7.26 (m, 5H), 5.92 (s, 1H), 5.52 (s, 2H), 4.33 (s, 2H), 3.02 (t, *J* = 6.4 Hz, 2H), 2.23 (d, *J* = 7.1 Hz, 2H), 2.14-2.07 (m, 1H), 3.00-1.80 (m, 9H), 1.78-1.72 (m, 1H), 0.96 (d, *J* = 6.7 Hz, 6H), 0.86 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.6, 159.8, 135.0, 129.2, 128.7, 128.3, 127.0, 74.9, 72.0, 64.2, 53.8, 48.2, 43.0, 28.5, 25.6, 22.5, 20.1, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.9, -7.7, -10.4, -12.2, -15.4, -16.8; HRMS (ESI, positive) for $C_{22}H_{38}B_{10}N_4O_3$ (m/z): calculated 551.3609 $(M+Na)^+$, found 551.3611.

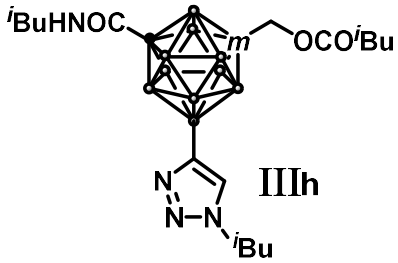
Synthesis of 1-phenylacetoxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIIg)



IIIg

This compound was prepared from **26d** (3.9 mg, 7.64 μmol) using the procedure described before for **IIIa** in quantitative yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$):δ 7.40 (s, 1H), 7.35-7.32 (m, 2H), 7.29-7.27 (m, 2H), 5.88 (s, 1H), 4.36 (s, 2H), 4.14 (d, *J* = 7.2 Hz, 2H), 3.66 (s, 2H), 3.03 (t, *J* = 6.4 Hz, 2H), 3.20-1.80 (m, 9H), 2.24-2.19 (m, 1H), 1.78-1.73 (m, 1H), 0.95 (d, *J* = 6.7 Hz, 6H), 0.88 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.1, 159.8, 133.1, 129.6, 128.8, 127.54, 127.48, 74.9, 71.8, 64.6, 57..3, 48.2, 41.1, 29.9, 28.5, 20.1, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.8, -7.1, -10.4, -12.1; HRMS (ESI, positive) for $C_{22}H_{38}B_{10}N_4O_3$ (m/z): calculated 551.3609 $(M+Na)^+$, found 551.3633.

Synthesis of 1-(3-methylbutanoyl)oxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIIh)



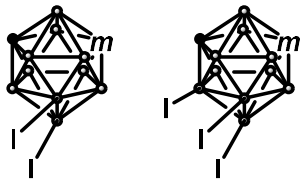
IIIh

This compound was prepared from **26d** (16.5 mg, 32.3 μmol) and isovaleryl chloride using the procedure described before for **IIIa** in quantitative yield as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$):δ 7.42 (s, 1H), 5.93 (s, 1H), 4.35 (s, 2H), 4.14 (d, *J* = 7.3 Hz, 2H), 3.04 (d, *J* = 7.3 Hz, 2H), 3.00-1.80 (m, 9H), 2.24 (d, *J* = 7.1 Hz, 2H), 2.23-2.17 (m, 1H), 2.15-2.07 (m, 1H), 1.79-1.73 (m, 1H), 0.97 (d, *J* = 6.7 Hz, 6H), 0.95 (d, *J* = 6.7 Hz, 6H), 0.87 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.7, 159.9, 127.5, 74.9, 72.0, 64.2, 57.3, 48.2, 43.0, 29.9, 28.5, 25.6, 22.5, 20.1, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.8, -7.3, -10.2, -12.2, -15.0, -16.7; HRMS (ESI, positive) for $C_{19}H_{40}B_{10}N_4O_3$ (m/z): calculated 517.3752 $(M+Na)^+$, found 517.3733.

- **Synthesis of scaffold IV-type compounds**

Synthesis of 9,10-diiodo-1,7-dicarba-*closo*-dodecaborane (27) and 4,9,10-triiodo-1,7-dicarba-*closo*-dodecaborane (27')



27 **27'**

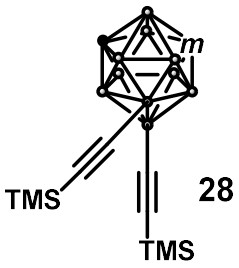
To a solution of *m*-carborane (72.1 mg, 0.500 mmol) and I_2 (133.1 mg, 0.525 mmol) in acetic acid (1.5 mL) was added a 1:1 mixture of concentrated H_2SO_4 and concentrated HNO_3 (0.375 mL). The resulting mixture was stirred at 100 °C for 40 min with microwave. Then, the colorless orange mixture was cooled to room temperature and poured into ice wa*ter*the white precipitate was filtered, washed with water, and the resulting mixture was purified by column chromatography on silica gel (15% EtOAc in hexane) to give white solids **27** (166.7 mg, 0.42 mmol, 84%) and **27'** (26.6 mg, 0.051 mmol, 10%).

(**27**)^{1}H NMR (500 MHz; $CDCl_3$): δ 3.18 (s, 2H).

The NMR spectra of this compound is consistent with previous report.[21]

(**27'**)^{1}H NMR (500 MHz; $CDCl_3$): δ 3.61 (s, 1H), 3.48 (s, 1H), 4.00-2.50 (m, 7H); ^{13}C NMR (125 MHz; $CDCl_3$): 58.9, 54.0; δ ;^{11}B NMR (160 MHz; $CDCl_3$): δ -4.1, -9.9, -11.8, -15.7, -18.0; HRMS (ESI, positive) for $C_2H_9B_{10}I_3$ (m/z): calculated 546.8665 $(M+Na)^+$, found 546.8665.

Synthesis of 9,10-di((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (28)



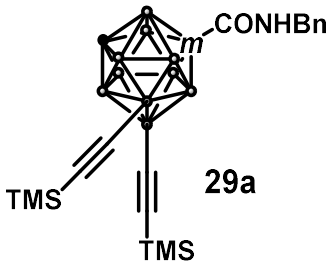
To a solution of TMS acetylene (1.46 ml, 10.6 mmol) in THF (6 ml), add ethyl magnesium bromide 3.0 M in THF (3.51 ml, 10.6 mmol) under argon; reflux at 60 °C for 2 hours.

To a solution of **27** (837.7 mg, 2.11 mmol) in THF (14 ml), add dichlorobis(triphenylphosphine)-palladium (147.3 mg, 0.21 mmol) under argon and degas. Add the previously adjusted TMS acetylene magnesium bromide and reflux at 60°C overnight. The reaction was quenched with NH_4Cl and the brown mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) afforded **28** (666.7 mg, 1.98 mmol, 94%) as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ 2.83(s, 2H), 0.17 (s, 18H).

The NMR spectra of this compound is consistent with previous report.[21]

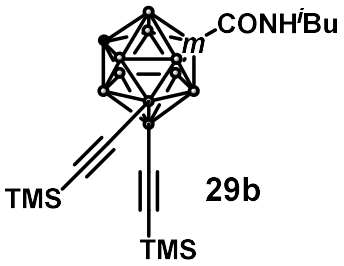
Synthesis of 1-benzylaminocarboxyl-9,10-di((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (29a)



To a solution of **28** (0.952 g, 2.50 mmol) in THF (10 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (1.75 mL, 2.75 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 2 h, CO_2 balloon was attached. Then, the resulting mixture was stirred at 0 ℃ for 2 h under CO_2 atmosphere. The reaction was quenched with 1 M HCl and the mixture was extracted with Et_2O, washed with brine, dried over sodium sulfate, and concentrated

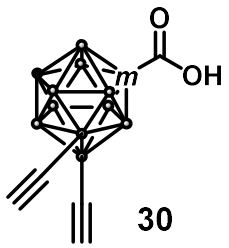
under vacuum. The crude materials were used to the next reaction without further purification. To a solution of the crude material in THF (4 mL), benzylamine (328 μL, 3.00 mmol), COMU (1.18 g, 2.75 mmol) and DIEA (871 μL, 5.00 mmol) were added. The resulting mixture was stirred at room temperature under argon atmosphere for 25 min until complete conversion of the starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was extracted with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc) to afford **29a** (484.4 mg, 1.03 mmol, 41% 2 steps) as a white solid; m.p. 127-128 °C; ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.35-7.16 (m, 5H), 6.15 (t, *J*=5.3 Hz, 1H), 4.35(d, *J* = 5.7 Hz, 2H), 2.96(s, 1H), 0.18 (s, 18H) ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.6, 136.7, 129.1, 128,1, 127.6, 107.2, 72.0, 51.4, 45.0 0.22 ^{11}B NMR (160 MHz; $CDCl_3$): δ -6.32, -9.63, -13.63, -18.77; HRMS (ESI, positive) for $C_{20}H_{35}B_{10}NOSi_2$ (m/z): calculated 494.3114 $(M+Na)^+$, found 494.3117.

Synthesis of 1-isobutylaminocarboxyl-9,10-di((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (29b)



This compound was prepared from **28** (1.01 g, 2.65 mmol) and isobutylamine using the procedure described before for **29a** (572.4 mg, 1.31 mmol, 48% 2 steps) as a white solid; m.p. 166-167 °C; ^{1}H NMR (400 MHz; $CDCl_3$): δ 5.85 (t, *J* = 5.5 Hz, 1H), 3.00 (t, *J* = 6.5 Hz, 2H), 2.93 (s, 1H), 1.78-1.68 (m, *J* = 6.7 Hz, 1H), 0.86 (d, *J* = 6.7 Hz, 6H), 0.16 (s, 18H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.6, 107.0, 72.4, 51.4, 48.2, 28.5, 20.0, 0.24; ^{11}B NMR (160 MHz; $CDCl_3$): δ -6.52, -9.73, -13.75, -18.90; HRMS (ESI, positive) for $C_{17}H_{37}B_{10}NOSi_2$ (m/z): calculated 460.3268 $(M+Na)^+$, found 460.3265.

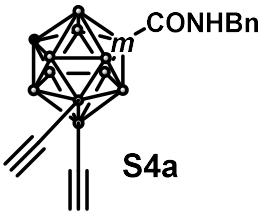
Synthesis of 1-benzylaminocarboxyl-9,10-di((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (30)



30

To a solution of **28** (460 mg, 1.37 mmol) in THF (11 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (957 μL, 1.50 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 2 h, CO_2 balloon was attached. Then, the resulting mixture was stirred at 0 ℃ for 2 h under CO_2 atmosphere. The reaction was quenched with 1M HCl and the mixture was extracted with Et_2O, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude materials were used to the next reaction without further purification.
To a solution of the crude material in MeOH (10 mL), potassium carbonate (568 mg, 4.11 mmol) was added. The resulting mixture was stirred at room temperature for 30 min until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. Hexane was added after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, to the aqueous layer, were added 6 M HCl and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum to afford **30** (97 mg, 0.411 mmol, 30% 2 steps) as a white solid; m.p. 166-167 ℃; ^{1}H NMR (400 MHz; $CDCl_3$): δ 5.85 (t, *J* = 5.5 Hz, 1H), 3.00 (t, *J* = 6.5 Hz, 2H), 2.93 (s, 1H), 1.78-1.68 (m, *J* = 6.7 Hz, 1H), 0.86 (d, *J* = 6.7 Hz, 6H), 0.16 (s, 18H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.6, 107.0, 72.4, 51.4, 48.2, 28.5, 20.0, 0.24; ^{11}B NMR (160 MHz; $CDCl_3$): δ -6.52, -9.73, -13.75, -18.90; HRMS (ESI, positive) for $C_{17}H_{37}B_{10}NOSi_2$ (m/z): calculated 460.3268 $(M+Na)^+$, found 460.3265.

Synthesis of 1-benzylaminocarboxyl-9,10-diethynyl-1,7-dicarba-*closo*-dodecaborane (S4a)

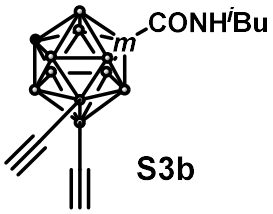


S4a

To a solution of **29a** (484.4 mg, 1.03 mmol) in MeOH (10 mL), potassium carbonate (1.40 g, 10.3 mmol) was added. The resulting mixture was stirred at room temperature for 30 min until the full

conversion of a starting material was observed. To the resulting mixture was added DCM after which the product was filtered with celite. The material was purified by short column chromatography on silica gel (DCM 100%) to afford **S4a** (309.1 mg, 0.95 mmol, 92%) as a white solid; m.p. 141-142 °C; ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.28-7.08 (m, 5H), 6.09 (t, *J* = 4.8 Hz, 1H), 4.29(d, *J* = 5.7 Hz, 2H), 2.97(s, 1H), 2.33 (s, 2H); ^{13}C NMR (125 MHz; $CDCl_3$): δ159.2, 136.6, 129.0, 128.128.1, 128.0, 87.7, 72.6, 52.0, 45.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.33, -6.62, -9.88, -12.02, -13.42, -18.39; HRMS (ESI, positive) for $C_{14}H_{19}B_{10}NO$ (m/z): calculated 350.2312 $(M+Na)^+$, found 350.2318.

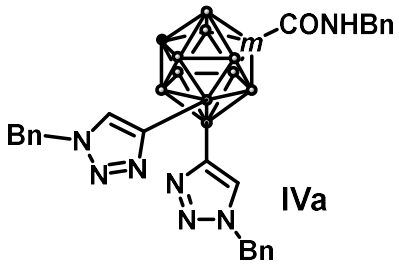
Synthesis of 1-isobutylaminocarboxyl-9,10-diethynyl-1,7-dicarba-*closo*-dodecaborane (S3b)



S3b

This compound was prepared from **29b** (572.4 mg, 1.31 mmol) using the procedure described before for **S4a** (383.0 mg, 1.31 mmol, quant.) as a white solid; m.p. 148-149 °C; ^{1}H NMR (400 MHz; $CDCl_3$): δ 5.90 (t, *J* = 4.9 Hz, 1H), 3.06 (s, 1H), 3.02 (t, *J* = 6.4 Hz, 2H), 2.40 (s, 2H), 1.80-1.70 (m, *J* = 6.7 Hz, 1H), 0.86 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.1, 87.7, 77.4, 77.1, 76.8, 72.9, 51.9, 48.1, 28.3, 19.8; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.28, -6.81, -9.95, -12.03, -13.47, -18.47; HRMS (ESI, positive) for $C_{11}H_{21}B_{10}NO$ (m/z): calculated 316.2464 $(M+Na)^+$, found 316.2465.

Synthesis of 1-benzylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVa)

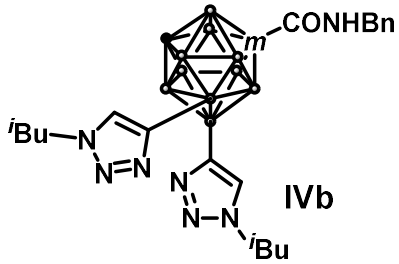


IVa

To a solution of **S4a** (148 mg, 0.455 mmol) in DMF:H_2O (1.6 mL, 4:1, v/v), were added CuI (8.6 mg, 45 μmol), sodium ascorbate (18 mg, 91 μmol) and azide (114 μL, 0.910 mmol). The mixture was stirred at room temperature for 24 h under argon atmosphere. The reaction was quenched with water and the mixture was extracted with EtOAc, washed with brine, dried over sodium

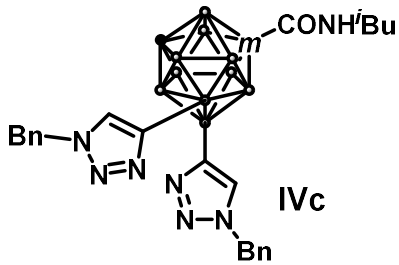
sulfate, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (80% EtOAc) to afford **IVa** (220 mg, 0.372 mmol, 82%) as a white solid; m.p. 70-72 °C; ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.33 (s, 2H), 7,28-7.10 (m, 15H), 6.66 (t, *J* = 5.7 Hz, 1H), 5.31 (s, 4H), 4.34 (d, *J* = 5.8 Hz, 2H), 3.18 (s, 1H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.7, 142.5, 136.8, 134.9, 128.9, 128.4, 127.8, 127.7, 127.6, 127.4, 72.9, 53.3, 52.2, 44.7; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.01, -11.27; HRMS (ESI, positive) for $C_{28}H_{33}B_{10}N_7O$ (m/z): calculated 616.3607 $(M+Na)^+$, found 616.3596.

Synthesis of 1-benzylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)isobutylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVb)



This compound was prepared from **S4a** (161 mg, 0.494 mmol) using the procedure described before for **IVa** (75 mg, 0.143 mmol, 29%) as a white solid; m.p. 76-77 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.60 (s, 2H), 7.28-7.22 (m, 5H), 6.88 (t, *J* = 5.0 Hz, 1H), 4.38 (d, *J* = 5.9 Hz, 2H), 4.04 (d, *J* = 7.2 Hz, 4H), 3.28 (s, 1H), 2.17-2.09 (m, 2H), 0.85 (d, *J* = 6.7 Hz, 12H); ^{13}C NMR (125 MHz; $CDCl_3$): δ159.6, 137.1, 128.8, 128.7, 127.7, 127.6, 73.3, 57.4, 52.5, 44.7, 29.5, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.07, -12.35, -17.16; HRMS (ESI, positive) for $C_{22}H_{37}B_{10}N_7O$ (m/z): calculated 548.3914 $(M+Na)^+$, found 548.3916.

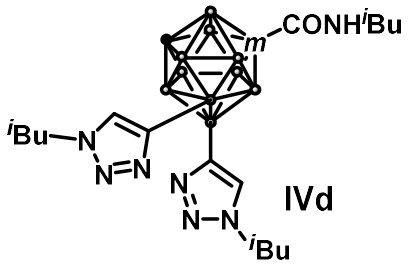
Synthesis of 1-isobutylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVc)



This compound was prepared from **S4b** (192 mg, 0.660 mmol) using the procedure described before for **IVa** (277 mg, 0.497 mmol, 75%) as a white solid; m.p. 176-177 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34 (s, 2H), 7.30-7.13 (m, 10H), 6.24 (t, *J* = 5.7 Hz, 1H), 5.35 (s, 4H), 3.22 (s, 1H), 3.01 (t, *J* = 6.5 Hz, 2H), 1.78-1.70 (m, 1H), 0.83 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz;

$CDCl_3$): δ159.6, 128.7, 128.2, 127.7, 127.6, 73.1, 53.2, 52.1, 47.9, 28.1, 19.7; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.70, -13.10, -17.48; HRMS (ESI, positive) for $C_{25}H_{35}B_{10}N_7O$ (m/z): calculated 582.3761 $(M+Na)^+$, found 582.3759.

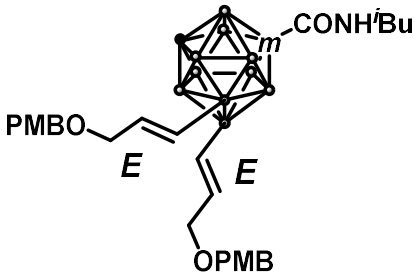
Synthesis of 1-isobutylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)isobutylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVd)



IVd

This compound was prepared from **S4b** (191 mg, 0.654 mmol) using the procedure described before for **IVa** (123 mg, 0.251 mmol, 38%) as a white solid; m.p. 103-105 °C; ^1H NMR (500 MHz; $CDCl_3$): δ 7.51 (s, 2H), 6.20 (t, J = 5.3 Hz, 1H), 4.10 (d, J = 7.5 Hz, 4H), 3.22 (s, 1H), 3.05 (t, J = 6.5 Hz, 2H), 2.22-2.13 (m, 2H), 1.85-1.77 (m, 1H), 0.89 (d, J = 6.7 Hz, 12H), 0.88 (d, J = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ159.5, 128.5, 73.81, 57.5, 52.0, 48.2, 29.5, 28.4, 20.0, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.81, -11.24, -17.19; HRMS (ESI, positive) for $C_{19}H_{39}B_{10}N_7O$ (m/z): calculated 514.4067 $(M+Na)^+$, found 514.4064.

Synthesis of 9,10-bis((*E*)-3-((4-methoxybenzyl)oxy)prop-1-en-1-yl)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (32)



32

To a solution of 9,10-diiodo-1,7-dicarba-*closo*-dodecaborane (**27**) (1.03 g, 2.60 mmol), silver phosphate (2.72 g, 6.50 mmol), and Herrmann's catalyst (trans-di-μ-acetatobis[2-(di-o-tolylphosphino)-benzyl]dipalladium)(II) (244 mg, 0.26 mmol) in dry DMF (15 mL) was added 1-((allyloxy)methyl)-4-methoxybenzene (1.02 g, 5.72 mmol). The mixture was heated to 120 °C and stirred for 3 h. The resulting mixture was diluted with methylene cloride and filtered through a pad of Celite. The filtrate was wash with ice water to remove DMF and dried over sodium

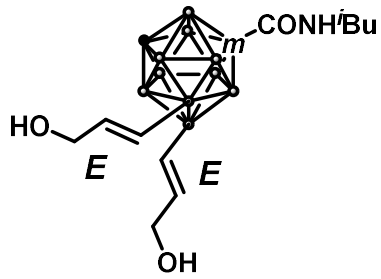
sulfate and concentrated under vacuum. pressure. The crude material was purified by column chromatography on silica gel (10% Et_2O in Hexane) to use in the next step without further purification.

To a solution of the crude material in THF (2 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (180 μL, 0.281 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 2 h, CO_2 balloon was attached. Then, the resulting mixture was stirred at room temperature for 2 h under CO_2 atmosphere. The reaction was quenched with 1M HCl and the mixture was extracted with Et_2O, washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of the crude material in DMF (1 mL), isobutylamine (31 μL, 0.306 mmol), and COMU (131 mg, 0.306 mmol) was added DIEA (130 μL, 0.765 mmol). The resulting mixture was stirred at room temperature under argon atmosphere for 25 min until complete conversion of the starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was extracted with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% EtOAc in Hexane) to afford **32** (26 mg, 0.0440 mmol, 2% 3 steps) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.25 (d, *J* = 8.6 Hz, 4H), 6.86 (d, *J* = 8.6 Hz, 4H), 5.99 (Ddd, *J* = 5.5 Hz, 17.6 Hz, 2H), 5.84 (s, 1H), 5.77 (d, *J* = 17.6 Hz, 2H), 4.42 (s, 4H), 3.98 (Dd, *J* = 1.4 Hz, 5.5 Hz, 4H), 3.79 (s, 6H), 3.02 (t, *J* = 6.4 Hz, 2H), 2.95 (s, 1H), 3.00-2.00 (m, 8H), 1.78-1.73 (m, 1H), 0.87 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 159.3, 139.4, 130.6, 129.6, 113.9, 74.7, 72.6, 71.8, 55.4, 51.6, 48.1, 28.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ –2.2, -6.6, -7.7, -13.8, -19.3.

Synthesis of 9,10-bis((*E*)-3-hydroxyprop-1-en-1-yl)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (33)

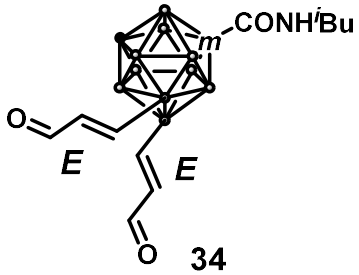


33

To a solution of **32** (26 mg, 0.0440 mmol) and NaH_2PO_4 (11 mg, 0.0880 mmol) in DCM/H_2O (500 mL, 10:1, v/v) was added DDQ (30 mg, 0.132 mmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (80% EtOAc in Hexane) to afford **33** (11 mg, 0.0315 mmol, 72%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 6.04 (Ddd, *J* = 5.0 Hz, 17.6 Hz, 2H), 5.87 (s, 1H), 5.75 (d, *J* = 17.6 Hz, 2H), 4.14 (Dd, *J* = 1.6 Hz, 5.1 Hz, 4H), 3.03 (t, *J* = 6.4 Hz, 2H), 2.97 (s, 1H), 3.00-2.00 (m, 8H), 1.79-1.74 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 142.0, 120.6, 72.6, 65.5, 51.7, 48.1, 28.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ –2.3, -6.3, -7.9, -12.2, -13.7, -19.1.

Synthesis of 9,10-bis((*E*)-3-oxoprop-1-en-1-yl)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (34)

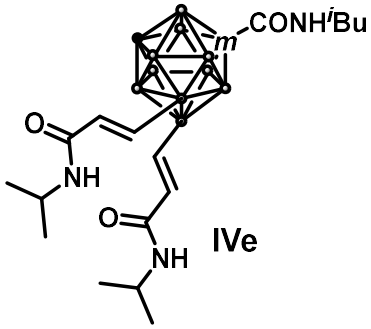


34

To a solution of **33** (11 mg, 30.9 μmol) in DCM (500 μL) was added Dess-Martin periodinane (20 mg, 46.4 μmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. To the resulting mixture were added $NaHCO_3$ aqueous and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% EtOAc in Hexane) to afford **34** (7.8 mg, 22.2 μmol, 72%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 9.48 (d, *J* = 7.7 Hz, 2H), 6.91 (d, *J* = 17.6 Hz, 2H), 6.43 (Ddd, *J* = 7.6 Hz, 17.6 Hz, 2H), 5.88 (s, 1H), 3.19 (s, 1H), 3.07 (t, *J* = 6.4 Hz, 2H), 2.95 (s, 1H), 3.00-2.00 (m, 8H), 1.82-1.76 (m, 2H), 0.90 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 194.3, 159.1, 143.0, 74.1, 53.0, 48.3, 28.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -3.47, -6.09, -7.65, -11.7, -13.1, -17.4.

Synthesis of 9,10-bis((*E*)-3-(isopropylamino)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IVe)



IVe

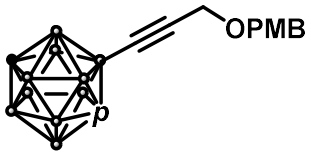
To a solution of **34** (3.5 mg, 10.0 μmol) and 2-methyl-2-butene (21 μL, 200 μmol) in acetone (500 μL) was slowly added a freshly prepared solution of NaH_2PO_4 (19 mg, 120 μmol) and $NaClO_2$ (9.0 mg, 100 μmol) in water (500 μL) at 0 °C. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with brine, which was partitioned between the aqueous and organic layers. The aqueous layer was washed with DCM and combined organic layers were dried over sodium sulfate and concentrated under vacuum to use in the next step without further purification.

To a solution of the crude material in DMF (500 μL), isopropylamine (2.0 μL, 22.0 μmol), COMU (9.4 mg, 22.0 μmol), and DIEA (5.1 μL, 30.0 μmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (80% EtOAc in Hexane) to afford **IVe** (3.8 mg, 7.62 μmol, 76% 2 steps) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 6.85 (d, *J* = 17.5 Hz, 2H), 6.04 (d, *J* = 17.5 Hz, 2H), 5.85 (s, 1H), 5.50 (d, *J* = 7.6 Hz, 2H), 4.16-4.12 (m, 2H), 3.05-3.03 (m, 3H), 3.00-2.00 (m, 8H), 1.80-1.73 (m, 2H), 1.19 (d, *J* = 6.5 Hz, 12H), 0.88 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 164.8, 159.7, 135.3, 73.5, 52.4, 48.2, 41.6, 29.8, 28.5, 22.9, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -2.87, -6.07, -7.52, -13.4, -18.3.

- **Synthesis of scaffold V-type compounds**

Synthesis of 2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaborane (36)

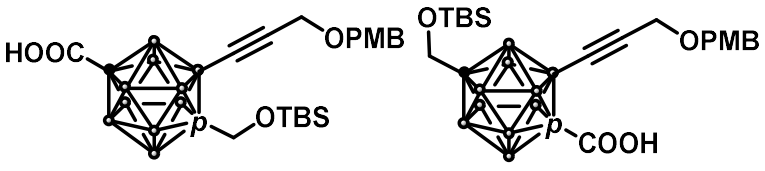


36

To a solution of 1-methoxy-4-((prop-2-yn-1-yloxy)methyl)benzene (**11**) (1.59 g, 9.00 mmol) in toluene:piperidine (13.5 mL, 2:1, v/v), were added CuI (57.0 mg, 0.300 mmol), $PdCl_2(PPh_3)_2$ (210 mg, 0.300 mmol) and 2-iodo-1,12-dicarba-*closo*-dodecaborane (**35**) (810 mg, 3.00 mmol) prepared according to the reported literature.[21] The mixture was stirred at 80 °C under argon atmosphere for 40 min with microwave synthesizer. The reaction was quenched with 1N HCl aq. and Et_2O. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O, and the mixture was extracted with Et_2O, washed with brine, dried over sodium sulfate, and concentrated under vacuum. The residue was purified by column chromatography on silica gel (5% to 10% EtOAc in Hexane) to afford **36** as a colorless oil (50 mg, 0.157 mmol, 49%).

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.28 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 4.53 (s, 2H), 4.13 (s, 2H), 3.80 (s, 3H), 3.01 (s, 1H), 2.78 (s, 1H), 2.90-1.40 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.5, 130.0, 129.6, 93.56, 71.4, 66.3, 63.2, 57.6, 55.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.3, -14.3, -15.1, -16.8; HRMS (ESI, positive) for $C_{13}H_{22}B_{10}O_2$ (m/z): calculated 343.2464 $(M+Na)^+$, found 343.2472.

Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaboranyl-1-carboxylic acid (38a) and 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaboranyl-1-carboxylic acid (38b)



38a **38b**

To a solution of **36** (202 mg, 0.634 mmol) in THF (2.5 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (400 μL, 0.634 mmol) at -78 ℃. After the resulting mixture was stirred at -

78 ℃ under argon atmosphere for 2 h, paraformaldehyde (23 mg, 0.761 mmol) was added at -78 ℃. Then, the resulting mixture was warmed up to room temperature and stirred for 12 h under argon atmosphere. The reaction mixture was quenched with aq. 1N HCl. The resulting mixture was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc, and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (10% to 20% EtOAc in Hexane) to afford the mixture of regioisomer to use in the next step without further purification.

To a solution of the mixture (**37**) and 2,6-lutidine (56 μL, 0.482 mmol) in DCM (1.2 mL) was slowly added TBSOTf (89 μL, 0.386 mmol) at 0 ℃ under argon atmosphere. The resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford the mixture of regioisomer to use in the next step without further purification.

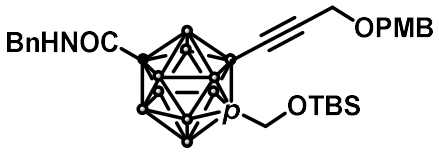
To a solution of the mixture in THF (1 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (185 μL, 0.288 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 2 h, CO_2 balloon was attached. Then, the resulting mixture was warmed up to room temperature and stirred for 12 h under CO_2 atmosphere. After that, the reaction mixture was concentrated under pressure. Hexane was added after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, to the aqueous layer, were added 6 M HCl and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by reverse-phase HPLC (40% to 100% MeCN in H_2O) to afford 60.5 mg of faster eluting isomer **38a** as colorless oil, 43.6 mg of slower eluting isomer **38b** as a white solid, and 10.5 mg of the mixture (total yield 114.6 mg, 0.226 mmol, 37%, 3 steps).

38a: ^{1}H NMR (400 MHz; $CDCl_3$): major isomer δ 7.28 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 4.53 (s, 2H), 4.15 (s, 2H), 3.81 (s, 3H), 3.63 (s, 2H), 3.00-1.70 (br, 9H), 0.85 (s, 9H), -0.01 (s, 6H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 166.3, 159.7, 130.0, 129.4, 114.1, 96.3, 85.2, 74.8, 71.3, 65.0, 57.5, 55.5, 25.8, 18.3, -5.4; ^{11}B NMR (128 MHz; $CDCl_3$): δ -13.2; HRMS (ESI, positive) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 531.3346 $(M+Na)^+$, found 531.3342.

38b: ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.28 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 4.50 (s, 2H), 4.10 (s, 2H), 3.81 (s, 3H), 3.44 (s, 2H), 3.00-1.70 (br, 9H), 0.85 (s, 9H), -0.03 (s, 6H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 164.1, 159.6, 130.2, 129.5, 114.1, 71.0, 66.5, 57.4, 55.5, 25.8, 18.3, -5.5; ^{11}B NMR (128 MHz; $CDCl_3$): δ -13.2; HRMS (ESI, positive) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z):

calculated 531.3346 $(M+Na)^+$, found 531.3345.

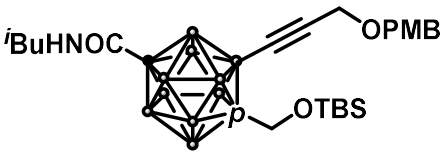
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (39a)



39a

To a solution of **38a** (25.2 mg, 49.7 μmol) in THF (1.0 mL), benzyllamine (6.5 μL, 59.7 μmol), EDCI (11 mg, 59.7 μmol), HOBt (11 mg, 74.6 μmol) and DIEA (25 μL, 0.149 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **39a** (20.2 mg, 33.9 μmol, 68%) as a colorless oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.33-7.27 (m, 5H), 7.15-7.13 (m, 2H), 6.88 (d, J = 8.7 Hz, 2H), 5.88 (s, 1H), 4.53 (s, 2H), 4.30 (d, J = 5.7 Hz, 2H), 4.15 (s, 2H), 3.81 (s, 3H), 3.65 (s, 2H), 3.10-1.70 (br, 9H), 0.85 (s, 9H), -0.01 (s, 6H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 160.8, 159.6, 137.1, 129.9, 129.6, 129.0, 128.0, 127.5, 114.1, 71.4, 64.9, 57.6, 55.4, 44.8, 25.8, 18.3, -5.4; ^{11}B NMR (128 MHz; $CDCl_3$): δ -13.1; HRMS (ESI, positive) for $C_{28}H_{45}B_{10}NO_4Si$ (m/z): calculated 620.3982 $(M+Na)^+$, found 620.3990.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (39b)

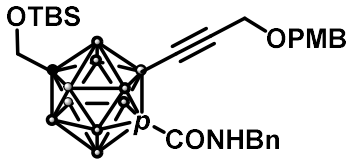


39b

To a solution of **38a** (35.3 mg, 69.7 μmol) in THF (1.0 mL), isobutylamine (8.4 μL, 83.6 μmol), EDCI (16 mg, 83.6 μmol), HOBt (16 mg, 0.105 mmol) and DIEA (36 μL, 0.209 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic

layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **39b** (18.3 mg, 32.6 µmol, 47%) as a colorless oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.28 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 5.64 (s, 1H), 4.53 (s, 2H), 4.15 (d, *J* = 5.7 Hz, 2H), 3.81 (s, 3H), 3.65 (s, 2H), 2.94 (t, *J* = 6.4 Hz, 2H), 3.10-1.70 (br, 9H), 1.75-1.65 (m, 1H), 0.85 (s, 9H), 0.84 (d, *J* = 6.7 Hz, 6H), -0.01 (s, 6H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 160.7, 159.6, 129.9, 129.6, 114.0, 83.9, 79.4, 71.3, 64.9, 57.6, 55.4, 48.0, 29.8, 28.4, 25.8, 19.9, 18.3, -5.4; ^{11}B NMR (128 MHz; $CDCl_3$): δ -13.3; HRMS (ESI, positive) for $C_{25}H_{47}B_{10}NO_4Si$ (m/z): calculated 586.4136 $(M+Na)^+$, found 586.4136.

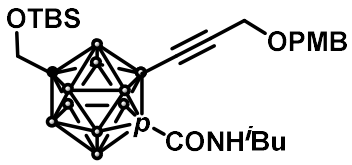
Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (43a)



43a

This compound was prepared from **38b** (18.4 mg, 36.3 µmol) using the procedure described before for **39a** in 73% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.28-7.26 (m, 5H), 7.19-7.17 (m, 2H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.42 (s, 1H), 4.46 (s, 2H), 4.34 (d, *J* = 5.6 Hz, 2H), 3.94 (s, 2H), 3.82 (s, 3H), 3.48 (s, 2H), 3.00-1.70 (br, 9H), 0.87 (s, 9H), -0.01 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 159.6, 137.1, 130.0, 129.4, 128.9, 127.8, 127.7, 114.0, 83.8, 79.7, 71.4, 66.3, 57.4, 55.4, 44.8, 29.8, 25.8, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, positive) for $C_{28}H_{45}B_{10}NO_4Si$ (m/z): calculated 620.3982 $(M+Na)^+$, found 620.3987.

Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (43b)

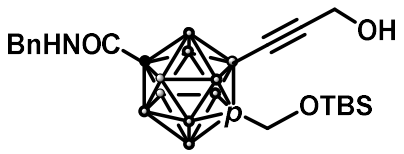


43b

This compound was prepared from **38b** (23.1 mg, 45.6 µmol) using the procedure described

before for **39b** in 83% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.28 (d, *J* = 8.6 Hz, 2H), 6.88 (d, *J* = 8.6 Hz, 2H), 6.10 (s, 1H), 4.52 (s, 2H), 4.12 (s, 2H), 3.81 (s, 3H), 3.46 (s, 2H), 2.96 (t, *J* = 6.4 Hz, 2H), 3.00-1.80 (br, 9H), 1.72-1.64 (m, 1H), 0.85 (s, 9H), 0.82 (d, *J* = 6.7 Hz, 6H), -0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.2, 159.6, 130.0, 129.4, 114.0, 96.9, 83.6, 80.2, 71.4, 66.3, 57.5, 55.4, 48.1, 28.4, 25.8, 20.0, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, positive) for $C_{25}H_{47}B_{10}NO_4Si$ (m/z): calculated 586.4136 $(M+Na)^+$, found 586.4136.

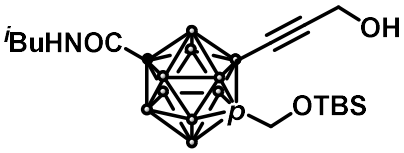
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-hydroxyprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (40a)



40a

To a solution of **39a** (20 mg, 33.6 μmol) and NaH_2PO_4 (8.1 mg, 67.1 μmol) in DCM/H_2O (500 μL, 10:1, v/v) was added DDQ (15 mg, 67.1 mmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% to 40% EtOAc in Hexane) to afford **40a** (14 mg, 29.4 mmol, 88%) as white solid. m.p. 113-114 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34-7.28 (m, 3H), 7.13 (d, *J* = 7.0 Hz, 2H), 5.88 (s, 1H), 4.30 (d, *J* = 5.8 Hz, 2H), 4.28 (s, 2H), 3.63 (s, 2H), 3.00-1.80 (br, 9H), 0.86 (s, 9H), -0.00 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.7, 137.0, 129.0, 128.0, 127.5, 84.1, 78.9, 64.8, 51.8, 44.8, 25.8, 18.3, -5.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, positive) for $C_{20}H_{37}B_{10}NO_3Si$ (m/z): calculated 500.3398 $(M+Na)^+$, found 500.3399.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-hydroxyprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (40b)

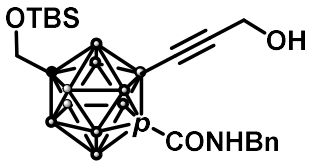


40b

This compound was prepared from **39b** (18.3 mg, 32.6 μmol) using the procedure described before for **40a** in 89% yield as white solid. m.p. 122-123 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.62

(s, 1H), 4.29 (s, 2H), 3.63 (s, 2H), 2.94 (t, J = 6.4 Hz, 2H), 2.90-1.70 (br, 9H), 1.72-1.66 (m, 1H), 0.86 (s, 9H), 0.83 (d, J = 6.7 Hz, 6H), 0.00 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.7, 83.8, 79.3, 64.8, 51.8, 48.0, 28.4, 25.8, 19.9, 18.3, -5.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.1, -13.6, -14.5; HRMS (ESI, positive) for $C_{17}H_{39}B_{10}NO_3Si$ (m/z): calculated 466.3552 $(M+Na)^+$, found 466.3552.

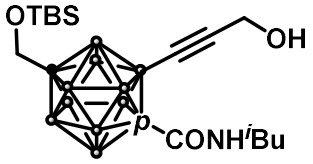
Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-hydroxyprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (44a)



44a

This compound was prepared from **43a** (15.0 mg, 25.2 μmol) using the procedure described before for **40a** in 73% yield as white solid. m.p. 141-142 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.28 (m, 3H), 7.24-7.22 (m, 2H), 6.42 (s, 1H), 4.35 (d, J = 5.6 Hz, 2H), 4.00 (s, 2H), 3.45 (s, 2H), 3.00-1.70 (br, 9H), 0.84 (s, 9H), -0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 137.3, 128.9, 128.00, 127.97, 83.9, 79.6, 66.3, 51.5, 44.9, 25.8, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.5; HRMS (ESI, positive) for $C_{20}H_{37}B_{10}NO_3Si$ (m/z): calculated 500.3398 $(M+Na)^+$, found 500.3392.

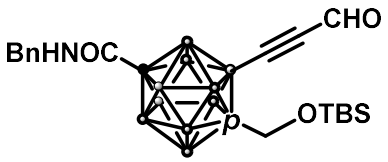
Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-hydroxyprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (44b)



44b

This compound was prepared from **43b** (20.0 mg, 35.6 μmol) using the procedure described before for **40a** in 92% yield as white solid. m.p. 111-112 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.07 (s, 1H), 4.26 (s, 2H), 3.45 (s, 2H), 2.99 (t, J = 6.4 Hz, 2H), 3.00-1.80 (br, 9H), 1.76-1.70 (m, 1H), 0.87 (d, J = 6.7 Hz, 6H), 0.84 (s, 9H), -0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 83.6, 66.3, 51.7, 48.1, 28.5, 25.8, 20.0, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.5; HRMS (ESI, positive) for $C_{17}H_{39}B_{10}NO_3Si$ (m/z): calculated 466.3552 $(M+Na)^+$, found 466.3550.

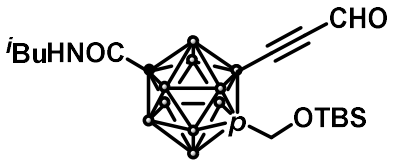
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (41a)



41a

To a solution of **40a** (12.0 mg, 25.2 μmol) in DCM (1 mL) was added Dess-Martin periodinane (21.0 mg, 50.5 μmol) at 0 °C under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. To the resulting mixture were added $NaHCO_3$ aqueous and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **41a** (9.6 mg, 20.3 μmol, 62%) as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.19 (s, 1H), 7.34-7.28 (m, 3H), 7.14 (d, J = 7.2 Hz, 2H), 5.88 (s, 1H), 4.30 (d, J = 5.6 Hz, 2H), 3.62 (s, 2H), 3.10-1.80 (br, 9H), 0.86 (s, 9H), -0.00 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 176.4, 160.3, 136.9, 129.0, 128.0, 127.5, 84.4, 79.5, 64.8, 44.9, 25.7, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.7, -14.1; HRMS (ESI, positive) for $C_{20}H_{35}B_{10}NO_3Si$ (m/z): calculated 498.3241 $(M+Na)^+$, found 498.3249.

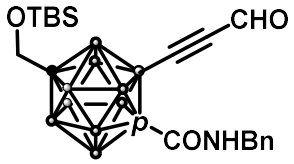
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (41b)



41b

This compound was prepared from **40b** (10.0 mg, 22.6 μmol) using the procedure described before for **41a** in quantitative yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.20 (s, 1H), 5.62 (s, 1H), 3.61 (s, 2H), 2.95 (t, J = 6.4 Hz, 2H), 3.00-1.80 (br, 9H), 1.73-1.68 (m, 1H), 0.86 (s, 9H), 0.84 (d, J = 6.7 Hz, 6H), 0.00 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 176.4, 160.2, 84.2, 79.9, 64.8, 48.0, 28.4, 25.7, 19.9, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6, -14.2; HRMS (ESI, positive) for $C_{17}H_{39}B_{10}NO_3Si$ (m/z): calculated 466.3552 $(M+Na)^+$, found 466.3552.

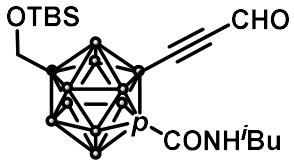
Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (45a)



45a

This compound was prepared from **44a** (8.0 mg, 16.8 μmol) using the procedure described before for **41a** in 29% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.95 (s, 1H), 7.32-7.27 (m, 3H), 7.21-7.19 (m, 2H), 6.03 (s, 1H), 4.35 (d, *J* = 5.7 Hz, 2H), 3.46 (s, 2H), 3.00-1.80 (br, 9H), 0.85 (s, 9H), -0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 176.4, 159.4, 136.9, 129.0, 128.1, 127.8, 84.5, 80.0, 66.2, 45.0, 25.8, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.4; HRMS (ESI, positive) for $C_{20}H_{35}B_{10}NO_3Si$ (m/z): calculated 498.3241 $(M+Na)^+$, found 498.3242.

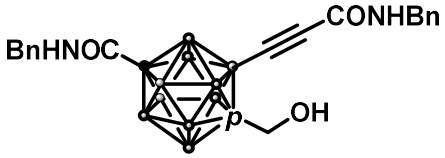
Synthesis of 12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (45b)



45b

This compound was prepared from **44b** (12.0 mg, 27.2 μmol) using the procedure described before for **41a** in 98% yield as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 9.15 (s, 1H), 5.78 (s, 1H), 3.46 (s, 2H), 3.00 (t, *J* = 6.4 Hz, 2H), 3.00-1.80 (br, 9H), 1.76-1.70 (m, 1H), 0.86 (d, *J* = 6.8 Hz, 6H), 0.85 (s, 9H), -0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 176.4, 159.4, 84.3, 80.5, 66.2, 48.2, 28.5, 25.8, 20.0, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.4; HRMS (ESI, positive) for $C_{17}H_{37}B_{10}NO_3Si$ (m/z): calculated 464.3395 $(M+Na)^+$, found 466.3401.

Synthesis of 1-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (42a)



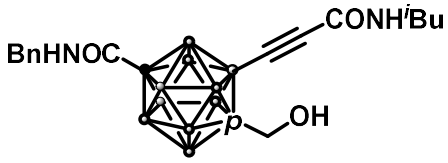
42a

To a solution of **41a** (63.3 mg, 0.134 mmol) and 2-methyl-2-butene (283 μL, 2.68 mmol) in acetone (1 mL) was slowly added a freshly prepared solution of NaH_2PO_4 (250 mg, 1.60 mmol) and $NaClO_2$ (121 mg, 1.33 mmol) in water (1 mL) at 0 ℃. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with brine, which was partitioned between the aqueous and organic layers. The aqueous layer was washed with DCM and combined organic layers were dried over sodium sulfate and concentrated under vacuum to use in the next step without further purification.

To a solution of the crude material in THF (1 mL), benzylamine (18 μL, 0.161 mmol), EDCI (30.9 mg, 0.161 mmol), HOBt (30.8 mg, 0.201 mmol) and DIEA (68 μL, 0.402 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were dried over sodium sulfate and concentrated under vacuum to use in the next step without further purification.

To a solution of the crude material in CH_2Cl_2 (500 μL), was added 4N HCl in Dioxane (500 μL). The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After the reaction mixture was concentrated under pressure, the crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **42a** (50.6 mg, 0.109 mmol, 81%) as white solid. m.p. 64-66 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.38-7.29 (m, 8H), 7.13 (d, J = 7.0 Hz, 2H), 6.26 (bs, 1H), 5.84 (bs, 1H), 4.47 (d, J = 5.8 Hz, 2H), 4.29 (d, J = 5.6 Hz, 2H), 3.66 (s, 2H), 3.00-1.50 (br, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.2, 152.5, 137.0, 136.9, 129.1, 129.1, 129.0, 128.2, 128.1, 128.06, 127.5, 84.6, 79.4, 64.7, 44.9, 44.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -14.1; HRMS (ESI, positive) for $C_{21}H_{28}B_{10}N_2O_3$ (m/z): calculated 489.2954 $(M+Na)^+$, found 489.2951.

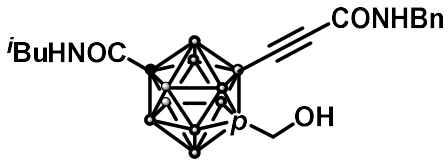
Synthesis of 1-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (42b)



42b

This compound was prepared from **41a** (63.3 mg, 0.134 mmol) and isobutylamine instead of benzylamine using the procedure described before for **42a** in 83% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.29 (m, 3H), 7.13 (d, *J* = 6.8 Hz, 2H), 6.03 (s, 2H), 5.86 (s, 1H), 4.30 (d, *J* = 5.6 Hz, 2H), 3.13 (t, *J* = 6.5 Hz, 2H), 3.00-1.80 (br, 9H), 1.84-1.79 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 152.7, 136.9, 129.1, 128.1, 127.5, 84.6, 79.4, 64.8, 47.4, 44.9, 28.5, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -14.1; HRMS (ESI, positive) for $C_{18}H_{30}B_{10}N_2O_3$ (m/z): calculated 455.3107 $(M+Na)^+$, found 455.3107.

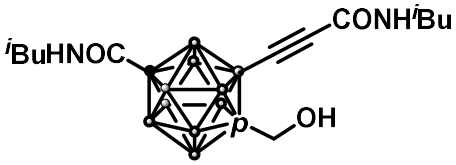
Synthesis of 1-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (42c)



42c

This compound was prepared from **41b** (54.5 mg, 0.124 mmol) using the procedure described before for **42a** in 85% yield as white solid. m.p. 135-136 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.37-7.29 (m, 5H), 6.42 (s, 1H), 5.61 (s, 1H), 4.47 (d, *J* = 5.8 Hz, 2H), 3.64 (s, 2H), 3.00-1.80 (br, 9H), 2.93 (t, *J* = 6.4, 2H), 1.72-1.66 (m, 1H), 0.83 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.2, 152.7, 137.0, 129.0, 128.2, 128.1, 79.8, 64.6, 48.0, 44.1, 29.8, 28.4, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, positive) for $C_{18}H_{30}B_{10}N_2O_3$ (m/z): calculated 455.3107 $(M+Na)^+$, found 455.3092.

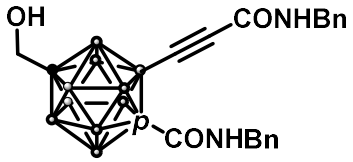
Synthesis of 1-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (42d)



42d

This compound was prepared from **41b** (54.5 mg, 0.124 mmol) and isobutylamine instead of benzylamine using the procedure described before for **42a** in 82% yield as white solid. m.p. 128-129 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.16 (s, 1H), 5.62 (s, 1H), 3.67 (s, 2H), 3.13 (t, *J* = 6.5, 2H), 2.94 (t, *J* = 6.4, 2H), 3.00-1.80 (br, 9H), 1.84-1.79 (m, 1H), 1.73-1.67 (m, 1H), 0.94 (d, *J* = 6.7 Hz, 6H), 0.84 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 160.3, 152.9, 84.5, 79.8, 64.6, 48.1, 47.4, 28.5, 28.4, 20.2, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -14.0; HRMS (ESI, positive) for $C_{15}H_{32}B_{10}N_2O_3$ (m/z): calculated 421.3260 $(M+Na)^+$, found 421.3261.

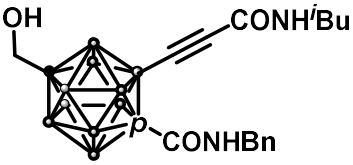
Synthesis of 12-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (46a)



46a

This compound was prepared from **35a** (50.8 mg, 0.107 mmol) using the procedure described before for **32a** in 55% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.16 (m, 10H), 6.18 (s, 1H), 6.04 (s, 1H), 4.38 (d, *J* = 5.9 Hz, 2H), 4.32 (d, *J* = 5.8 Hz, 2H), 3.49 (s, 2H), 3.00-1.60 (br, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.8, 152.6, 137.0, 129.0 127.9, 127.5, 127.49, 84.3, 80.1, 65.9, 47.3, 44.9, 28.5, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.8; HRMS (ESI, positive) for $C_{18}H_{30}B_{10}N_2O_3$ (m/z): calculated 489.2954 $(M+Na)^+$, found 489.2947.

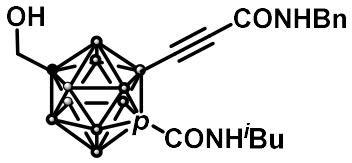
Synthesis of 12-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (46b)



46b

This compound was prepared from **45a** (50.8 mg, 0.107 mmol) and isobutylamine instead of benzylamine using the procedure described before for **42a** in 63% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.30-7.25 (m, 3H), 7.19 (d, *J* = 7.1 Hz, 2H), 6.24 (d, *J* = 5.4 Hz, 2H), 5.88 (d, *J* = 5.2 Hz, 2H), 4.35 (d, *J* = 5.4 Hz, 2H), 3.50 (s, 2H), 3.03 (t, *J* = 6.6 Hz, 2H), 3.00-2.00 (br, 9H), 1.76-1.71 (m, 1H), 0.89 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.8, 152.6, 137.0, 129.0, 127.9, 127.5, 127.49, 84.3, 80.1, 65.9, 47.3, 44.9, 28.5, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.8; HRMS (ESI, positive) for $C_{18}H_{30}B_{10}N_2O_3$ (m/z): calculated 455.3107 $(M+Na)^+$, found 455.3106.

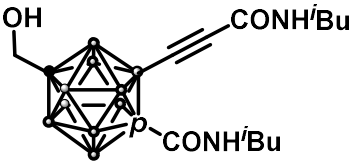
Synthesis of 12-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (46c)



46c

This compound was prepared from **45b** (43.6 mg, 0.094 mmol) using the procedure described before for **42a** in 68% yield as white solid. m.p. 134-135 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.27 (m, 5H), 6.23 (s, 1H), 5.84 (s, 1H), 4.45 (d, *J* = 5.9 Hz, 2H), 3.51 (d, *J* = 7.3 Hz, 2H), 2.97 (t, *J* = 4.3 Hz, 2H), 3.00-1.90 (br, 9H), 1.73-1.67 (m, 1H), 0.82 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.5, 152.2, 137.1, 129.0, 128.2, 128.1, 83.8, 66.0, 48.3, 44.1, 28.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.4, -14.1; HRMS (ESI, positive) for $C_{18}H_{30}B_{10}N_2O_3$ (m/z): calculated 455.3107 $(M+Na)^+$, found 455.3099.

Synthesis of 12-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (46d)

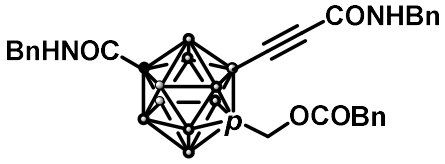


46d

This compound was prepared from **45b** (43.6 mg, 0.094 mmol) and isobutylamine instead of benzylamine using the procedure described before for **42a** in 78% yield as white solid. m.p. 157-158 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.92 (s, 1H), 5.87 (s, 1H), 3.52 (s, 2H), 3.11 (t, *J* = 6.5 Hz, 2H), 3.00 (t, *J* = 6.4 Hz, 2H), 3.00-1.90 (br, 9H), 1.82-1.71 (m, 2H), 0.93 (d, *J* = 6.7 Hz, 6H), 0.86 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.5, 152.2, 137.1, 129.0, 128.2, 128.1,

83.8, 66.0, 48.3, 44.1, 28.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.4, -14.1; HRMS (ESI, positive) for $C_{15}H_{32}B_{10}N_2O_3$ (m/z): calculated 421.3260 $(M+Na)^+$, found 421.3264.

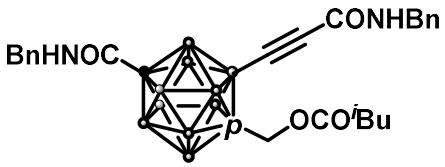
Synthesis of 1-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Va)



Va

To a solution of **42a** (25.3 mg, 54.5 μmol) and triethylamine (22 μL, 164 μmol) in CH_2Cl_2 (200 μL), was slowly added phenylacetyl chloride (8.6 μL, 65.4 μmol) in CH_2Cl_2 at 0 ℃. After the resulting mixture was stirred at room temperature for 9 h, the resulting mixture was concentrated under vacuum. The crude materials were filtered through basic Al_2O_3 pad and purified by column chromatography on silica gel (20% to 40% EtOAc in hexane) afforded **Va** (22.1 mg, 37.9 μmol, 70%) as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34-7.24 (m, 11H), 7.19 (d, J = 6.6 Hz, 2H), 7.12 (d, J = 6.8 Hz, 2H), 6.47 (s, 1H), 5.86 (s, 1H), 4.39 (d, J = 5.9 Hz, 2H), 4.28 (d, J = 5.7 Hz, 2H), 3.58 (s, 2H), 3.10-1.60 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.4, 159.9, 152.2, 137.2, 136.8, 133.0, 129.5, 129.0, 128.9, 128.8, 128.1, 128.0, 127.8, 127.52, 127.46, 92,9, 80.1, 79.9, 63.6, 44.8, 43.9, 40.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, negative) for $C_{29}H_{34}B_{10}N_2O_4$ (m/z): calculated 619.3178 $(M+Cl)^-$, found 619.3187.

Synthesis of 1-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12- benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vb)

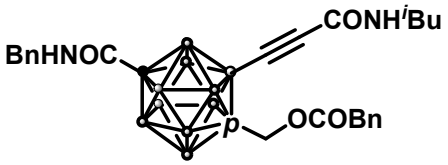


Vb

This compound was prepared from **42a** (25.3 mg, 54.5 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 74% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36-7.28 (m, 8H), 7.12 (d, J = 7.1 Hz, 2H), 6.62 (s, 1H), 5.88 (s, 1H), 4.47 (d, J = 5.9 Hz, 2H), 4.29 (d, J = 5.7 Hz, 2H), 4.21 (s, 2H), 2.13 (d, J = 7.2 Hz, 2H), 1.97-1.92 (m, 1H), 3.00-1.80 (m, 9H), 0.88 (d, J = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.9, 160.0, 152.3, 137.3, 136.8, 129.0, 128.9, 128.2, 128.1, 127.9, 127.5, 127.3, 93.0, 80.2, 80.1, 63.1, 44.9, 44.0, 42.9, 25.5, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, negative)

for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 585.3333 $(M+Cl)^-$, found 585.3344.

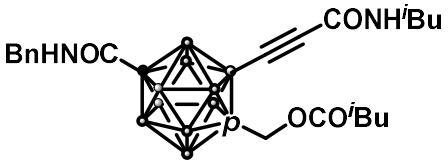
Synthesis of 1-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vc)



Vc

This compound was prepared from **42b** (23.9 mg, 55.5 μmol) using the procedure described above for **Va** in 55% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.35-7.26 (m, 6H), 7.23 (d, *J* = 6.7 Hz, 2H), 7.13 (d, *J* = 6.7 Hz, 2H), 6.23 (s, 1H), 5.86 (s, 1H), 4.29 (d, *J* = 5.7 Hz, 2H), 4.25 (s, 2H), 3.62 (s, 2H), 3.04 (t, *J* = 6.5 Hz, 2H), 1.73-1.68 (m, 1H), 3.00-1.80 (m, 9H), 0.88 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.5, 160.0, 152.5, 136.8, 133.0, 129.6, 129.1, 128.8, 128.1, 127.6, 127.5, 93.4, 80.1, 90.0, 63.6, 47.2, 44.9, 41.0, 18.4, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, negative) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 549.3559 $(M+Cl)^-$, found 549.3566.

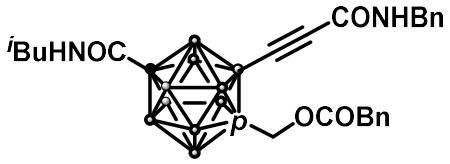
Synthesis of 1-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12- benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vd)



Vd

This compound was prepared from **42b** (23.9 mg, 55.5 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 55% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.33-7.26 (m, 3H), 7.23 (d, *J* = 6.7 Hz, 2H), 7.13 (d, *J* = 6.9 Hz, 2H), 6.36 (s, 1H), 5.88 (s, 1H), 4.30 (d, *J* = 5.6 Hz, 2H), 4.24 (s, 2H), 3.12 (t, *J* = 6.5 Hz, 2H), 2.19 (t, *J* = 7.2 Hz, 2H), 2.07-2.03 (m, 1H), 1.83-1.78 (m, 1H), 3.00-1.80 (m, 9H), 0.945 (d, *J* = 6.7 Hz, 6H), 0.939 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.8, 160.0, 152.6, 136.8, 129.1, 128.1, 127.5, 93.3, 80.2, 80.1, 63.1, 47.3, 44.9, 43.0, 28.5, 25.6, 22.5, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.5; HRMS (ESI, negative) for $C_{23}H_{38}B_{10}N_2O_4$ (m/z): calculated 551.3487 $(M+Cl)^-$, found 551.3502.

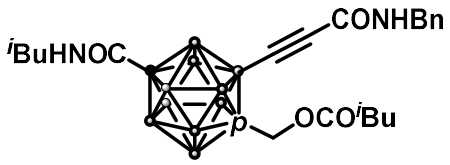
Synthesis of 1-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Ve)



Ve

This compound was prepared from **42c** (24.1 mg, 56.0 μmol) using the procedure described above for **Va** in 71% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34-7.18 (m, 10H), 6.49 (s, 1H), 5.61 (s, 1H), 4.40 (d, *J* = 5.9 Hz, 2H), 4.21 (s, 2H), 3.58 (s, 2H), 2.93 (t, *J* = 6.4 Hz, 2H), 3.05-1.60 (m, 9H), 1.72-1.66 (m, 1H), 0.83 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.4, 160.0, 152.2, 137.3, 133.0, 129.5, 128.9, 128.8, 128.1, 127.9, 127.6, 92.9, 80.5, 79.7, 63.6, 48.0, 43.9, 41.0, 28.4, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.7; HRMS (ESI, negative) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 585.3344 $(M+Cl)^-$, found 585.3334.

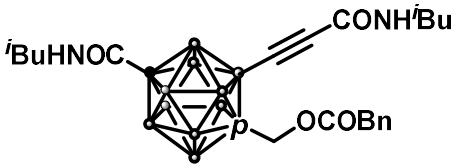
Synthesis of 1-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vf)



Vf

This compound was prepared from **42c** (24.1 mg, 56.0 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 62% yield as white solid. m.p. 84-86 °C; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.33-7.28 (m, 5H), 6.64 (s, 1H), 5.62 (s, 1H), 4.48 (d, *J* = 5.9 Hz, 2H), 4.21 (s, 2H), 2.94 (t, *J* = 6.4 Hz, 2H), 2.13 (t, *J* = 7.2 Hz, 2H), 1.97-1.92 (m, 1H), 1.72-1.67 (m, 1H), 3.05-1.60 (m, 9H), 1.72-1.66 (m, 1H), 0.89 (d, *J* = 6.7 Hz, 6H), 0.83 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.9, 160.0, 152.3, 137.3, 128.9, 128.2, 127.9, 92.9, 80.5, 80.0, 63.1, 48.0, 44.0, 42.9, 28.4, 25.5, 22.4, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, negative) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 551.3487 $(M+Cl)^-$, found 551.3510.

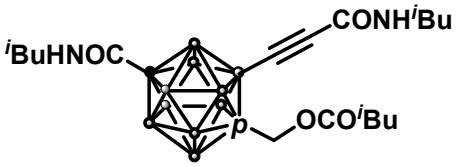
Synthesis of 1-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vg)



Vg

This compound was prepared from **42d** (21.2 mg, 53.5 μmol) using the procedure described above for **Va** in 54% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.29-7.22 (m, 5H), 6.26 (s, 1H), 5.62 (s, 1H), 4.25 (s, 2H), 3.62 (s, 2H), 3.04 (t, *J* = 6.5 Hz, 2H), 2.94 (t, *J* = 6.4 Hz, 2H), 1.74-1.66 (m, 2H), 3.05-1.60 (m, 9H), 0.88 (d, *J* = 6.7 Hz, 6H), 0.88 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.5, 160.0, 152.5, 133.0, 129.6, 128.8, 127.6, 93.3, 80.5, 79.8, 63.6, 48.0, 47.2, 41.0, 28.40, 28.36, 20.2, 19.9, ; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.7; HRMS (ESI, negative) for $C_{23}H_{38}B_{10}N_2O_4$ (m/z): calculated 551.3498 $(M+Cl)^-$, found 551.3485.

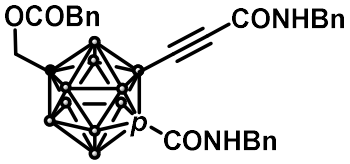
Synthesis of 1-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vh)



Vh

This compound was prepared from **42d** (21.2 mg, 53.5 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 48% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.37 (s, 1H), 5.61 (s, 1H), 4.24 (s, 2H), 3.13 (t, *J* = 6.5 Hz, 2H), 2.94 (t, *J* = 6.4 Hz, 2H), 2.20 (d, *J* = 7.2 Hz, 2H), 2.09-2.03 (m, 1H), 1.84-1.79 (m, 1H), 1.73-1.68 (m, 1H), 3.00-1.50 (m, 9H), 0.95 (d, *J* = 6.7 Hz, 6H), 0.94 (d, *J* = 6.7 Hz, 6H), 0.84 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.9, 160.0, 152.6, 93.3, 80.5, 80.0, 63.1, 48.1, 47.3, 43.0, 28.5, 28.4, 25.6, 22.5, 20.2, 19.9; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.6; HRMS (ESI, negative) for $C_{20}H_{40}B_{10}N_2O_4$ (m/z): calculated 517.3641 $(M+Cl)^-$, found 517.3640.

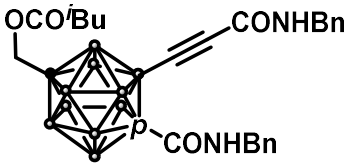
Synthesis of 12-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vi)



Vi

This compound was prepared from **46a** (13.8 mg, 29.6 μmol) using the procedure described above for **Va** in quantitative yield as white solid. m.p. 126-128 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34-7.15 (m, 10), 6.20 (s, 1H), 6.15 (s, 1H), 4.38 (d, *J* = 6.0 Hz, 2H), 4.30 (d, *J* = 5.8 Hz, 2H), 3.98 (s, 2H), 3.57 (s, 2H), 3.00-1.80 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.0, 159.4, 152.1, 137.1, 136.9, 133.0, 129.5, 129.0, 128.9, 128.8, 128.1, 128.0, 127.9, 127.63, 127.57, 127.5, 92.7, 80.7, 79.3, 65.1, 60.5, 44.9, 44.0, 41.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.9; HRMS (ESI, negative) for $C_{29}H_{34}B_{10}N_2O_4$ (m/z): calculated 619.3178 $(M+Cl)^-$, found 619.3186.

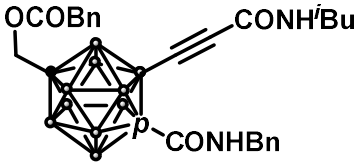
Synthesis of 12-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1- benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vj)



Vj

This compound was prepared from **46a** (13.8 mg, 29.6 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 49% yield as white solid. m.p. 133-135 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.28-7.17 (m, 10), 6.12 (s, 1H), 5.90 (s, 1H), 4.40 (d, *J* = 6.0 Hz, 2H), 4.33 (d, *J* = 5.8 Hz, 2H), 3.98 (s, 2H), 2.16 (d, *J* = 7.1 Hz, 2H), 2.08-2.03 (m, 1H), 3.00-1.50 (m, 9H), 0.94 (d, *J* = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.5, 159.4, 152.1, 137.1, 137.0, 129.01, 128.96, 128.1, 128.01, 127.95, 127.7, 127.5, 92.8, 80.7, 79.5, 64.8, 44.9, 44.0, 42.9, 25.6, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.8; HRMS (ESI, negative) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 549.3559 $(M-H)^-$, found 549.3561.

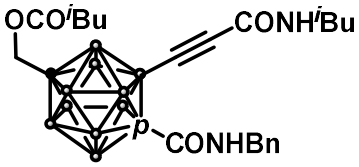
Synthesis of 12-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vk)



Vk

This compound was prepared from **46b** (14.5 mg, 33.6 μmol) using the procedure described above for **Va** in 83% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.29-7.18 (m, 10H), 6.15 (s, 1H), 5.74 (s, 1H), 4.35 (d, *J* = 5.7 Hz, 2H), 3.99 (s, 2H), 3.59 (s, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 1.78-1.73 (m, 1H), 3.00-1.50 (m, 9H), 0.90 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.0, 159.4, 152.3, 136.9, 133.0, 129.5, 129.0, 128.8, 127.9, 127.6, 127.5, 93.1, 80.8, 79.3, 65.1, 47.3, 44.9, 41.0, 28.5, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.5; HRMS (ESI, negative) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 585.3333 $(M+Cl)^-$, found 585.3342.

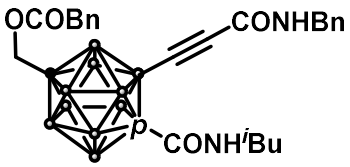
Synthesis of 12-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1- benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vl)



Vl

This compound was prepared from **46c** (14.5 mg, 33.6 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 44% yield as amorphous. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.33-7.20 (m, 3H), 7.20 (d, *J* = 7.1 Hz, 2H), 6.14 (s, 1H), 5.71 (s, 1H), 4.36 (d, *J* = 5.8 Hz, 2H), 3.99 (s, 2H), 3.05 (t, *J* = 6.6 Hz, 2H), 2.17 (d, *J* = 7.2 Hz, 2H), 2.10-2.03 (m, 1H), 1.78-1.72 (m, 1H), 3.00-1.50 (m, 9H), 0.94 (d, *J* = 6.7 Hz, 6H), 0.90 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.5, 159.5, 152.3, 136.9, 129.0, 127.9, 127.6, 93.1, 80.7, 79.5, 64.8, 47.3, 44.9, 42.9, 28.5, 25.6, 22.5, 20.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.5; HRMS (ESI, negative) for $C_{23}H_{38}B_{10}N_2O_4$ (m/z): calculated 551.3487 $(M+Cl)^-$, found 551.3506.

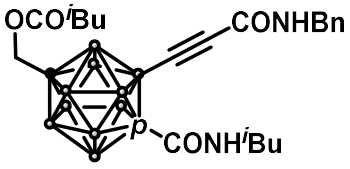
Synthesis of 12-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vm)



Vm

This compound was prepared from **46c** (13.8 mg, 32.1 μmol) using the procedure described above for **Va** in 51% yield as white solid. m.p. 112-115 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.37-7.23 (m, 10H), 6.16 (s, 1H), 5.82 (s, 1H), 4.46 (d, *J* = 5.9 Hz, 2H), 3.99 (s, 2H), 3.58 (s, 2H), 2.96 (t, *J* = 6.4 Hz, 2H), 1.74-1.67 (m, 1H), 3.00-1.80 (m, 9H), 0.81 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.0, 159.2, 152.1, 137.1, 133.0, 129.5, 129.0, 128.8, 128.2, 128.1, 127.6, 92.5, 81.3, 79.1, 48.2, 44.1, 41.0, 28.4, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.8; HRMS (ESI, negative) for $C_{26}H_{36}B_{10}N_2O_4$ (m/z): calculated 585.3333 $(M+Cl)^-$, found 585.3339.

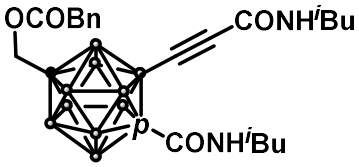
Synthesis of 12-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vn)



Vn

This compound was prepared from **46d** (13.8 mg, 32.1 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 42% yield as white solid. m.p. 116-118 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36-7.27 (m, 5H), 6.16 (s, 1H), 5.83 (s, 1H), 4.46 (d, *J* = 5.9 Hz, 2H), 3.98 (s, 2H), 3.58 (s, 2H), 2.96 (t, *J* = 6.4 Hz, 2H), 2.16 (d, *J* = 7.1 Hz, 2H), 2.08-2.03 (m, 1H), 1.73-1.67 (m, 1H), 3.00-1.80 (m, 9H), 0.94 (d, *J* = 6.6 Hz, 6H), 0.82 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.5, 159.3, 152.1, 137.1, 129.0, 128.2, 128.1, 92.5, 81.2, 79.3, 64.7, 48.2, 44.1, 42.9, 28.5, 25.6, 22.5, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.3; HRMS (ESI, negative) for $C_{23}H_{38}B_{10}N_2O_4$ (m/z): calculated 551.3487 $(M+Cl)^-$, found 551.3498.

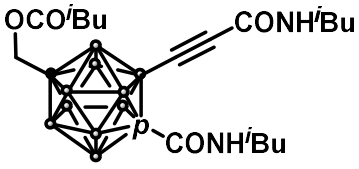
Synthesis of 12-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vo)



Vo

This compound was prepared from **46d** (14.6 mg, 36.8 μmol) using the procedure described above for **Va** in 67% yield as white solid. m.p. 115-119 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36-7.23 (m, 5H), 5.93 (s, 1H), 5.86 (s, 1H), 3.99 (s, 2H), 3.59 (s, 2H), 3.11 (t, *J* = 6.6 Hz, 2H), 2.99 (t, *J* = 6.4 Hz, 2H), 1.82-1.72 (m, 2H), 3.00-1.80 (m, 9H), 0.93 (d, *J* = 6.7 Hz, 6H), 0.85 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.0, 159.3, 152.4, 133.0, 129.5, 128.8, 127.6, 92.9, 81.3, 79.1, 65.1, 48.3, 47.3, 41.0, 28.51, 28.46, 21.2, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.9; HRMS (ESI, negative) for $C_{23}H_{38}B_{10}N_2O_4$ (m/z): calculated 551.3487 $(M+Cl)^-$, found 551.3495.

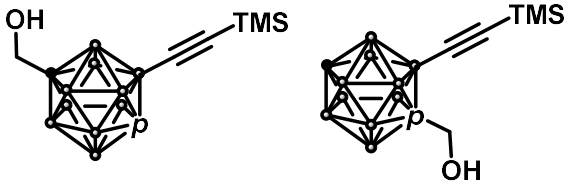
Synthesis of 12-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vp)



Vp

This compound was prepared from **46d** (14.6 mg, 36.8 μmol) and isovaleryl chloride instead of phenylacetyl chloride using the procedure described above for **Va** in 25% yield as white solid. m.p. 93-94 ℃; ^{1}H NMR (500 MHz; $CDCl_3$): δ 5.91 (s, 1H), 5.86 (s, 1H), 3.99 (s, 2H), 3.59 (s, 2H), 3.11 (t, *J* = 6.6 Hz, 2H), 3.00 (t, *J* = 6.4 Hz, 2H), 2.17 (d, *J* = 7.0 Hz, 2H), 2.10-2.04 (m, 1H), 1.82-1.71 (m, 2H), 3.00-1.80 (m, 9H), 0.94 (d, *J* = 6.7 Hz, 6H), 0.92 (d, *J* = 6.8 Hz, 6H), 0.86 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.5, 159.3, 152.4, 92.8, 81.2, 79.3, 64.8, 48.3, 47.3, 42.9, 28.51, 28.47, 25.6, 22.5, 20.2, 20.0; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.9; HRMS (ESI, negative) for $C_{20}H_{40}B_{10}N_2O_4$ (m/z): calculated 517.3641 $(M+Cl)^-$, found 517.3649.

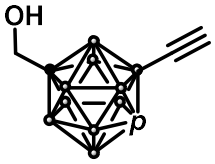
Synthesis of 1-(hydroxymethyl)-7-((trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (48a) and 1-(hydroxymethyl)-2-((trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (48b)



48a **48b**

To a solution of 2-(2-(trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (**47**) (172 mg, 0.713 mmol) in THF, was slowly added *n*-BuLi 1.6 M solution in hexane (506 μL, 0.784 mmol) at -78 °C. After the resulting mixture was stirred -78 °C under argon atmosphere for 1 h, paraformaldehyde (41.0 mg, 1.07 mmol) was added. Then, the resulting mixture was stirred at room temperature for 2 h. After that, the reaction mixture was concentrated under pressure. To the resulting mixture were added water and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude product was purified by column chromatography on silica gel (10 % Et_2O in hexane) afforded **48b** (74.9 mg, 0.277 mmol) as a faster eluting isomer and **48a** (72.8 mg, 0.267 mmol) as a slower eluting isomer totally in 86 % yield. **48a**: ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.46 (s, 2H), 2.98 (s, 1H), 1.77 (s, 1H), 2.90-1.60 (m, 9H), 0.16 (s, 9H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.8, -14.6, 15.2; ^{13}C NMR (125 MHz; $CDCl_3$): δ 105.5, 84.5, 66.6, 62.1, -0.013;
48b: ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.65 (s, 2H), 2.74 (s, 1H), 2.26 (s, 1H), 2.90-1.50 (m, 9H), 0.18 (s, 9H); ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.1, -13.4, -13.9, 14.6, 16.1; ^{13}C NMR (125 MHz; $CDCl_3$): δ 108.1, 86.0, 66.1, 59.3, -0.08.

Synthesis of 1-(hydroxymethyl)-7-ethynyl-1,12-dicarba-*closo*-dodecaborane (49)

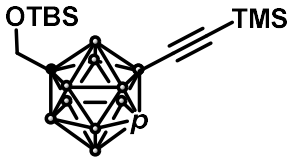


49

To a solution of **48a** (18.6 mg, 0.0688 mmol) in MeOH (500 μL), potassium carbonate (46 mg, 0.344 mmol) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. To the resulting mixture was added water and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc

and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) to afford **49** (11.3 mg, 0.0570 mmol, 83%) as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.48 (s, 2H), 2.99 (s, 1H), 2.21 (s, 1H), 3.00-1.60 (m, 9H), 1.66 (s, 1H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 85.8, 85.6, 84.8, 66.6, 62.1; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.2, -13.8, 15.2.

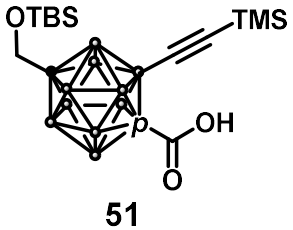
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-((trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (50)



50

To a solution of **48a** (43 mg, 0.159 mmol) in CH_2Cl_2 (500 μL) was added NaH (6.4 mg, 0.159 mmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred for 10 min, TBSCl (32 mg, 0.216 mmol) was slowly added. The resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. To the resulting mixture were added NH_4Cl aqueous and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum, which afforded **50** in quantitative yield. ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.42 (d, J = 3.7 Hz, 2H), 2.93 (s, 1H), 2.80-1.50 (m, 9H), 0.94 (s, 9H), 0.16 (s, 9H), -0.03 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 104.9, 84.8, 66.8, 61.9, 25.8, 18.3, 0.04, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.0, 13.9, 15.3.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-((trimethylsilyl)ethynyl)-12-carboxyl-1,12-dicarba-*closo*-dodecaborane (51)



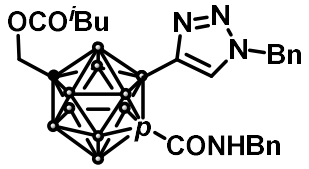
51

To a solution of **50** (90 mg, 0.234 mmol) in THF (4 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (166 μL, 0.257 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 1 h, CO_2 balloon was attached. Then, the resulting mixture was stirred at -

40 ℃ for 2 h under CO_2 atmosphere. After that, the reaction mixture was concentrated under pressure. To the resulting mixture was added hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, the aqueous layer was added 6 M HCl and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum to afford **51** (96 mg, 0.223 mmol, 95%) as a white solid.

^{1}H NMR (400 MHz; $CDCl_3$): δ 6.00 (br, 1H), 3.45 (s, 2H), 2.80-1.50 (m, 9H), 0.87 (s, 9H), 0.20 (s, 9H), -0.01 (s, 9H), -0.02 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 107.3, 83.5, 79.3, 66.4, 25.8, 18.3, -0.08, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -13.9.

Synthesis of 1-(3-methylbutanoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vq)



Vq

To a solution of **51** (44.4 mg, 0.104 mmol) in THF (1 mL), EDCI (24 mg, 0.125 mmol), HOBt (24 mg, 0.156 mmol) and DIEA (36 μL, 0.208 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. The resulting mixture was added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

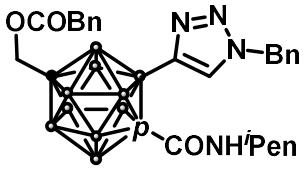
To a solution of the crude material, 4 M HCl in Dioxane (500 μL) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. The crude materials were used to the next reaction without further purification.

To a solution of the crude material in MeOH (500 μL), potassium carbonate (72 mg, 0.52 mmol) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. Then, the crude materials were shortly purified by column chromatography on silica gel (25% EtOAc in hexane) afforded the resulting mixture to use in the next step without further purification.

To a solution of the resulting mixture in CH_2Cl_2 (500 μL), were added EDCI (20 mg, 0.104 mmol),

a catalytic amount of DMAP and isovaleric acid (11 μL, 0.104 mmol). The mixture was stirred at room temperature under argon atmosphere for 8 h. The reaction was quenched with water and the mixture was extracted with CH_2Cl_2, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was shortly purified by column chromatography on silica gel (20% EtOAc) to afford the resulting mixture to use in the next step without further purification.
To a solution of the resulting mixture in DMF:H_2O (4:1, v/v, 500 μL), were added $CuSO_4$ (1.0 mg, 1.7 μmol), sodium ascorbate (1.0 mg, 3.4 μmol) and benzylazide (12 mg, 0.0866 mmol). The mixture was stirred at room temperature for 12 h. The reaction was quenched with water and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (25% EtOAc) to afford **Vq** (19 mg, 0.0350 mmol, 34% 5 steps) as a white solid.
^{1}H NMR (500 MHz; $CDCl_3$): δ 7.52 (s, 1H), 7.37-7.35 (m, 3H), 7.23-7.22 (m, 4H), 6.93-6.91 (m, 2H), 6.48 (s, 1H), 5.45 (s, 1H), 4.08 (d, *J* = 5.8 Hz, 2H), 4.02 (s, 2H), 2.14 (d, *J* = 7.2 Hz, 2H), 2.08-2.02 (m, 1H), 3.20-1.80 (m, 9H) 0.92 (d, *J* = 6.6 Hz, 2H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.6, 160.0, 137.0, 134.6, 129.4, 129.3, 128.9, 128.7, 128.2, 127.6, 127.4, 81.2, 80.5, 65.0, 53.9, 44.5, 42.9, 25.5, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ-8.04, -13.4.

Synthesis of 1-(phenylacetoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-12-(3-methylbutyl)aminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vr)



Vr

To a solution of **51** (24 mg, 0.056 mmol) in THF (500 μL), 3-metylbutylamine (6 μL, 0.0672 mmol), EDCI (13 mg, 0.0672 mmol), HOBt (13 mg, 0.084 mmol) and DIEA (22 μL, 0.122 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.
To a solution of the crude material, 4 M HCl in Dioxane (500 μL) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. The crude materials were used to the next reaction without further purification.

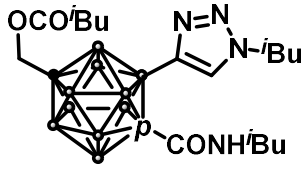
To a solution of the crude material in MeOH (500 μL), potassium carbonate (38 mg, 0.28 mmol) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. To the resulting mixture were added water and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of the crude material and triethylamine (17 μL, 0.168 mmol) in CH_2Cl_2 (500 μL), was slowly added phenylacetyl chloride (16 μL, 0.112 mmol) in CH_2Cl_2 at 0 ℃. After the resulting mixture was stirred 0 ℃ under argon atmosphere for 1 h, aq. $NaHCO_3$ and CH_2Cl_2 were added. The product was partitioned between the aqueous and organic layers. The aqueous layer was washed with CH_2Cl_2 and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of the crude material in DMF:H_2O (4:1, v/v, 500 μL), were added $CuSO_4$ (1.0 mg, 1.7 μmol), sodium ascorbate (1.0 mg, 3.4 μmol) and benzylazide (6.0 μL, 0.034 mmol). The mixture was stirred at room temperature for 12 h. The reaction was quenched with water and the mixture was extracted with EtOAc, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (30% EtOAc) to afford **Vr** (6.5 mg, 0.0116 mmol, 21%) as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.56 (s, 1H), 7.39-7.31 (m, 2H), 7.31-7.21 (m, 8H), 6.16 (s, 1H), 5.53 (s, 2H), 4.02 (s, 2H), 3.57 (s, 2H), 2.90 (q, *J* = 8.4 Hz, 2H), 2.90-1.60 (m, 9H), 1.34-1.29 (m, 1H), 1.07 (q, *J* = 9.0 Hz, 2H), 0.78 (d, *J* = 8.4 Hz, 2H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 170.1, 159.7, 134.6, 133.2, 129.5, 129.3, 129.0, 128.7, 128.2, 127.4, 81.7, 80.2, 65.3, 54.0, 41.0, 39.1, 37.5, 25.7, 22.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -7.48, -13.2.

Synthesis of 1-(3-methylbutanoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-12-(3-methylbutyl)aminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vs)



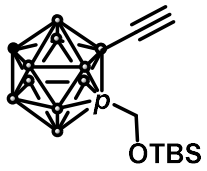
Vs

This compound was prepared from alcohol **51** (51.3 mg, 0.120 mmol) using the procedure described for **Vq** in 76% yield as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.63 (s, 1H), 6.29 (s, 1H), 4.15 (d, *J* = 7.2 Hz, 2H), 2.76 (t, *J* =

6.5 Hz, 2H), 2.23-2.19 (m, 1H), 2.16 (d, *J* = 7.2 Hz, 2H), 2.09-2.03 (m, 1H), 1.53-1.47 (m, 1H), 0.94 (d, *J* = 2.7 Hz, 6H), 0.93 (d, *J* = 2.7 Hz, 6H), 0.66 (d, *J* = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.6, 159.9, 129.9, 81.7, 80.3, 77.4, 65.0, 57.3, 48.0, 42.9, 31.7, 29.8, 28.2, 25.5, 22.8, 33.5, 20.0, 19.8, 14.2; ^{11}B NMR (160 MHz; $CDCl_3$): δ -8.14, -13.3.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-ethynyl-1,12-dicarba-*closo*-dodecaborane (53)

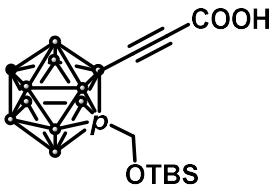


53

To a solution of **48b** (240 mg, 0.887 mmol) and 2,6-lutidine (310 µL, 2.66 mmol) in DCM (4 mL) was slowly added TBSOTf (306 µL, 1.33 mmol) at 0 °C under argon atmosphere. The resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was quenched with a small amount of aq. $NaHCO_3$ and filtered on celite pad. The filtrate was concentrated under vacuum. The crude material was used in the next step without further purification.

To a solution of the crude material in MeOH (1.2 mL), potassium carbonate (148 mg, 1.07 mmol) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. Then, the crude materials were shortly purified by column chromatography on silica gel (hexane) afforded **53** (102 mg, 0.325 mmol, 91% 2 steps). ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.62 (s, 2H), 2.73 (s, 1H), 3.00-1.60 (m, 9H), 2.28 (s, 1H), 0.86 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 87.2, 85.9, 65.3, 59.4, 25.8, 18.3, -5.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.4, -13.2, -14.9, -15.9, -16.5.

Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-carboxyethynyl-1,12-dicarba-*closo*-dodecaborane (54)

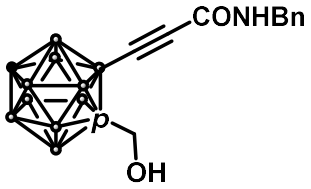


54

To a solution of **53** (101 mg, 0.325 mmol) in THF (3 mL), was slowly added *n*-BuLi 1.6 M solution in hexane (207 µL, 0.325 mmol) at -78 °C. After the resulting mixture was stirred at -

78 °C under argon atmosphere for 1 h, CO_2 balloon was attached. Then, the resulting mixture was stirred at room temperature for 2 h under CO_2 atmosphere. After that, the reaction mixture was concentrated under pressure. To the resulting mixture were added and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane. Then, to the aqueous layer was added 6 M HCl and hexane after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with hexane and combined organic layers were dried over sodium sulfate and concentrated under vacuum to afford **54** (116 mg, 0.325 mmol, quant.). ^{1}H NMR (500 MHz; $CDCl_3$): δ 6.47 (br, 1H), 3.60 (s, 2H), 2.79 (s, 2H), 3.00-1.60 (m, 9H), 0.86 (s, 9H), 0.00 (s, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 156.8, 86.3, 65.3, 59.8, 25.8, 18.3, -5.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.6, -14.3, -15.5.

Synthesis of 1-hydroxymethyl-7-(3-3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaborane (55)



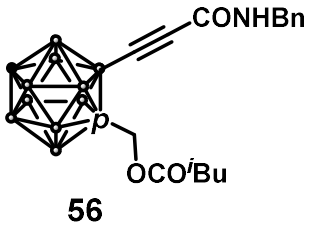
55

To a solution of **54** (73 mg, 0.205 mmol) in THF (1 mL), benzylamine (27 μL, 0.246 mmol), EDCI (47 mg, 0.246 mmol), HOBt (47 mg, 0.307 mmol) and DIEA (104 μL, 0.614 mmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude materials were used to the next reaction without further purification.

To a solution of the crude material in CH_2Cl_2 (1 mL), 4 M HCl in dioxane (1 mL) was added. The resulting mixture was stirred at room temperature until the full conversion of a starting material was observed. After that, the reaction mixture was concentrated under pressure. Then, the crude material was purified by column chromatography on silica gel (10% EtOAc in Hexane) afforded **55** as a solid (88.7 mg, 0.139 mmol, 68%). ^{1}H NMR (400 MHz; CD_3OD): δ 7.35-7.24 (m, 5H), 4.39 (s, 2H), 3.60 (s, 2H), 3.35 (s, 1H), 3.10-1.40 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 154.9, 139.2, 129.6, 128.7, 128.4, 88.1, 65.4, 61.4, 44.3; ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.7, -15.9.

Synthesis of 1-(3-methylbutanoxymethyl)-7-(3-3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1,12-

dicarba-*closo*-dodecaborane (56)

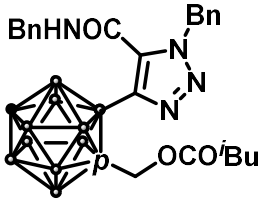


56

To a solution of **55** (8.2 mg, 0.025 mmol) in CH_2Cl_2 (500 μL), were added EDCI (7.0 mg, 0.037 mmol), a small amount of DMAP and isovaleric acid (4.0 μL, 0.0375 mmol). The mixture was stirred at room temperature under argon atmosphere for 8 h. The reaction was quenched with water and the mixture was extracted with CH_2Cl_2, washed with brine, dried over sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography on silica gel (20% EtOAc) to afford **56** (10 mg, 0.024 mmol, 96%) as a white solid.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.36-7.29 (m, 5H), 6.66 (s, 1H), 4.48 (d, J = 5.8 Hz, 2H), 4.19 (s, 2H), 3.49 (s, 2H), 2.81 (s, 1H), 2.13 (d, J = 7.2 Hz, 2H), 1.99-1.92 (m, 1H), 2.80-1.50 (m, 9H), 0.88 (d, J = 6.7 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 172.0, 152.5, 137.4, 128.9, 128.2, 127.9, 92.2, 82.0, 63.7, 60.4, 44.0, 43.0, 25.5, 22.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -12.7, -13.6, -14.8., -15.9.

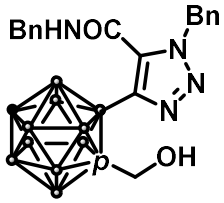
Synthesis of 1-(3-methylbutanoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-7-(1-benzyl-5-1,12-dicarba-closo-dodecaborane (Vt)



Vt

To a solution of **56** (184 mg, 0.443 mmol) in toluene (1 mL), was added excess amount of benzylazide. After the resulting mixture was stirred at 90 ℃ for 48 h, the reaction mixture was concentrated under pressure. Then, the crude material was purified by column chromatography on silica gel (40% EtOAc in Hexane) afforded **Vt** in quantitative yield. ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.31-7.15 (m, 10H), 6.38 (s, 1H), 5.71 (s, 2H), 4.50 (d, J = 5.8 Hz, 2H), 3.91 (s, 2H), 3.00-1.60 (m, 9H), 2.87 (s, 1H), 1.95 (d, J = 6.7 Hz, 2H), 1.91-1.84 (m, 1H), 0.83 (d, J = 6.6 Hz, 6H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 171.8, 160.0, 137.4, 136.5, 135.1, 129.0, 128.5, 128.48, 128.3, 128.1, 82.7, 63.7, 61.5, 52.8, 44.4, 42.7, 25.4, 22.4; ^{11}B NMR (160 MHz; $CDCl_3$): δ -8.35, 12.7, -14.8, -15.7.

Synthesis of 1-hydroxymethyl-2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaborane (S5)

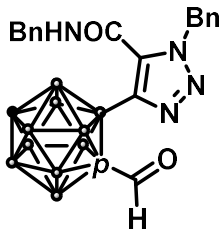


S5

To a solution of **Vt** (180 mg, 0.328 mmol) in MeOH (2 mL), was added NaOH (52 mg, 1.31 mmol). After the resulting mixture was stirred at room temperature under argon atmosphere for 24 h , the reaction mixture was concentrated under vacuum. The resulting mixture was added brine and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was extracted with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum.The crude material was purified by column chromatography on silica gel (40% EtOAc in Hexane) to afford **S5** (140 mg, 0.302 mmol, 92%) as colorless oil.

^{1}H NMR (500 MHz; $CDCl_3$): δ 7.34-7.26 (m, 8H), 7.15-14 (m, 2H), 6.31 (t, *J* = 5.4 Hz, 1H), 5.71 (s, 2H), 5.43 (s, 2H), 4.52 (d, *J* = 5.8 Hz, 2H), 3.53 (s, 2H), 2.72 (s, 1H), 3.00-1.80 (m, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 159.5, 153.6, 136.7, 136.3, 134.6, 129.1, 129.0, 128.8, 128.6, 128.4, 128.2, 126.1, 87.9, 65.7, 59.3, 53.6, 53.1, 44.5; ^{11}B NMR (160 MHz; $CDCl_3$): δ -9.6, -11.8, -12.7, -14.5, -15.5, -16.6.

Synthesis of 1-formyl-2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaborane (S6)

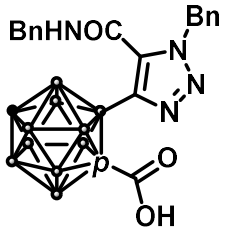


S6

To a solution of **S5** (32 mg, 68.9 μmol) in DCM (1 mL) was added Dess-Martin periodinane (58 mg, 138 μmol) at 0 ℃ under argon atmosphere. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. To the resulting mixture were added $NaHCO_3$ aqueous and Et_2O after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were dried over sodium

sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% EtOAc in Hexane) to afford **S6** (28.8 mg, 62.3 μmol, 90%) as colorless oil. ^{1}H NMR (500 MHz; $CDCl_3$): δ 8.89 (s, 1H), 7.31-7.28 (m, 6H), 7.24-7.21 (m, 2H), 7.16-7.14 (m, 2H), 6.44 (t, *J* = 5.5 Hz, 1H), 5.68 (s, 1H), 4.52 (d, *J* = 5.8 Hz, 2H), 2.95 (s, 1H), 3.10-1.80 (br, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 185.7, 159.5, 136.4, 134.9, 129.0, 128.95, 128.6, 128.4, 128.1, 86.0, 63.7, 52.9, 44.3; ^{11}B NMR (160 MHz; $CDCl_3$): δ -8.2, -13.4, -15.2.

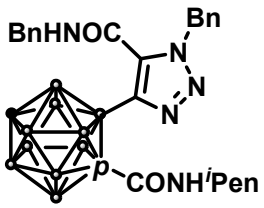
Synthesis of 2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaboranyl-1-carboxylic acid (S7)



S7

To a solution of **S6** (16.2 mg, 0.035 mmol) and 2-methyl-2-butene (74 μL, 0.70 mmol) in acetone (500 μL) was slowly added a freshly prepared solution of NaH_2PO_4 (66 mg, 0.42 mmol) and $NaClO_2$ (32 mg, 0.35 mmol) in water (500 μL) at 0 °C. After the resulting mixture was stirred at room temperature until the starting material disappeared as monitored by TLC analysis. Then, the reaction mixture was concentrated under pressure. Then, the reaction mixture was quenched with brine, which was partitioned between the aqueous and organic layers. The aqueous layer was washed with DCM and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by reverse-phase HPLC (50% to 100% MeCN in H_2O) to afford **S7** (13.4 mg, 0.028 mmol, 80%). ^{1}H NMR (500 MHz; $CDCl_3$): δ 7.65 (bs, 1H), 7.25-7.17 (m, 10H), 5.47 (s, 2H), 4.44 (d, *J* = 5.6 Hz, 2H), 2.76 (s, 1H), 3.00-2.00 (br, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 165.6, 160.6, 137.7, 136.7, 134.6, 128.8, 128.7, 128.5, 128.4, 128.36, 127.7, 89.4, 60.9, 52.8, 44.3; ^{11}B NMR (160 MHz; $CDCl_3$): δ -9.0, -13.0, 16.0.

Synthesis of 1-isopentylcarbamoyl-2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaborane (Vu)

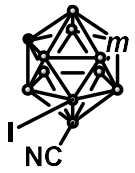


Vu

To a solution of **S7** (6.3 mg, 13.2 μmol) in THF (500 μL), isopentylamine (1.5 μL, 13.2 μmol), EDCI (3.0 mg, 15.8 μmol), HOBt (3.0 mg, 19.7 μmol) and DIEA (6.7 μL, 39.5 μmol) were slowly added. The resulting mixture was stirred at room temperature under argon atmosphere until the full conversion of a starting material was observed. To the resulting mixture were added aq. $NaHCO_3$ and EtOAc after which the product was partitioned between the aqueous and organic layers. The aqueous layer was washed with EtOAc and combined organic layers were washed with brine, dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (20% EtOAc in Hexane) to afford **Vu** (6.2 mg, 11.3 μmol, 86%) as a colorless oil. ^{1}H NMR (400 MHz; $CDCl_3$): δ 7.80 (t, J = 5.7 Hz, 1H), 7.29-7.14 (m, 10H), 5.98 (t, J = 5.5 Hz, 1H), 5.72 (s, 2H), 4.54 (d, J = 5.9 Hz, 1H), 2.98 (s, 1H), 2.94-2.88 (m, 2H), 3.00-1.80 (br, 9H), 1.39-1.32 (m, 1H), 1.10 (q, J = 7.2 Hz, 2H), 0.75 (d, J = 6.6 Hz, 6H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 160.7, 159.9, 137.1, 135.3, 128.9, 128.8, 128.4, 128.2, 127.6, 84.8, 63.4, 52.8, 44.0, 39.2, 37.6, 25.7, 22.6, 22.3; ^{11}B NMR (128 MHz; $CDCl_3$): δ -8.0, -14.0, -15.9.

- **Synthesis of scaffold IV-type compounds with different substituents**

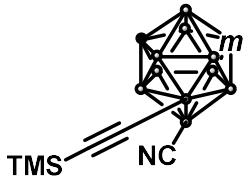
Synthesis of 9-cyano-10-iodo-1,7-dicarba-*closo*-dodecaborane (58)



58

To a solution of 9-cyano-1,7-dicarba-*closo*-dodecaborane (**57**) (532 mg, 3.14 mmol) and I_2 (399 mg, 1.57 mmol) in acetic acid (12.5 mL) was added a 1:1 mixture of concentrated H_2SO_4 and concentrated HNO_3 (4.7 mL). The resulting mixture was stirred at 100 °C for 2 h with microwave synthesizer. Then, the colorless mixture was allowed to cool to room temperature and poured into ice water. The white precipitate was filtered, washed with water and purified by column chromatography on silica gel (25% Et_2O in Hexane) afforded **58** as a white solid (640 mg, 2.17 mmol, 69%). ^{1}H NMR (400 MHz; $CDCl_3$): δ 3.41-1.65 (m, 8H), 3.08 (s, 2H); ^{13}C NMR (100 MHz; $CDCl_3$): δ 55.2; ^{11}B NMR (128 MHz; $CDCl_3$): δ -6.38, -9.97, -13.4, -15.4, -17.2.

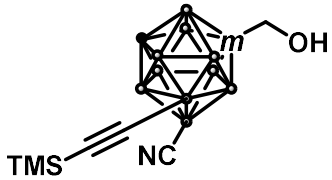
Synthesis of 9-cyano-10-iodo-1,7-dicarba-*closo*-dodecaborane (59)



59

To a solution of **58** (640 mg, 2.17 mmol) and dichlorobis(triphenylphosphine)palladium (70 mg, 0.10 mmol) in THF (3.3 mL), was added turbo Grignard reagent freshly prepared (trimethylsilyl)acetylene (1.0 mL, 7.50 mmol) and 1.3 M isopropylmagnesium chloride-lithium chloride complex in THF (7 mL). The resulting mixture was stirred at 50 °C for 36 h. Then, the resulting mixture was cooled to room temperature and quenched with water, which was filtered on celite pad. The filtrate was wash with brine and dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% EtOAc in Hexane) afforded **59** (206 mg, 0.774 mmol, 36%) as a white solid. ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.30-1.60 (m, 8H), 3.00 (s, 2H), 0.18 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 53.0, 0.06; ^{11}B NMR (160 MHz; $CDCl_3$): δ -5.65, -9.04, -12.9, -13.7, -14.2, -17.5, -19.6.

Synthesis of 1-hydroxymethyl-9-cyano-10-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (60)



60

To a solution of **59** (49 mg, 0.186 mmol) in THF (800 μL), was slowly added lithium diisopropylamide (LDA) 1.1 M solution in THF (211 μL, 0.223 mmol) at -78 ℃. After the resulting mixture was stirred at -78 ℃ under argon atmosphere for 1 h, paraformaldehyde was added. Then, the resulting mixture was stirred at room temperature for 2 h under argon atmosphere. After that, the reaction mixture was quenched with brine, which was partitioned between the aqueous and organic layers. The aqueous layer was washed with Et_2O and combined organic layers were dried over sodium sulfate and concentrated under vacuum. The crude material was purified by column chromatography on silica gel (25% to 40% EtOAc in Hexane) afforded **60** (9.0 mg, 0.0305 mmol, 16%) as a white solid. ^{1}H NMR (500 MHz; $CDCl_3$): δ 3.83 (s, 2H), 3.02 (s, 1H), 3.50-1.80 (m, 8H), 0.18 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$): δ 108.9, 75.9, 64.8, 52.7, 0.06; ^{11}B NMR (160 MHz; $CDCl_3$): δ -4.0, -8.3, -9.4, -11.6, -12.4, -13.1, -13.8, -14.6, -16.3, -18.6.

X-ray Crystallography

All data generated or analyzed were provided in supplementary information. CCDC 2110331, 2110336-2110341.

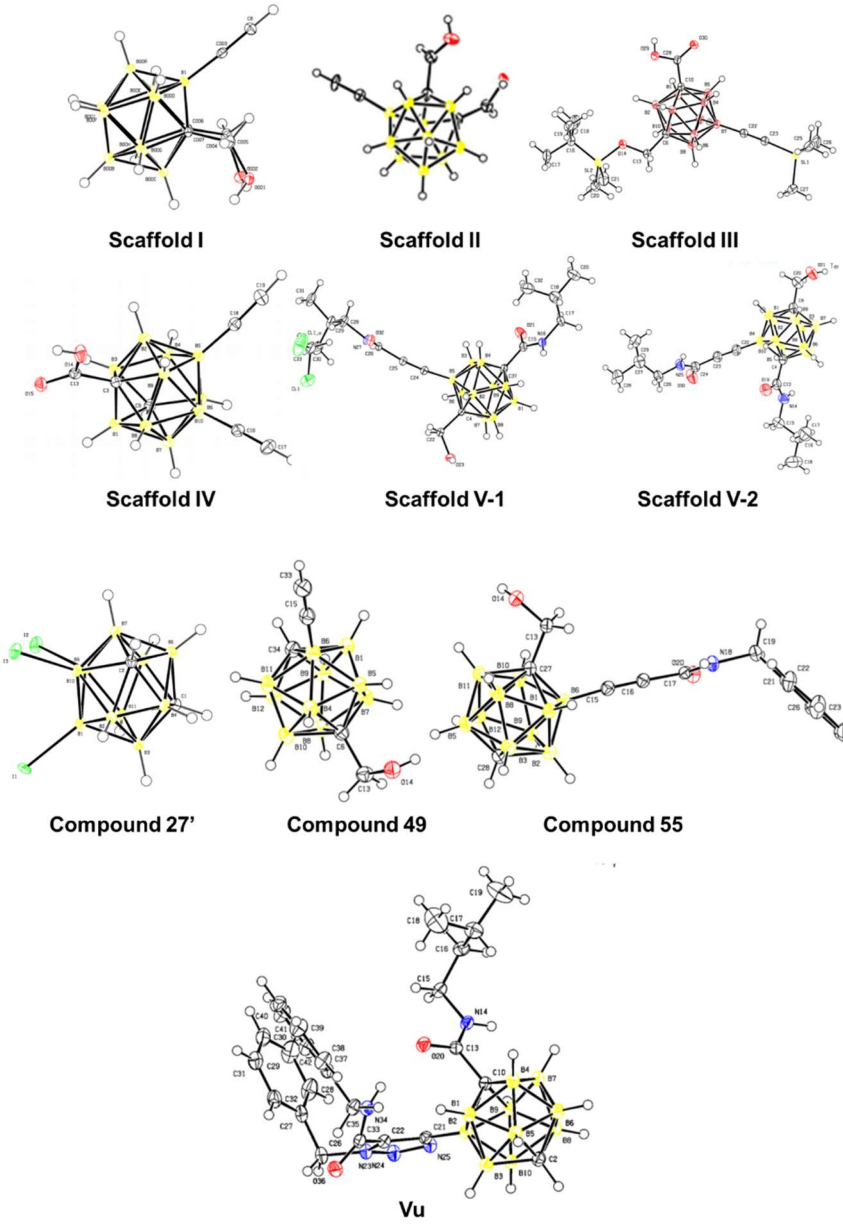


Table S1 Crystal data and structure refinement

Compound	**7** (Scaffold I)	**15** (Scaffold II)
Identification code	CRL-26-1263	CRL261328-2_autored
Empirical formula	$C_6H_{16}B_{10}O_2$	$C_6H_{16}B_{10}O_2$
Formula weight	228.29	228.29
Temperature/K	90	90
Crystal system	orthorhombic	monoclinic
Space group	$Pna2_1$	I2/a
a/Å	12.0547(4)	24.7677(3)
b/Å	13.7982(4)	15.2606(2)
c/Å	7.4135(2)	26.7524(3)
α/°	90	90
β/°	90	100.1710(10)
γ/°	90	90
Volume/Å^3	1233.11(6)	9952.7(2)
Z	4	32
ρ_{calc}g/cm^3	1.230	1.219
μ/mm^{-1}	0.510	0.506
F(000)	472.0	3776.0
Crystal size/mm^3	0.163 × 0.074 × 0.053	0.27 × 0.078 × 0.041
Radiation	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	9.742 to 153.358	6.694 to 153.06
Index ranges	-14 ≤ h ≤ 14, -15 ≤ k ≤ 17, -8 ≤ l ≤ 3	-31 ≤ h ≤ 31, -18 ≤ k ≤ 18, -33 ≤ l ≤ 33
Reflections collected	4182	72811
Independent reflections	1774 [R_{int} = 0.0262, R_{sigma} = 0.0301]	10199 [R_{int} = 0.0453, R_{sigma} = 0.0202]
Data/restraints/parameters	1774/326/165	10199/2105/822
Goodness-of-fit on F^2	1.081	1.133
Final R indexes [I>=2σ (I)]	R_1 = 0.0598, wR_2 = 0.1571	R_1 = 0.2344, wR_2 = 0.6794
Final R indexes [all data]	R_1 = 0.0615, wR_2 = 0.1586	R_1 = 0.2370, wR_2 = 0.6856
Largest diff. peak/hole / e Å^{-3}	1.00/-0.91	1.97/-1.92

Table S1 Crystal data and structure refinement (continued)

Compound	**24** (Scaffold III)	**30** (Scaffold IV)
Identification code	CRL-26-1114_3	CRL-26-1021
Empirical formula	$C_{15}H_{36}B_{10}O_3Si_2$	$C_7H_{12}B_{10}O_2$
Formula weight	428.72	236.27
Temperature/K	90	293(2)
Crystal system	monoclinic	triclinic
Space group	I2/a	P-1
a/Å	11.4845(4)	6.9823(4)
b/Å	12.7847(5)	7.3010(4)
c/Å	36.4855(13)	13.2381(8)
α/°	90	76.236(5)
β/°	95.462(3)	83.750(5)
γ/°	90	89.704(4)
Volume/Å^3	5332.7(3)	651.42(7)
Z	8	2
ρ_{calc}g/cm^3	1.068	1.205
μ/mm^{-1}	1.296	0.510
F(000)	1824.0	240.0
Crystal size/mm^3	0.297 × 0.053 × 0.046	0.121 × 0.119 × 0.071
Radiation	Cu Kα (λ = 1.54184)	CuKα (λ = 1.54184)
2Θ range for data collection/°	7.33 to 149.398	6.918 to 152.73
Index ranges	-14 ≤ h ≤ 14, -15 ≤ k ≤ 12, -44 ≤ l ≤ 43	-8 ≤ h ≤ 8, -9 ≤ k ≤ 8, -10 ≤ l ≤ 16
Reflections collected	16339	6996
Independent reflections	5168 [R_{int} = 0.0217, R_{sigma} = 0.0236]	2599 [R_{int} = 0.0265, R_{sigma} = 0.0309]
Data/restraints/parameters	5168/0/280	2599/0/217
Goodness-of-fit on F^2	1.052	1.069
Final R indexes [I>=2σ (I)]	R_1 = 0.0397, wR_2 = 0.1047	R_1 = 0.0360, wR_2 = 0.0957
Final R indexes [all data]	R_1 = 0.0435, wR_2 = 0.1073	R_1 = 0.0397, wR_2 = 0.0988
Largest diff. peak/hole / e Å^{-3}	0.59/-0.32	0.24/-0.32

Table S1 Crystal data and structure refinement (continued)

Compound	**42d** (Scaffold V-1)	**46d** (Scaffold V-2)
Identification code	CRL-26-1132_2	CRL-26-1134_4
Empirical formula	$C_{31}H_{66}B_{20}Cl_2N_4O_6$	$C_{15}H_{32}B_{10}N_2O_3$
Formula weight	877.97	396.52
Temperature/K	90	90
Crystal system	monoclinic	triclinic
Space group	C2/c	P-1
a/Å	29.1281(13)	9.1672(5)
b/Å	8.7234(4)	11.8780(5)
c/Å	20.1283(9)	12.9840(5)
α/°	90	105.803(4)
β/°	108.963(5)	102.443(4)
γ/°	90	111.614(4)
Volume/Å^3	4836.9(4)	1183.29(10)
Z	4	2
ρ_{calc}g/cm^3	1.206	1.113
μ/mm^{-1}	1.543	0.514
F(000)	1848.0	420.0
Crystal size/mm^3	0.186 × 0.177 × 0.124	0.342 × 0.1 × 0.055
Radiation	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	6.418 to 149.99	7.564 to 149.972
Index ranges	-36 ≤ h ≤ 34, -10 ≤ k ≤ 10, -25 ≤ l ≤ 22	-11 ≤ h ≤ 11, -14 ≤ k ≤ 13, -15 ≤ l ≤ 15
Reflections collected	14464	13384
Independent reflections	4740 [R_{int} = 0.0472, R_{sigma} = 0.0388]	4674 [R_{int} = 0.0308, R_{sigma} = 0.0373]
Data/restraints/parameters	4740/486/290	4674/0/285
Goodness-of-fit on F^2	1.041	1.075
Final R indexes [I>=2σ (I)]	R_1 = 0.0906, wR_2 = 0.2566	R_1 = 0.0552, wR_2 = 0.1535
Final R indexes [all data]	R_1 = 0.0939, wR_2 = 0.2612	R_1 = 0.0642, wR_2 = 0.1617
Largest diff. peak/hole / e Å^{-3}	1.71/-1.37	1.03/-0.32

Table S1 Crystal data and structure refinement (continued)

Compound	**27'**	**49**
Identification code	CRL64-1-2	NFT26_1002
Empirical formula	$C_2H_9B_{10}I_3$	$C_5H_{14}B_{10}O$
Formula weight	521.89	198.409
Temperature/K	293(2)	90
Crystal system	monoclinic	tetragonal
Space group	$P2_1/c$	$I4_1/a$
a/Å	8.6488(2)	23.9820(2)
b/Å	12.1509(2)	23.9820(2)
c/Å	12.6613(2)	16.1633(2)
α/°	90	90
β/°	89.949(2)	90
γ/°	90	90
Volume/Å^3	1330.58(4)	9296.10(16)
Z	4	32
ρ_{calc}g/cm^3	2.605	1.134
μ/mm^{-1}	54.923	0.406
F(000)	920.0	3271.2
Crystal size/mm^3	? × ? × ?	0.3 × 0.06 × 0.02
Radiation	Cu Kα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	10.09 to 151.506	6.6 to 150.16
Index ranges	-10 ≤ h ≤ 10, -9 ≤ k ≤ 15, -15 ≤ l ≤ 14	-29 ≤ h ≤ 29, -29 ≤ k ≤ 29, -20 ≤ l ≤ 18
Reflections collected	4669	108845
Independent reflections	2191 [R_{int} = 0.0321, R_{sigma} = 0.0331]	4684 [R_{int} = 0.0361, R_{sigma} = 0.0106]
Data/restraints/parameters	2191/270/136	4684/0/371
Goodness-of-fit on F^2	1.184	1.039
Final R indexes [I>=2σ (I)]	R_1 = 0.0560, wR_2 = 0.1538	R_1 = 0.0377, wR_2 = 0.1049
Final R indexes [all data]	R_1 = 0.0702, wR_2 = 0.1998	R_1 = 0.0401, wR_2 = 0.1069
Largest diff. peak/hole / e Å^{-3}	2.22/-3.33	0.28/-0.23

Table S1 Crystal data and structure refinement (continued)

Compound	**55**	**Vu**
Identification code	NFT26_991	CRL261491_auto
Empirical formula	$C_{13}H_{21}B_{10}NO_2$	$C_{25}H_{37}B_{10}N_5O_2$
Formula weight	331.41	547.69
Temperature/K	90	90
Crystal system	triclinic	triclinic
Space group	P-1	P-1
a/Å	6.93880(10)	10.6228(2)
b/Å	8.8473(2)	11.6938(2)
c/Å	14.7429(2)	13.23540(10)
α/°	82.9450(10)	101.0160(10)
β/°	85.523(2)	95.4480(10)
γ/°	84.214(2)	103.261(2)
Volume/Å^3	891.65(3)	1554.13(4)
Z	2	2
ρ_{calc}g/cm^3	1.234	1.170
μ/mm^{-1}	0.533	0.535
F(000)	344.0	576.0
Crystal size/mm^3	0.11 × 0.05 × 0.02	0.349 × 0.163 × 0.07
Radiation	CuKα (λ = 1.54184)	Cu Kα (λ = 1.54184)
2Θ range for data collection/°	10.118 to 148.444	6.876 to 151.988
Index ranges	-8 ≤ h ≤ 8, -10 ≤ k ≤ 10, -18 ≤ l ≤ 18	-13 ≤ h ≤ 13, -14 ≤ k ≤ 14, -16 ≤ l ≤ 14
Reflections collected	17485	47760
Independent reflections	3480 [R_{int} = 0.0175, R_{sigma} = 0.0087]	6209 [R_{int} = 0.0368, R_{sigma} = 0.0209]
Data/restraints/parameters	3480/0/276	6209/0/381
Goodness-of-fit on F^2	1.076	1.056
Final R indexes [I>=2σ (I)]	R_1 = 0.0365, wR_2 = 0.0995	R_1 = 0.0415, wR_2 = 0.1072
Final R indexes [all data]	R_1 = 0.0379, wR_2 = 0.1011	R_1 = 0.0444, wR_2 = 0.1096
Largest diff. peak/hole / e Å^{-3}	0.19/-0.28	0.29/-0.28

Biology

Cell culture

Human epithelioid cervical carcinoma (HeLa) cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). The cells were incubated with RPMI-1640 medium (FUJIFILM Wako Pure Chemical) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific) and 1% peniciline/streptomycine (FUJIFILM Wako Pure Chemical). Cells were incubated in a cell incubator with 5% CO_2 at 37 ℃. Neuro 2a (N2a) cells were incubated with D-MEM medium (FUJIFILM Wako Pure Chemical) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific) and 1% peniciline/streptomycine (FUJIFILM Wako Pure Chemical). Cells were incubated in a cell incubator with 5% CO_2 at 37 ℃

MTT assay

HeLa cells or N2a cells (5×10^3 cells per well of a 96-well plate) were incubated under 5% CO_2 at 37 ℃ in RPMI-1640 media (for HeLa cells) or D-MEM media (for N2a cells) containing 10% fetal bovine serum, 1% peniciline/streptomycine, and various concentrations of compound (10 mM in DMSO) for 72 h. After the incubation, 3'-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in PBS (5 mg/mL, 10 μL) were added, and the cells were further incubated at 37 ℃ for 2 h. After removal of the medium, DMSO (100 μL) was added and the absorbance at 590 nm was measured with a microplate reader. The IC_{50} value was determined from semilogarithmic dose-response plots.

Luciferase reporter gene assay for HIF transcriptional activity

HeLa cells transfected with hypoxia response element dependent firefly luciferase reporter construct (HRE-Luc) were seeded in a 96-well plate (2.5×10^4 cells per a well) and incubated for 6 h under 5% CO_2 at 37 ℃ 42 with RPMI-1640 media containing 10% fetal bovine serum, 1% peniciline/streptomycine. After the incubation, cells were treated with various concentration of compound (10 mM in DMSO) and incubated at the same condition for 1 h. Then the cells were incubated at normoxic condition (1% O_2, 5% CO_2, 37 ℃) for 12 h. At the same time, cells without compounds were incubated at hypoxic condition for 12 h. After removal of the media cells were washed with PBS and the luciferase reporter gene assay was performed with Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions. The compound concentration required to reduce relative luminescence units (RLU) by 50% (IC_{50}) was determined from semilogarithmic dose response plots.

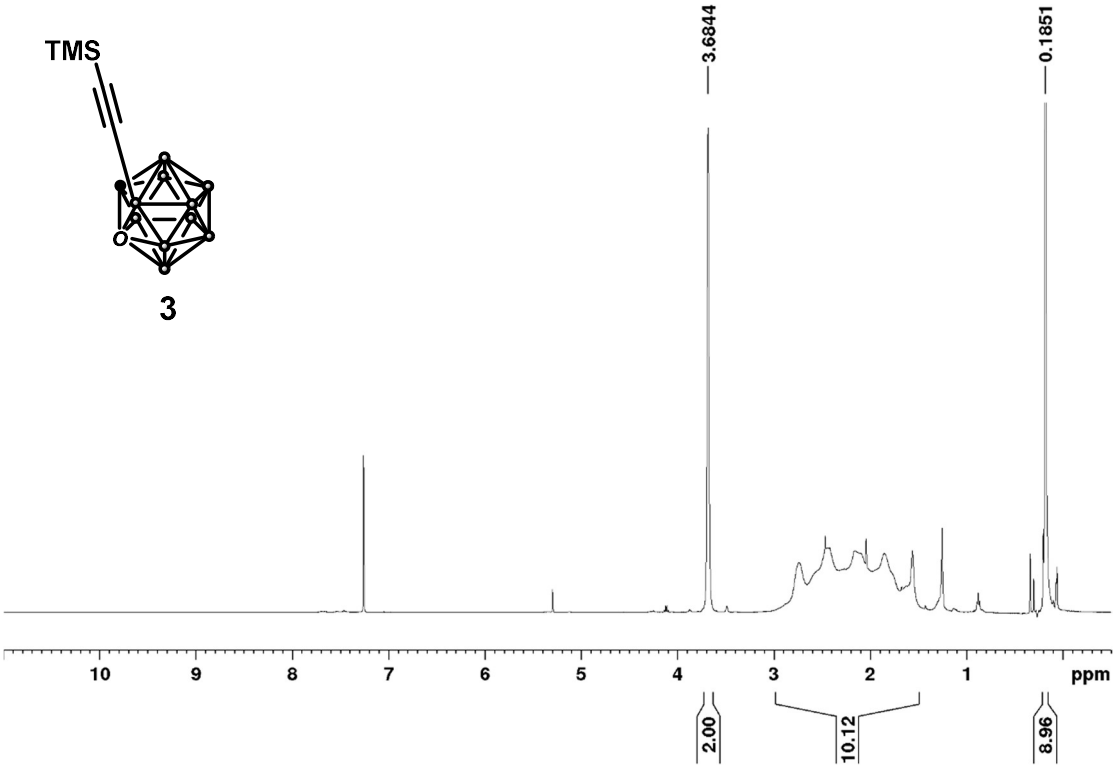
Luciferase reporter gene assay for antiviral activity against rabies virus

The recombinant rabies virus strain 1088 expressing gaussia luciferase (1088/GLuc) was generated as described previously.[60] The virus titer was determined using focus assay as reported[61] and expressed as focus forming units (FFU). N2a cells (4×10^4 cells per well) and 1088/GLuc (4×10^2 FFU per well) were prepared in E-MEM containing 10% fetal bovine serum and antibiotics, and the mixed solution was applied to a 96- well black plate with clear bottoms (Greiner). Subsequently, various concentrations of compounds were prepared in the medium and added to each well. The microplates were incubated at 37 ℃ under 5% CO_2 for 3 days, and then, a luciferase reporter gene assay was performed using Pierce Gaussia Luciferase Glow Assay Kit (Thermo Fisher Scientific). The substrate solution was added to each well, and RLU was immediately measured using a microplate luminometer LuMate (Awareness Technology). Based on the RLU value, IC_{50} was determined from semilogarithmic dose-response plots.

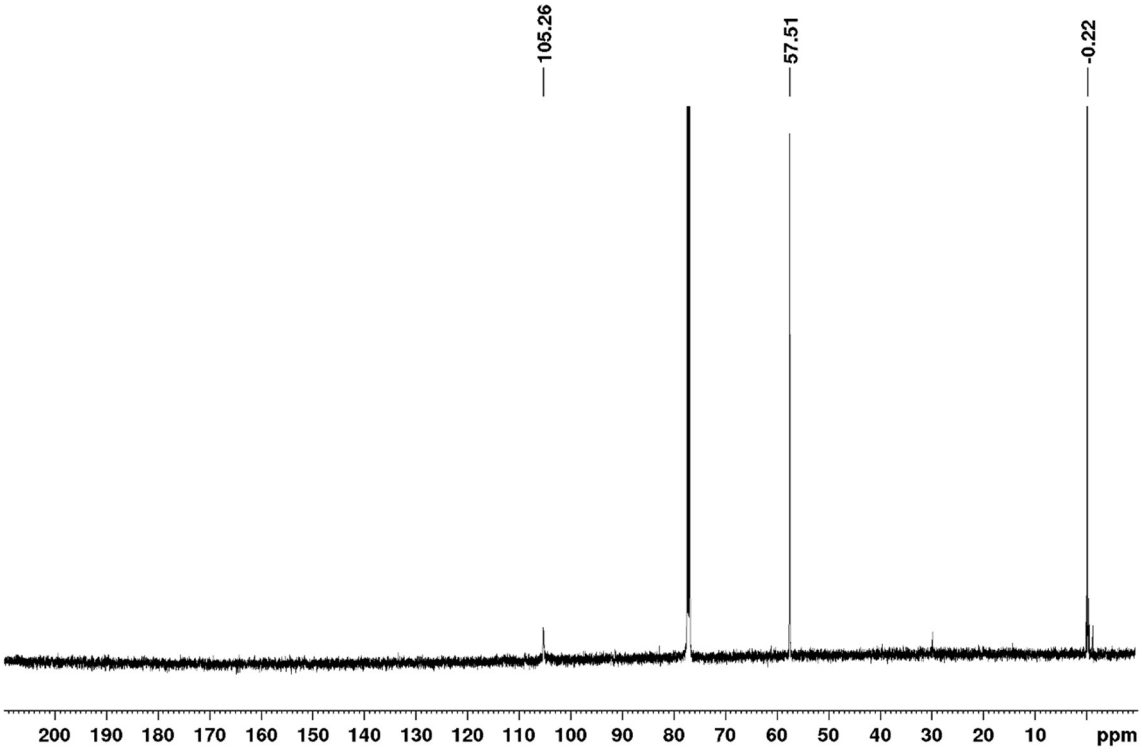
Spectra data

3-(2-(trimethylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (3)

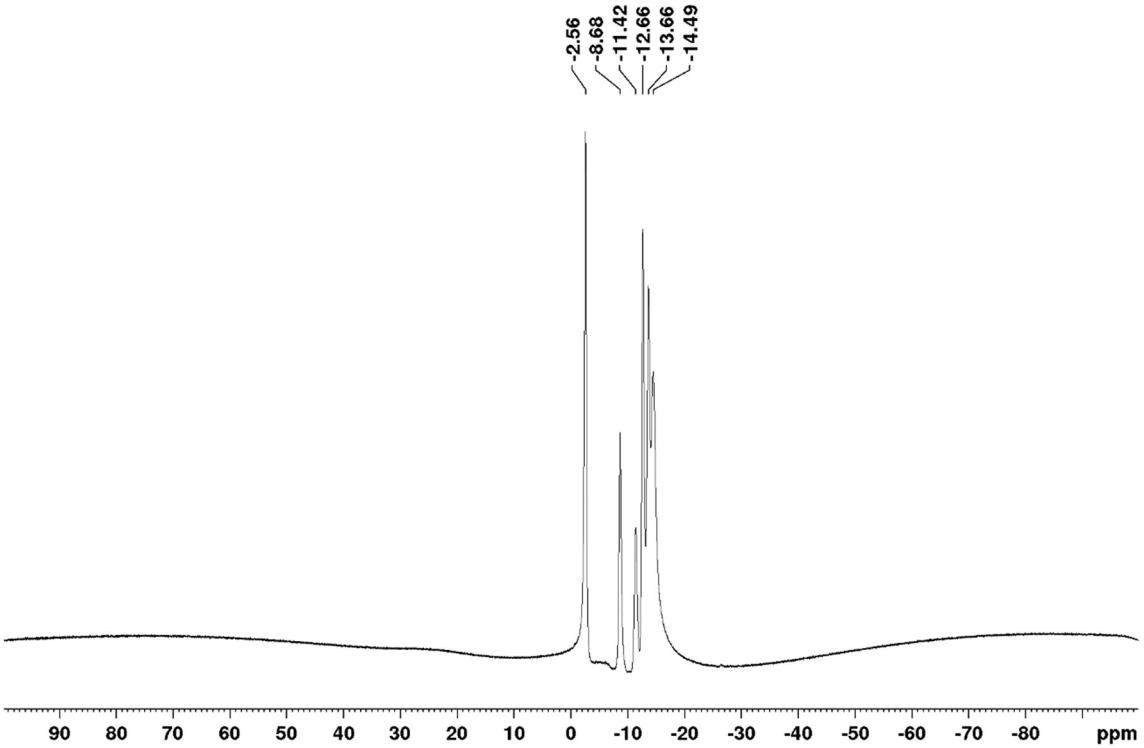
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

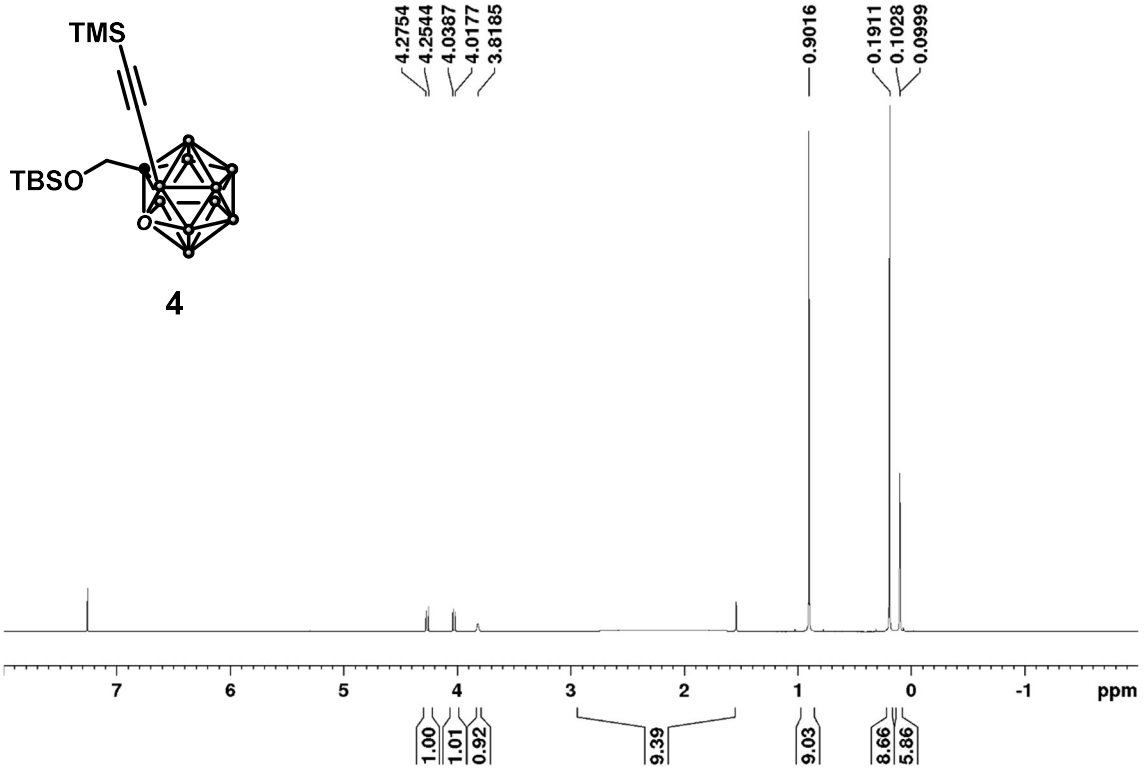


^{11}B NMR (160 MHz; $CDCl_3$)

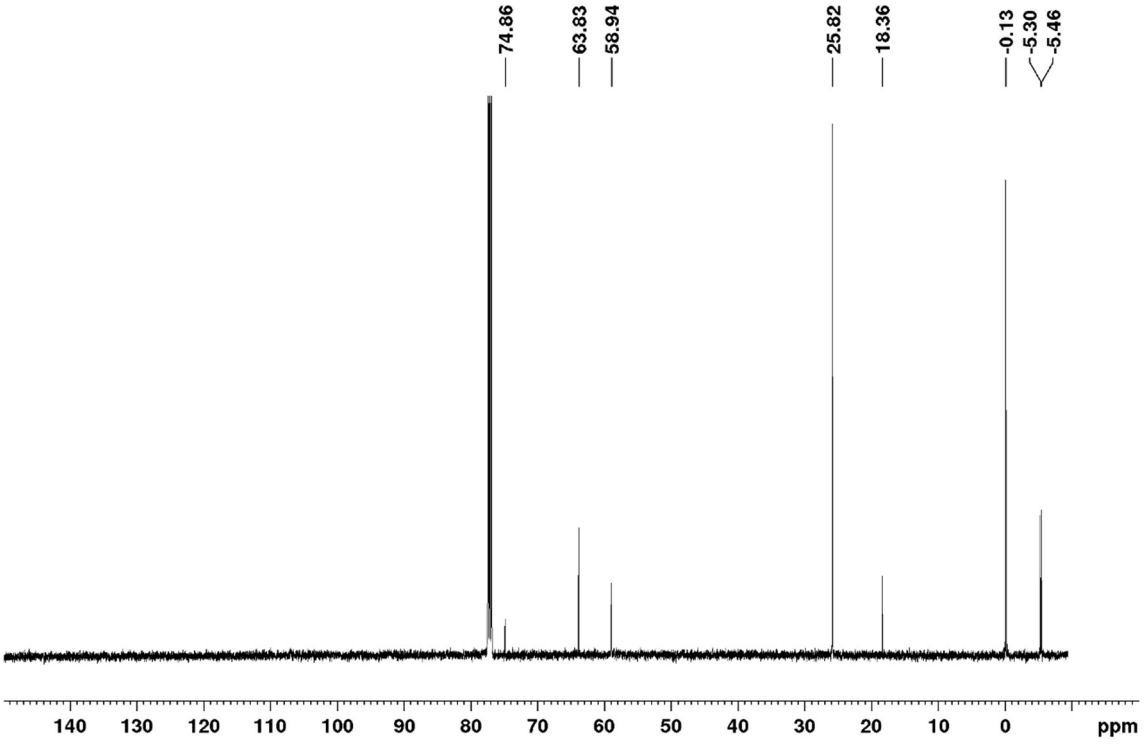


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-3-((trimethylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (4)

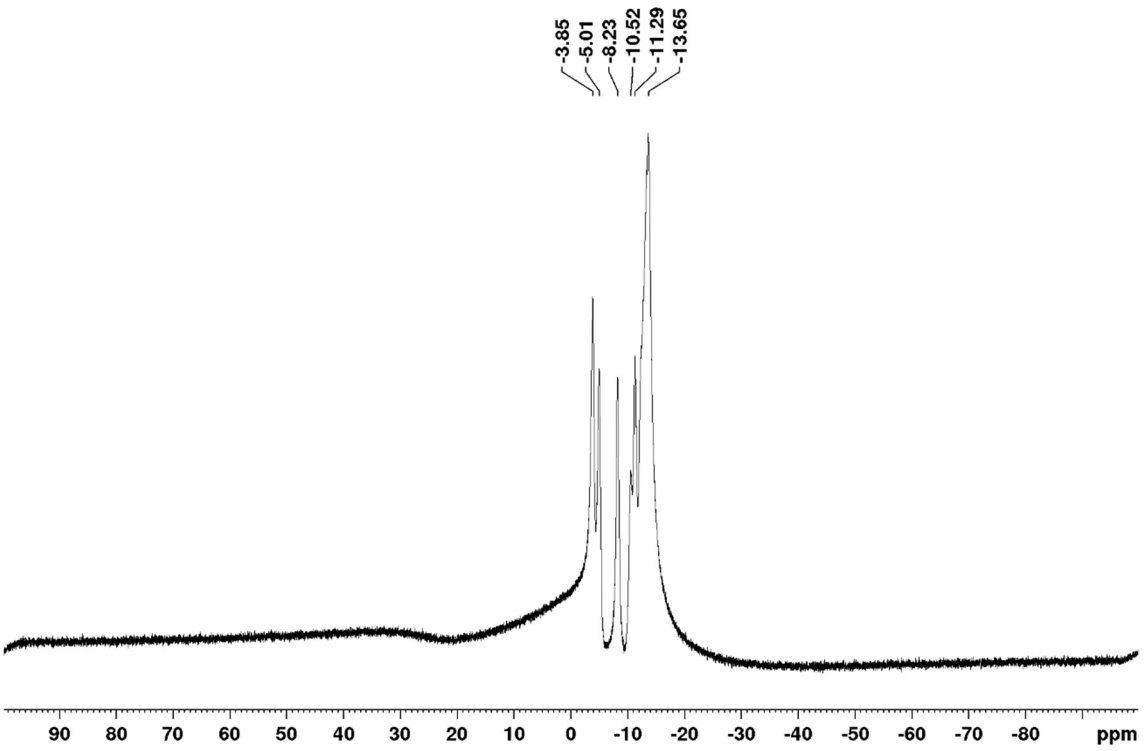
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

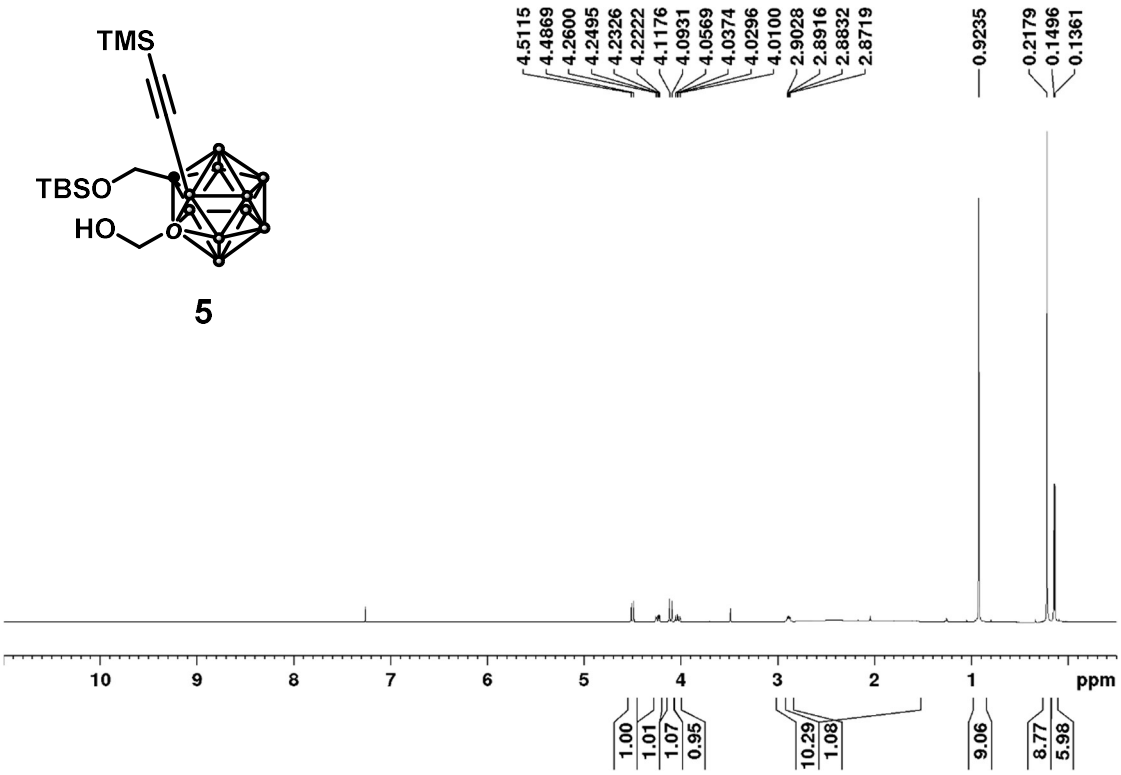


^{11}B NMR (160 MHz; $CDCl_3$)

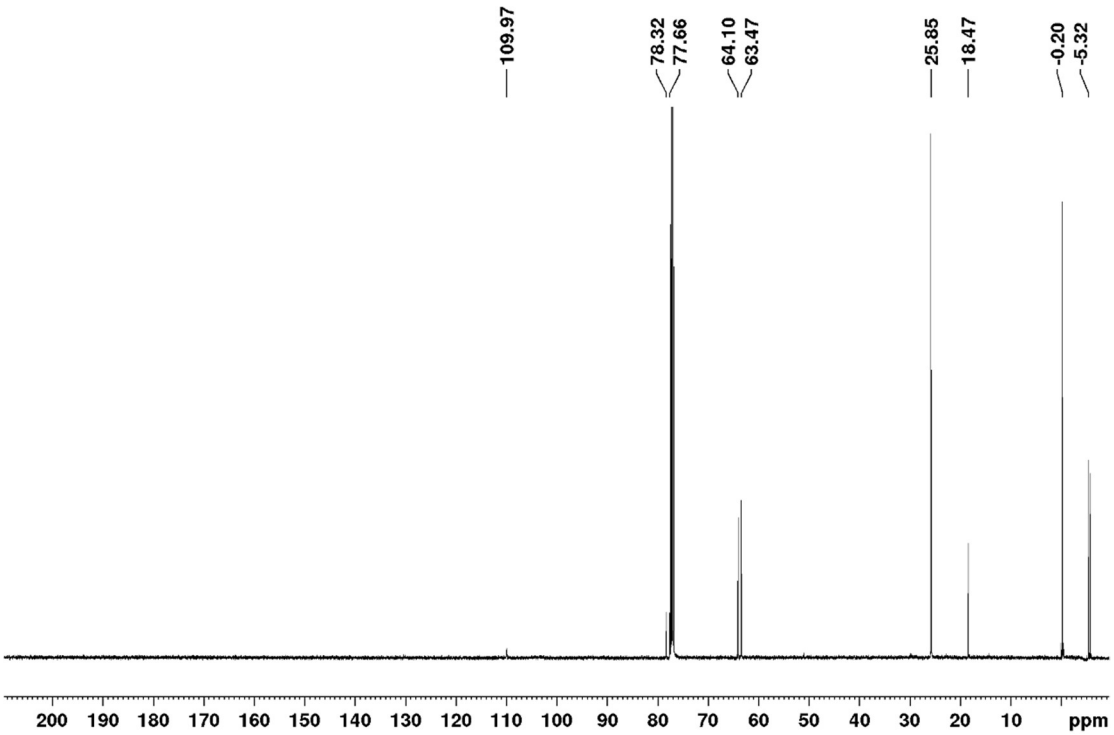


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-hydroxymethyl-3-((trimethylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (5)

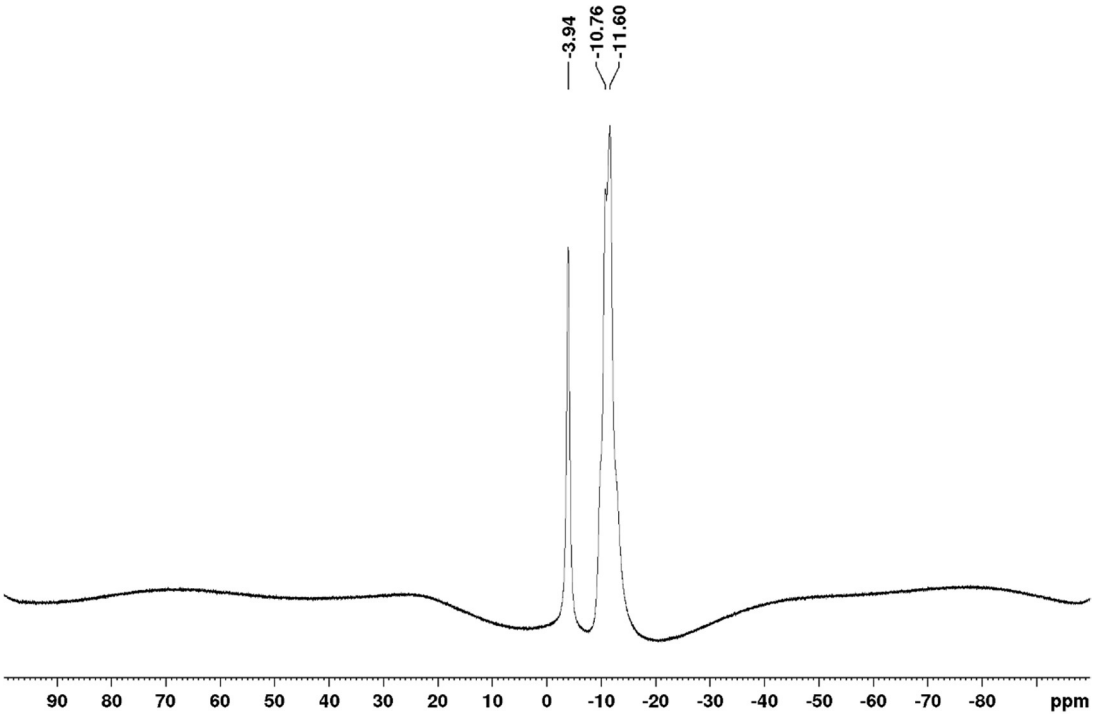
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)



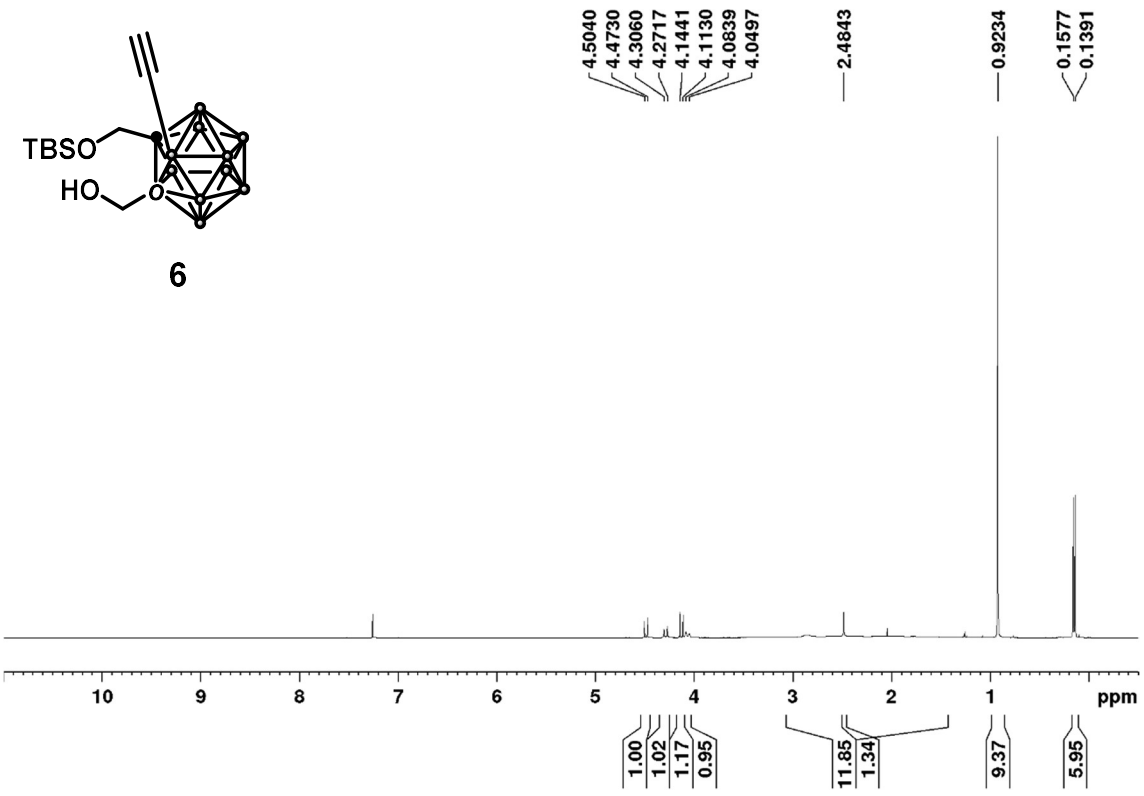
^{11}B NMR (160 MHz; $CDCl_3$)



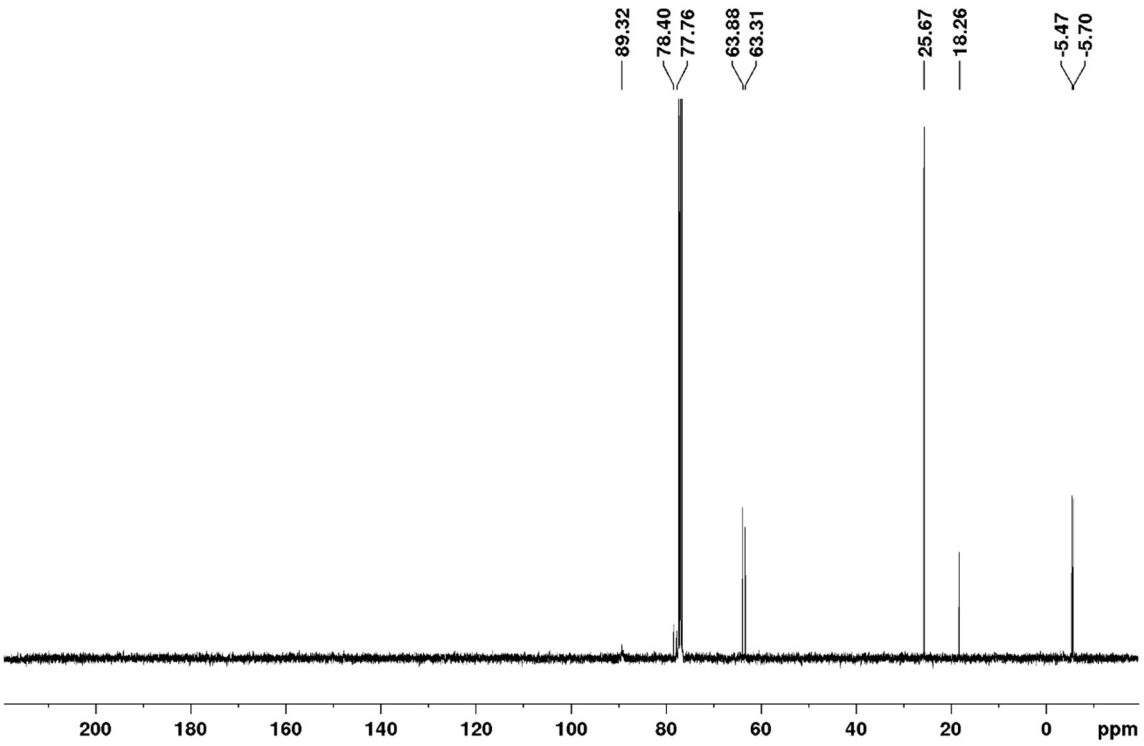
Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-hydroxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (6)

^{1}H NMR (400 MHz; $CDCl_3$)

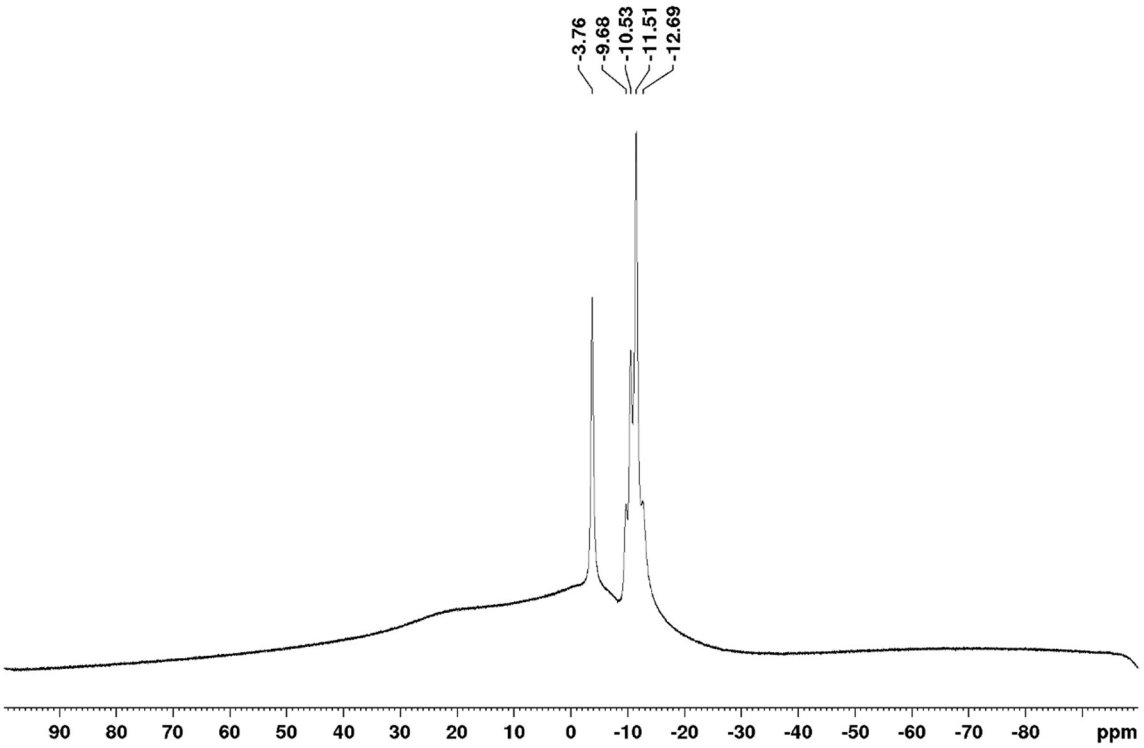
6



^{13}C NMR (100 MHz; $CDCl_3$)

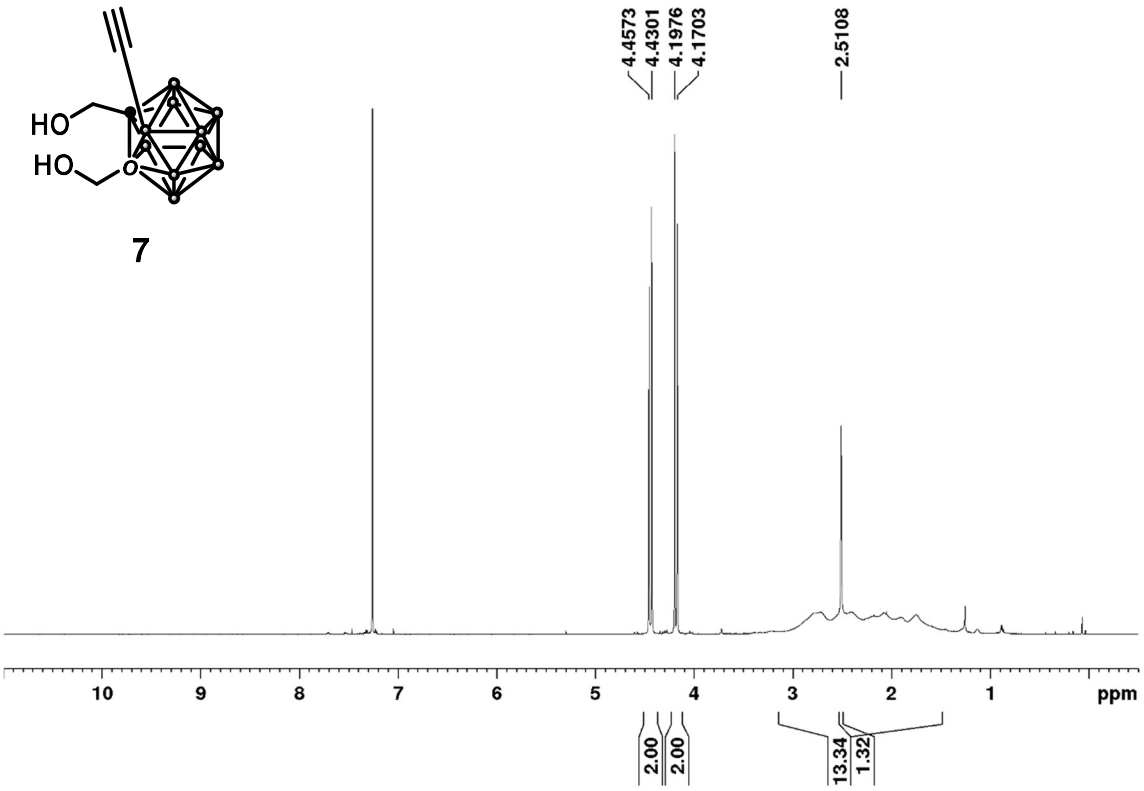


^{11}B NMR (128 MHz; $CDCl_3$)

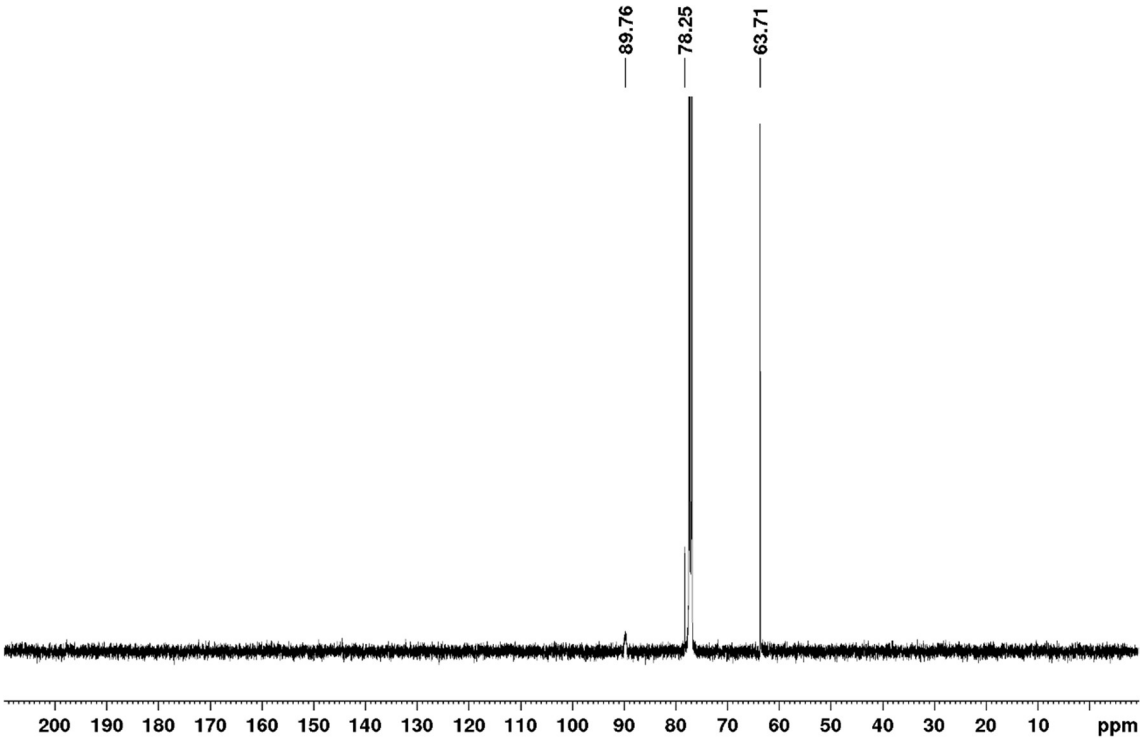


1,2-bis(hydroxymethyl)-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (7)

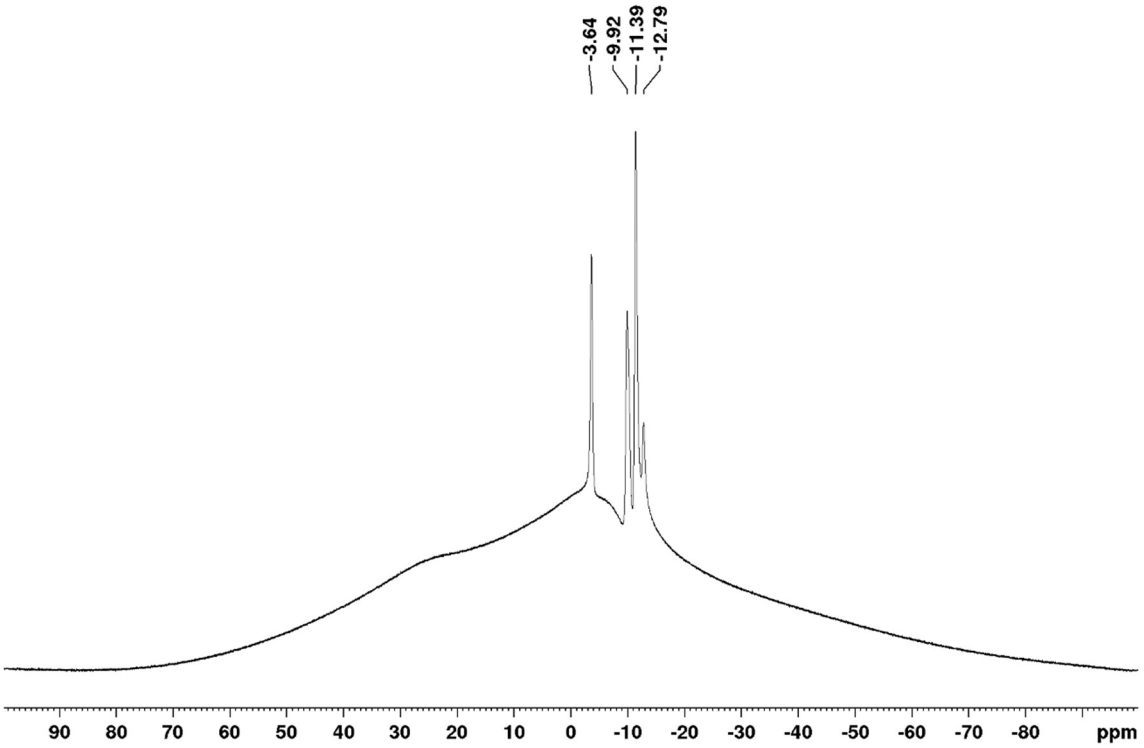
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

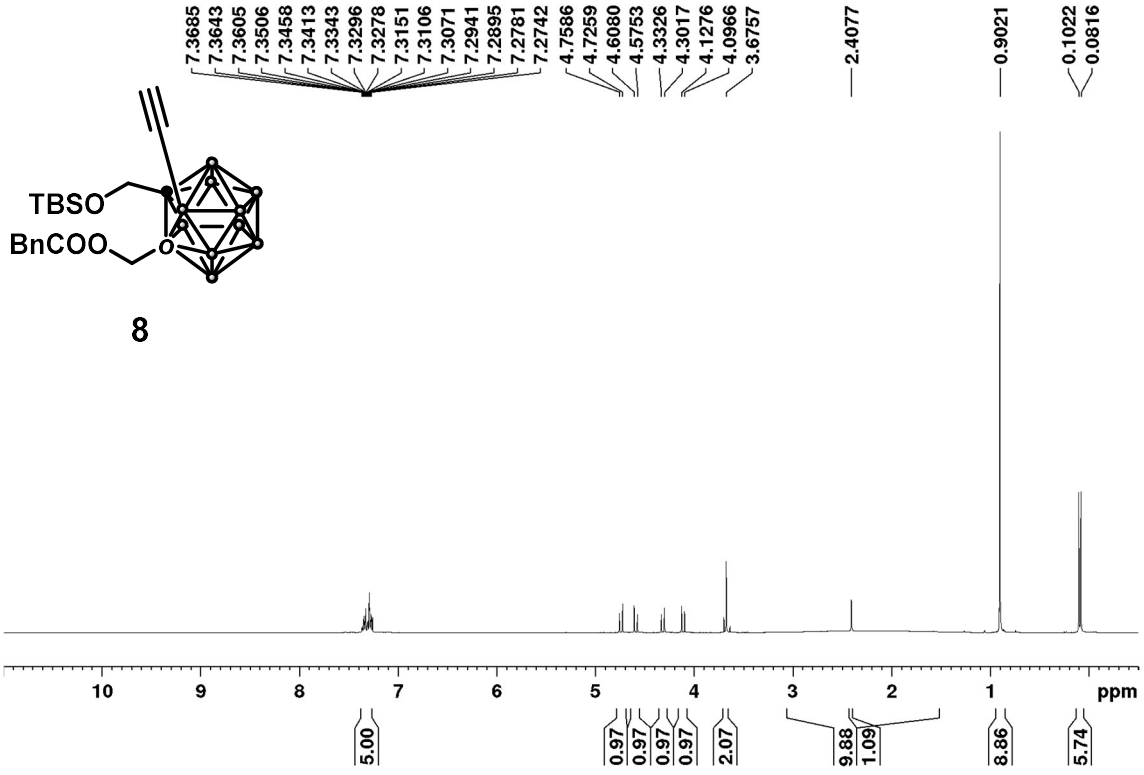


^{11}B NMR (160 MHz; $CDCl_3$)



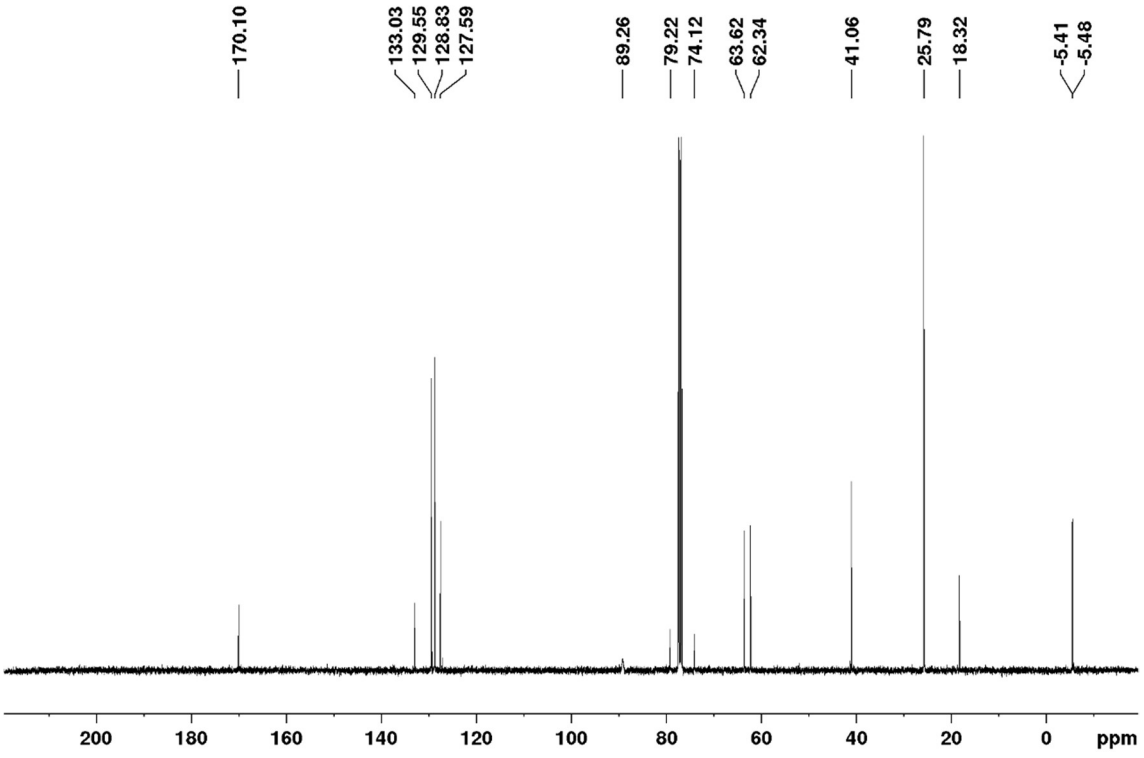
1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-phenylacetoxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (8)

^{1}H NMR (400 MHz; $CDCl_3$)

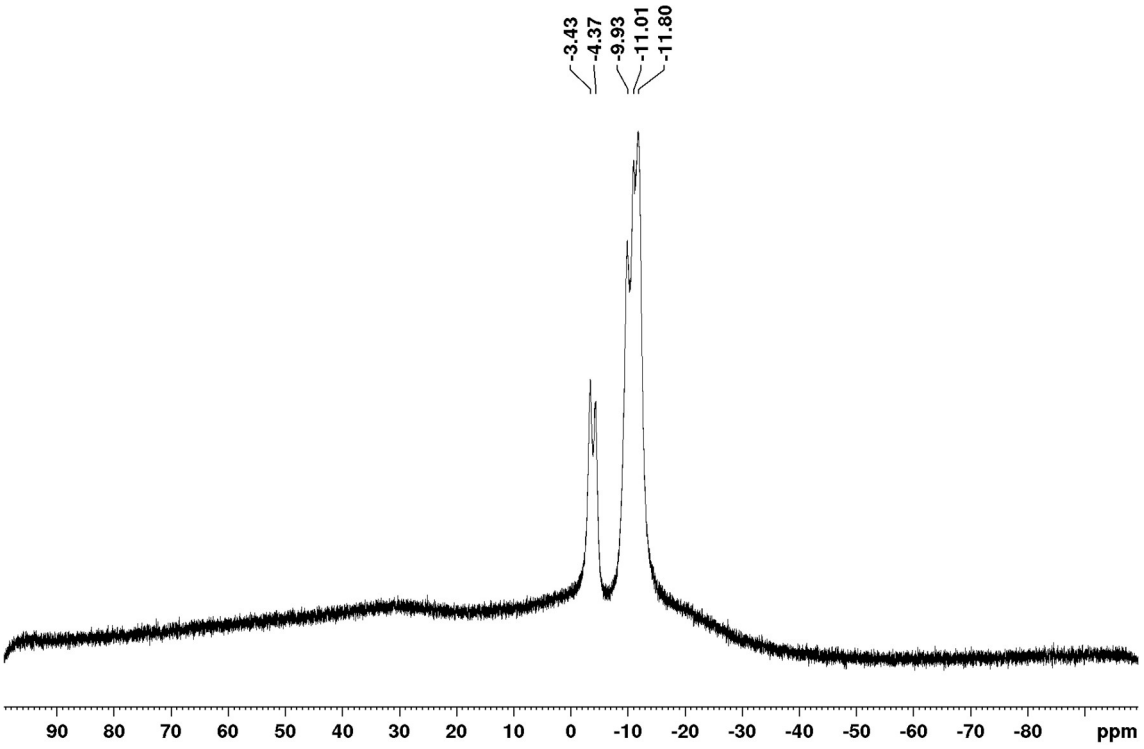


8

^{13}C NMR (100 MHz; $CDCl_3$)

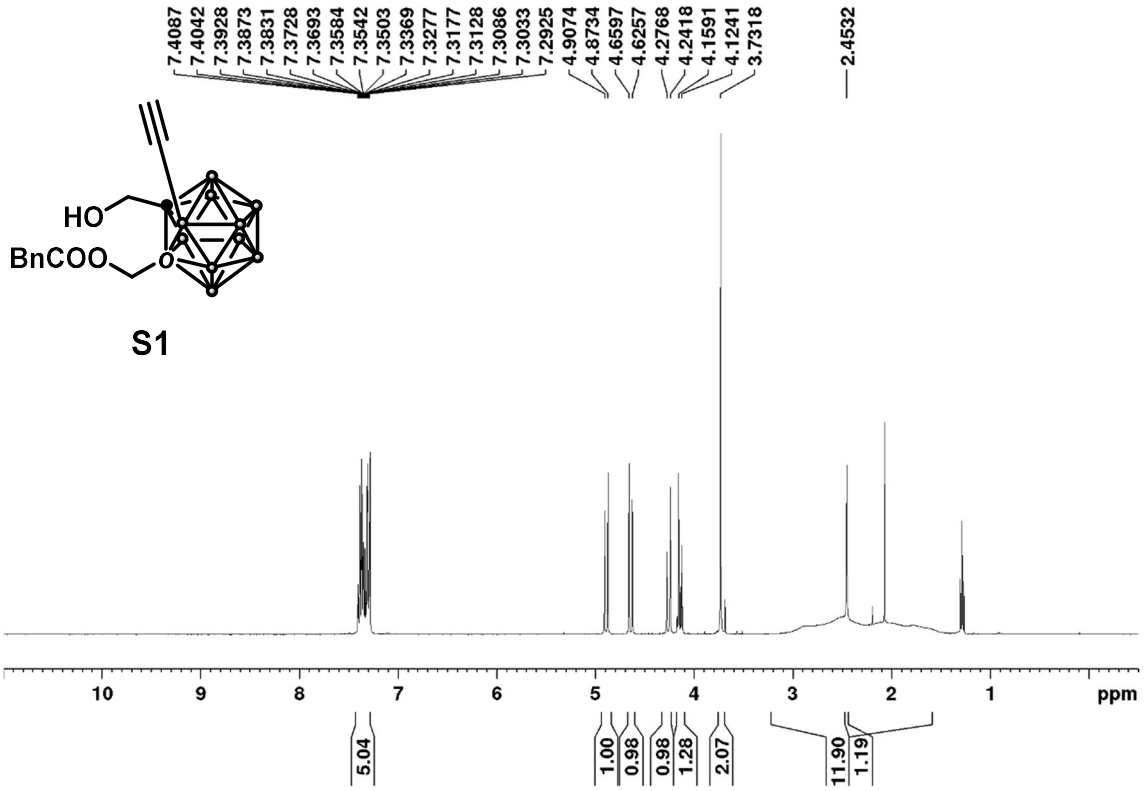


^{11}B NMR (128 MHz; $CDCl_3$)

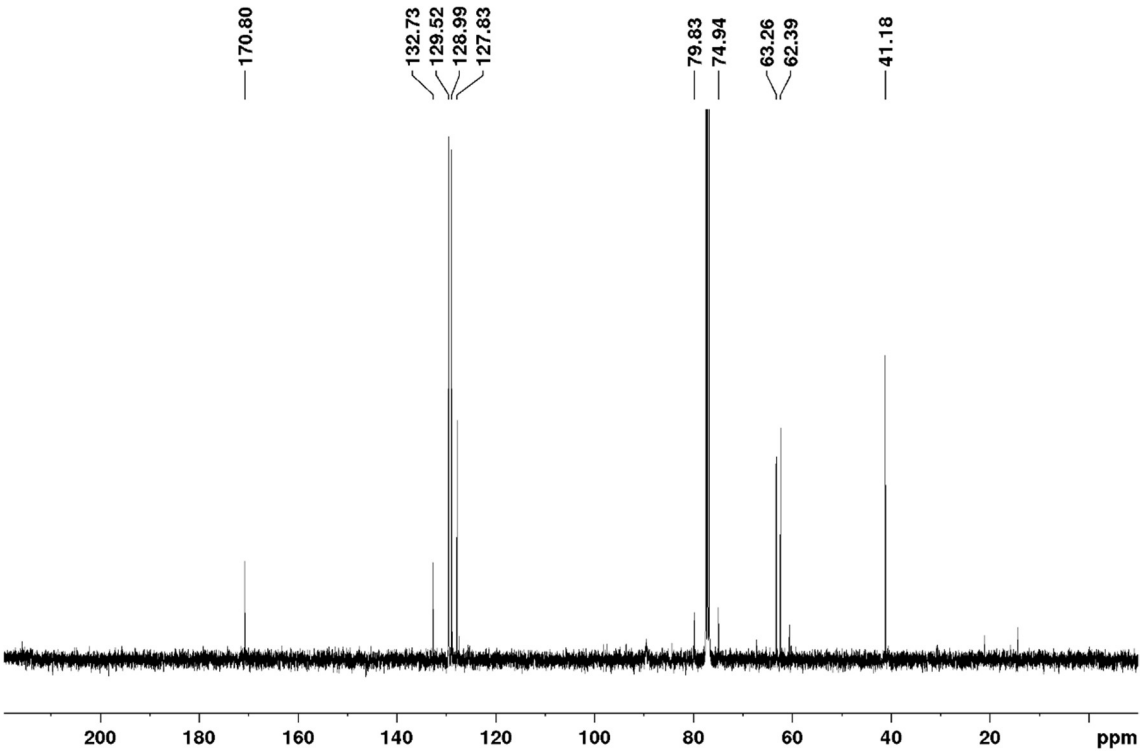


1-hydroxymethyl-2-phenylacetoxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (S1)

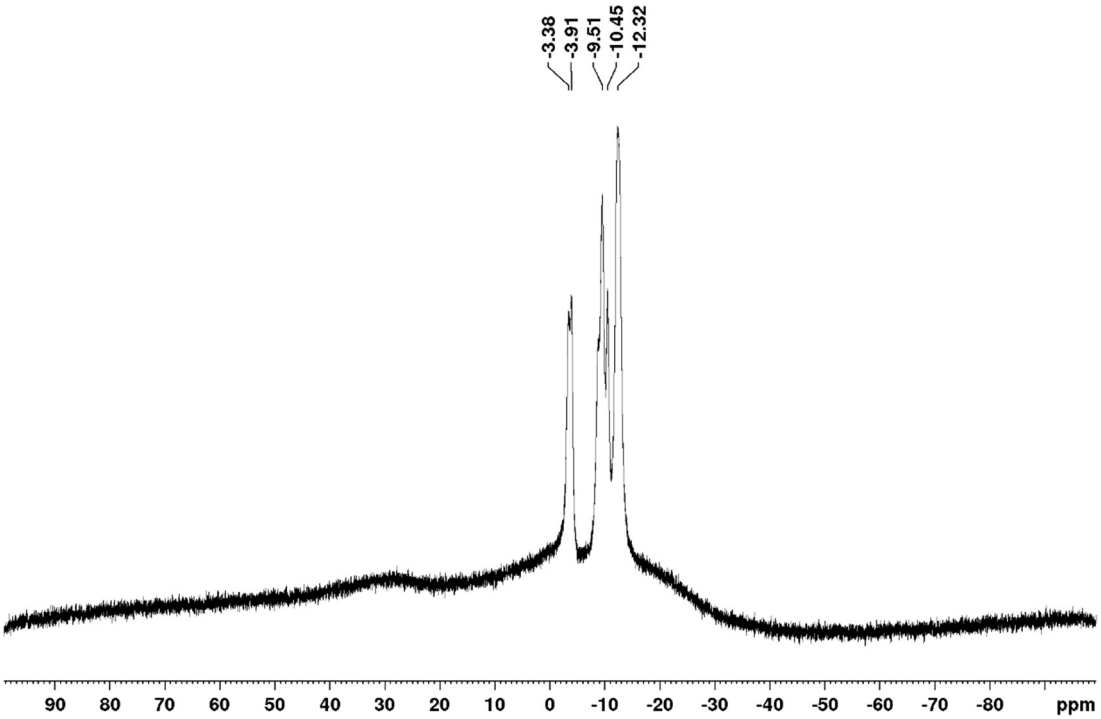
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

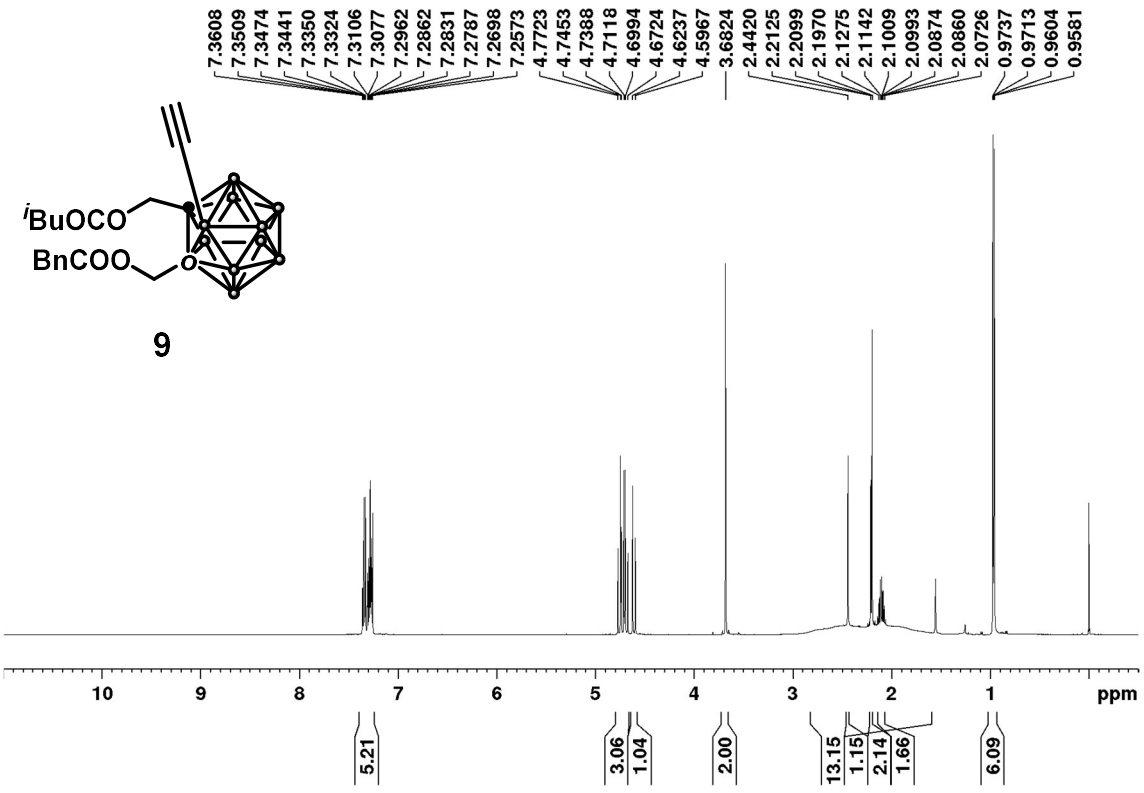


^{11}B NMR (128 MHz; $CDCl_3$)

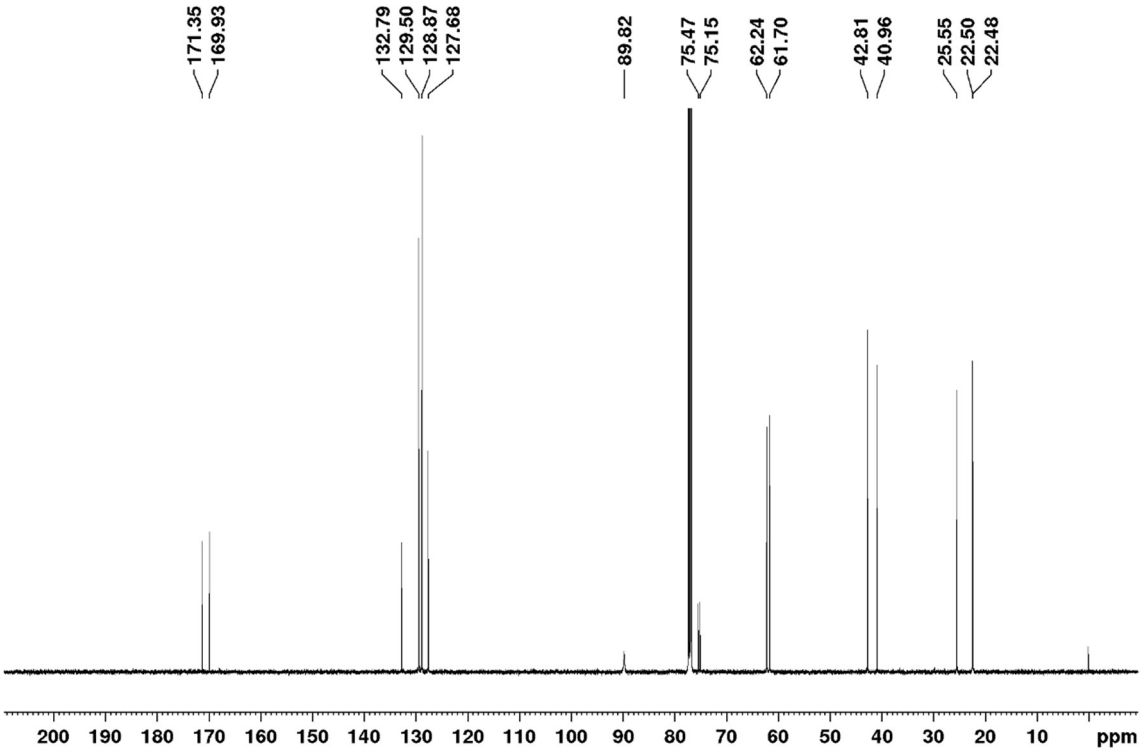


1-(3-methylbutanoyl)oxymethyl-2-phenylacetoxymethyl-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (9)

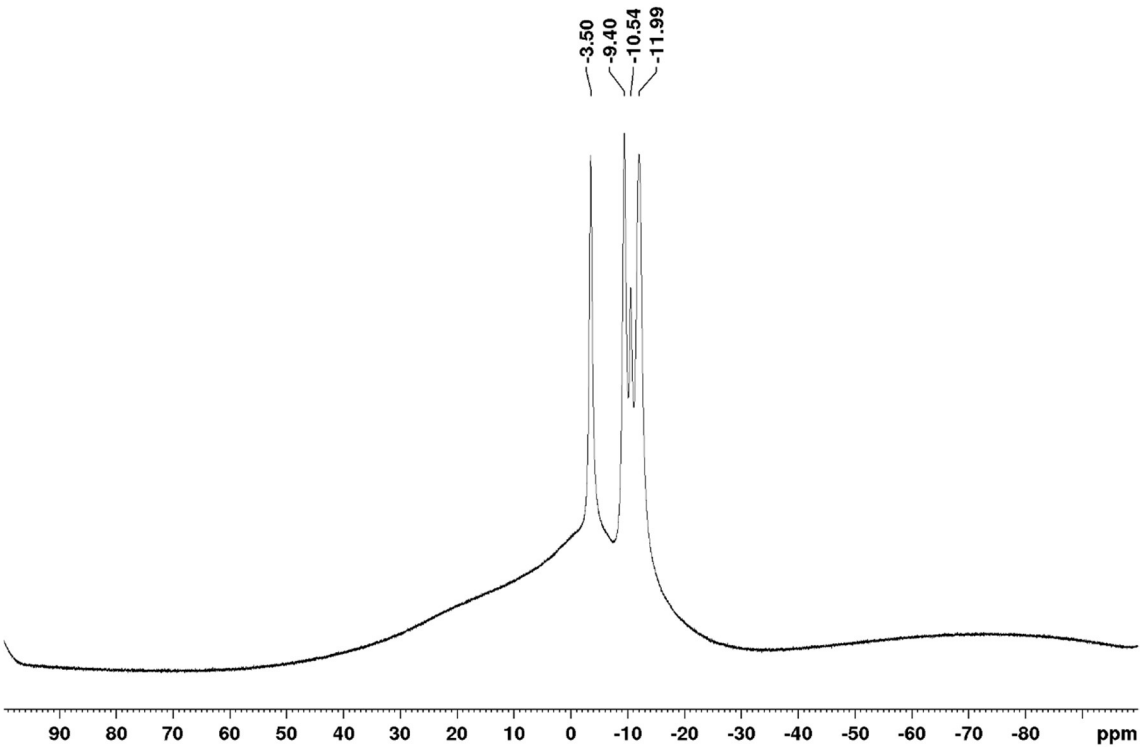
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

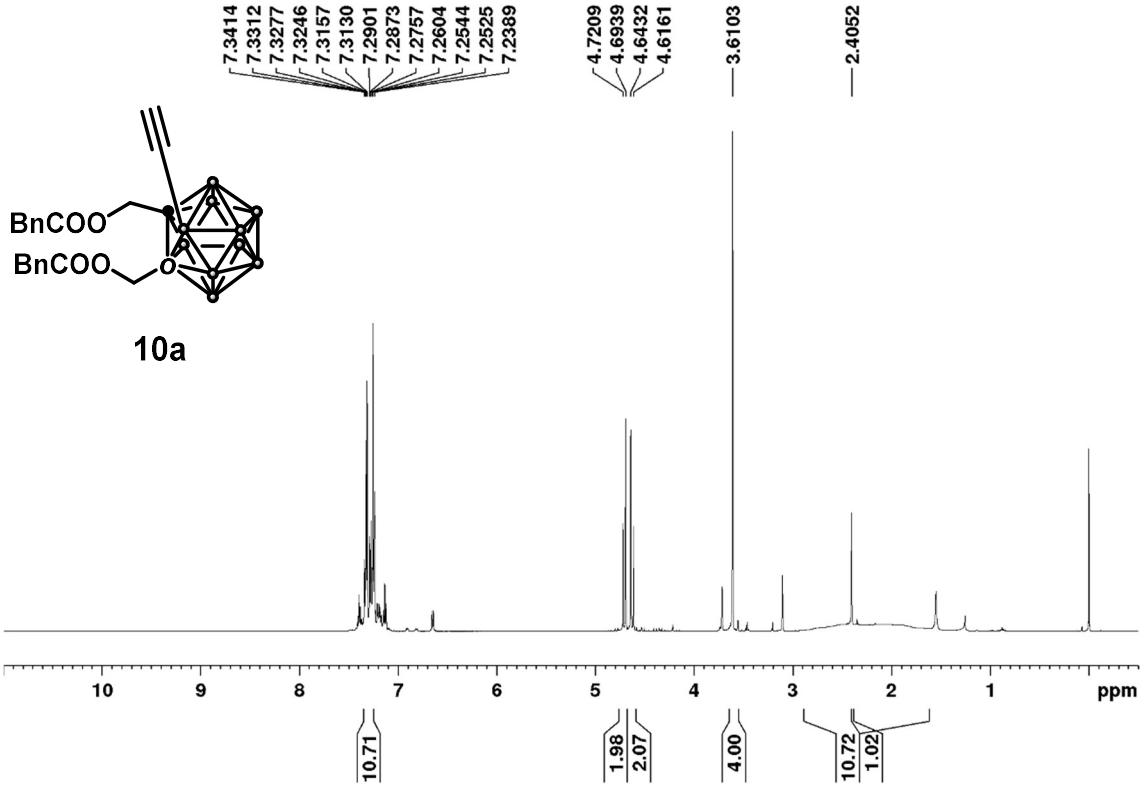


^{11}B NMR (160 MHz; $CDCl_3$)

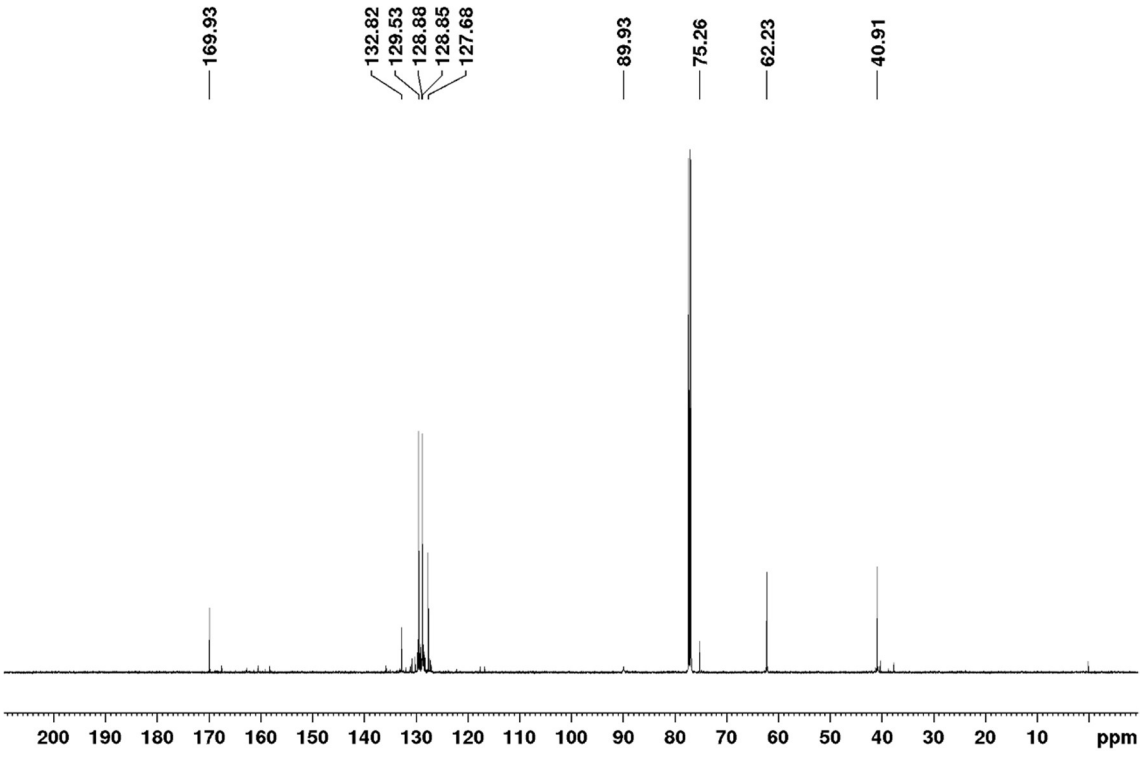


1,2-bis(phenylacetoxymethyl)-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (10a)

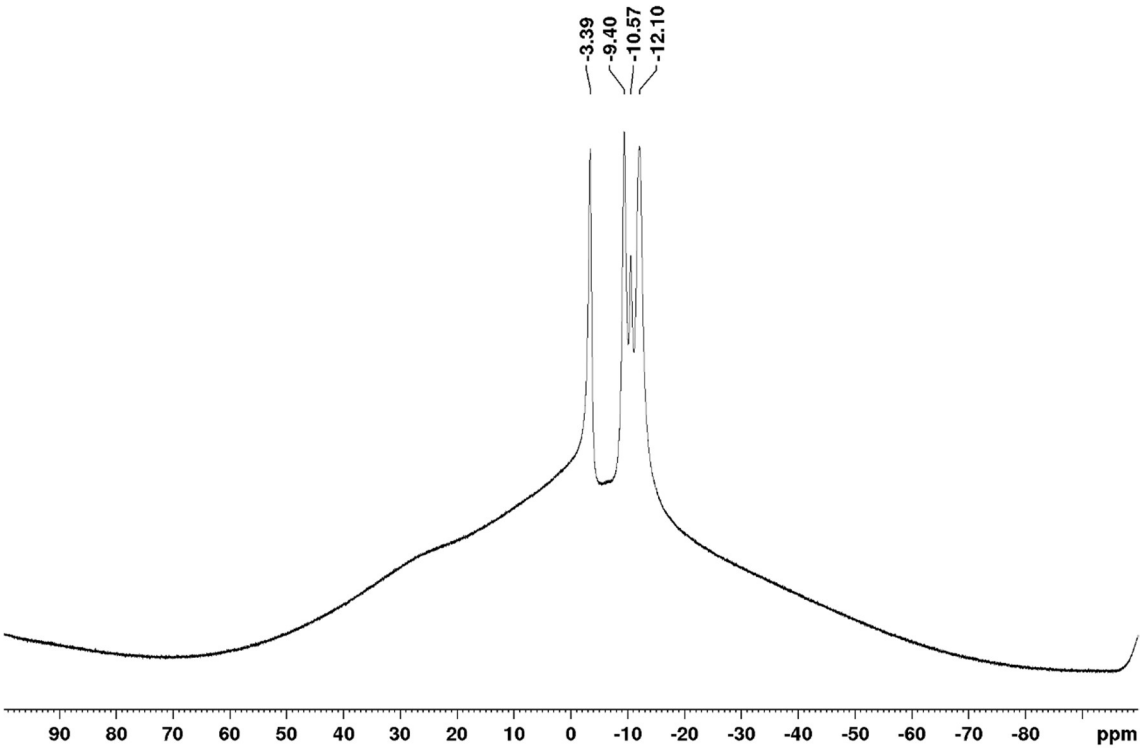
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)



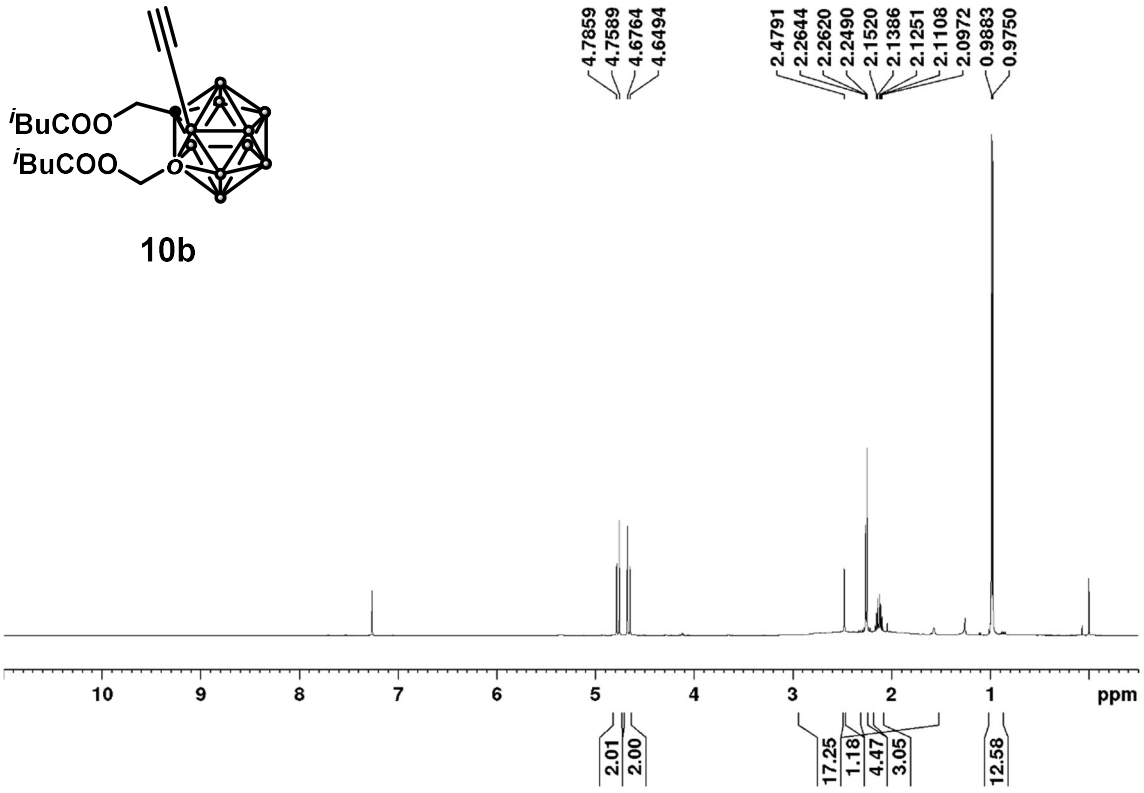
^{11}B NMR (160 MHz; $CDCl_3$)



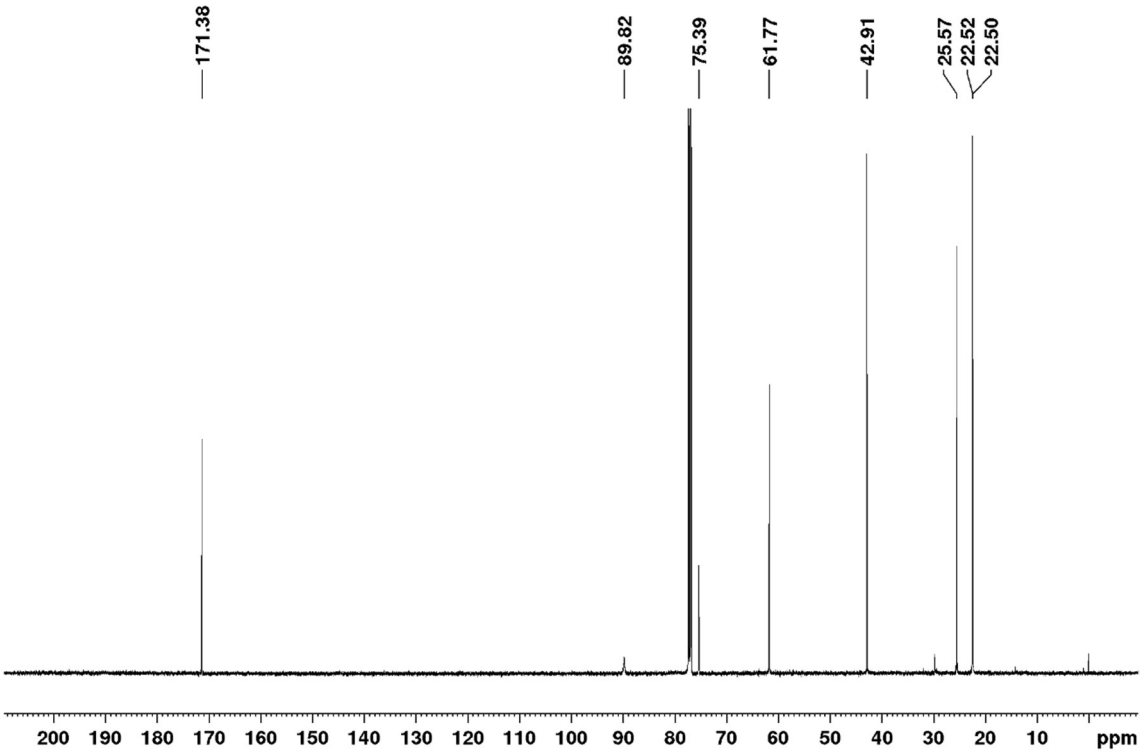
1,2-bis((3-methylbutanoyl)oxymethyl)-3-ethynyl-1,2-dicarba-*closo*-dodecaborane (10b)

^{1}H NMR (500 MHz; $CDCl_3$)

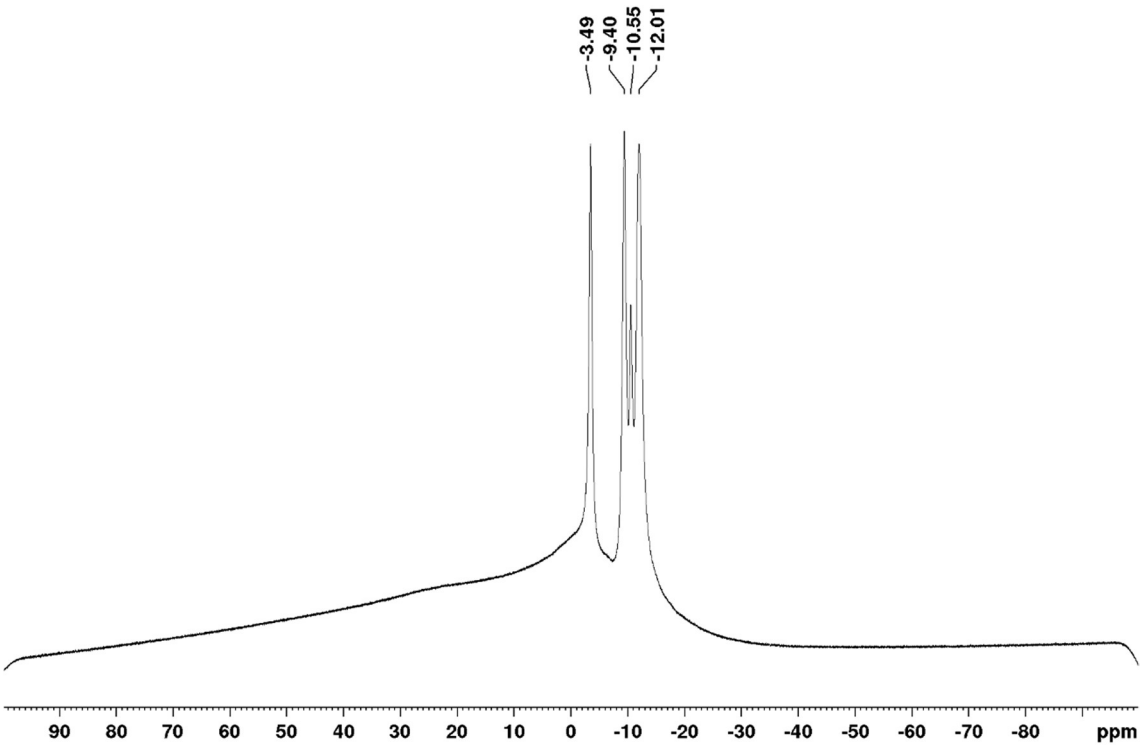
10b



^{13}C NMR (125 MHz; $CDCl_3$)

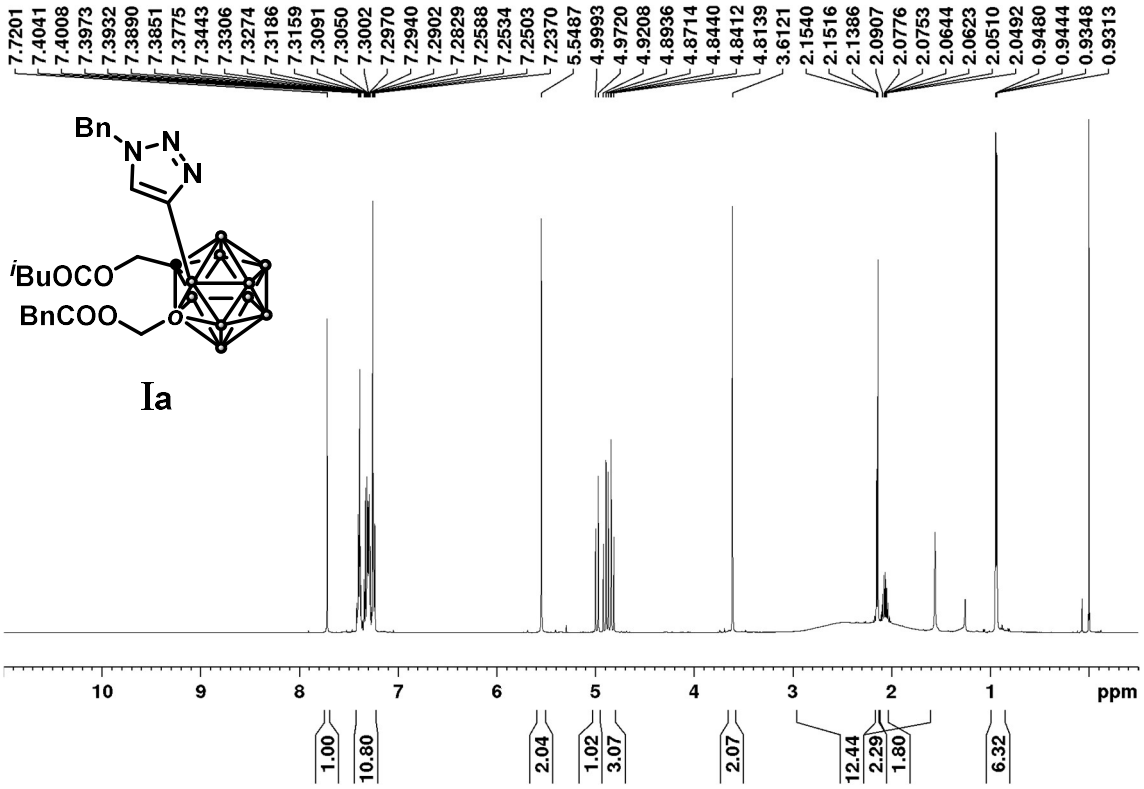


^{11}B NMR (160 MHz; $CDCl_3$)

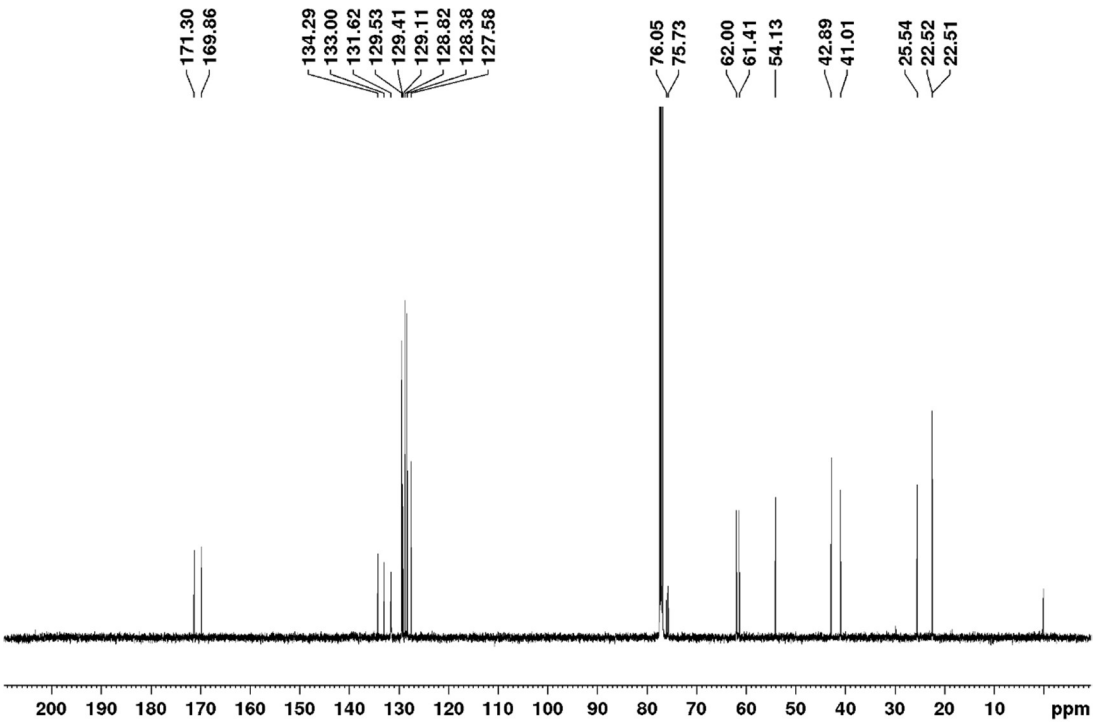


1-(3-methylbutanoyl)oxymethyl-2-phenylacetoxymethyl-3-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,2-dicarba-*closo*-dodecaborane (Ia)

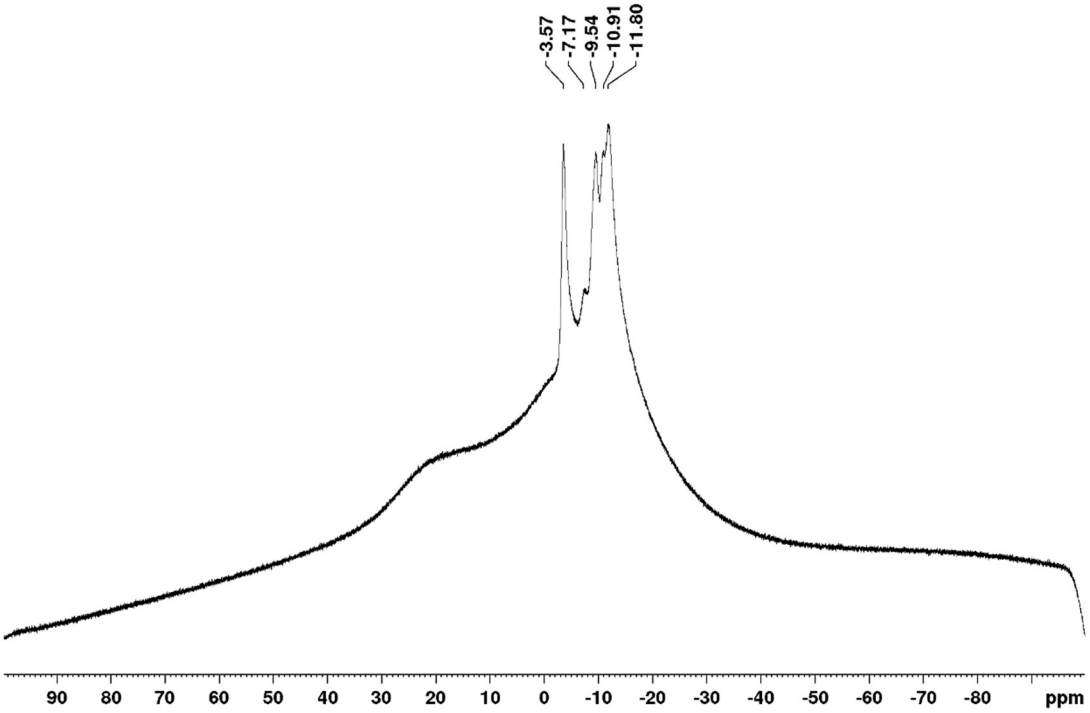
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

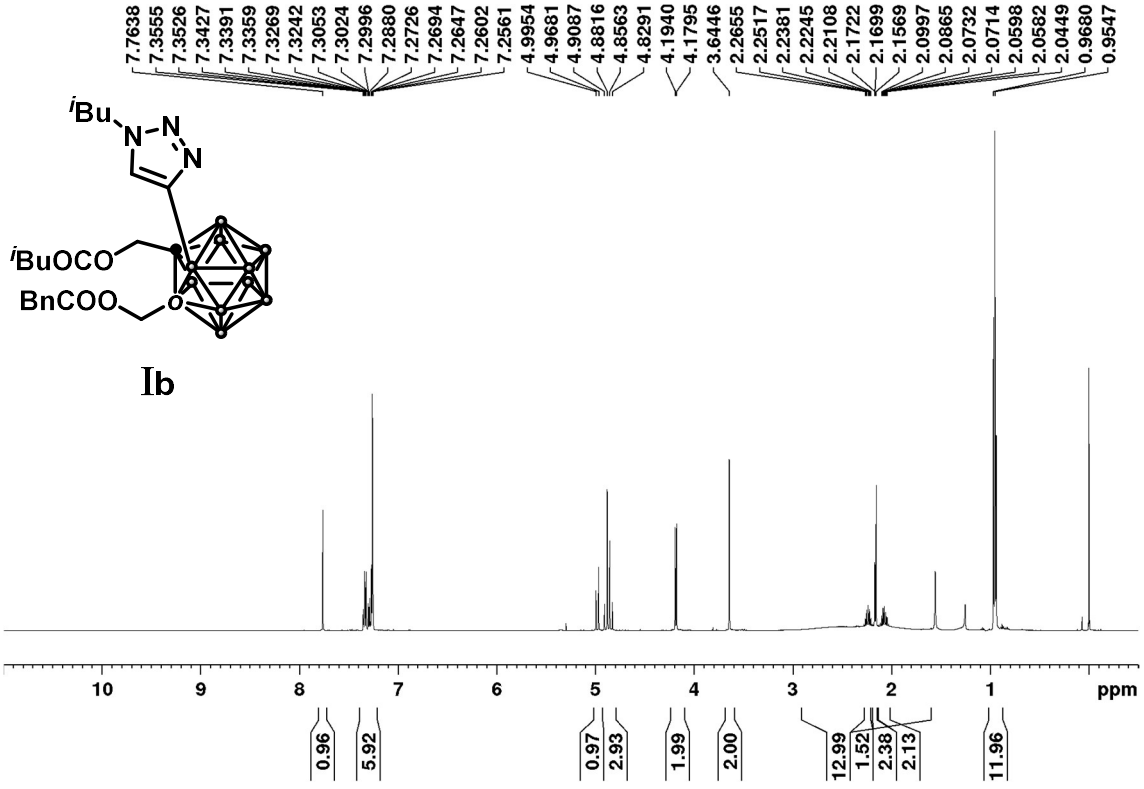


^{11}B NMR (160 MHz; $CDCl_3$)

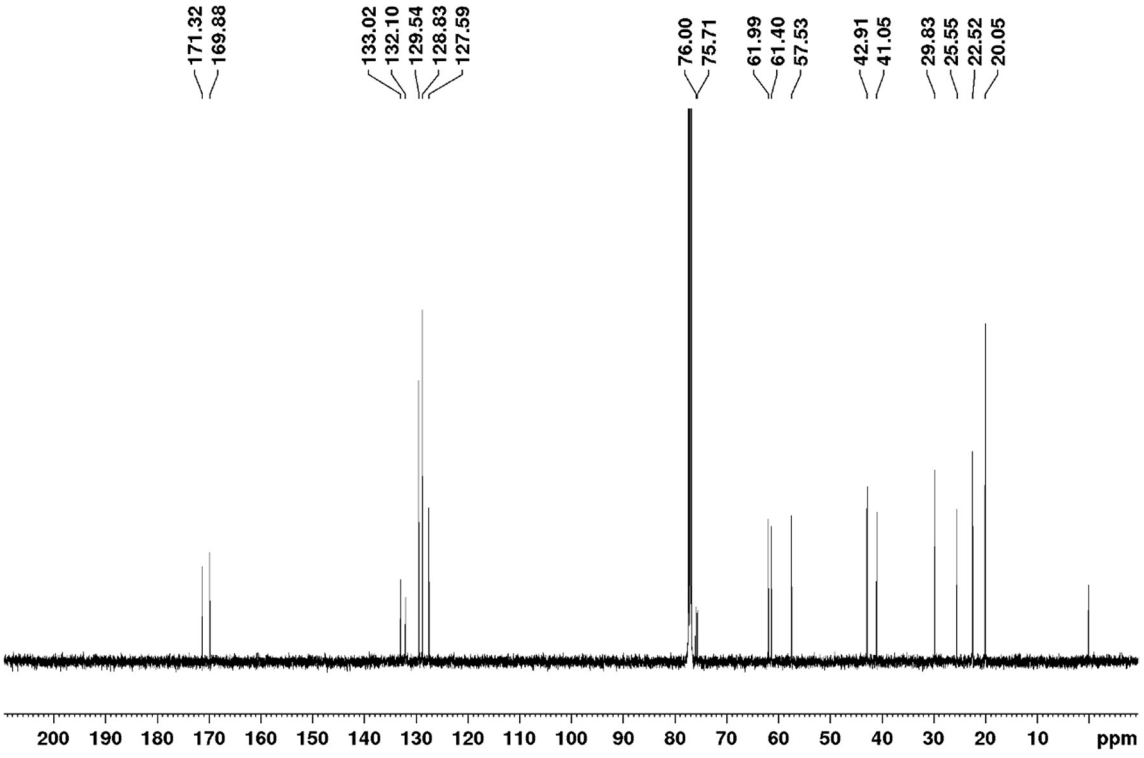


1-(3-methylbutanoyl)oxymethyl-2-phenylacetoxymethyl-3-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,2-dicarba-*closo*-dodecaborane (Ib)

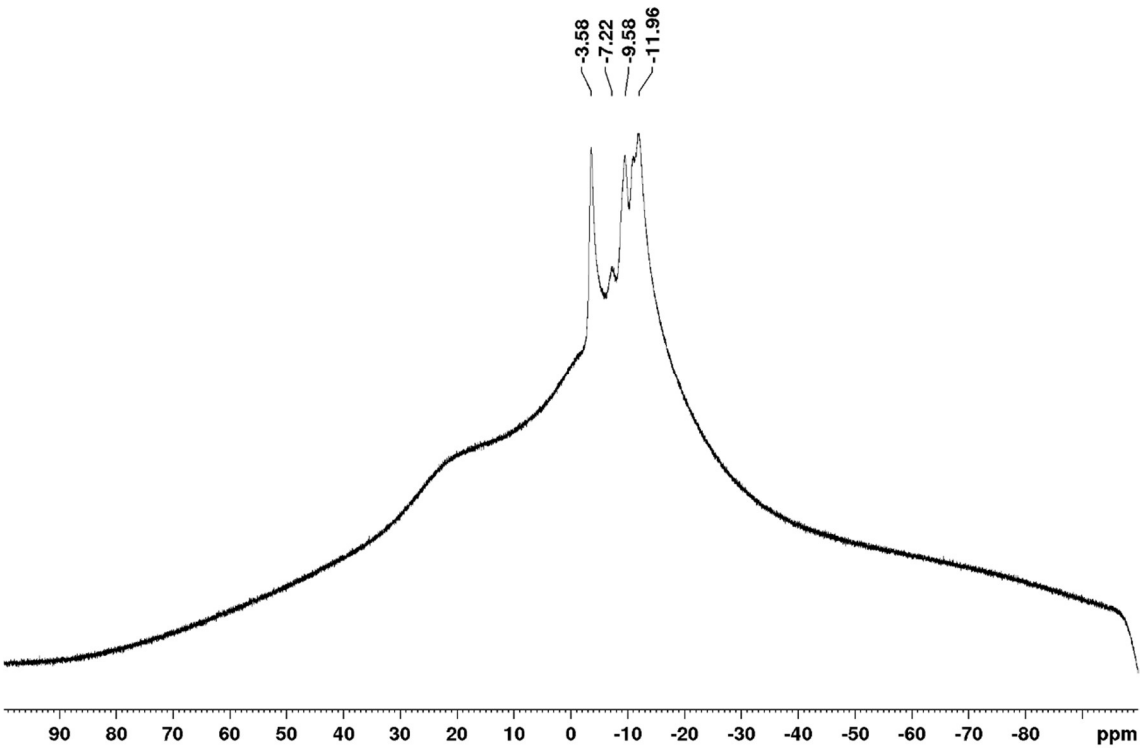
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

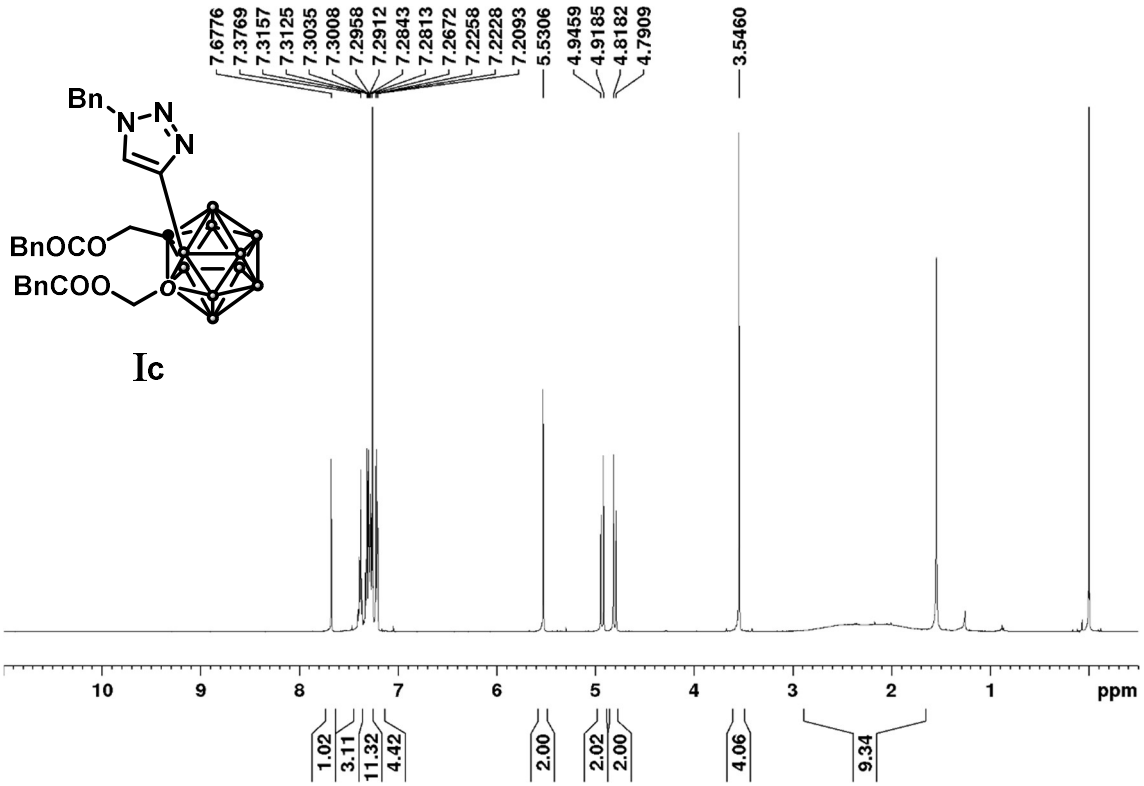


^{11}B NMR (160 MHz; $CDCl_3$)

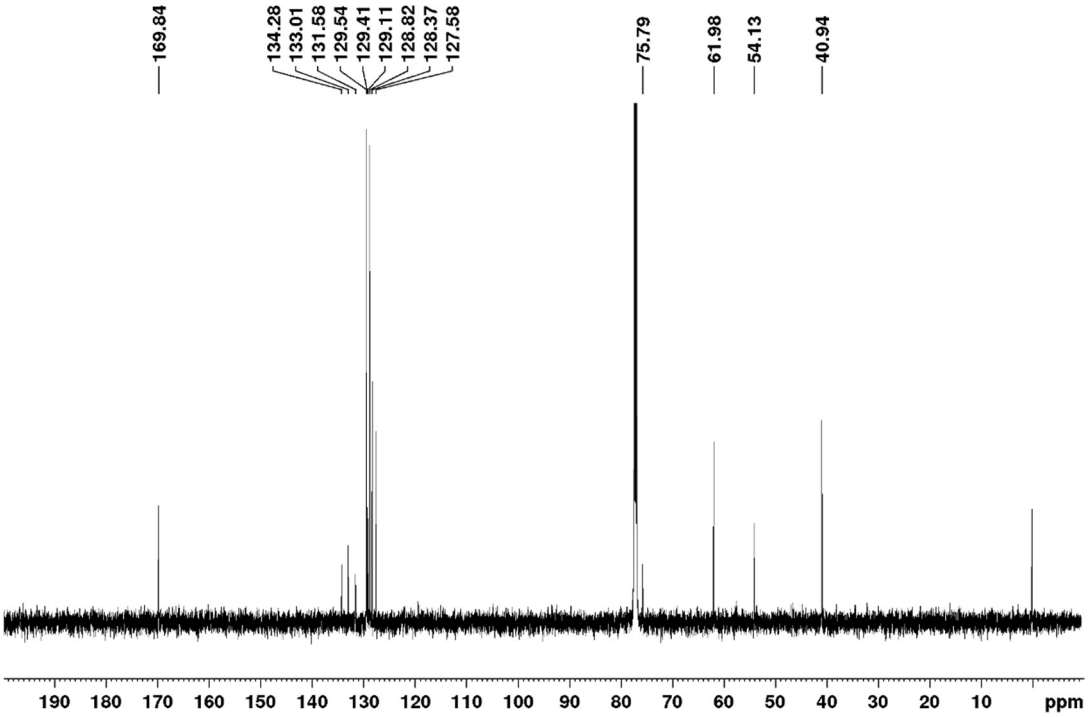


1,2-bis(phenylacetoxymethyl)-3-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,2-dicarba-*closo*-dodecaborane (Ic)

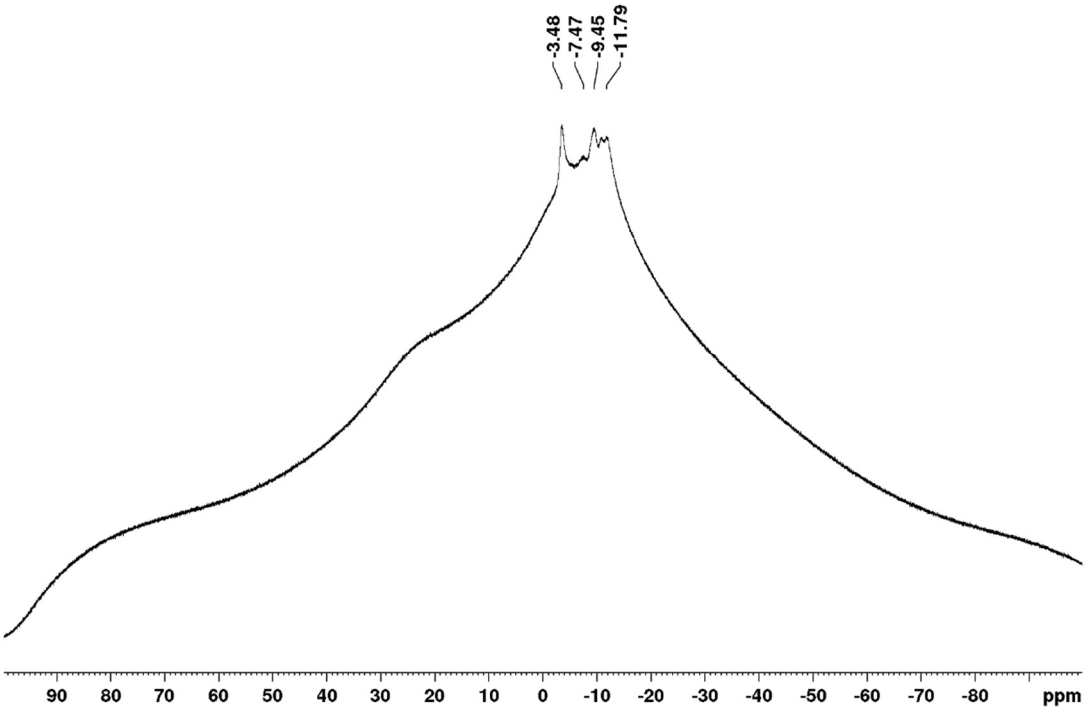
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

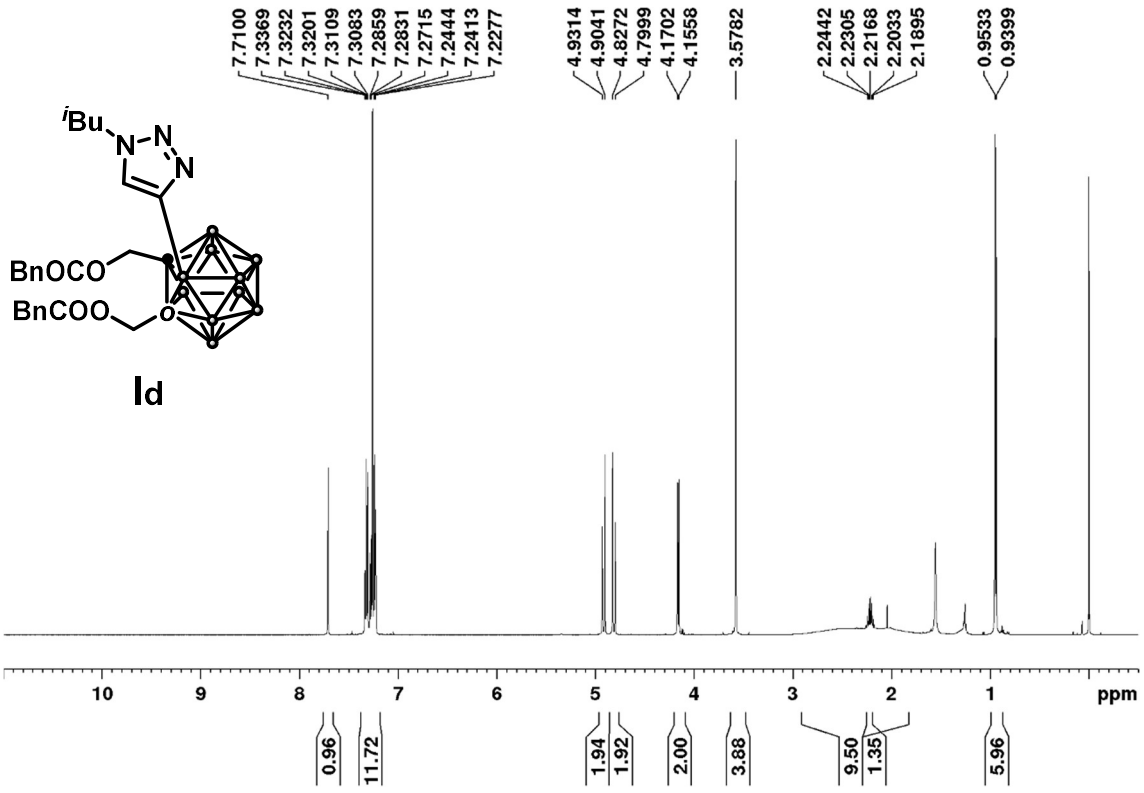


^{11}B NMR (160 MHz; $CDCl_3$)

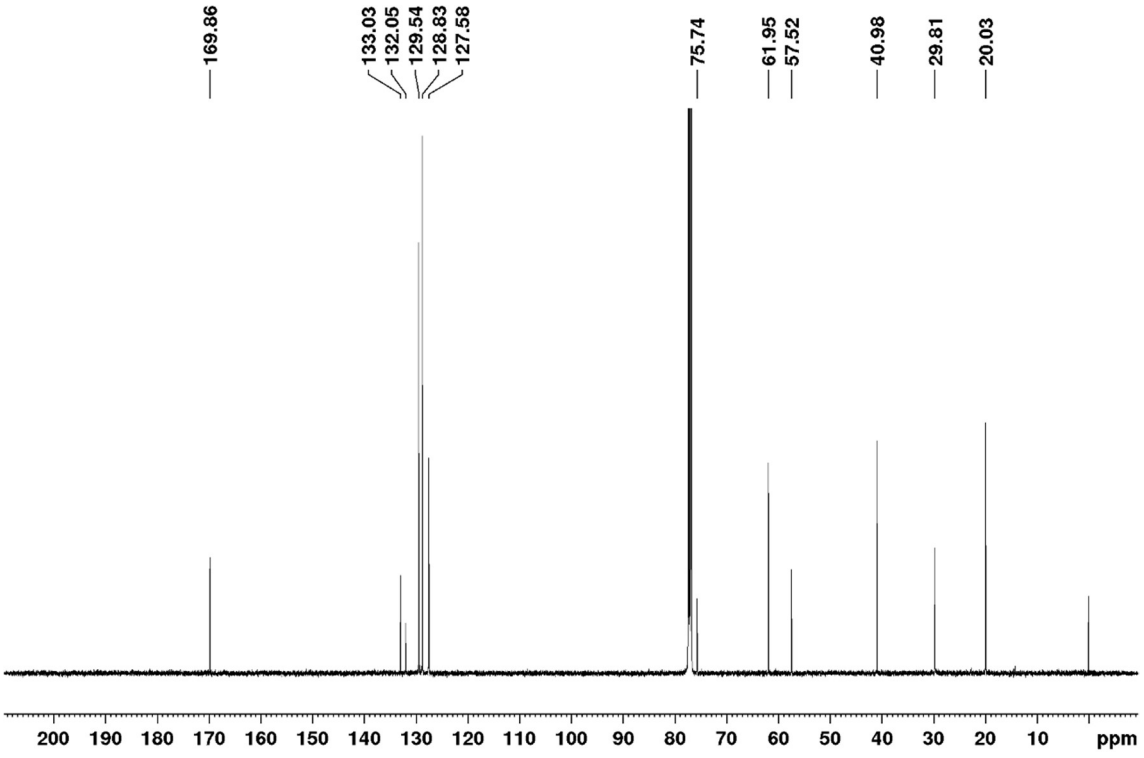


1,2-bis(phenylacetoxymethyl)-3-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,2-dicarba-*closo*-dodecaborane (Id)

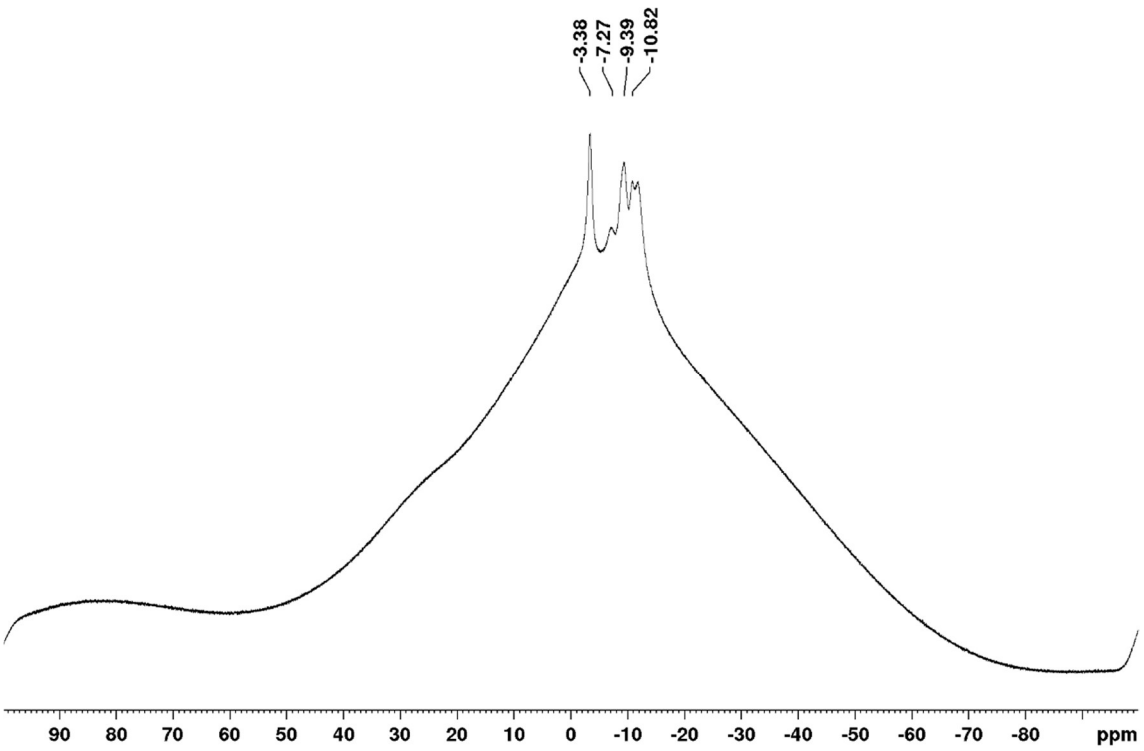
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

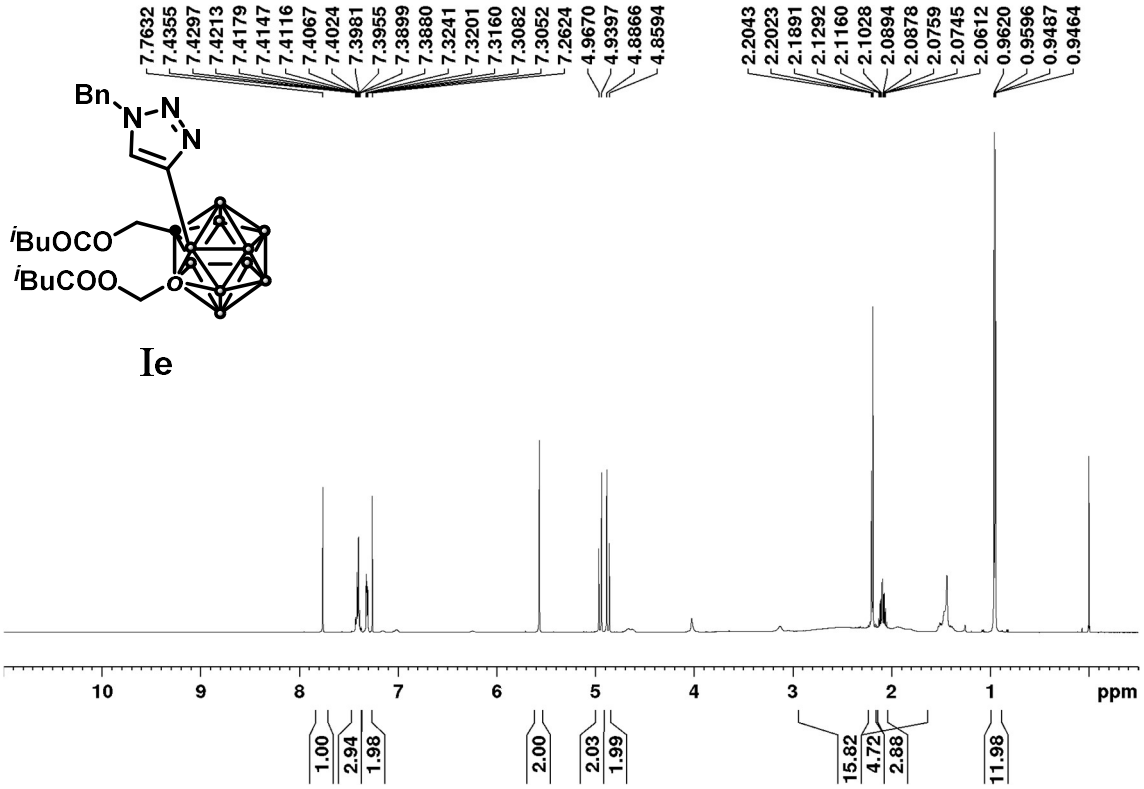


^{11}B NMR (160 MHz; $CDCl_3$)



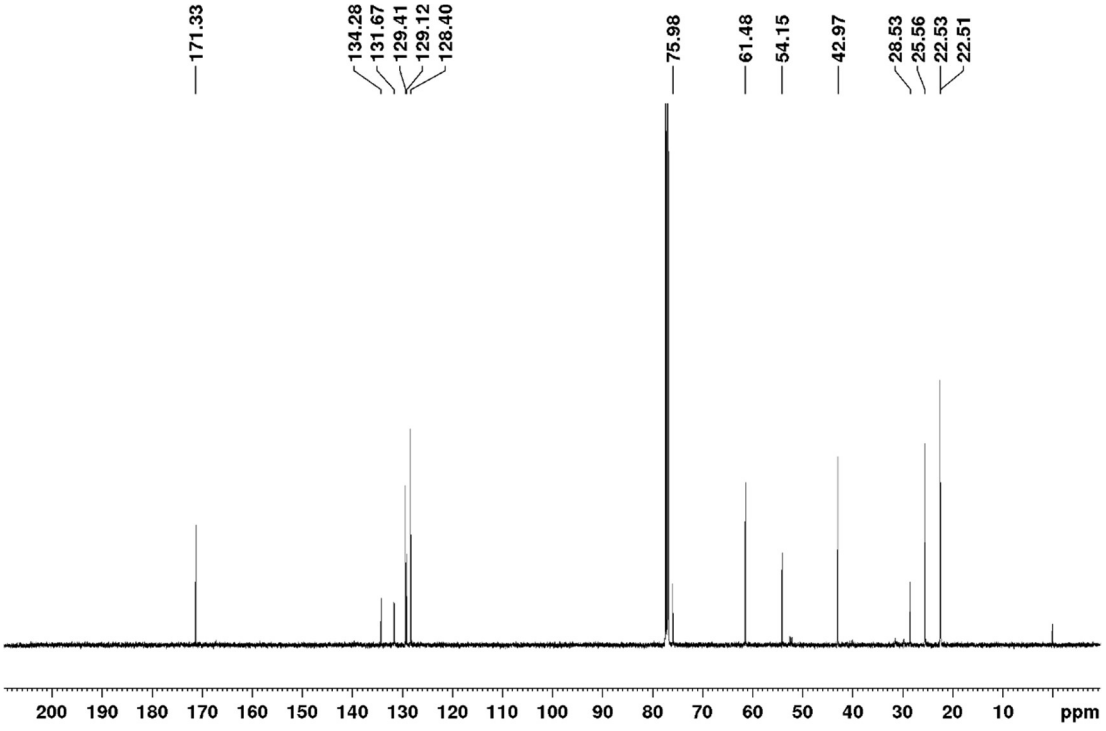
1,2-bis((3-methylbutanoyl)oxymethyl)-3-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,2-dicarba-*closo*-dodecaborane (Ie)

^{1}H NMR (500 MHz; $CDCl_3$)

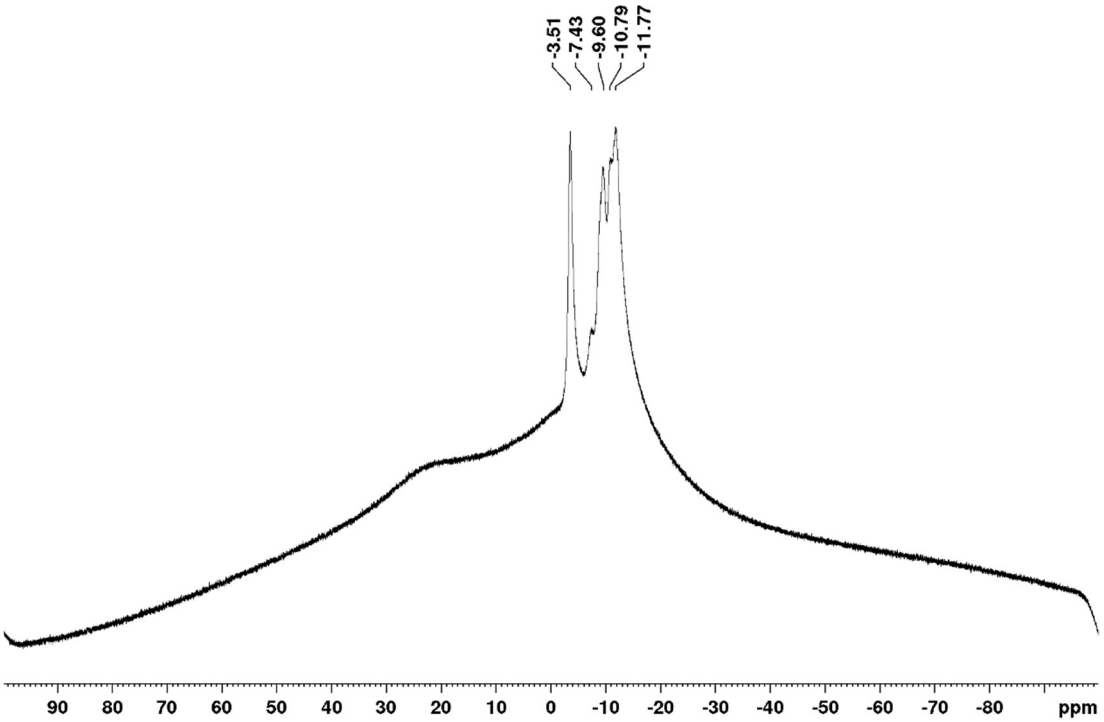


Ie

^{13}C NMR (125 MHz; $CDCl_3$)

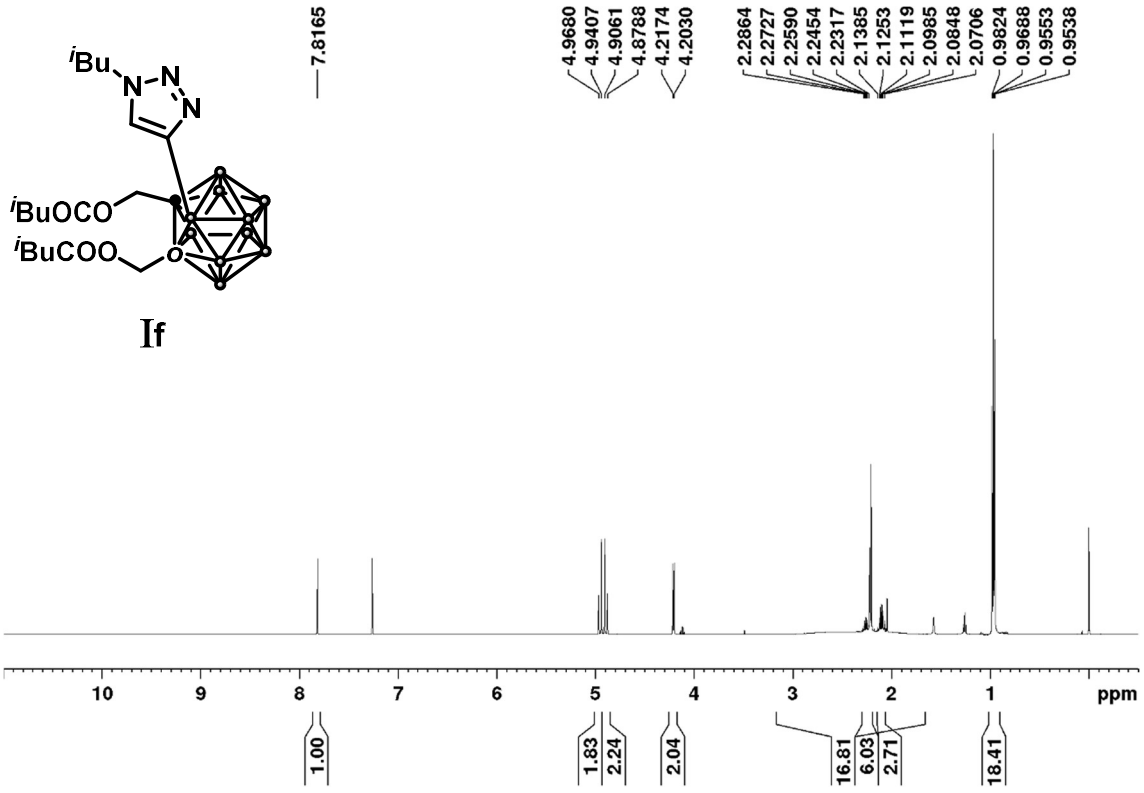


^{11}B NMR (160 MHz; $CDCl_3$)



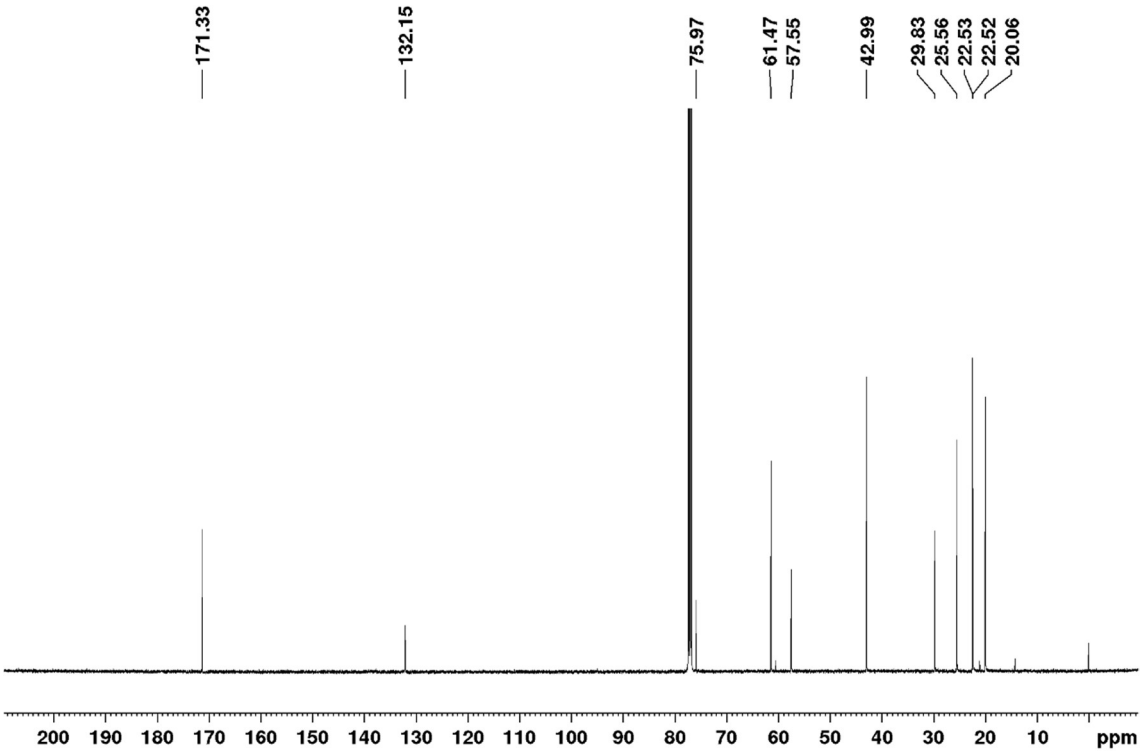
1,2-bis((3-methylbutanoyl)oxymethyl)-3-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,2-dicarba-*closo*-dodecaborane (If)

^{1}H NMR (500 MHz; $CDCl_3$)

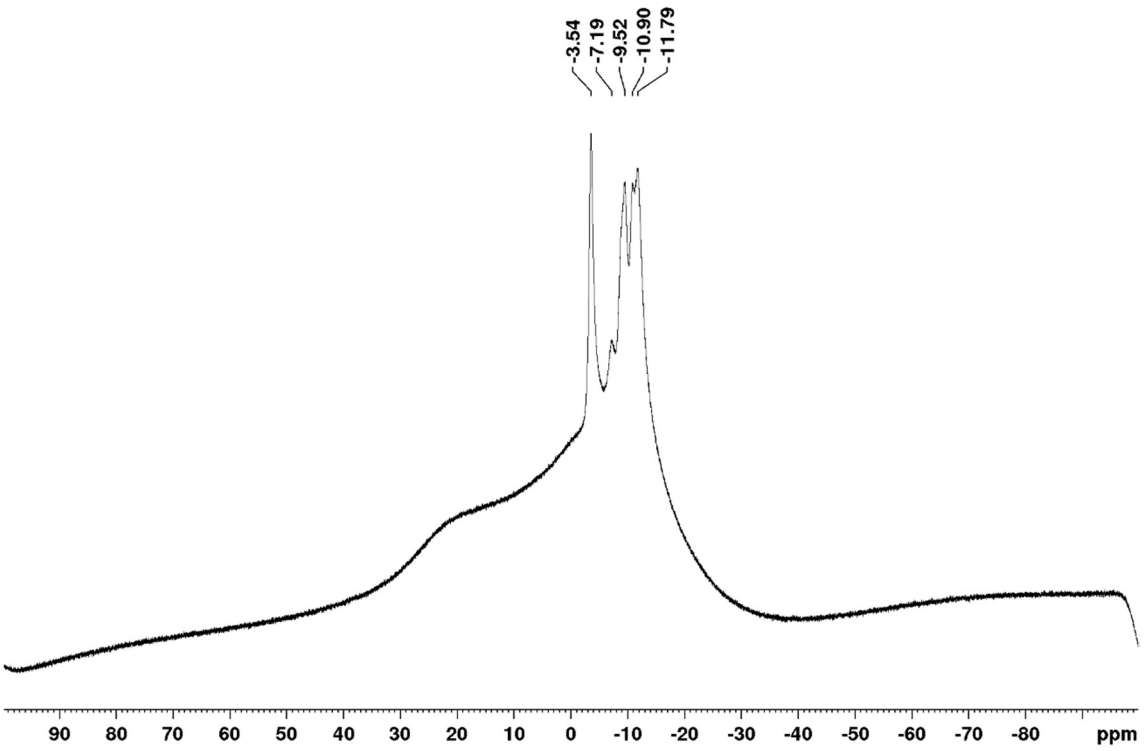


If

^{13}C NMR (125 MHz; $CDCl_3$)

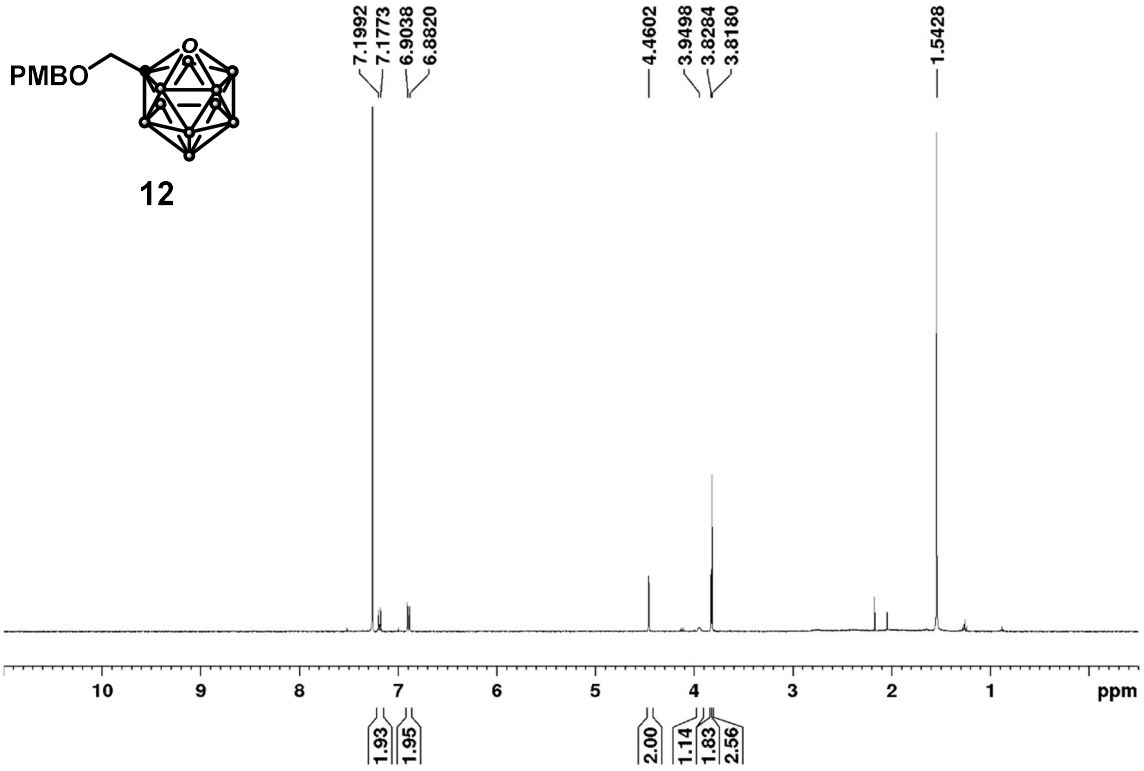


^{11}B NMR (160 MHz; $CDCl_3$)

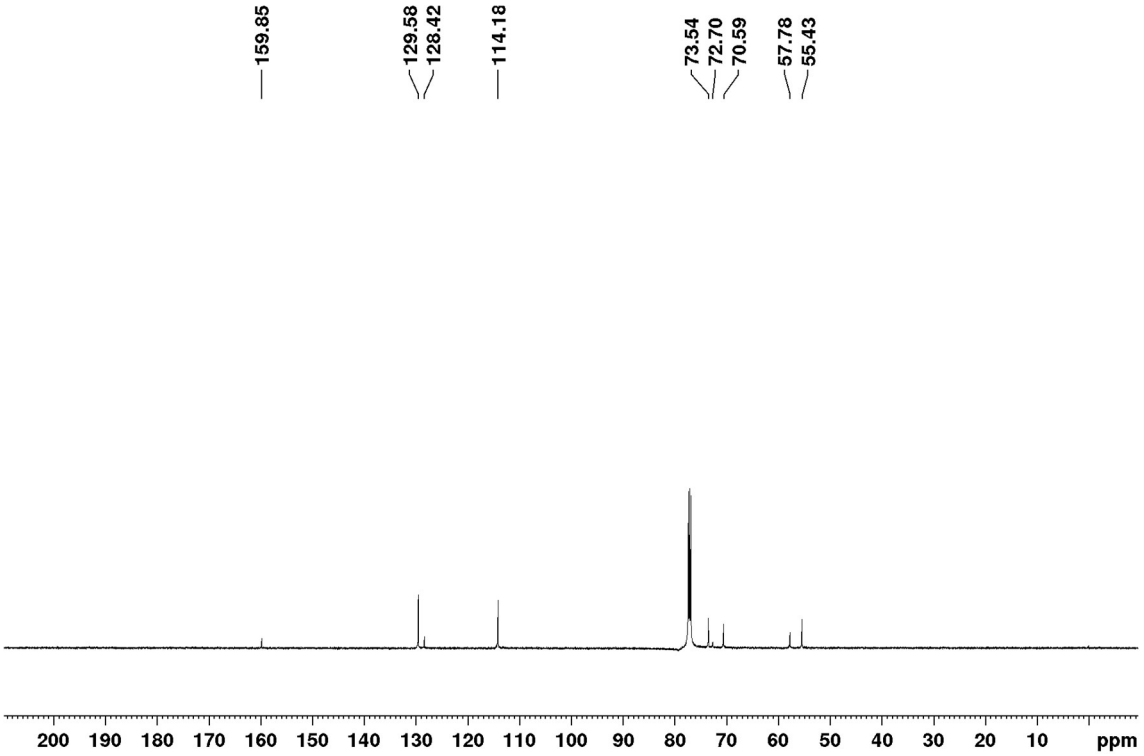


1-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (12)

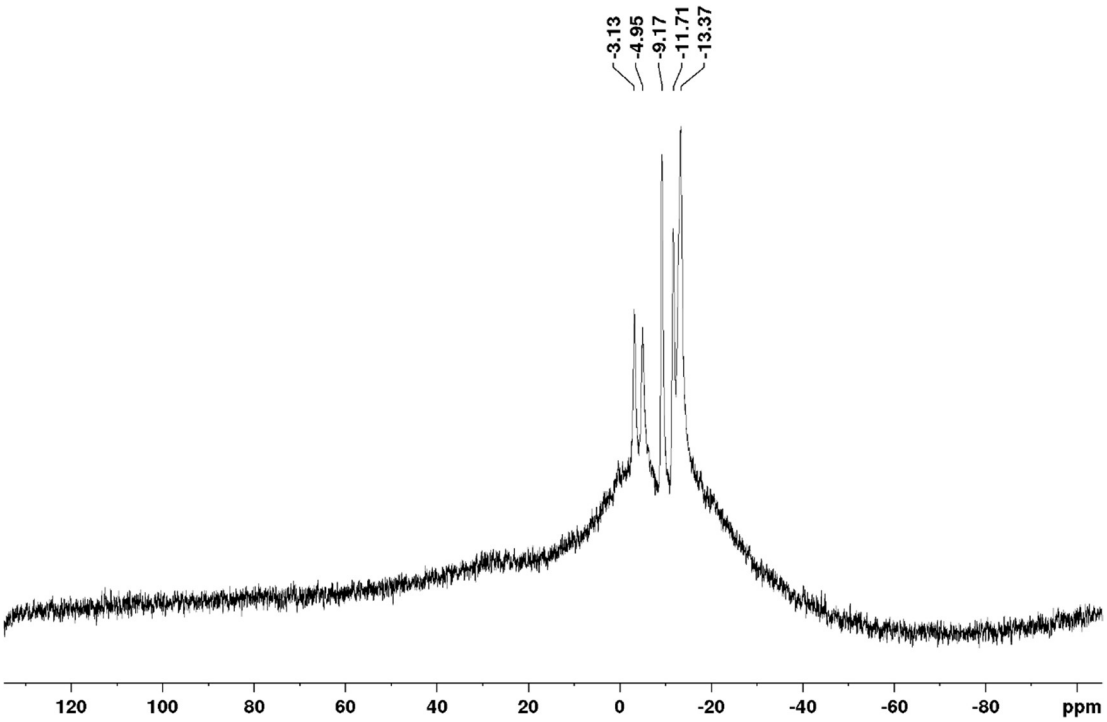
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

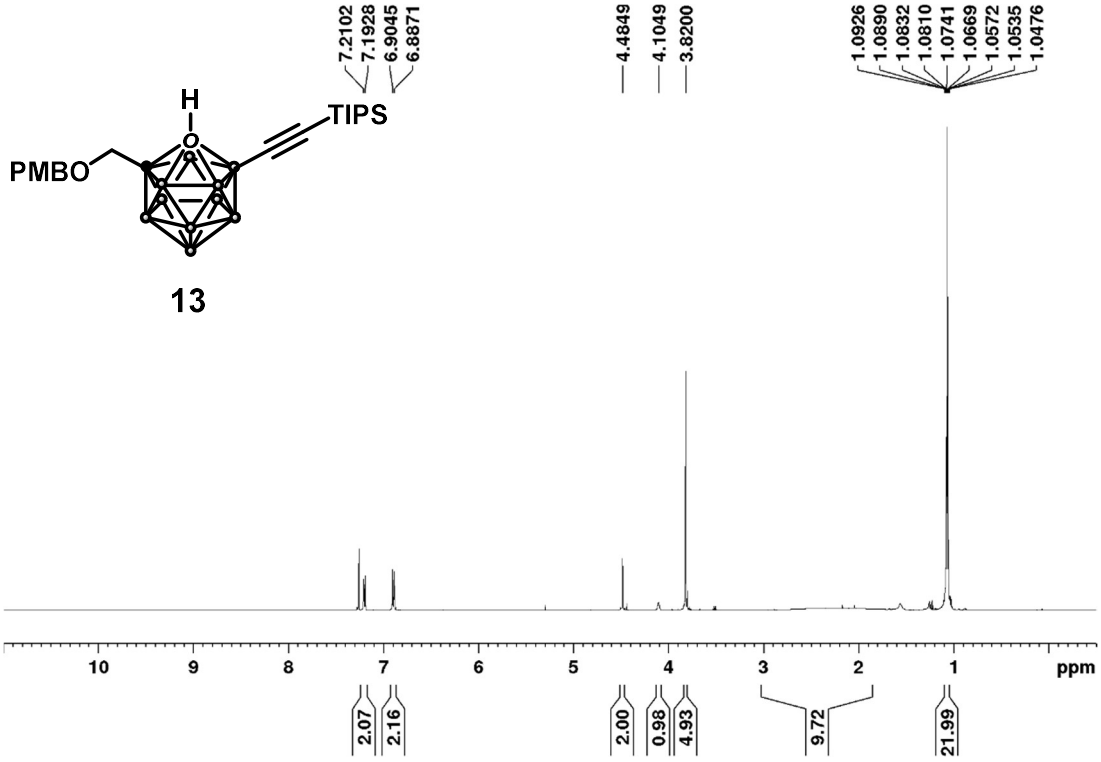


^{11}B NMR (160 MHz; $CDCl_3$)

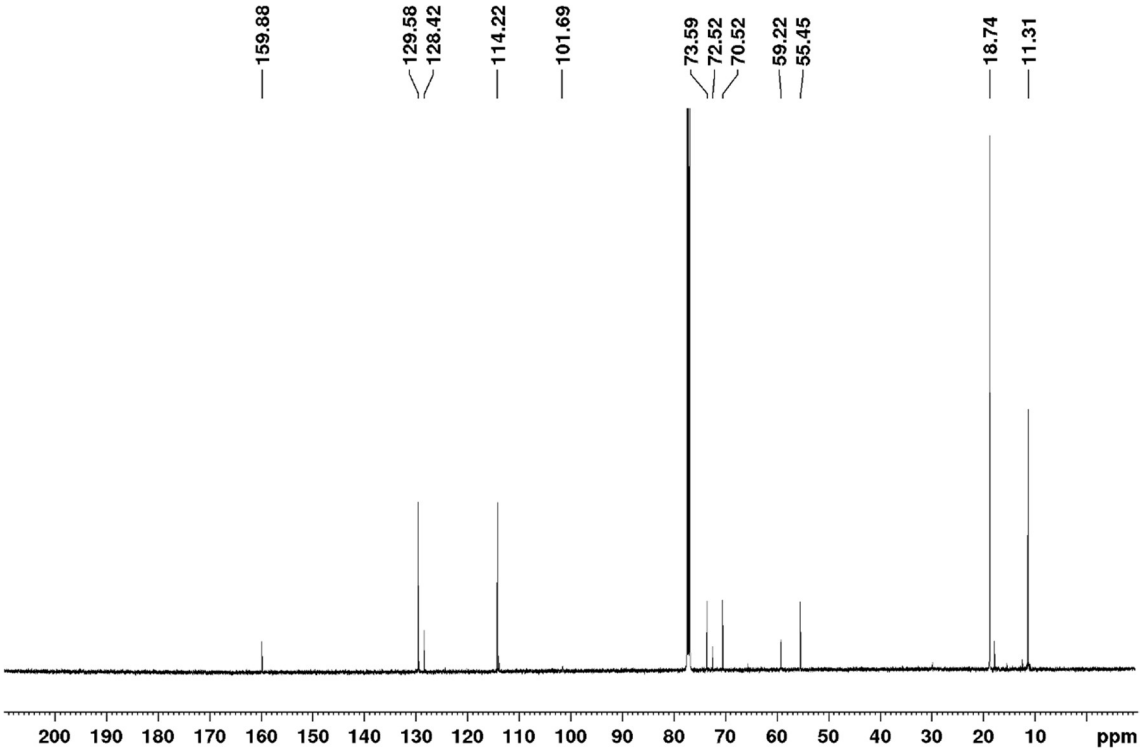


1-(3-(4-methoxybenzyl)hydroxymethyl)-4-((triisopropylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (13)

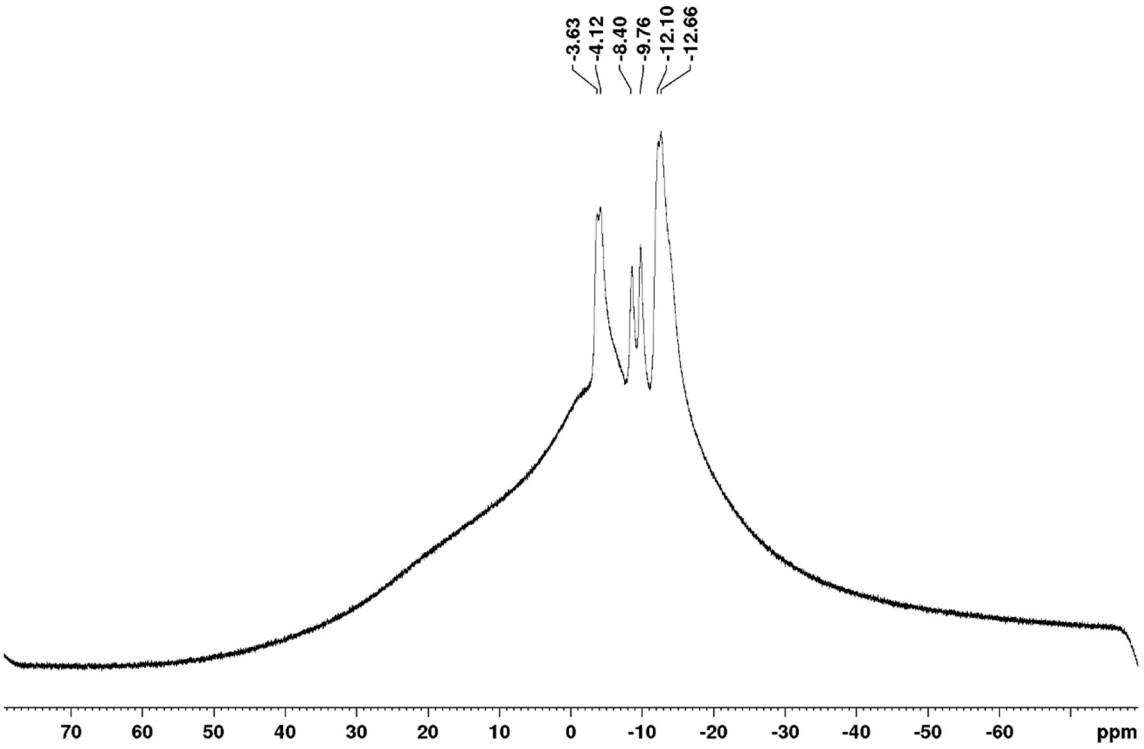
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

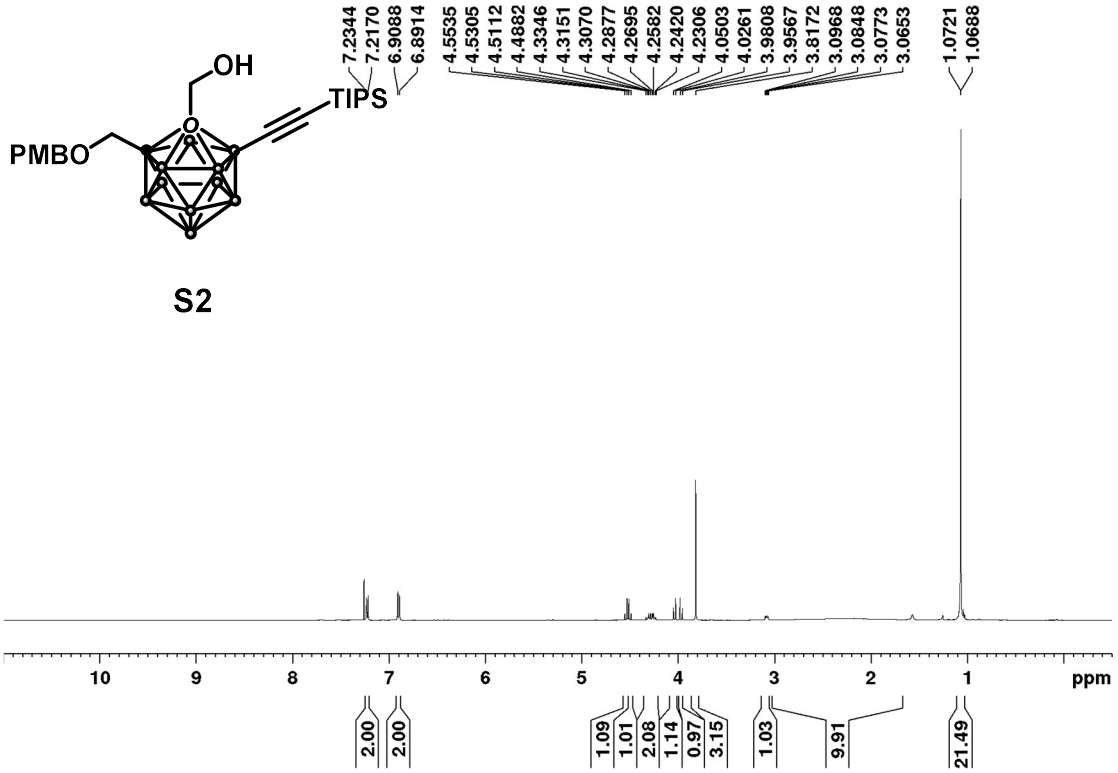


^{11}B NMR (160 MHz; $CDCl_3$)

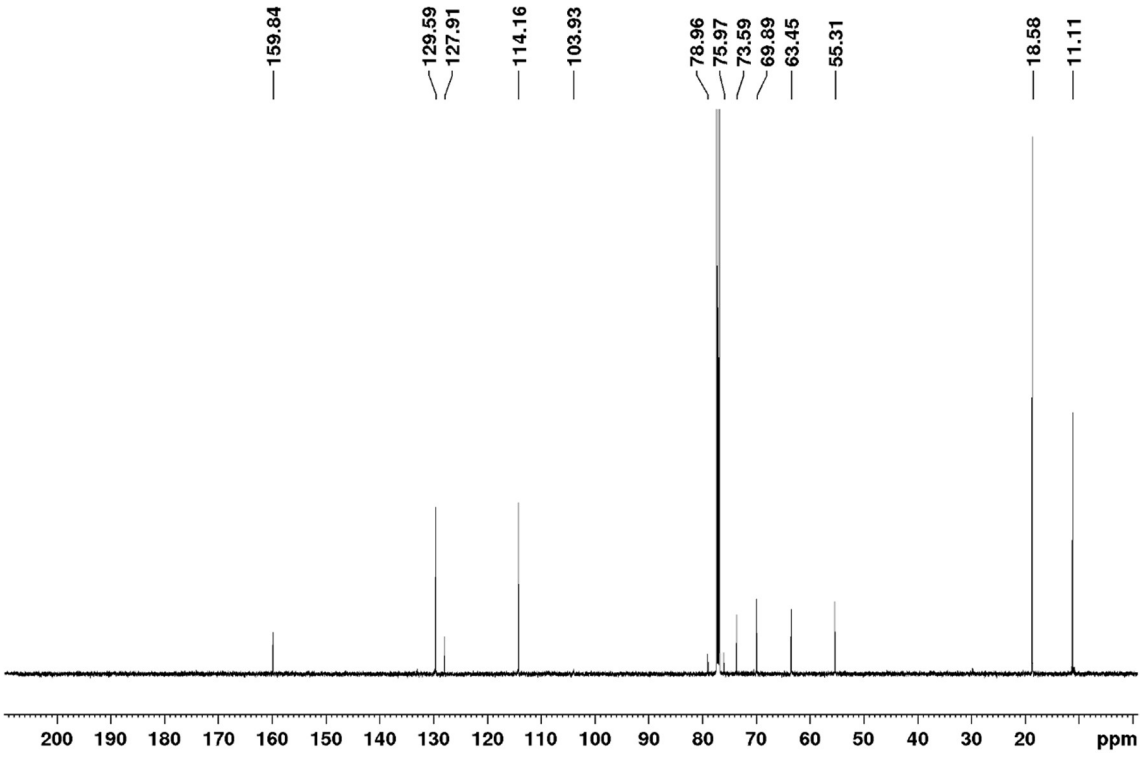


1-(3-(4-methoxybenzyl)hydroxymethyl)-2-(hydroxymethyl)-4-((triisopropylsilyl)ethynyl)-1,2-dicarba-*closo*-dodecaborane (S2)

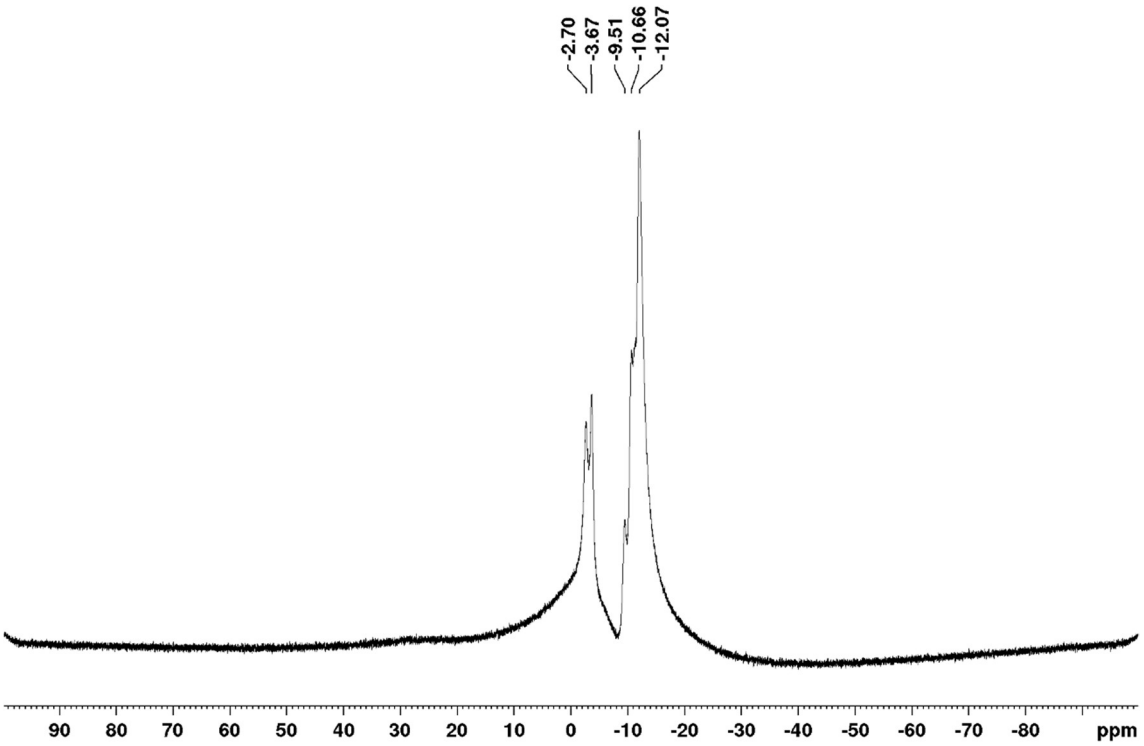
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

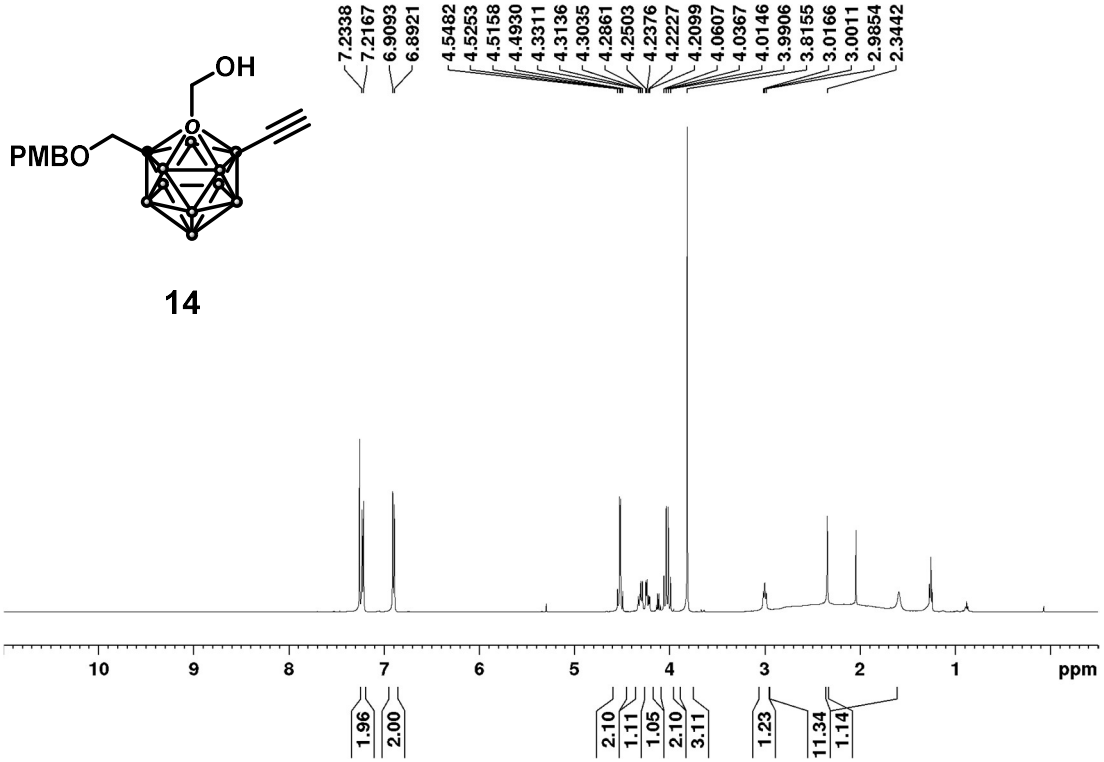


^{11}B NMR (160 MHz; $CDCl_3$)

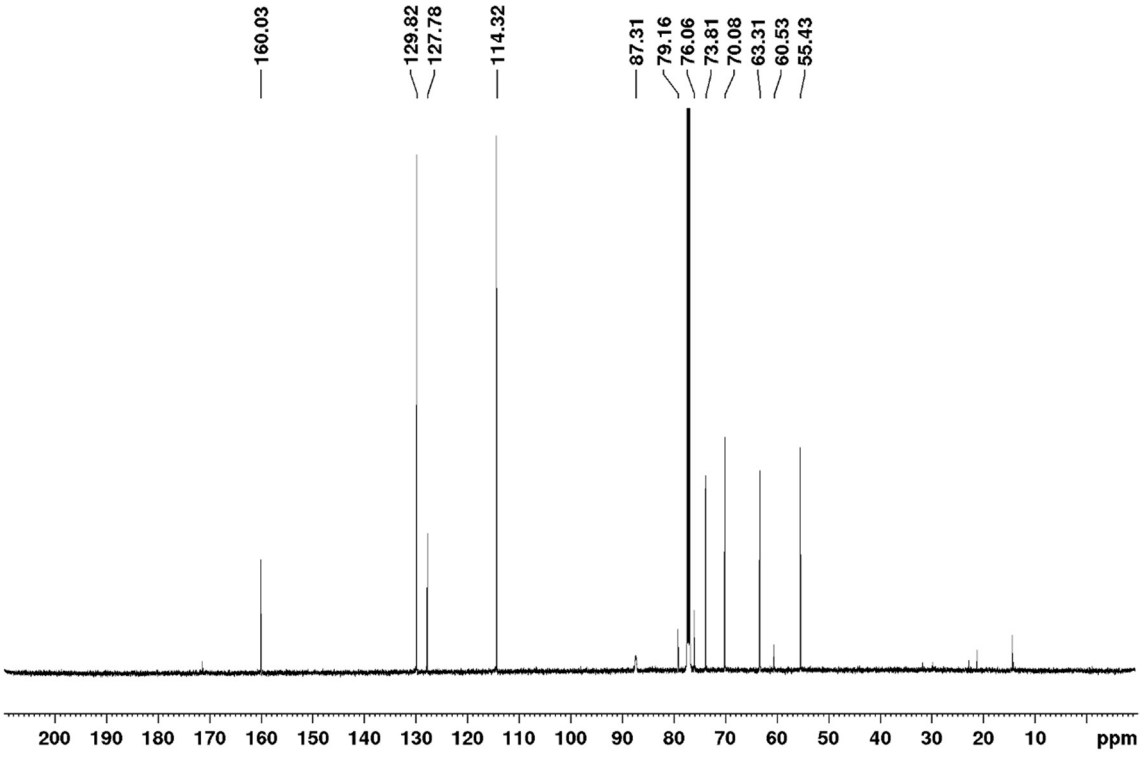


Synthesis of 1-(3-(4-methoxybenzyl)hydroxymethyl)-2-(hydroxymethyl)-4-ethynyl-1,2-dicarba-*closo*-dodecaborane (14)

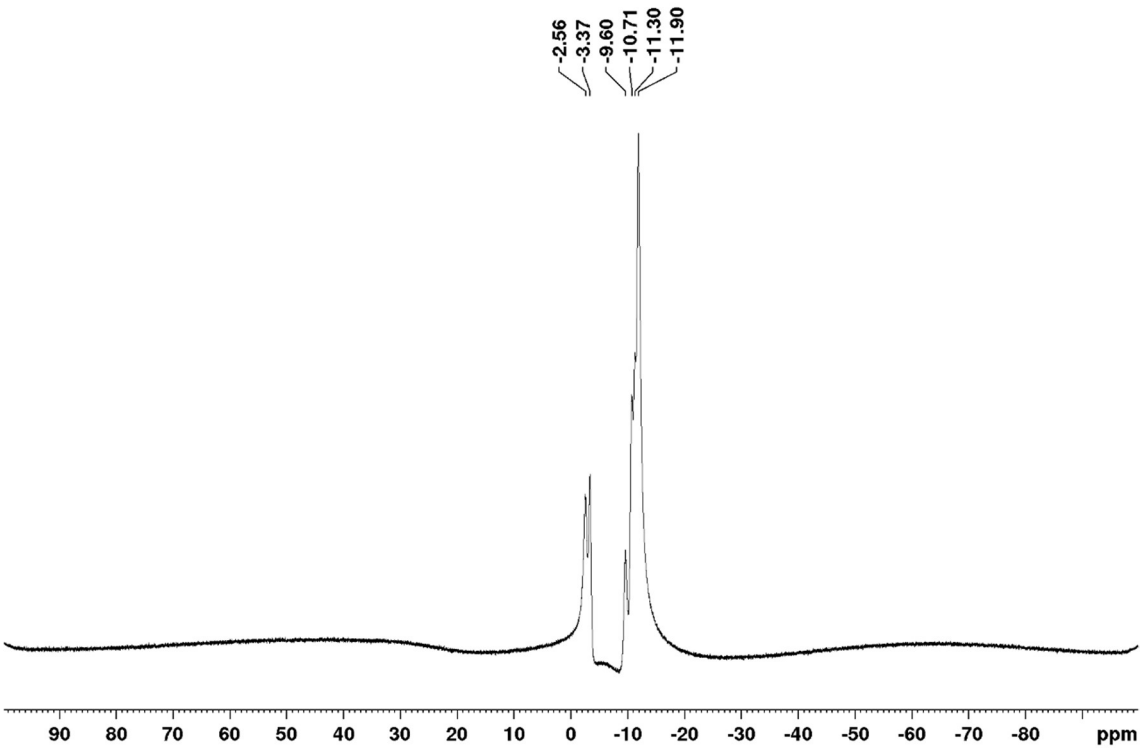
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

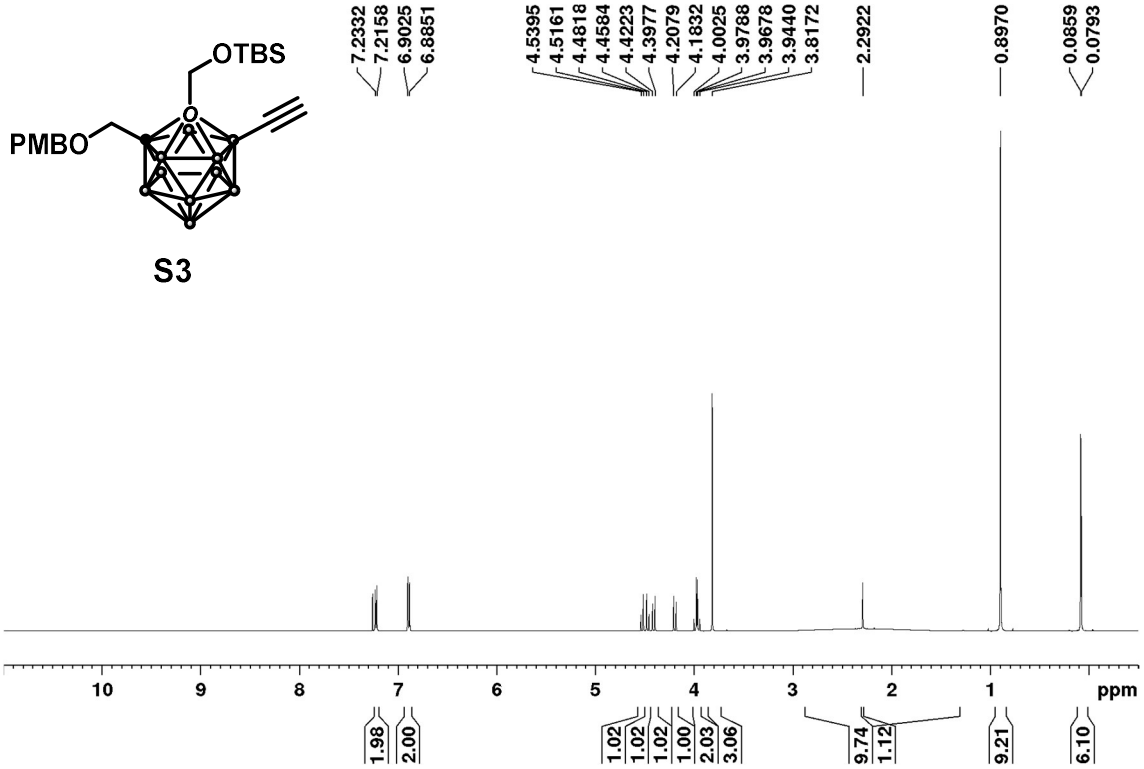


^{11}B NMR (160 MHz; $CDCl_3$)

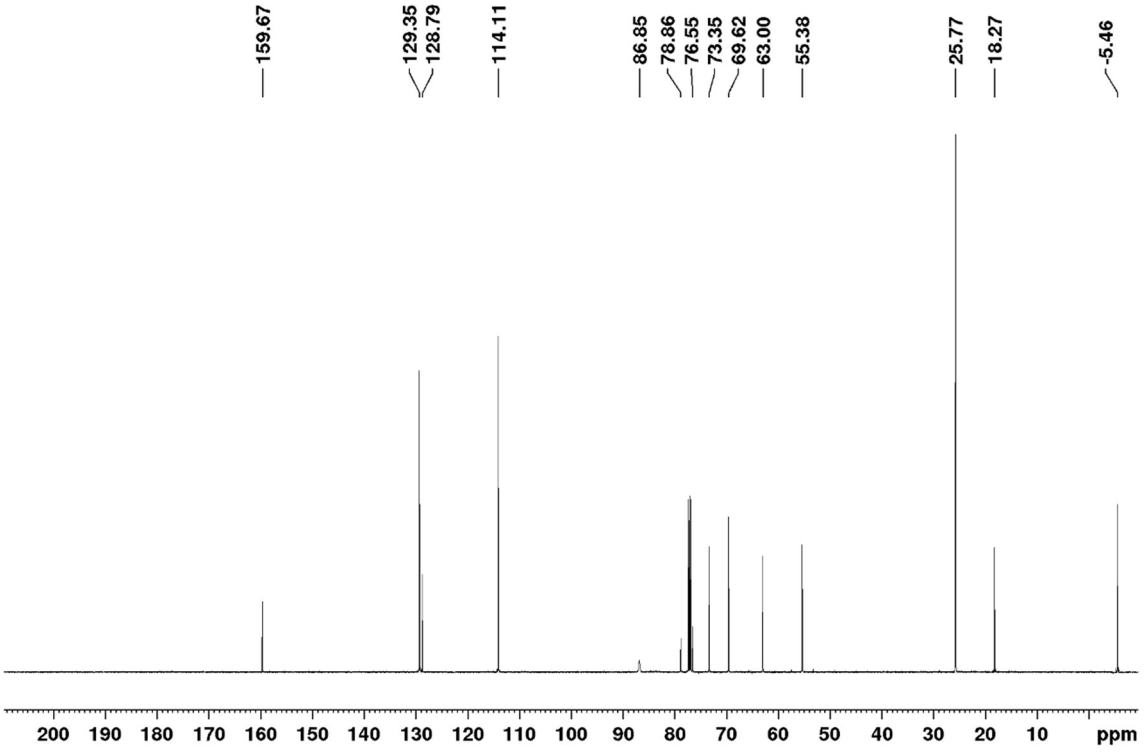


1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-ethynyl-1,2-dicarba-*closo*-dodecaborane (S3)

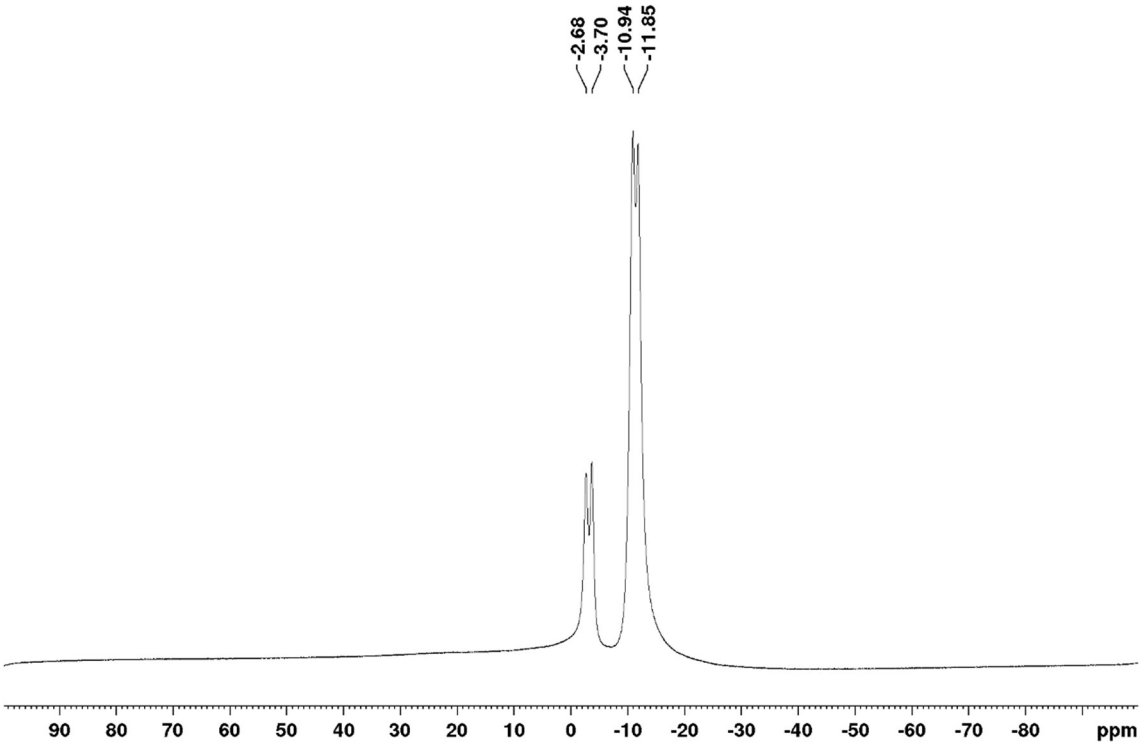
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

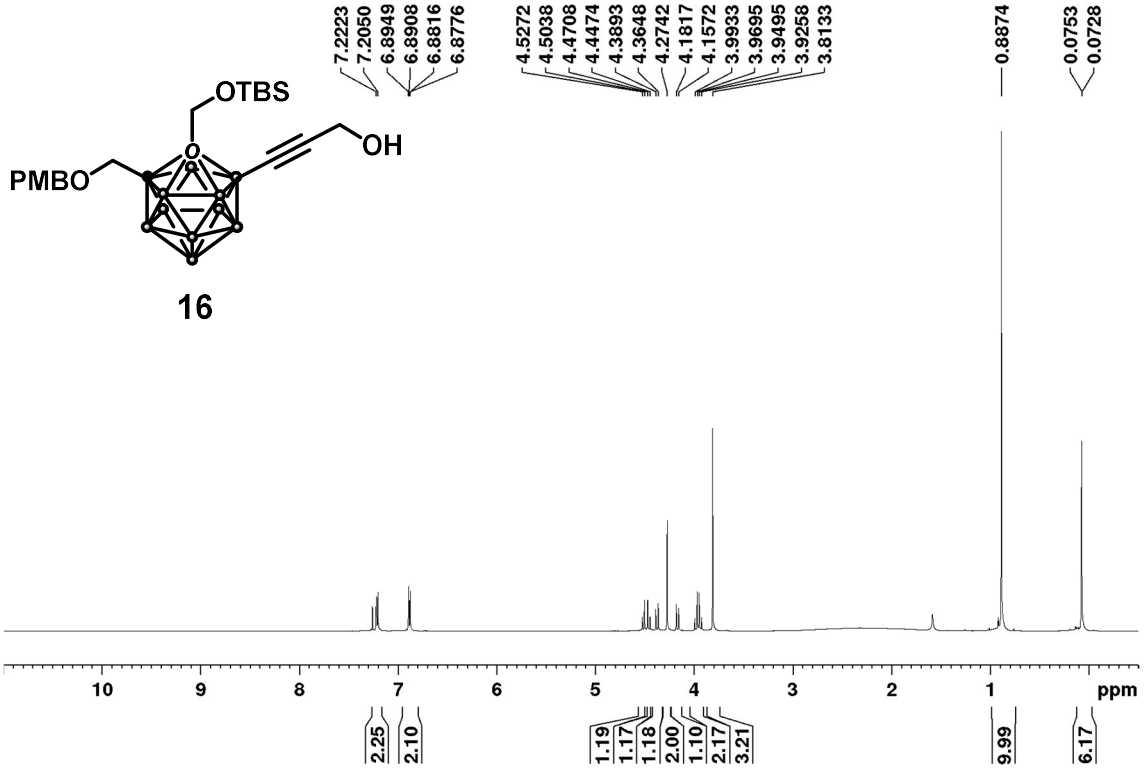


^{11}B NMR (160 MHz; $CDCl_3$)

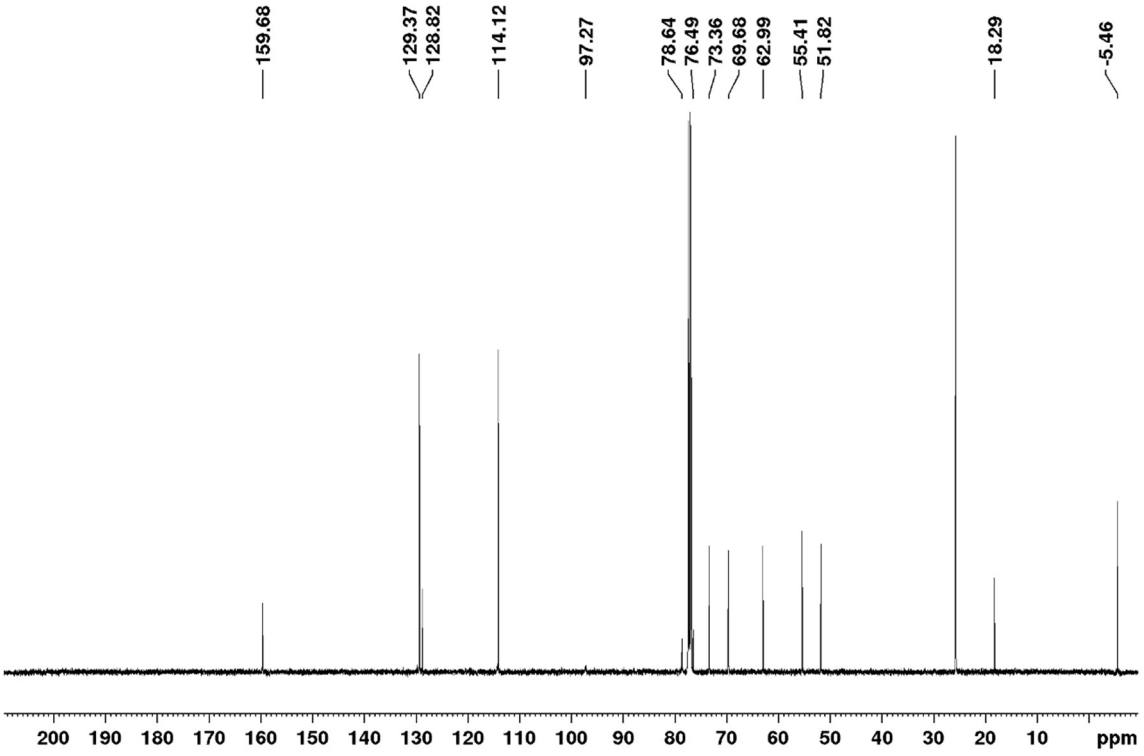


1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(3-oxoprop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (16)

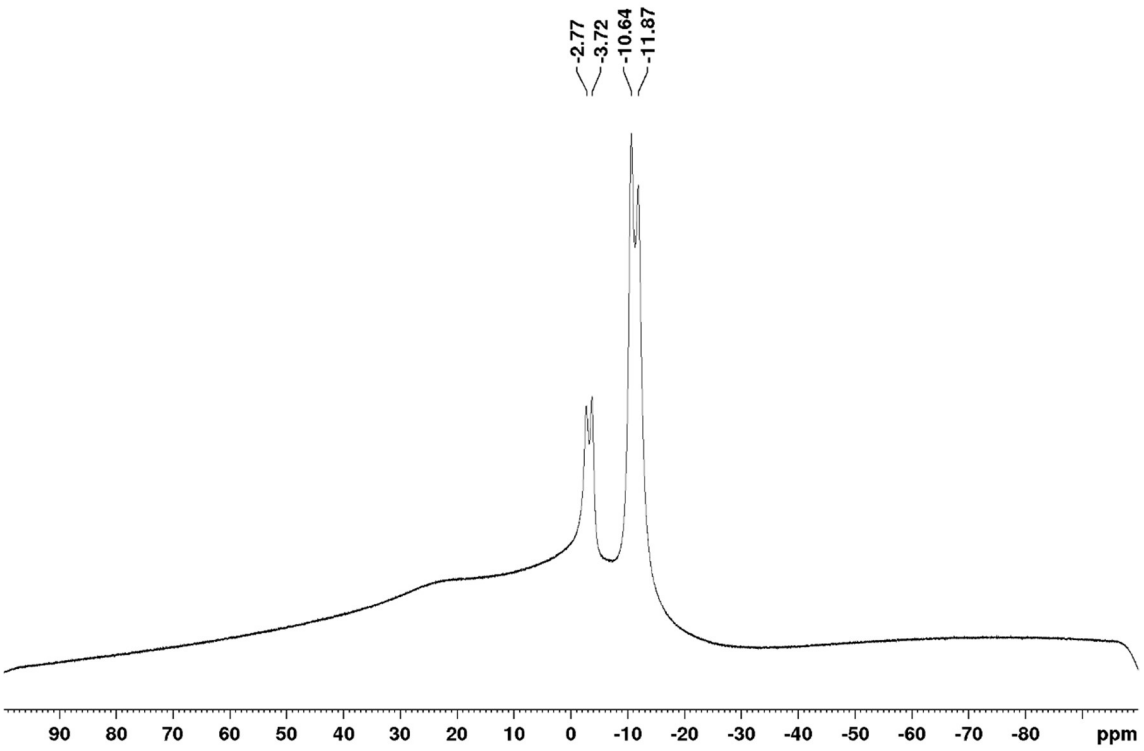
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

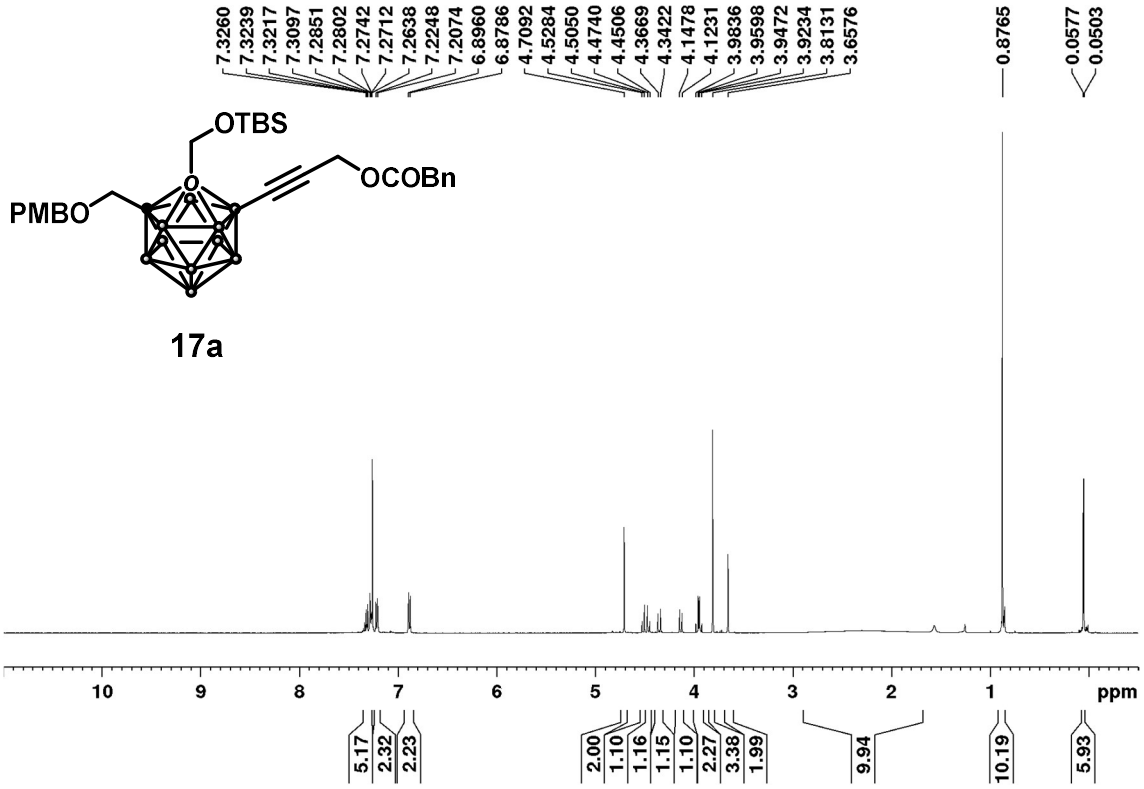


^{11}B NMR (160 MHz; $CDCl_3$)

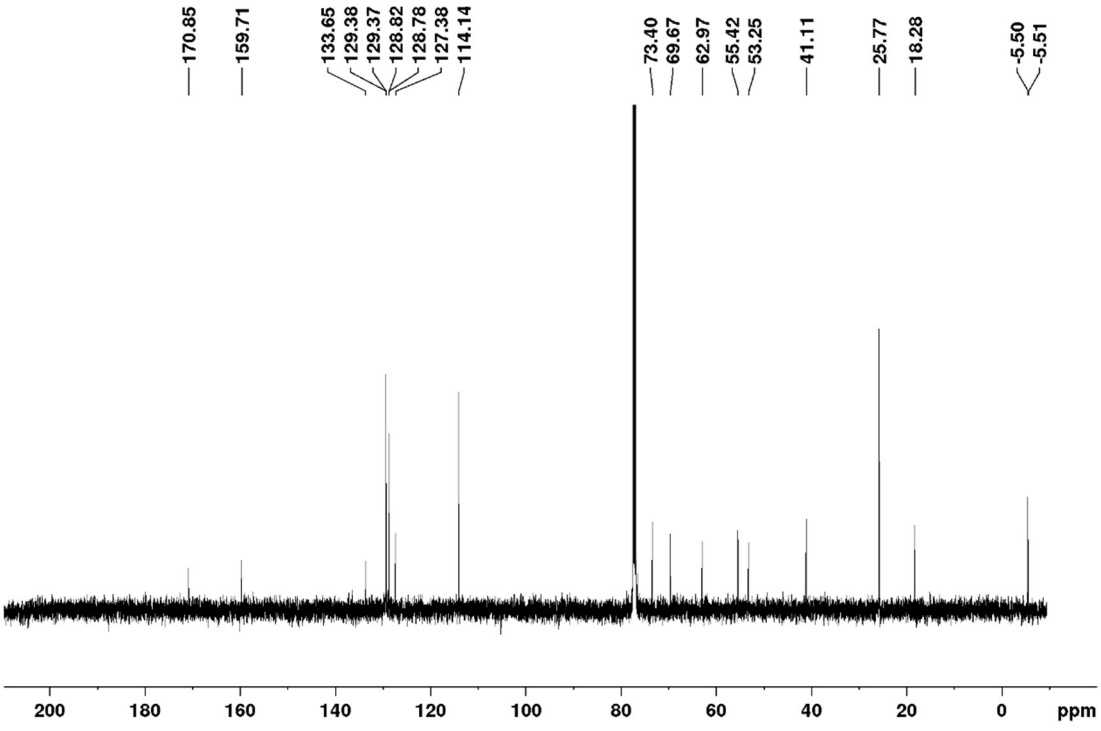


1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (17a)

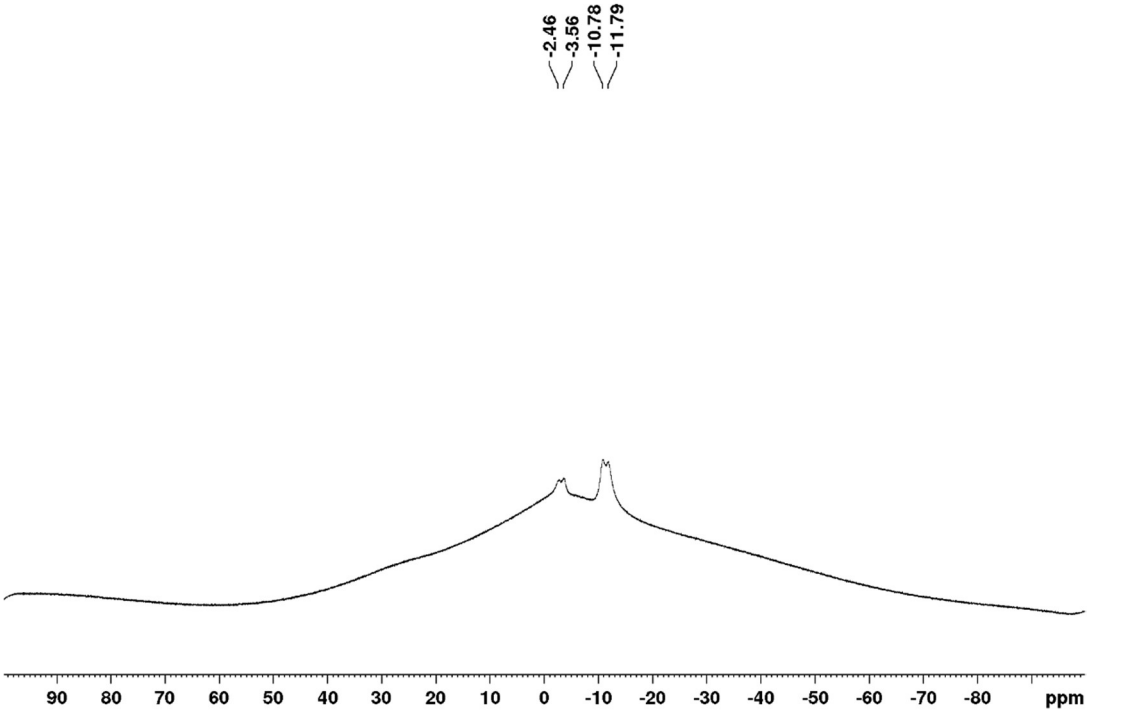
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

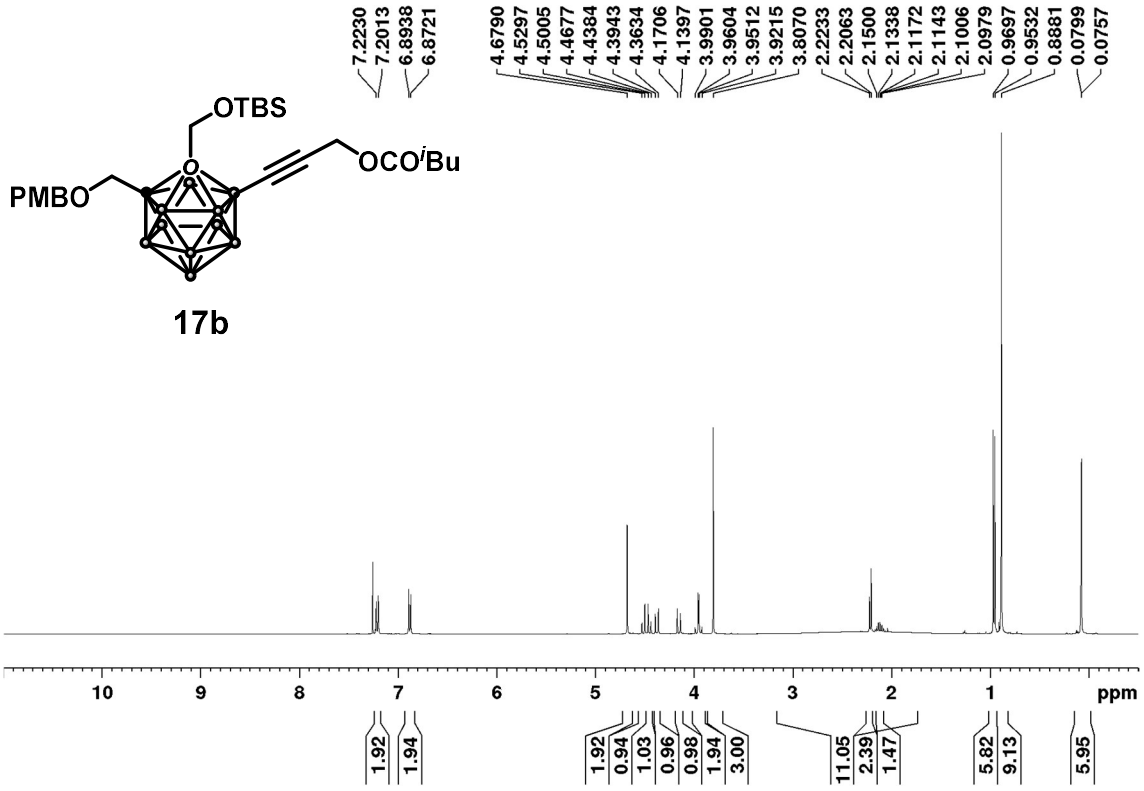


^{11}B NMR (160 MHz; $CDCl_3$)

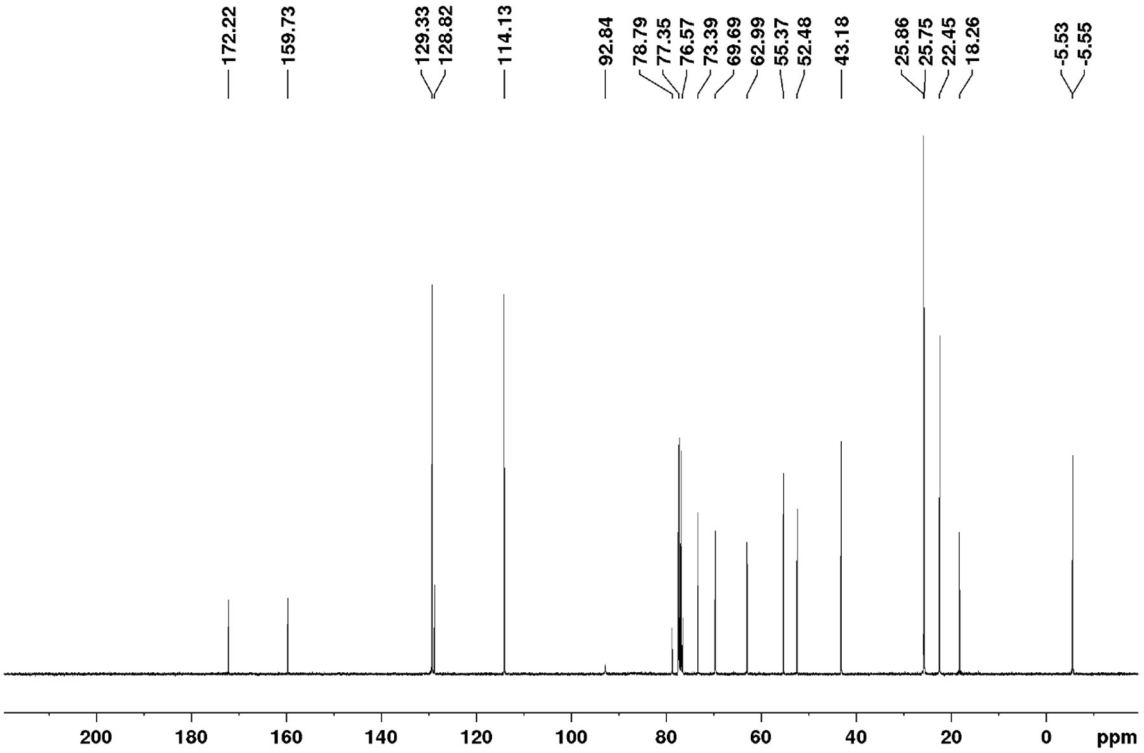


1-(3-(4-methoxybenzyl)hydroxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(3-((3-methylbutanoyl)oxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (17b)

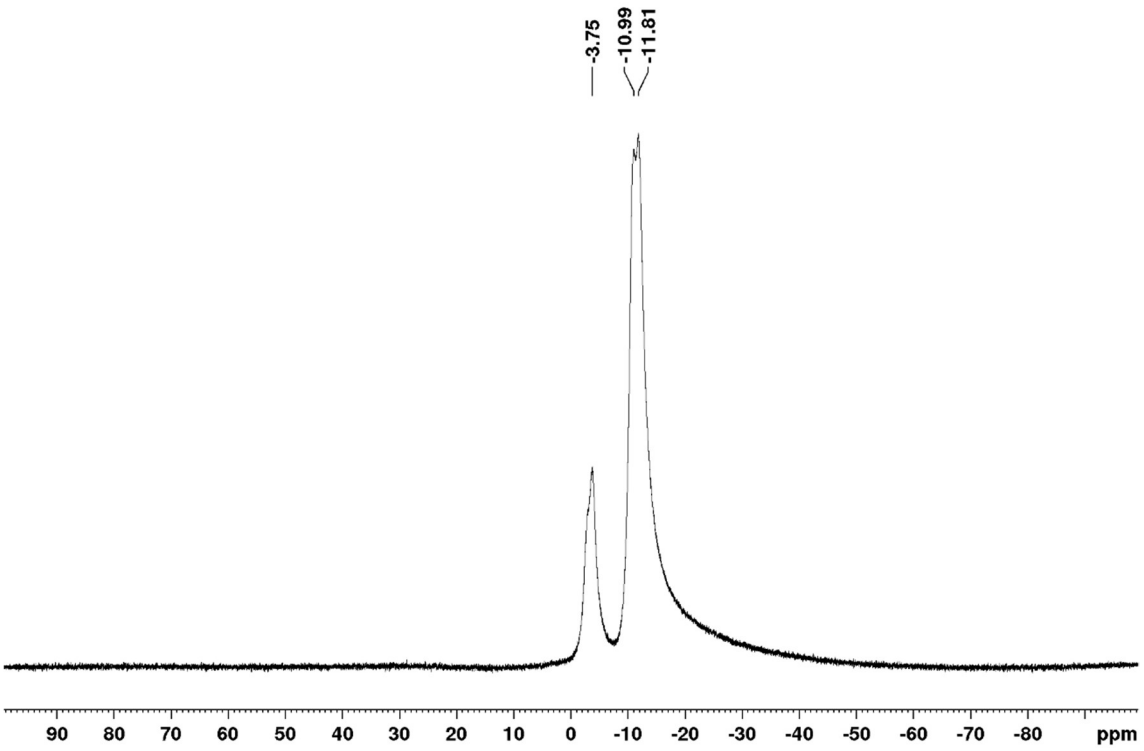
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

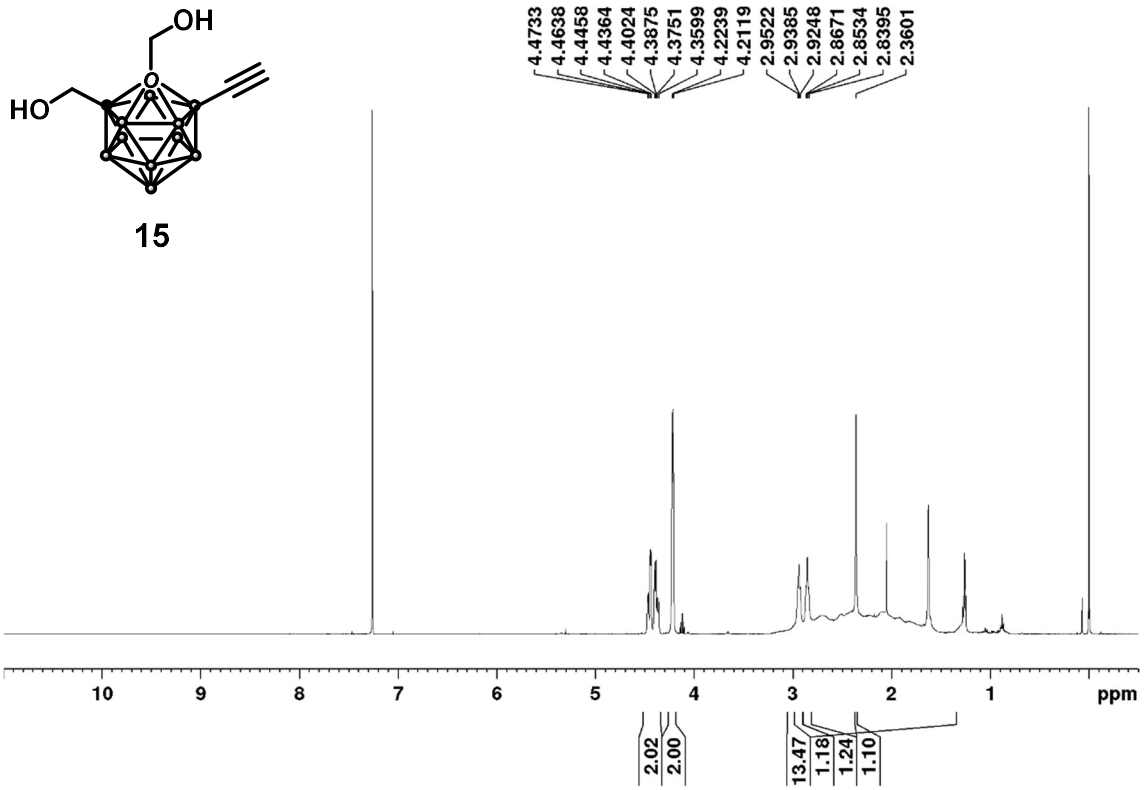


^{11}B NMR (128 MHz; $CDCl_3$)

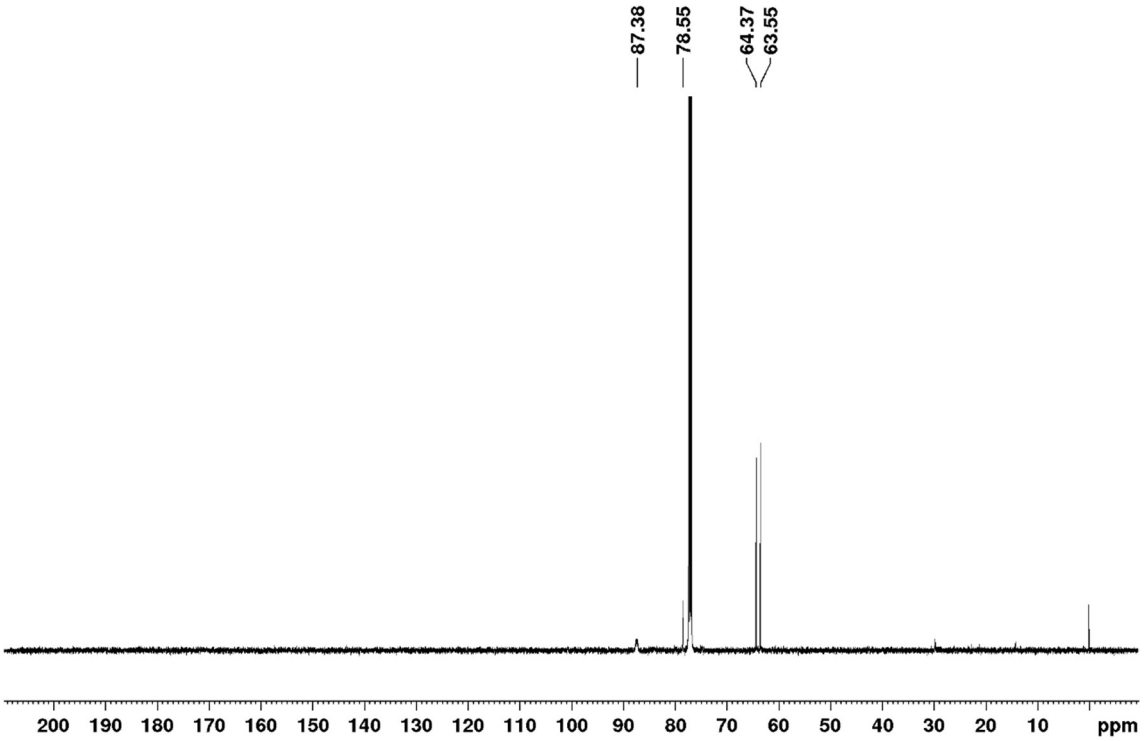


1,2-bis(hydroxymethyl)-4-ethynyl-1,2-dicarba-*closo*-dodecaborane (15)

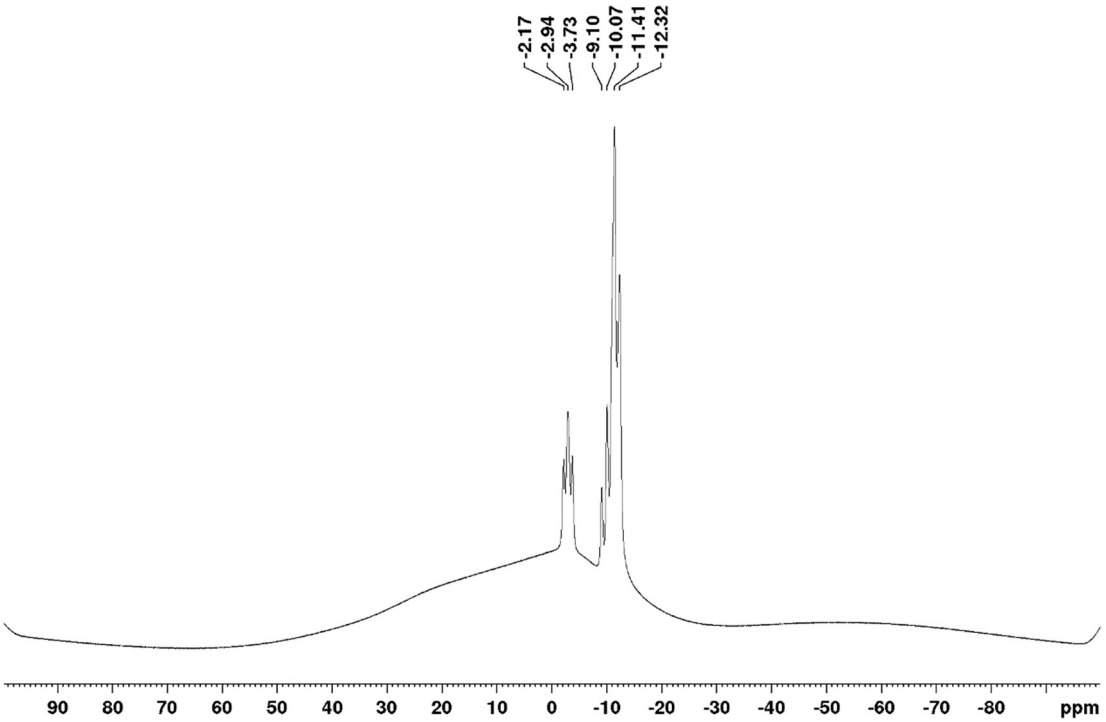
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

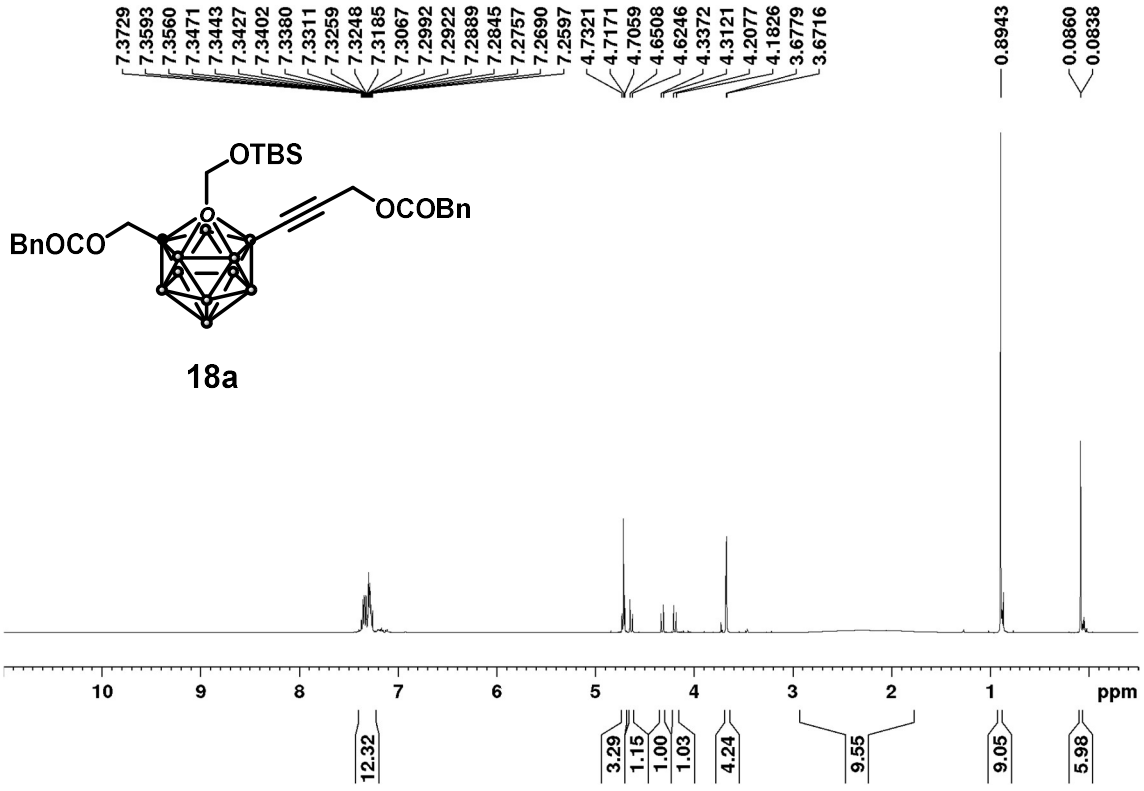


^{11}B NMR (160 MHz; $CDCl_3$)

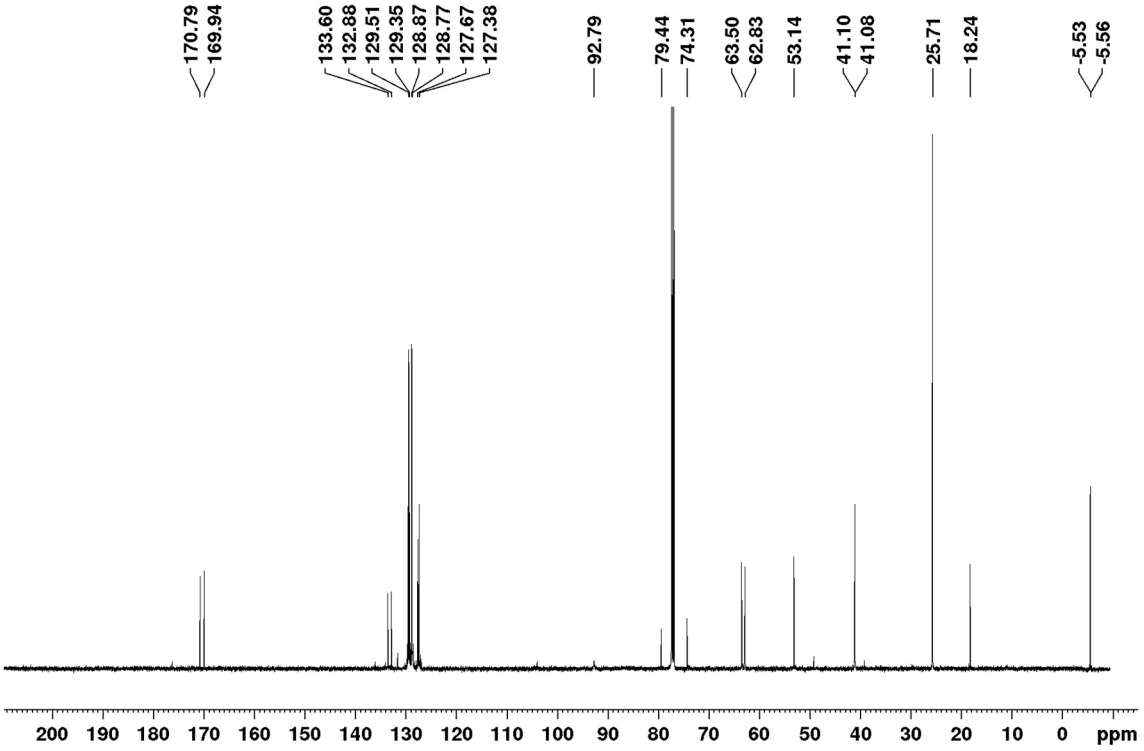


1-(2-phenylacetoxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18a)

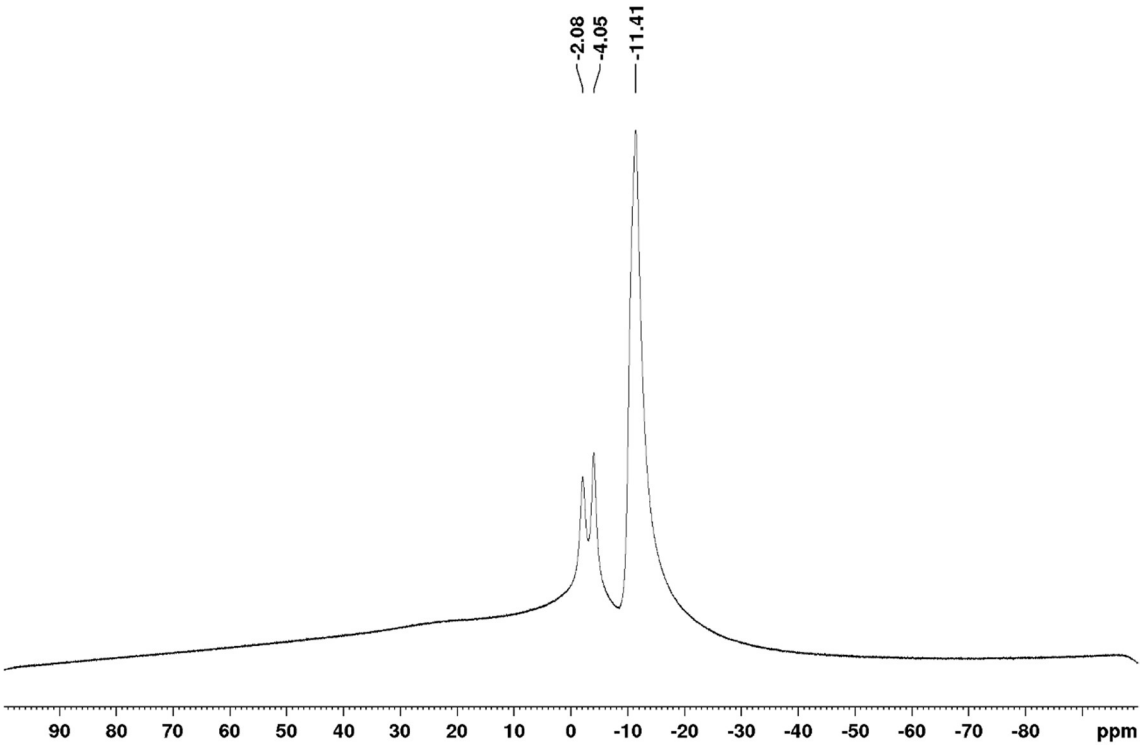
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

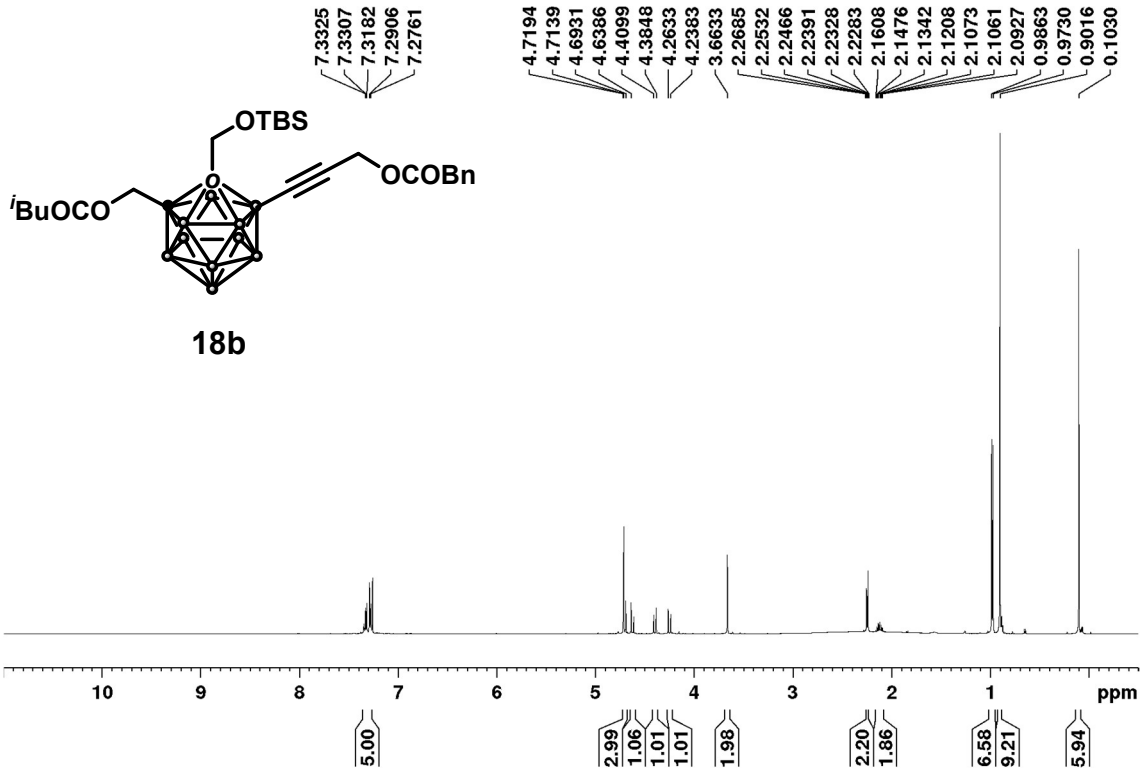


^{11}B NMR (160 MHz; $CDCl_3$)

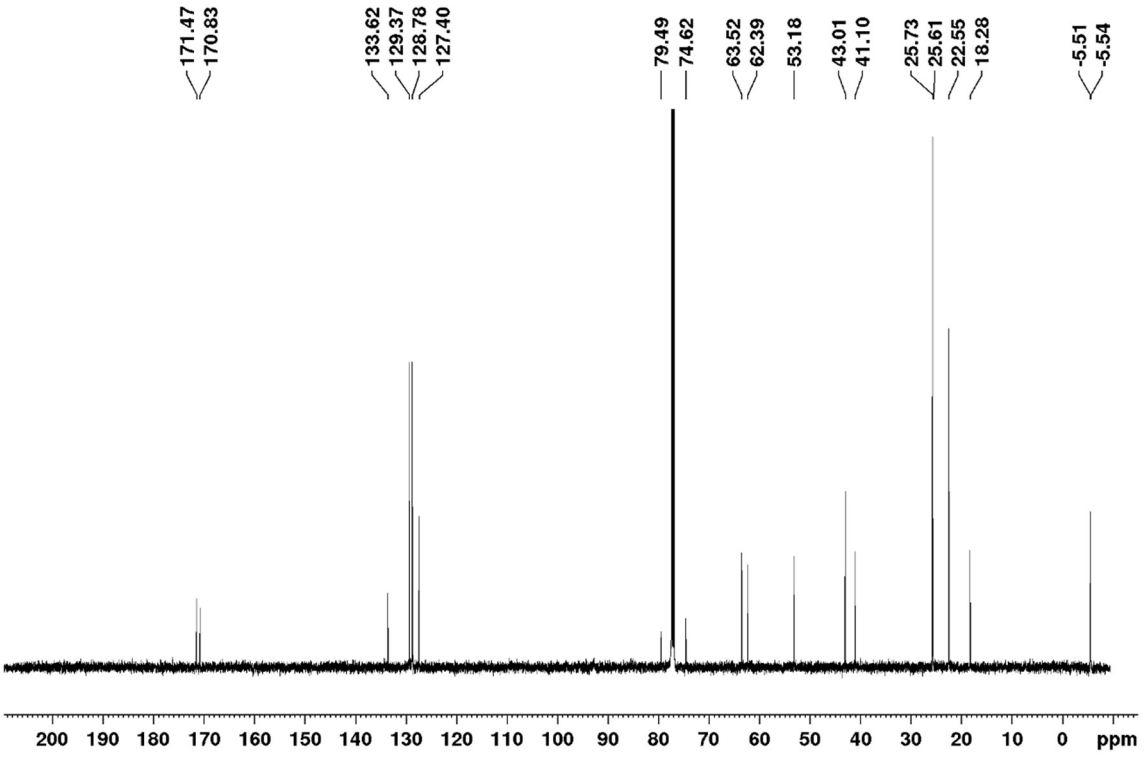


1-((3-methylbutanoyl)oxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18b)

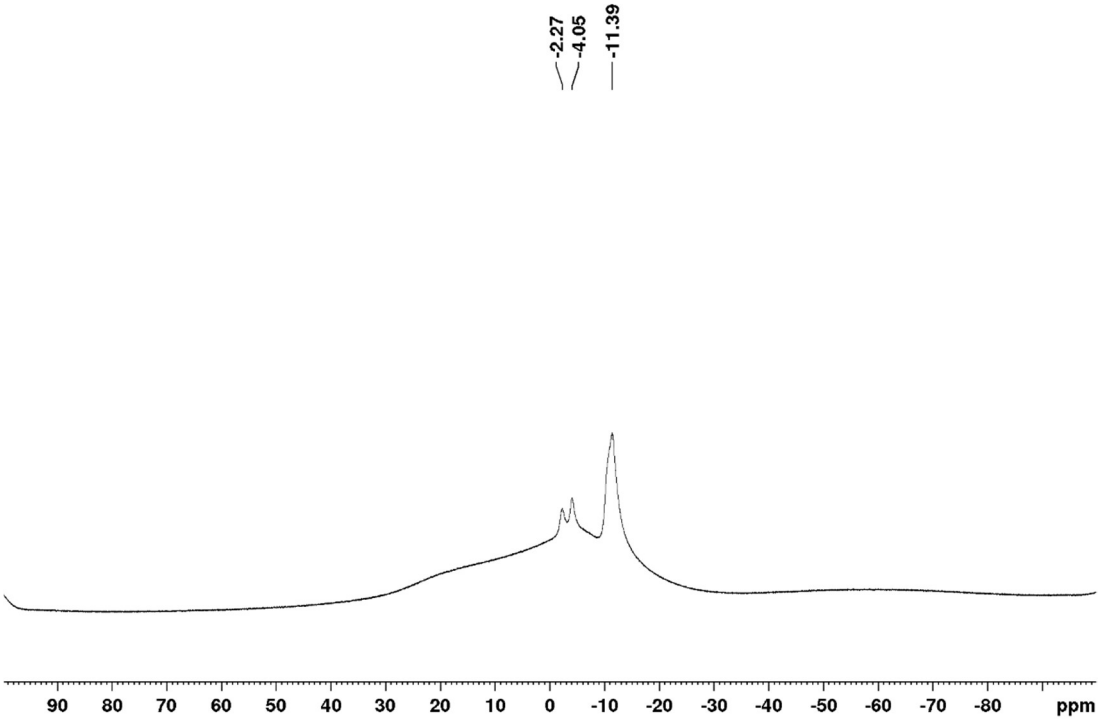
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

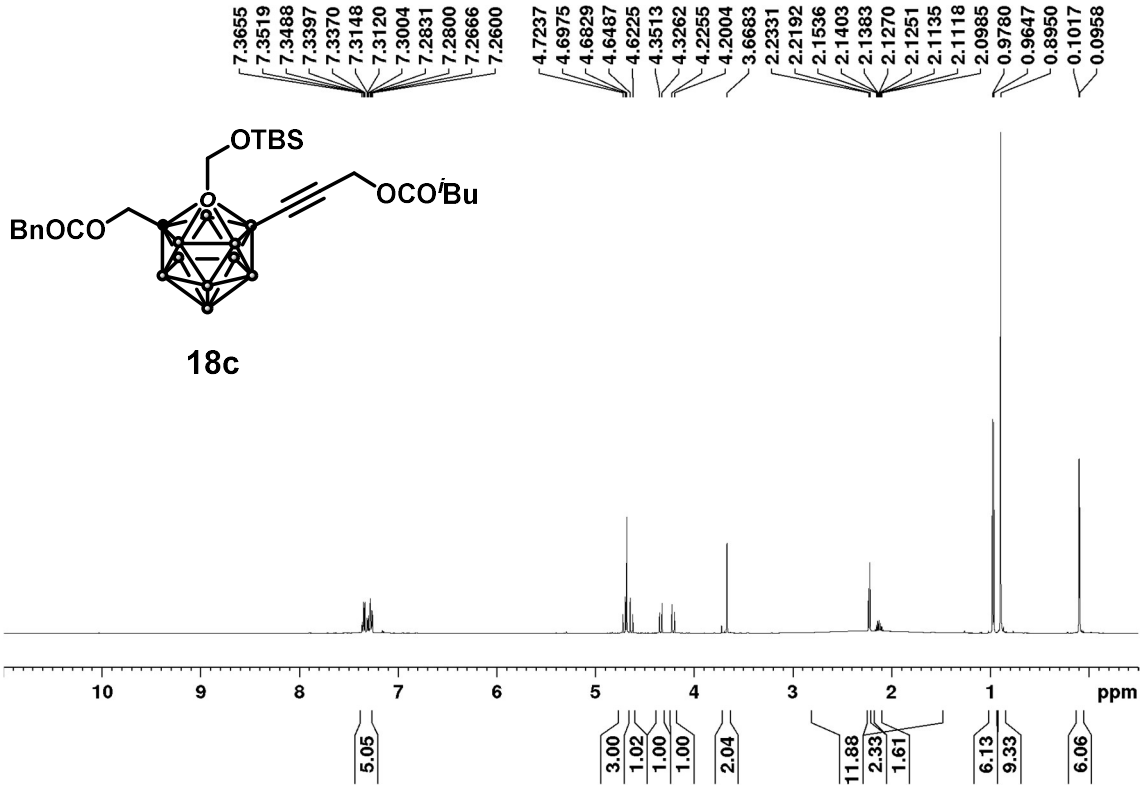


^{11}B NMR (160 MHz; $CDCl_3$)

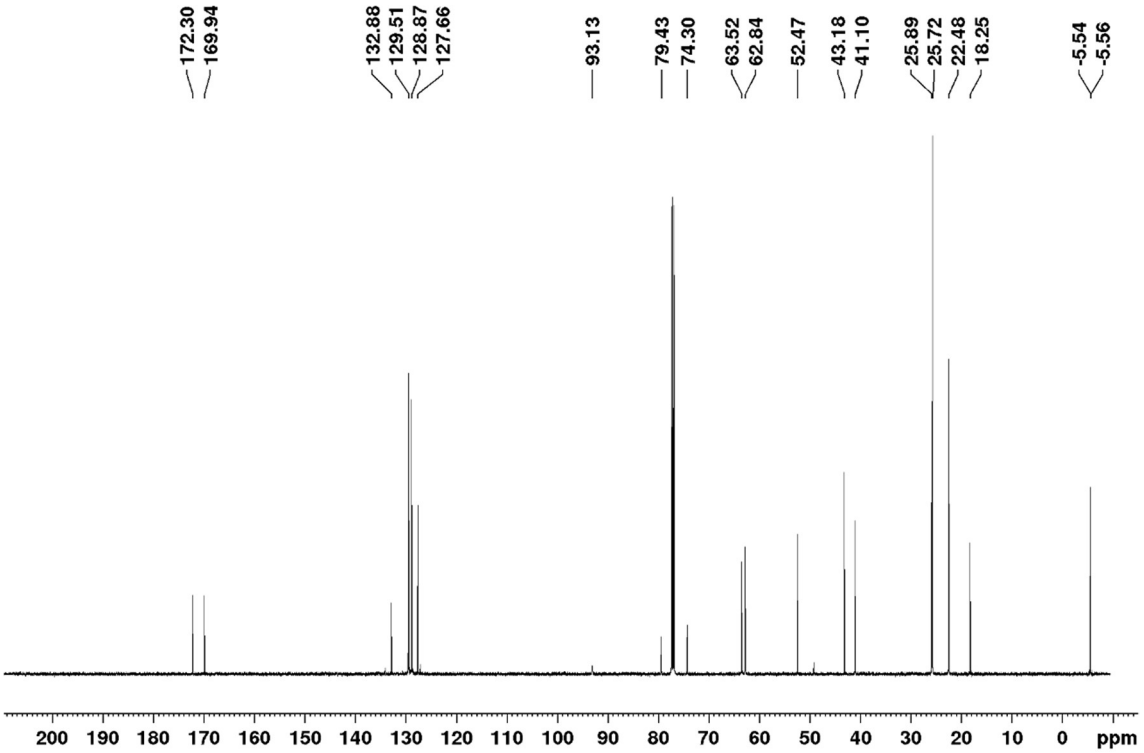


1-(2-phenylacetoxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18c)

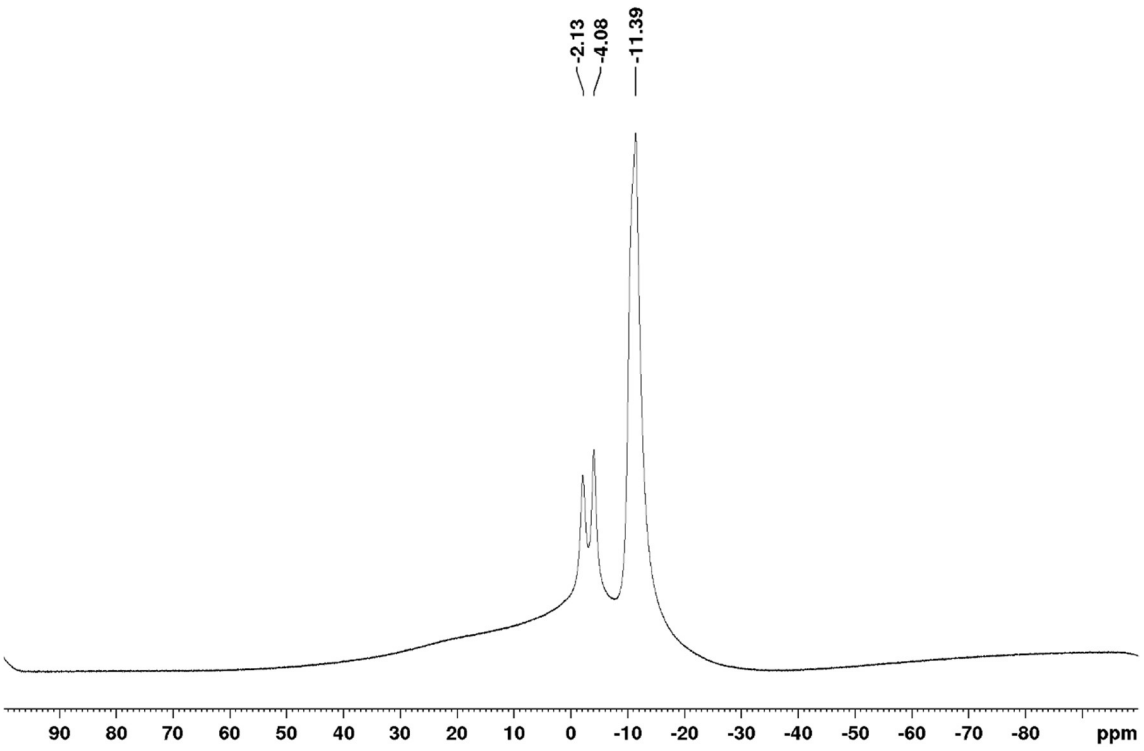
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

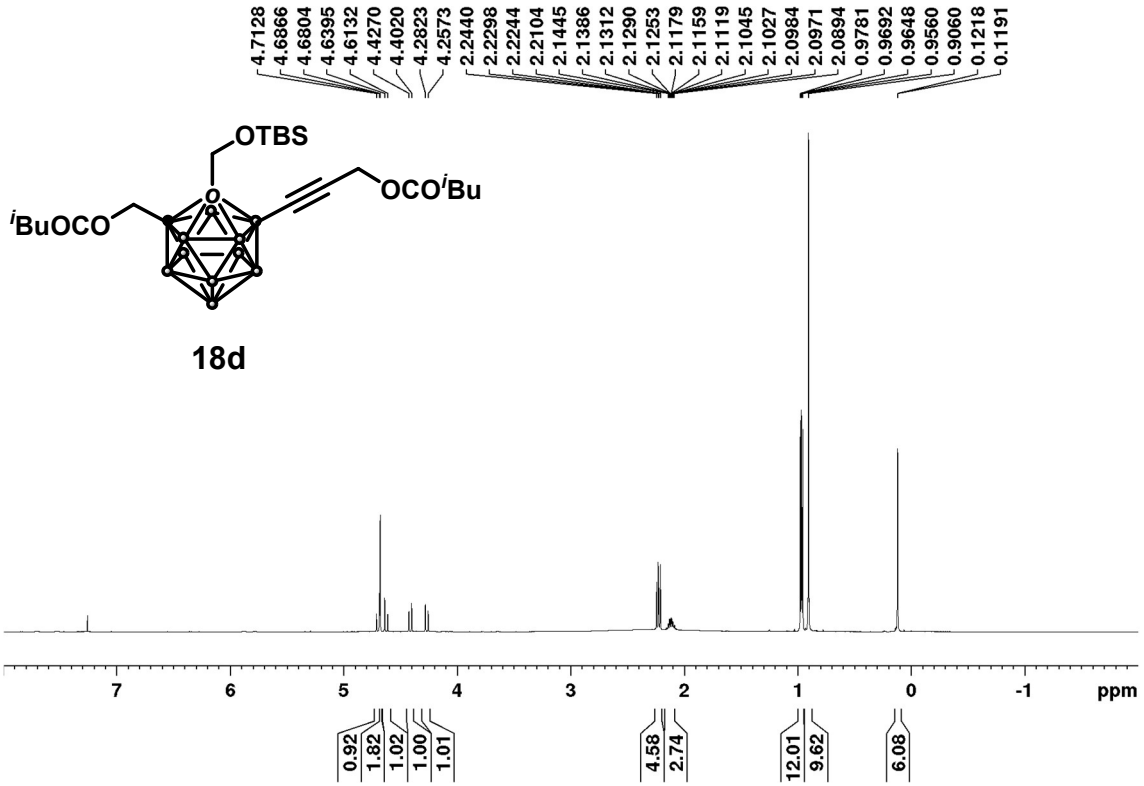


^{11}B NMR (160 MHz; $CDCl_3$)

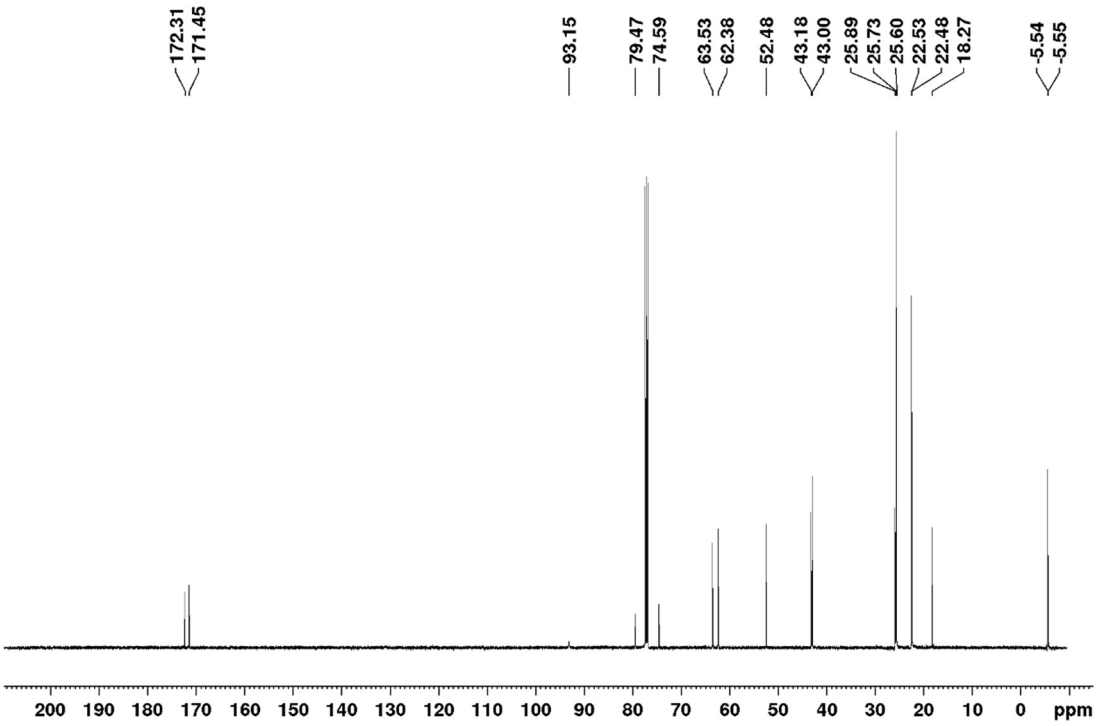


1-(3-methylbutanoyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (18d)

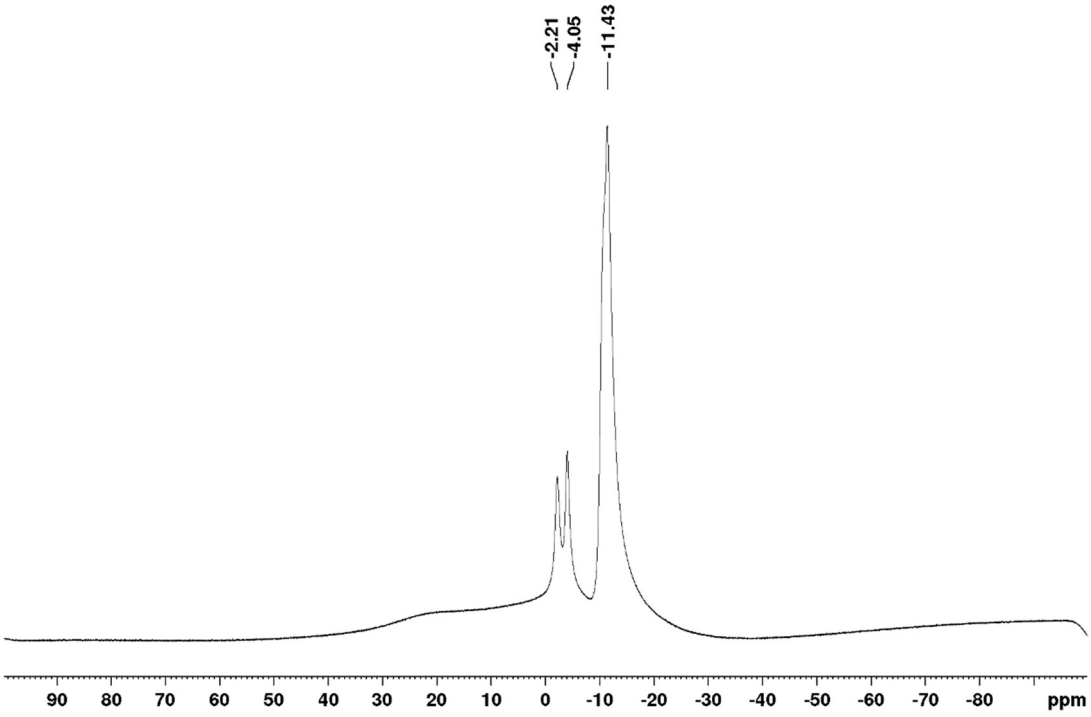
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

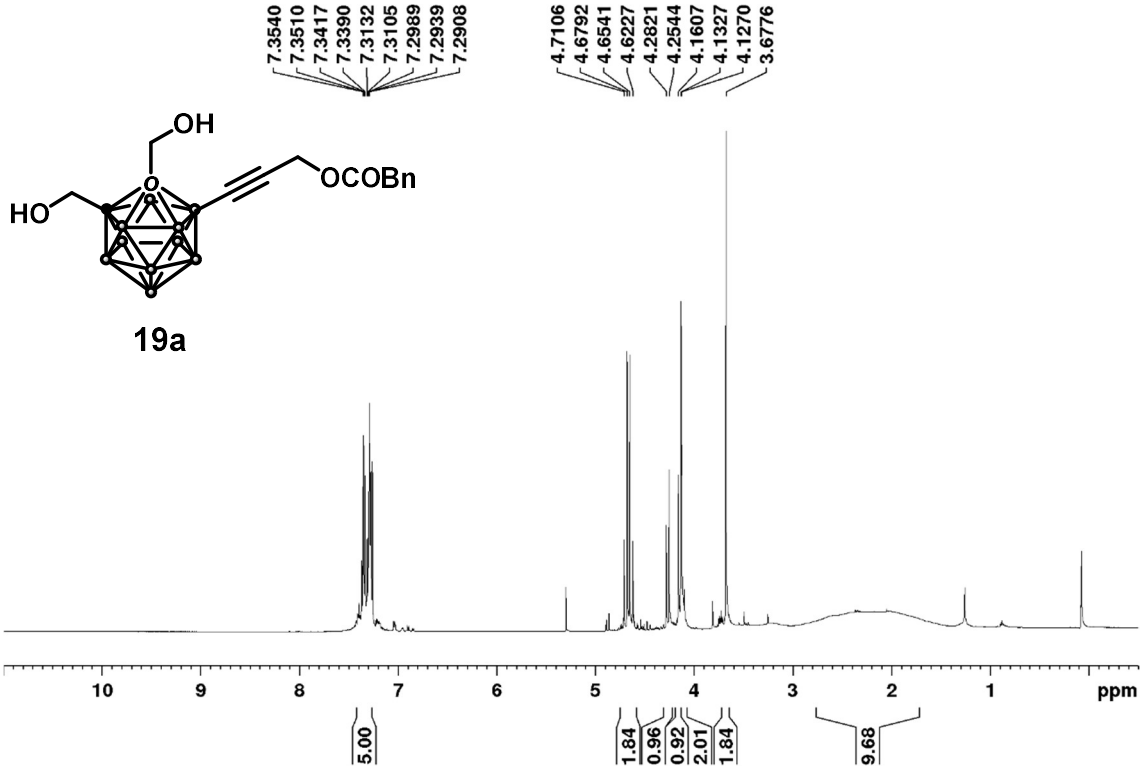


^{11}B NMR (160 MHz; $CDCl_3$)

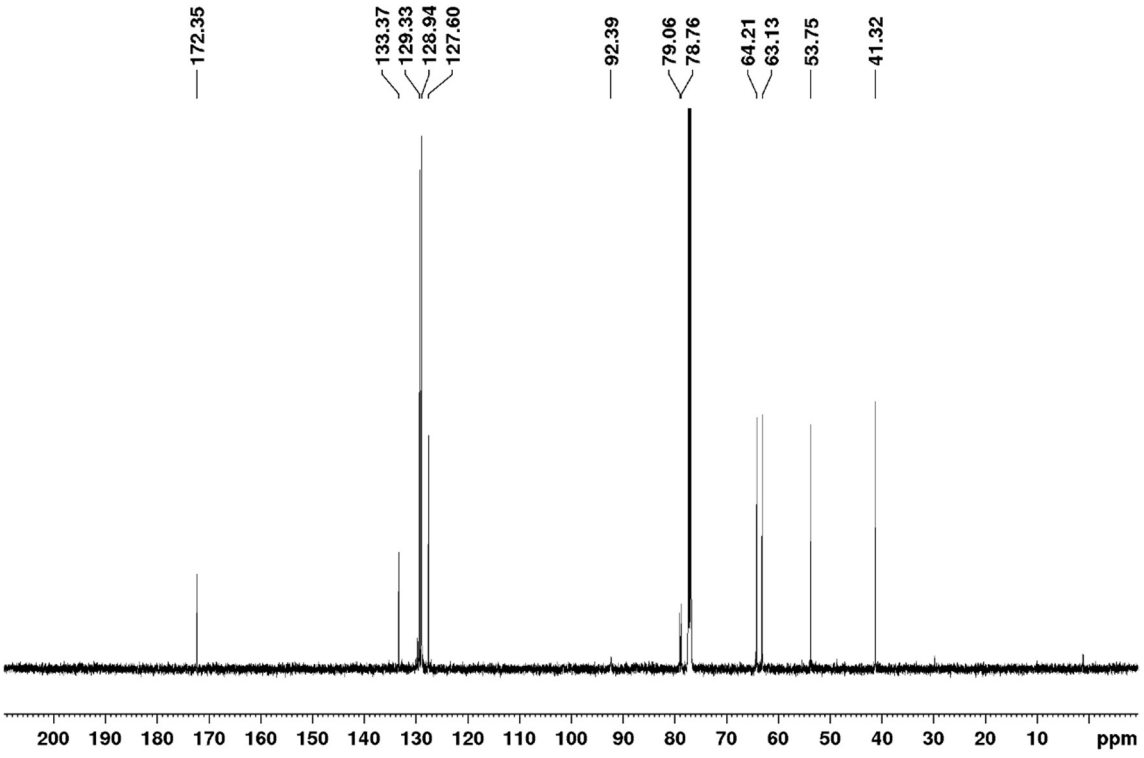


1,2-bis(hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (19a)

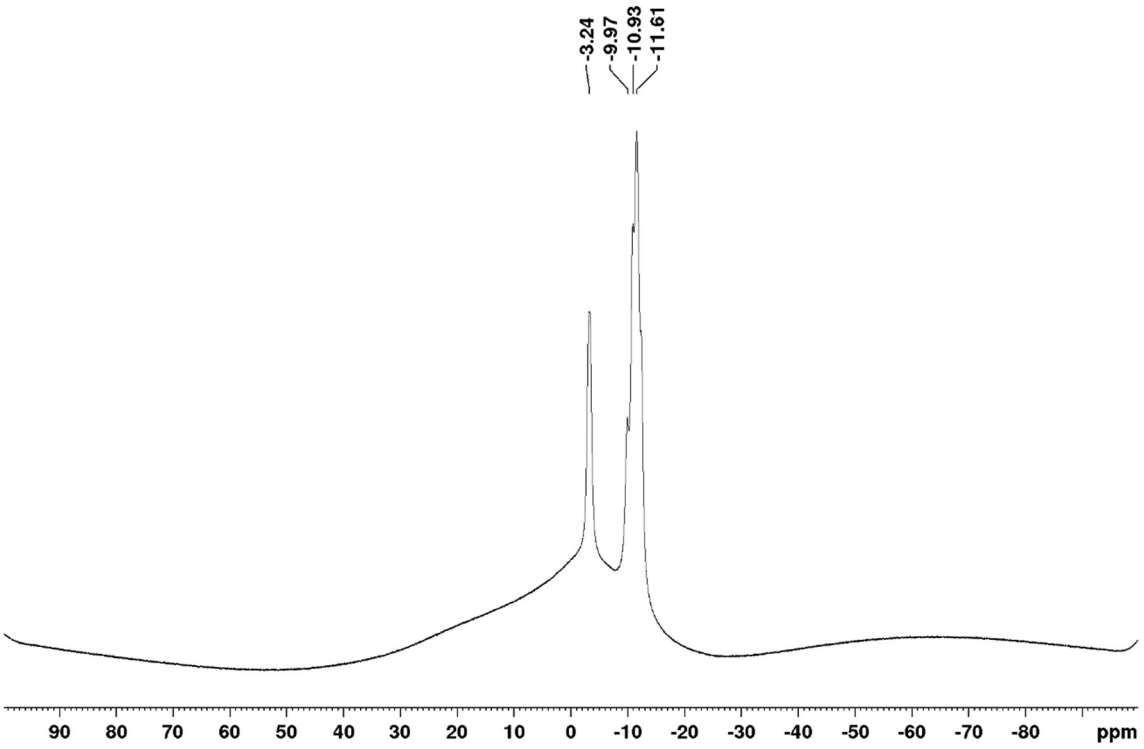
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

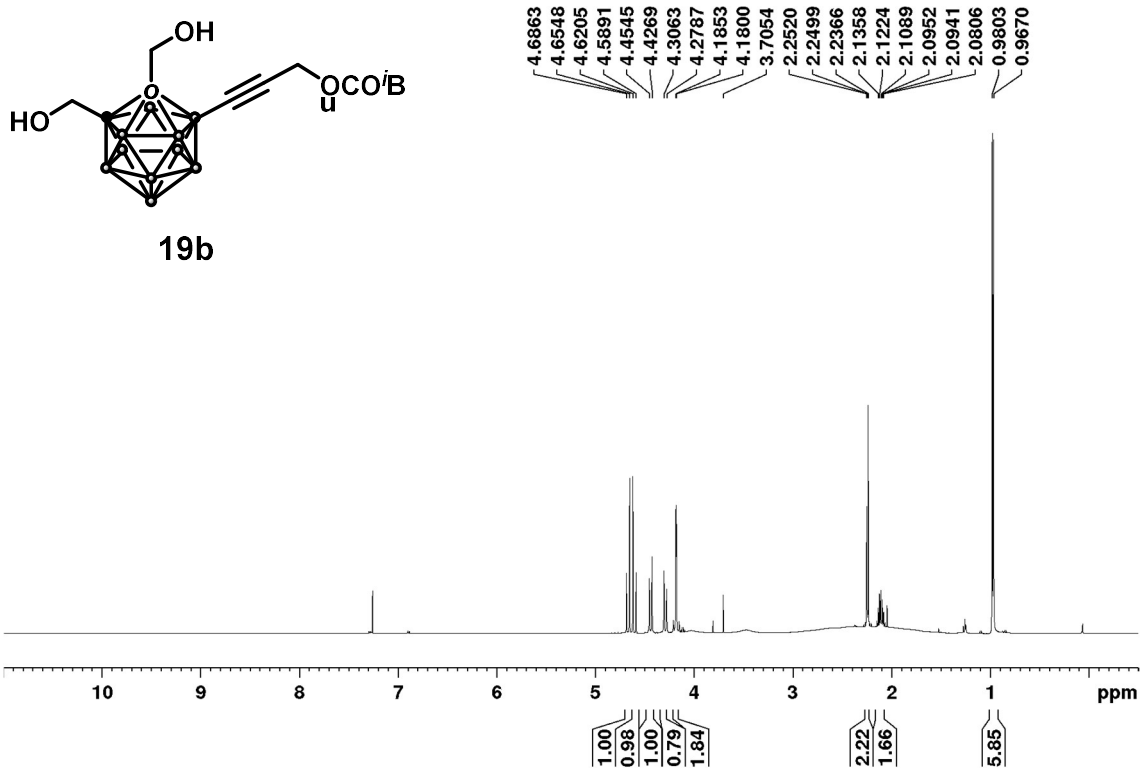


^{11}B NMR (160 MHz; $CDCl_3$)

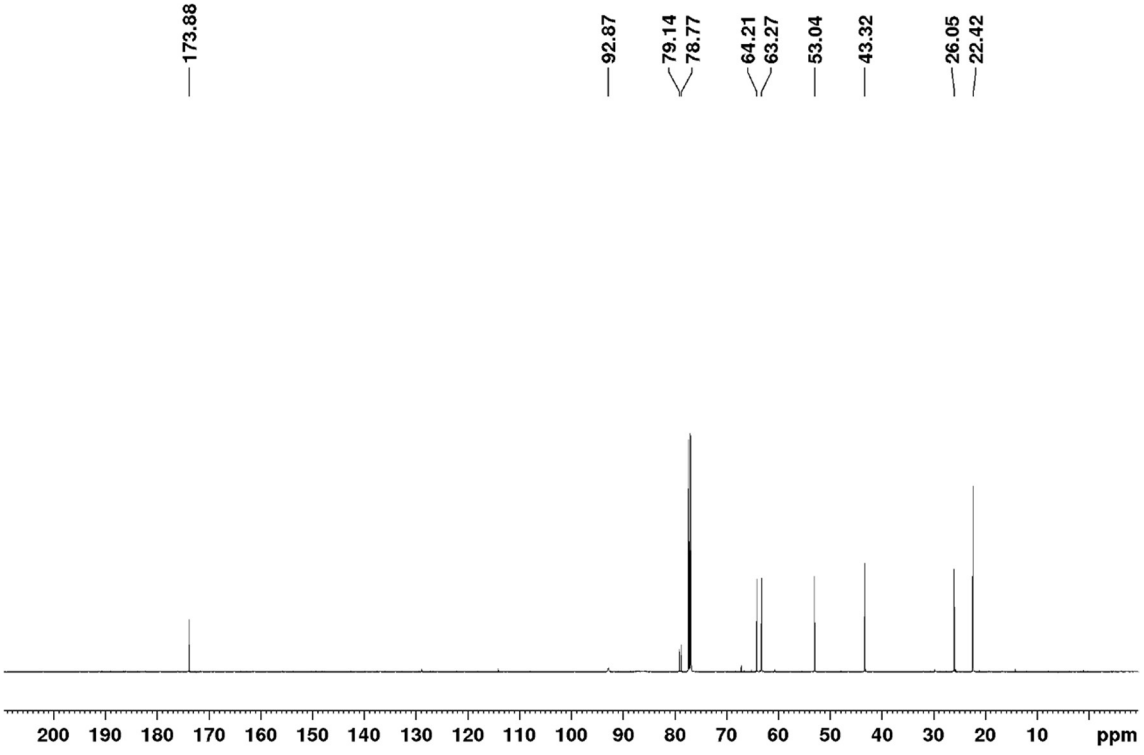


1,2-bis(hydroxymethyl)-4-(3-((3-methylbutanoyl)oxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (19b)

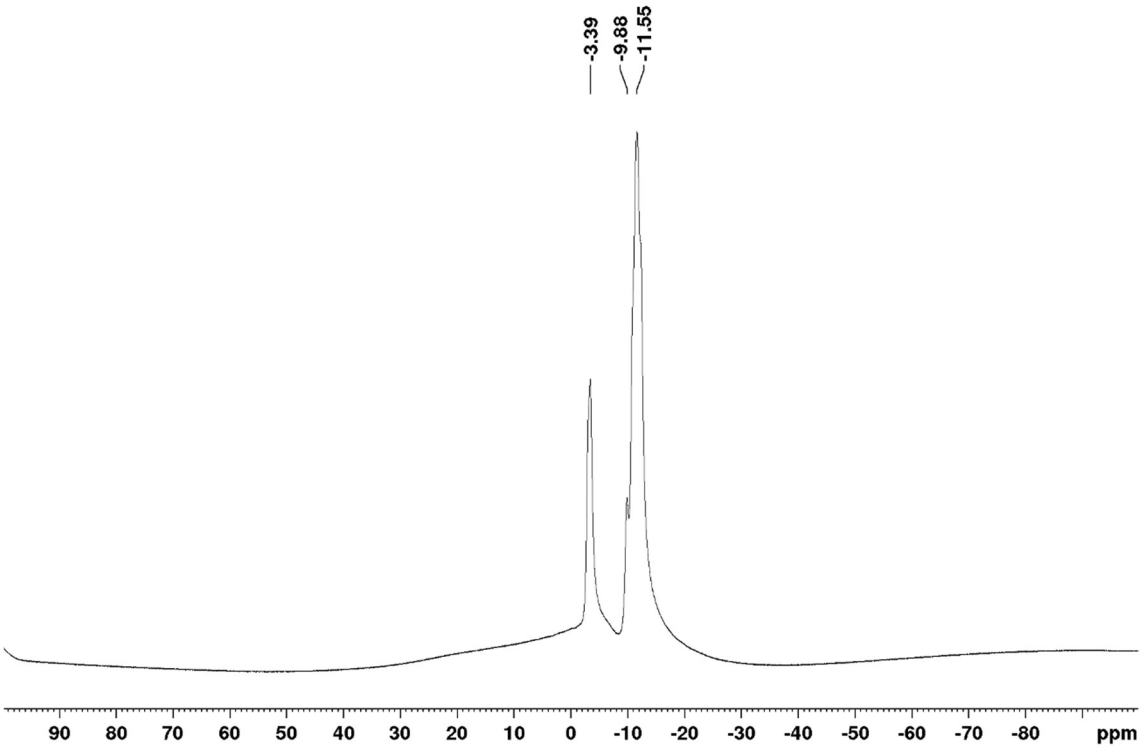
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

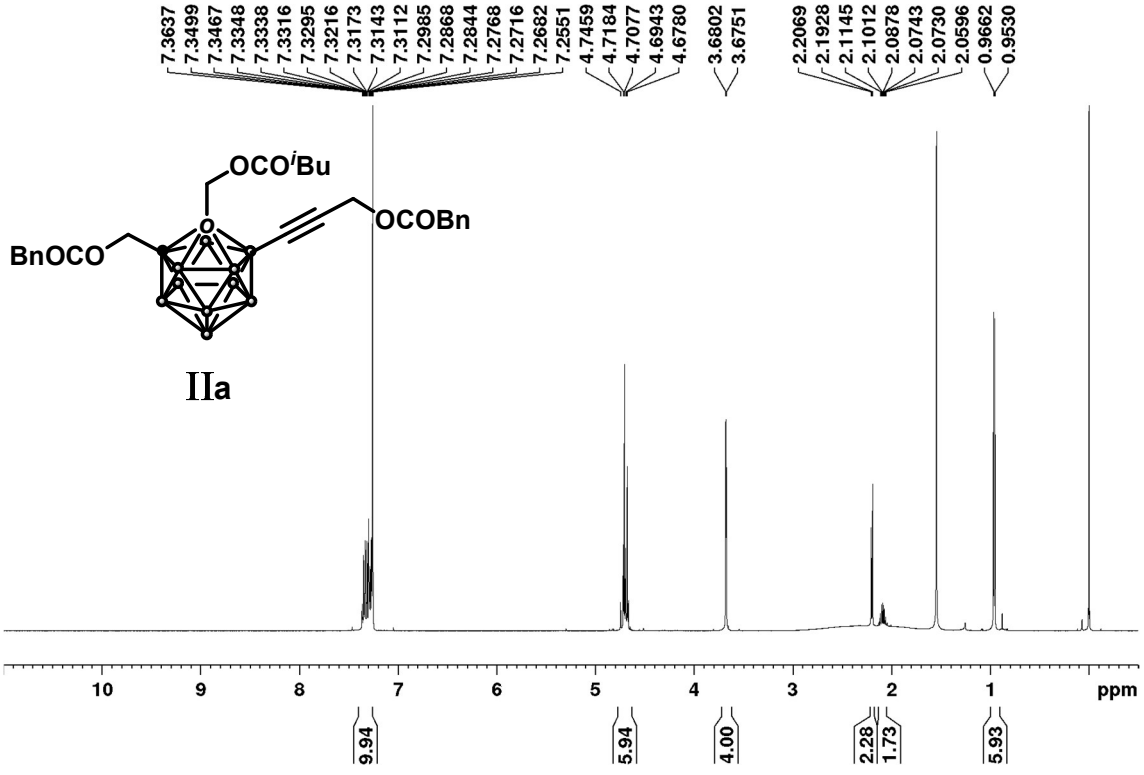


^{11}B NMR (160 MHz; $CDCl_3$)

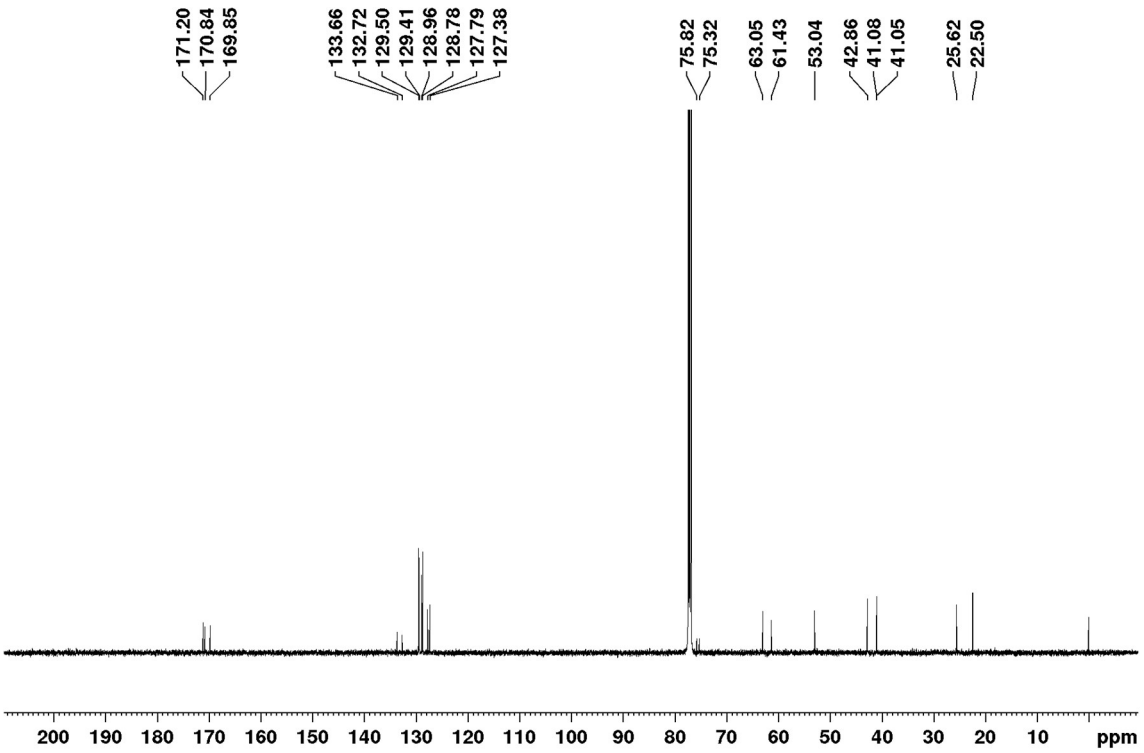


1-((3-methylbutanoyl)oxymethyl)-2-((*tert*-butyl)dimethylsilyl)-hydroxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIa)

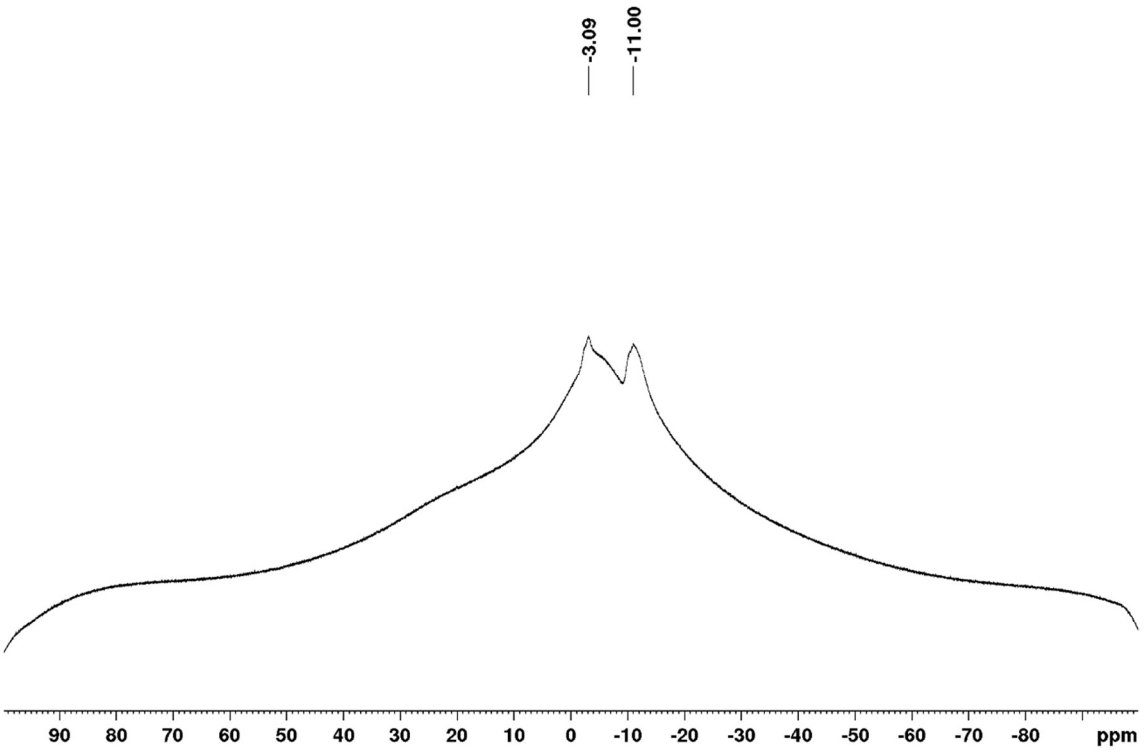
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

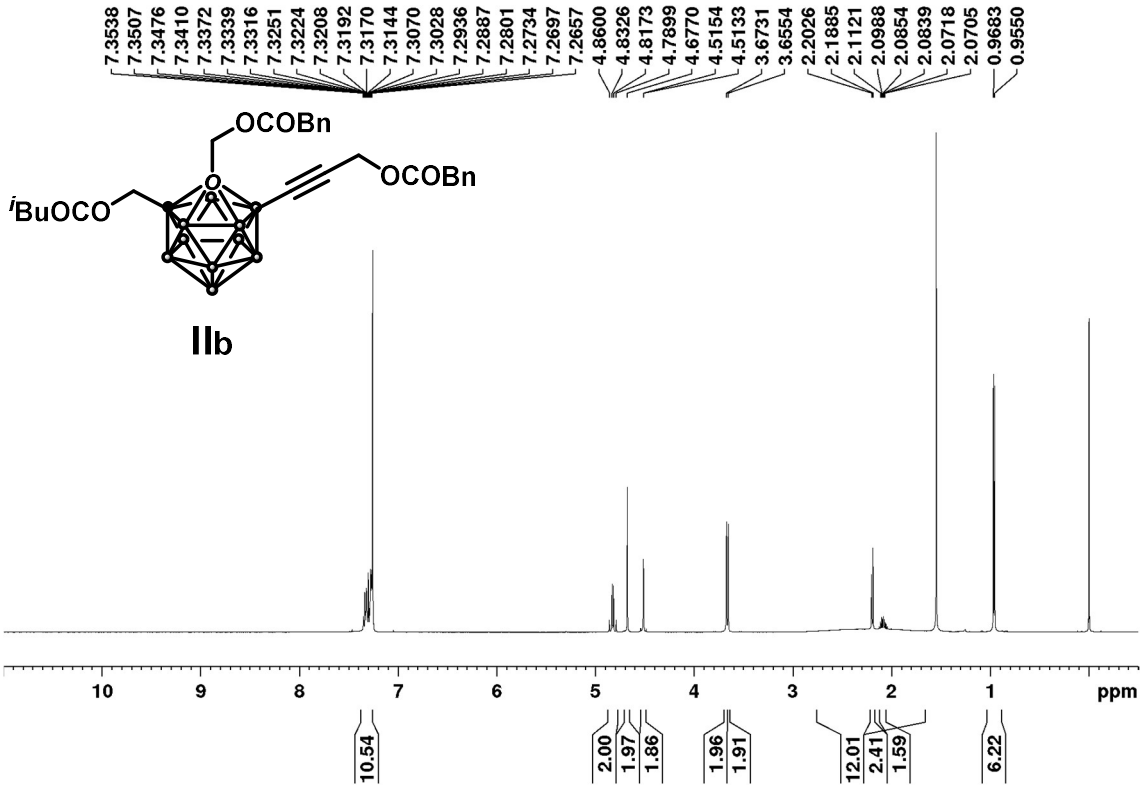


^{11}B NMR (160 MHz; $CDCl_3$)

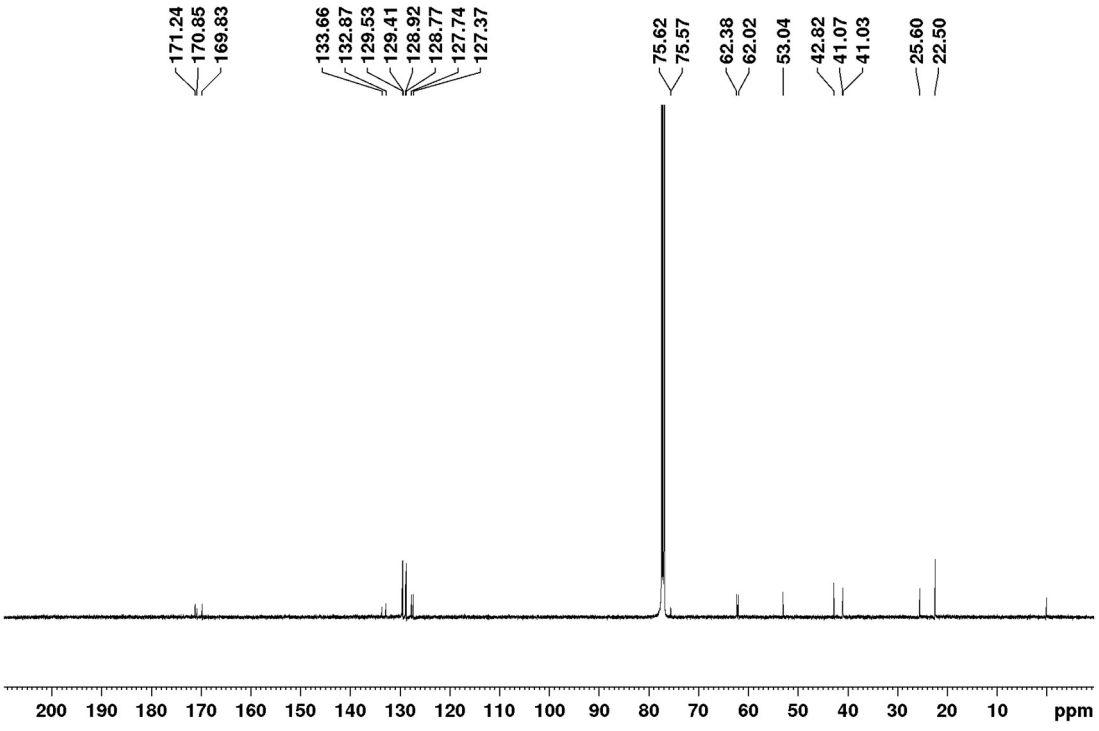


1-((3-methylbutanoyl)oxymethyl)-2-(2-phenylacetoxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIb)

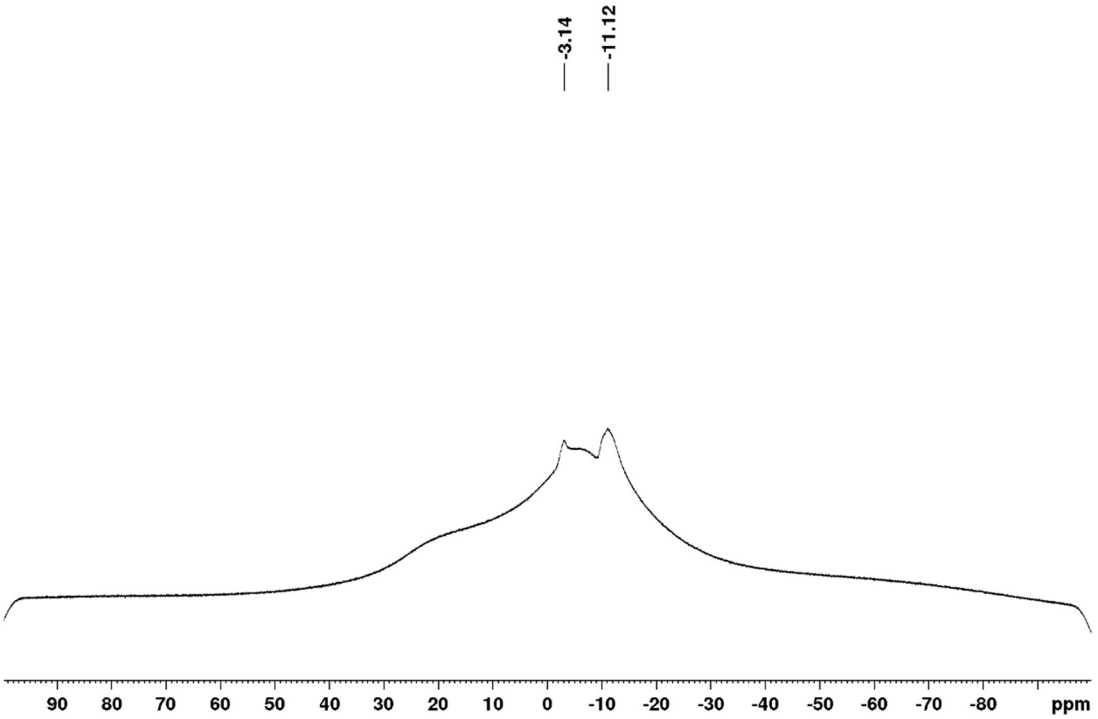
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

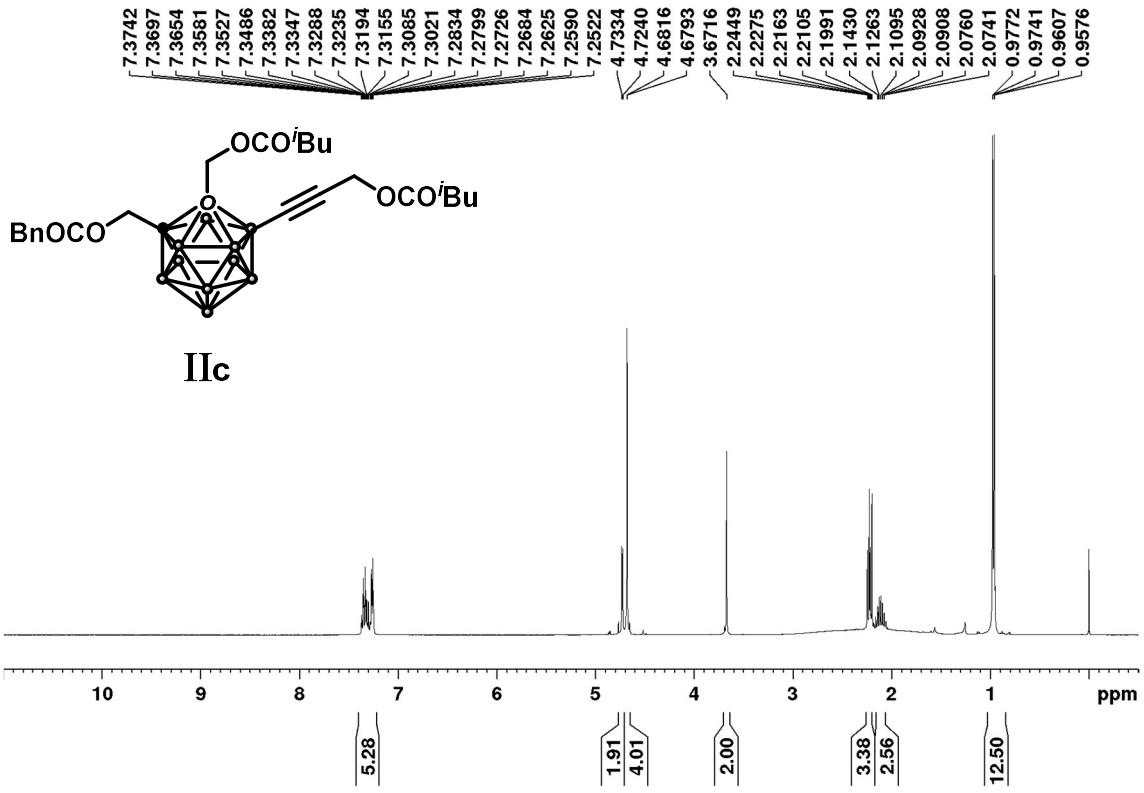


^{11}B NMR (160 MHz; $CDCl_3$)

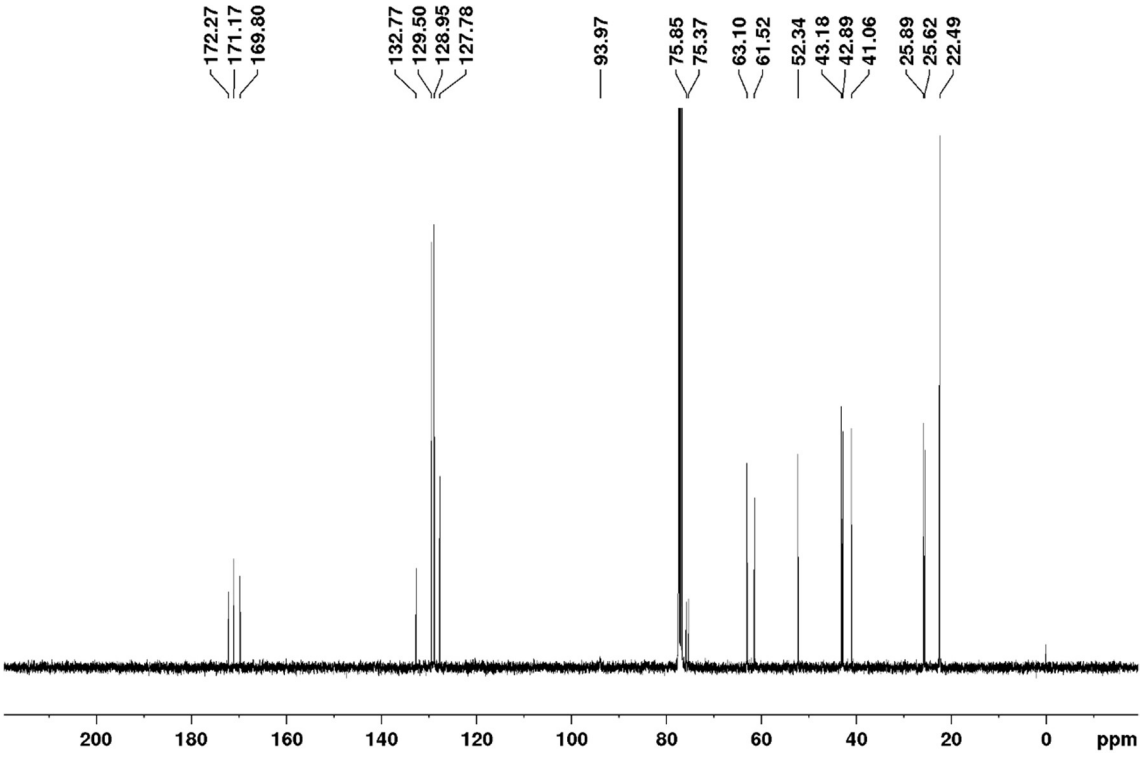


1-(2-phenylacetoxymethyl)-2-((3-methylbutanoyl)oxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIc)

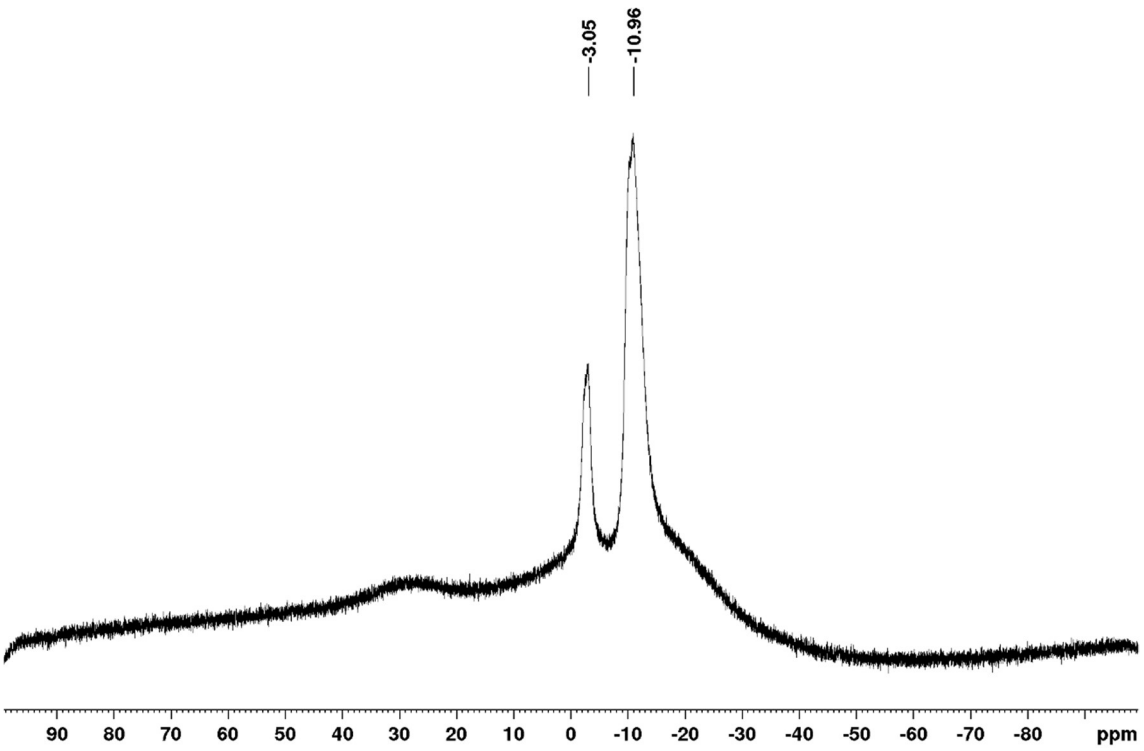
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

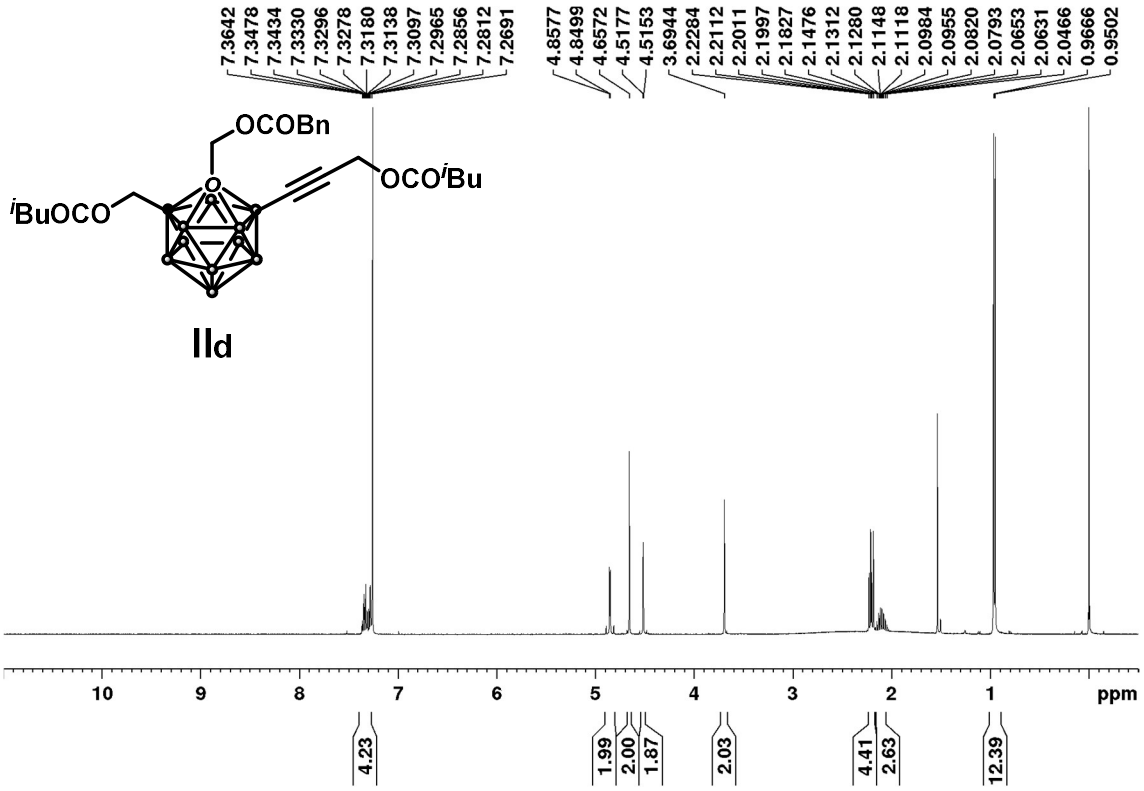


^{11}B NMR (160 MHz; $CDCl_3$)

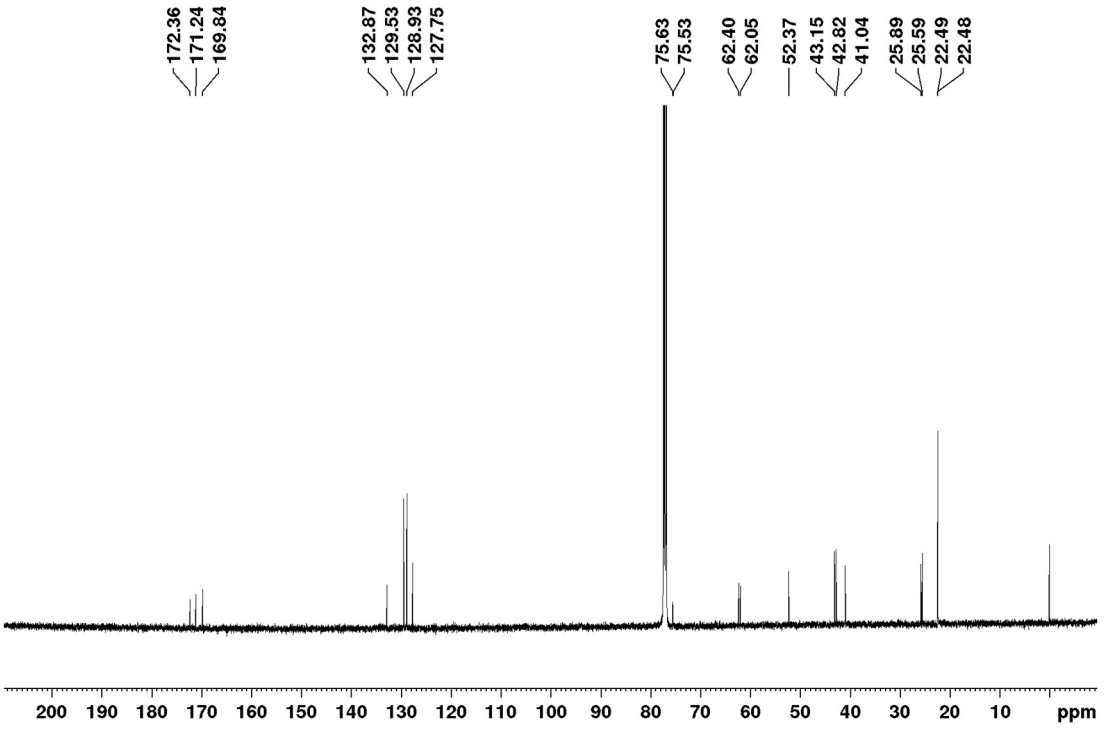


1-(3-methylbutanoyl)-2-(2-phenylacetoxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IId)

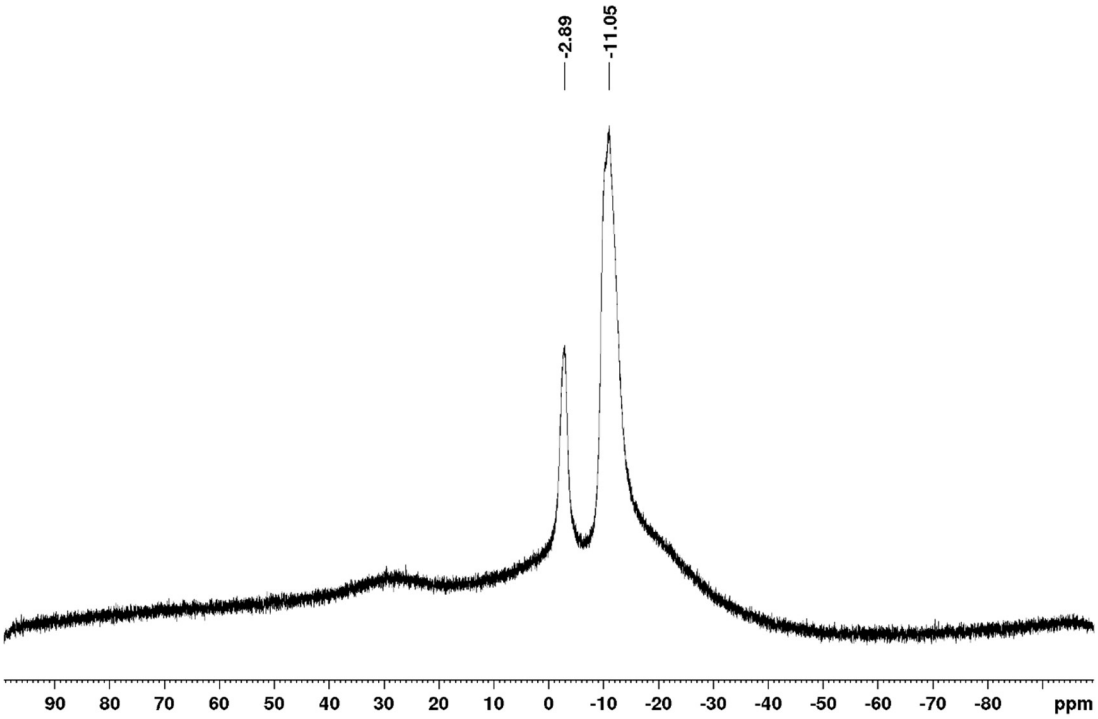
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

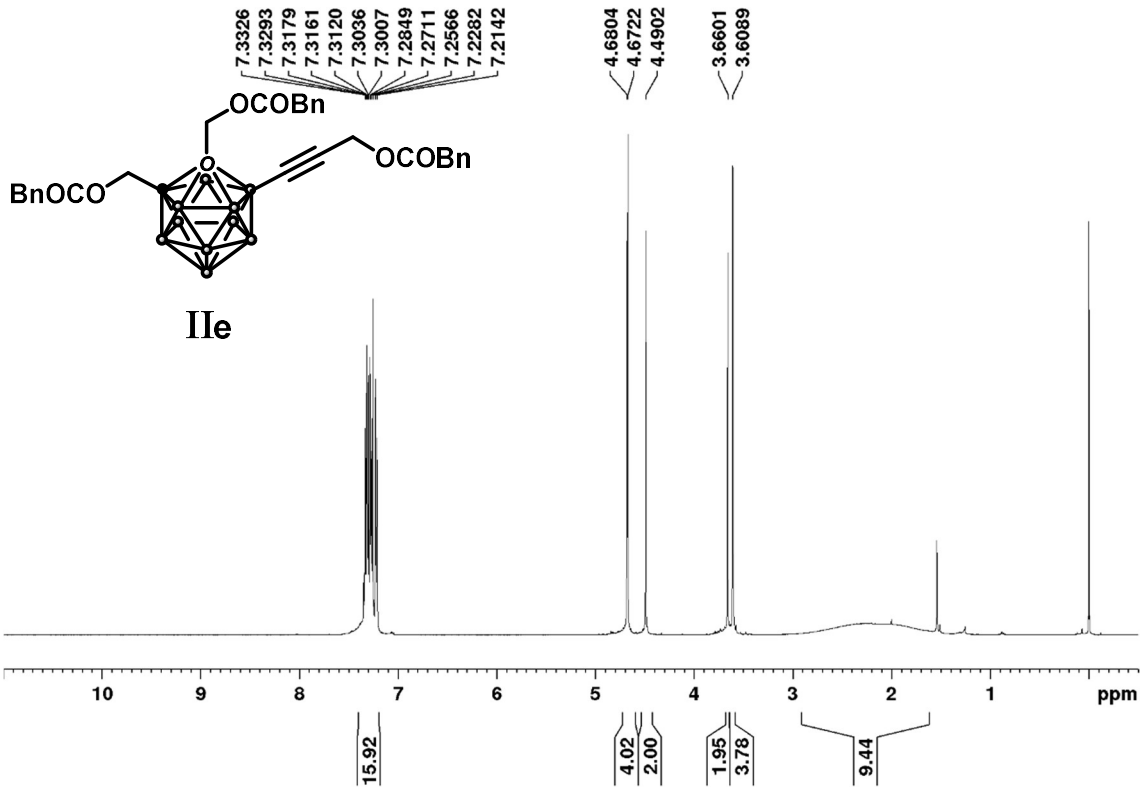


^{11}B NMR (160 MHz; $CDCl_3$)

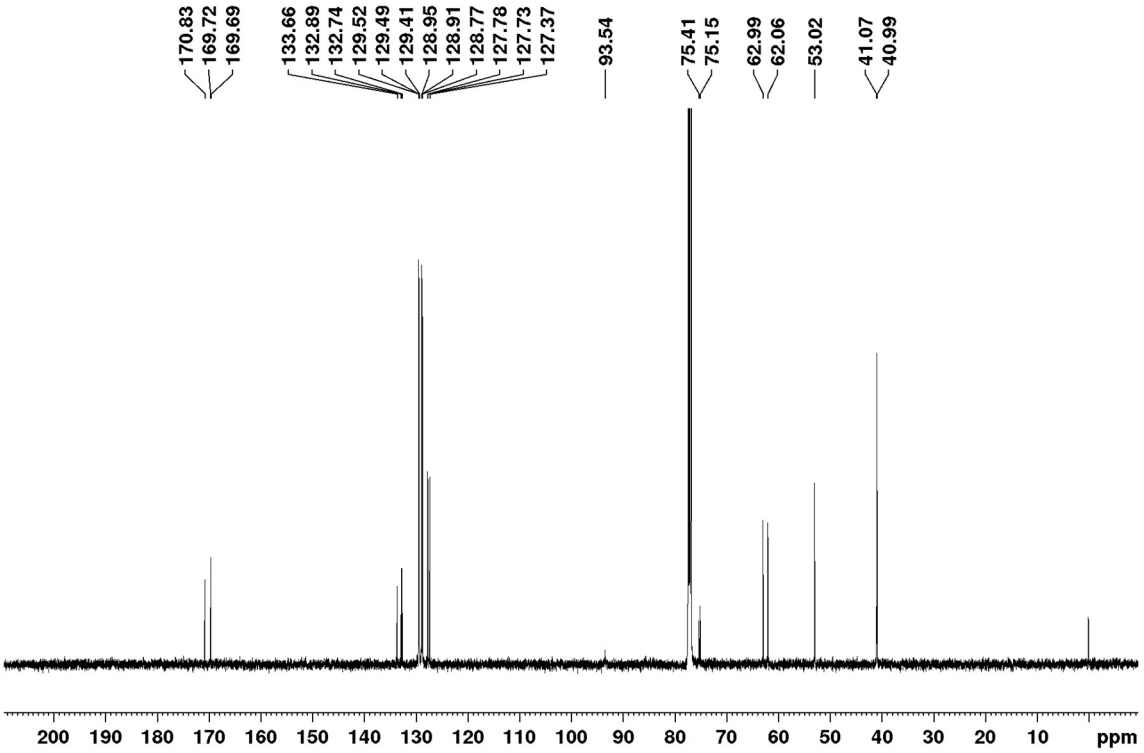


1,2-bis(phenylacetoxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIe)

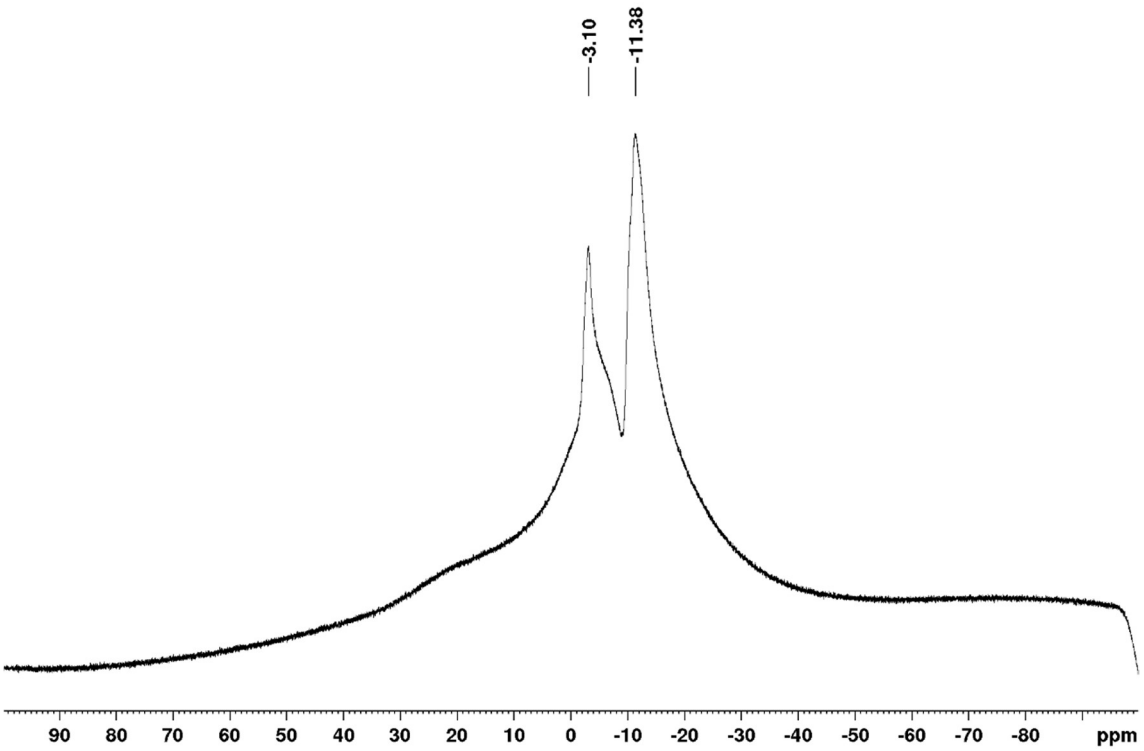
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

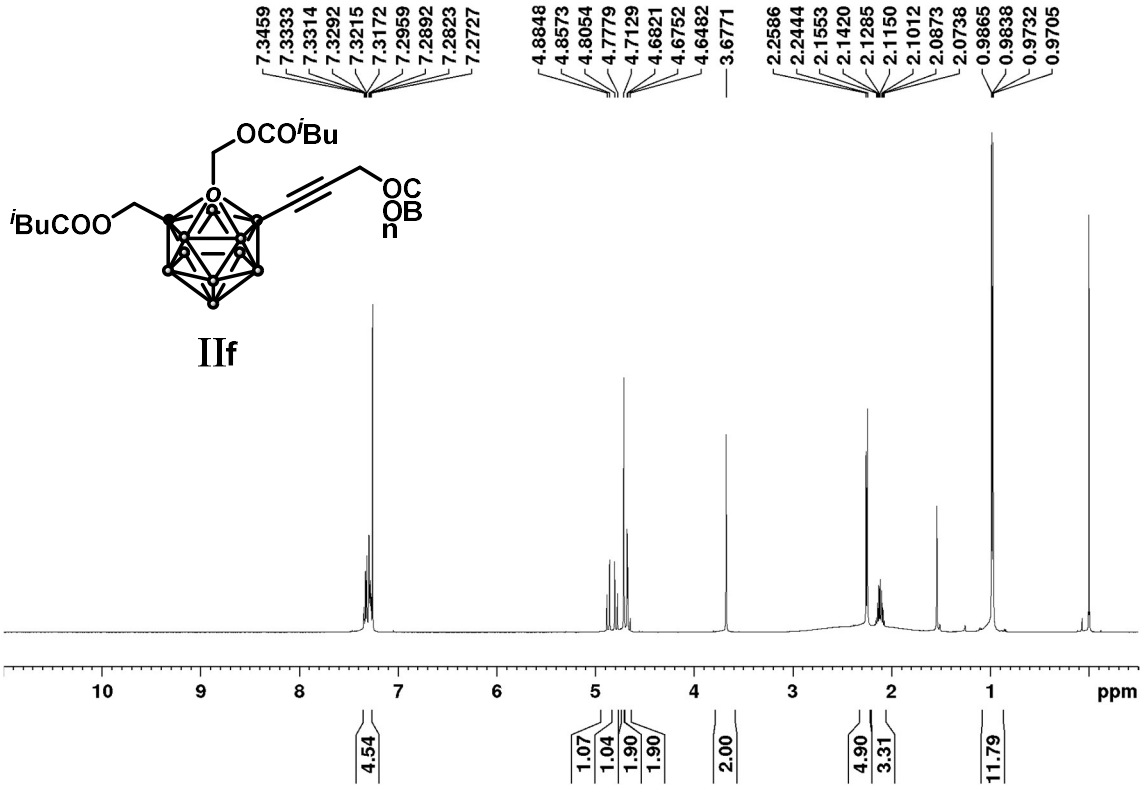


^{11}B NMR (160 MHz; $CDCl_3$)

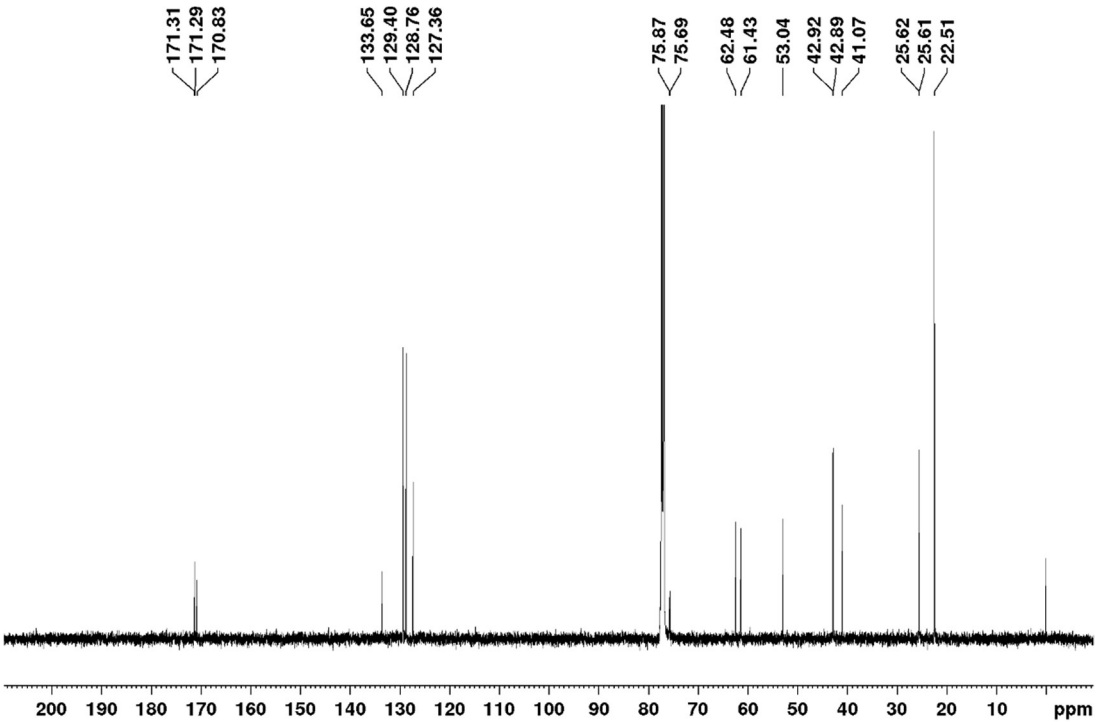


1,2-bis((3-methylbutanoyl)oxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIf)

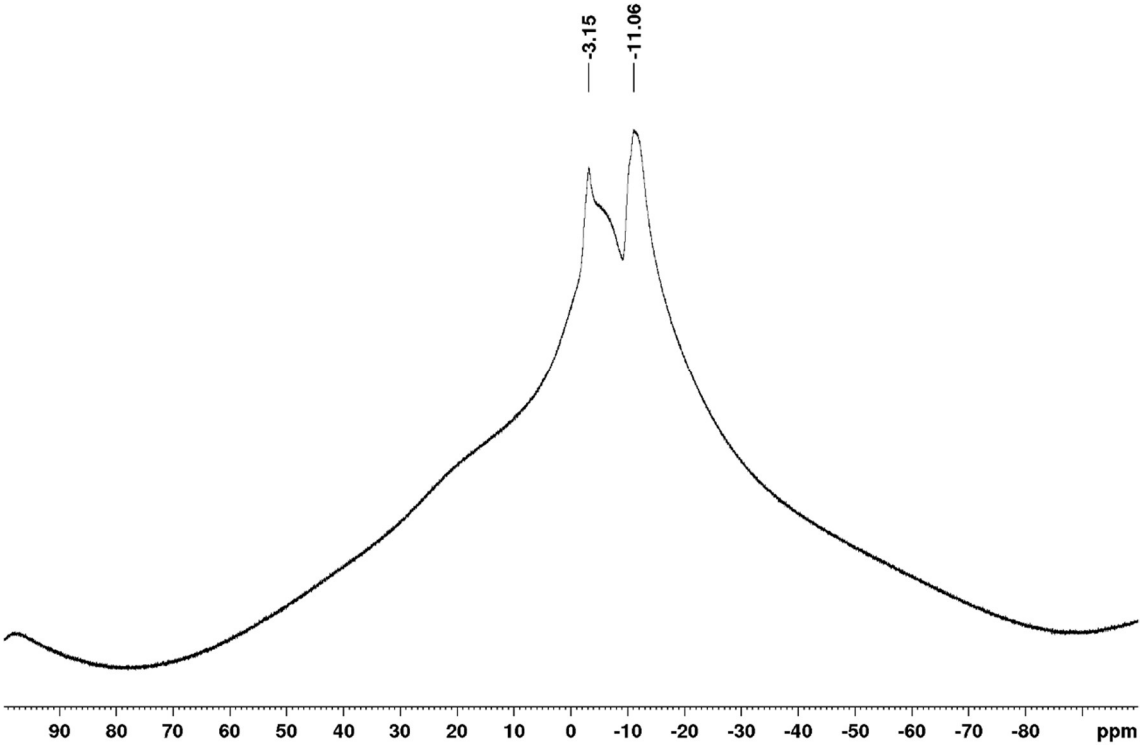
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

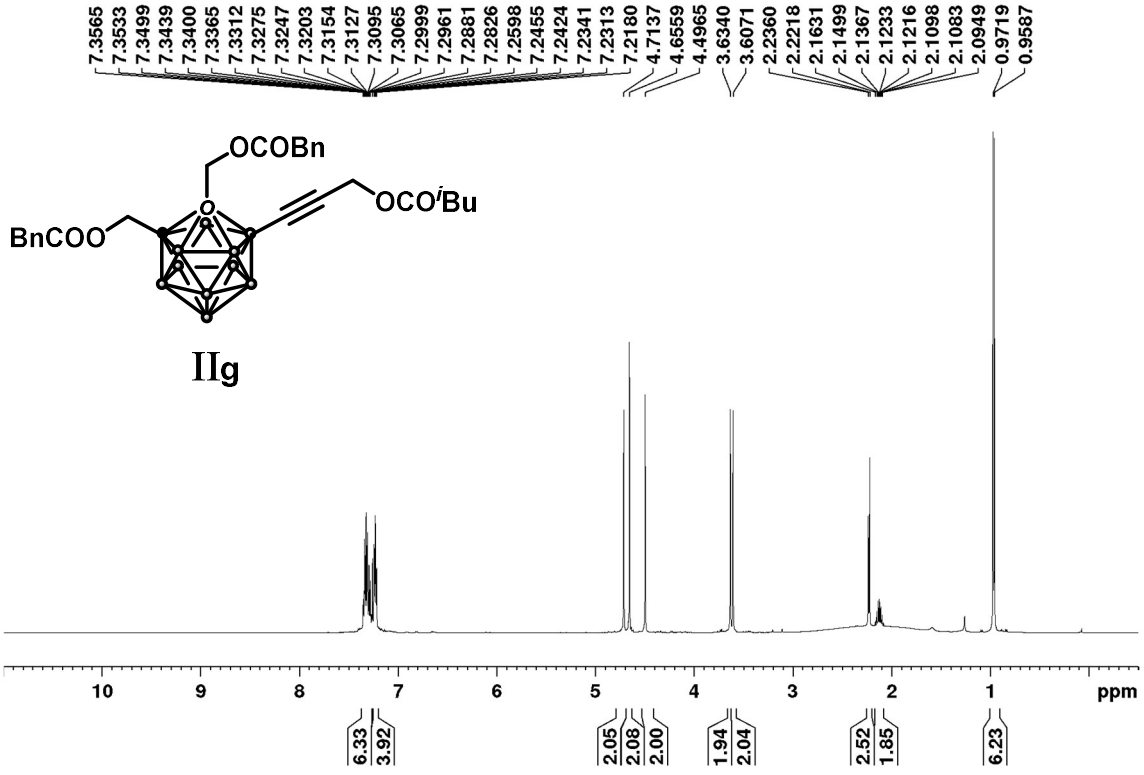


^{11}B NMR (160 MHz; $CDCl_3$)

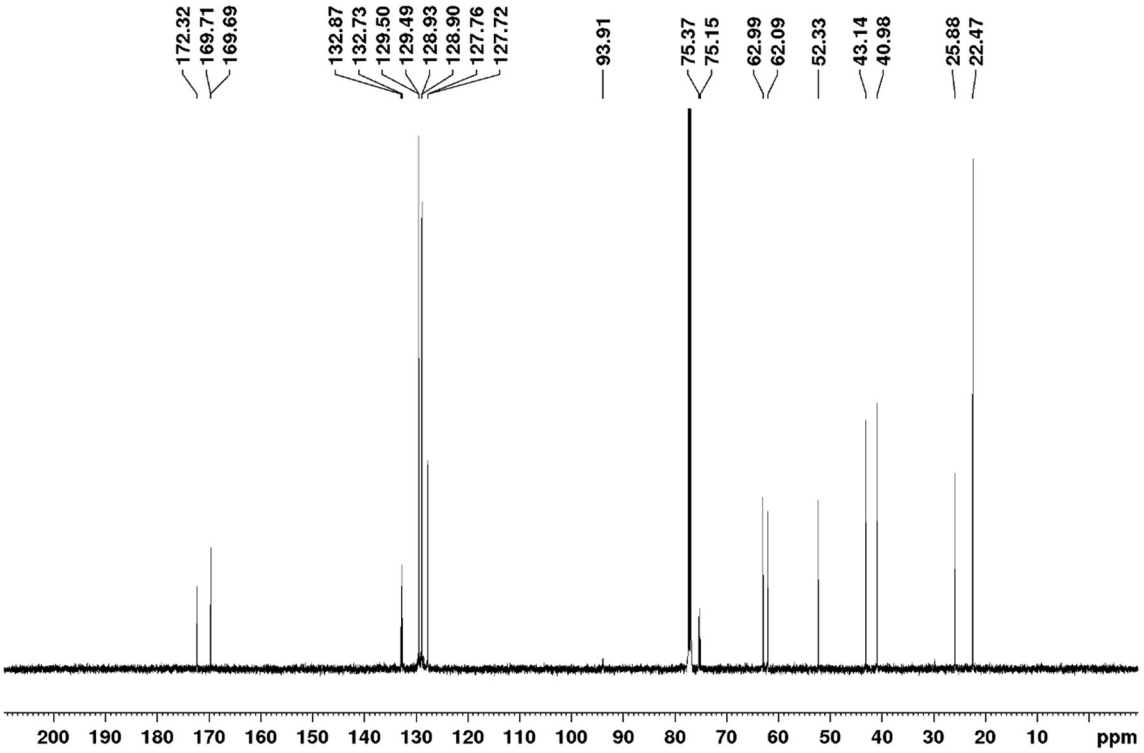


1,2-bis(phenylacetoxymethyl)-4-(2-phenylacetoxy)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIg)

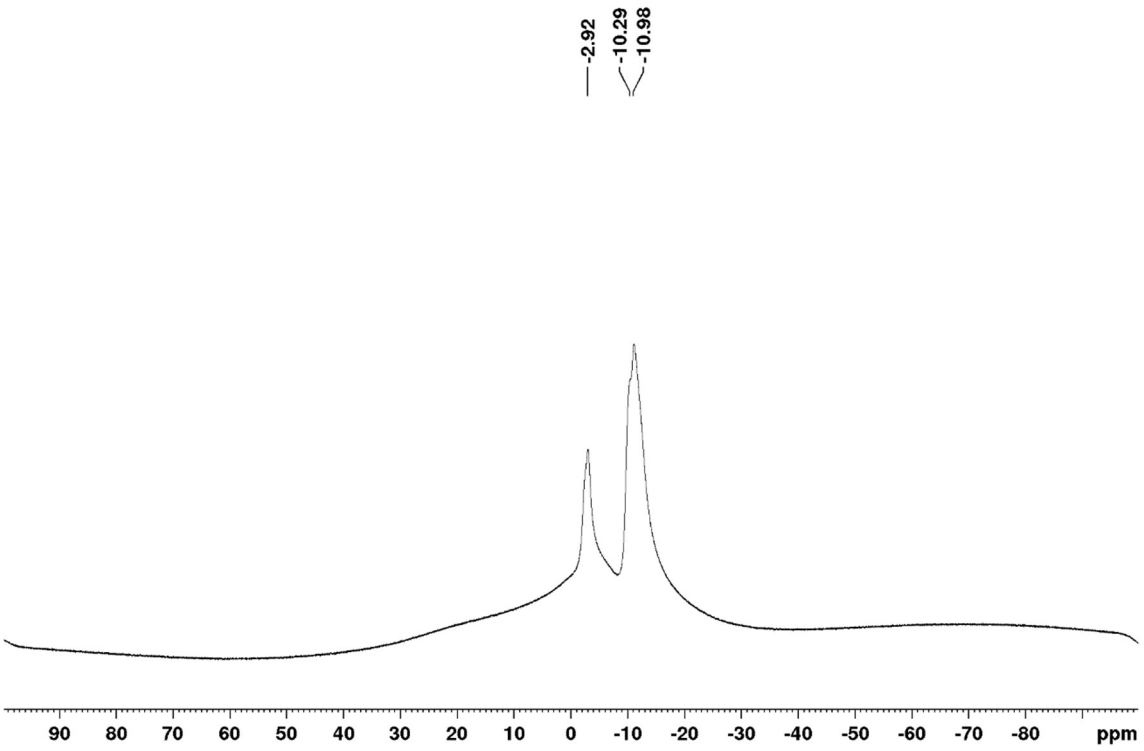
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

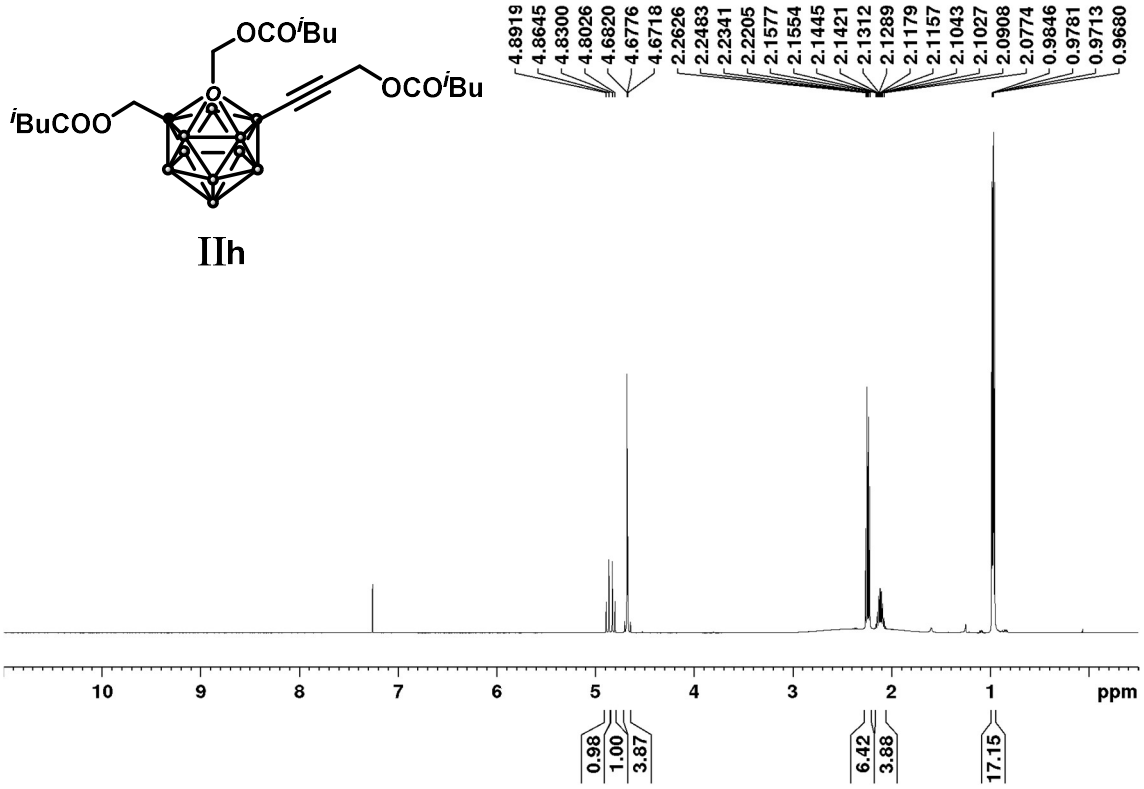


^{11}B NMR (160 MHz; $CDCl_3$)

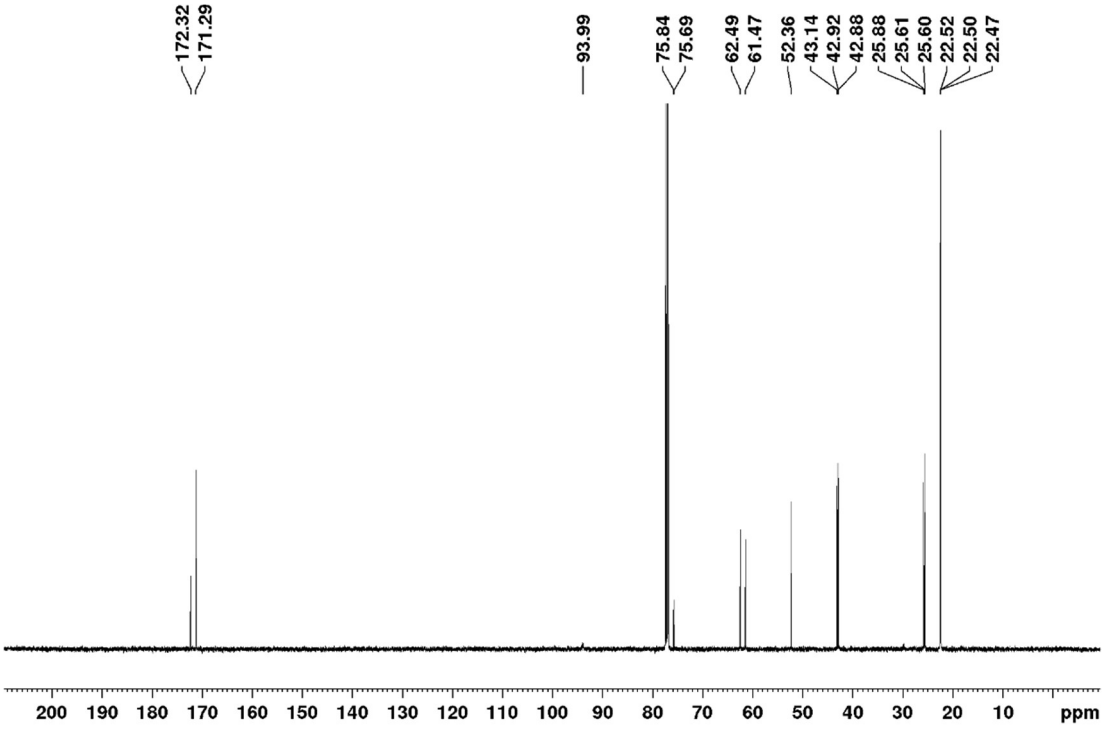


1,2-bis((3-methylbutanoyl)oxymethyl)-4-((3-methylbutanoyl)prop-1-yn-1-yl)-1,2-dicarba-*closo*-dodecaborane (IIh)

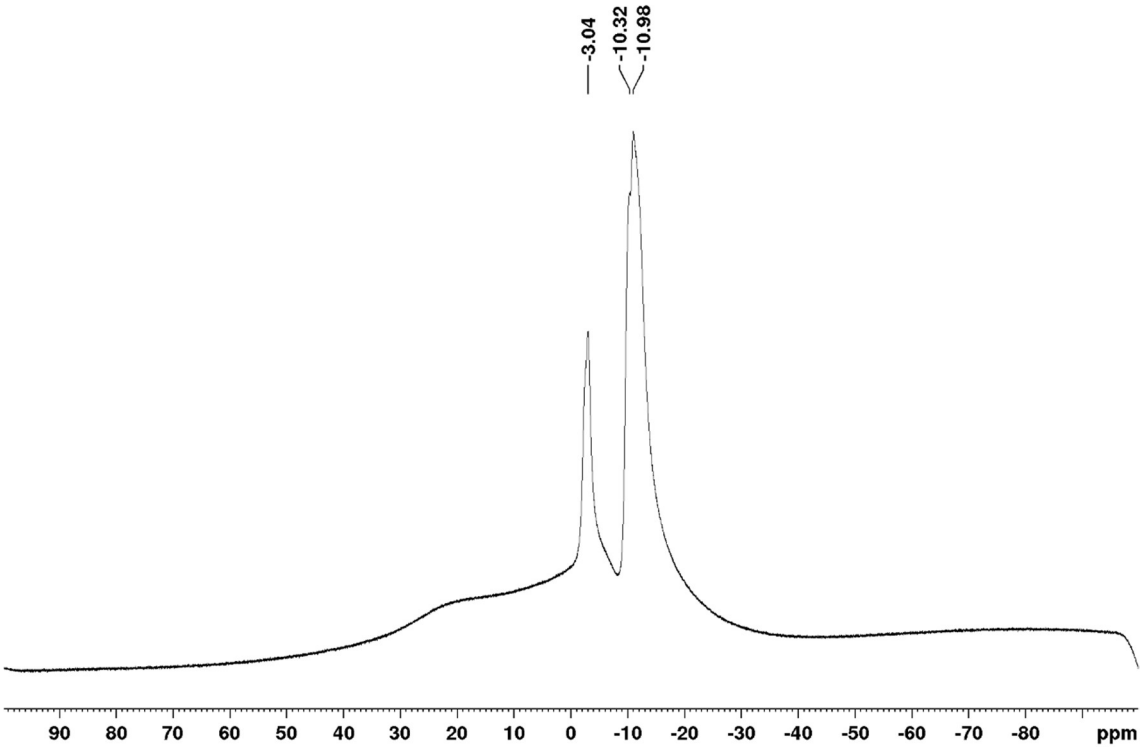
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

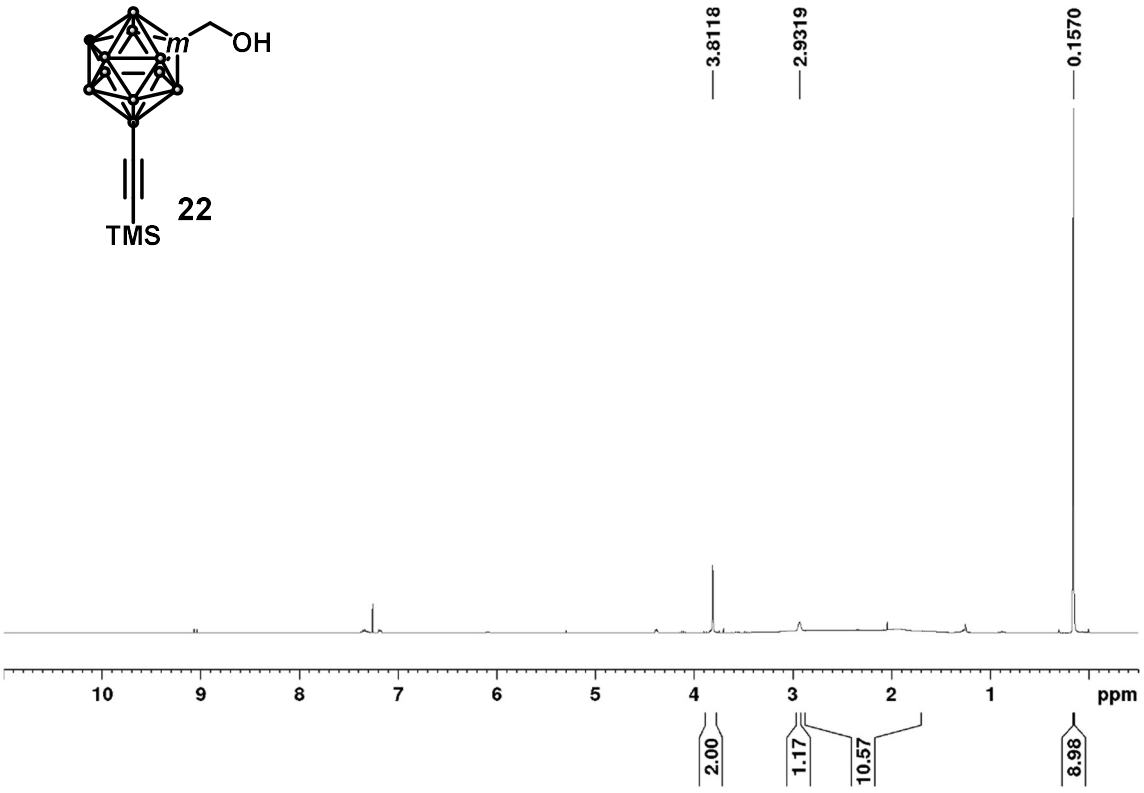


^{11}B NMR (160 MHz; $CDCl_3$)

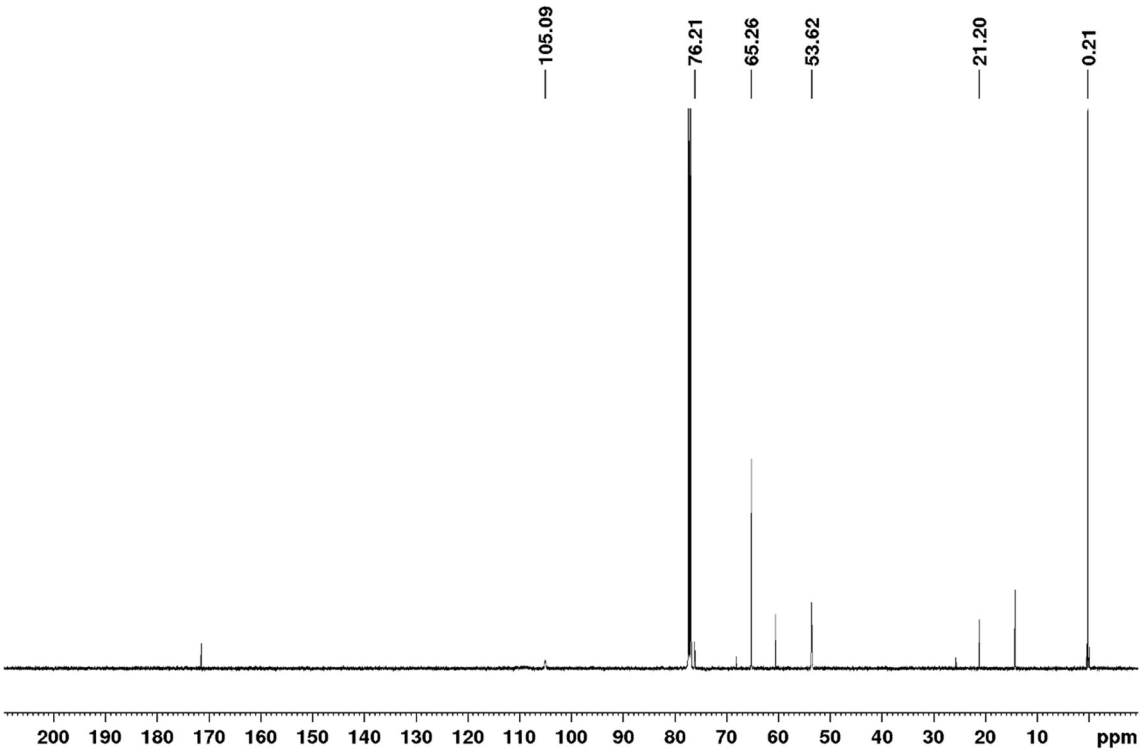


1-hydroxymethyl-9-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (22)

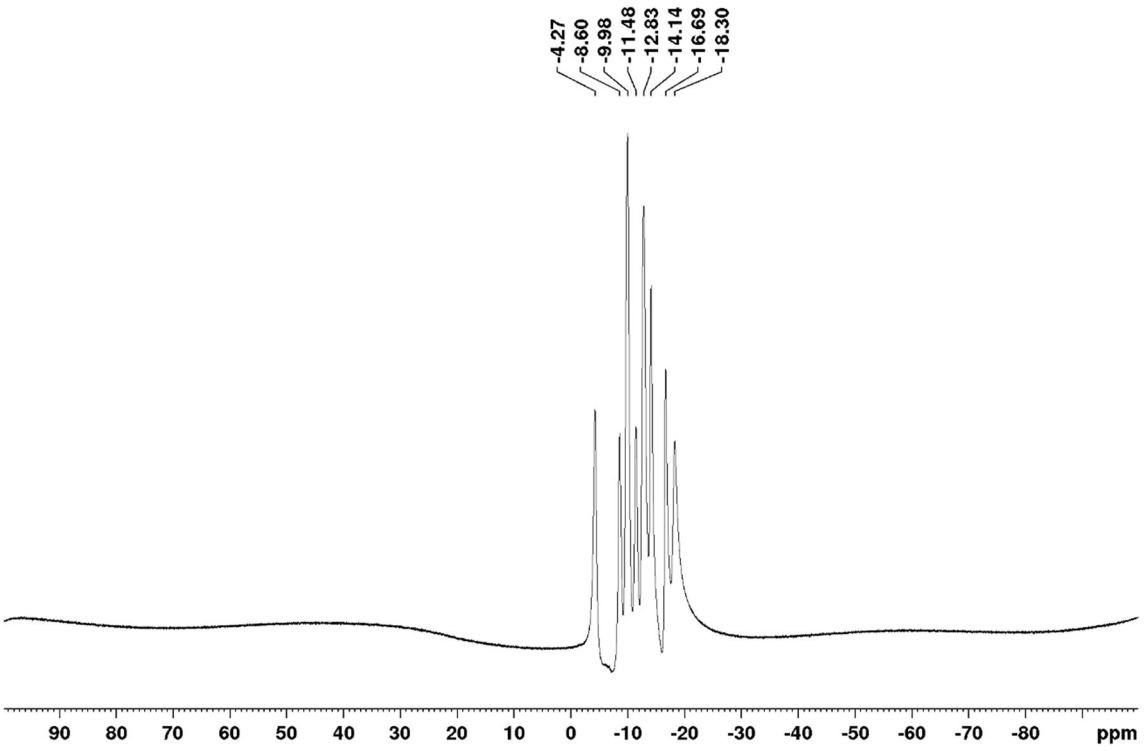
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

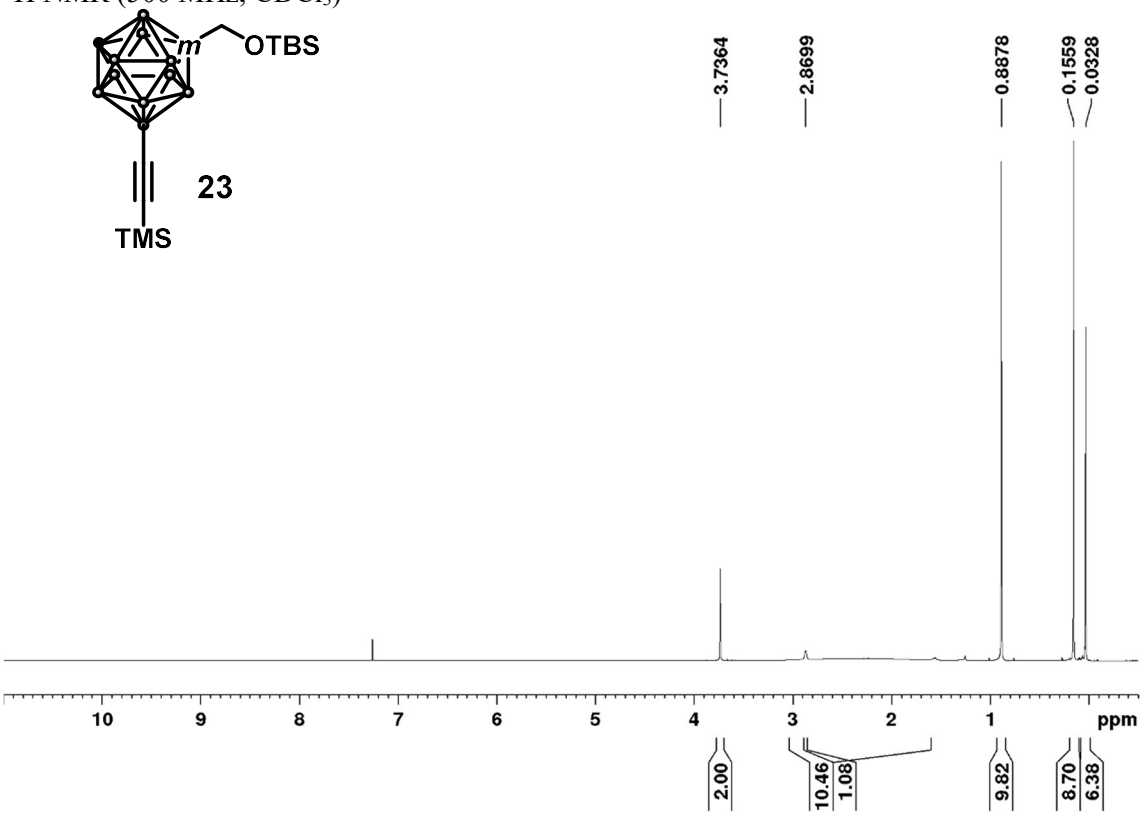


^{11}B NMR (160 MHz; $CDCl_3$)

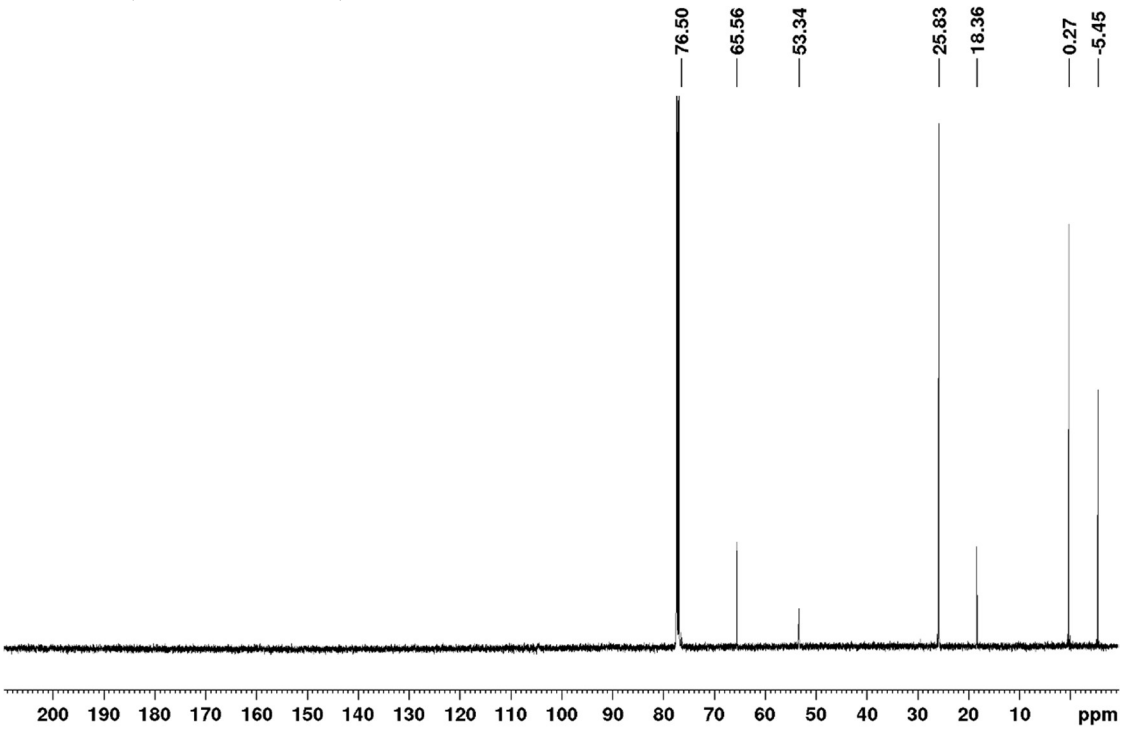


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-9-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (23)

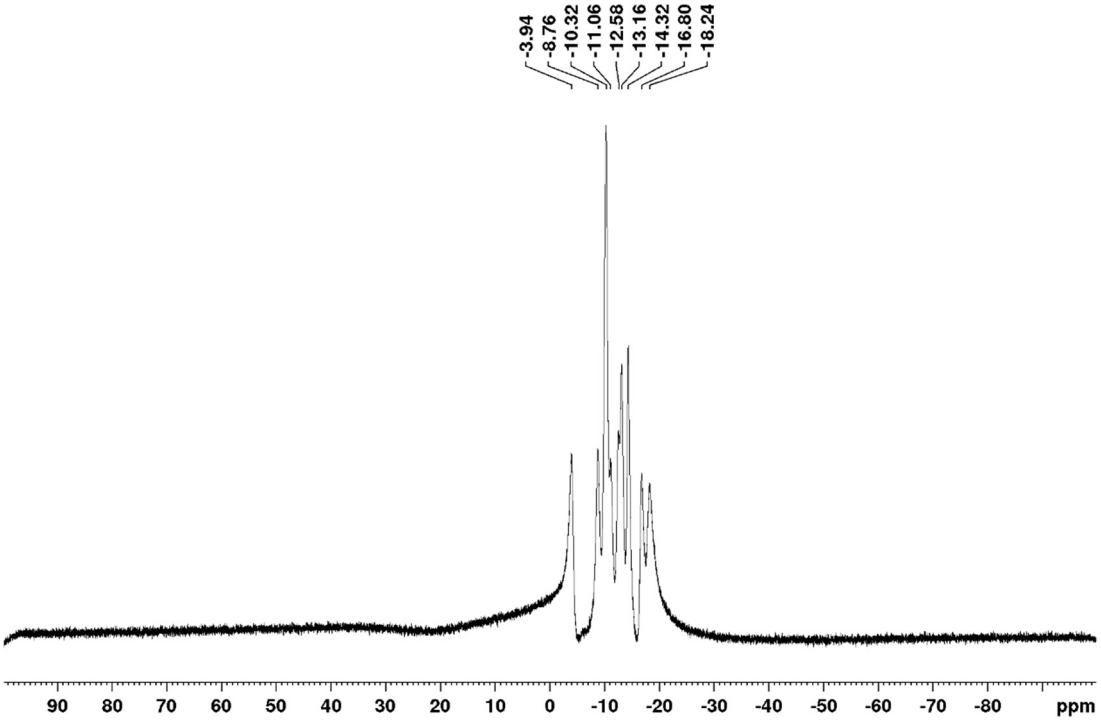
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

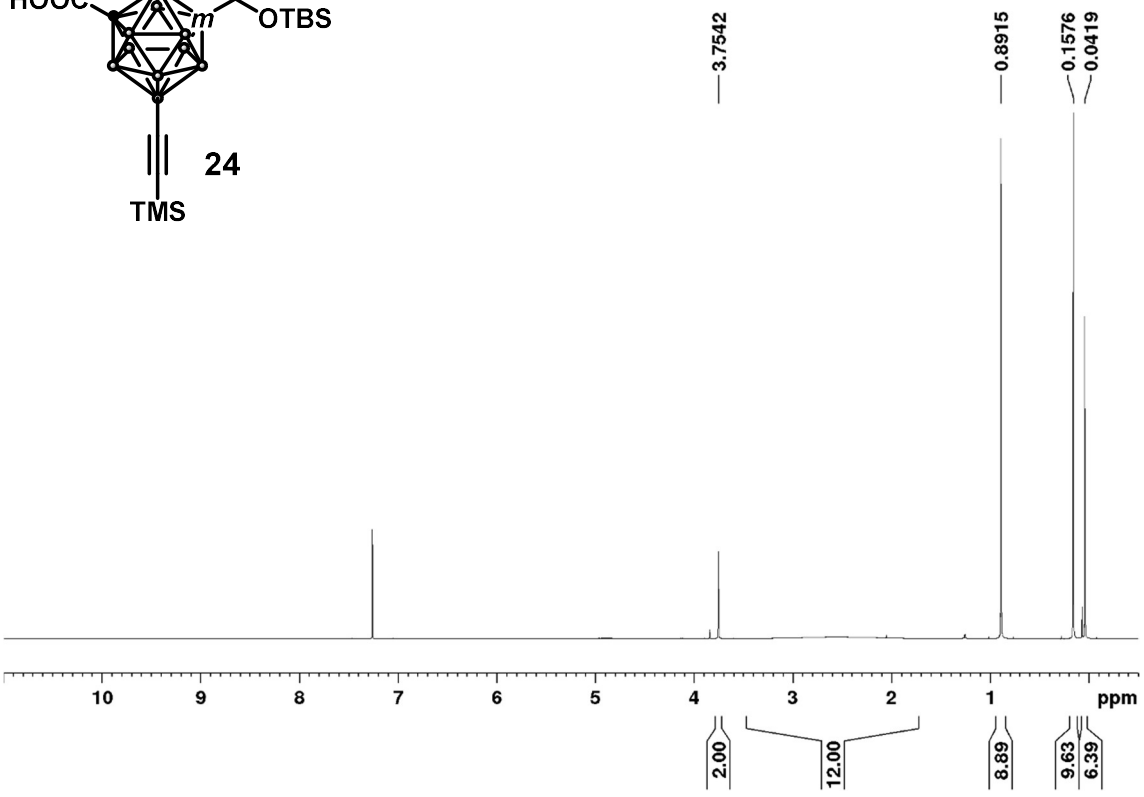


^{11}B NMR (160 MHz; $CDCl_3$)

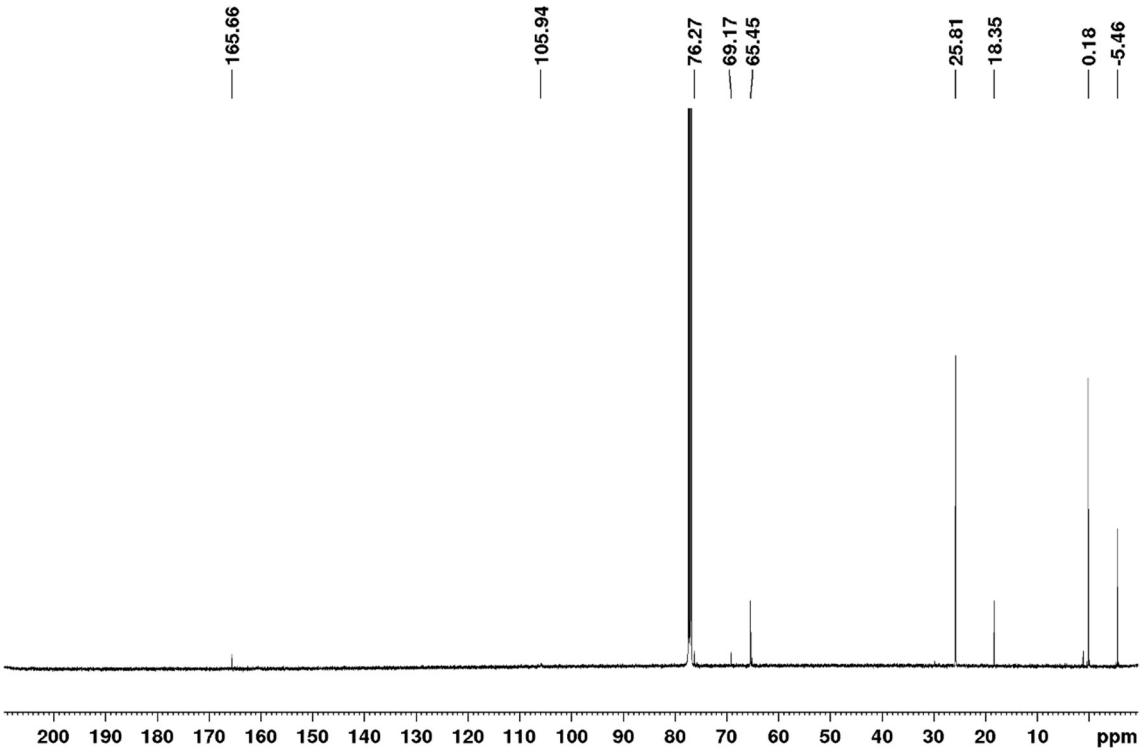


7-((*tert*-butyl)dimethylsilyl)hydroxymethyl-9-(trimethylsilyl)ethynyl-1,7-dicarba-*closo*-dodecaboranylcarboxylic acid (24)

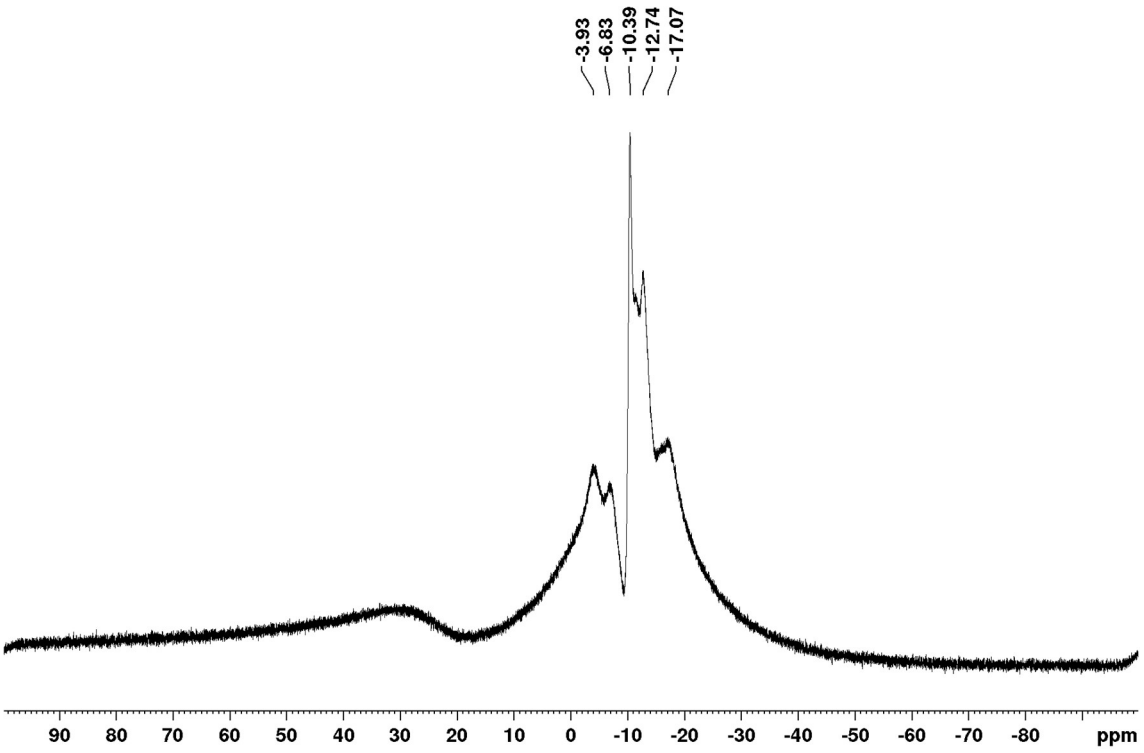
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

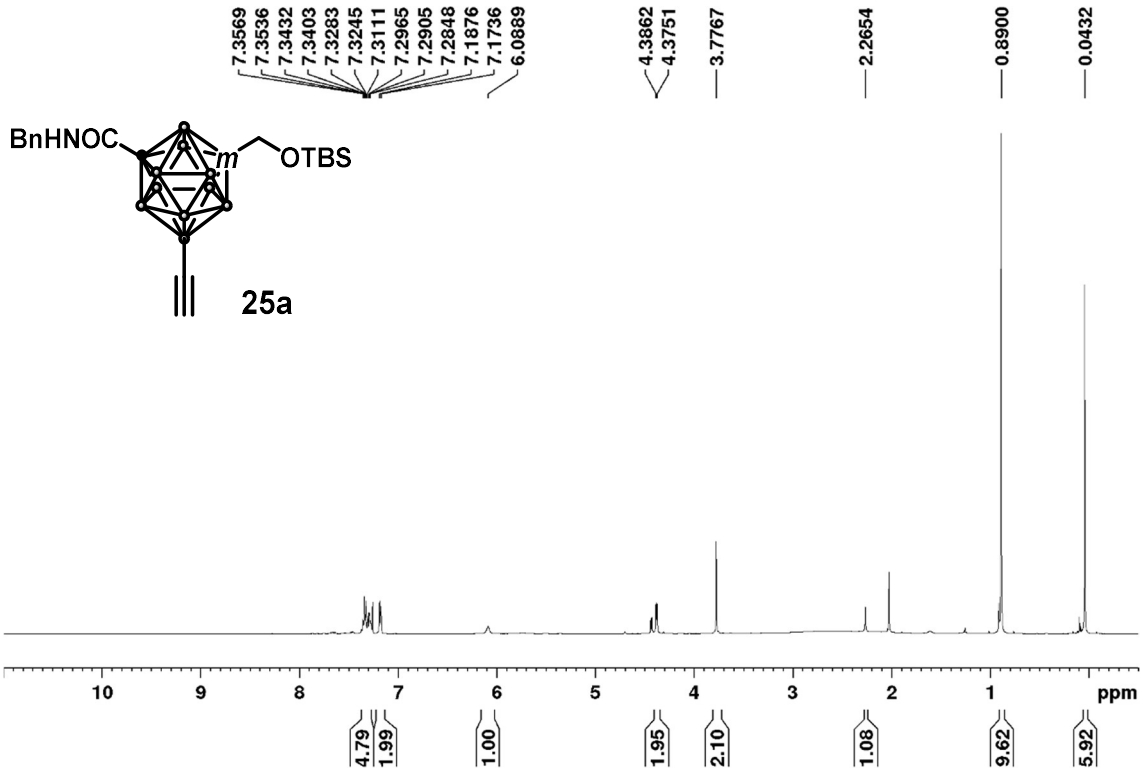


^{11}B NMR (160 MHz; $CDCl_3$)

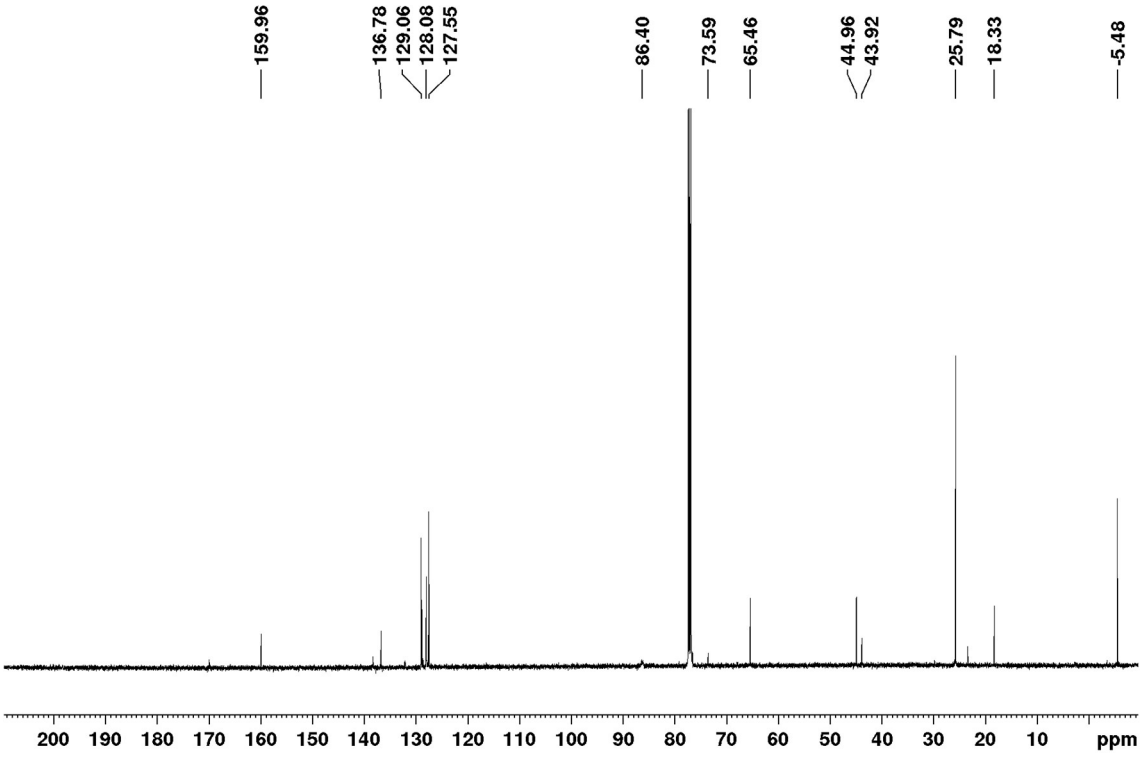


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-benzylaminocarboxyl-9-ethynyl-1,7-dicarba-*closo*-dodecaborane (25a)

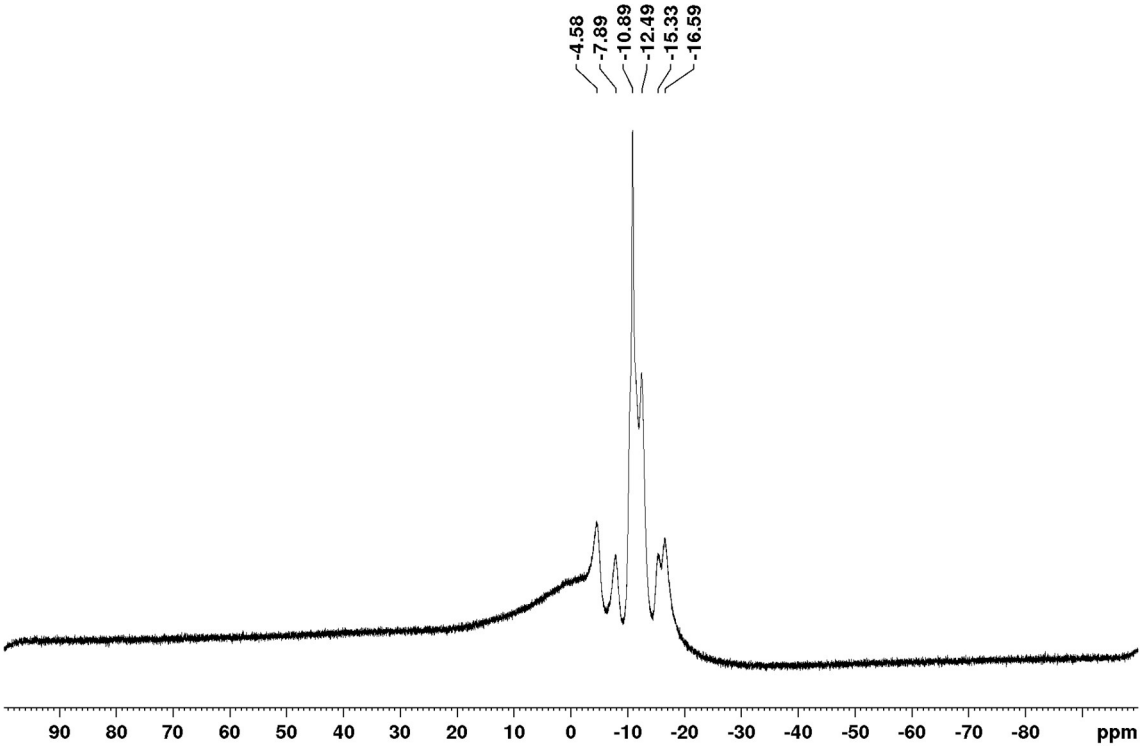
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

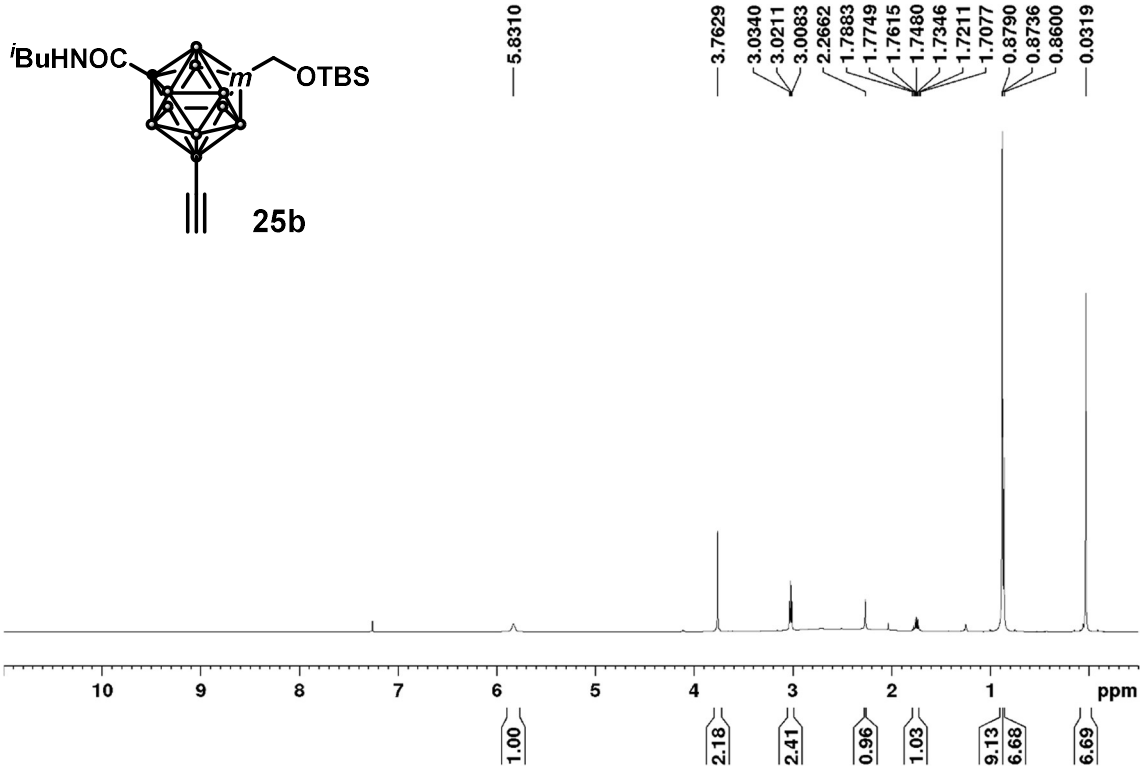


^{11}B NMR (160 MHz; $CDCl_3$)

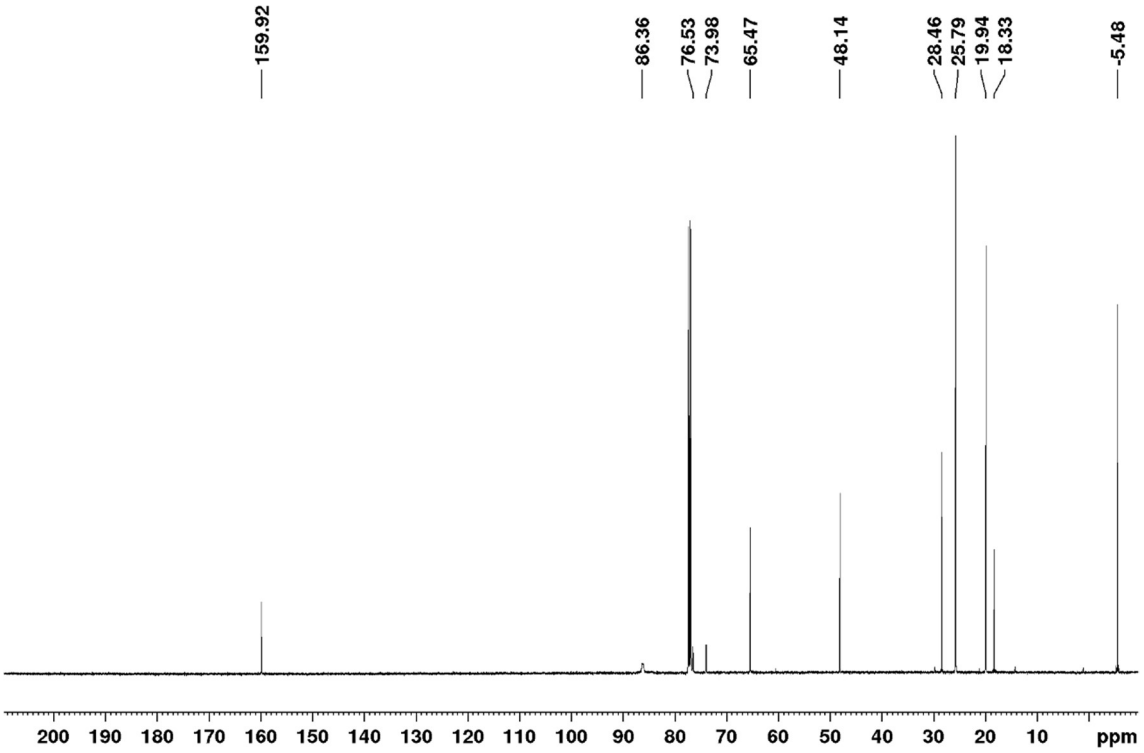


Synthesis of 1-((*tert*-butyl)dimethylsilyl)hydroxymethyl-7-isobutylcarbamoyl-9-ethynyl-1,7-dicarba-*closo*-dodecaborane (25b)

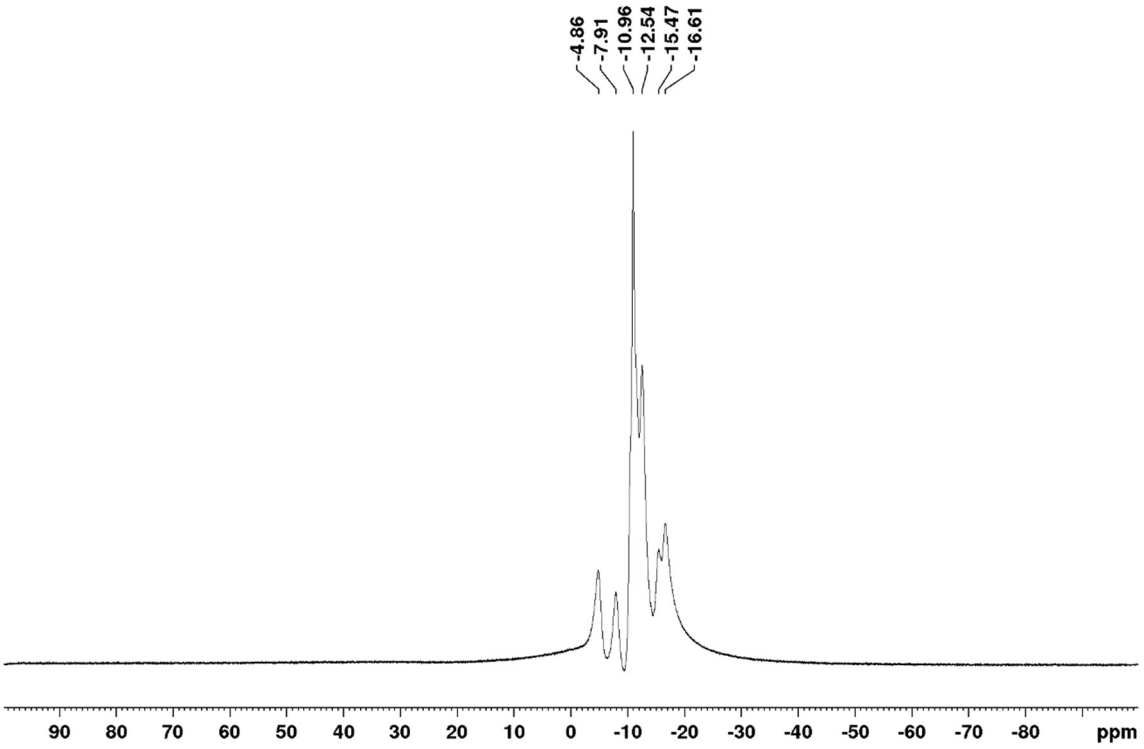
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

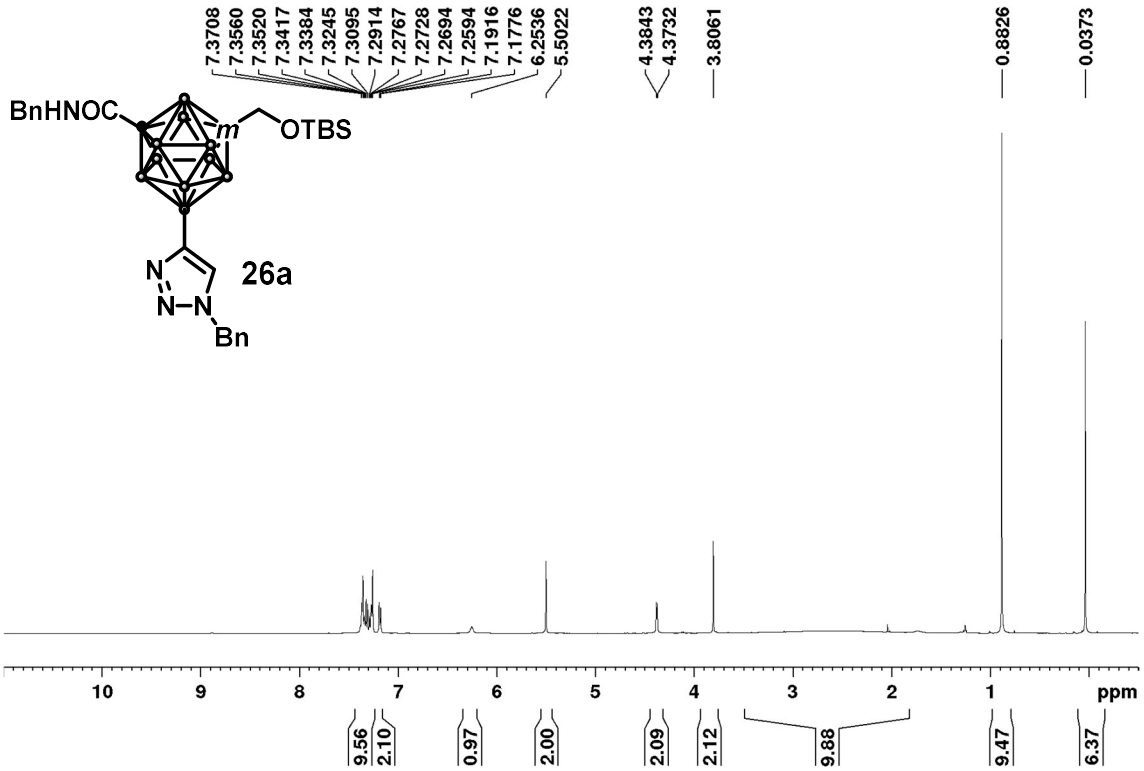


^{11}B NMR (160 MHz; $CDCl_3$)

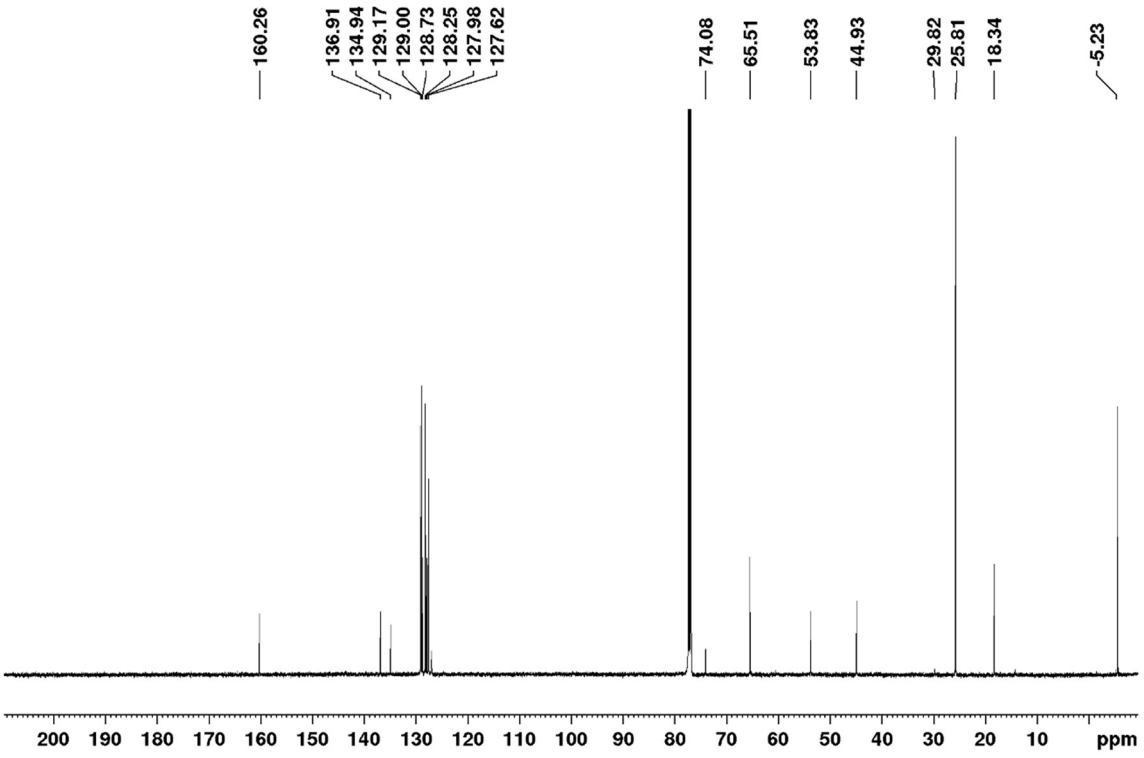


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-benzylaminocarboxyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (26a)

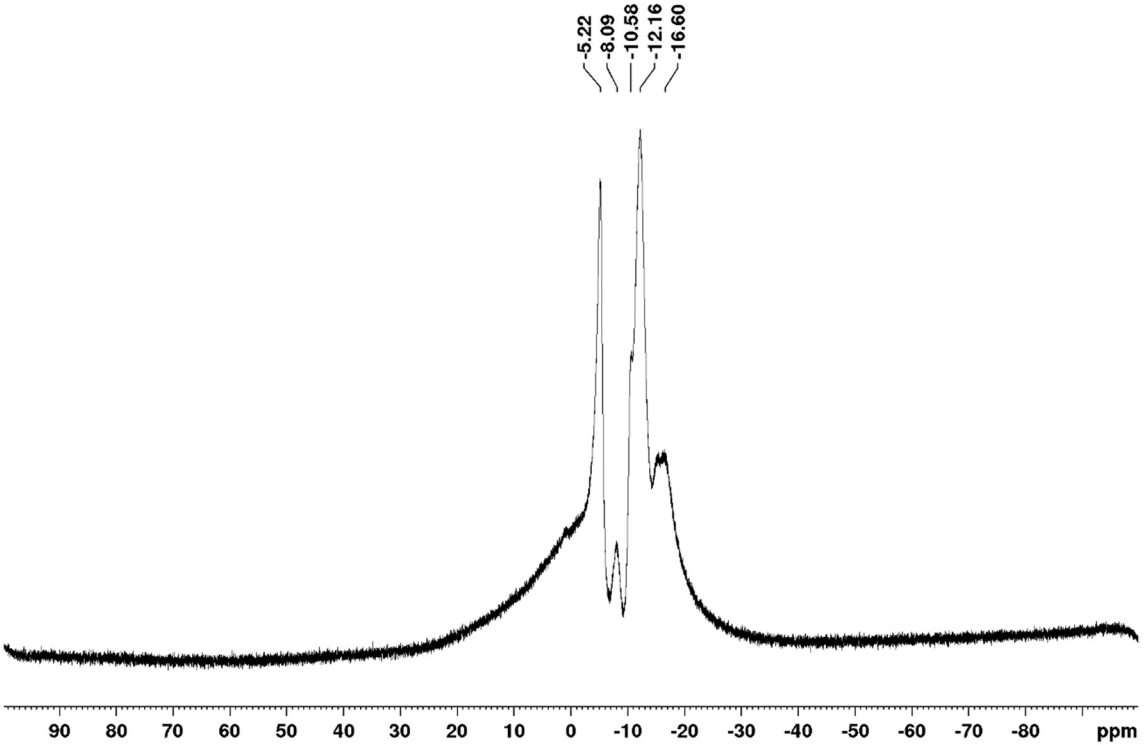
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

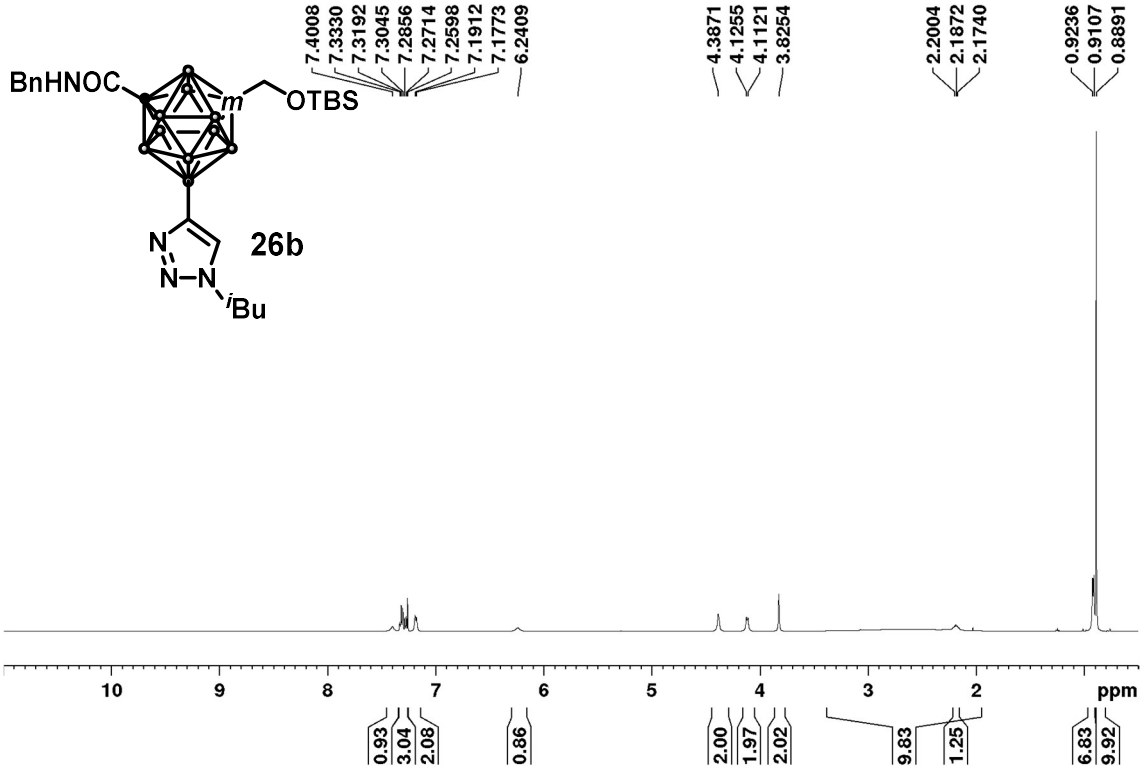


^{11}B NMR (160 MHz; $CDCl_3$)

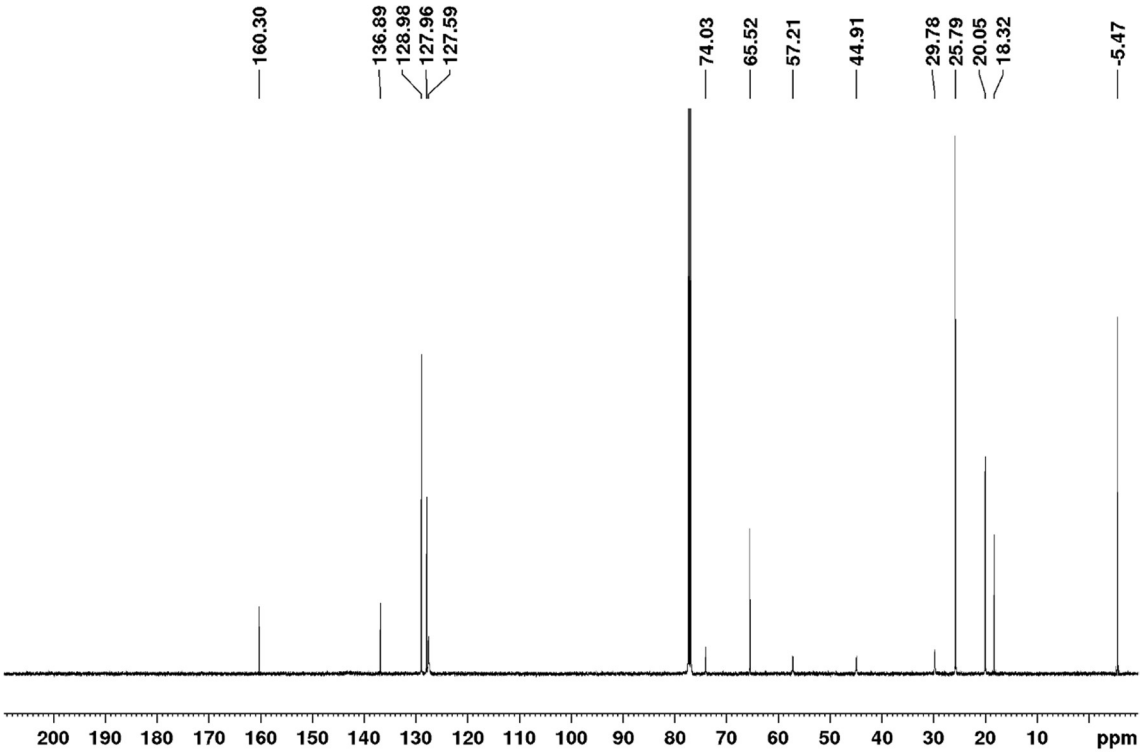


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (26b)

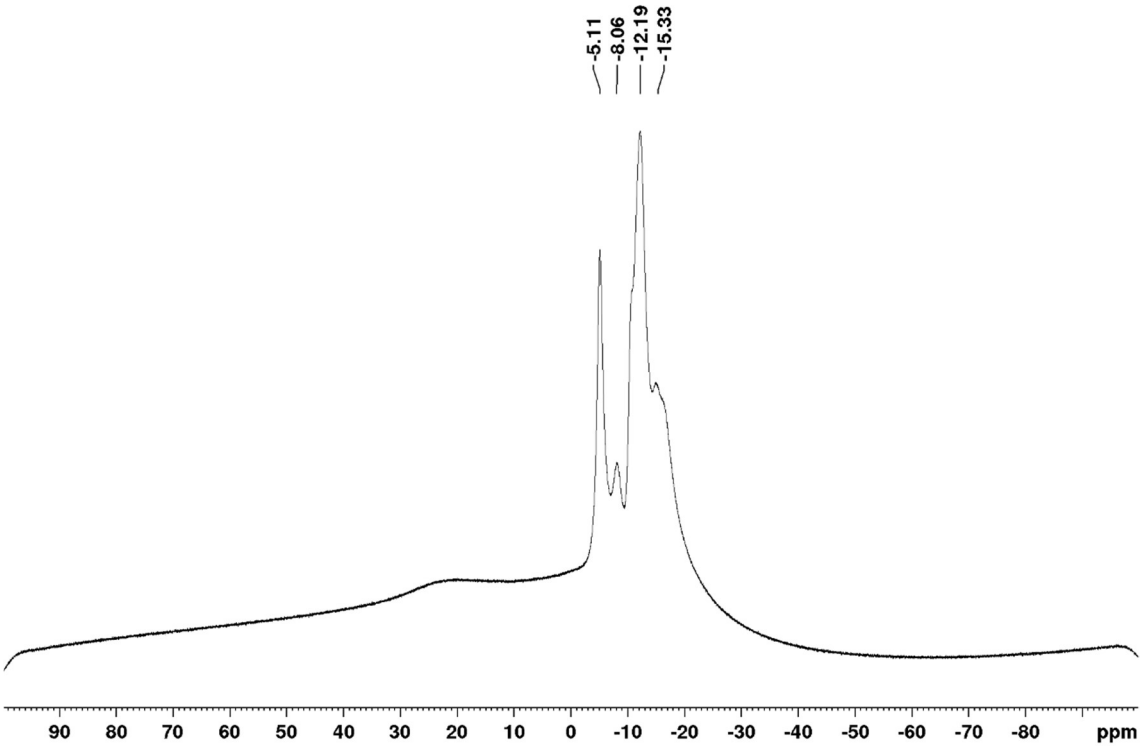
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

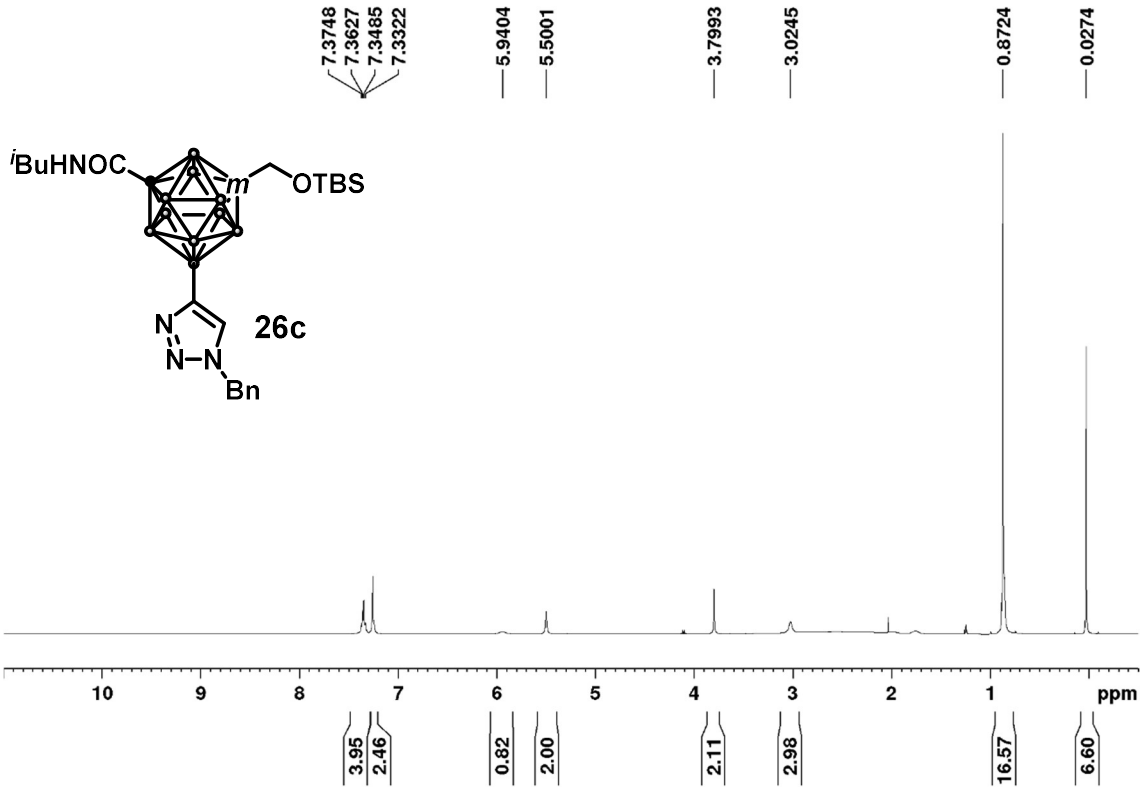


^{11}B NMR (160 MHz; $CDCl_3$)

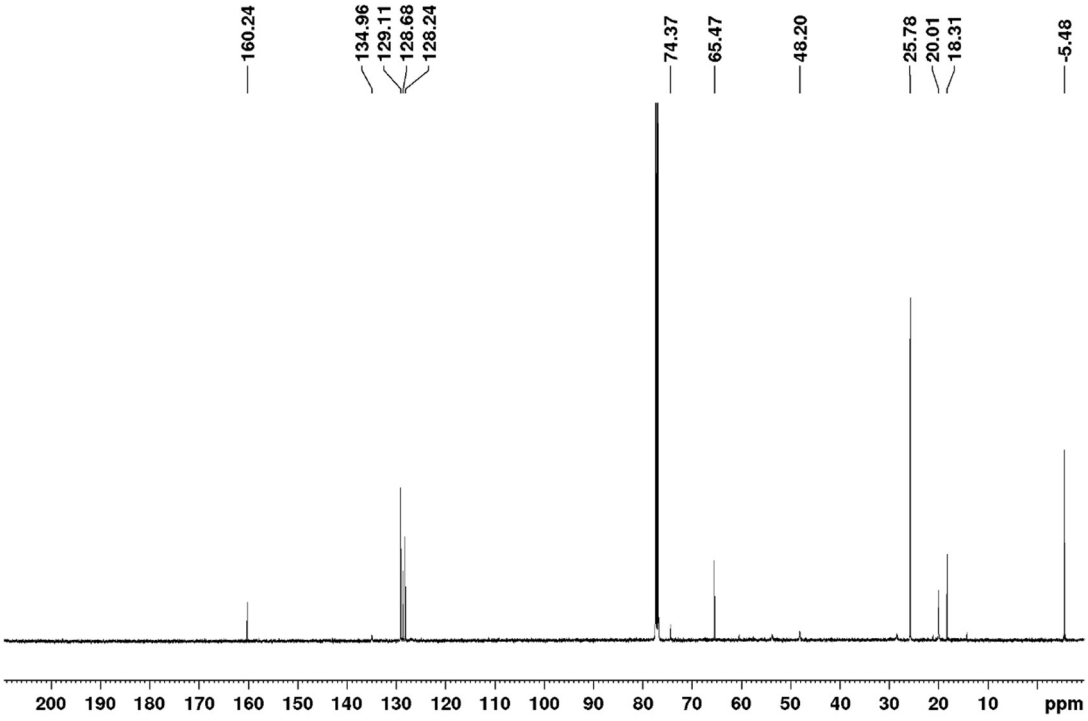


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-isobutylcarbamoyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (26c)

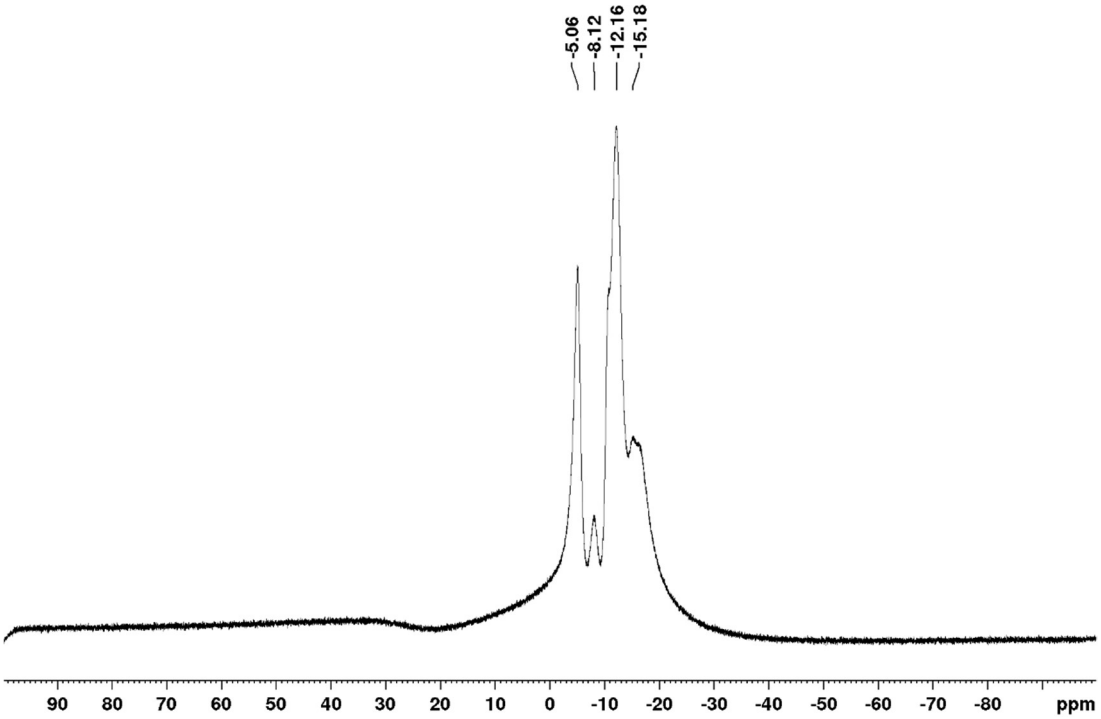
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

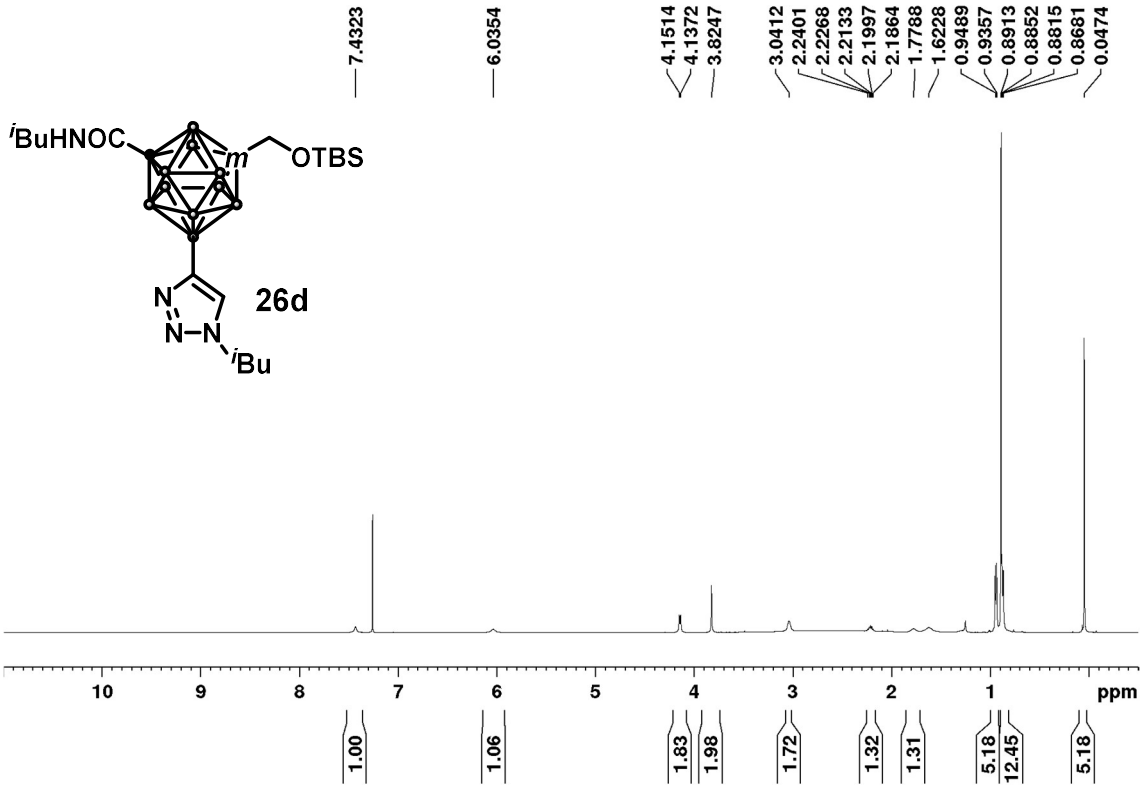


^{11}B NMR (160 MHz; $CDCl_3$)

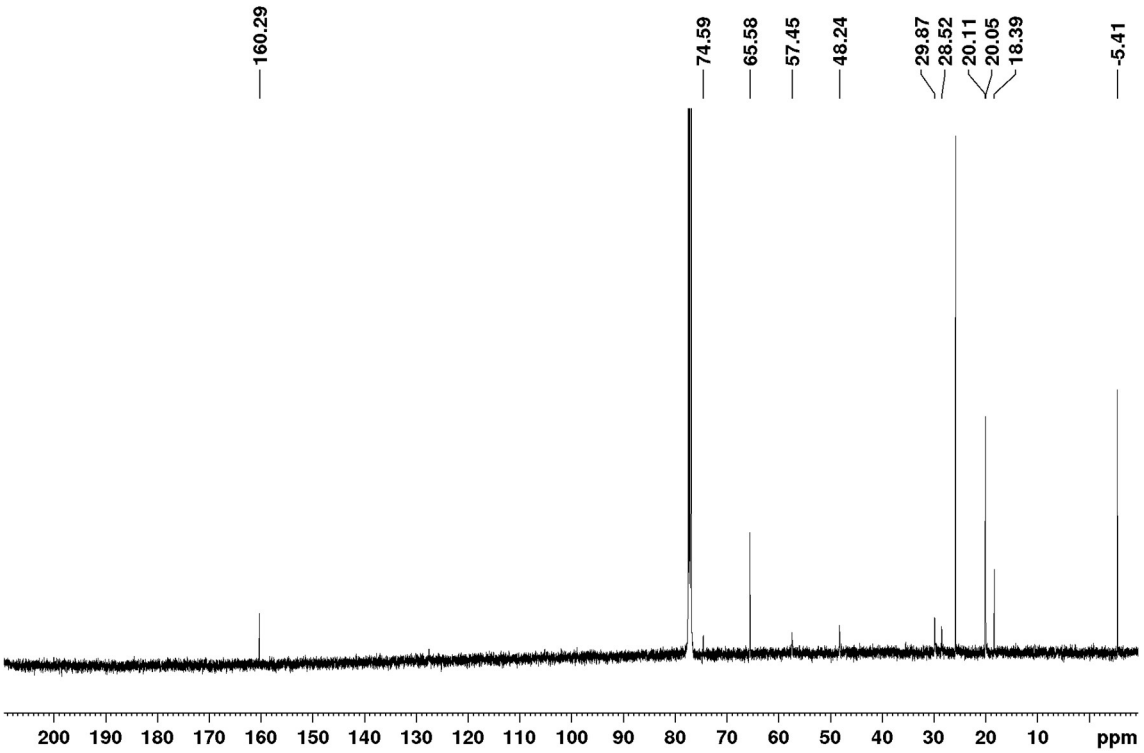


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-isobutylcarbamoyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (26d)

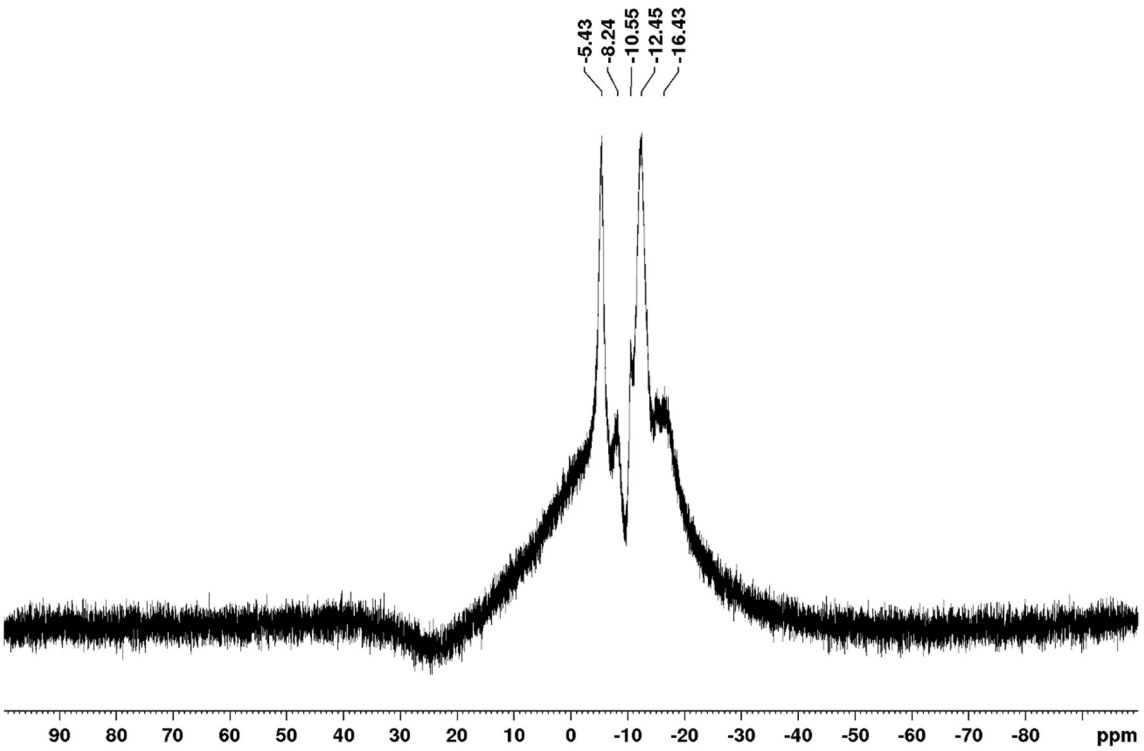
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

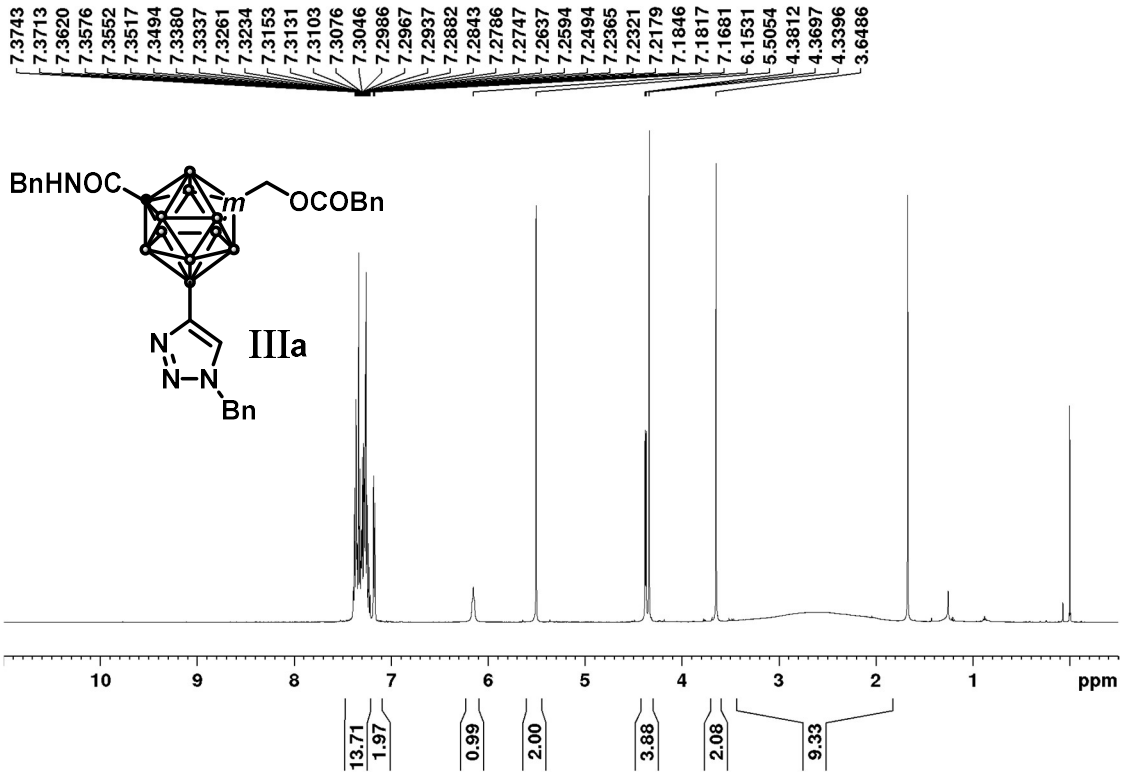


^{11}B NMR (160 MHz; $CDCl_3$)

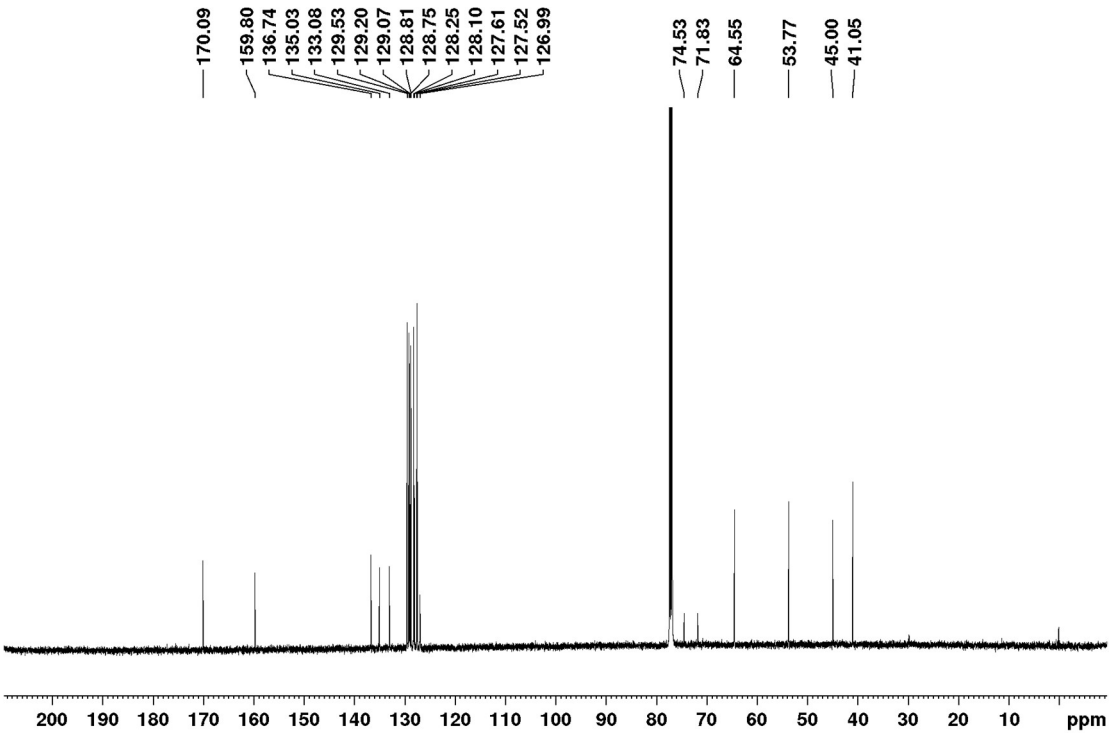


1-phenylacetoxymethyl-7-benzylaminocarboxyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIa)

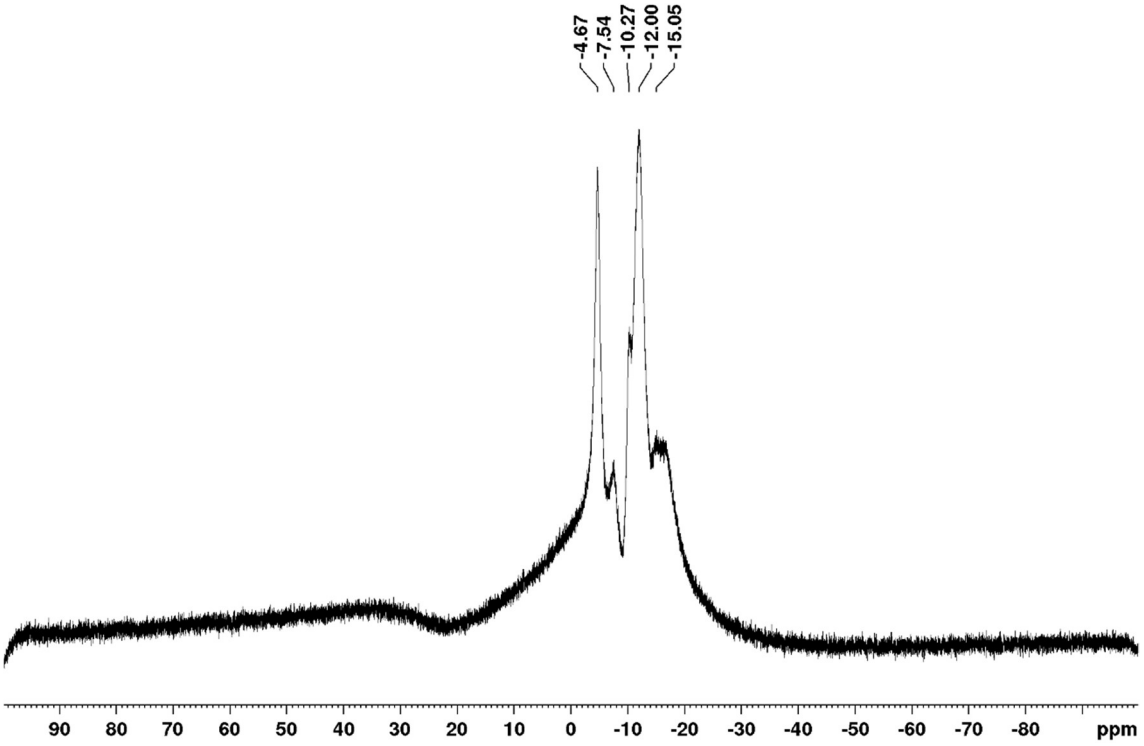
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

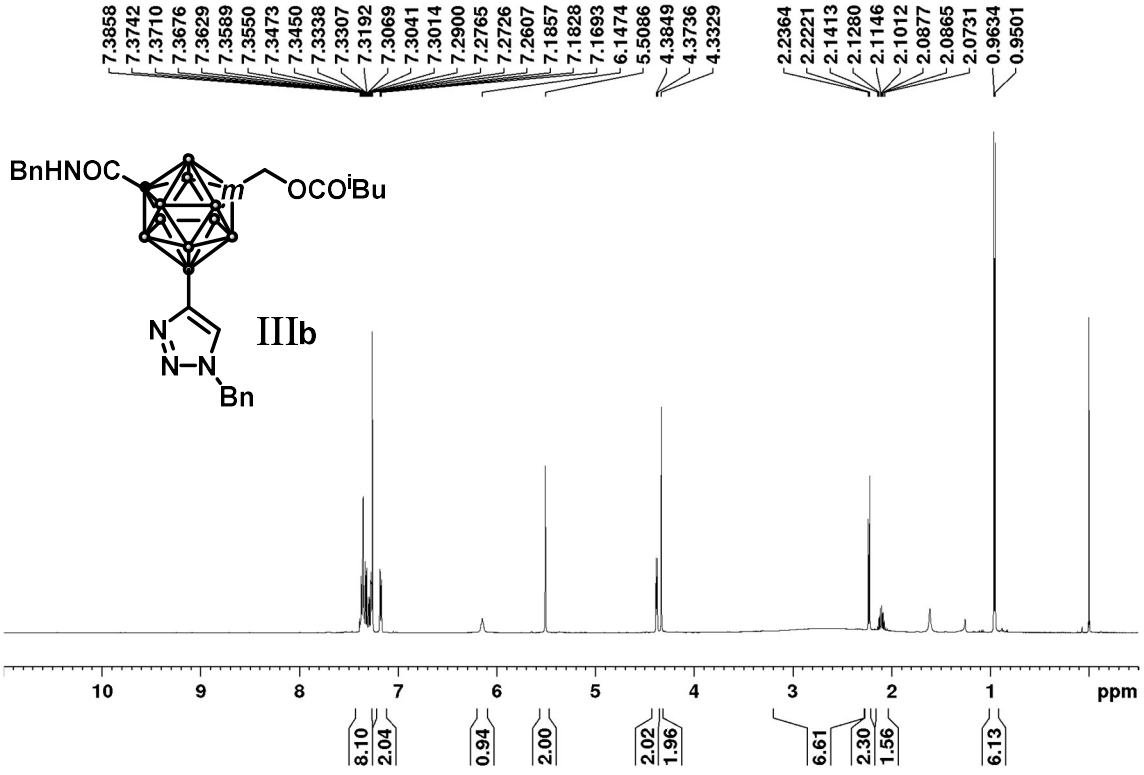


^{11}B NMR (160 MHz; $CDCl_3$)

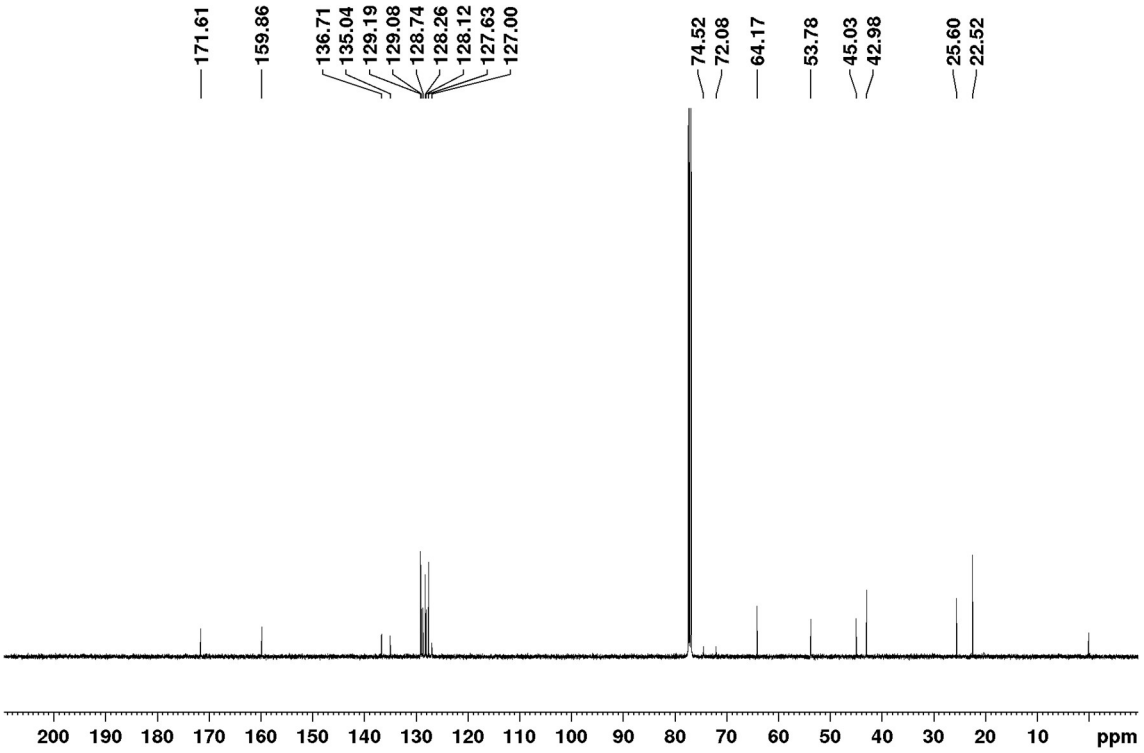


1-(3-methylbutanoyl)oxymethyl-7-benzylaminocarboxyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIb)

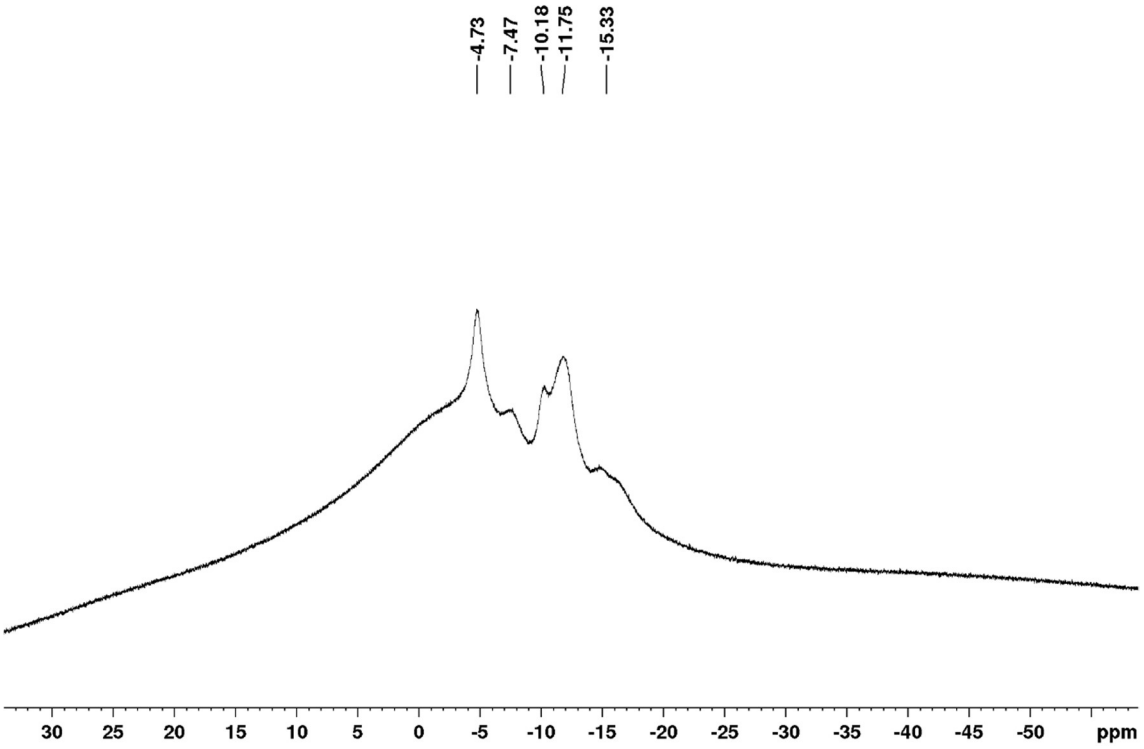
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

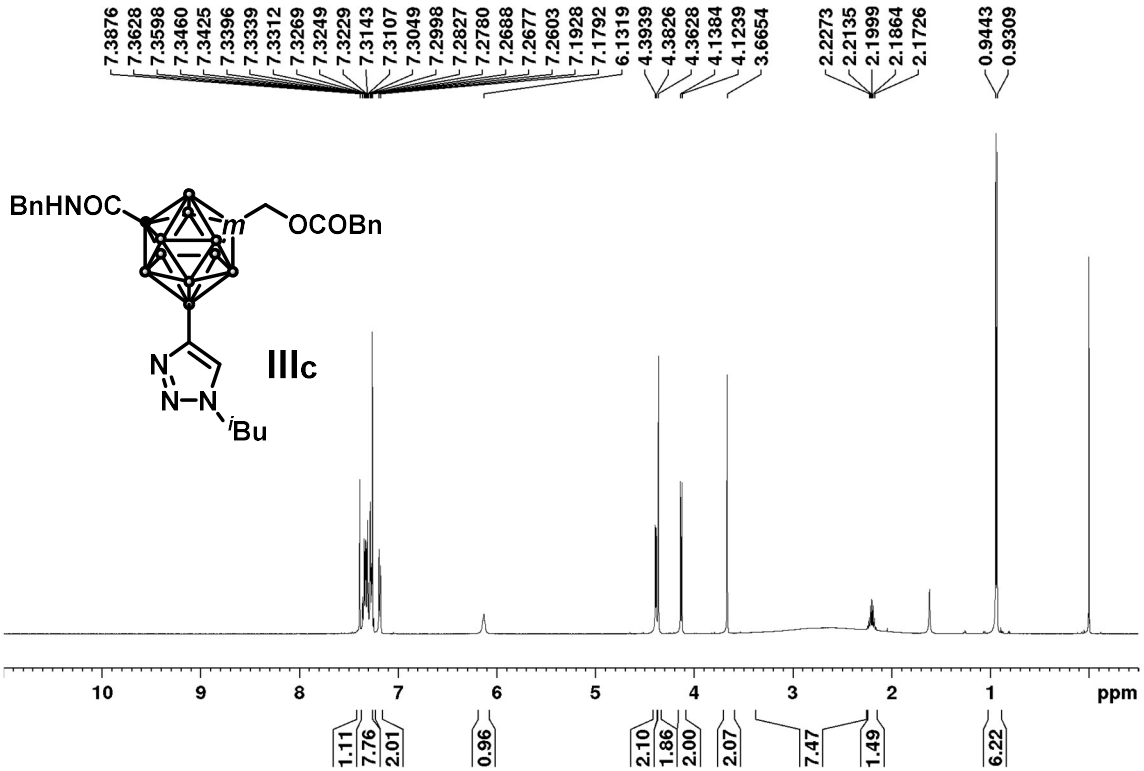


^{11}B NMR (160 MHz; $CDCl_3$)

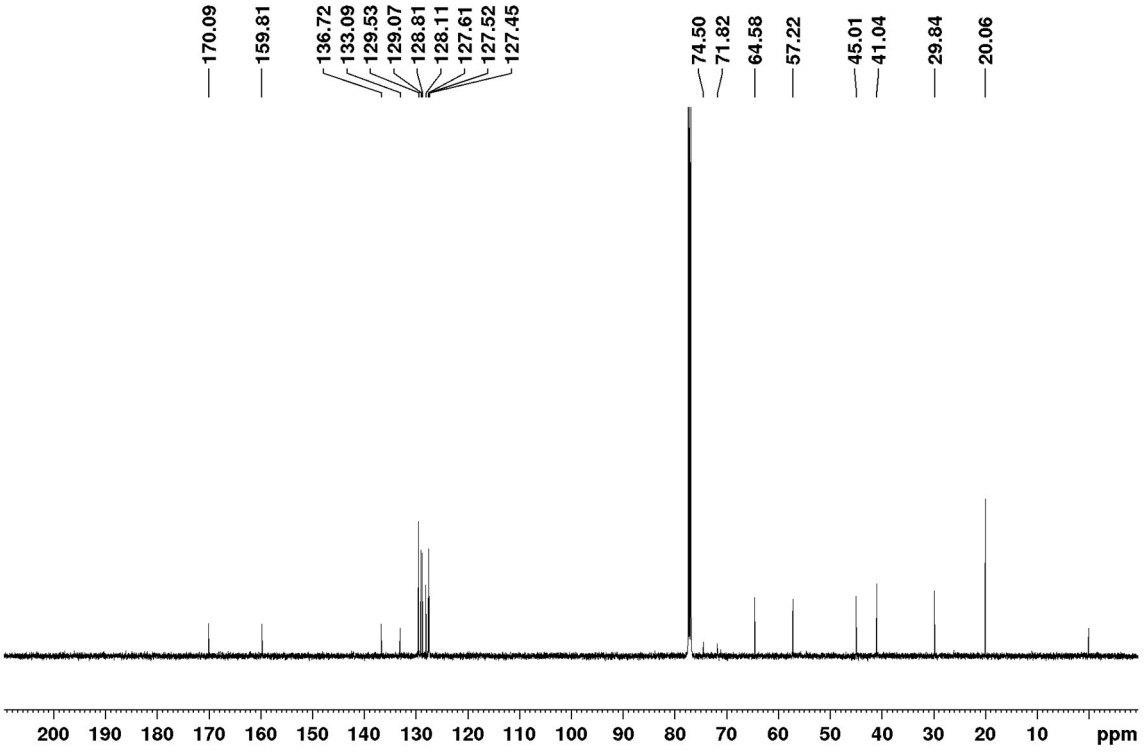


1-phenylacetoxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIIc)

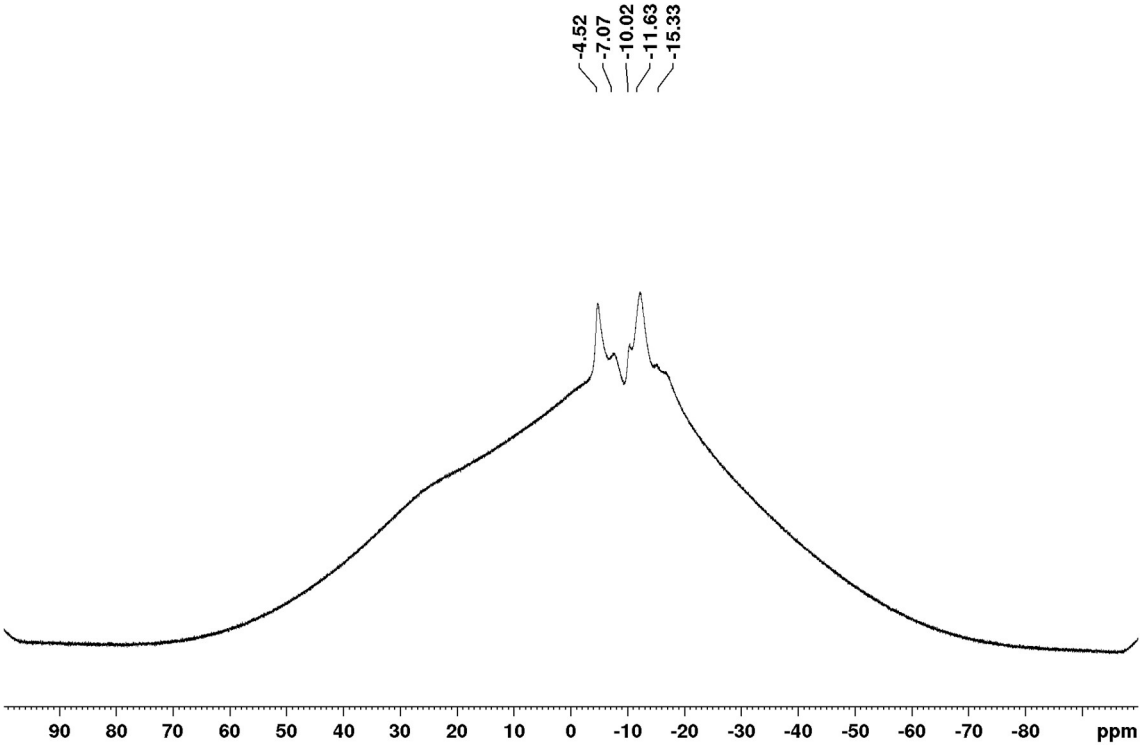
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

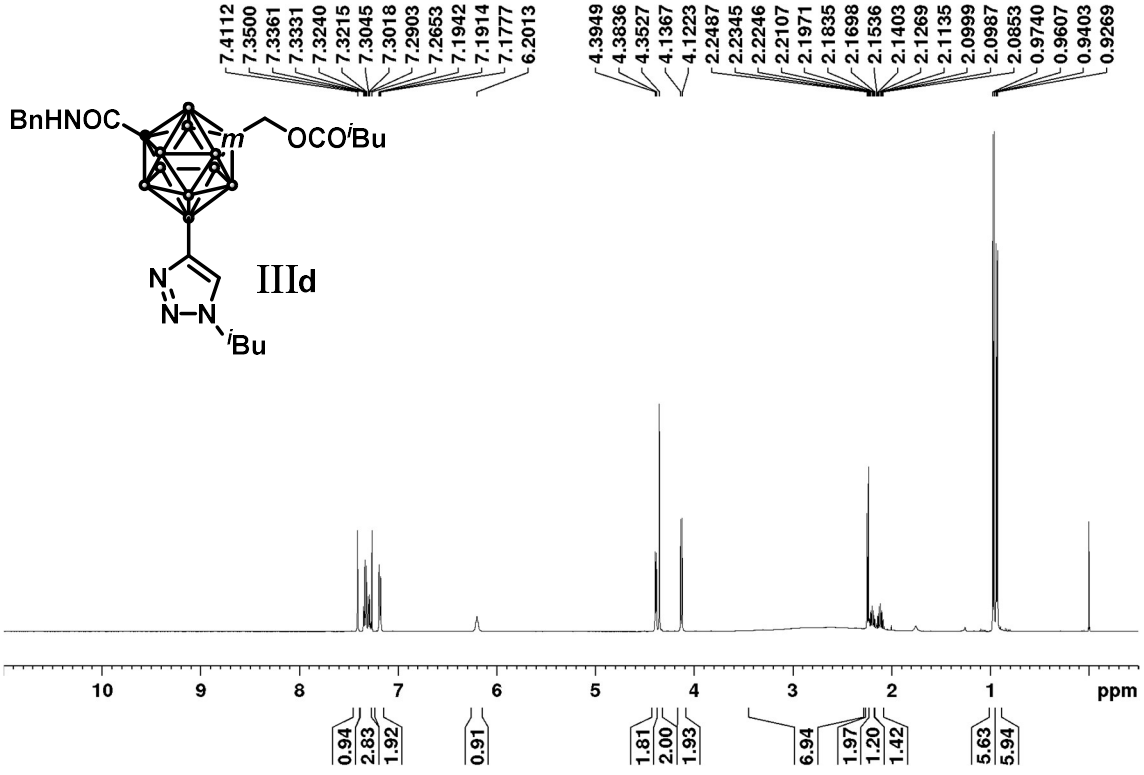


^{11}B NMR (160 MHz; $CDCl_3$)

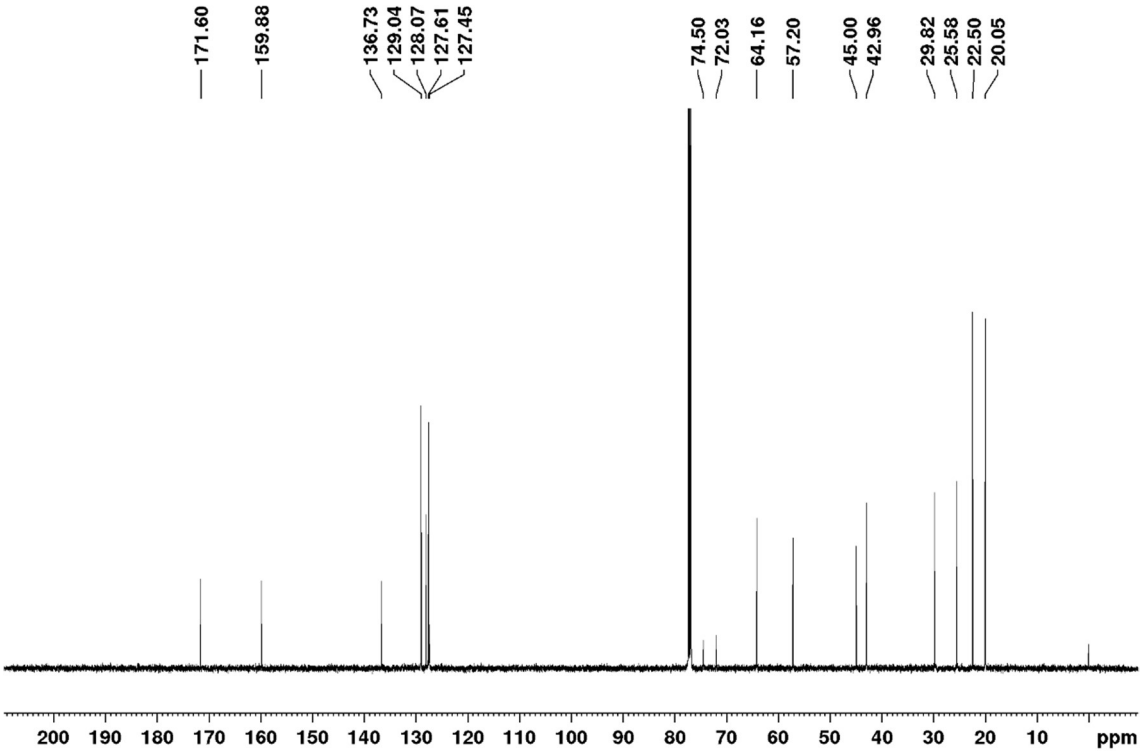


1-(3-methylbutanoyl)oxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIId)

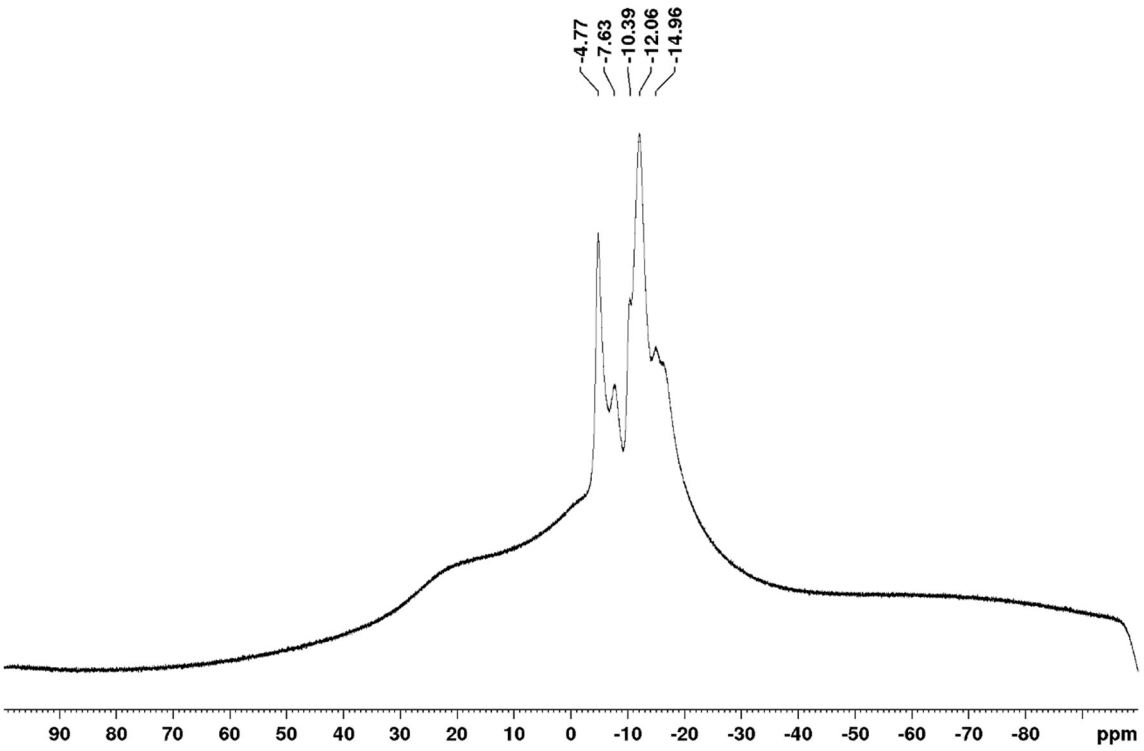
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

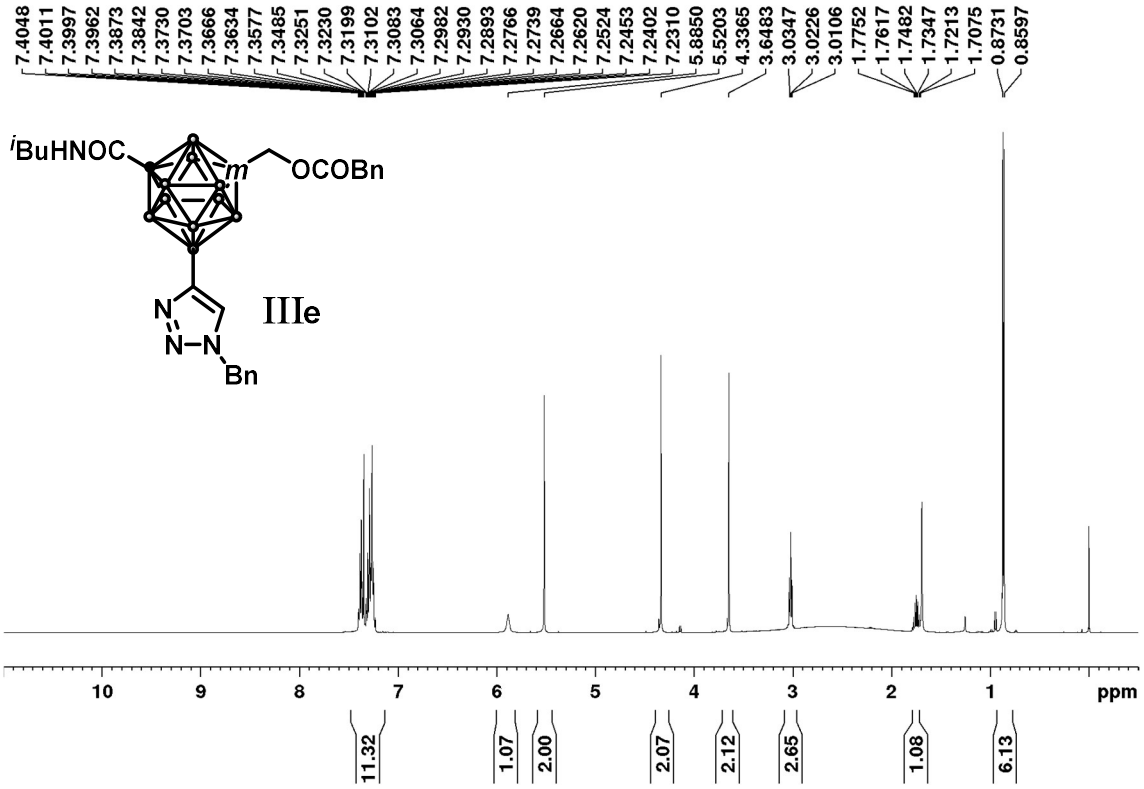


^{11}B NMR (160 MHz; $CDCl_3$)

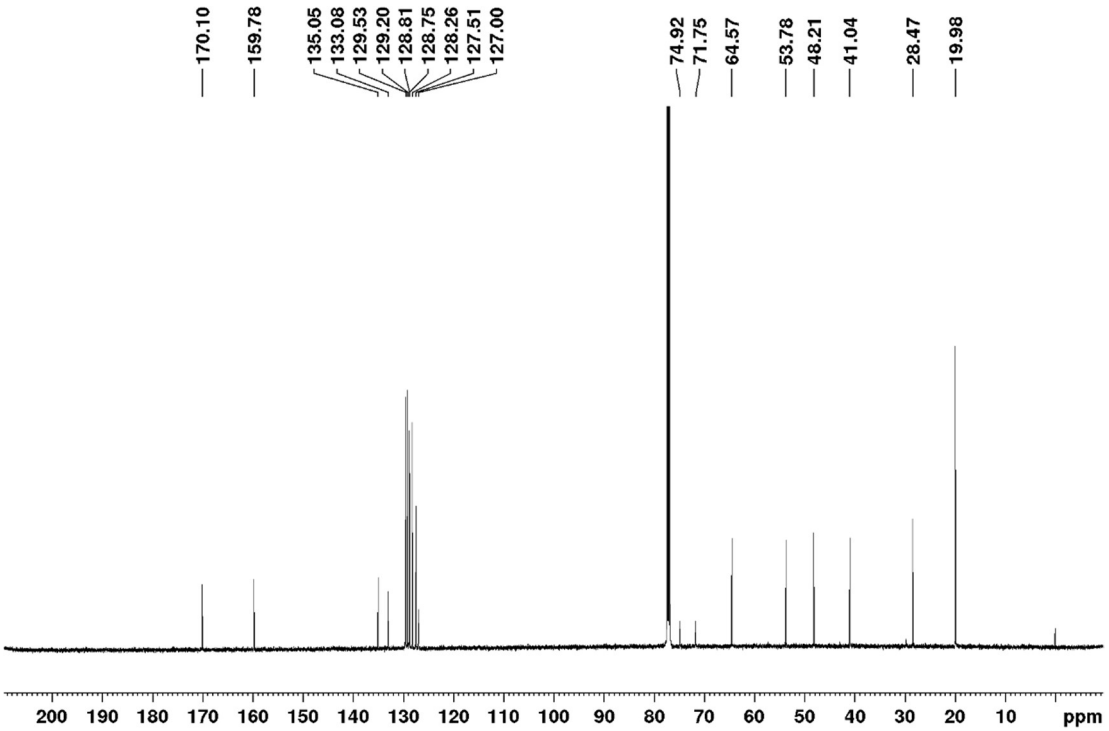


1-phenylacetoxymethyl-7-isobutylcarbamoyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIe)

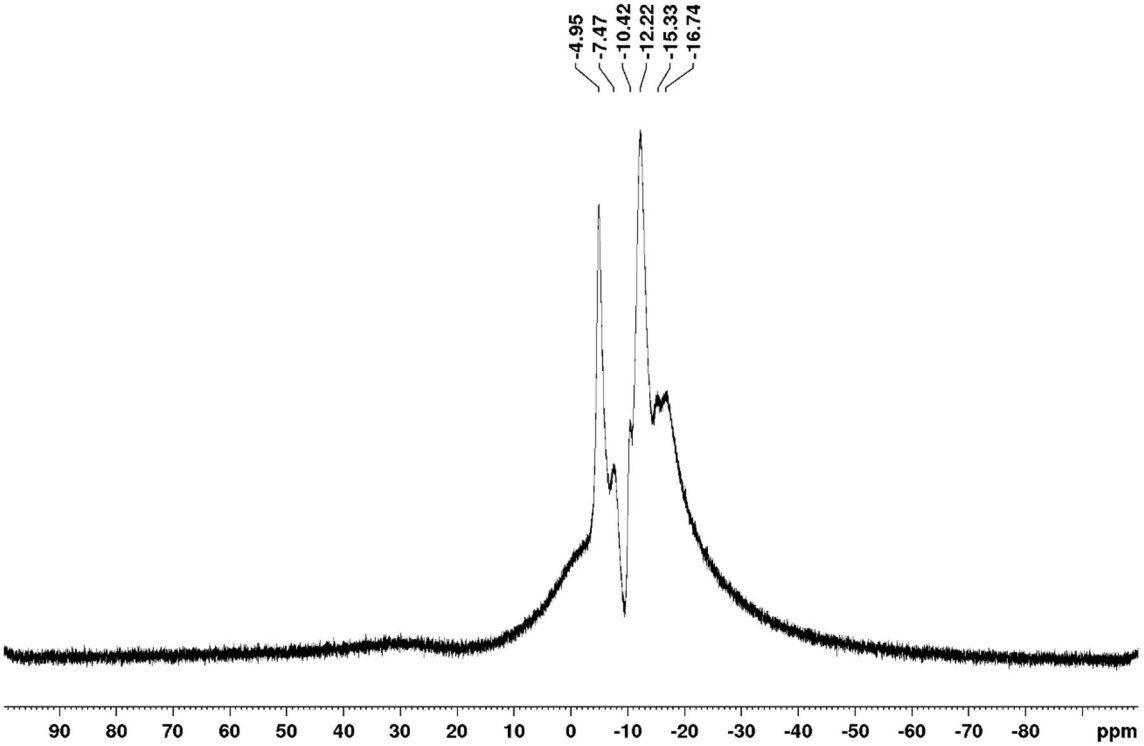
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

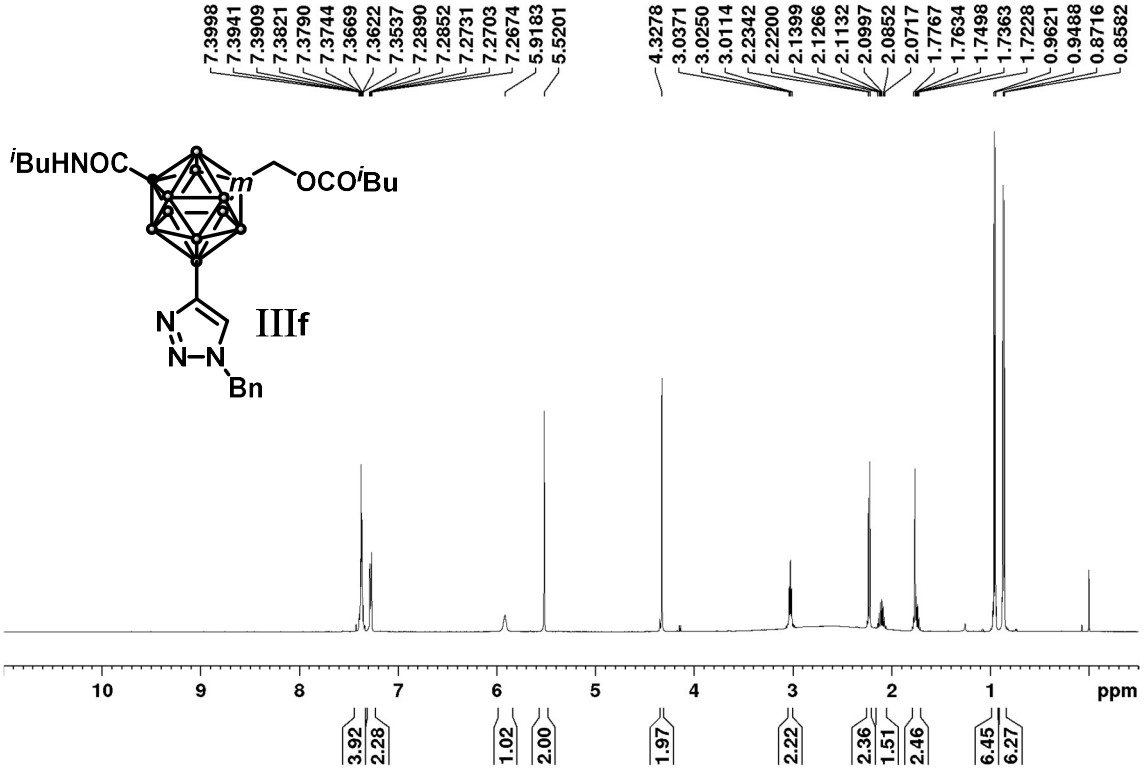


^{11}B NMR (160 MHz; $CDCl_3$)

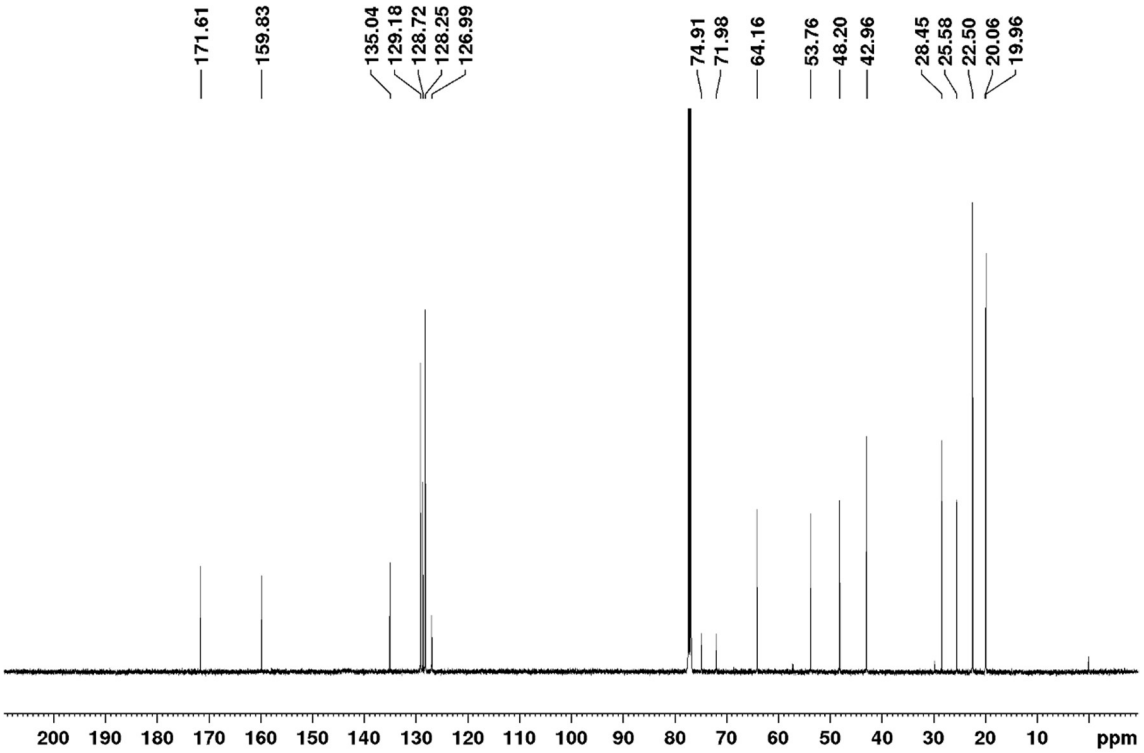


1-(3-methylbutanoyl)oxymethyl-7-isobutylcarbamoyl-9-(1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IIIf)

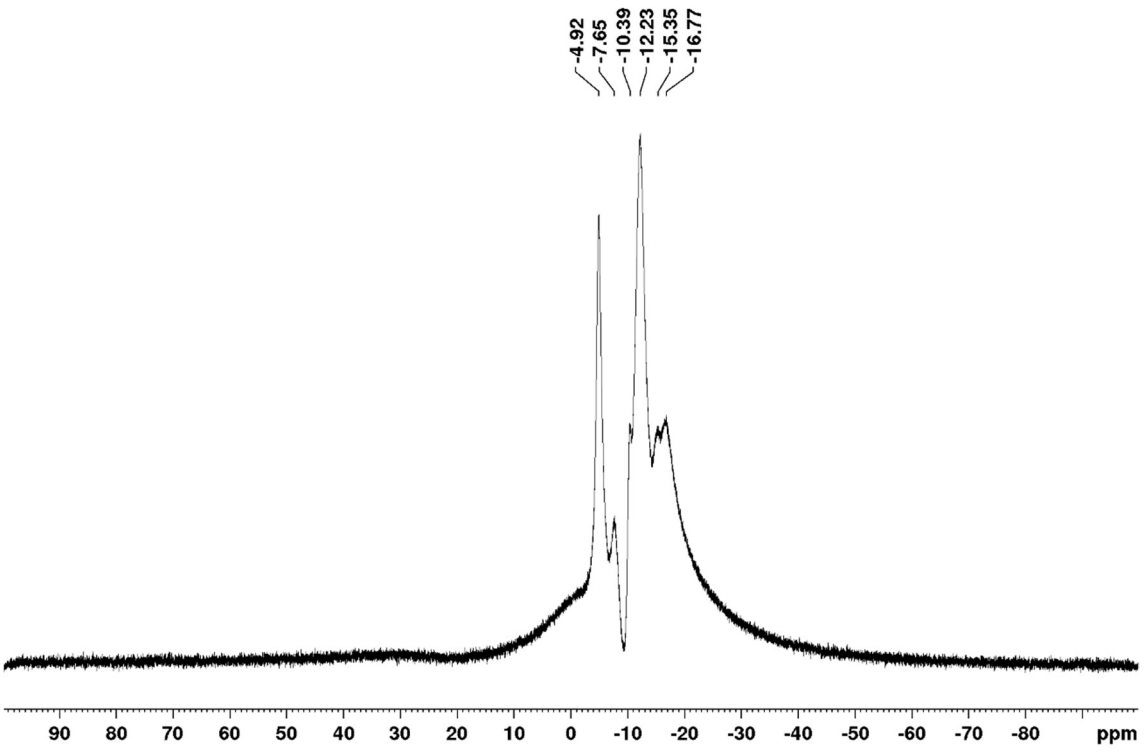
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

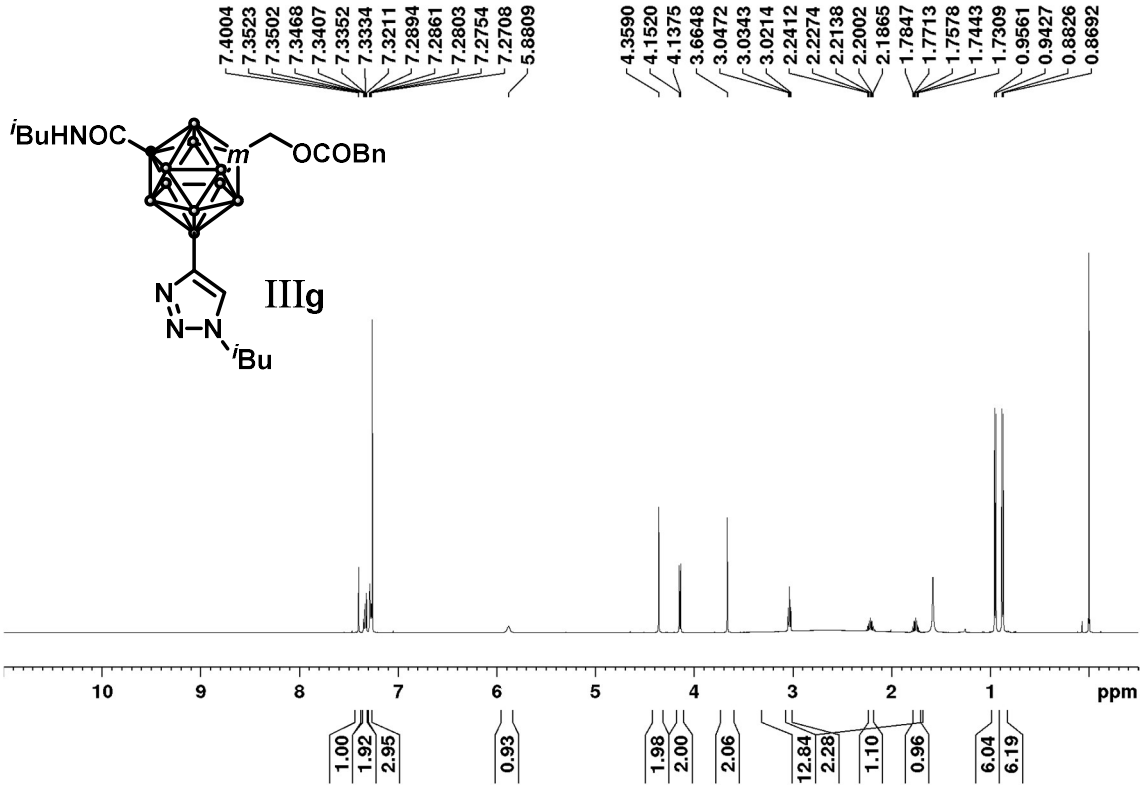


^{11}B NMR (160 MHz; $CDCl_3$)

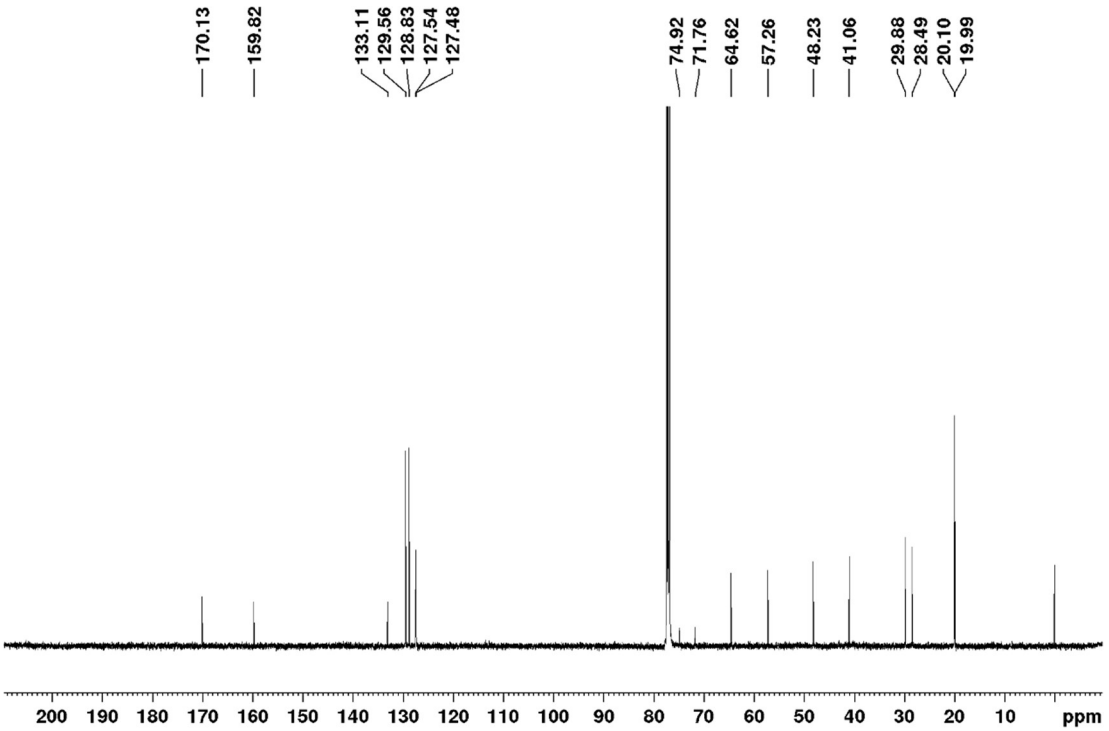


1-phenylacetoxymethyl-7-benzylaminocarboxyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIIg)

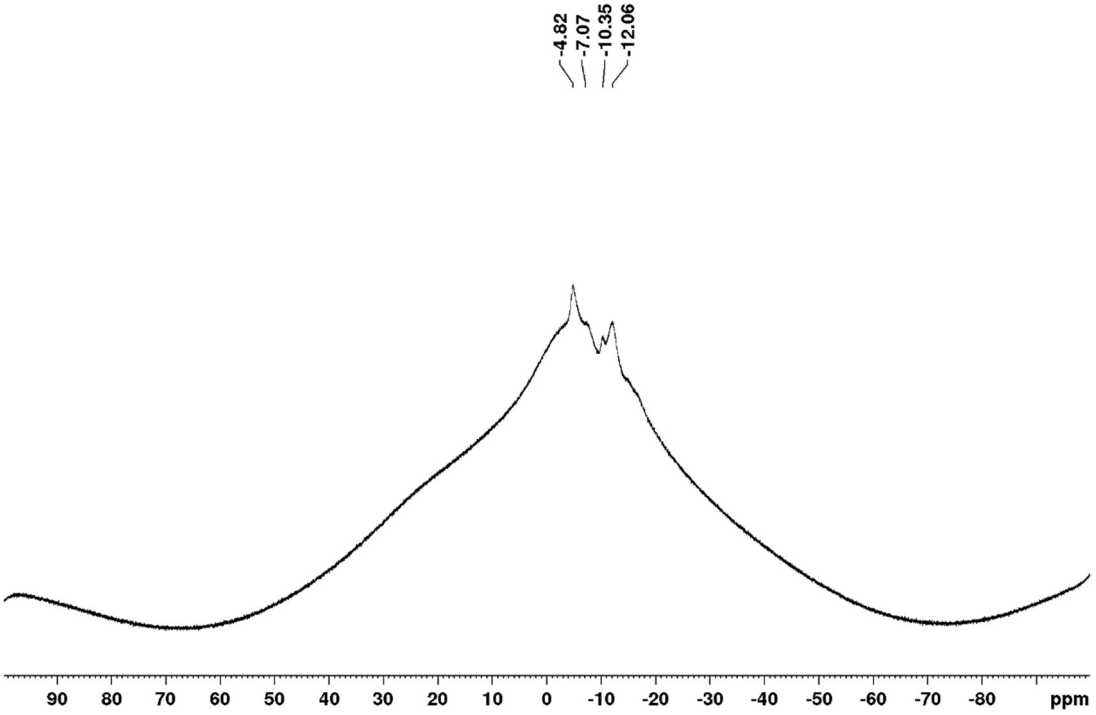
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

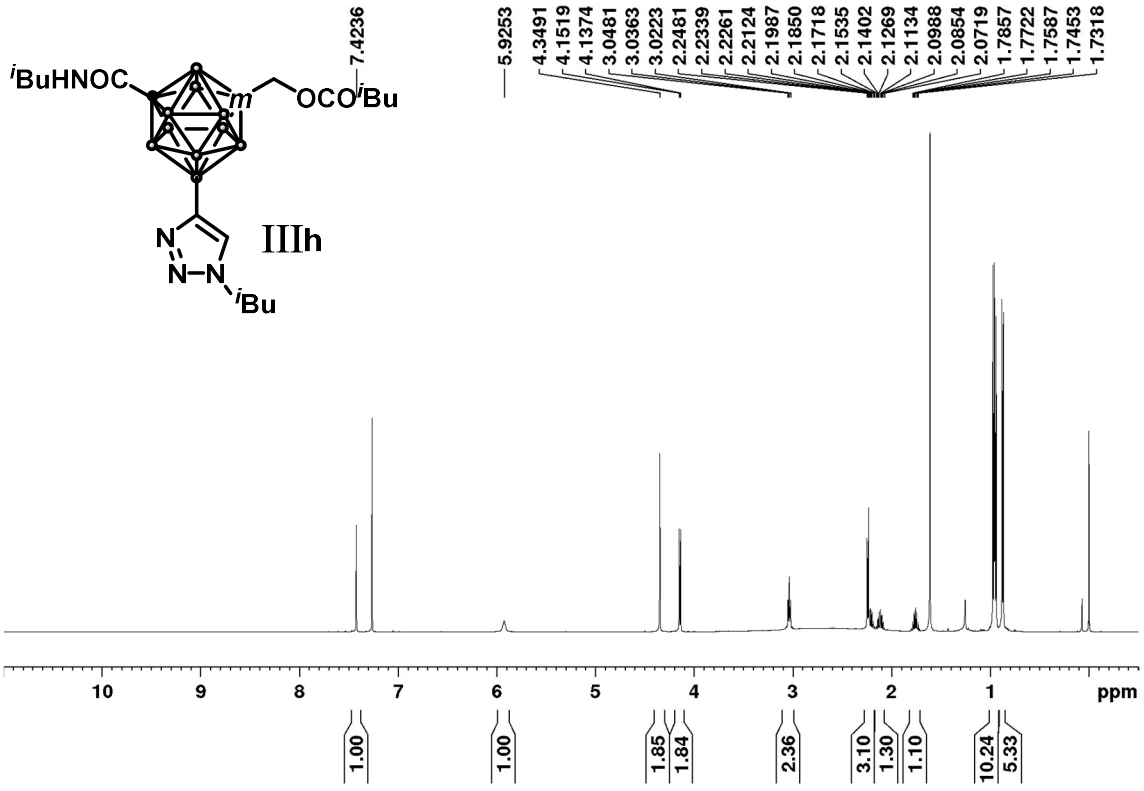


^{11}B NMR (160 MHz; $CDCl_3$)

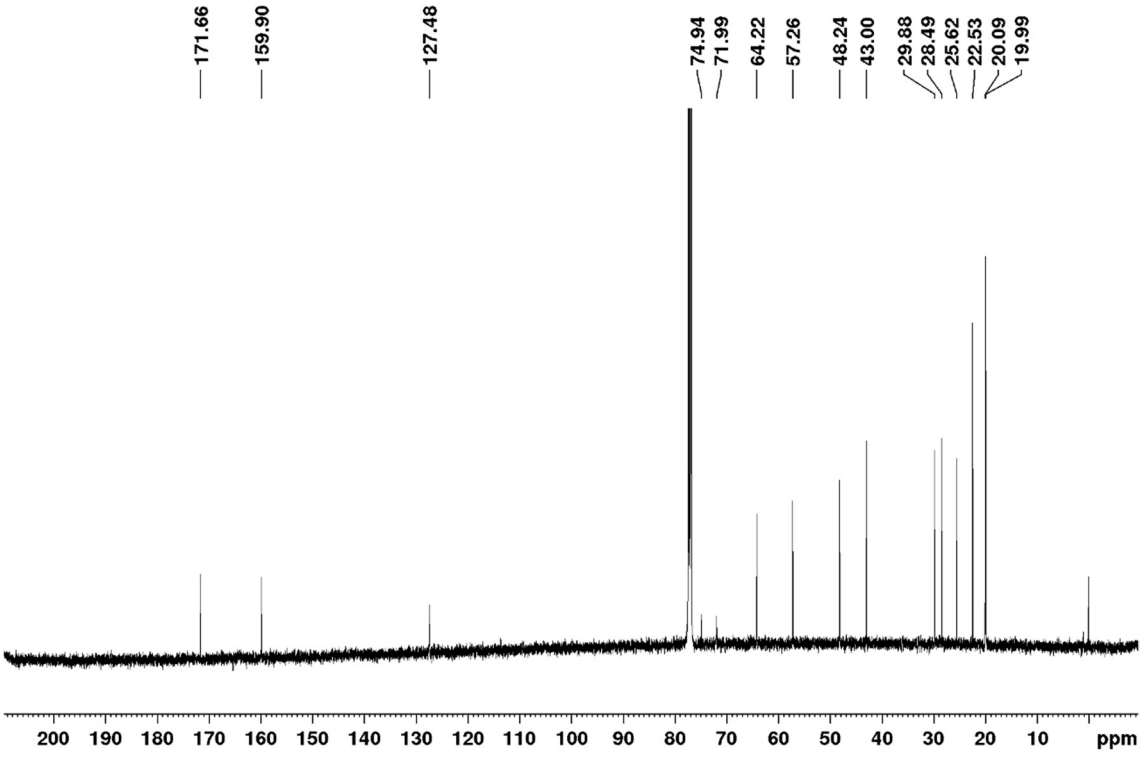


1-(3-methylbutanoyl)oxymethyl-7-isobutylcarbamoyl-9-((1*H*-1,2,3-triazol-4-yl)-3-methyl-butanoyl)-1,7-dicarba-*closo*-dodecaborane (IIIh)

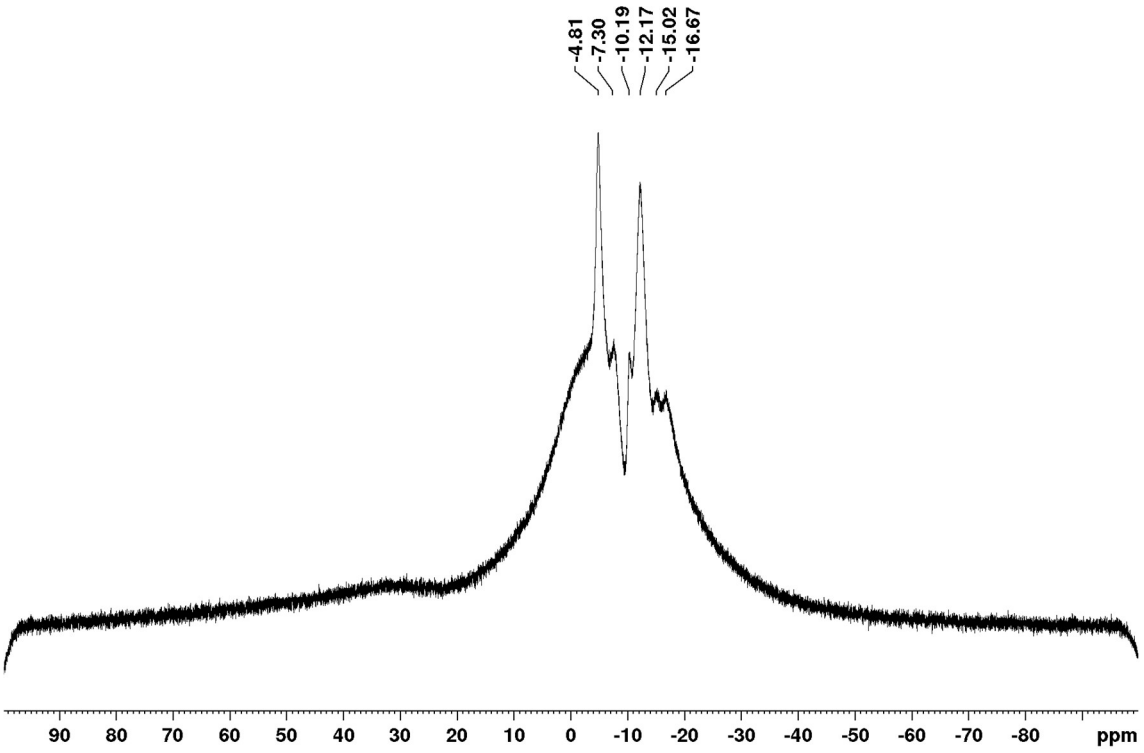
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)



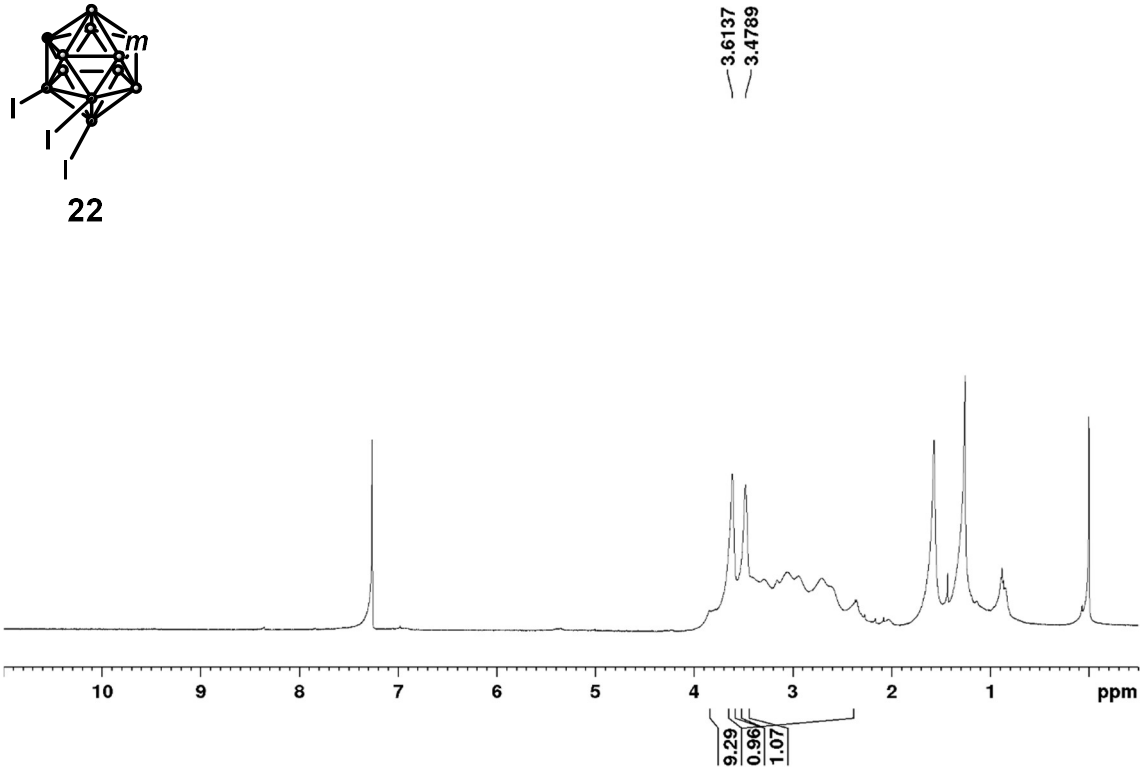
^{11}B NMR (160 MHz; $CDCl_3$)



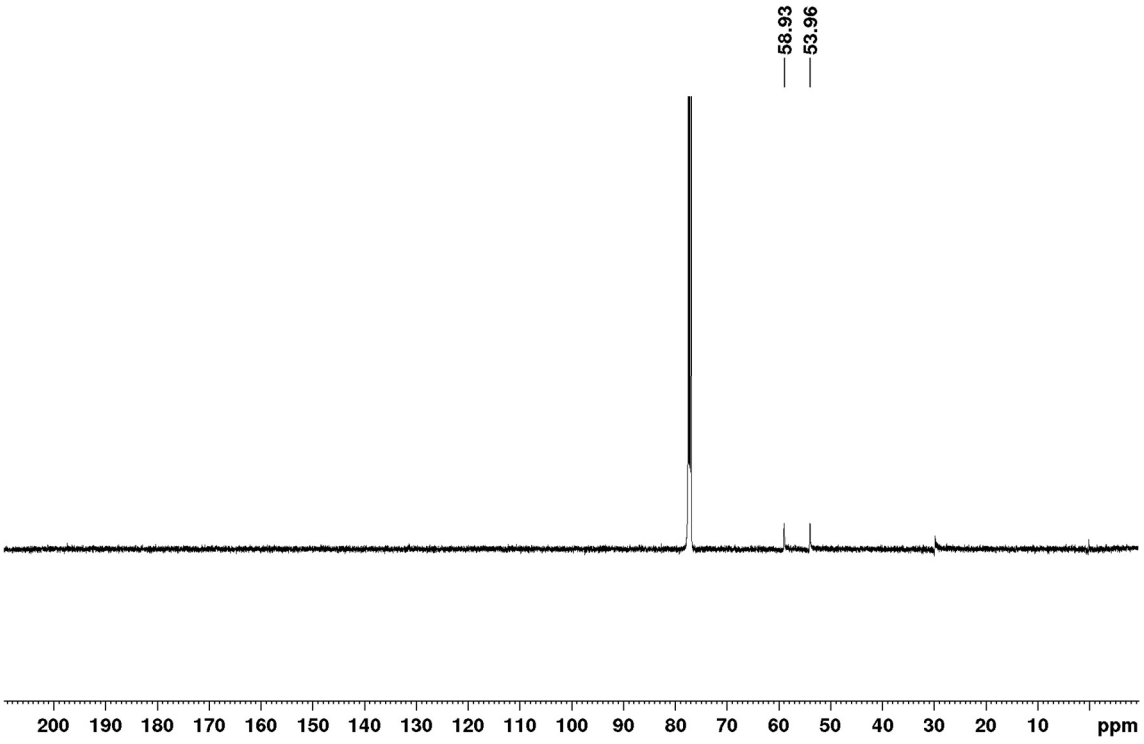
4,9,10-triiodo-1,7-dicarba-*closo*-dodecaborane (27')

^{1}H NMR (400 MHz; $CDCl_3$)

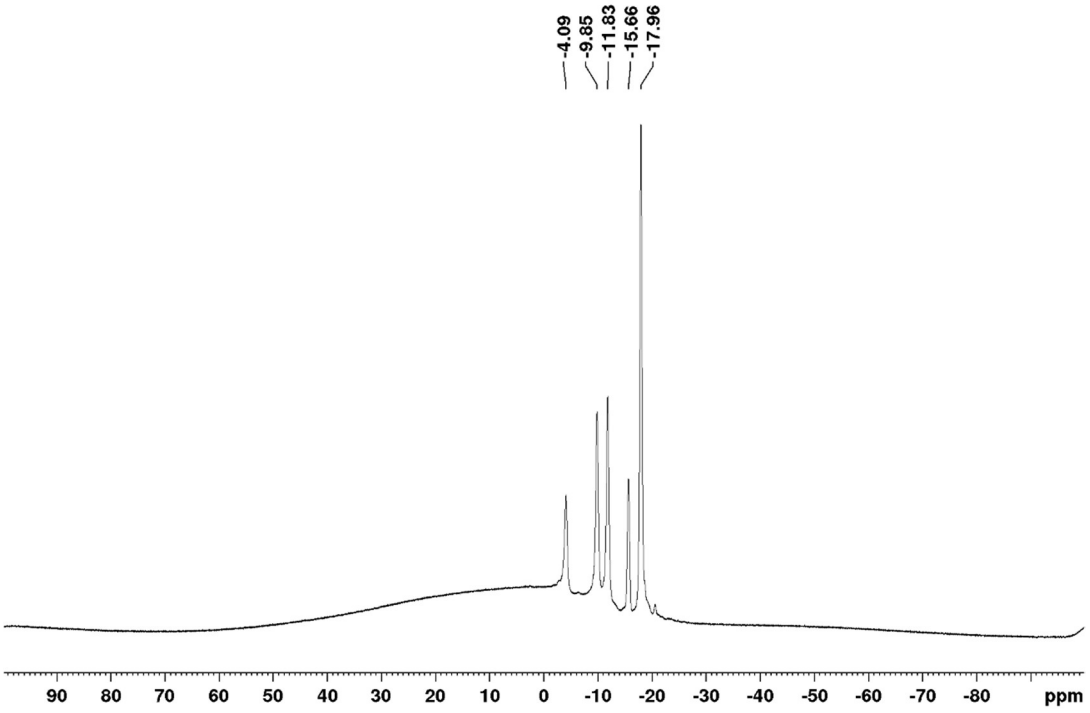
22



^{13}C NMR (125 MHz; $CDCl_3$)

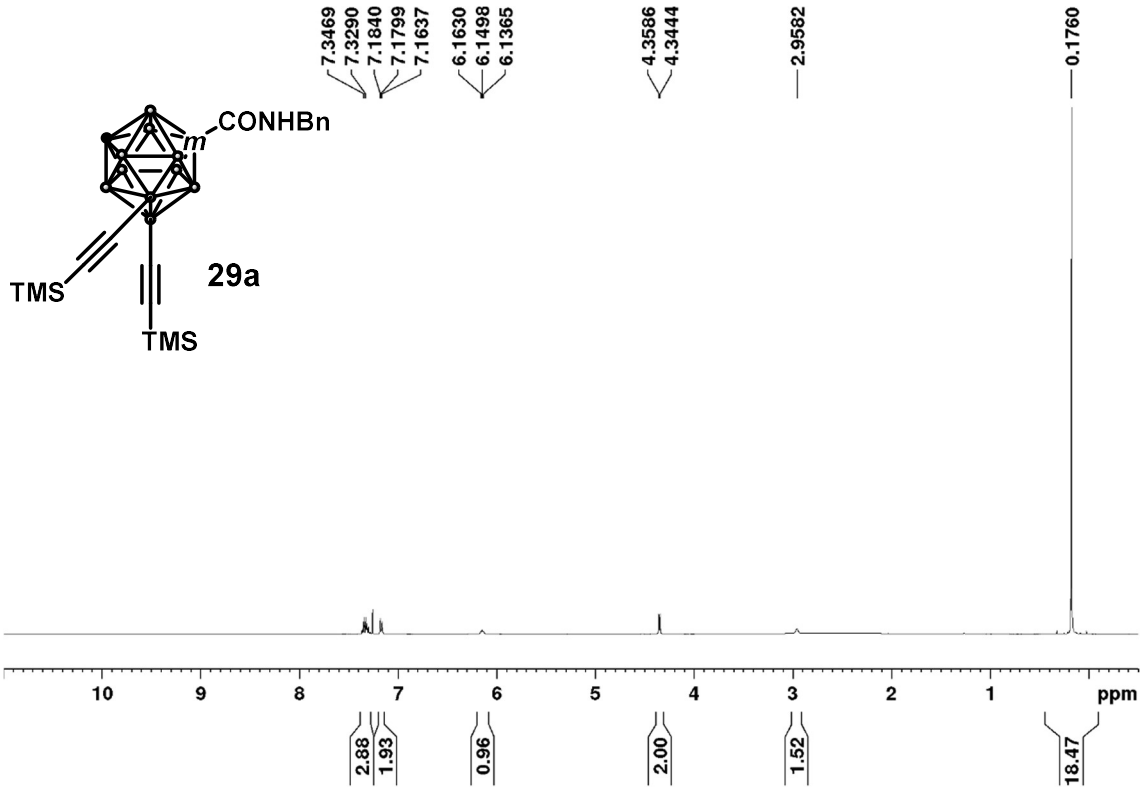


^{11}B NMR (160 MHz; CDCl3)

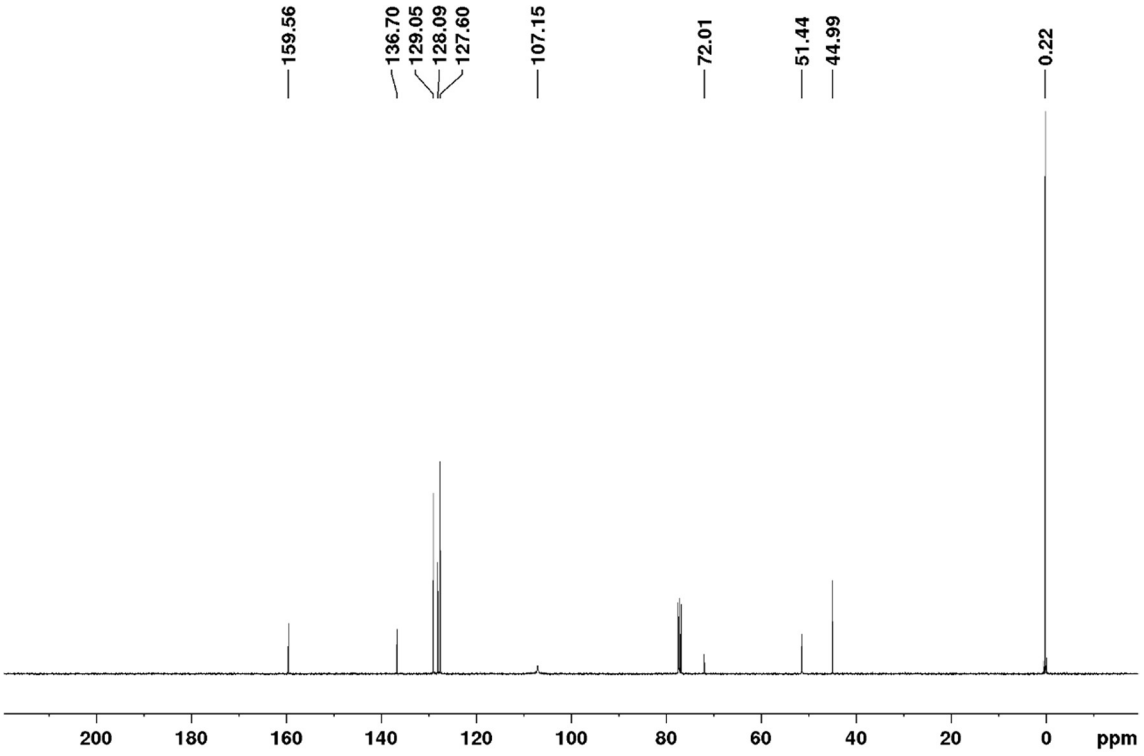


1-benzylaminocarboxyl-9,10-bis((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (29a)

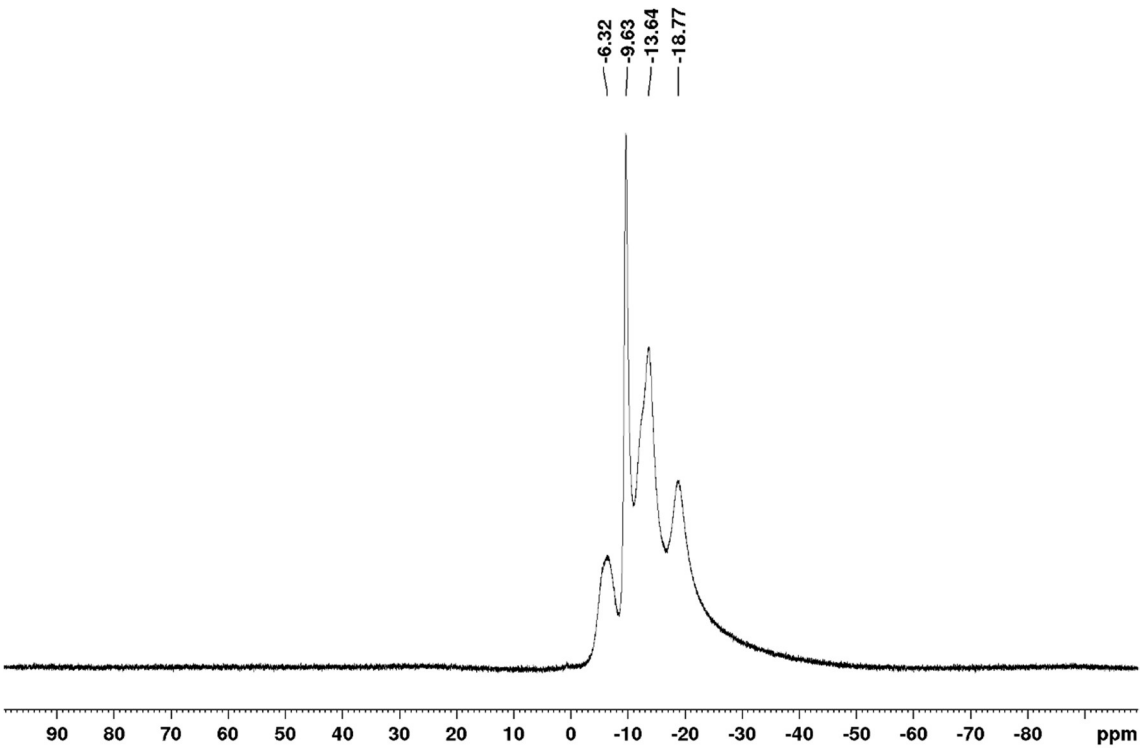
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

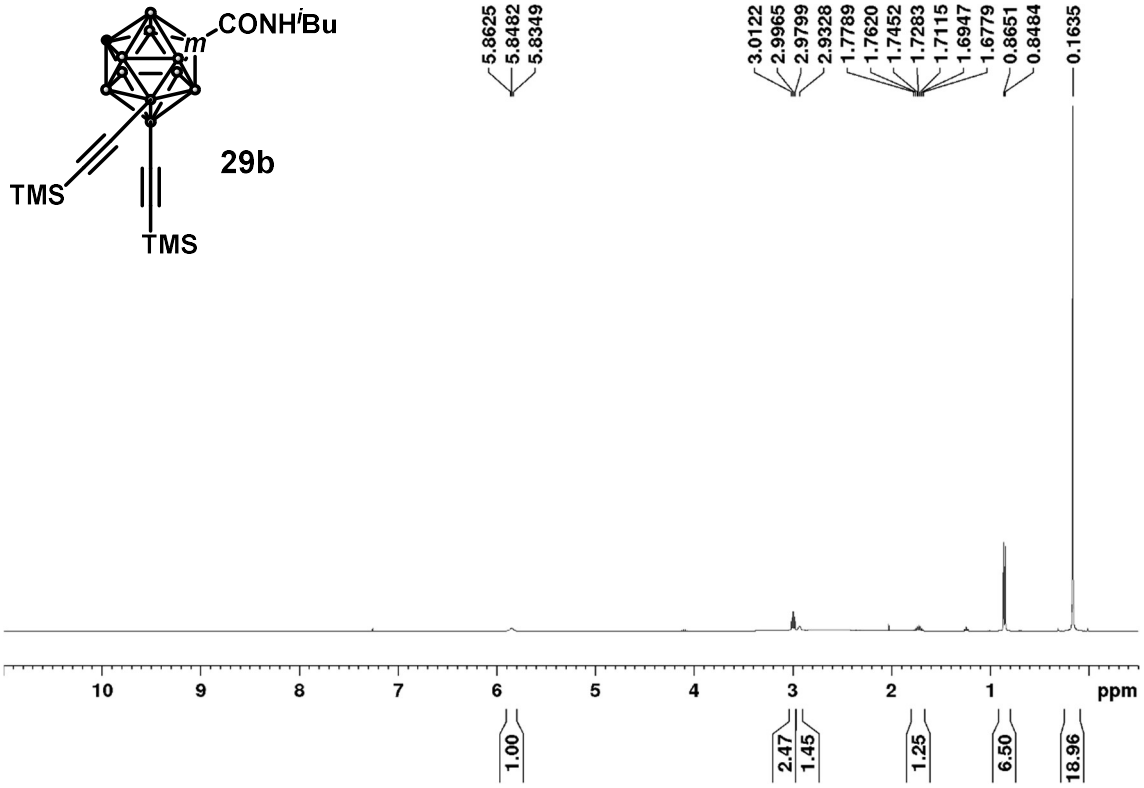


^{11}B NMR (160 MHz; CDCl3)

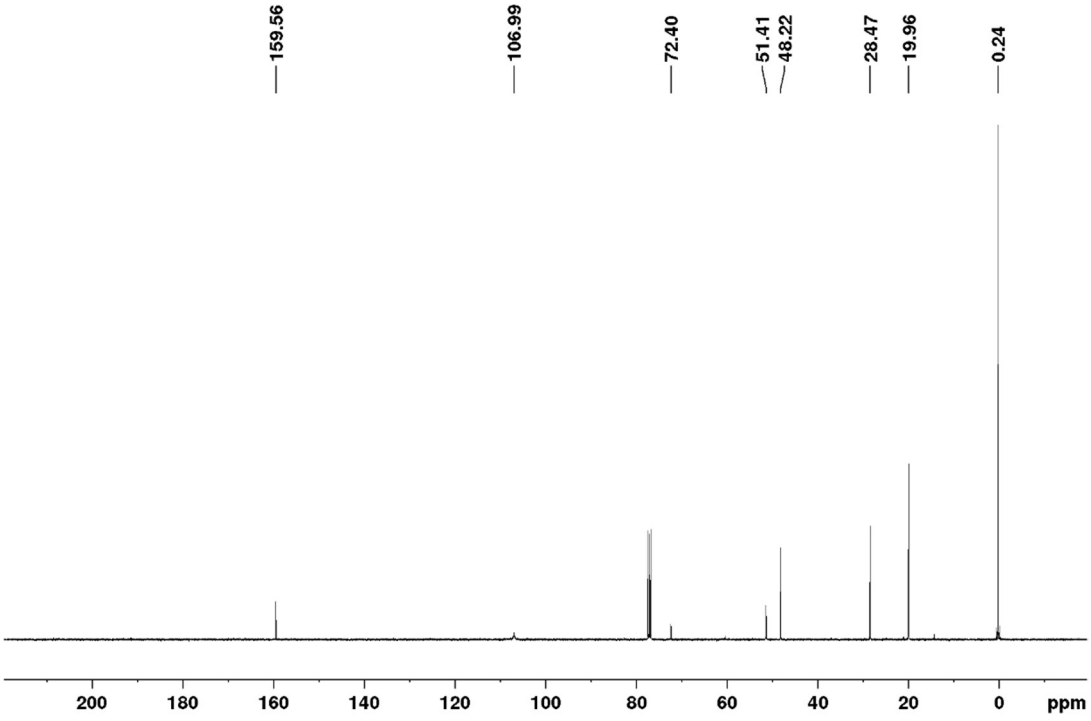


1-isobutylaminocarboxyl-9,10-bis((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (29b)

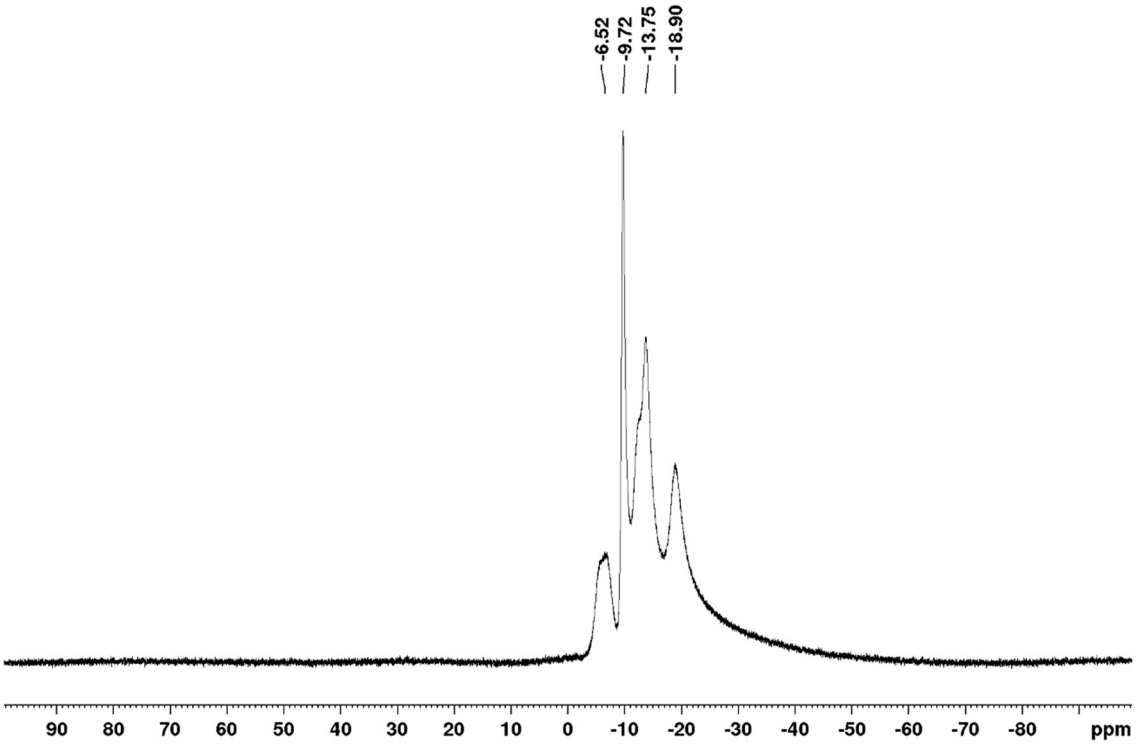
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

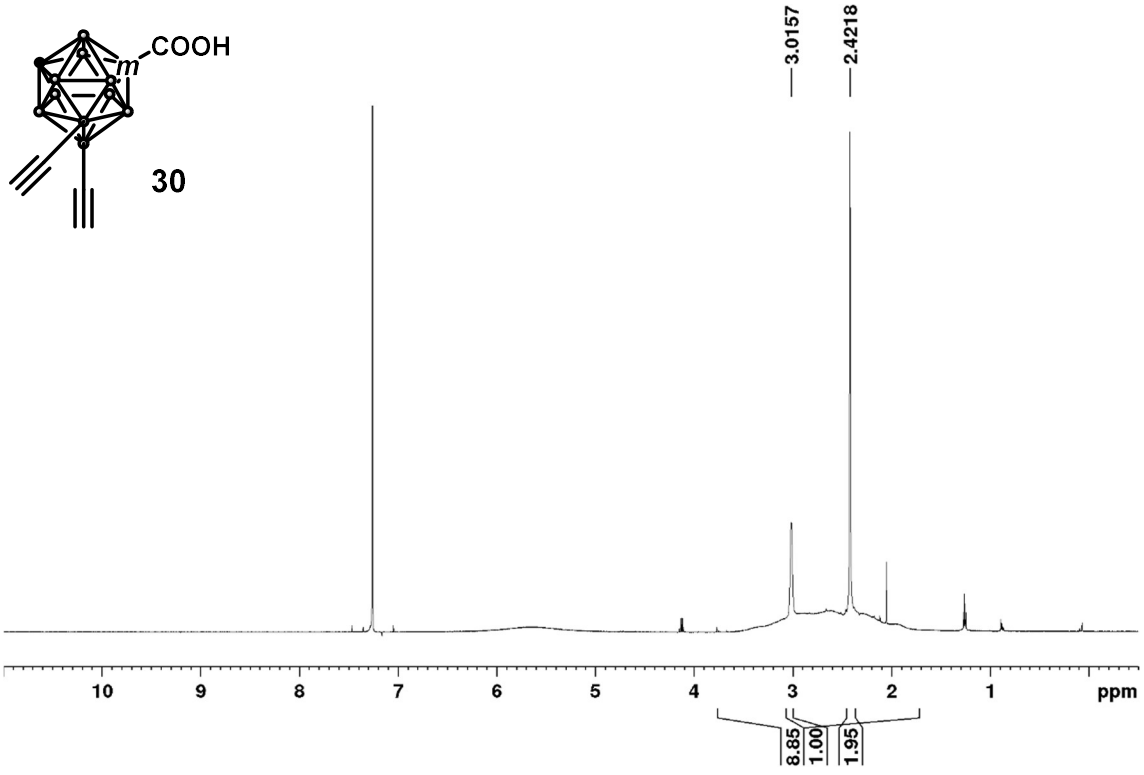


^{11}B NMR (160 MHz; CDCl3)

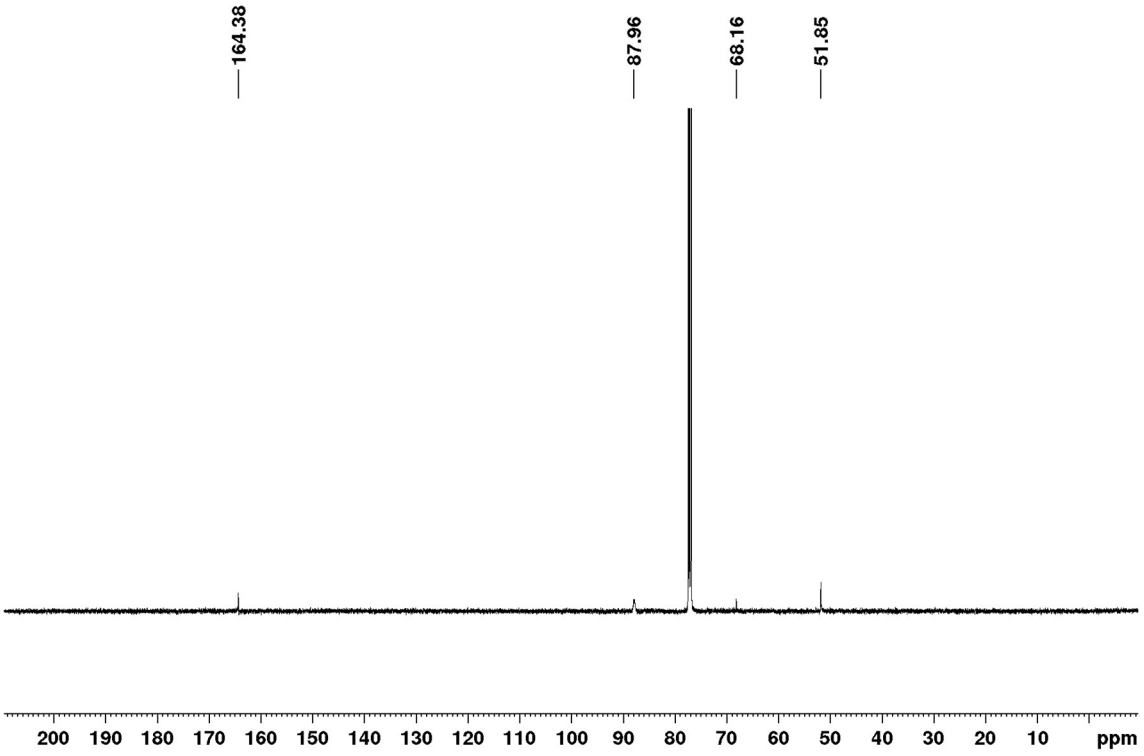


1-benzylaminocarboxyl-9,10-bis((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (30)

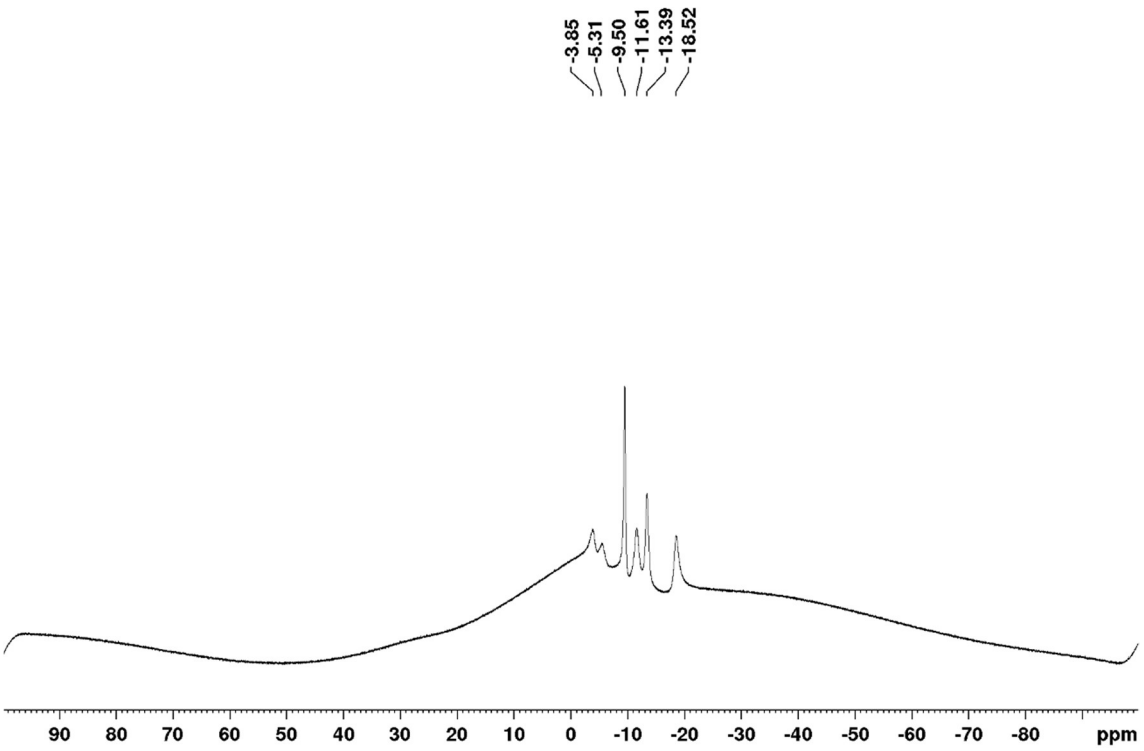
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

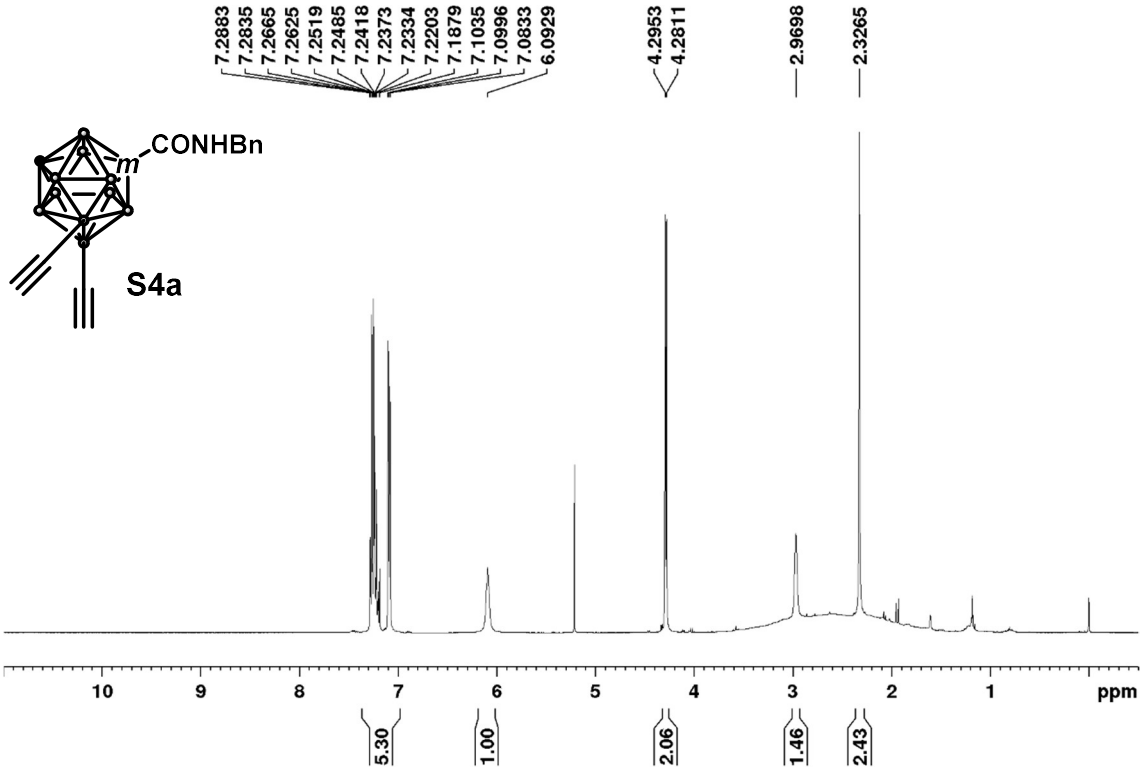


^{11}B NMR (160 MHz; CDCl3)

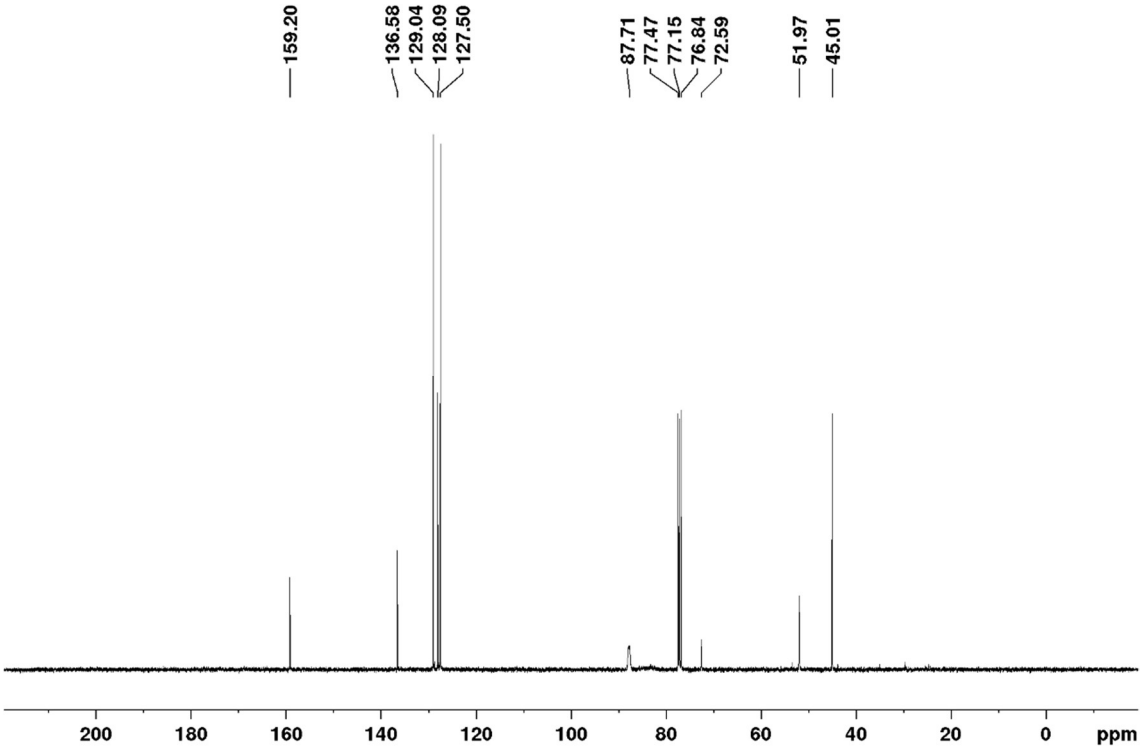


1-benzylaminocarboxyl-9,10-diethynyl-1,7-dicarba-*closo*-dodecaborane (S4a)

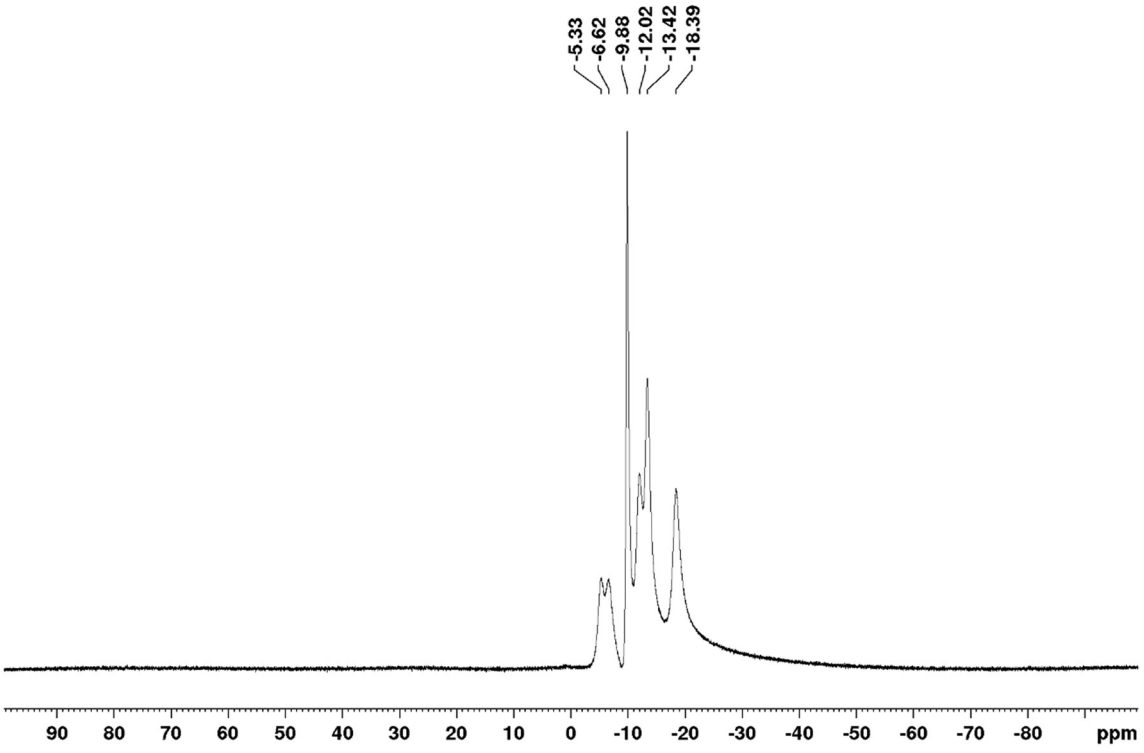
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

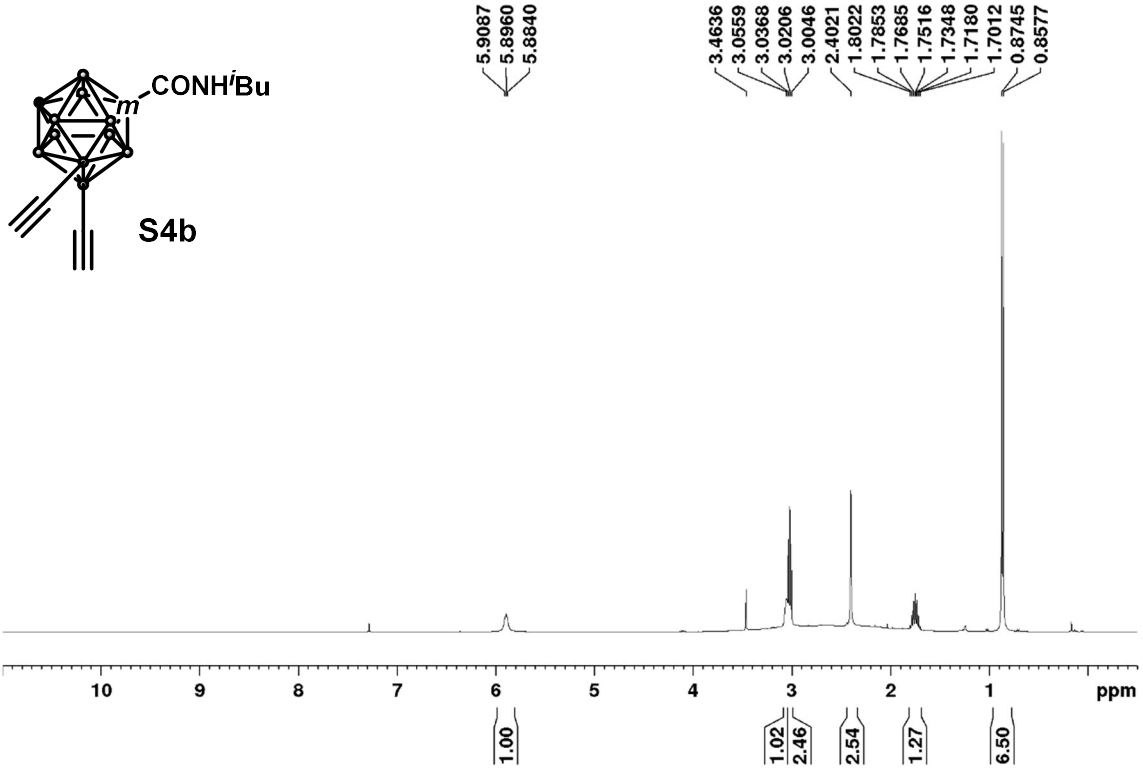


^{11}B NMR (160 MHz; CDCl3)

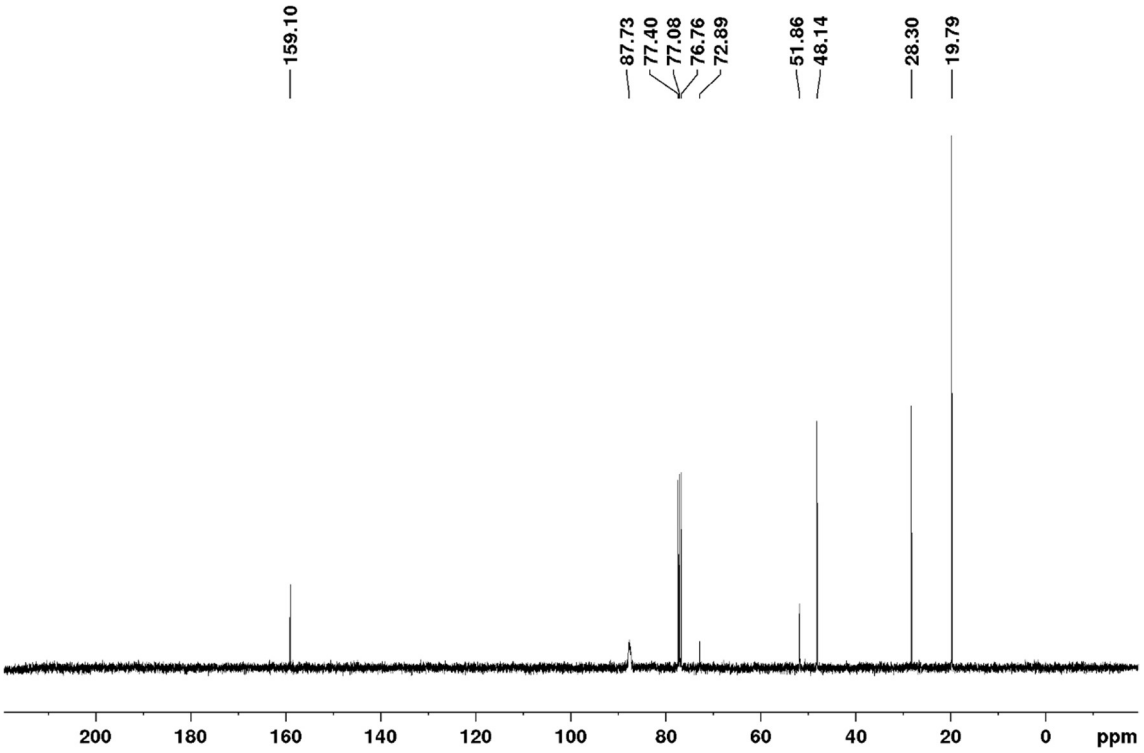


1-isobutylaminocarboxyl-9,10-diethynyl-1,7-dicarba-*closo*-dodecaborane (S4b)

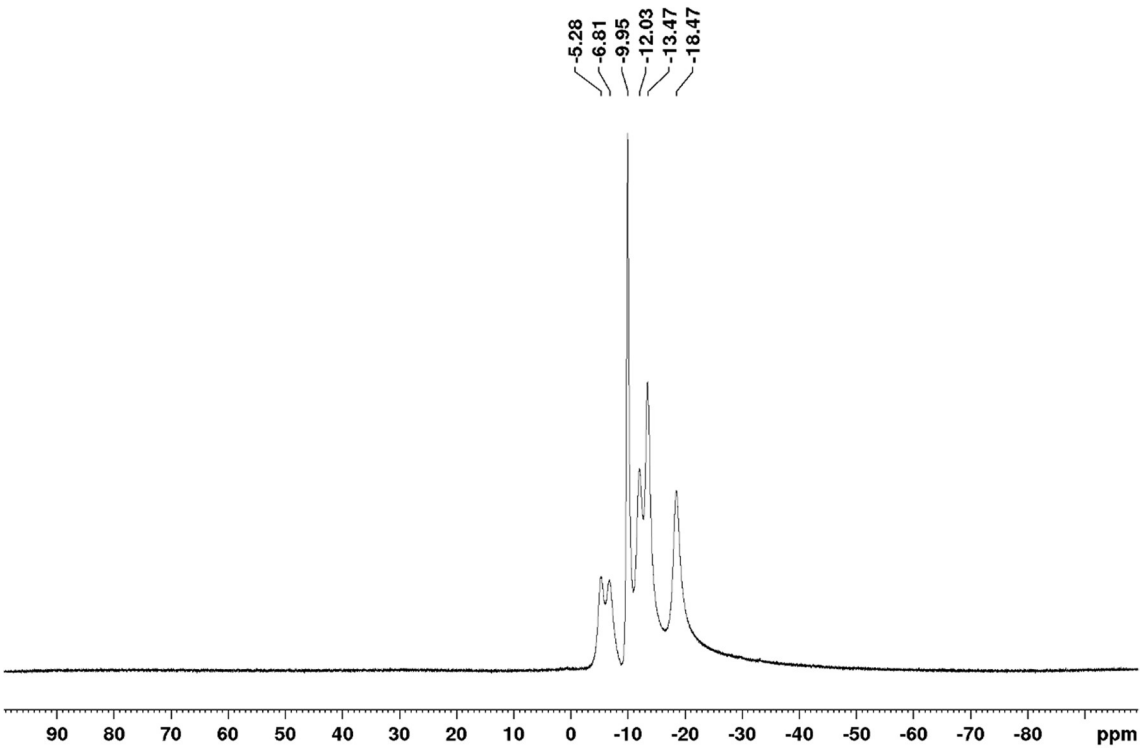
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

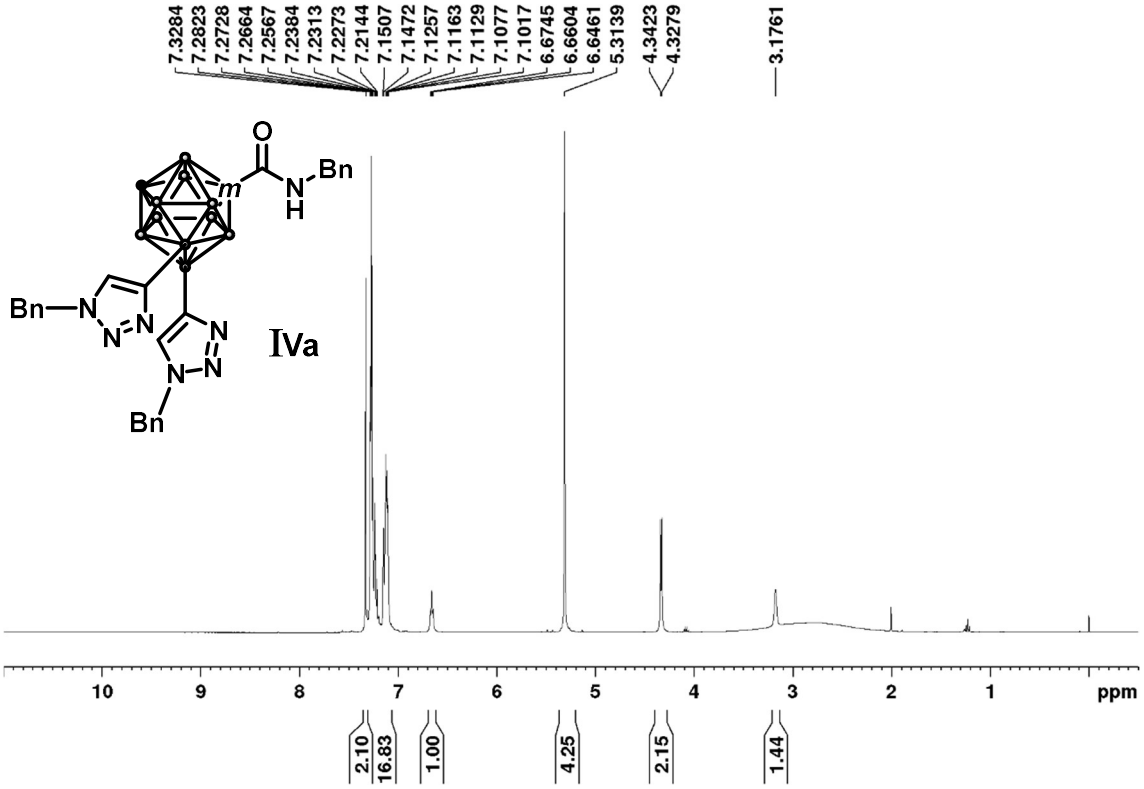


^{11}B NMR (160 MHz; CDCl3)

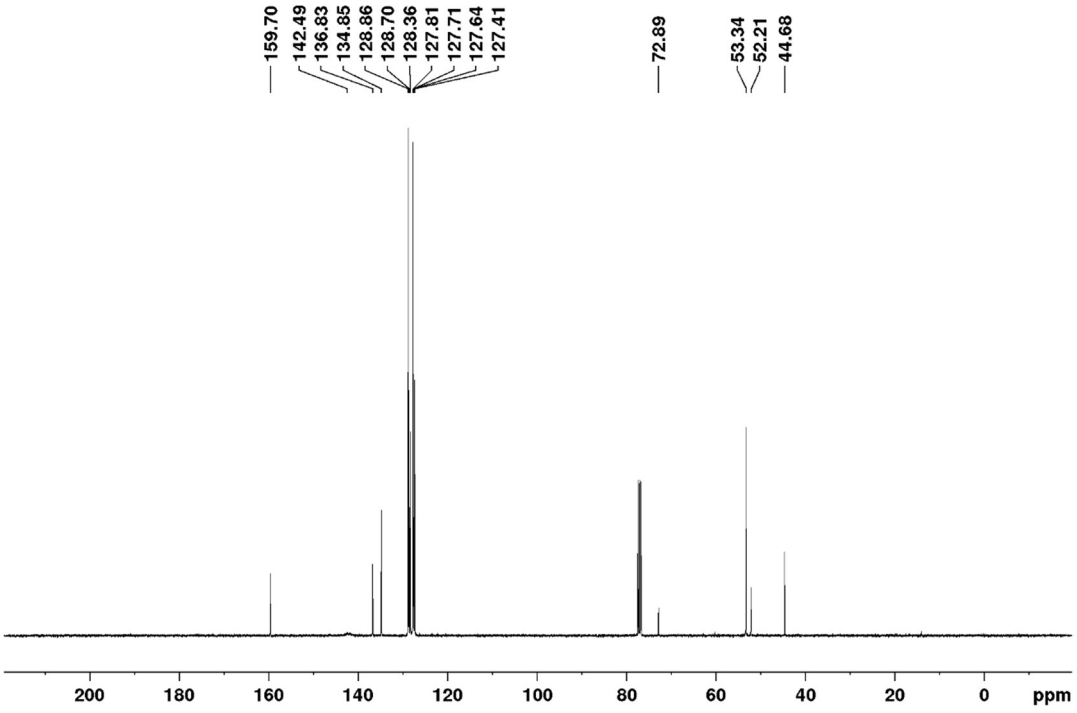


1-benzylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVa)

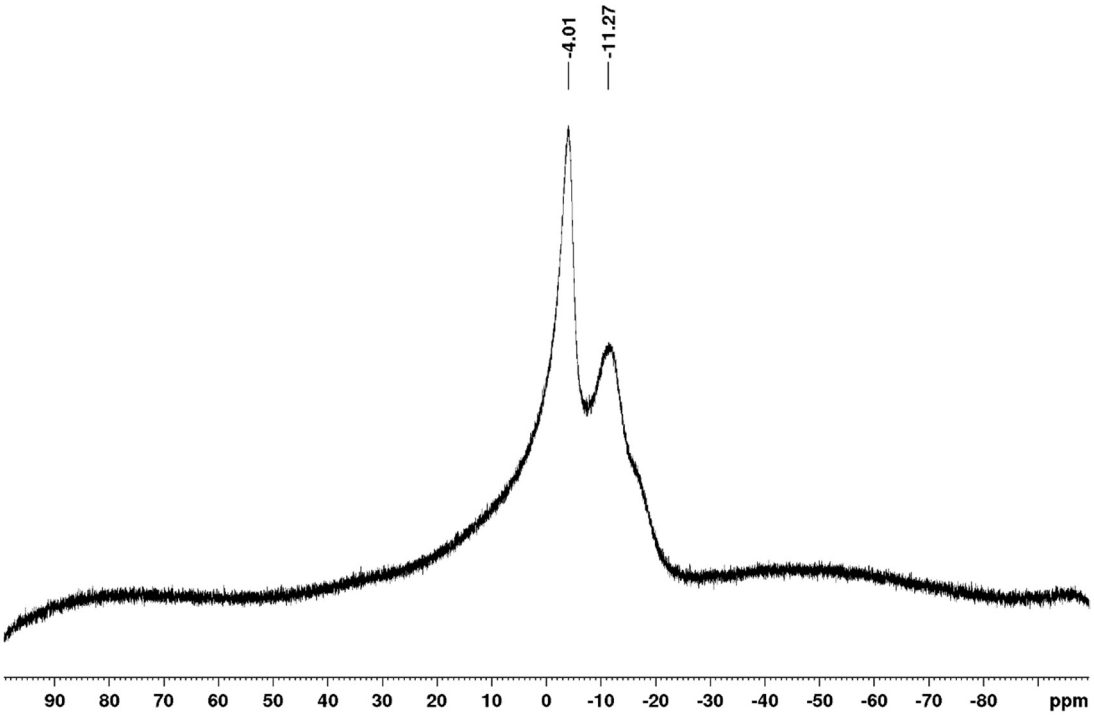
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

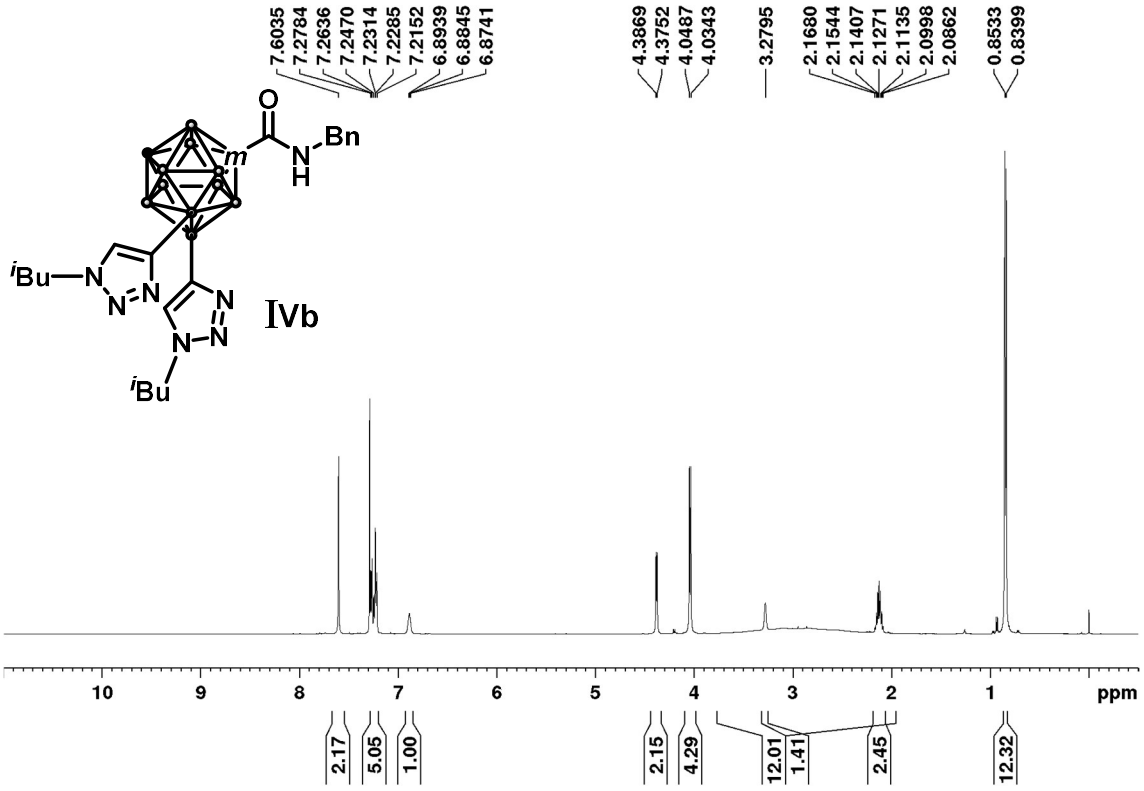


^{11}B NMR (160 MHz; CDCl3)

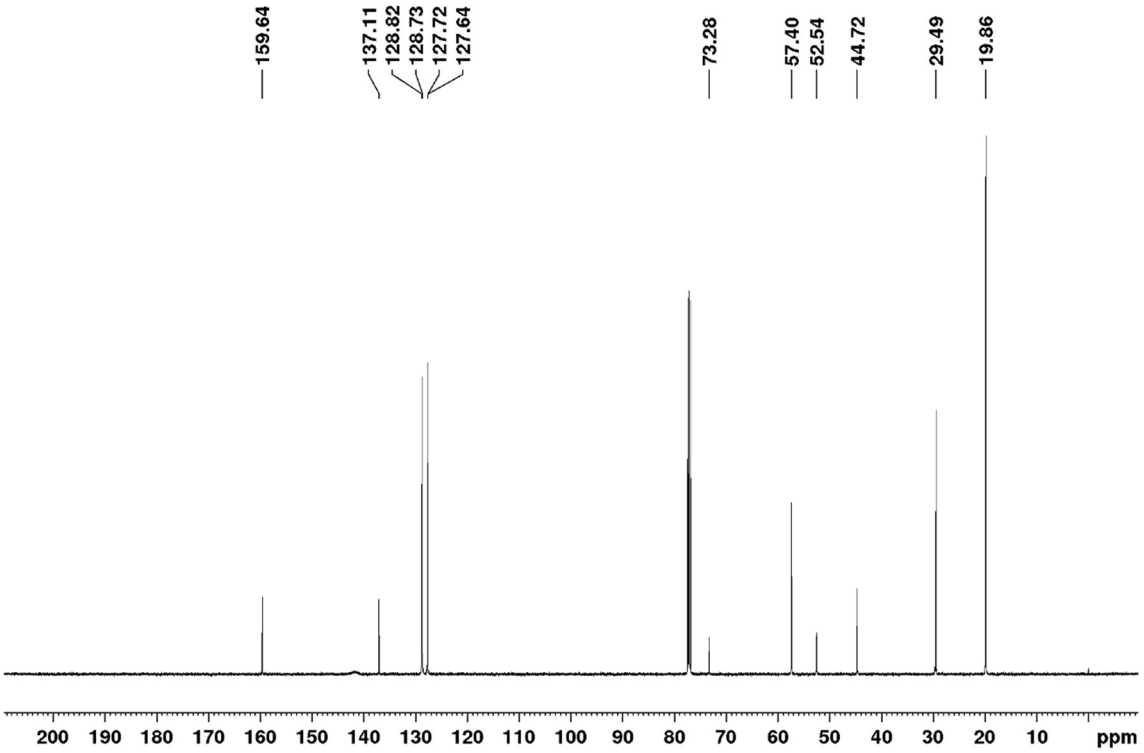


1-benzylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)isobutylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVb)

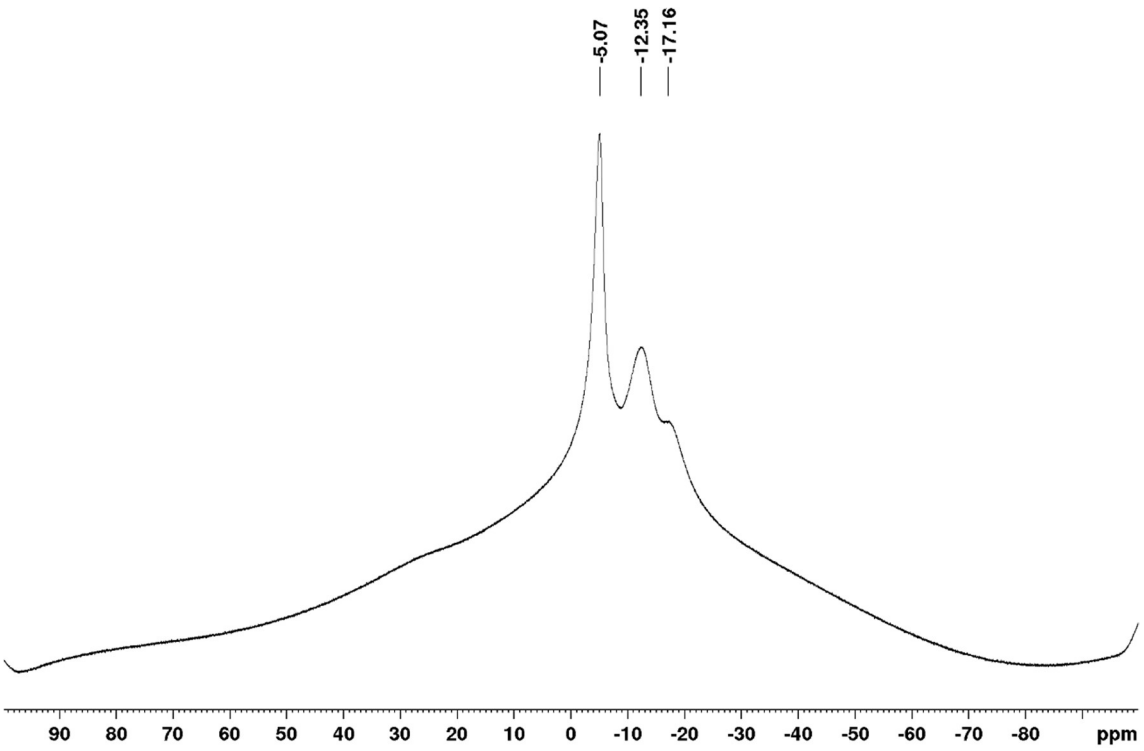
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

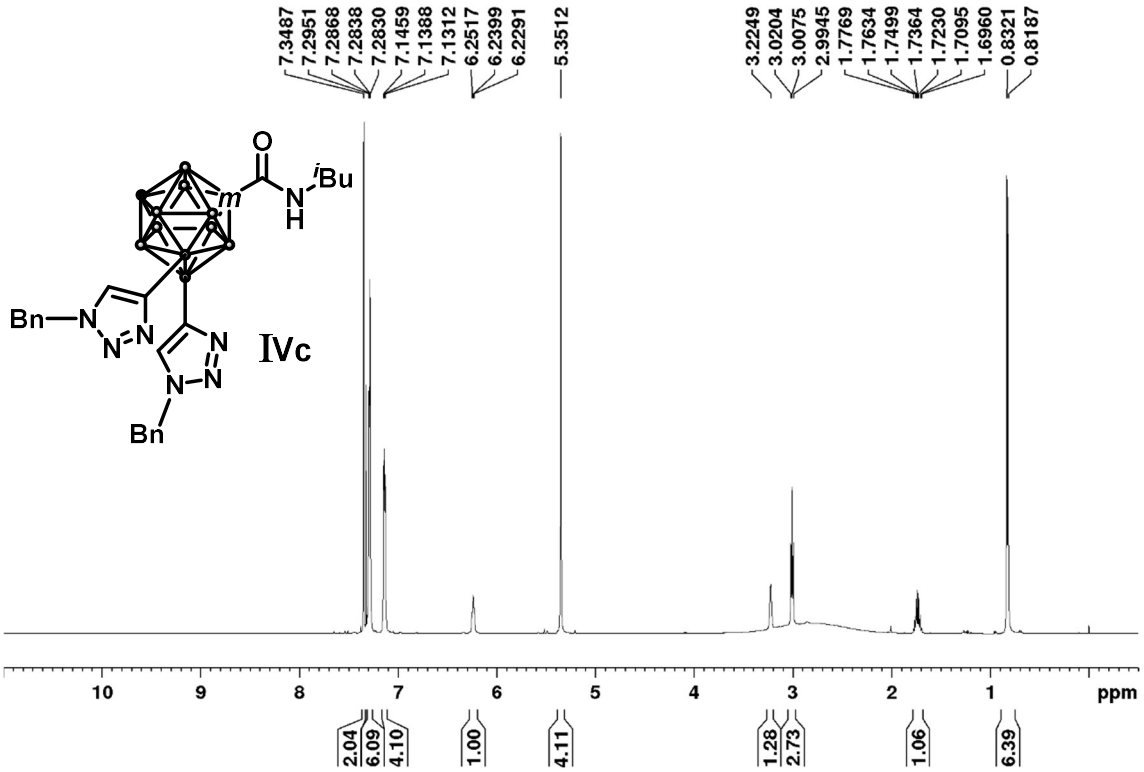


^{11}B NMR (160 MHz; CDCl3)

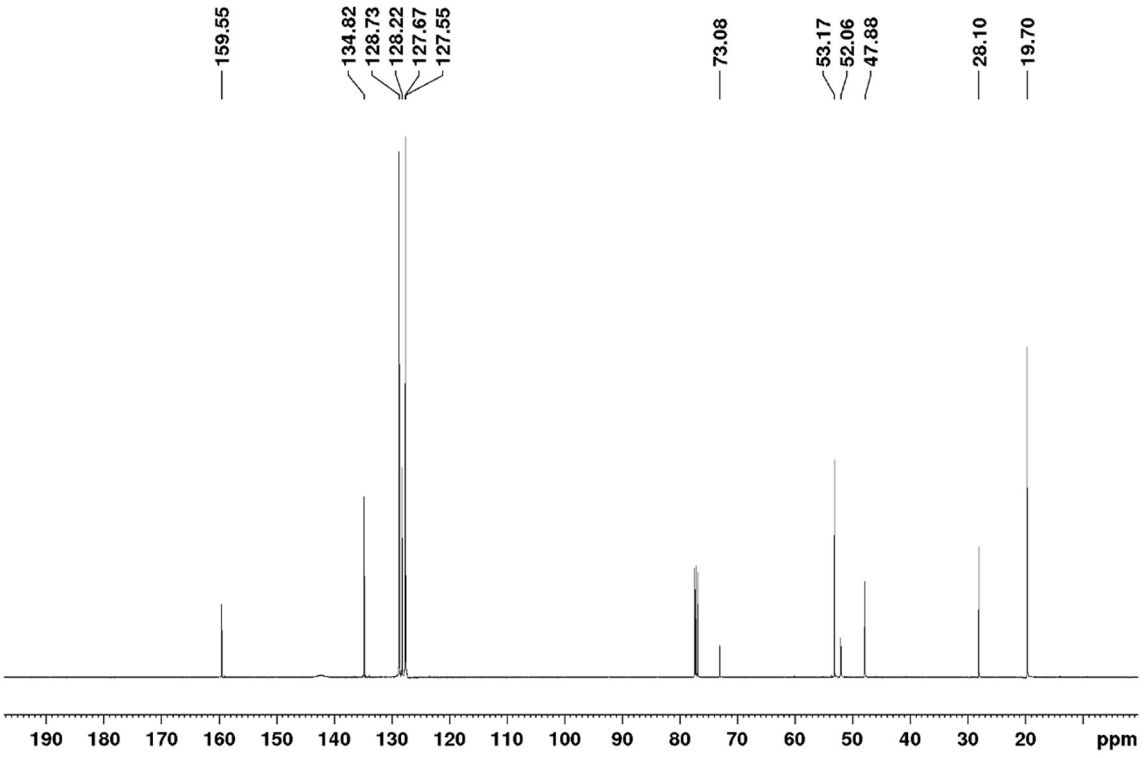


1-isobutylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVc)

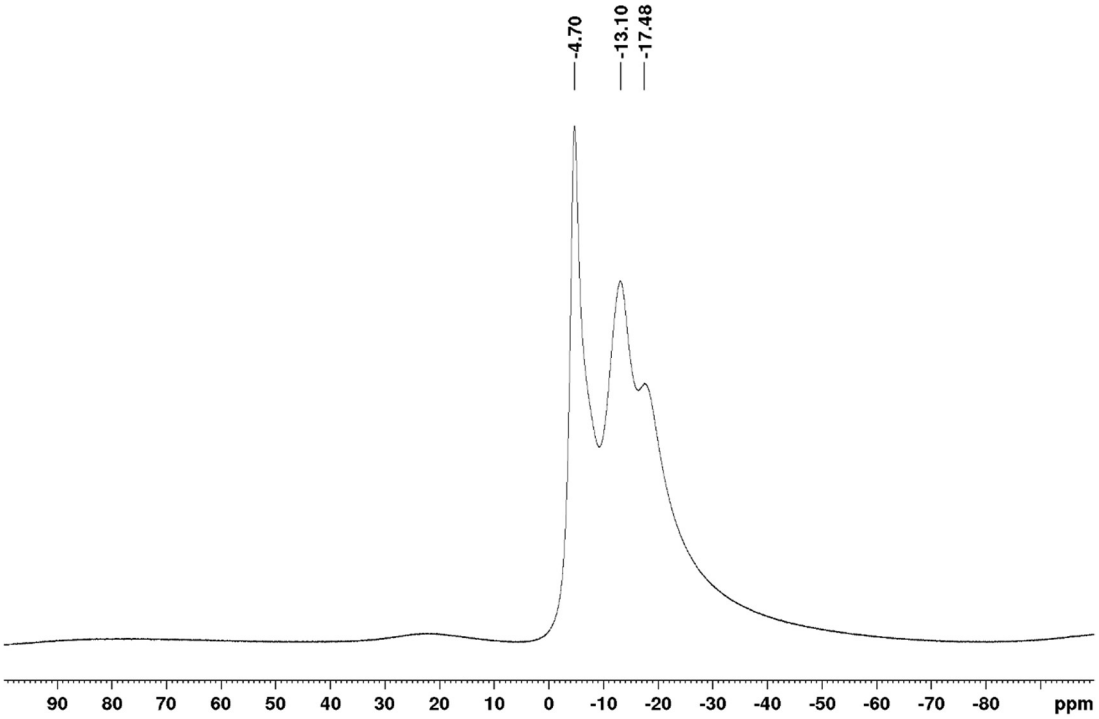
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

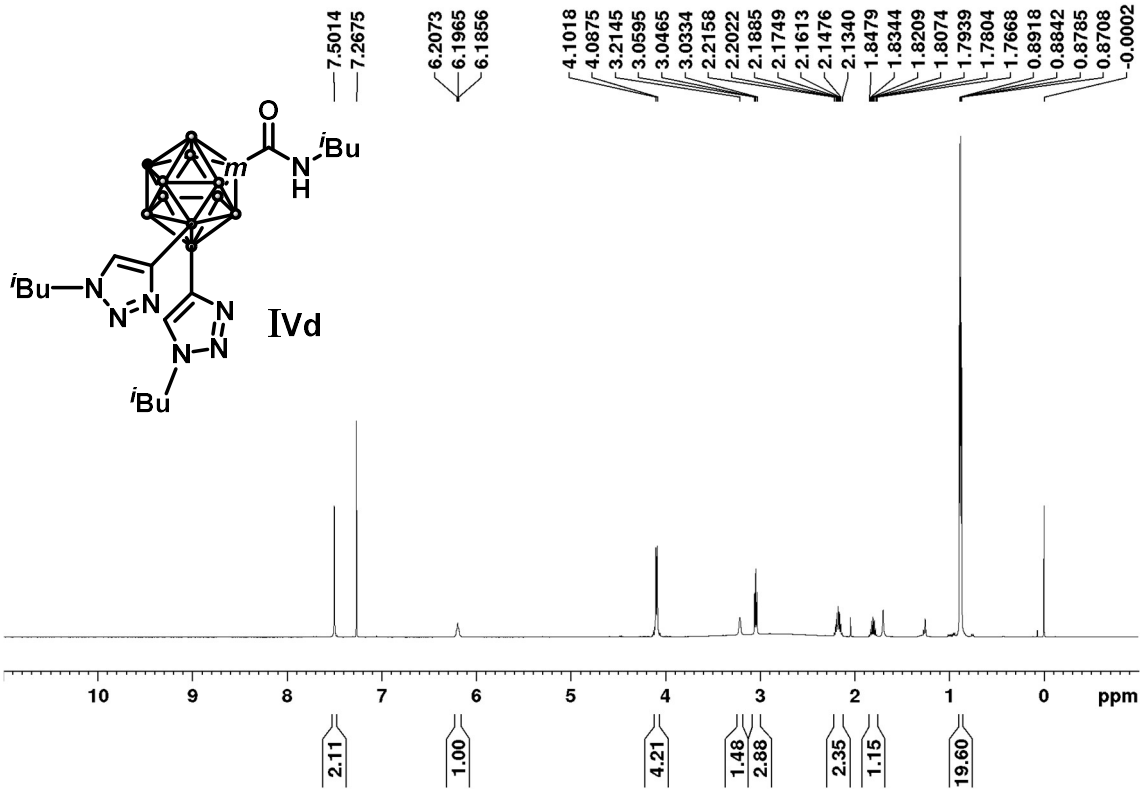


^{11}B NMR (160 MHz; CDCl3)

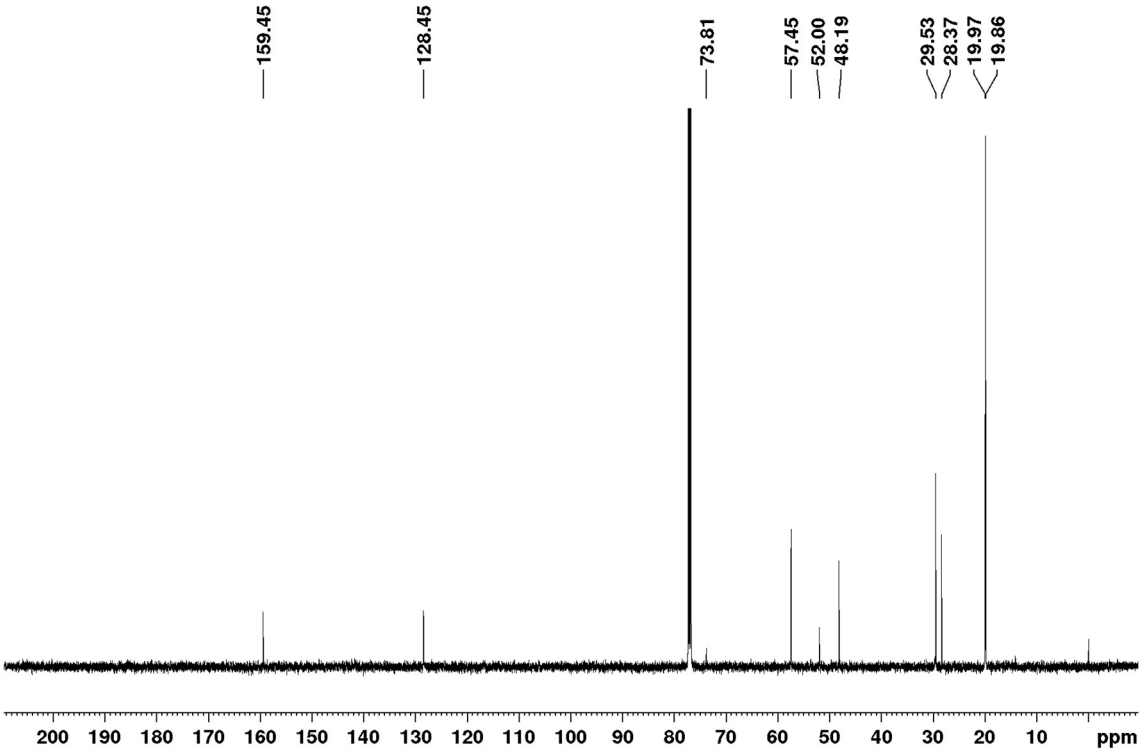


1-isobutylaminocarboxyl-9,10-bis((1*H*-1,2,3-triazol-4-yl)isobutylcarbamoyl)-1,7-dicarba-*closo*-dodecaborane (IVd)

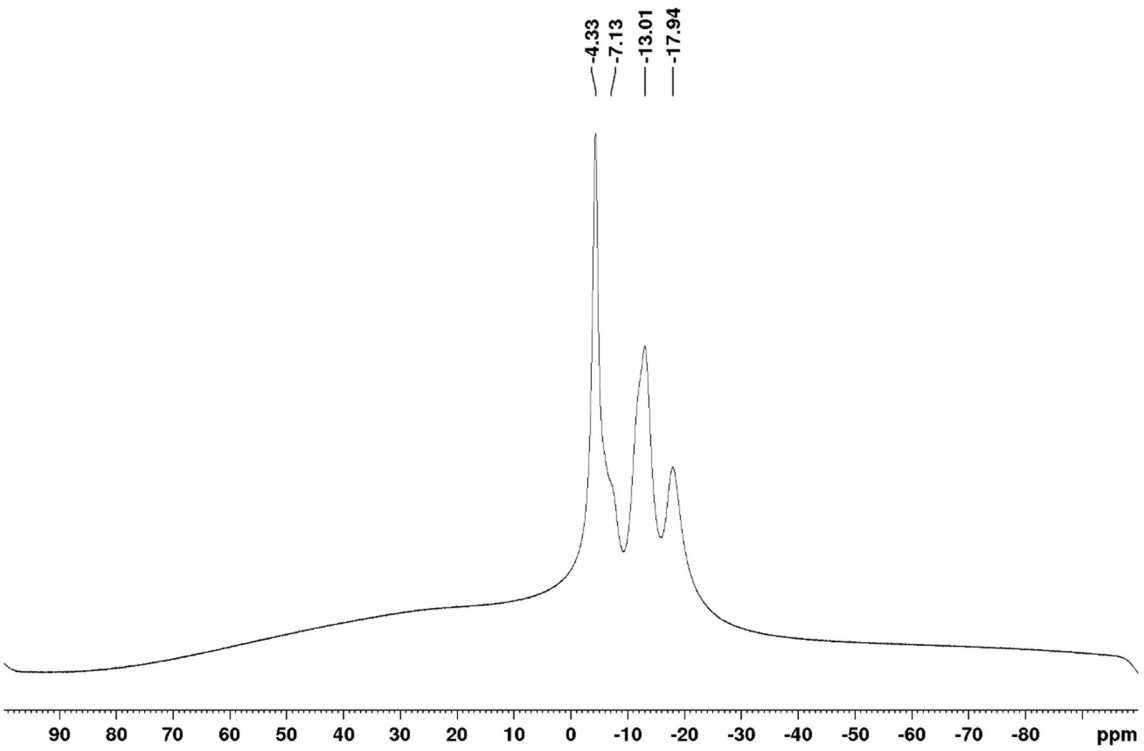
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

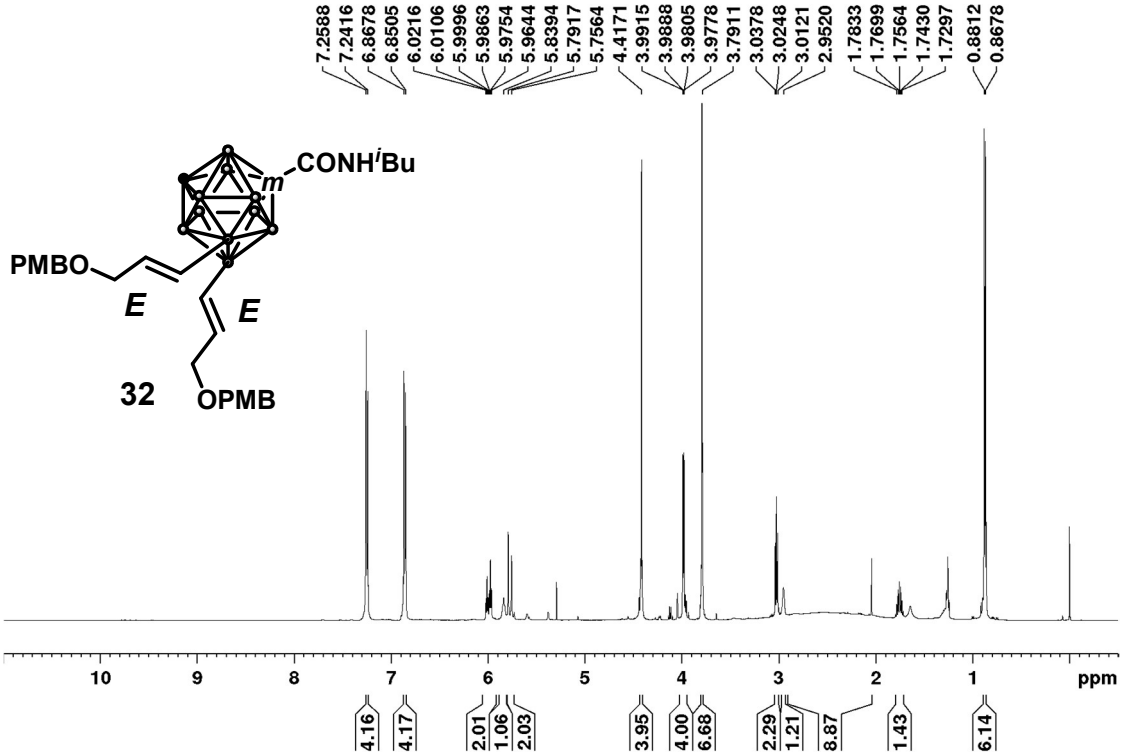


^{11}B NMR (160 MHz; CDCl3)

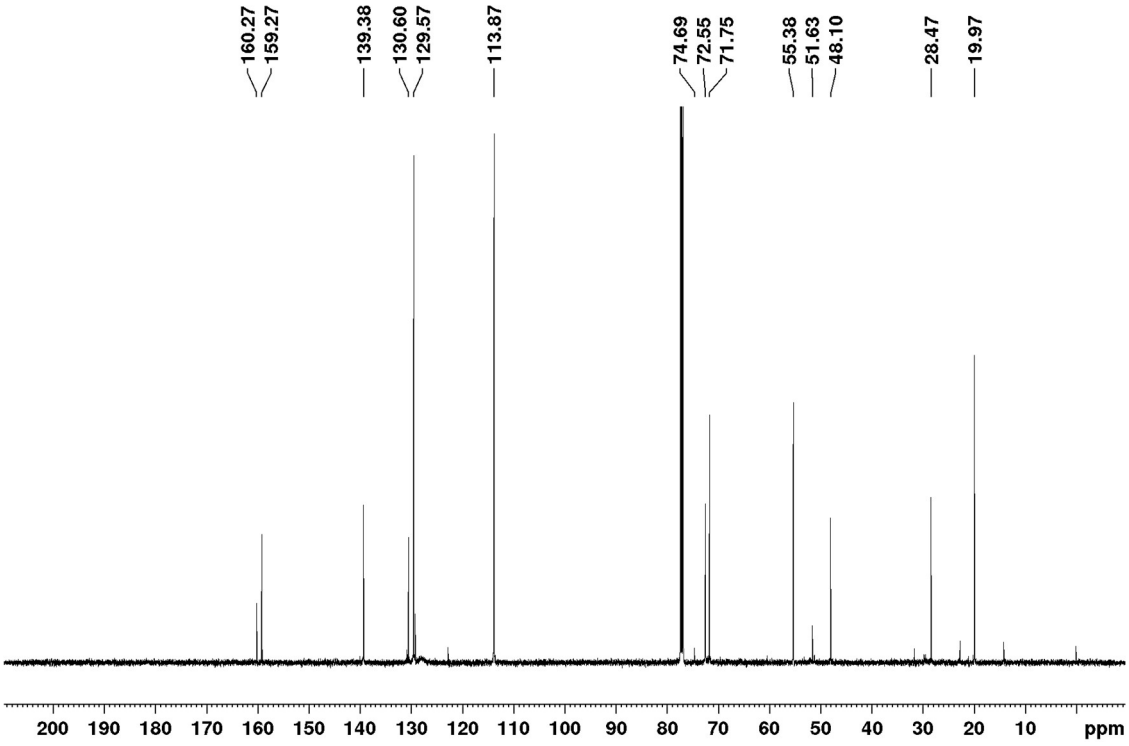


9,10-bis((*E*)-3-((4-methoxybenzyl)oxy)prop-1-en-1-yl)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (32)

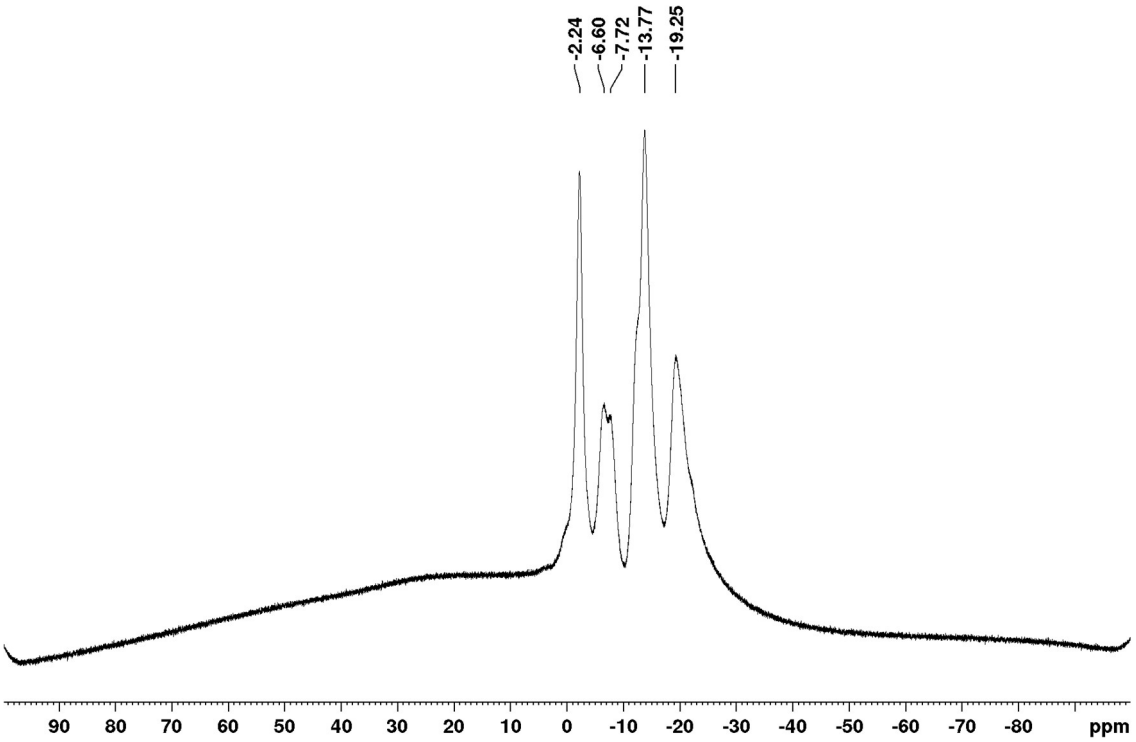
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

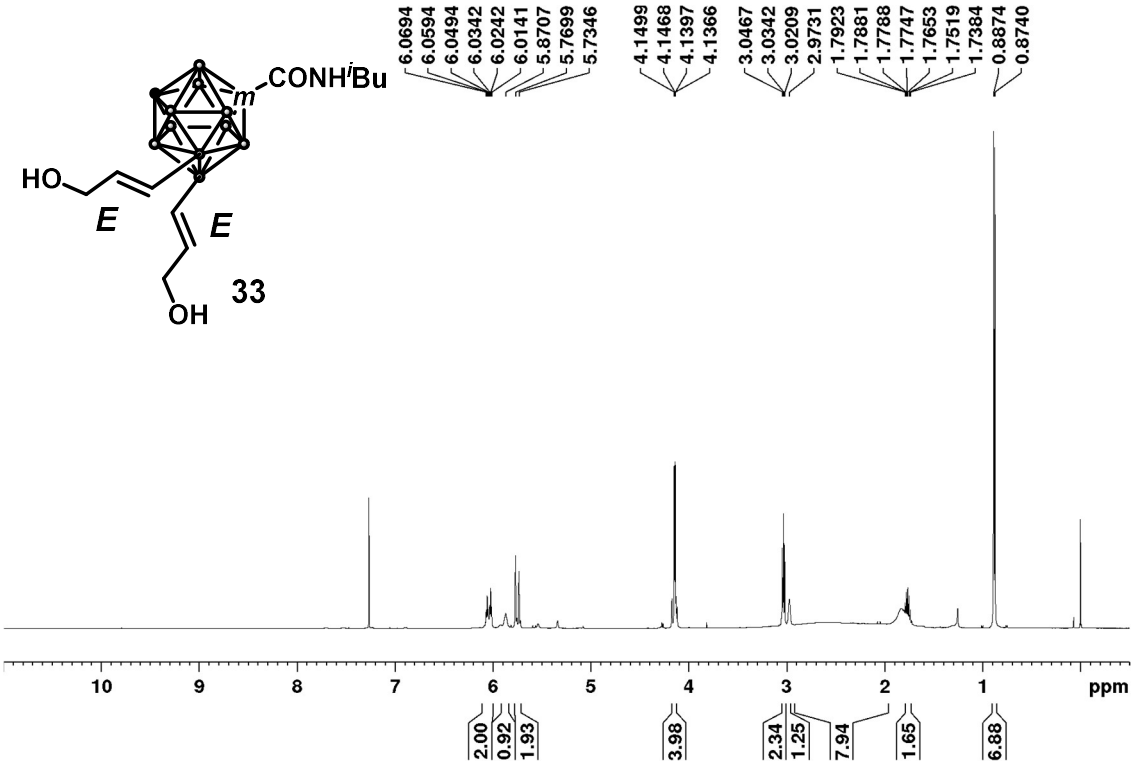


^{11}B NMR (160 MHz; $CDCl_3$)

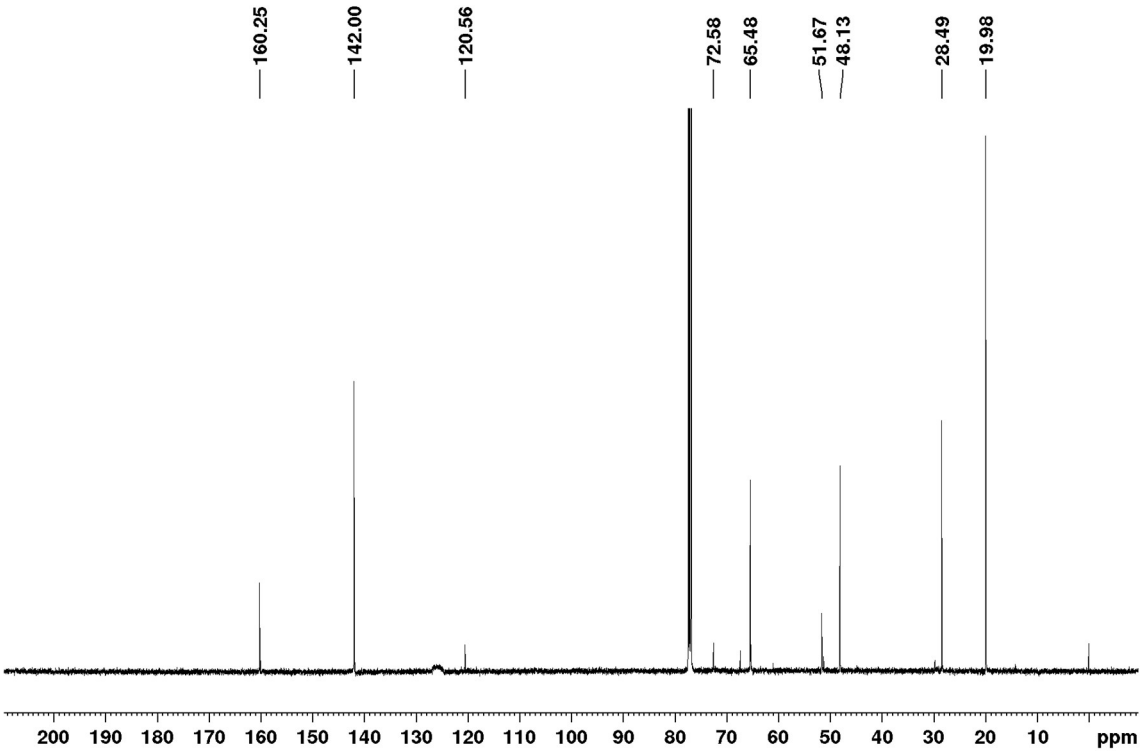


9,10-bis((*E*)-3-hydroxyprop-1-en-1-yl)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (33)

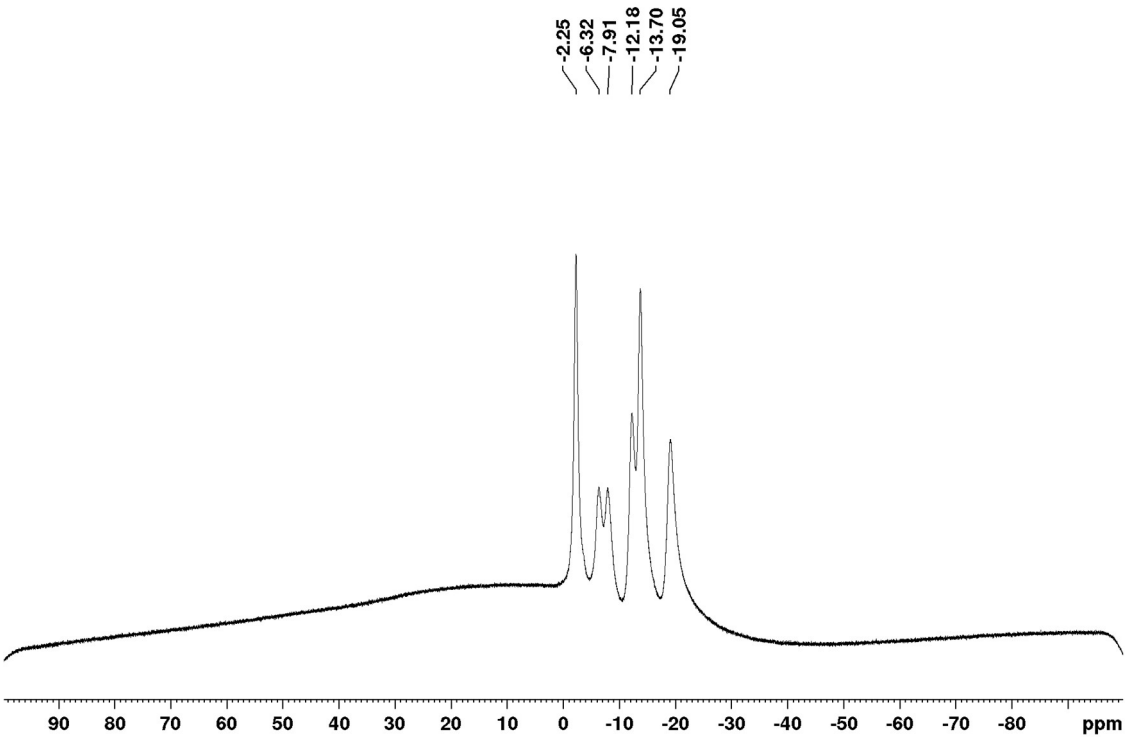
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

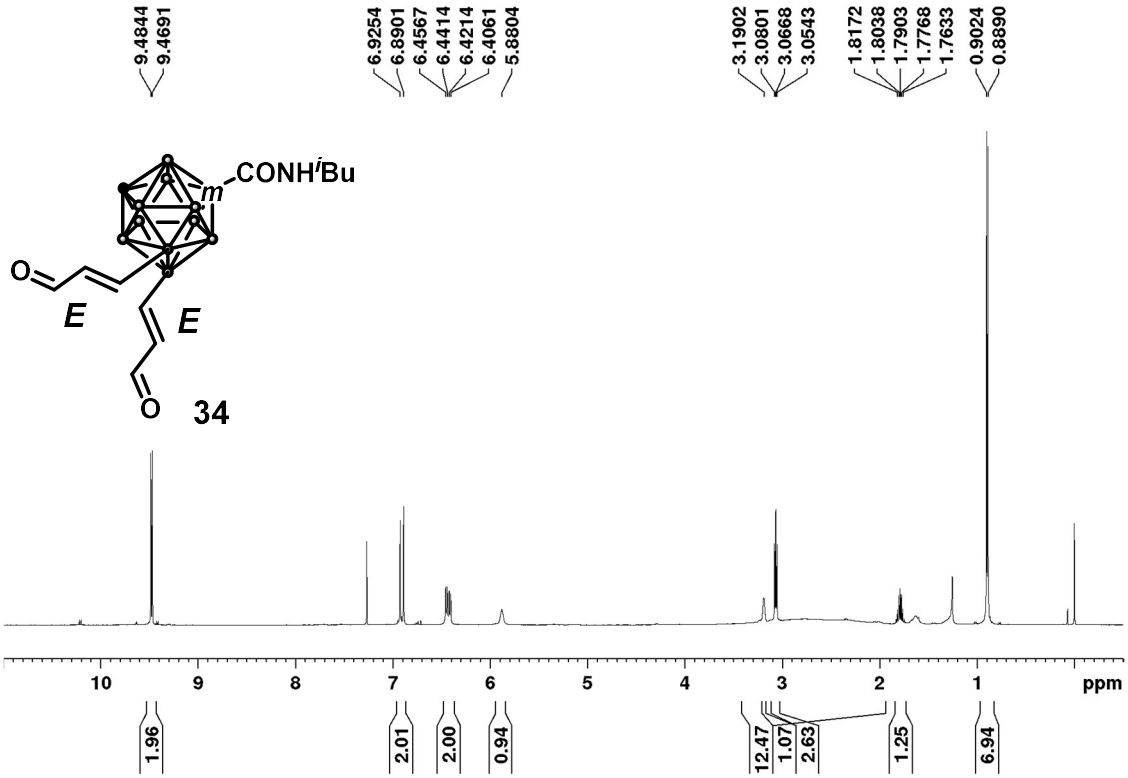


^{11}B NMR (160 MHz; $CDCl_3$)

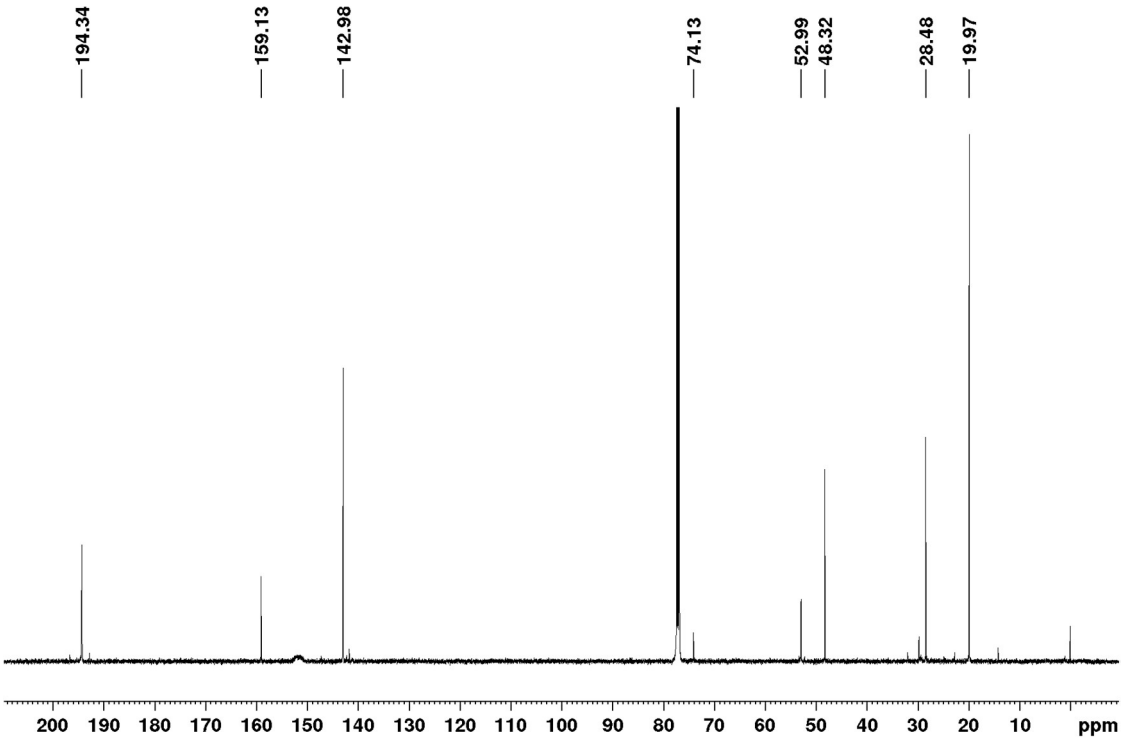


9,10-bis((*E*)-3-oxoprop-1-en-1-yl)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (34)

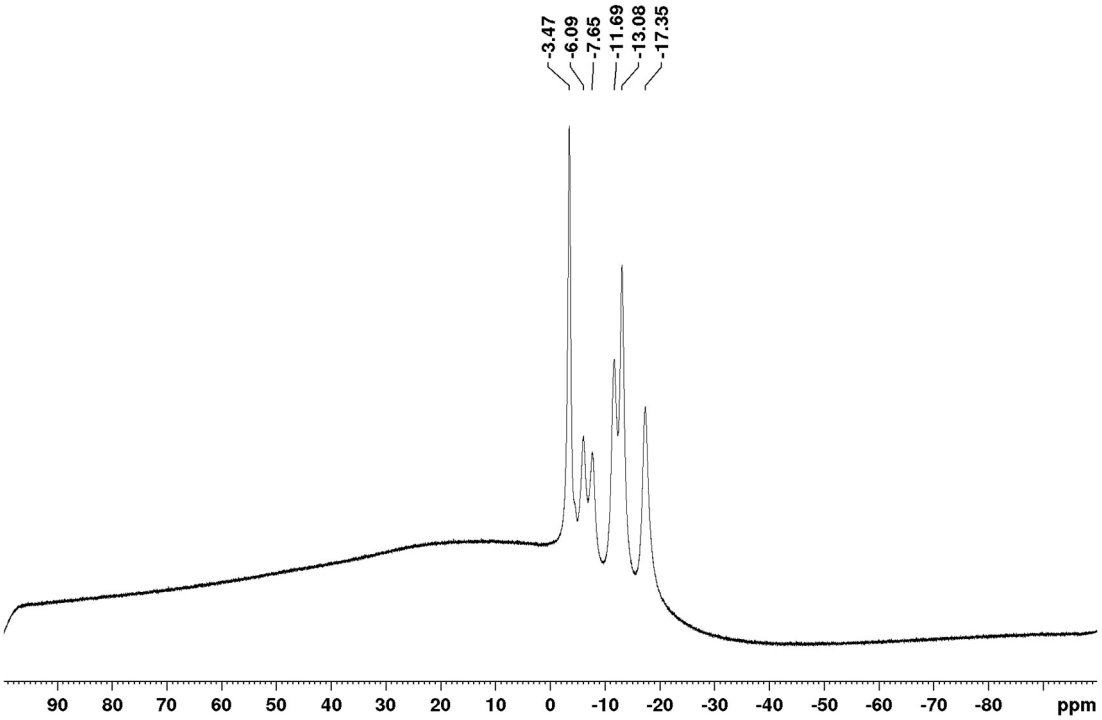
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

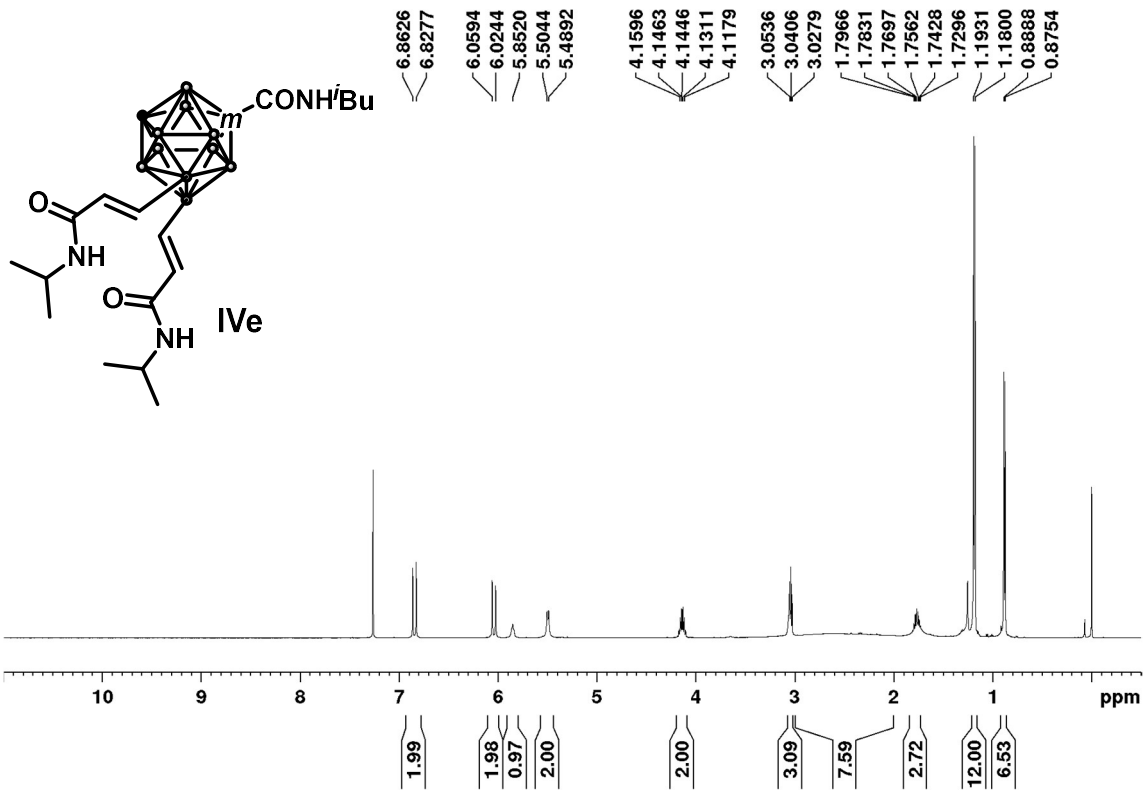


^{11}B NMR (160 MHz; $CDCl_3$)

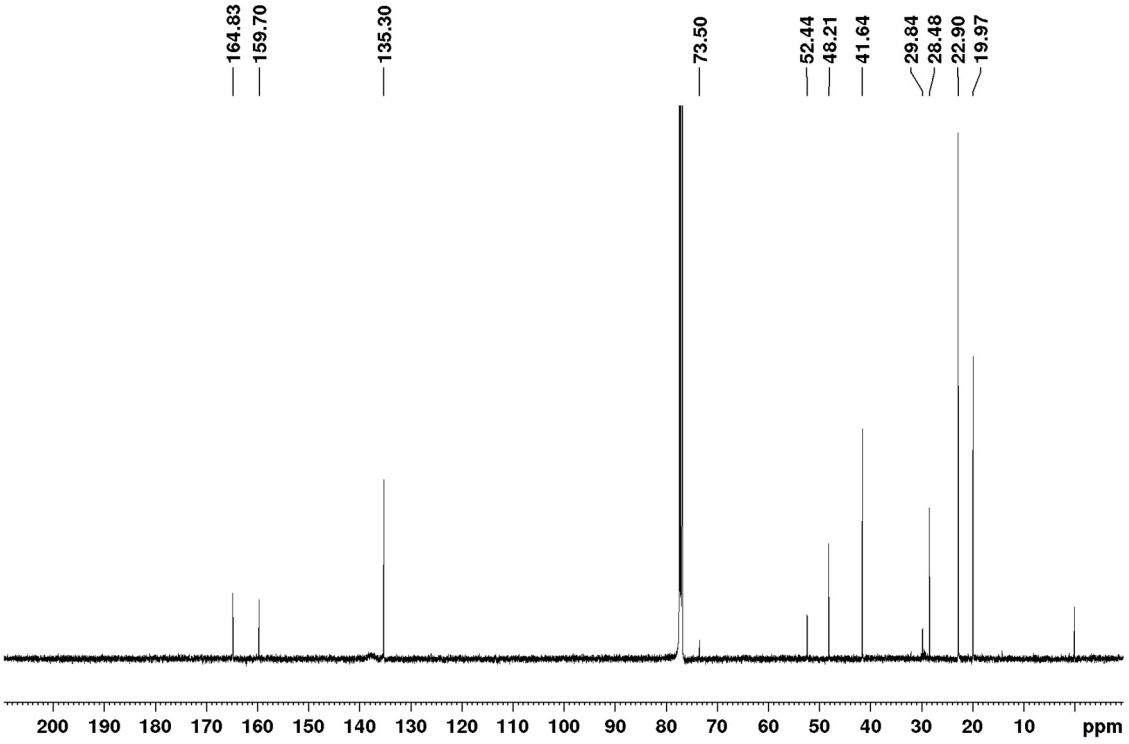


9,10-bis((E)-3-(isopropylamino)-1-isobutylcarbamoyl-1,7-dicarba-*closo*-dodecaborane (IVe)

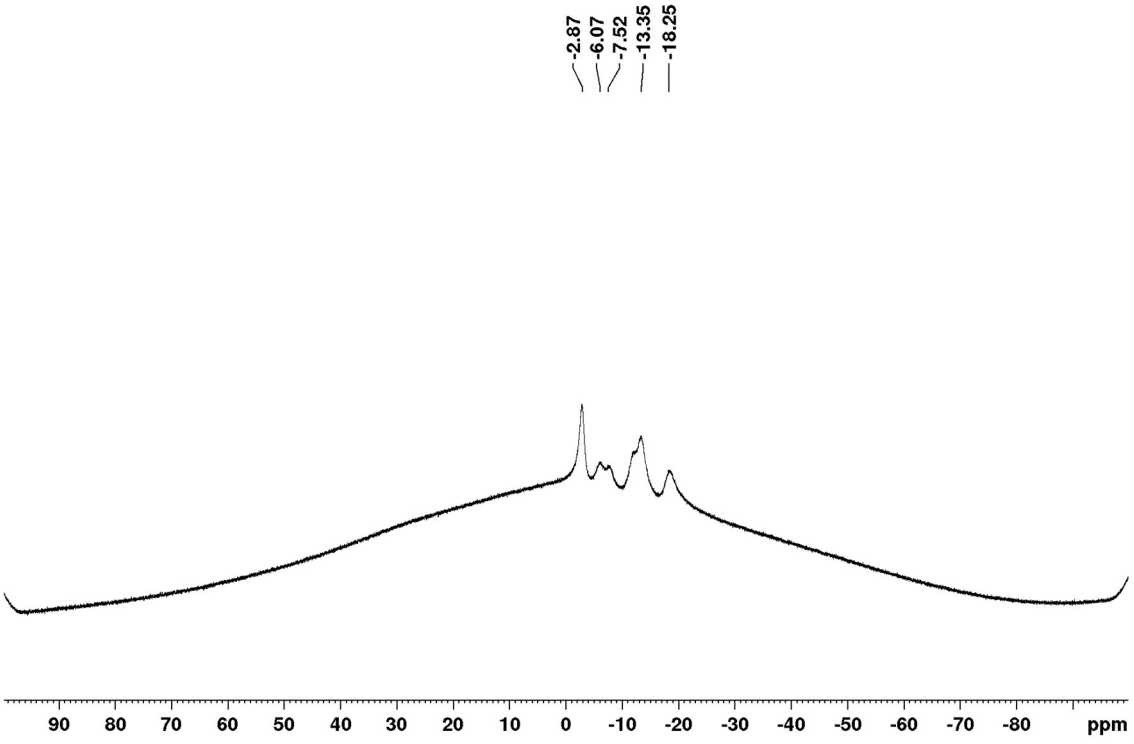
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

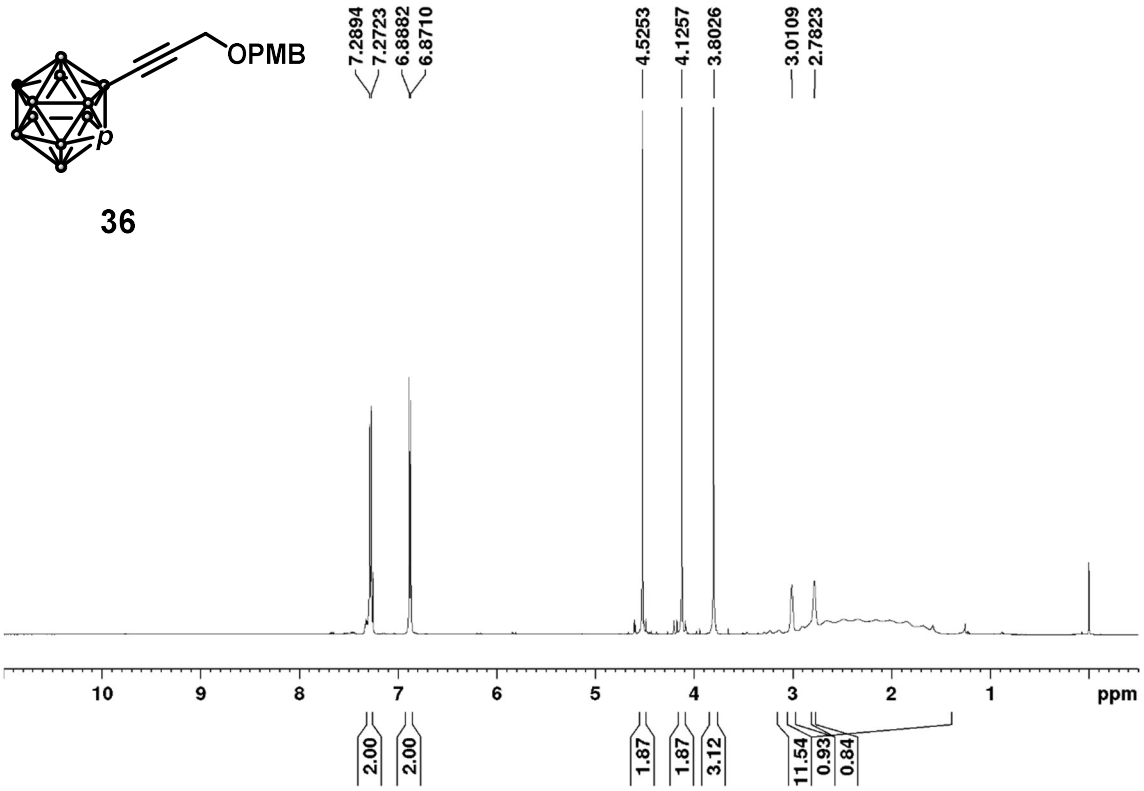


^{11}B NMR (160 MHz; $CDCl_3$)

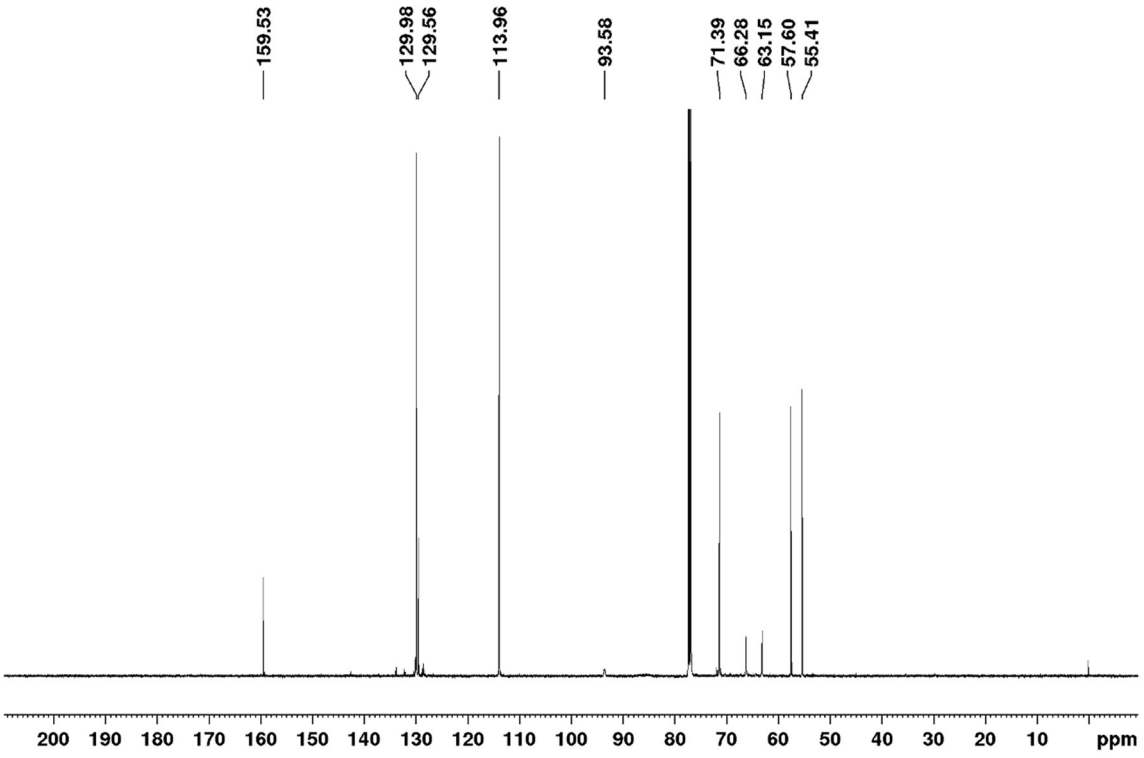


2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaborane (36)

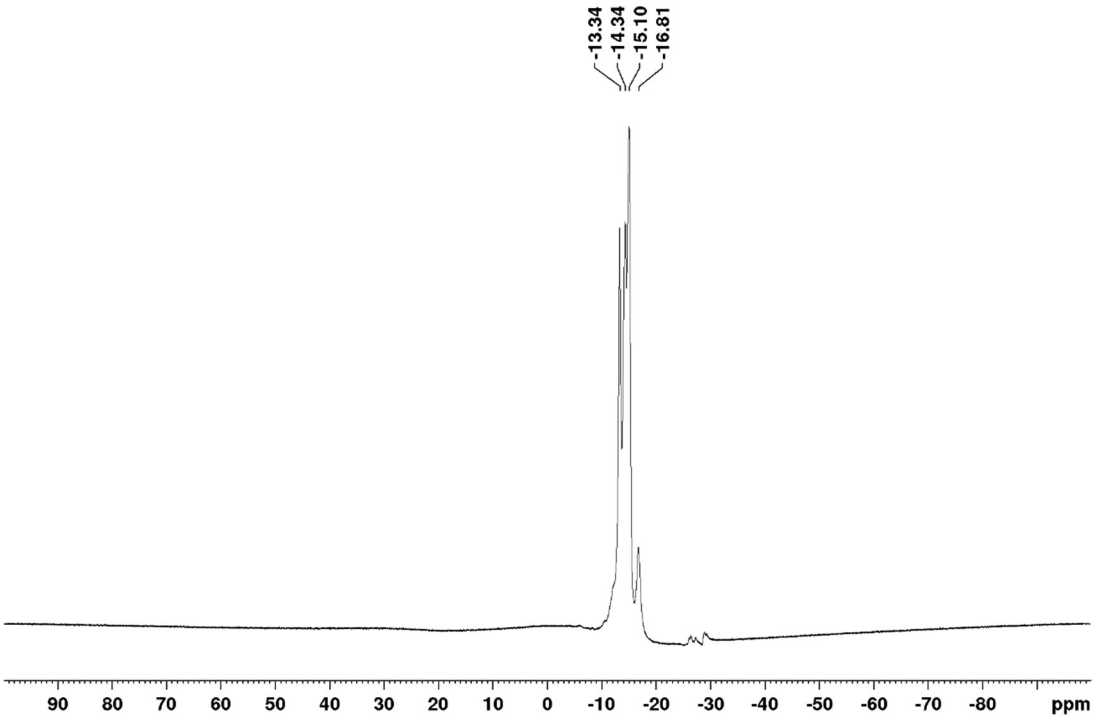
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

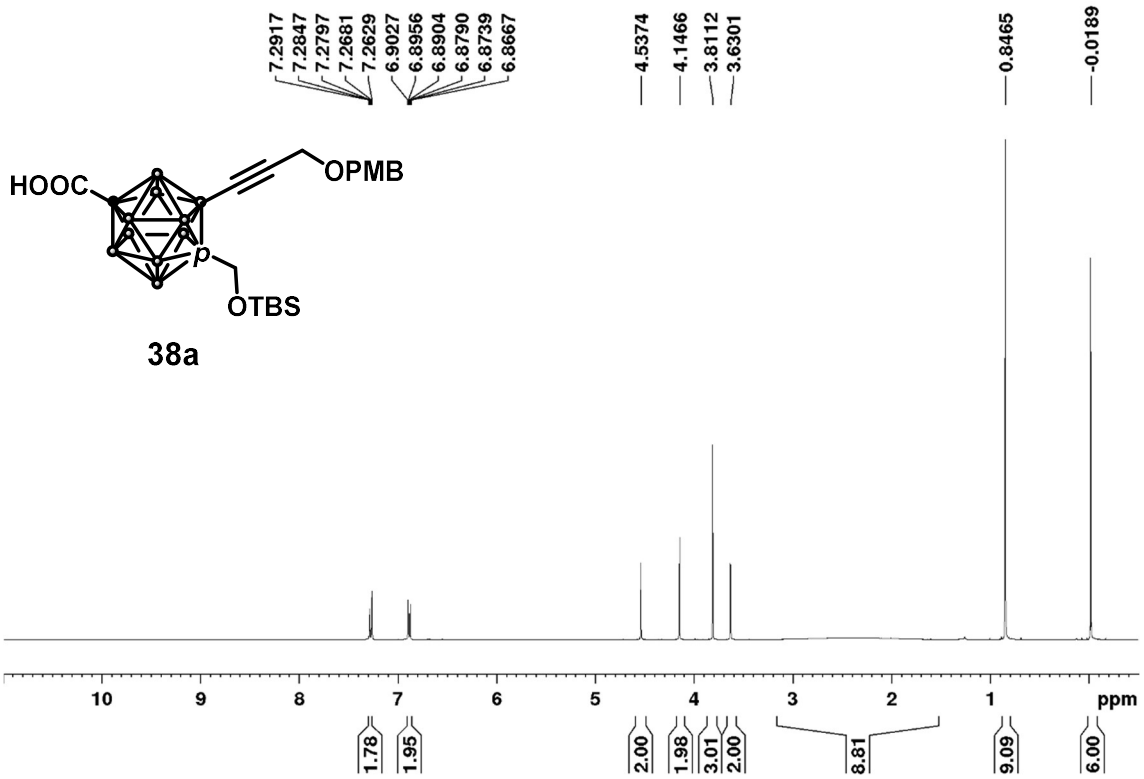


^{11}B NMR (160 MHz; $CDCl_3$)

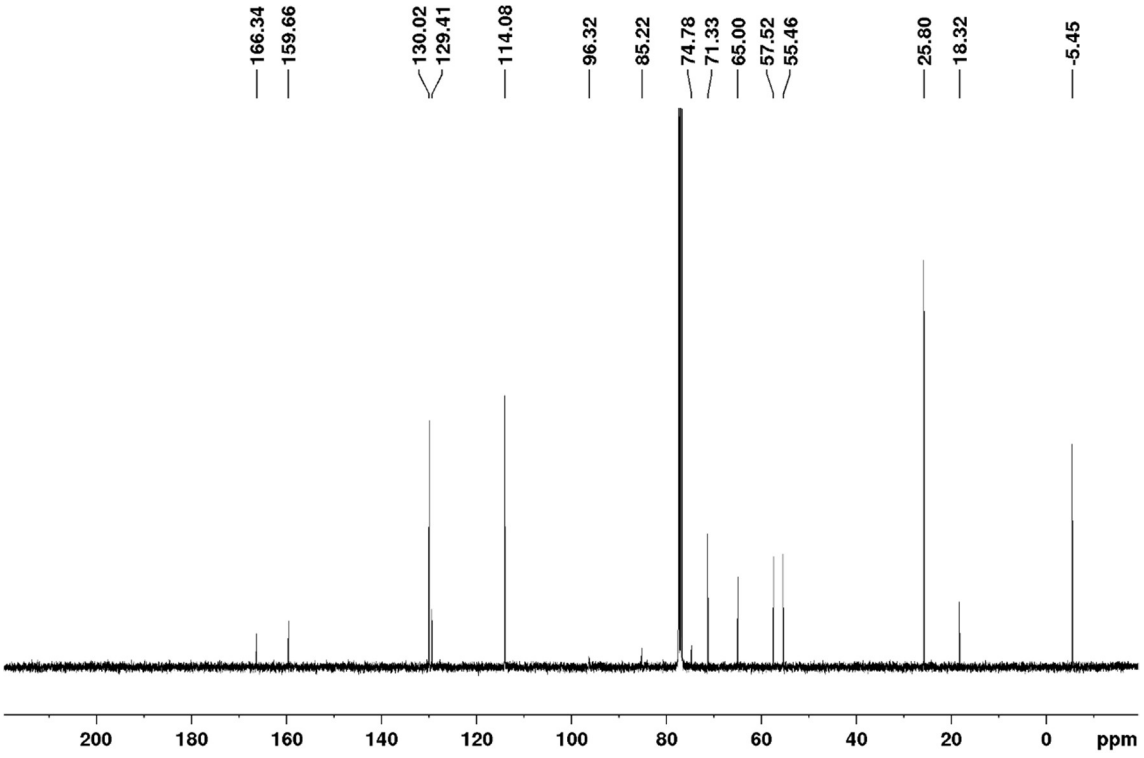


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaboranyl-1-carboxylic acid (38a)

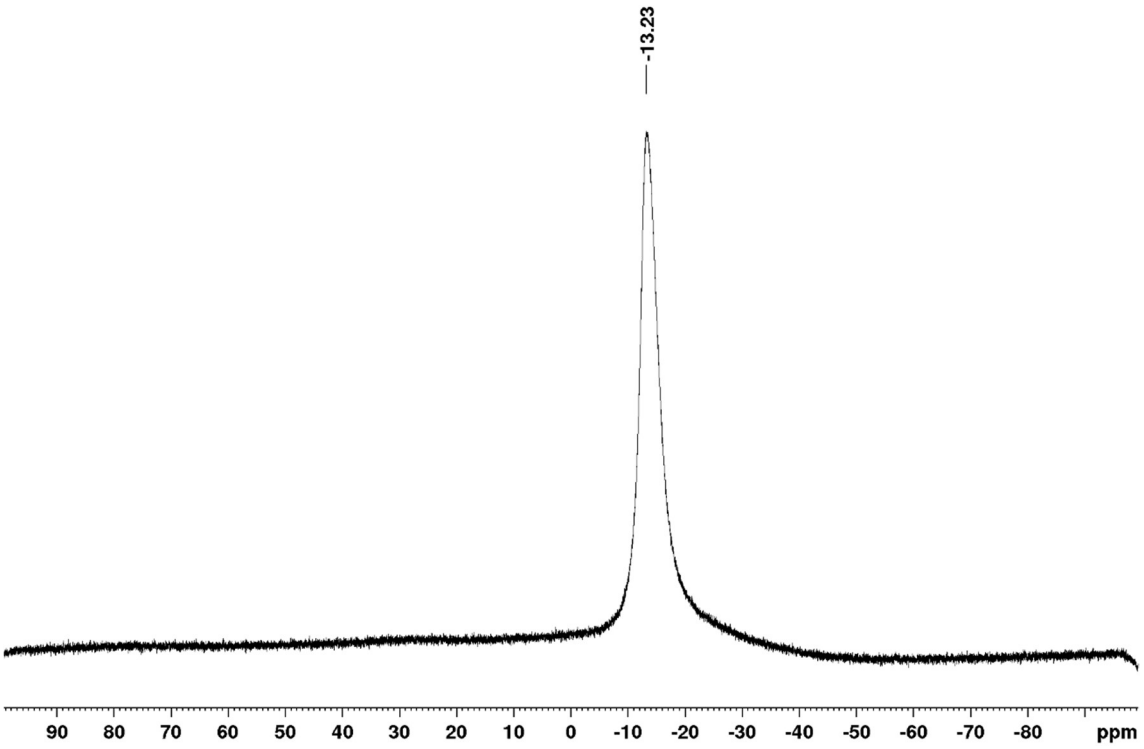
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

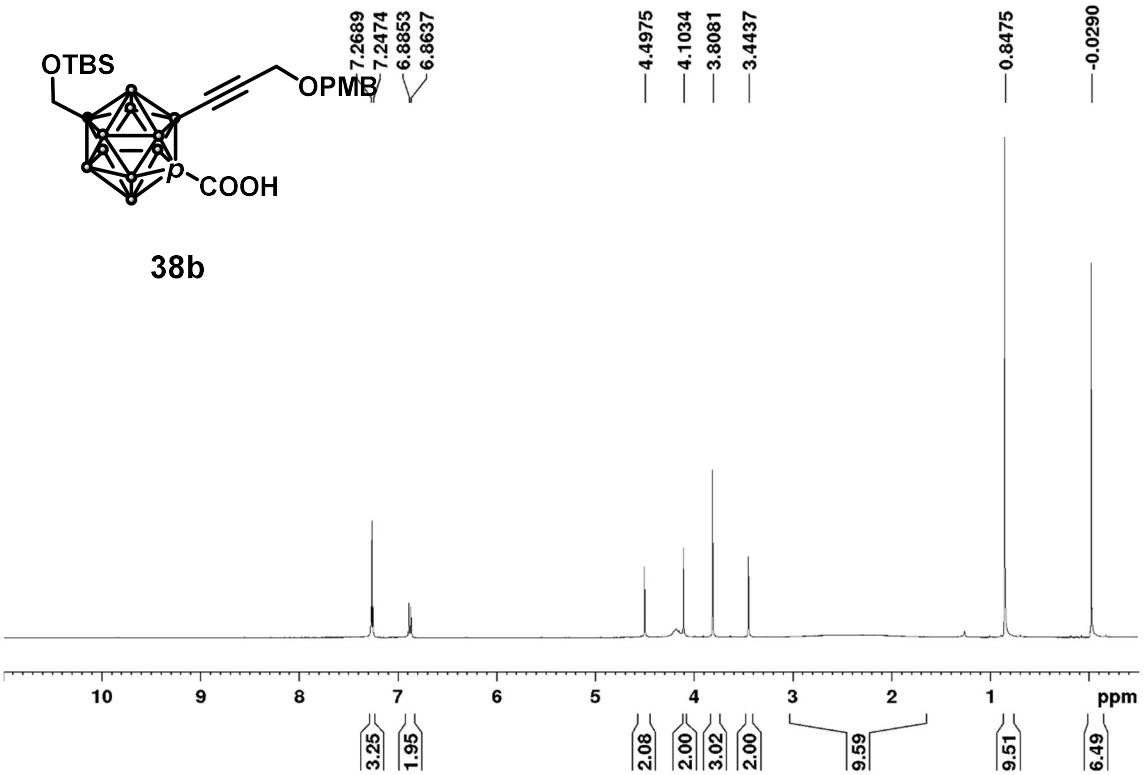


^{11}B NMR (128 MHz; $CDCl_3$)

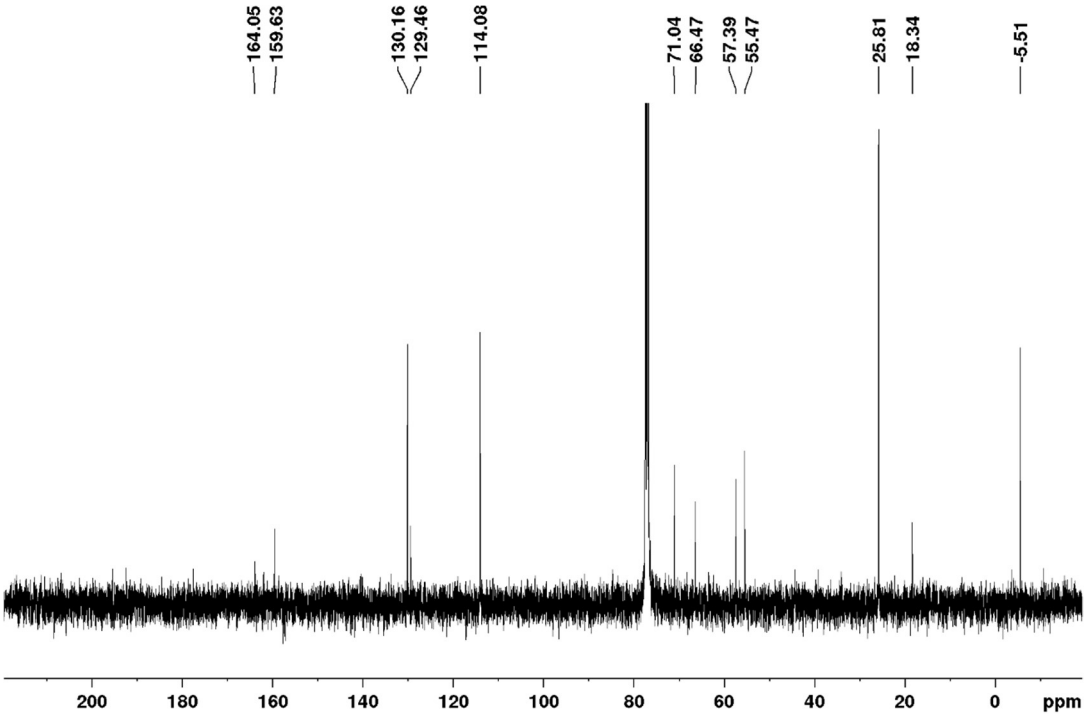


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaboranyl-1-carboxylic acid (38b)

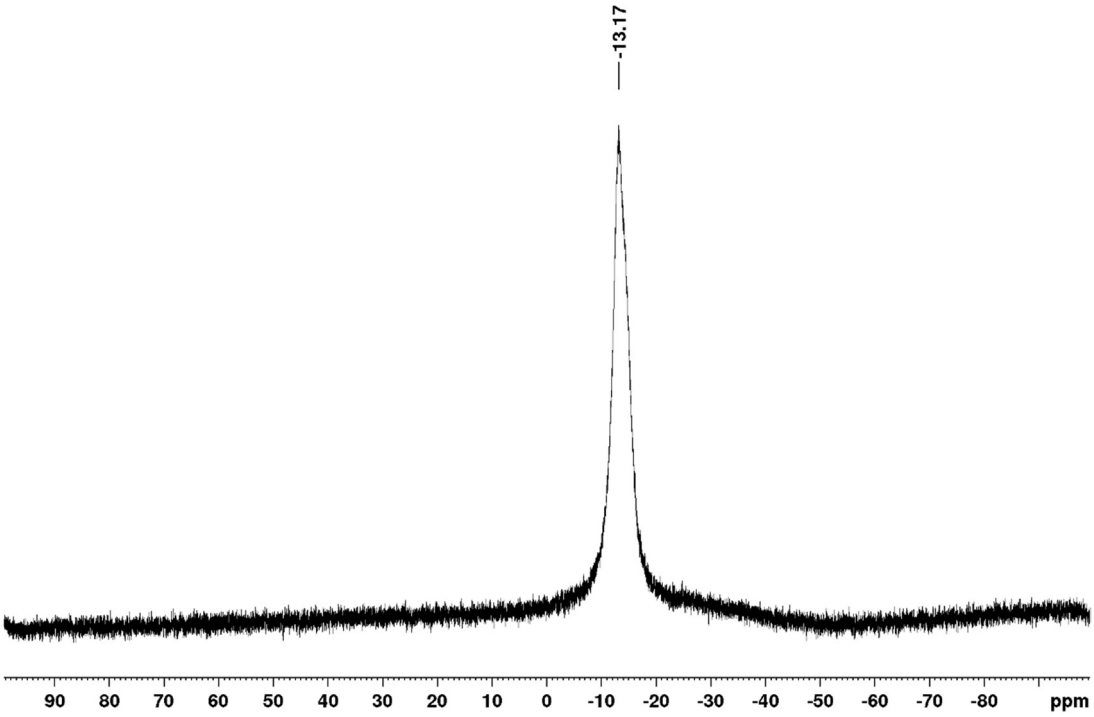
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

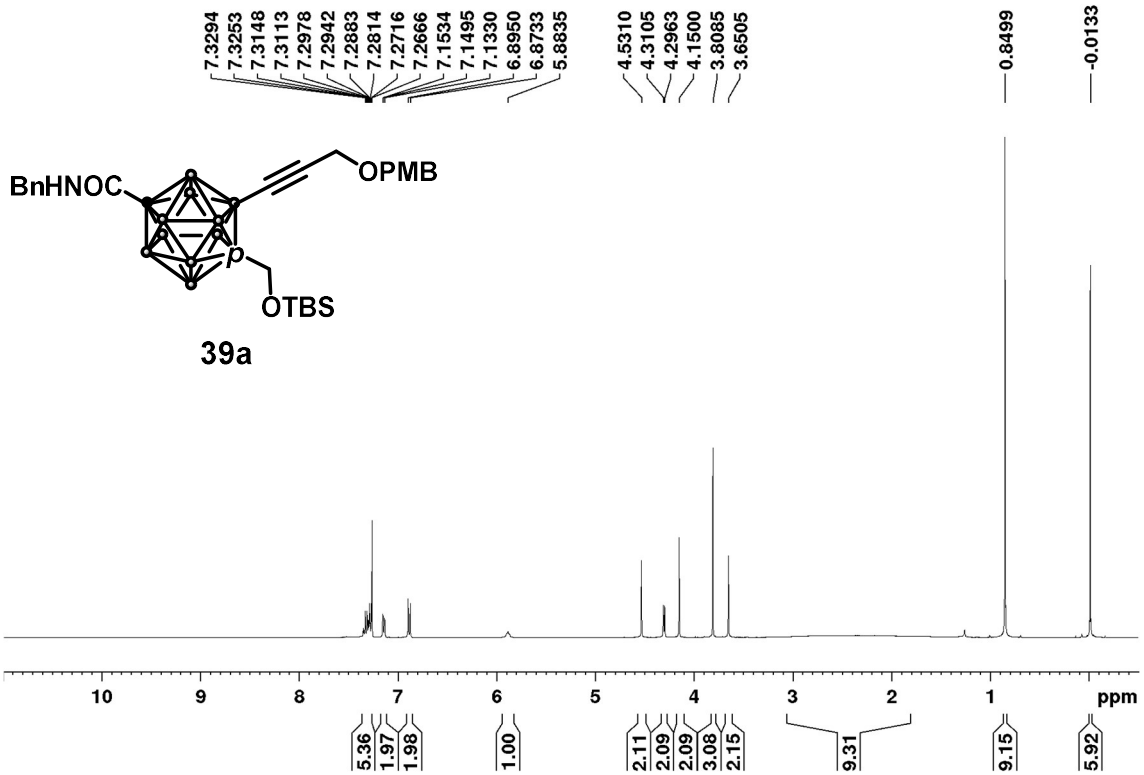


^{11}B NMR (128 MHz; $CDCl_3$)

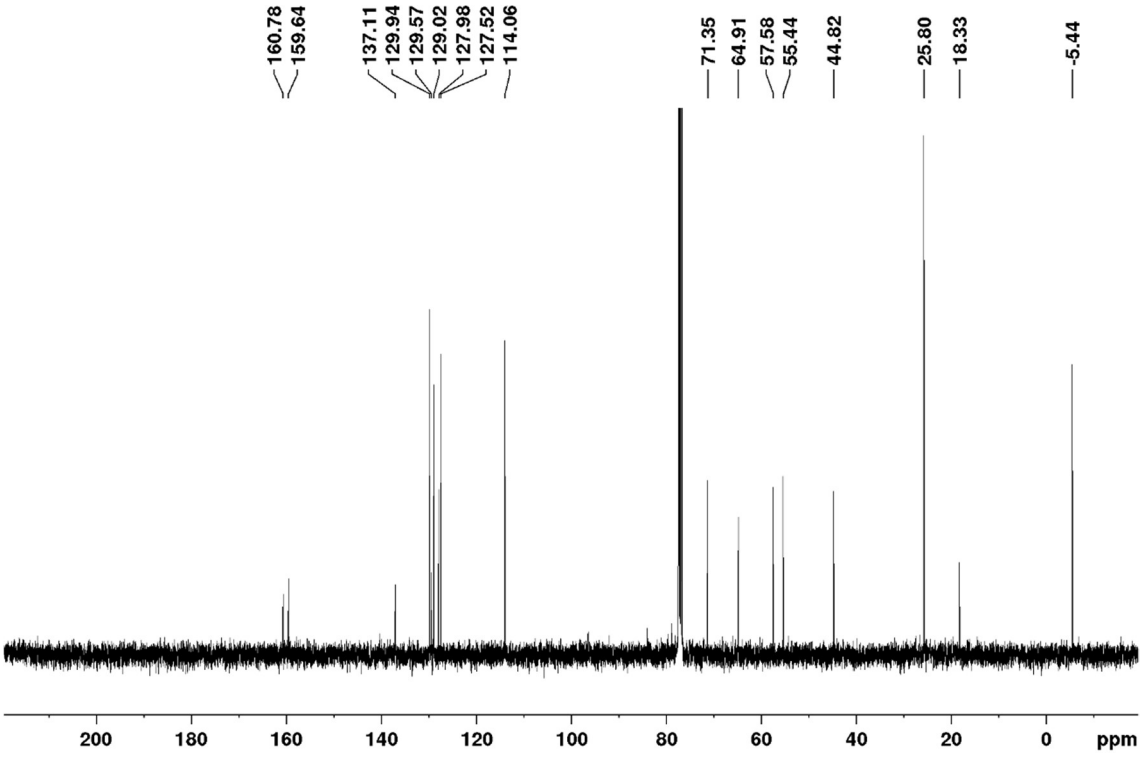


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-12- benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (39a)

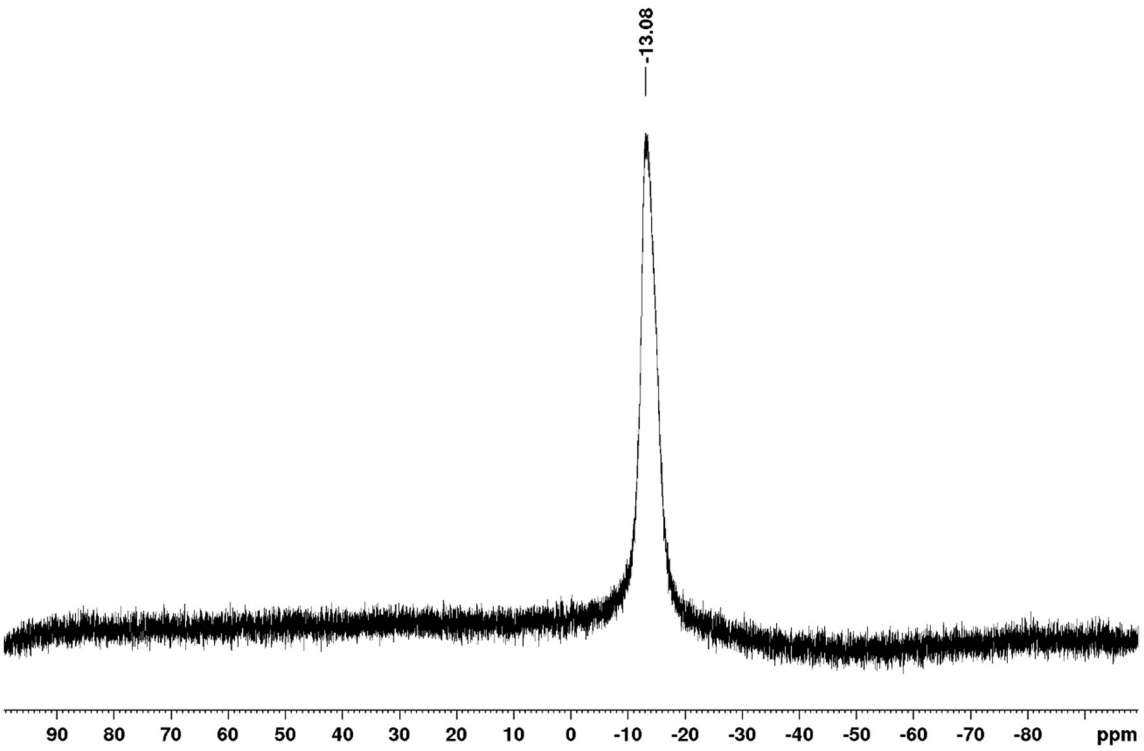
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

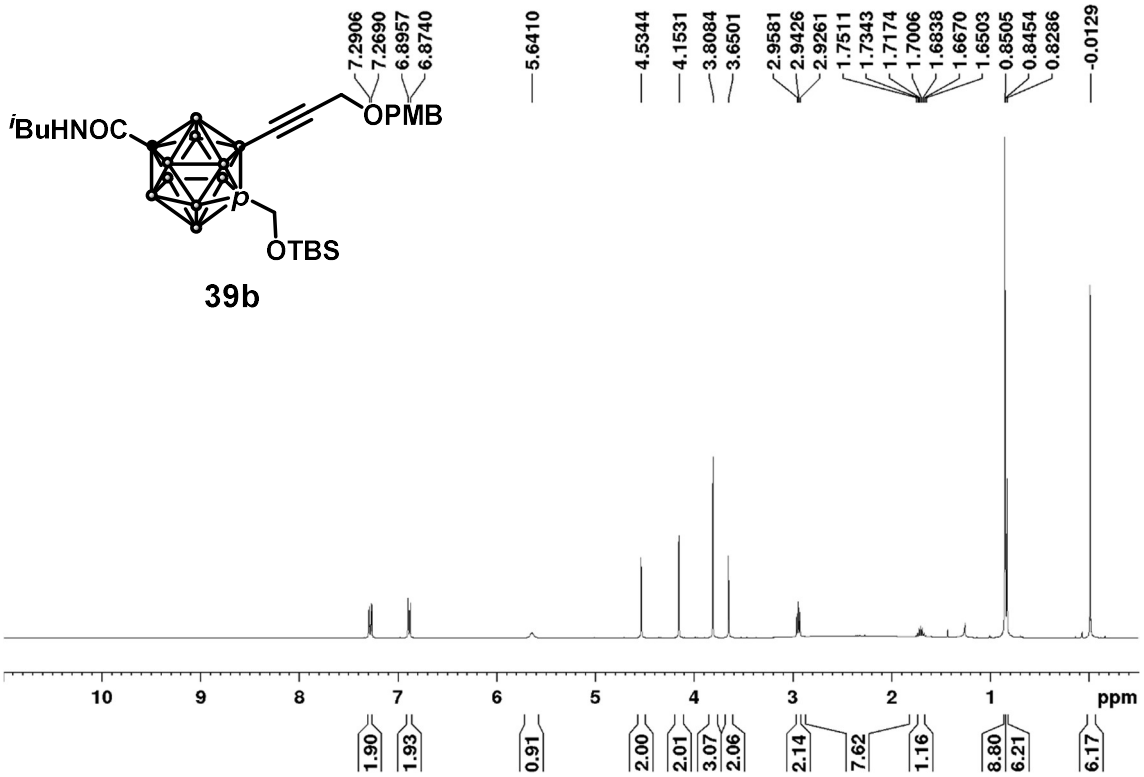


^{11}B NMR (128 MHz; $CDCl_3$)

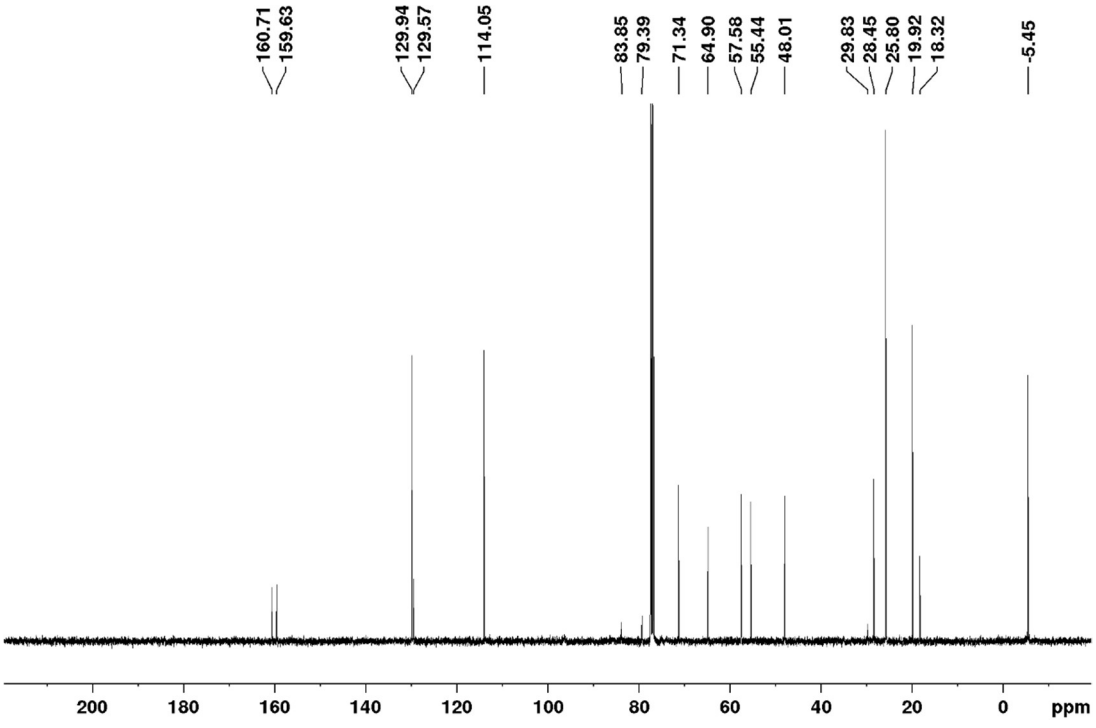


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (39b)

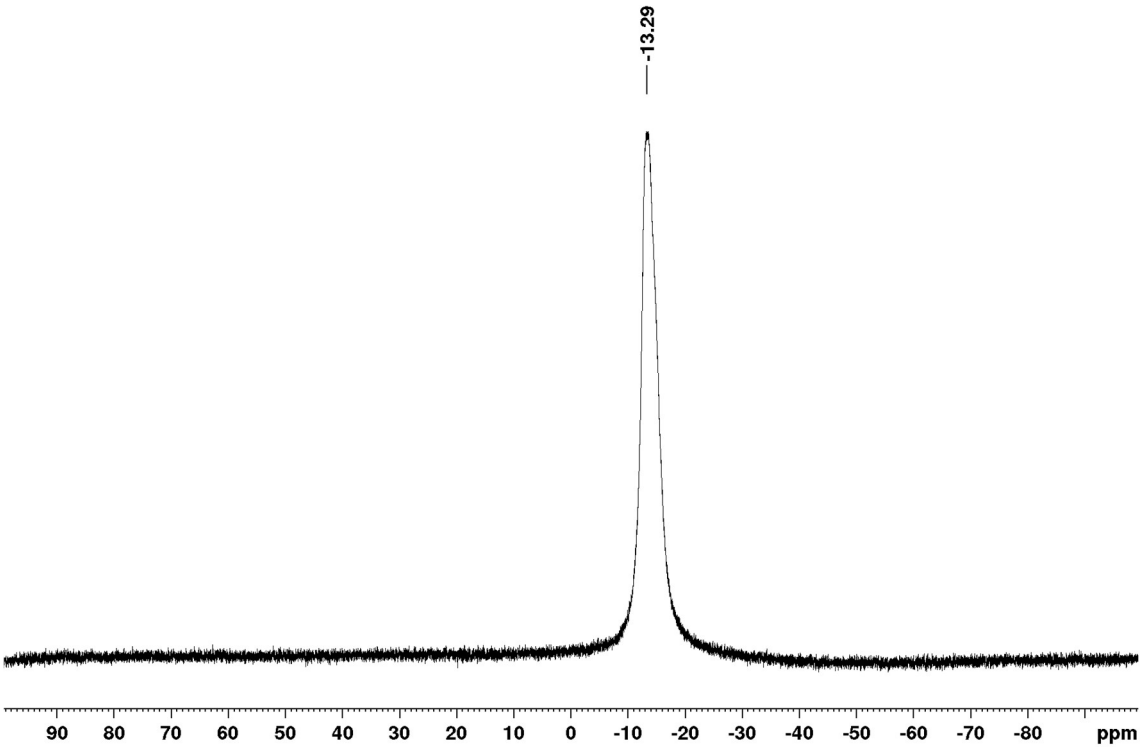
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

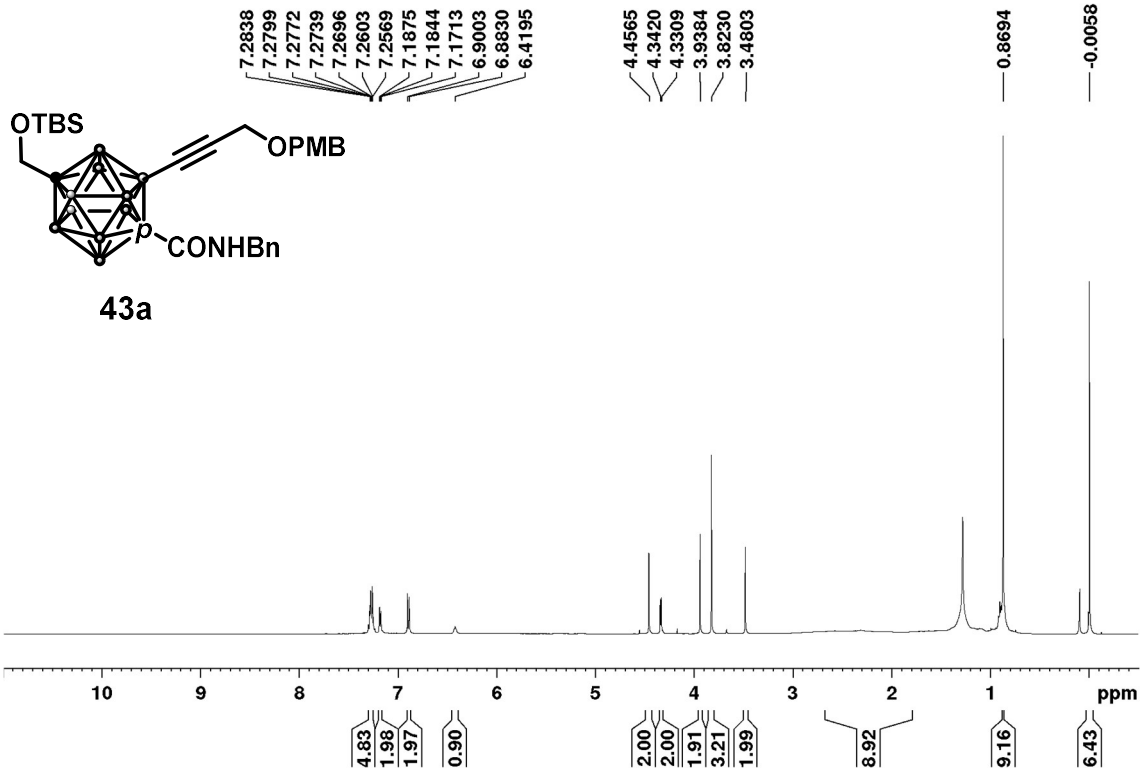


^{11}B NMR (128 MHz; $CDCl_3$)

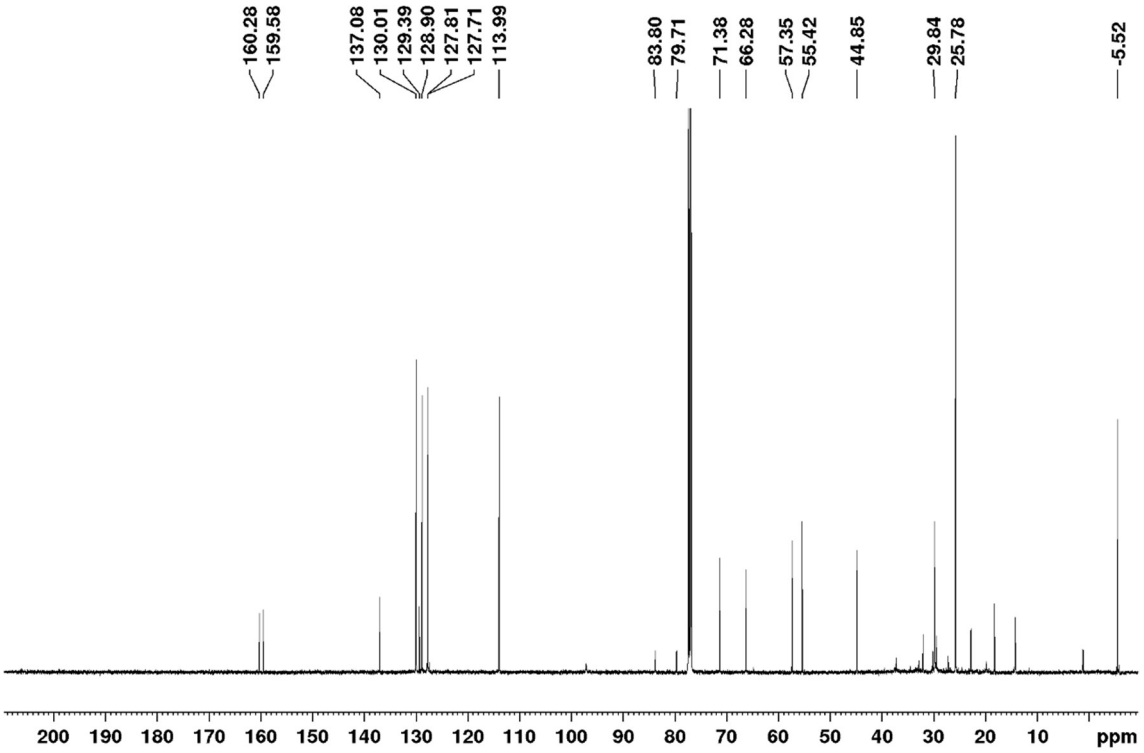


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (43a)

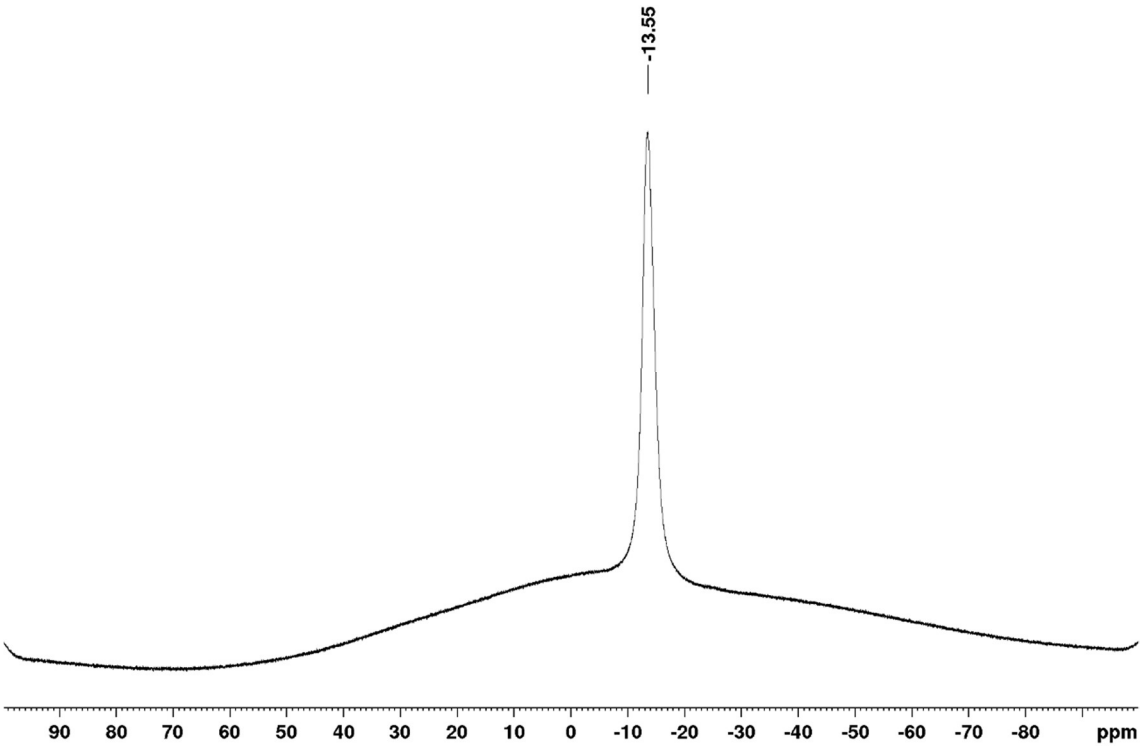
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

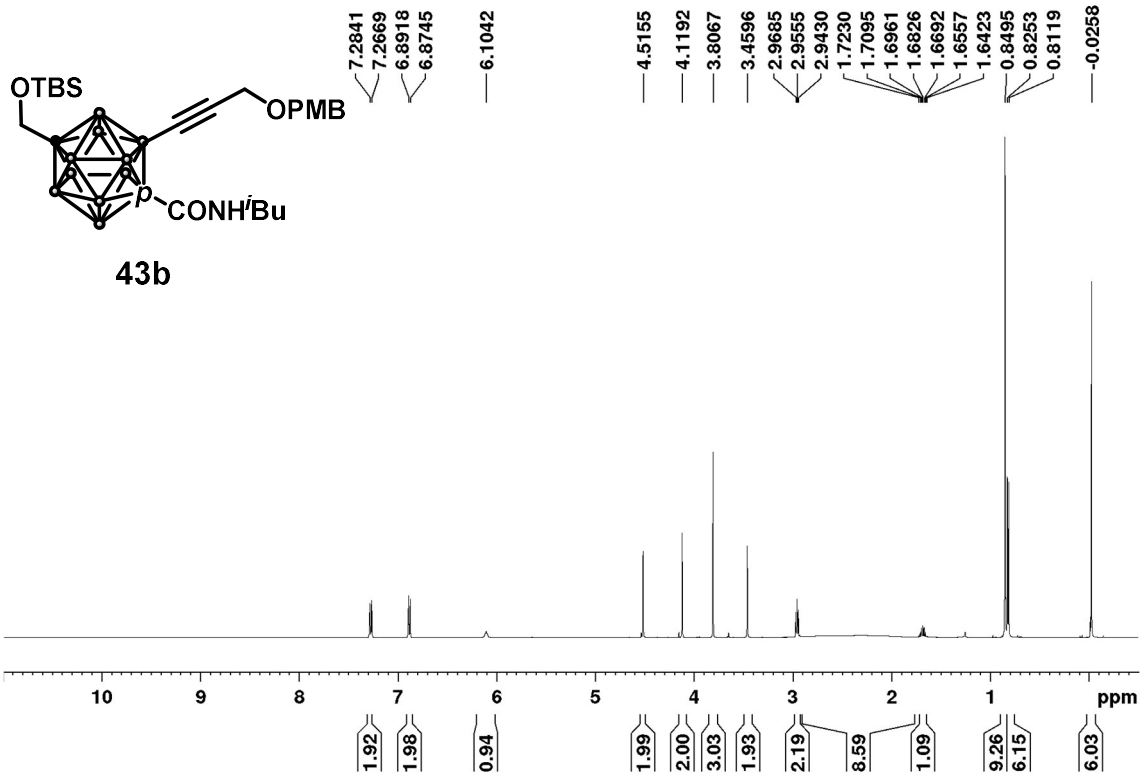


^{11}B NMR (160 MHz; $CDCl_3$)

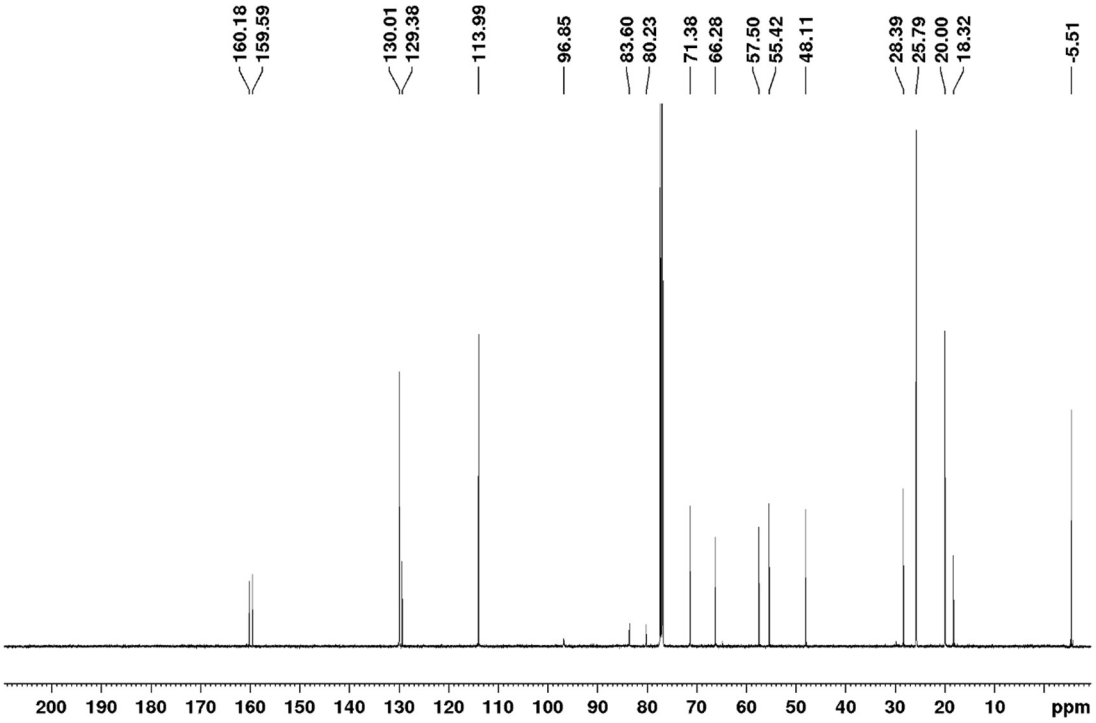


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-((4-methoxybenzyl)oxy)prop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (43b)

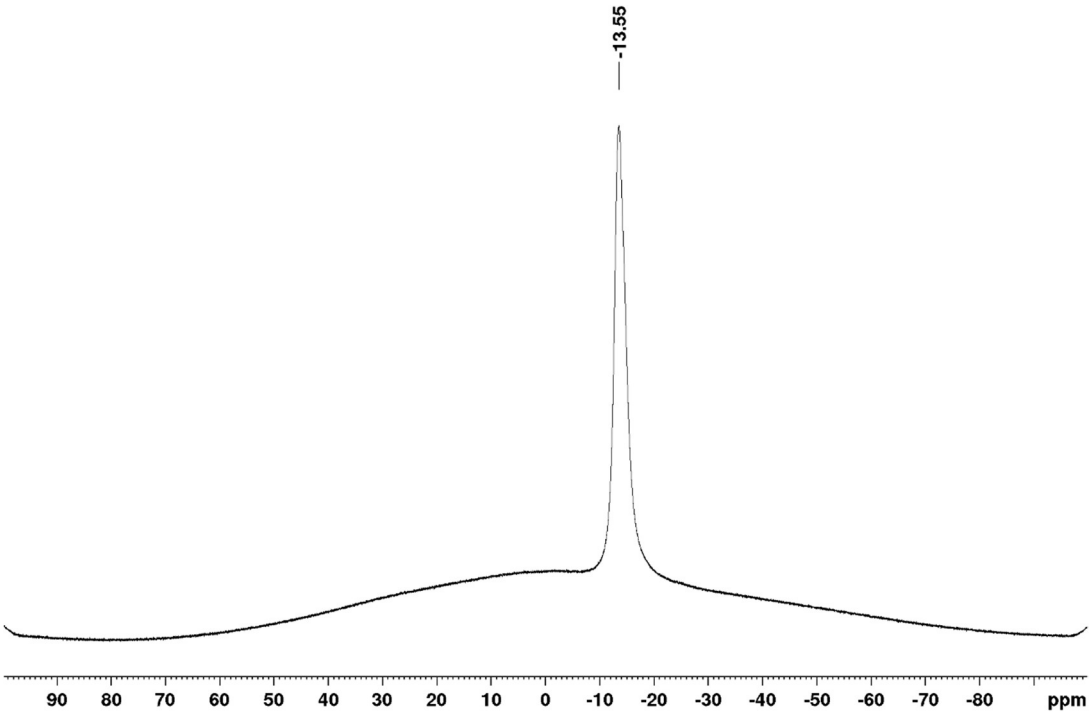
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

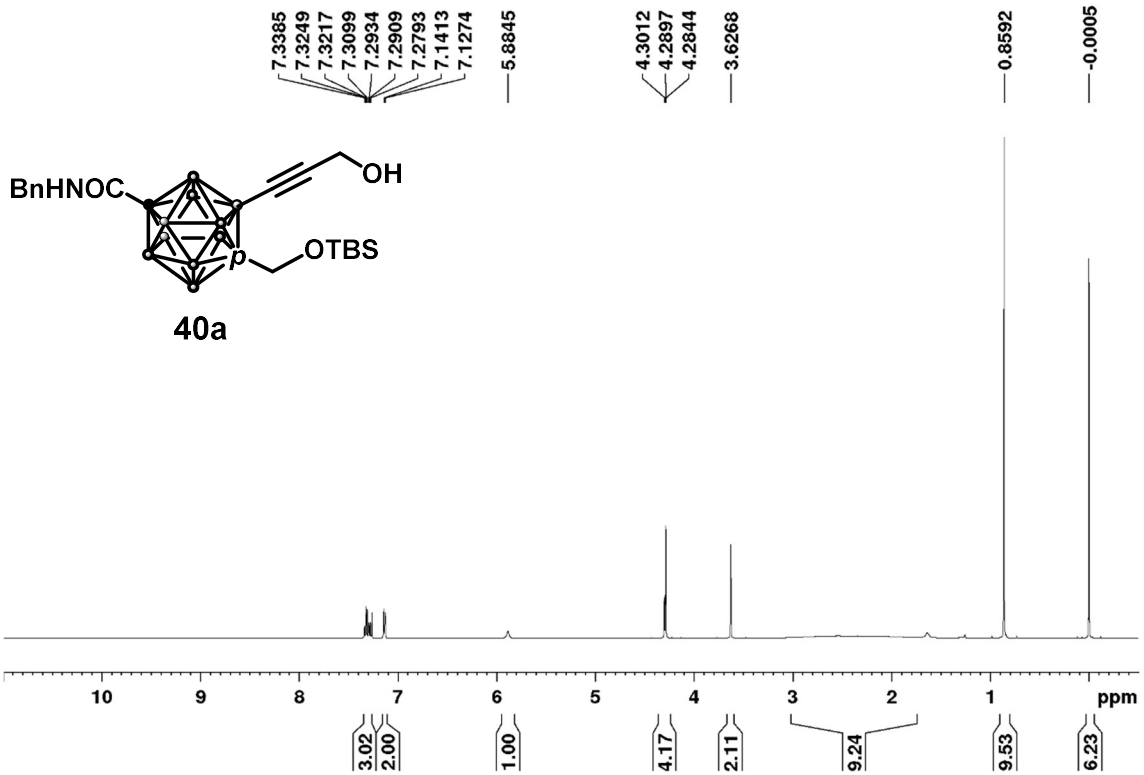


^{11}B NMR (160 MHz; $CDCl_3$)

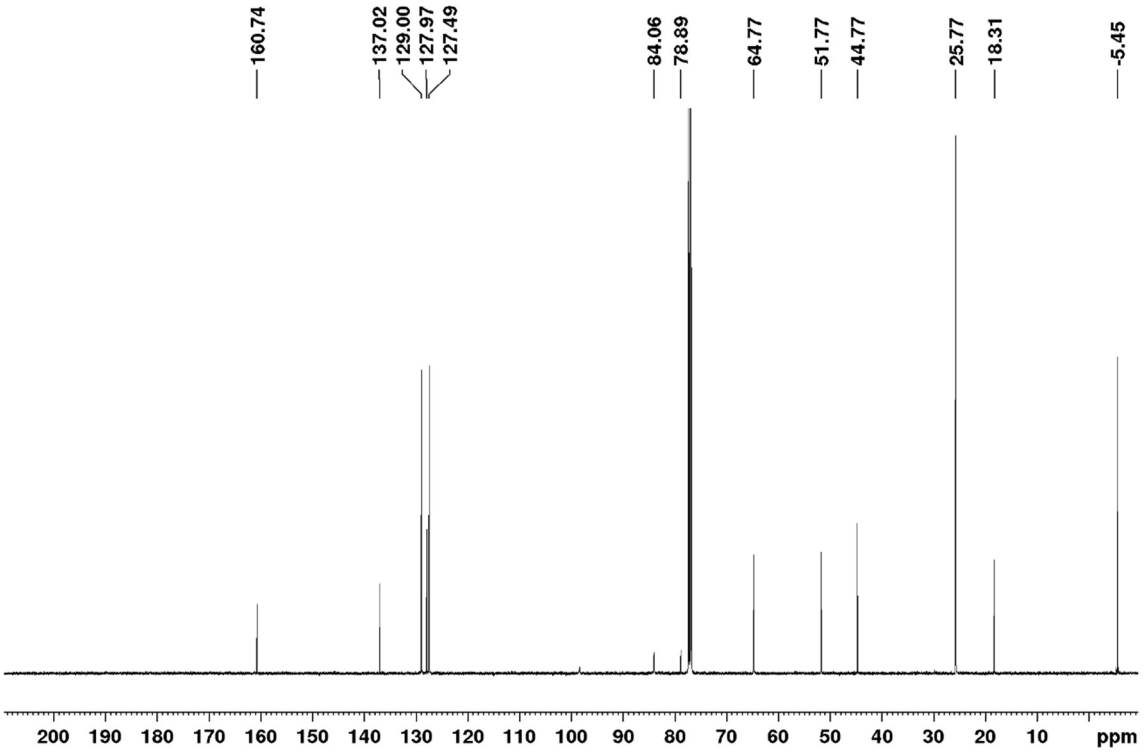


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (40a)

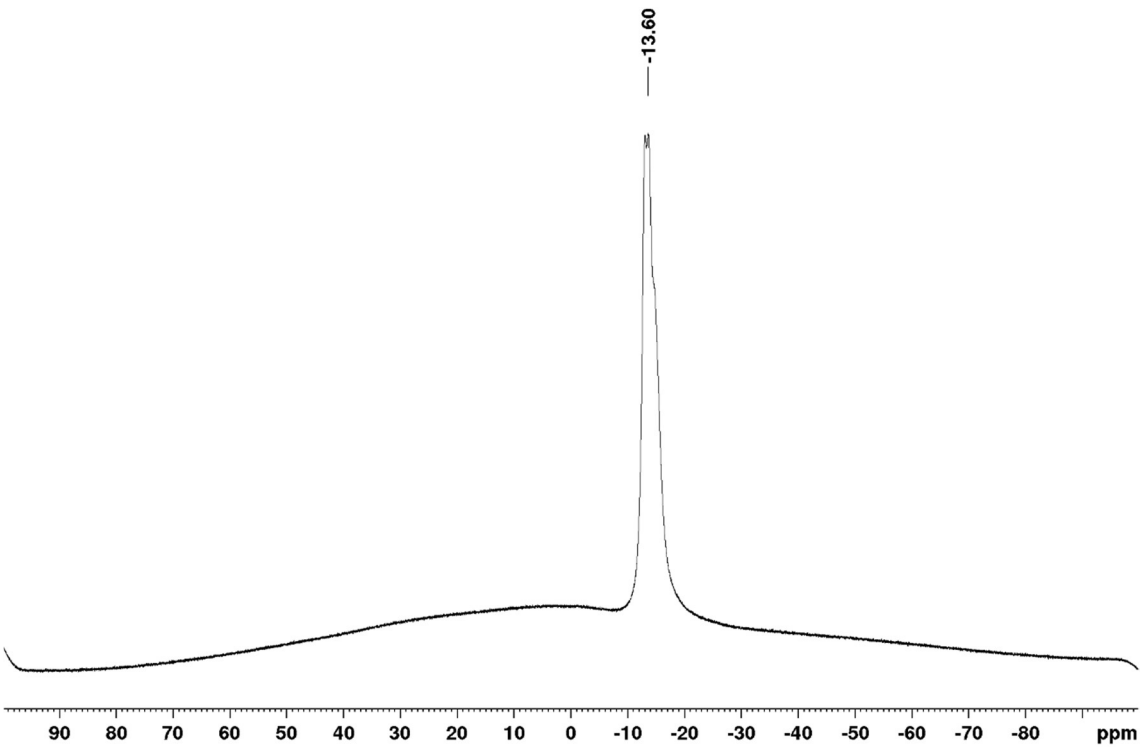
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

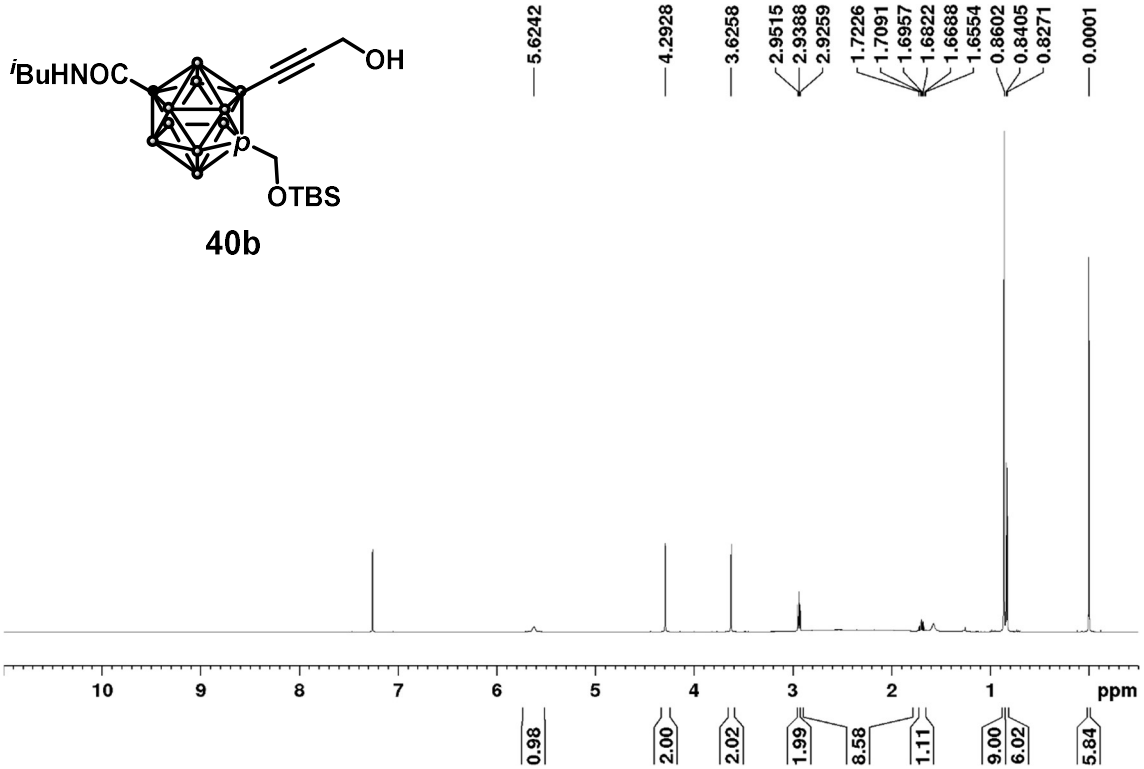


^{11}B NMR (160 MHz; $CDCl_3$)

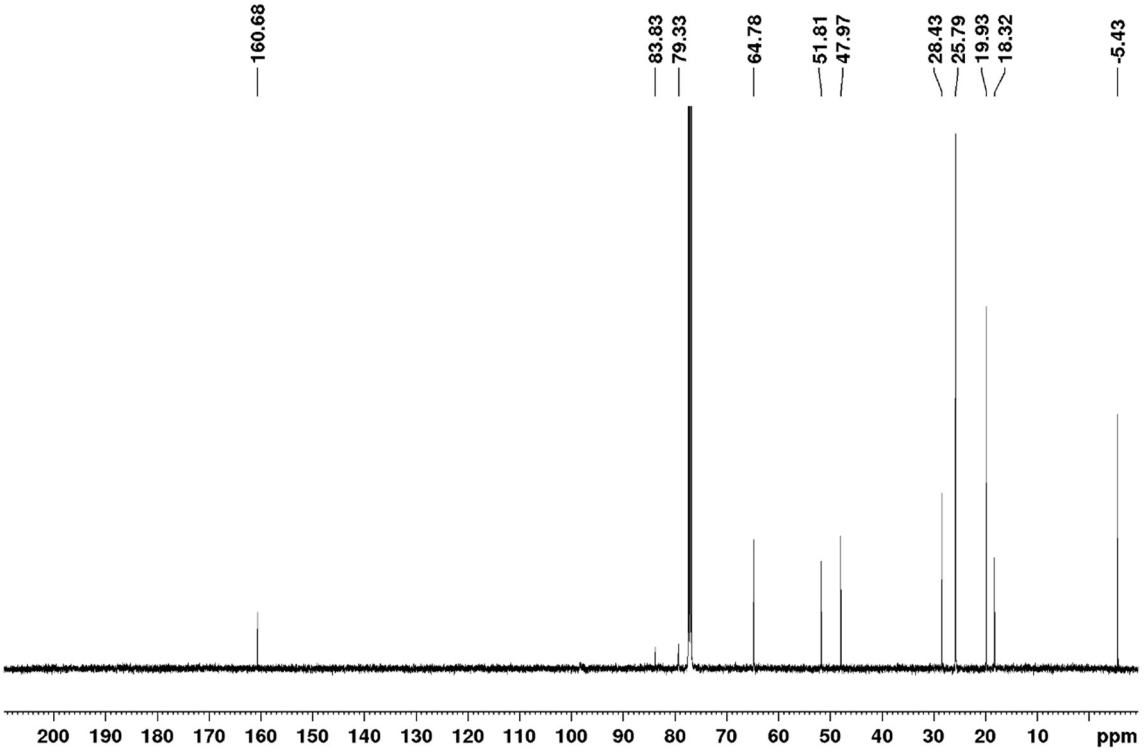


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (40b)

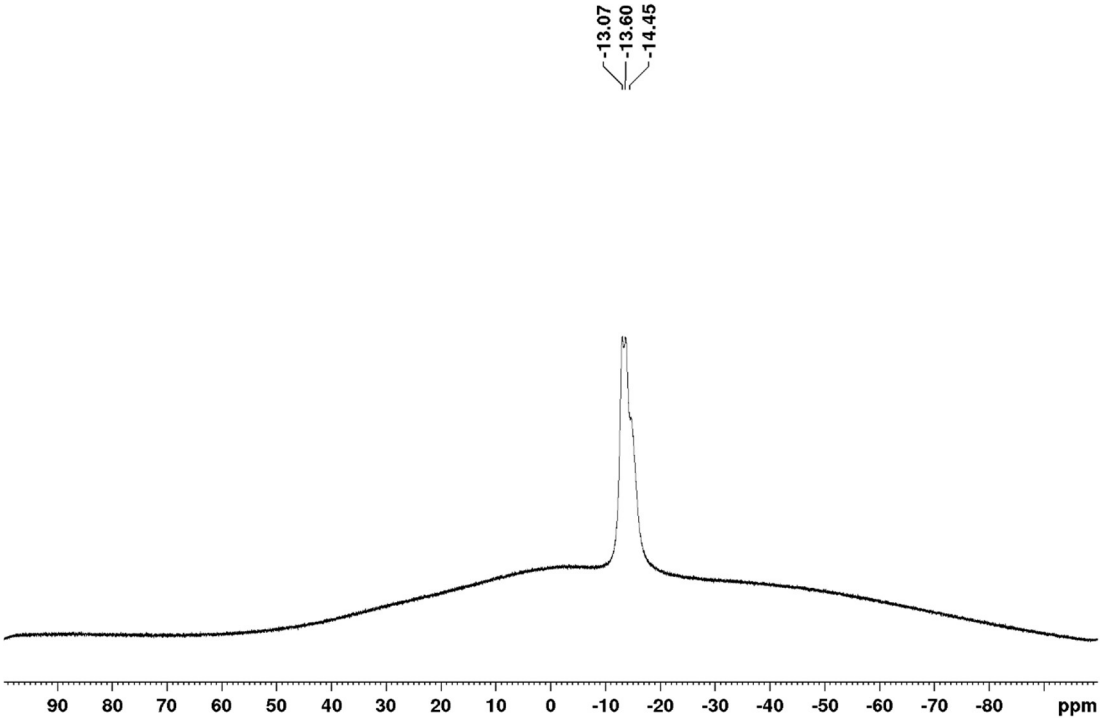
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

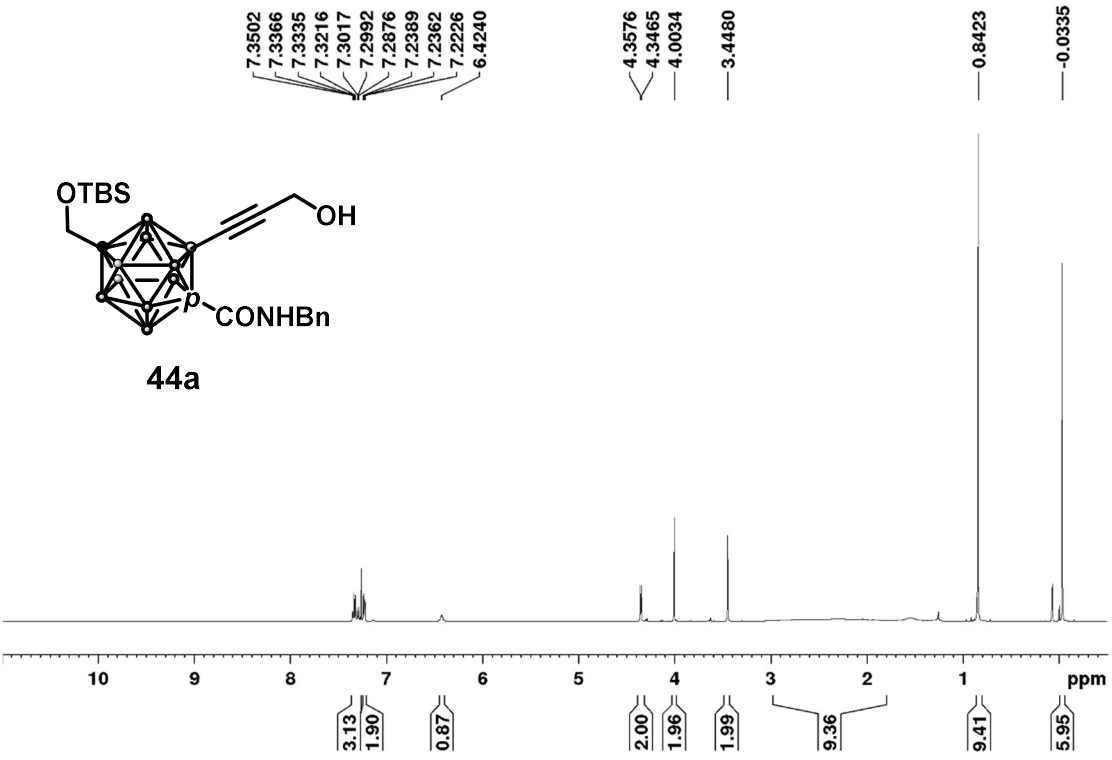


^{11}B NMR (160 MHz; $CDCl_3$)

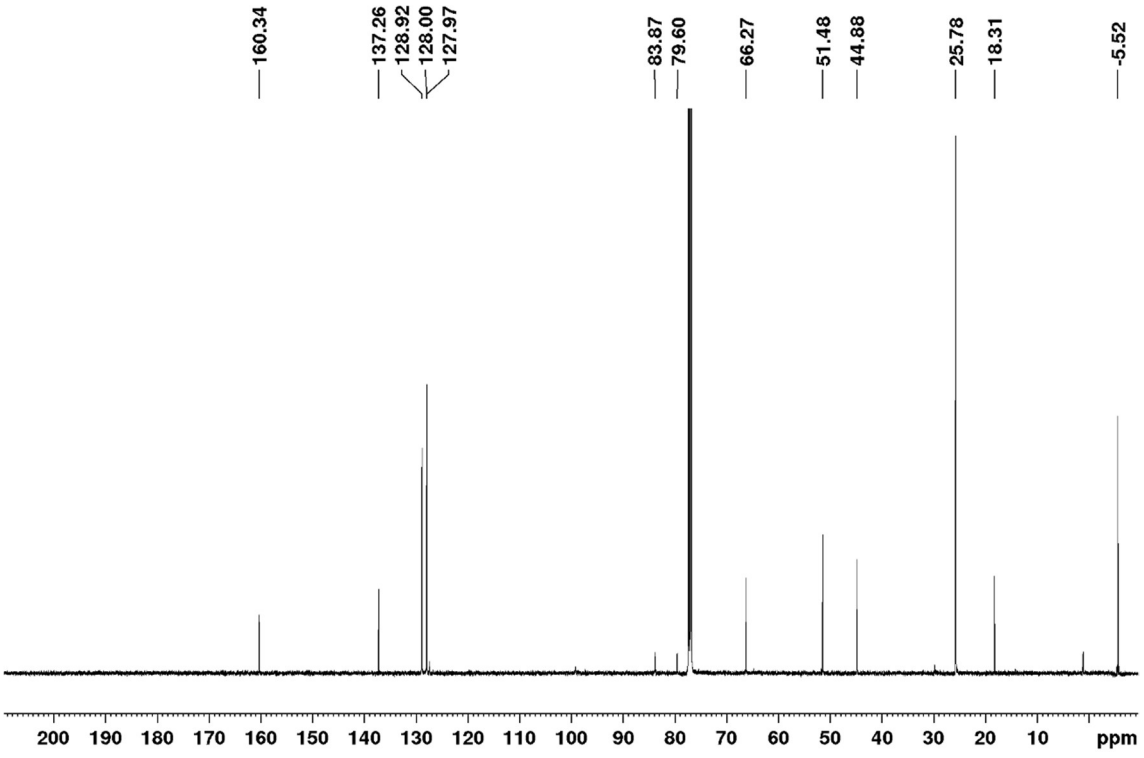


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (44a)

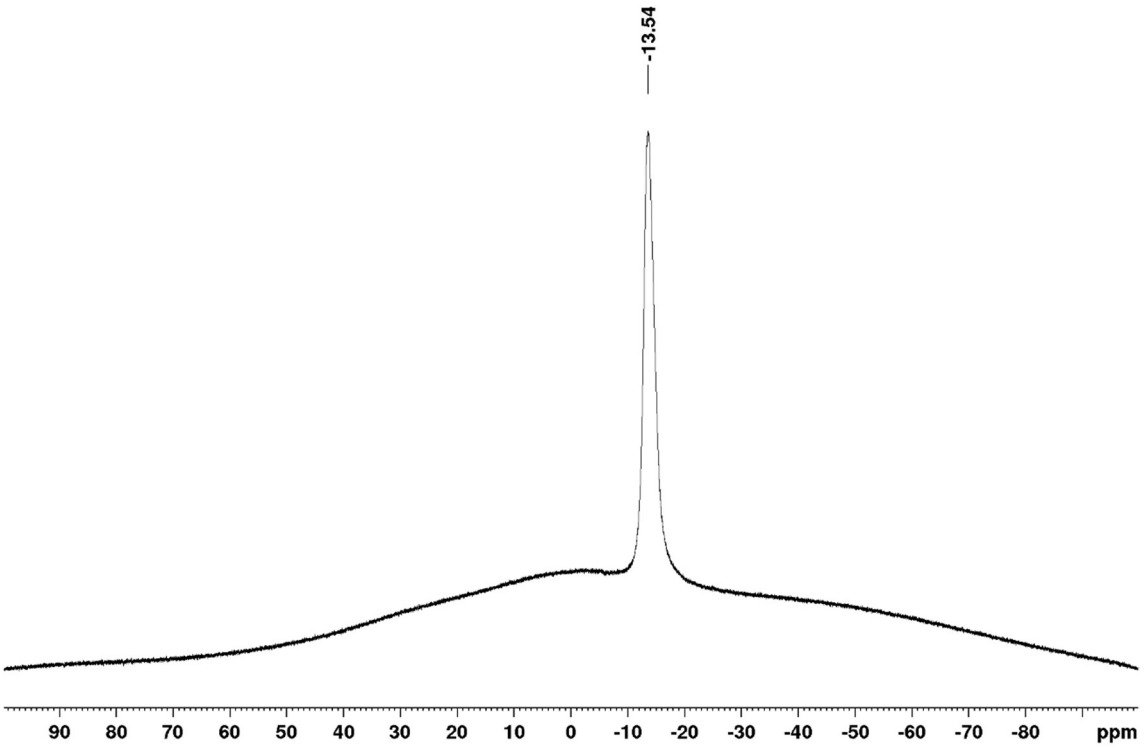
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

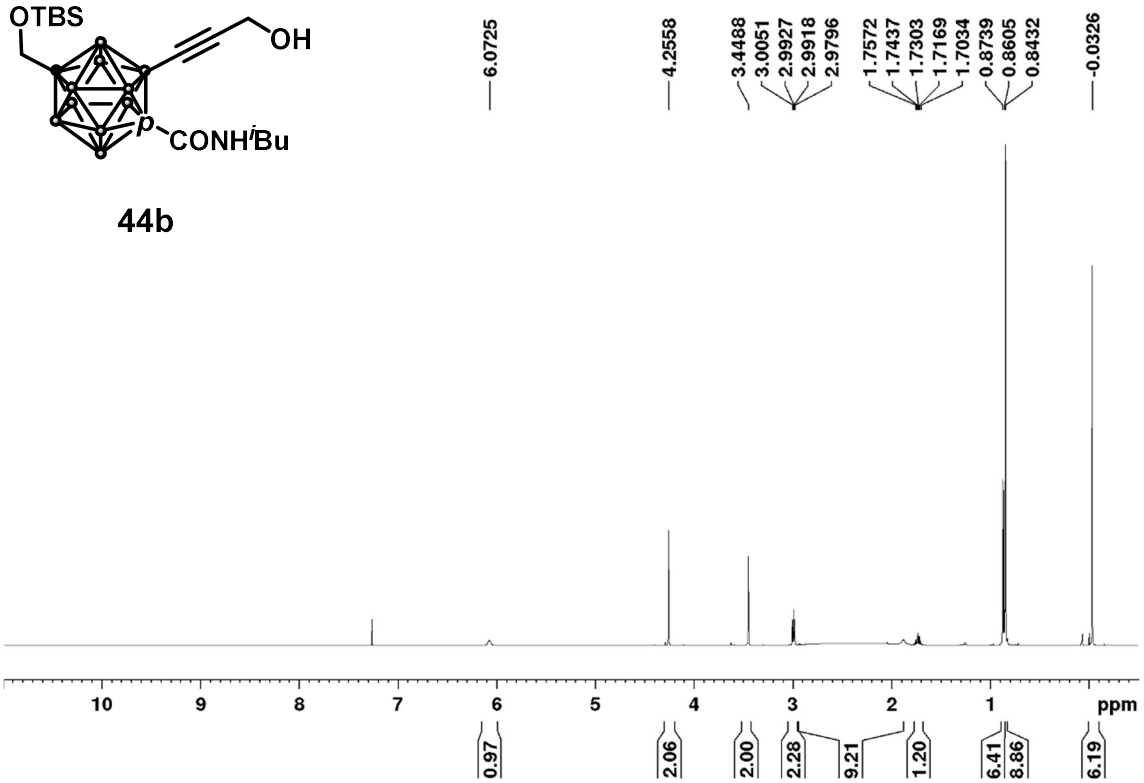


^{11}B NMR (160 MHz; $CDCl_3$)

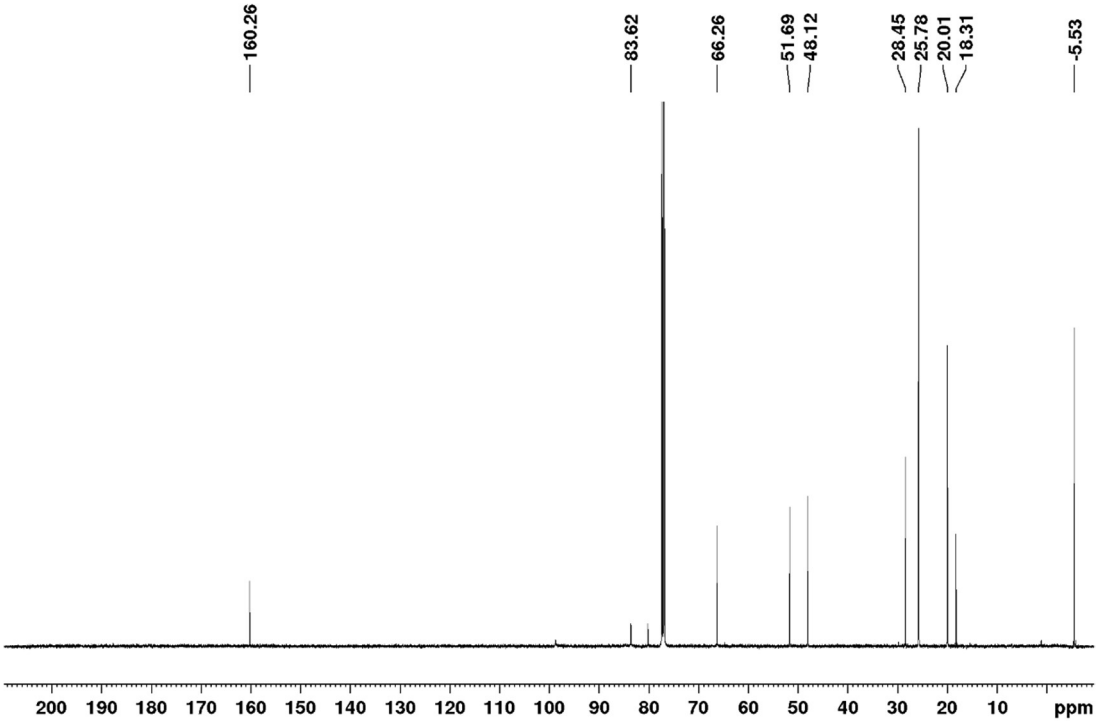


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (44b)

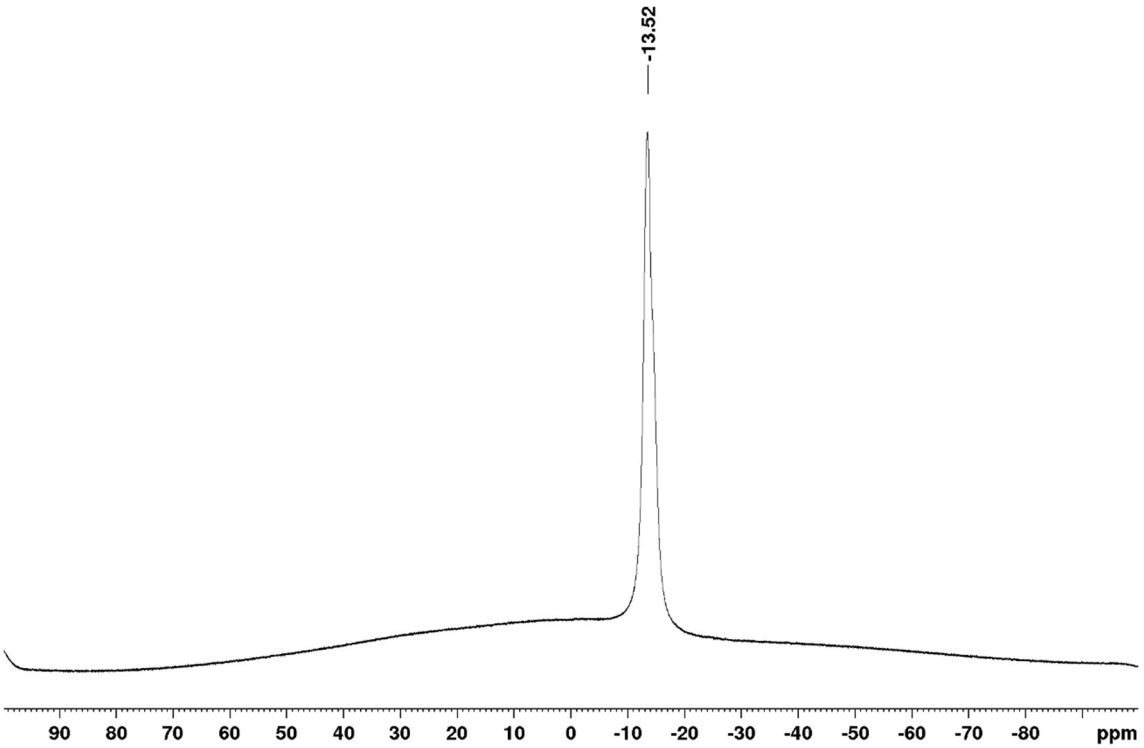
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

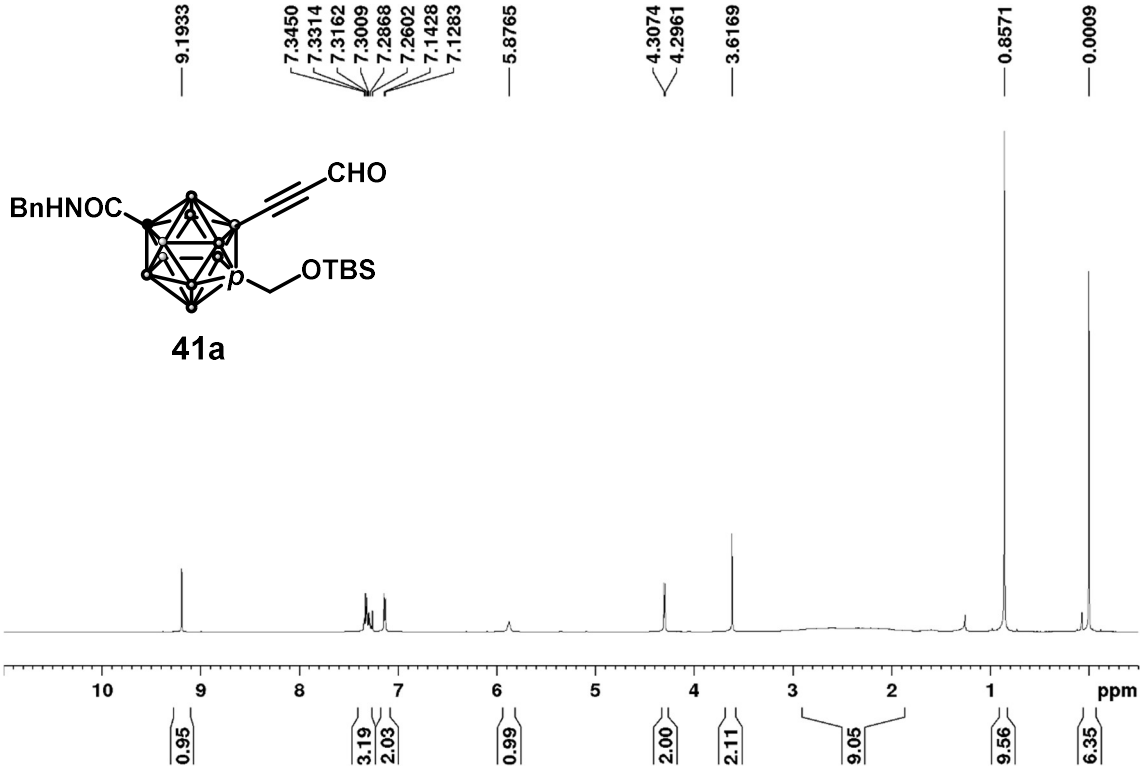


^{11}B NMR (160 MHz; $CDCl_3$)

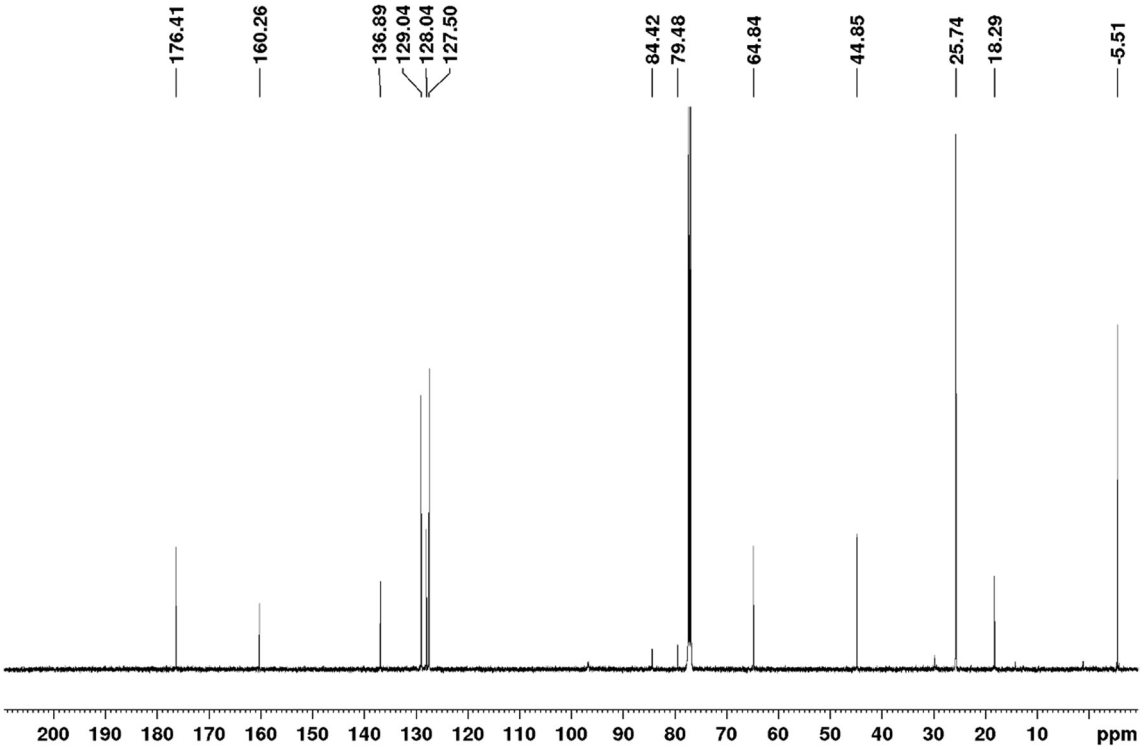


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (41a)

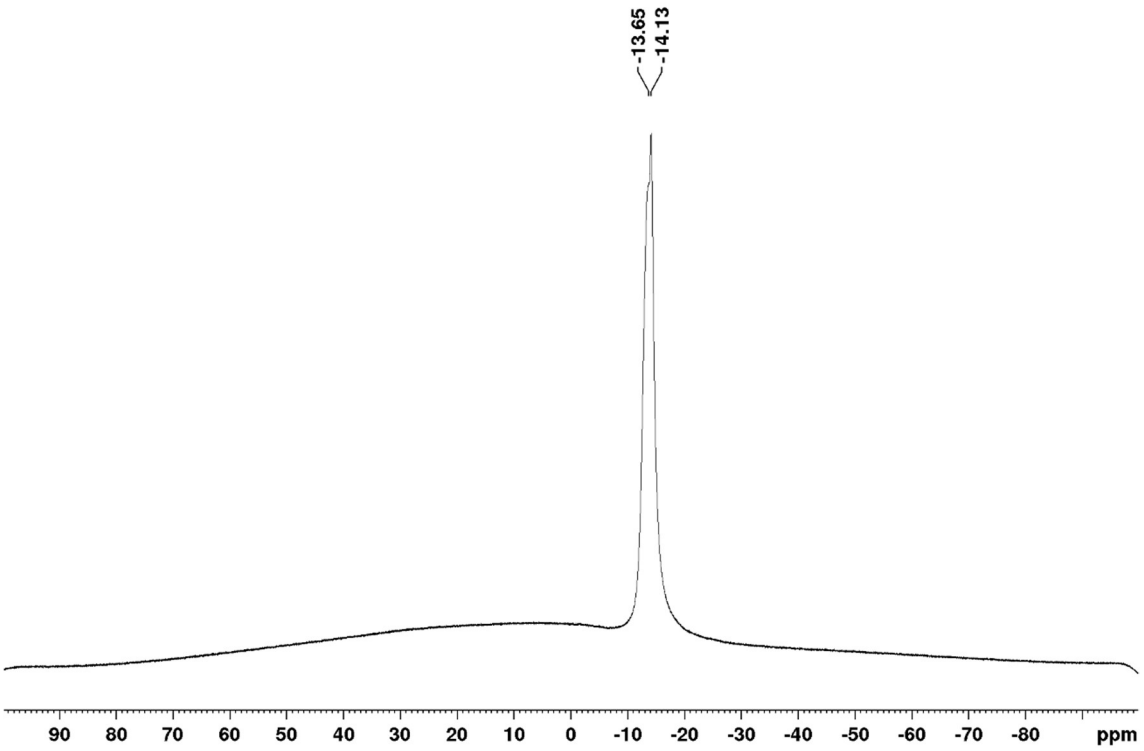
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

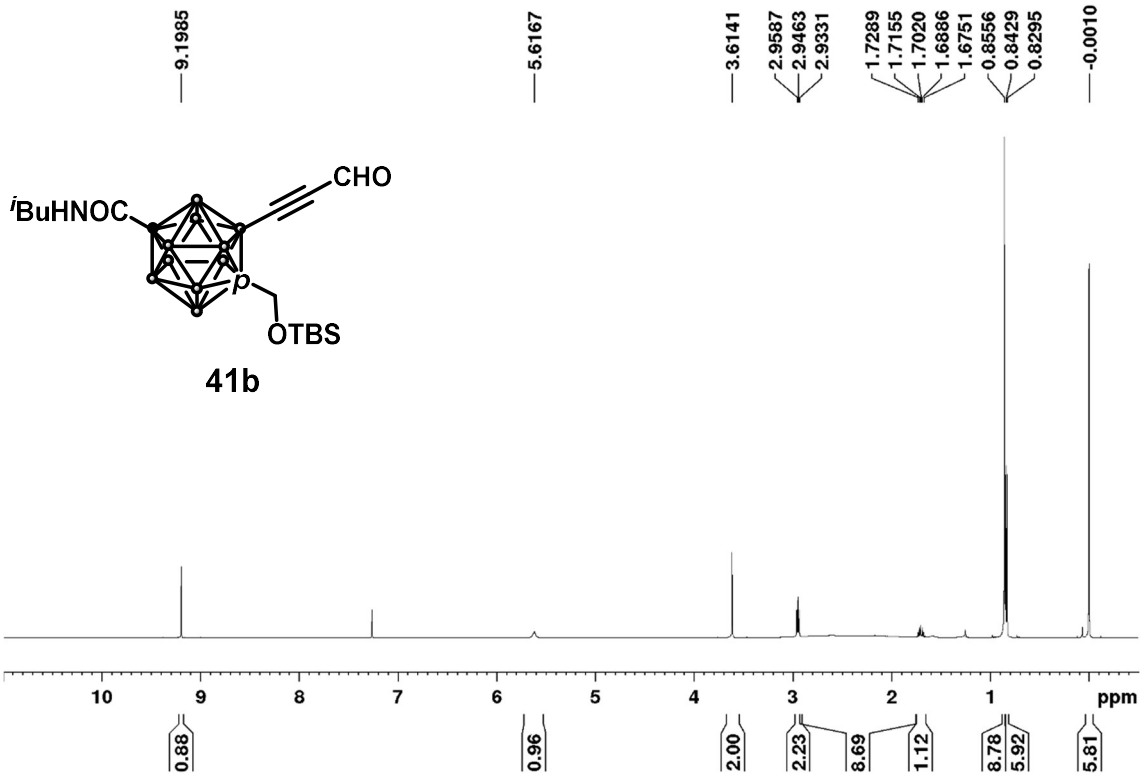


^{11}B NMR (160 MHz; $CDCl_3$)

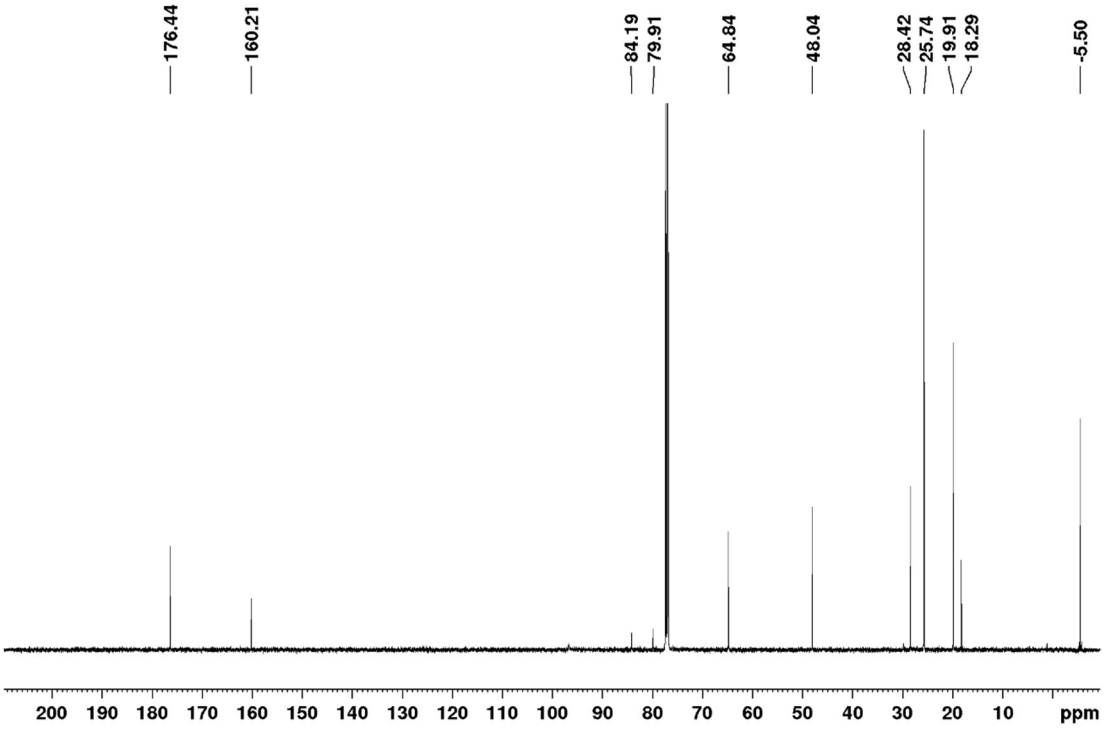


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (41b)

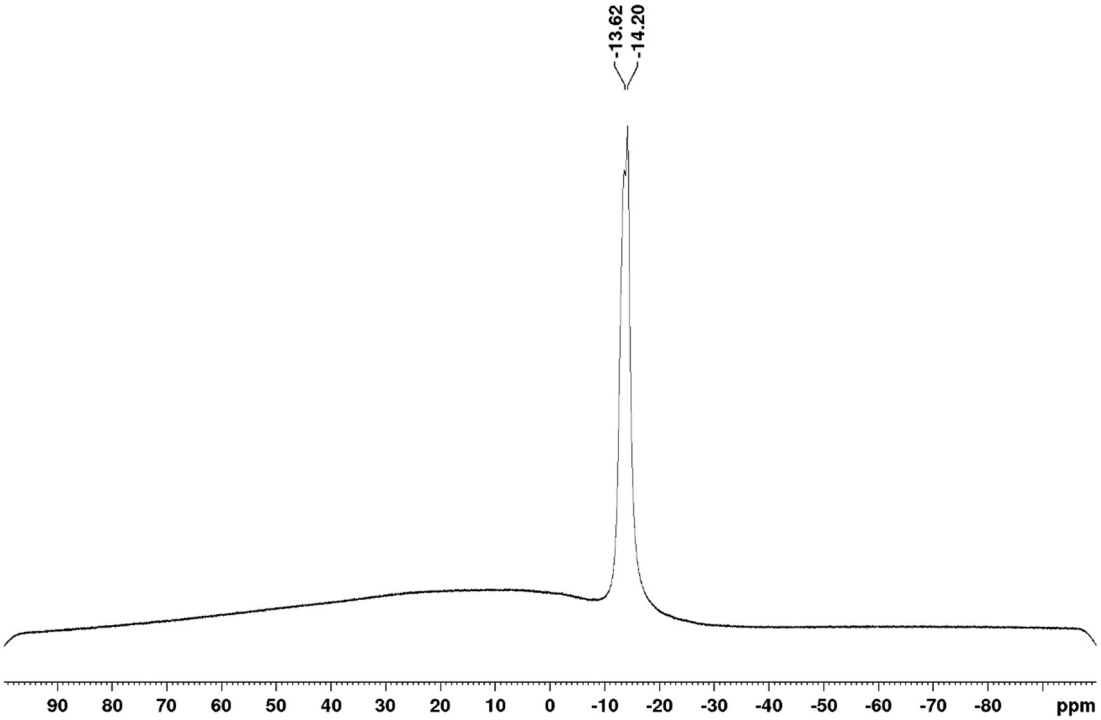
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

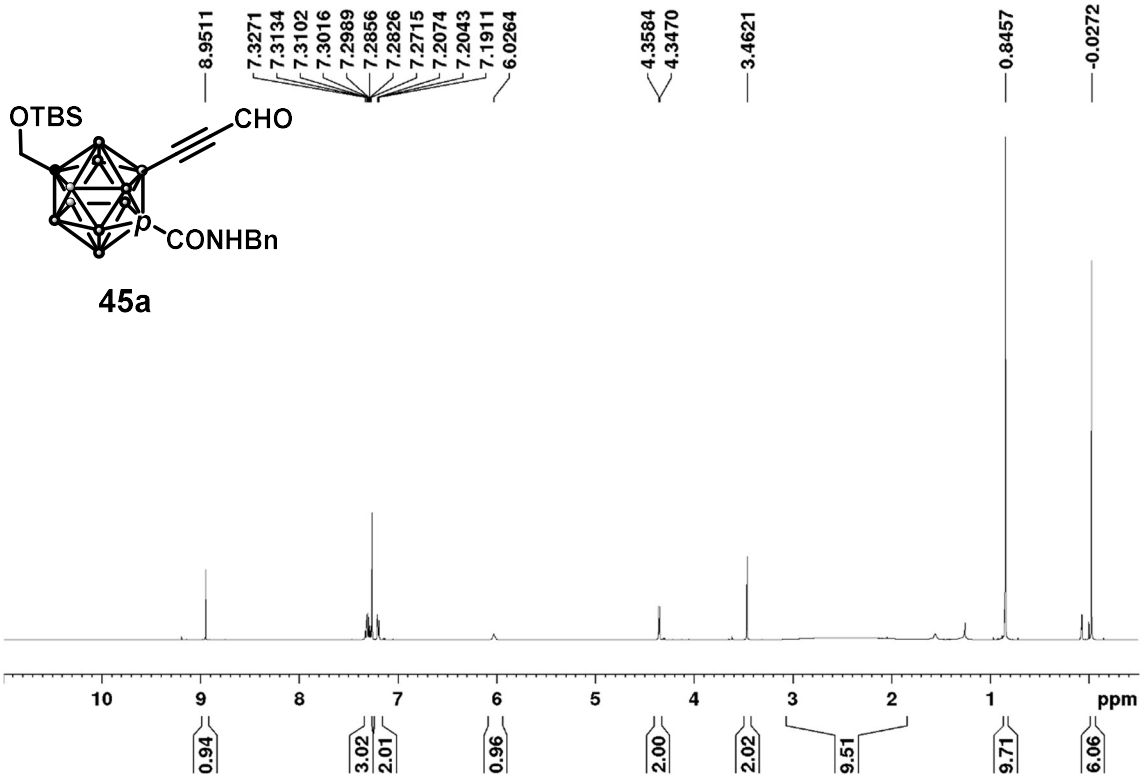


^{11}B NMR (160 MHz; $CDCl_3$)

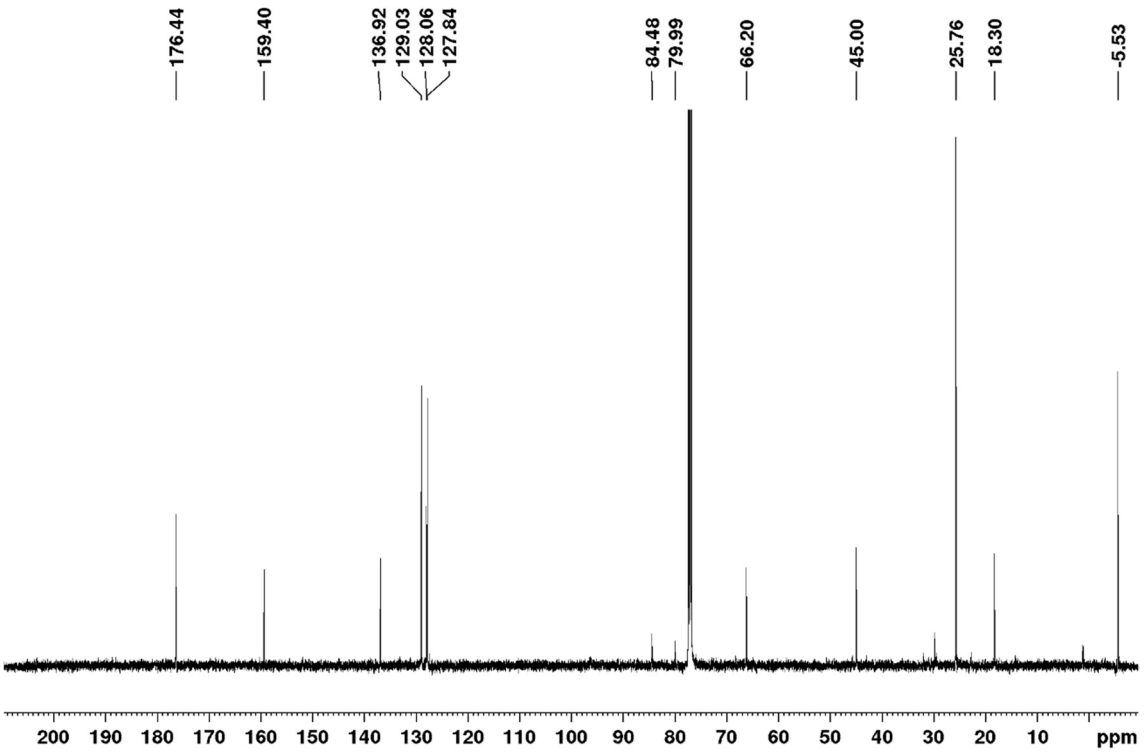


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (45a)

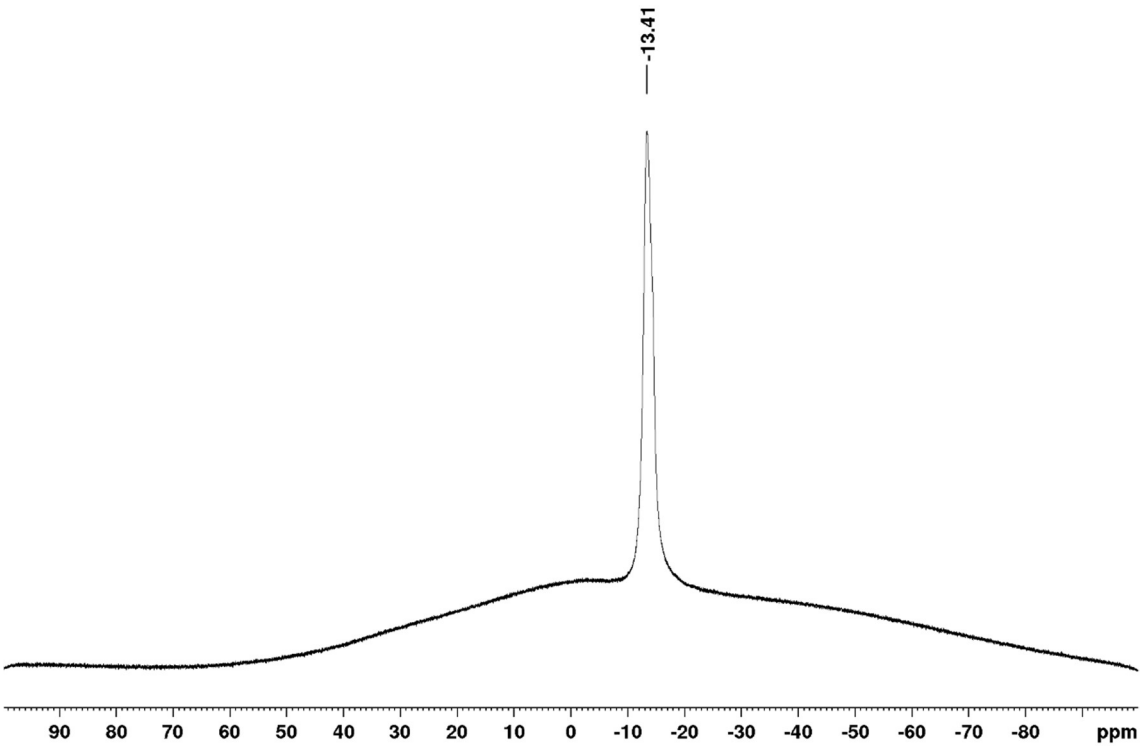
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

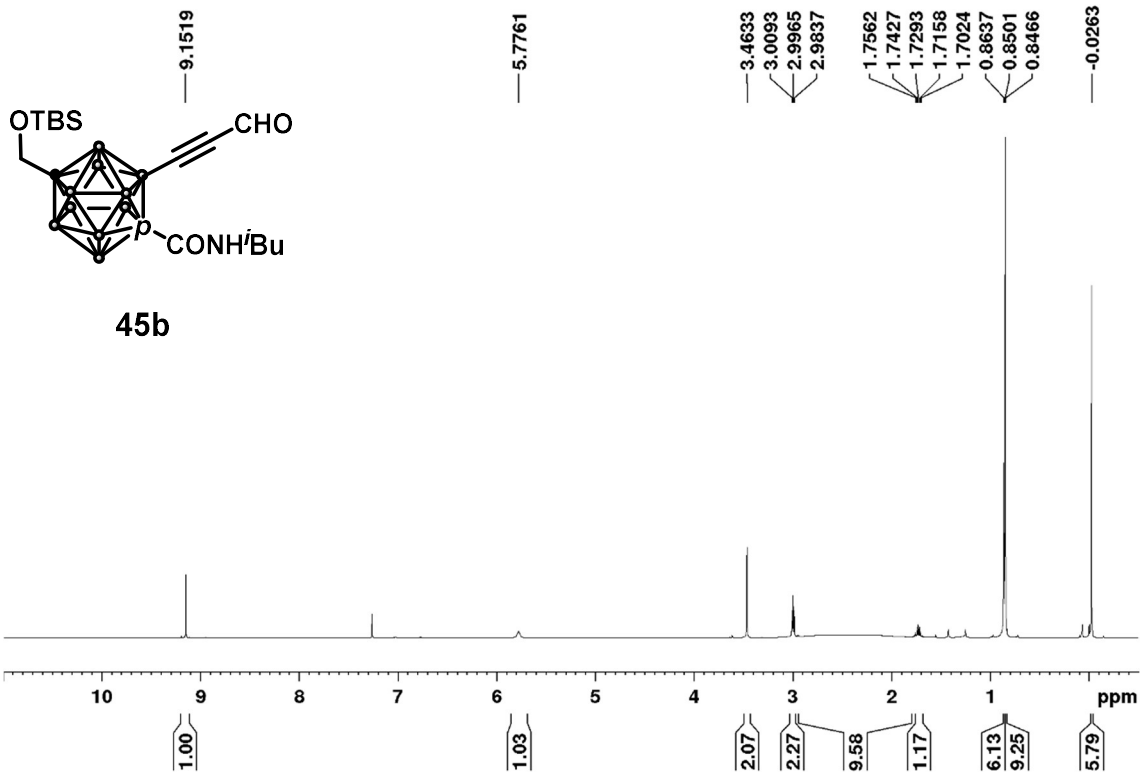


^{11}B NMR (160 MHz; $CDCl_3$)

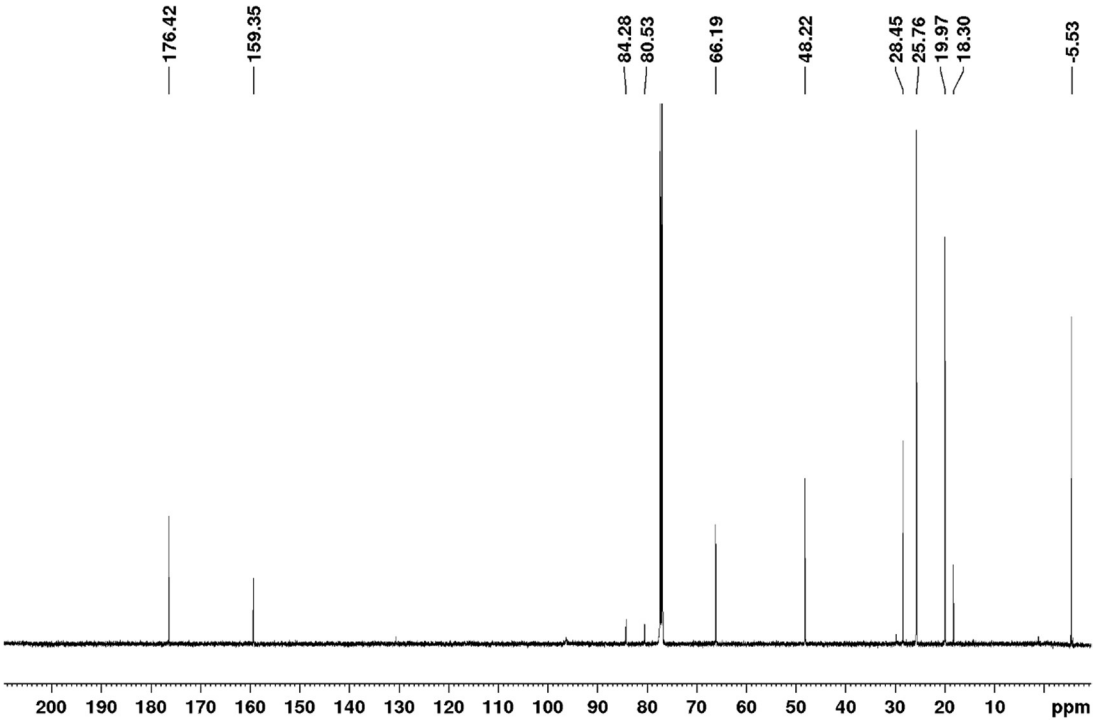


12-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-(3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (45b)

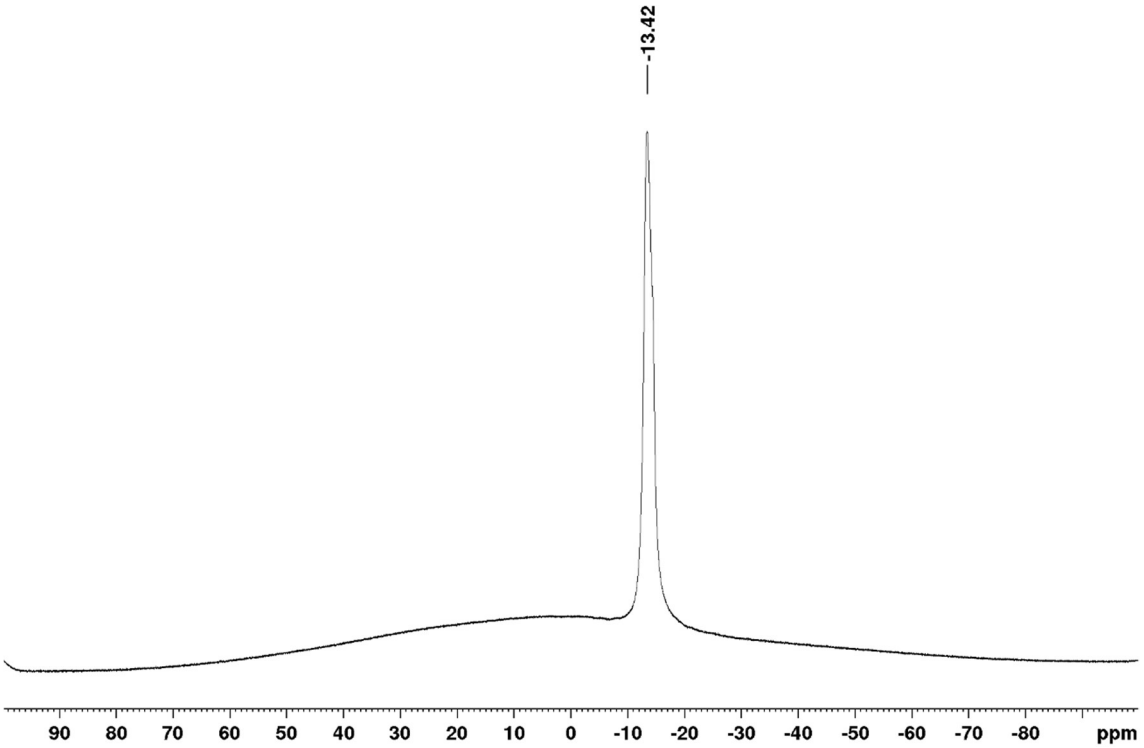
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

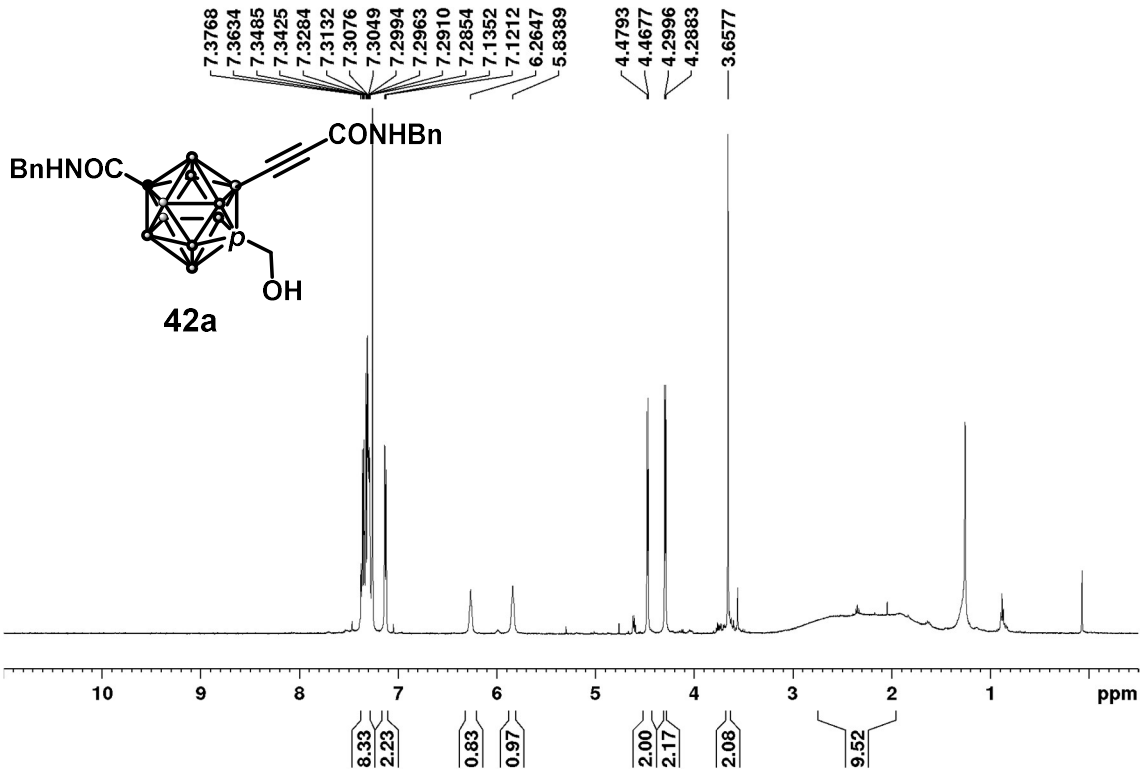


^{11}B NMR (160 MHz; $CDCl_3$)

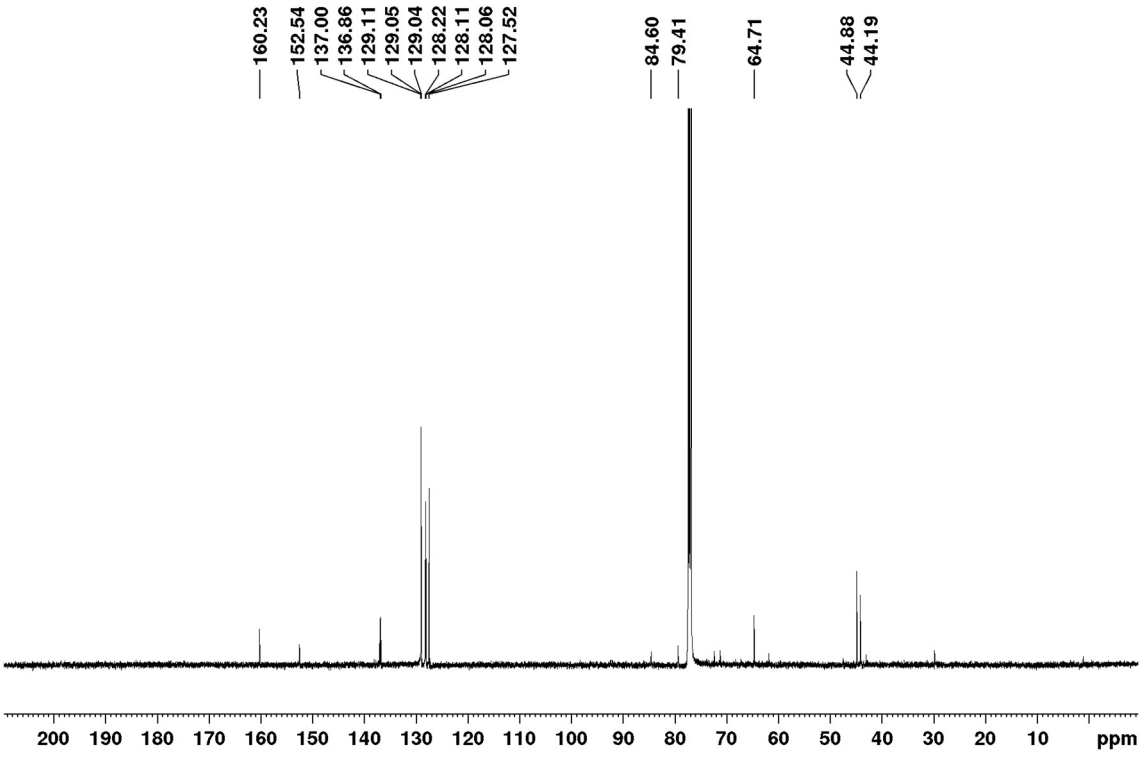


1-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (42a)

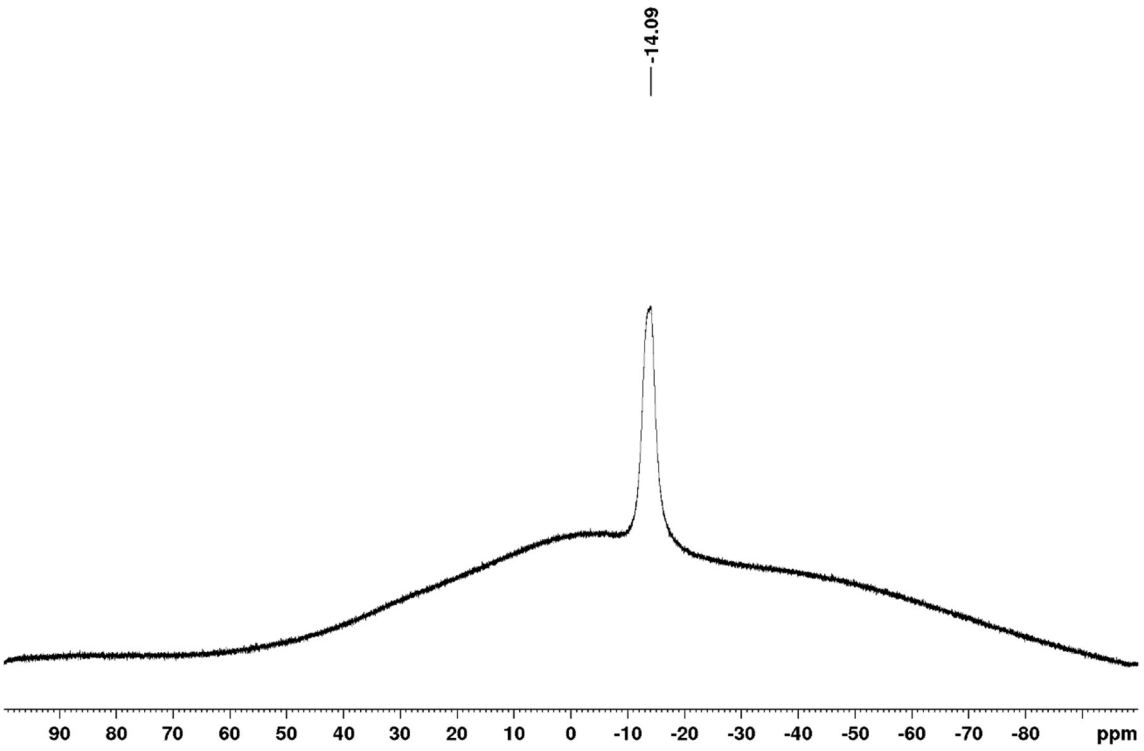
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

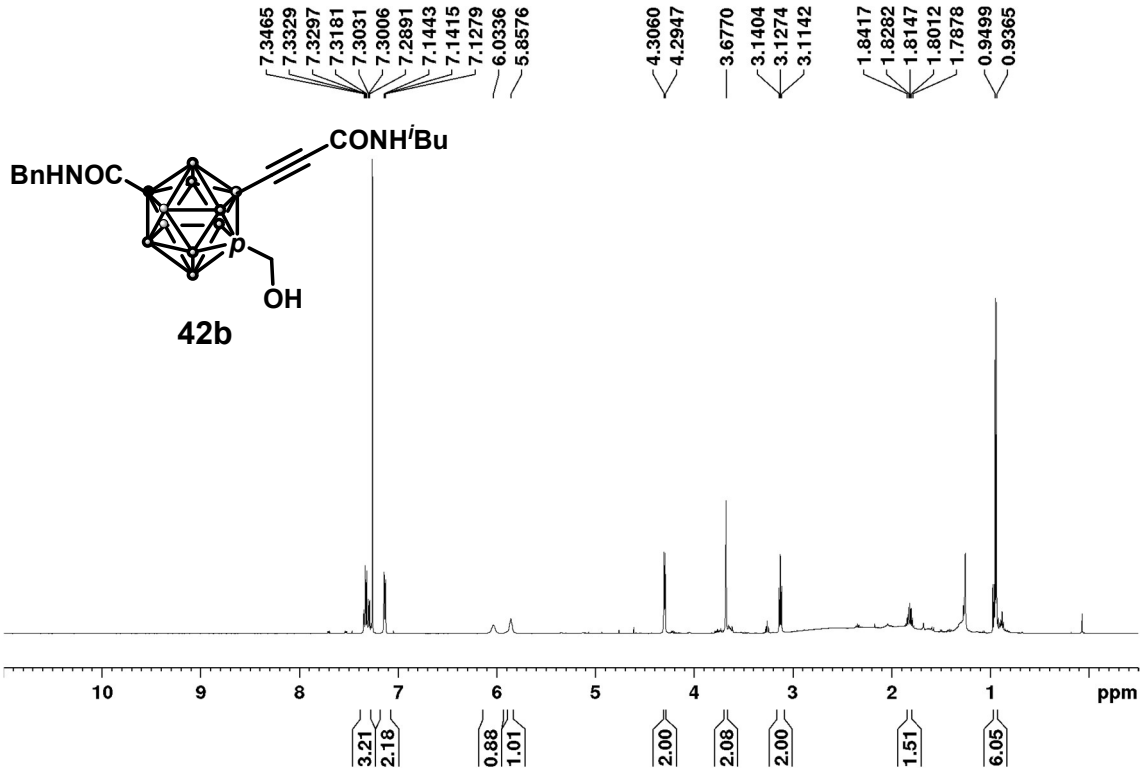


^{11}B NMR (160 MHz; $CDCl_3$)

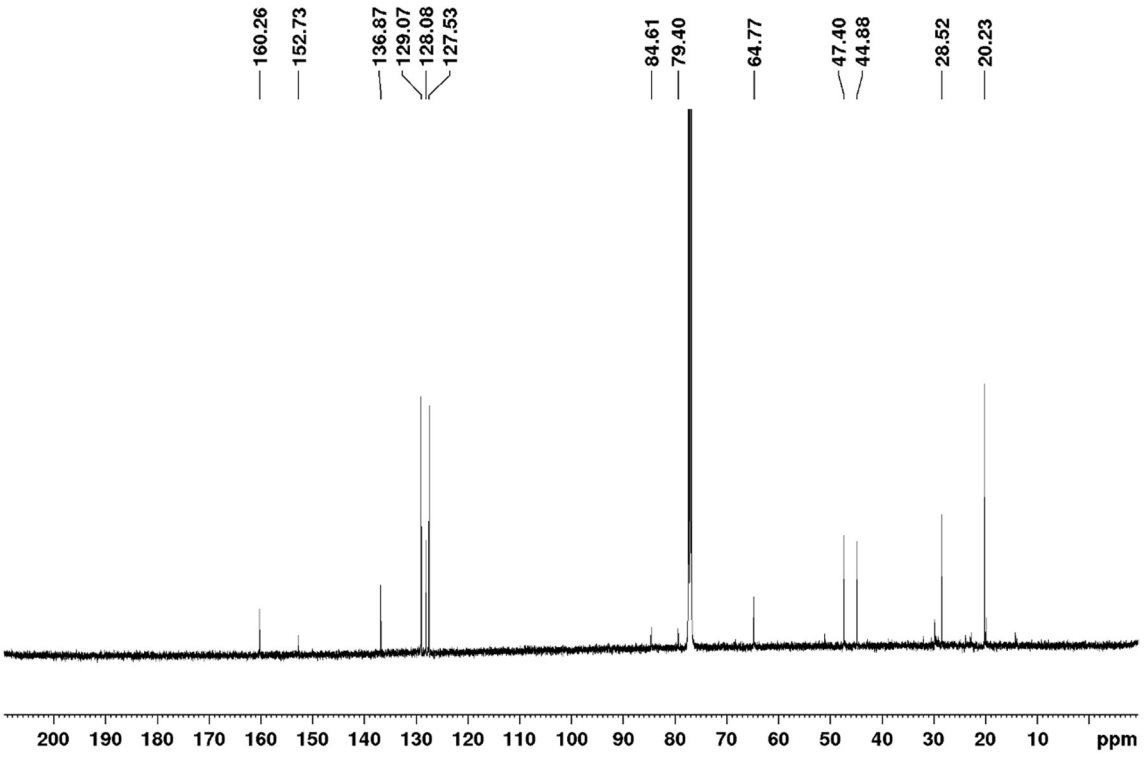


1-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (42b)

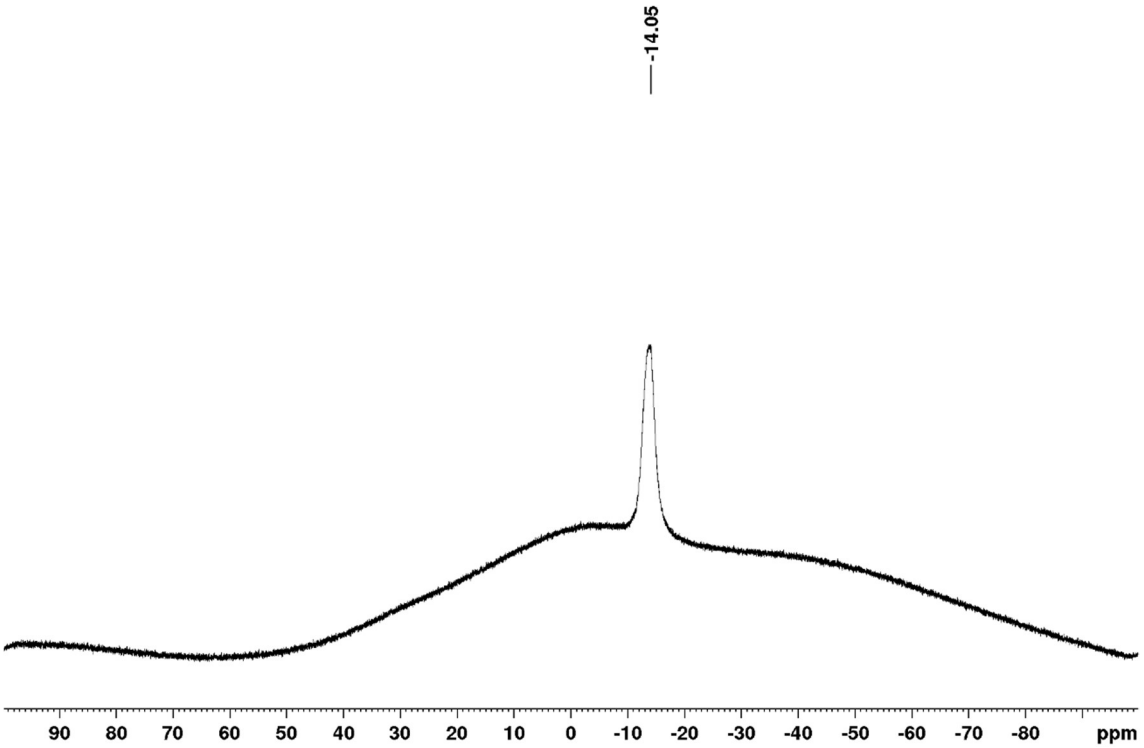
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

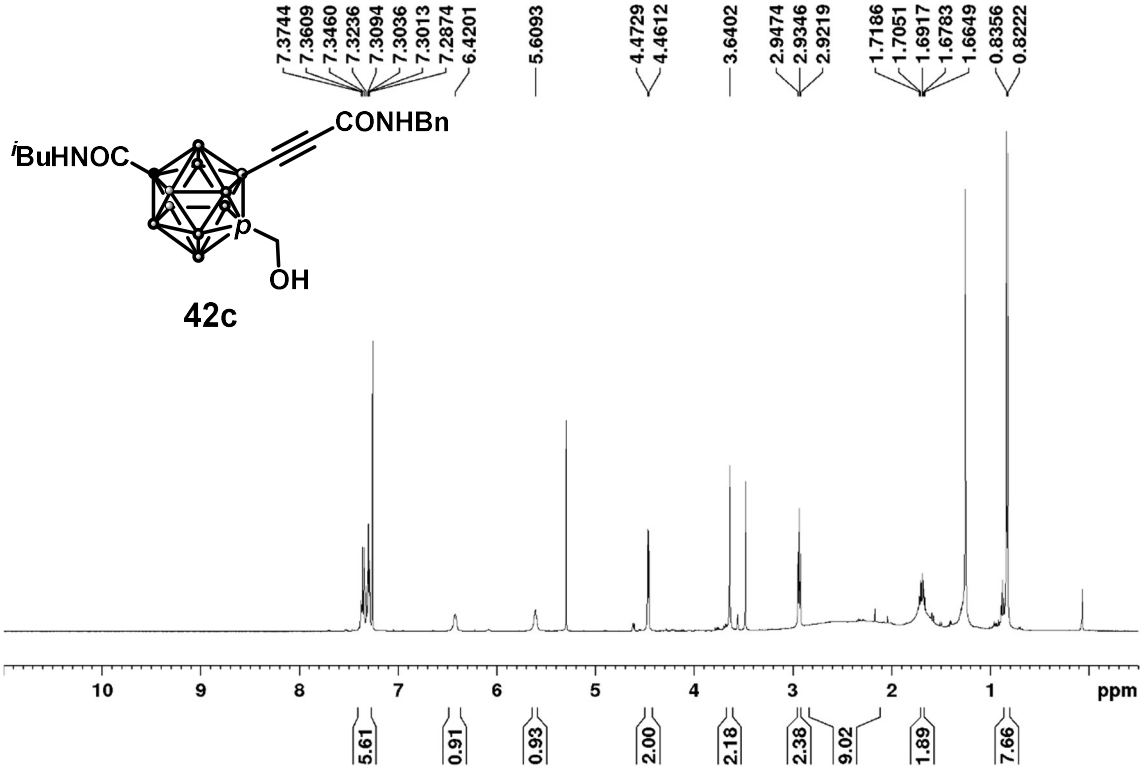


^{11}B NMR (160 MHz; $CDCl_3$)

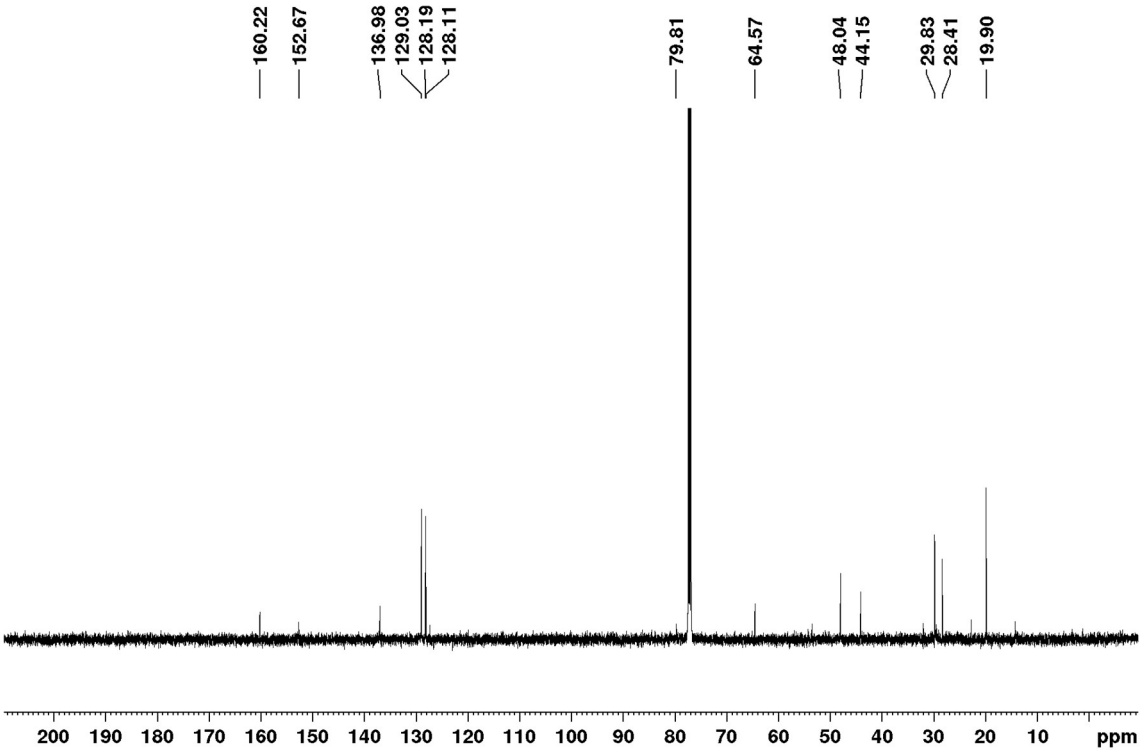


1-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (42c)

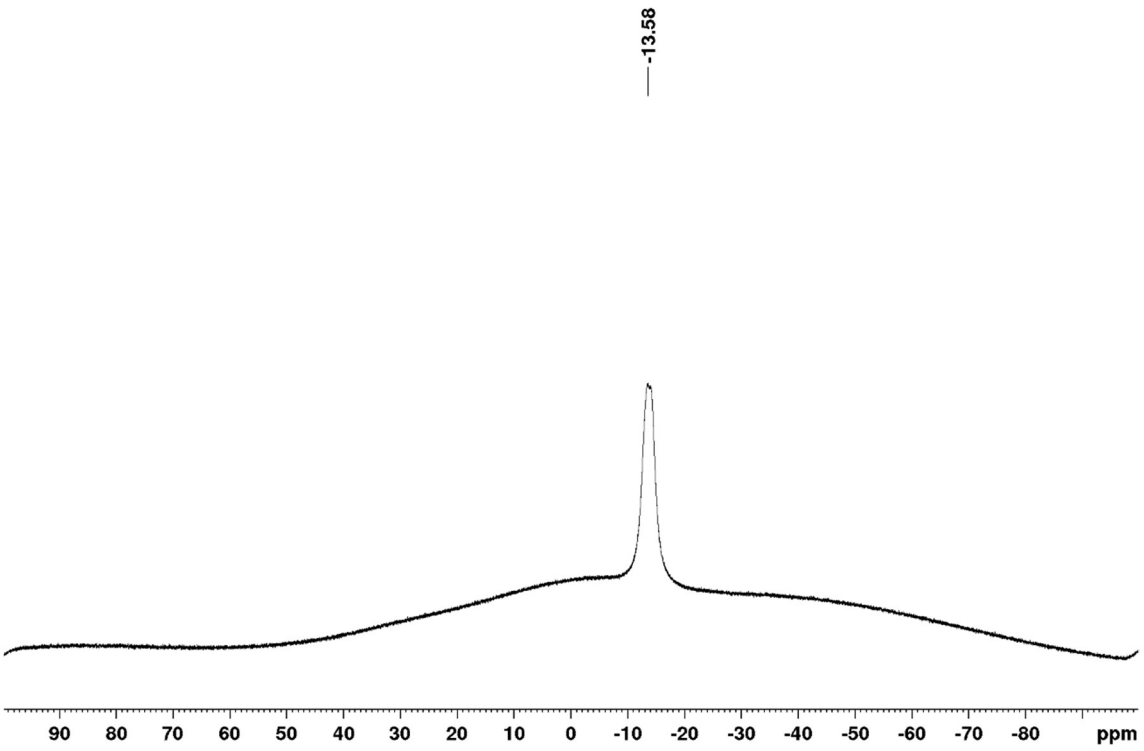
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

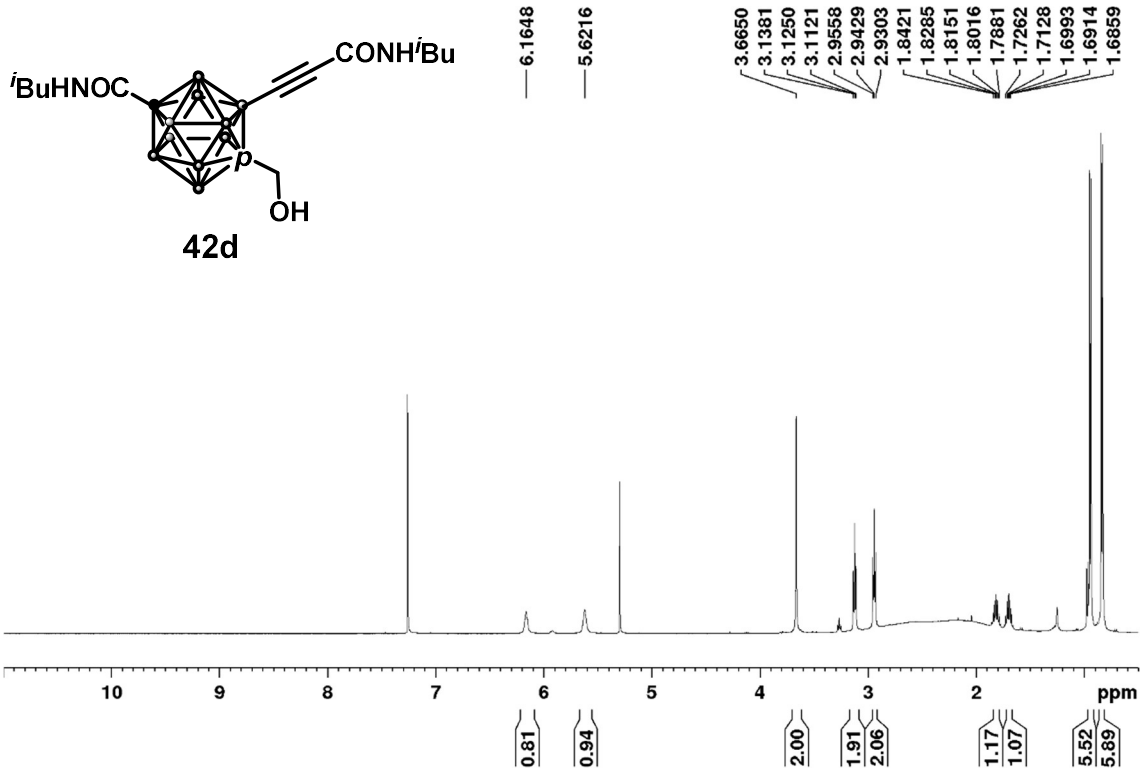


^{11}B NMR (160 MHz; $CDCl_3$)

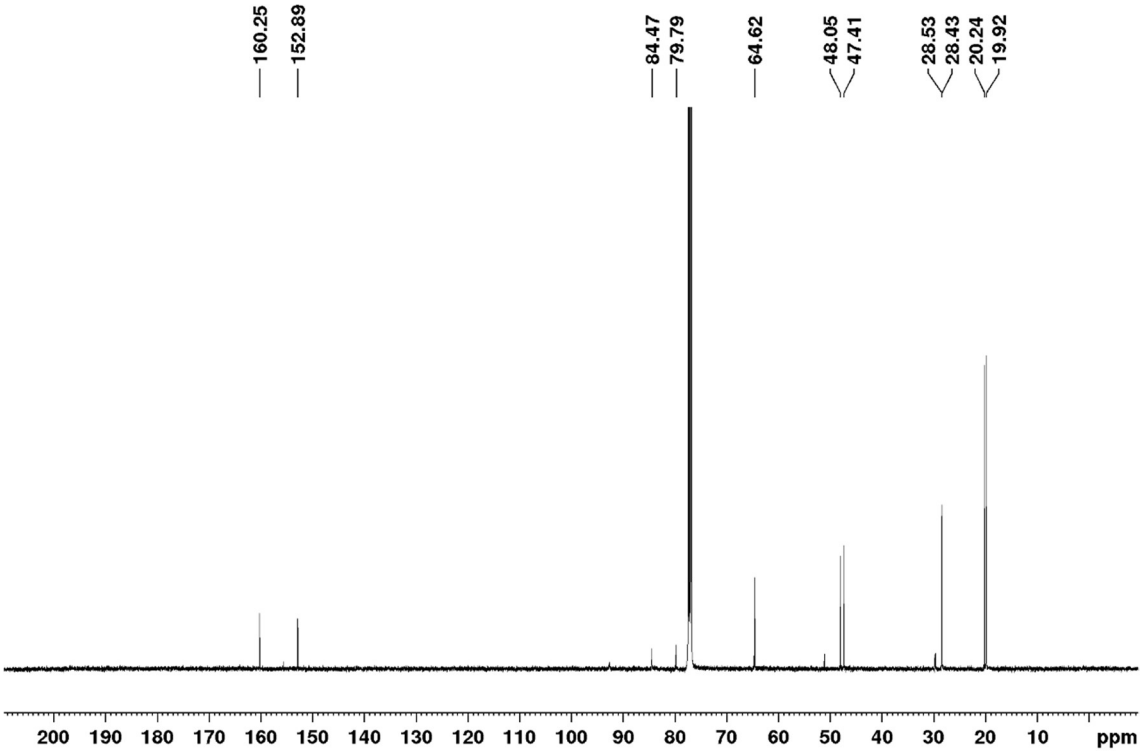


1-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (42d)

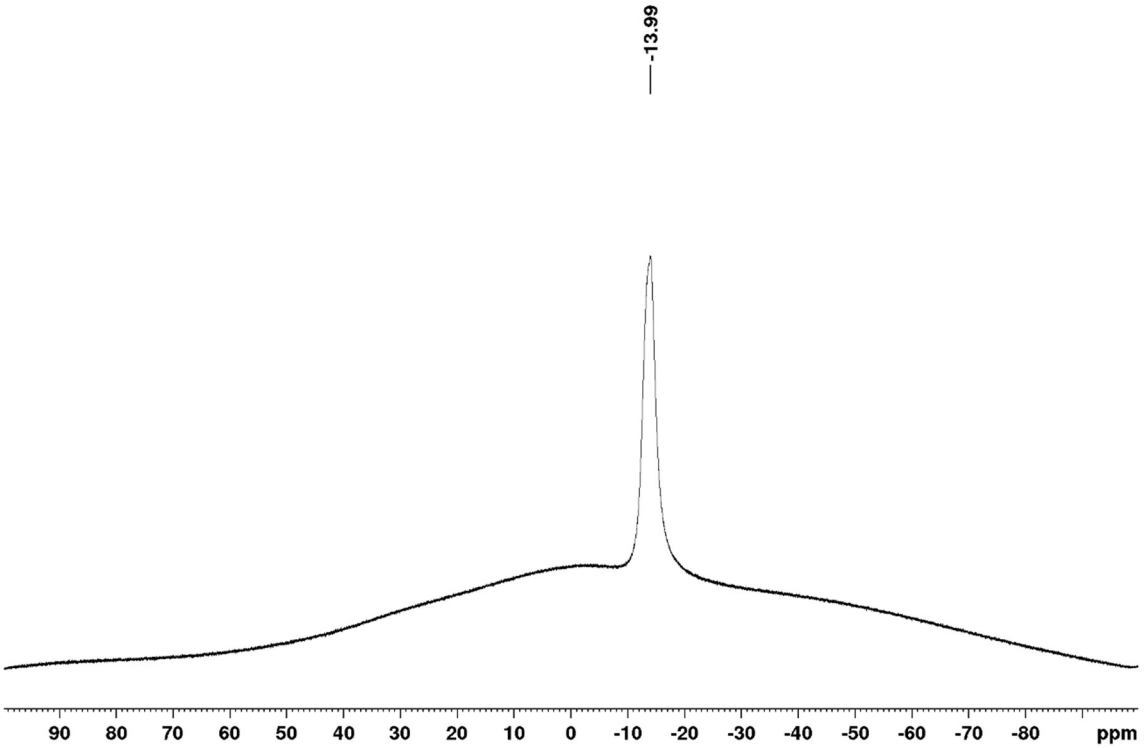
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

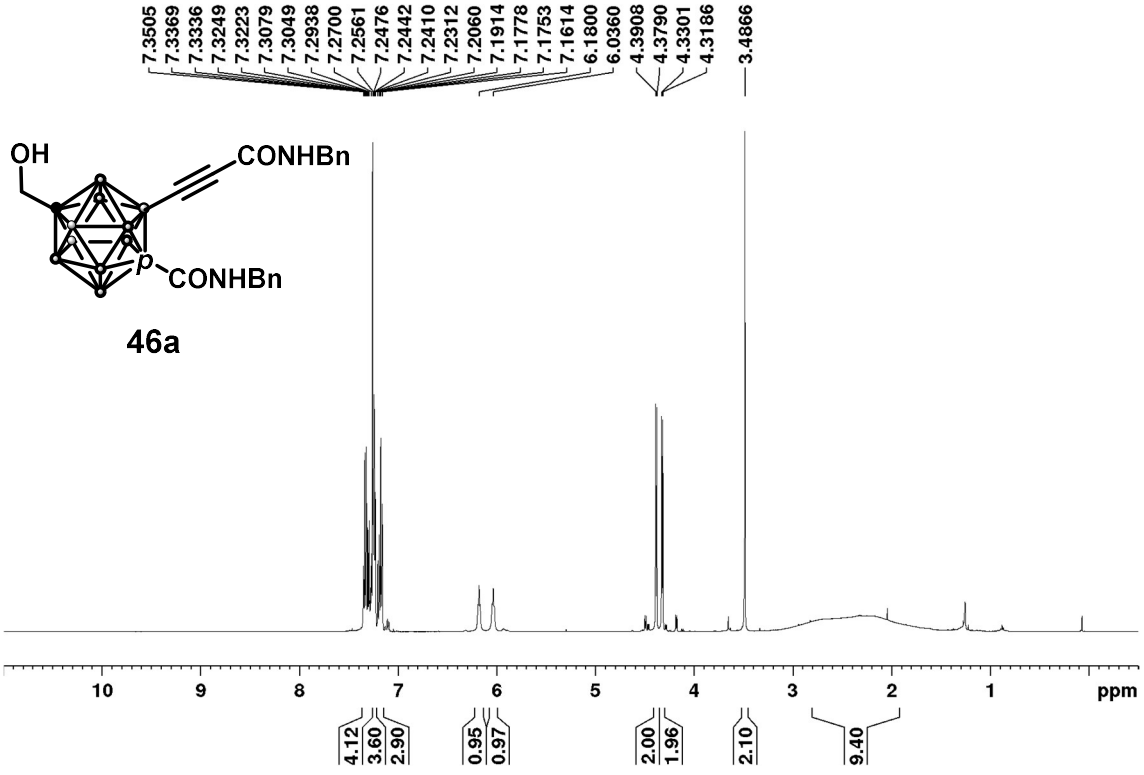


^{11}B NMR (160 MHz; $CDCl_3$)

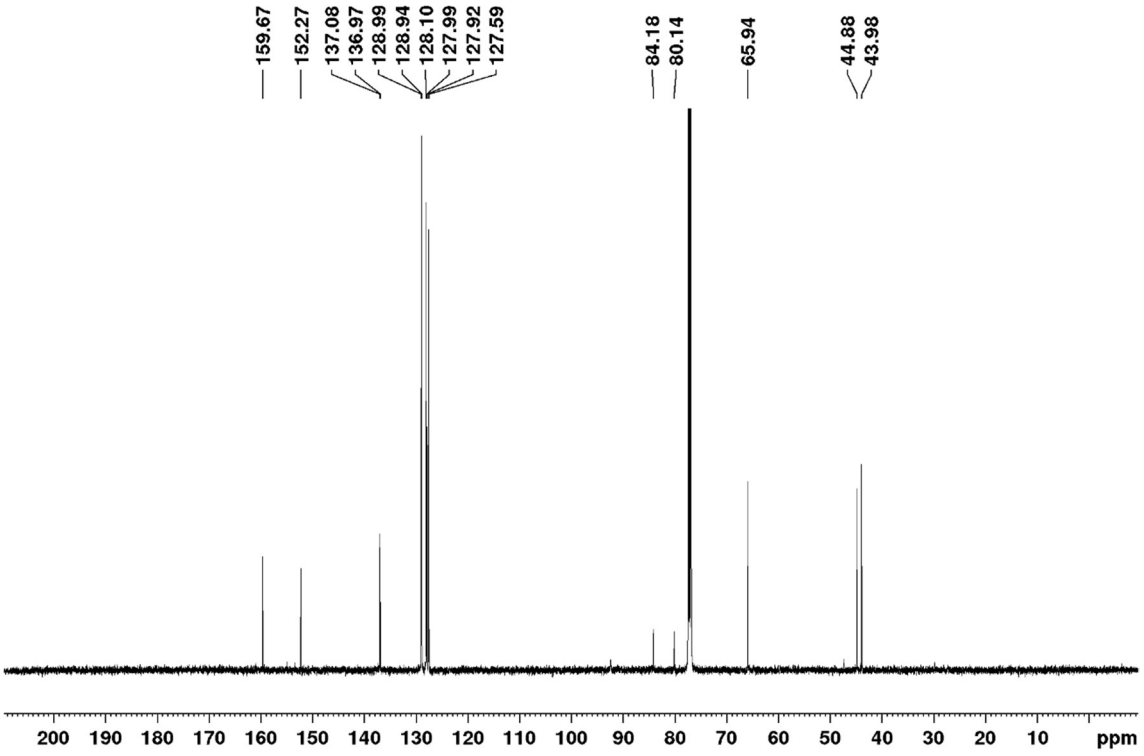


12-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (46a)

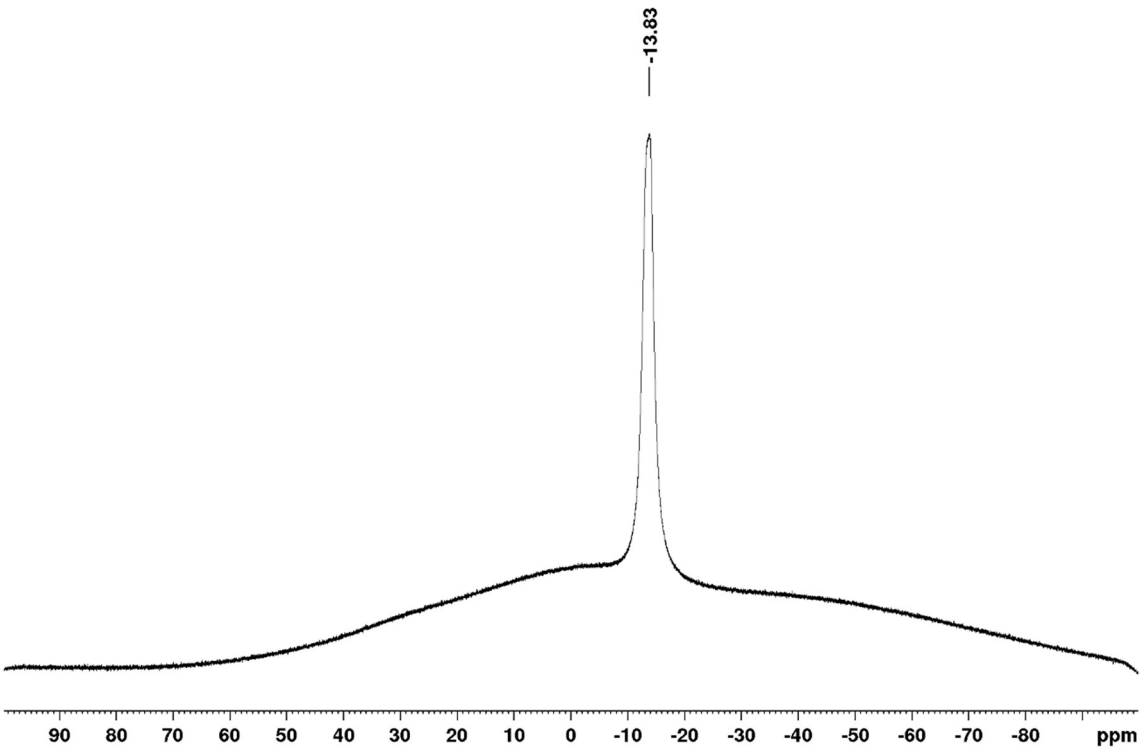
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

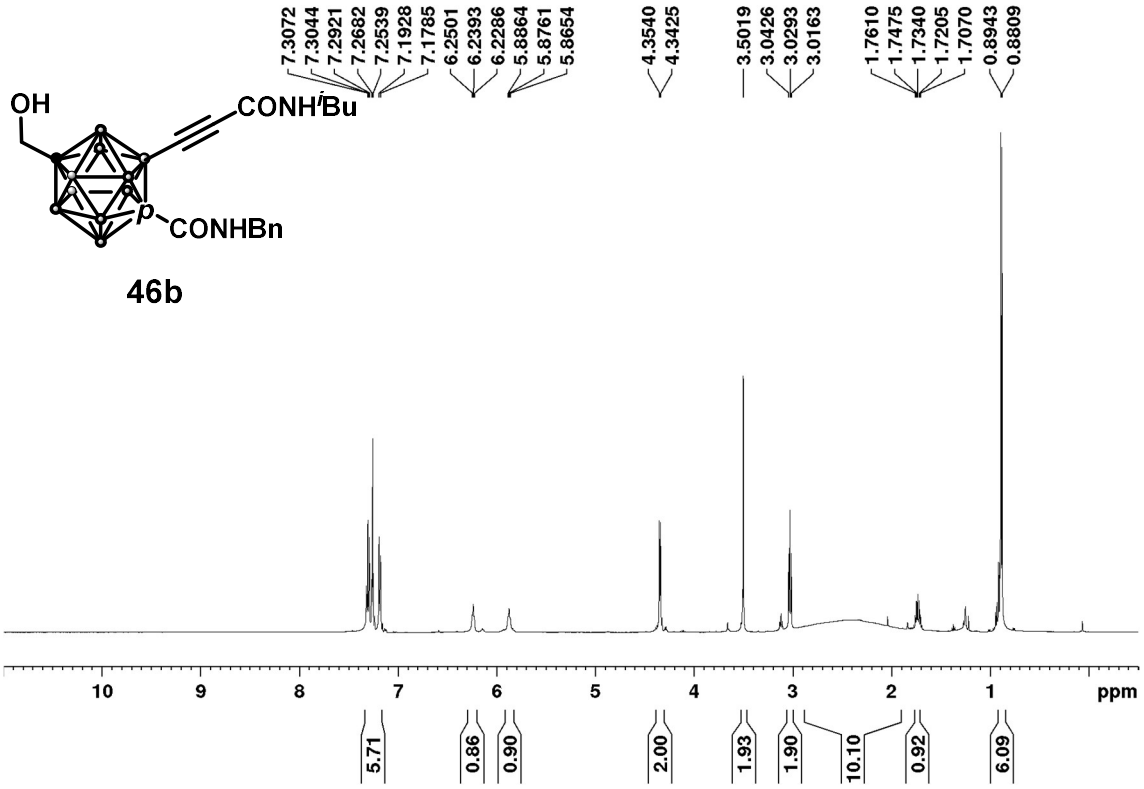


^{11}B NMR (160 MHz; $CDCl_3$)

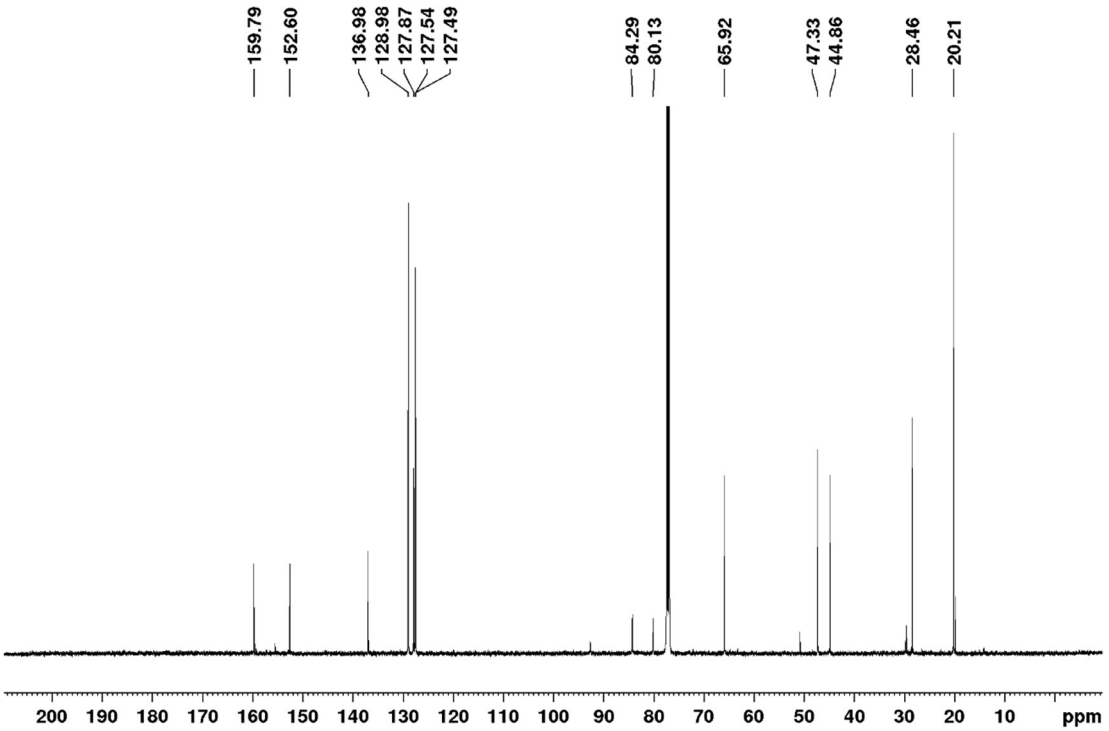


12-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (46b)

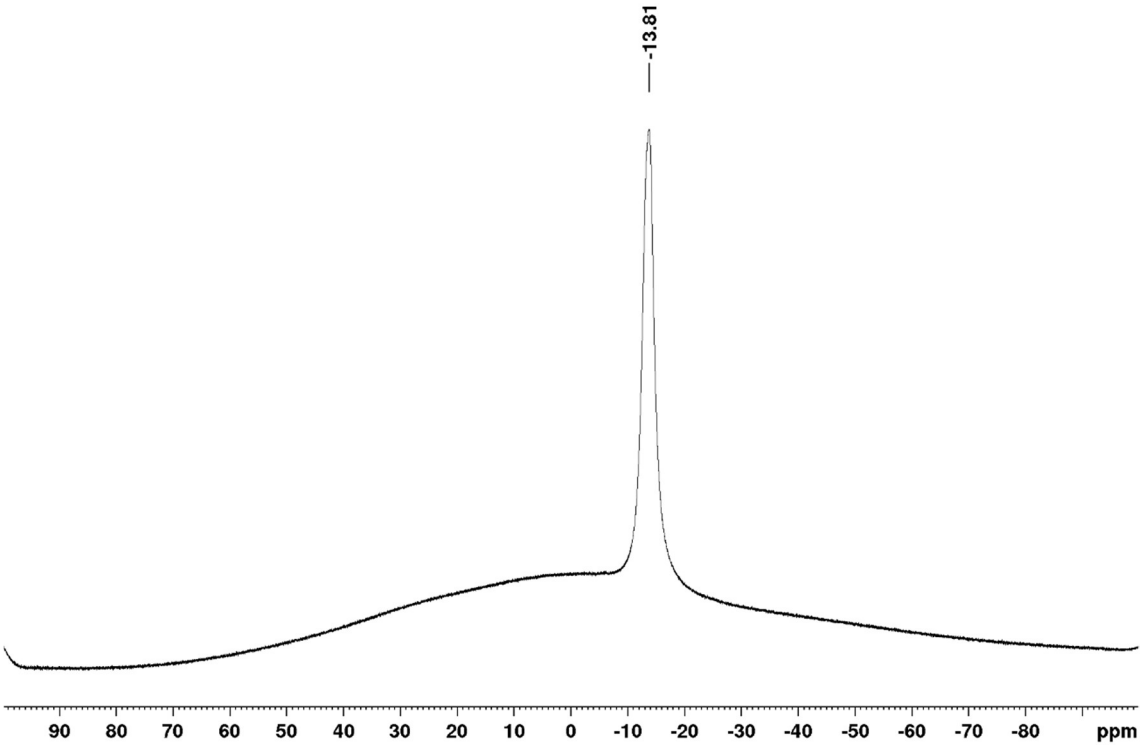
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

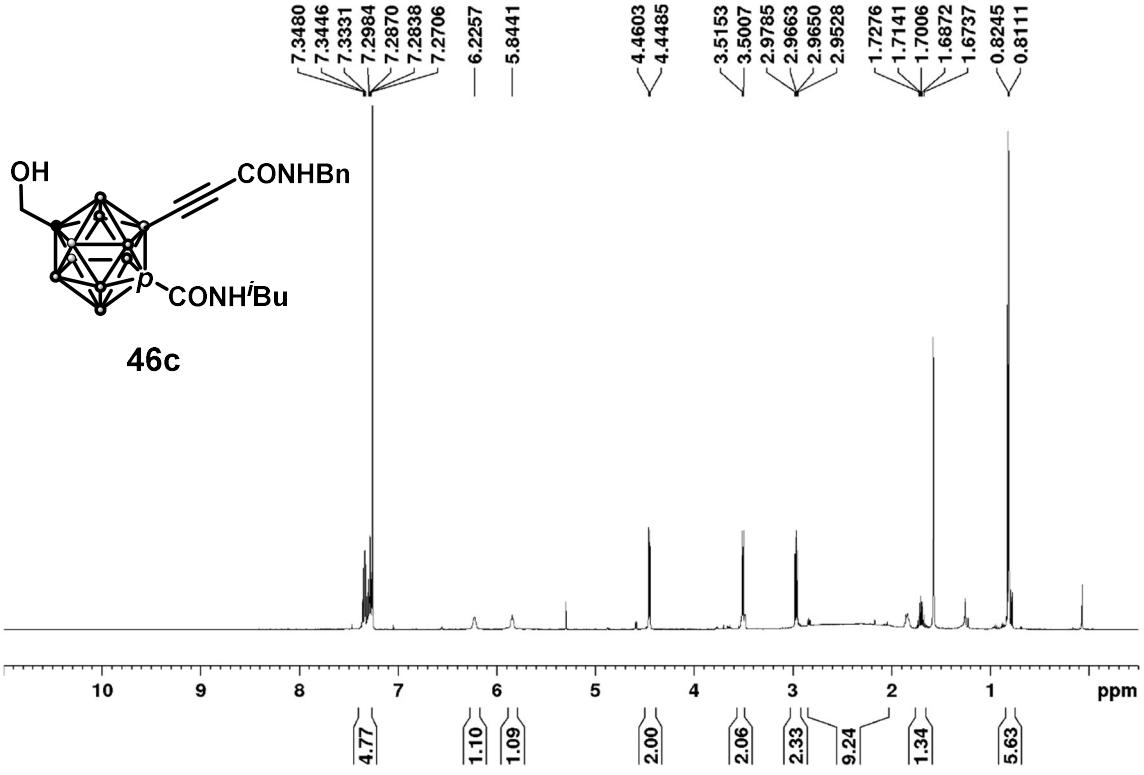


^{11}B NMR (160 MHz; $CDCl_3$)

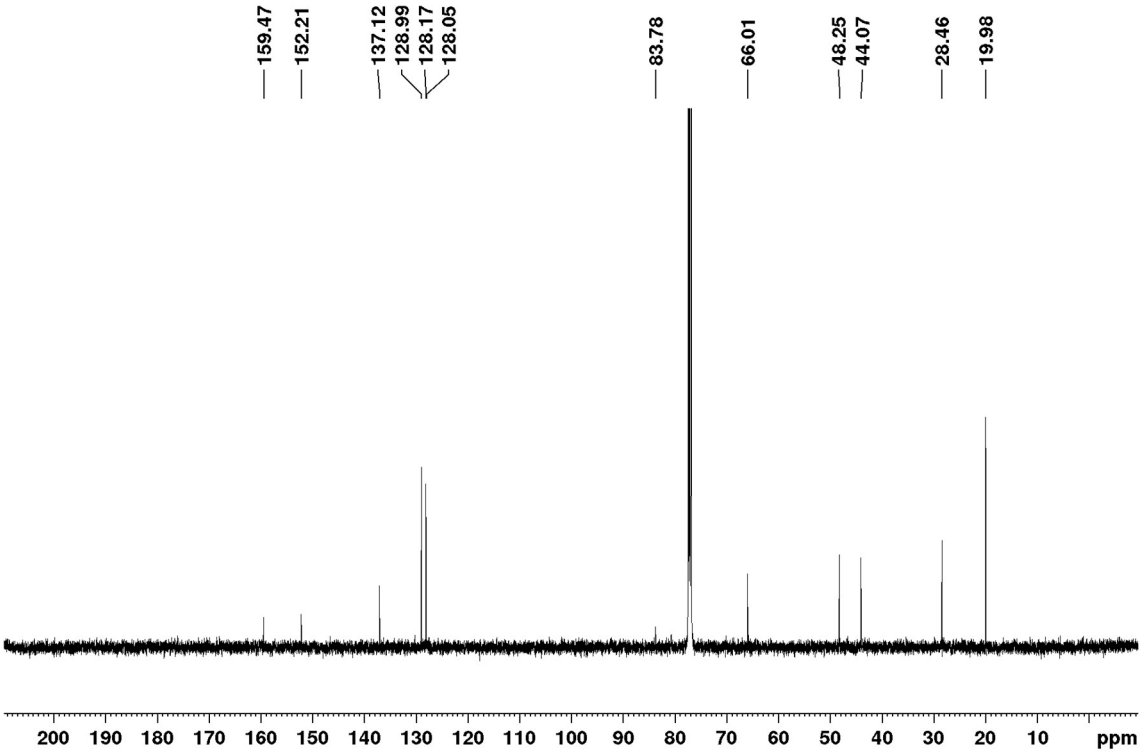


12-(hydroxymethyl)-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (46c)

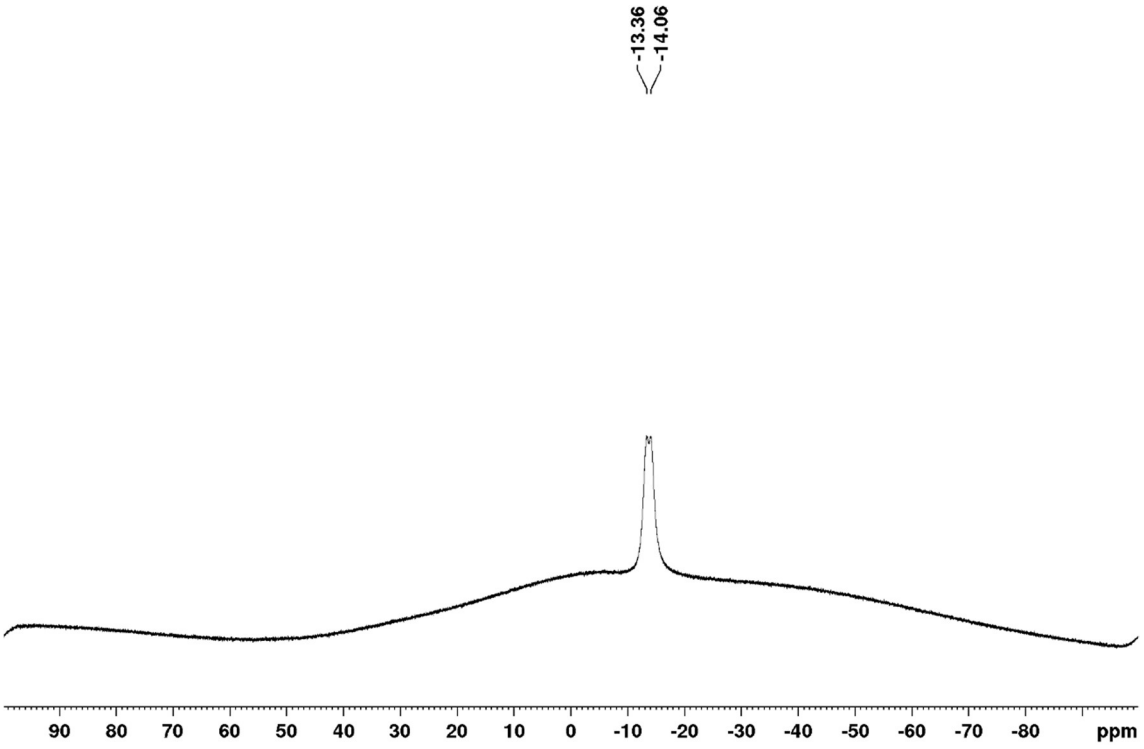
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

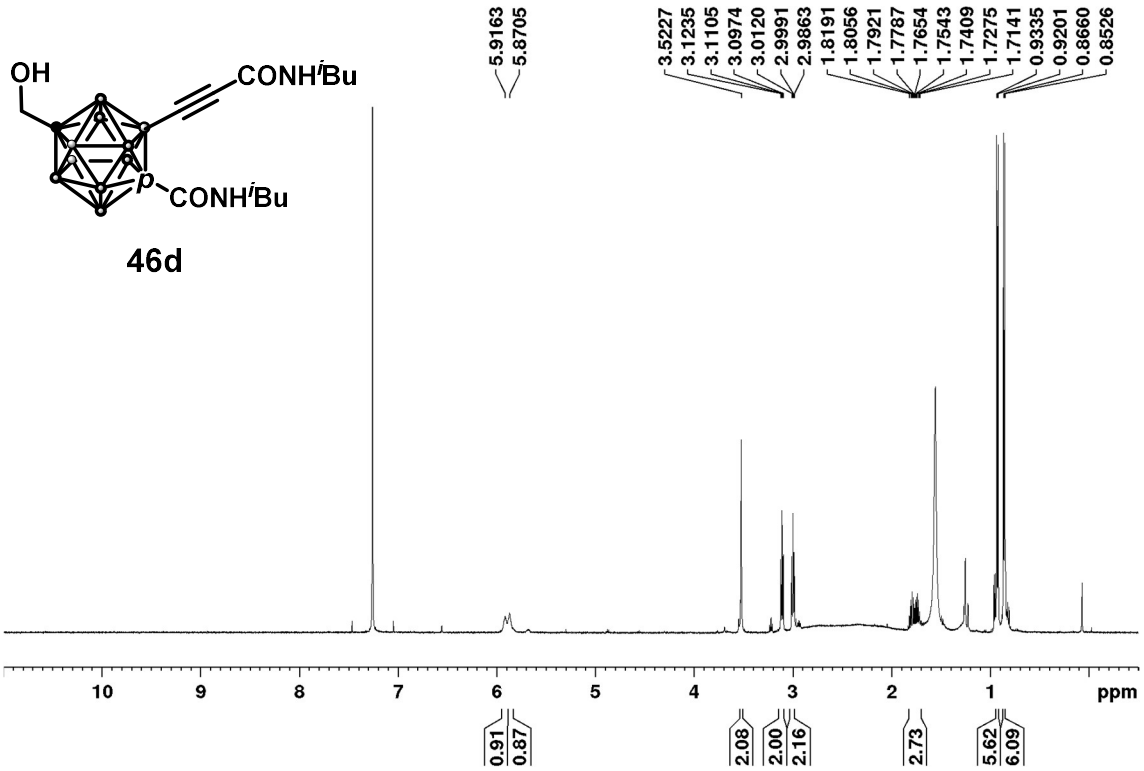


^{11}B NMR (160 MHz; $CDCl_3$)

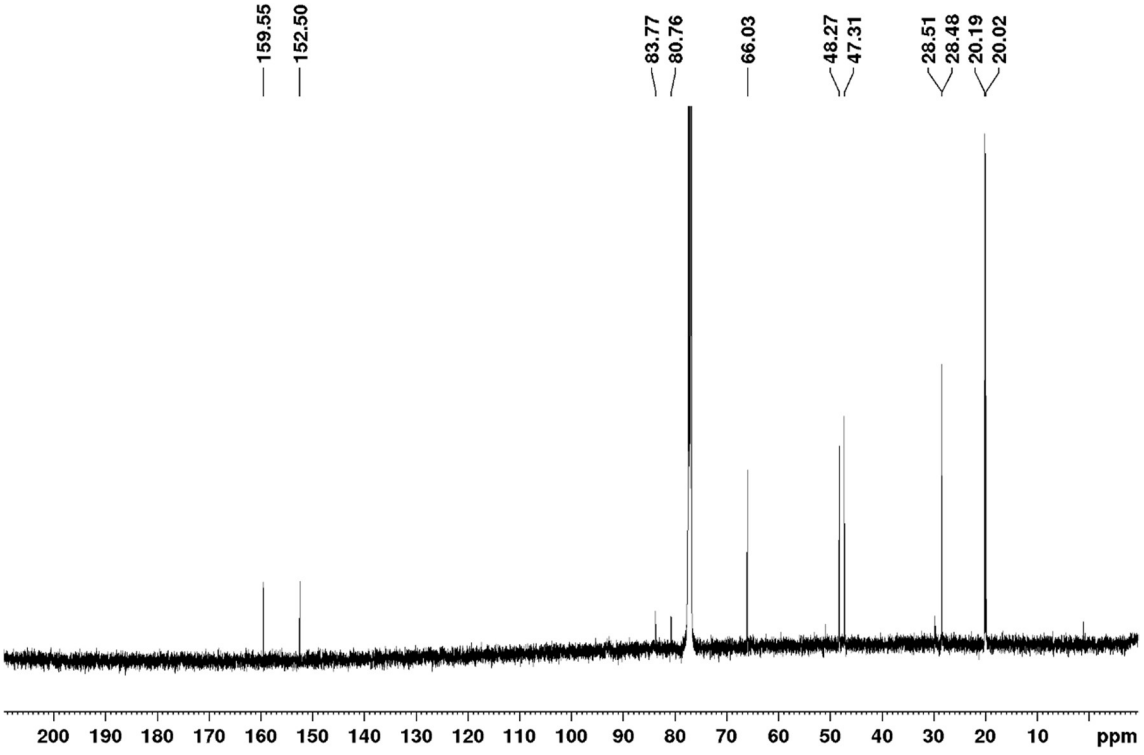


12-(hydroxymethyl)-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (46d)

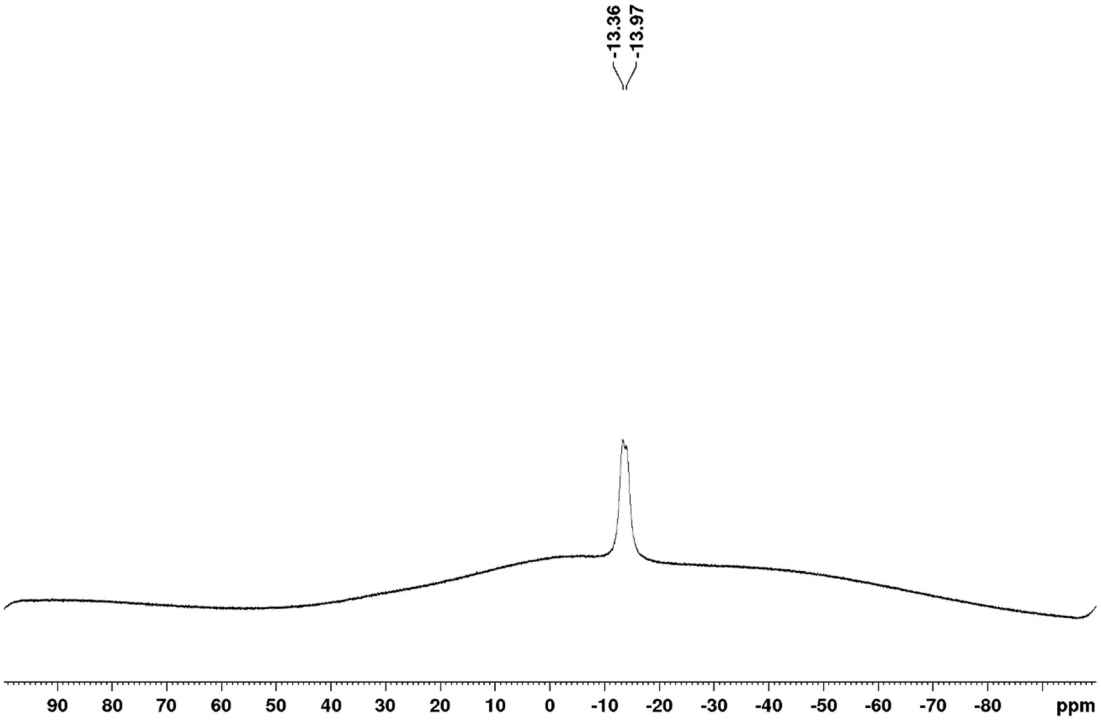
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

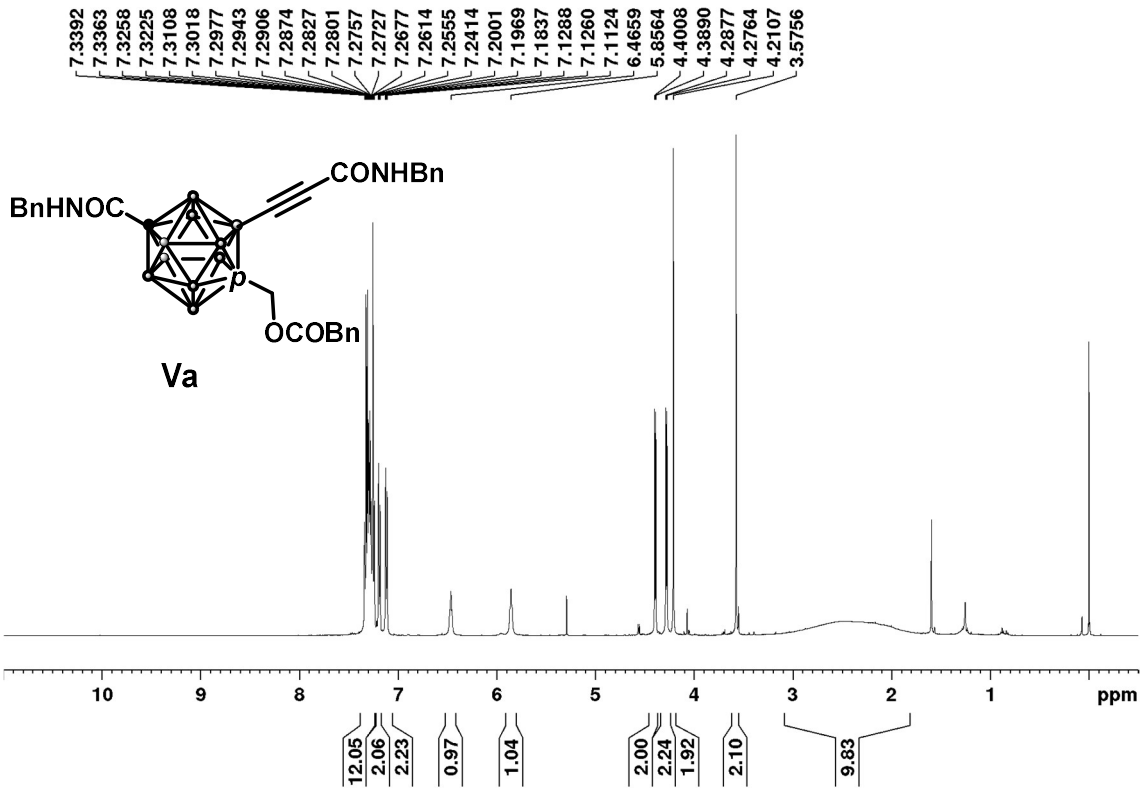


^{11}B NMR (160 MHz; $CDCl_3$)

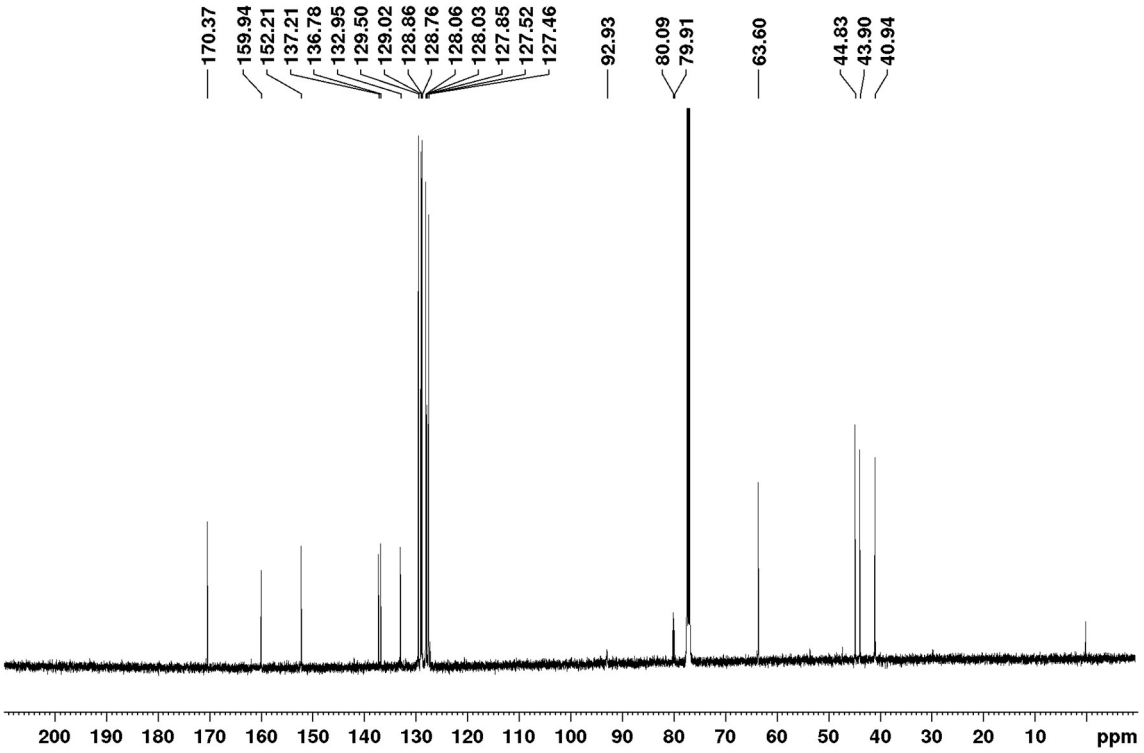


1-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Va)

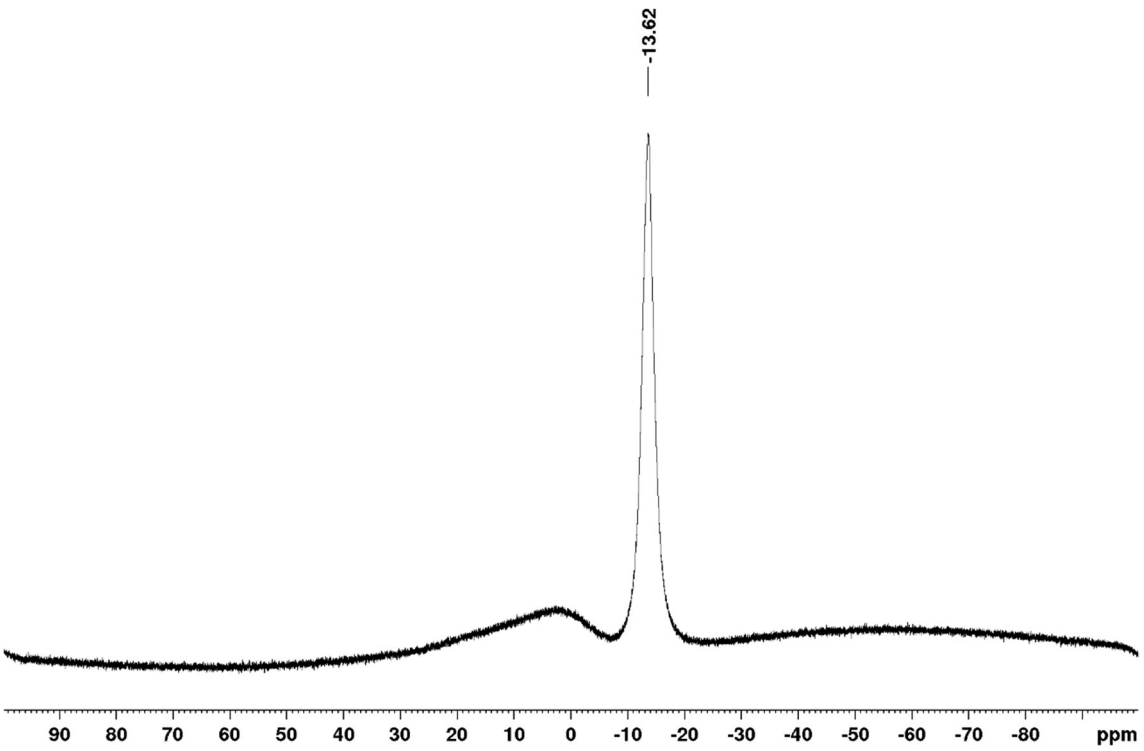
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

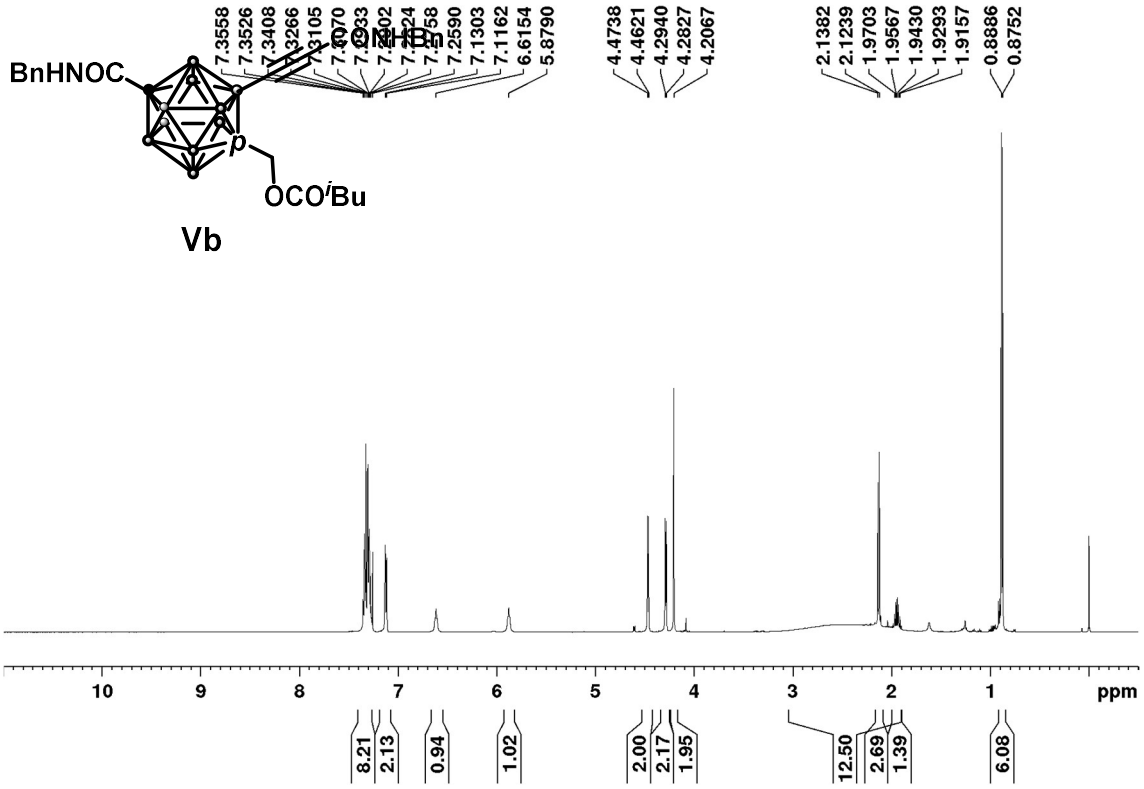


^{11}B NMR (160 MHz; $CDCl_3$)

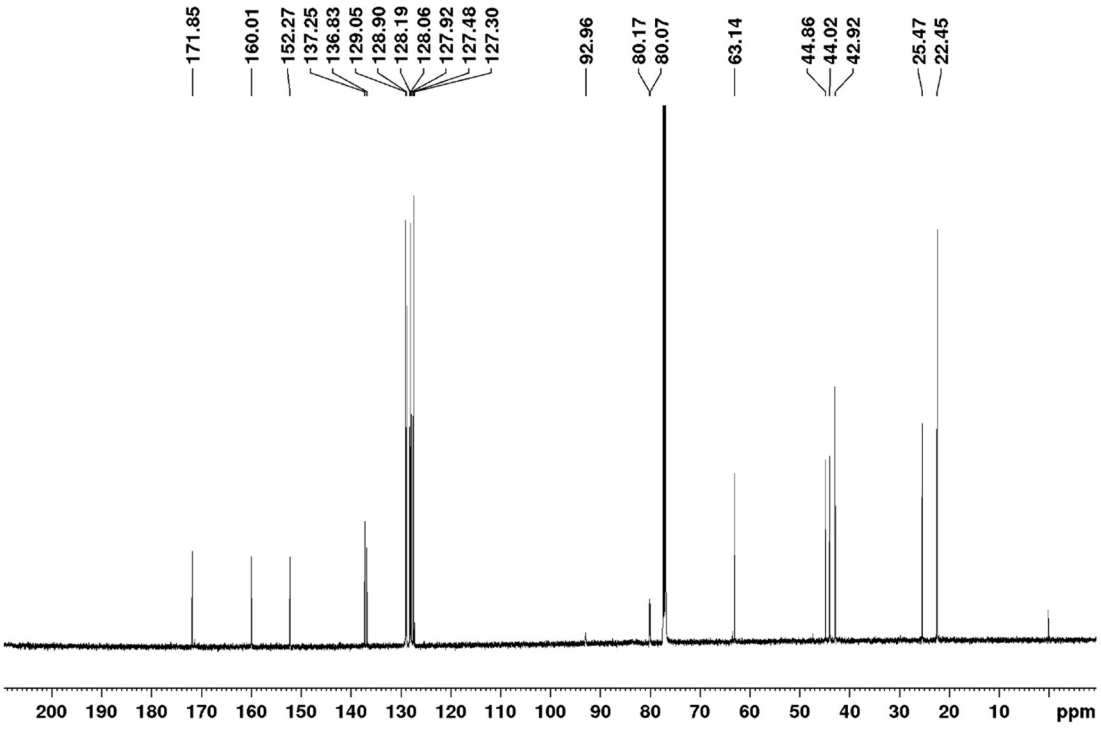


1-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vb)

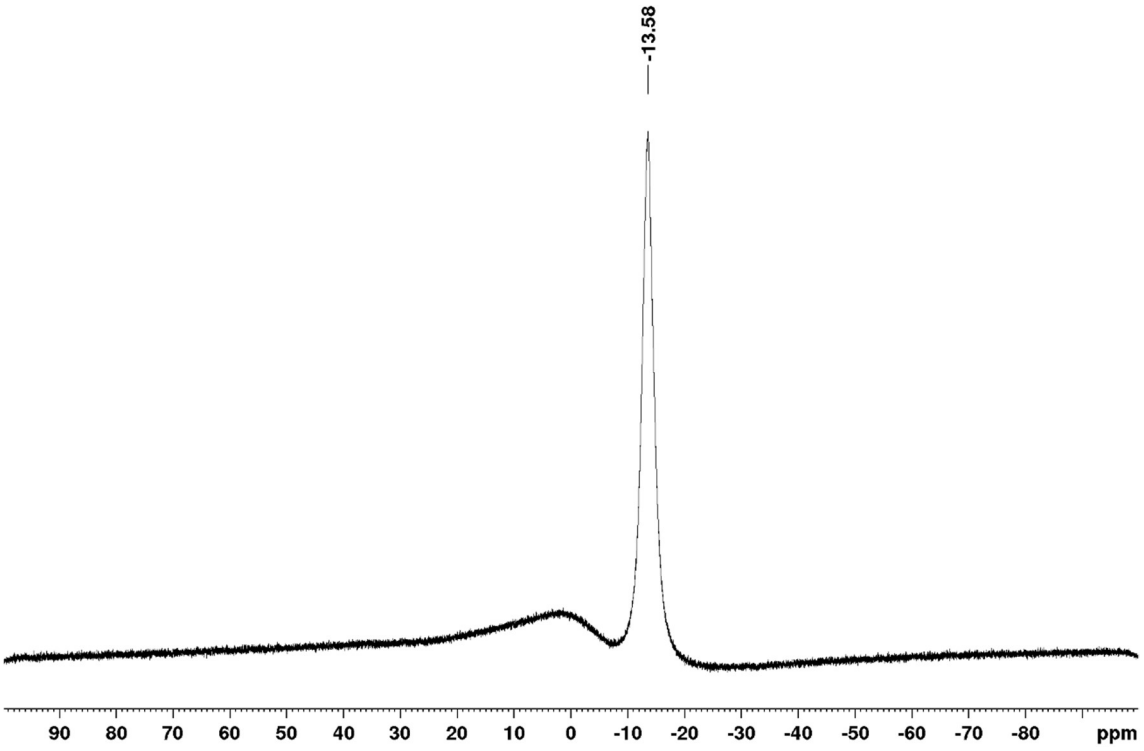
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

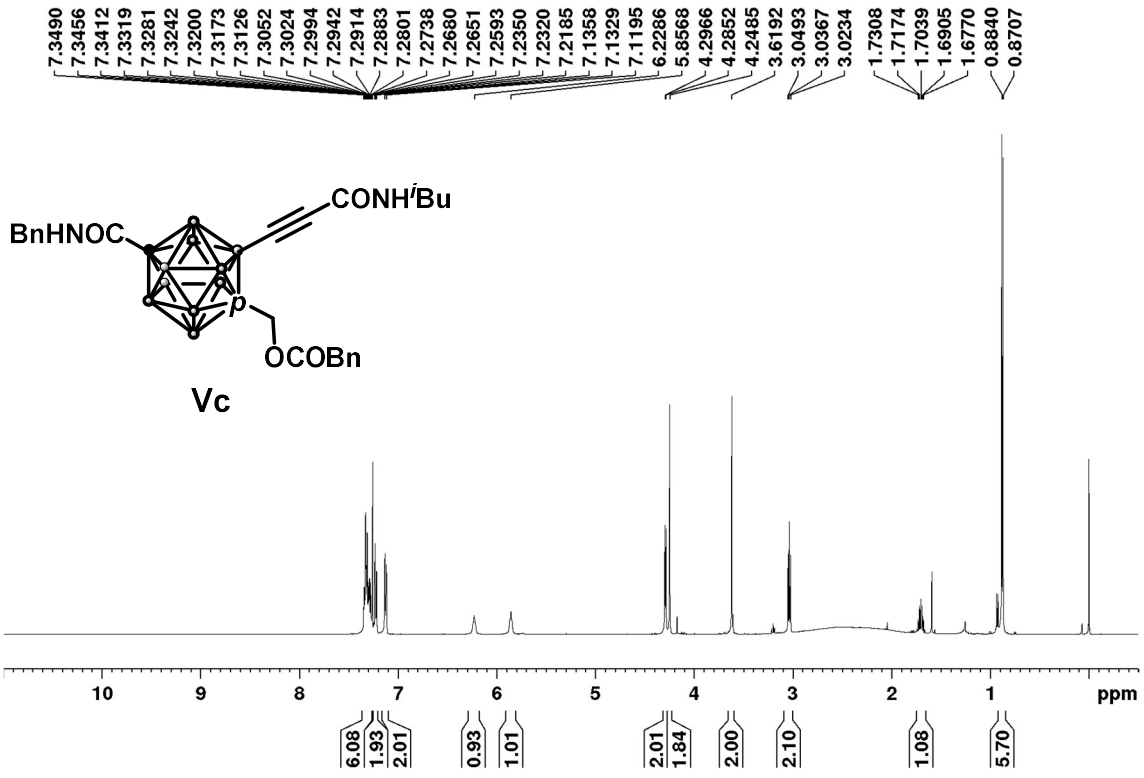


^{11}B NMR (160 MHz; $CDCl_3$)

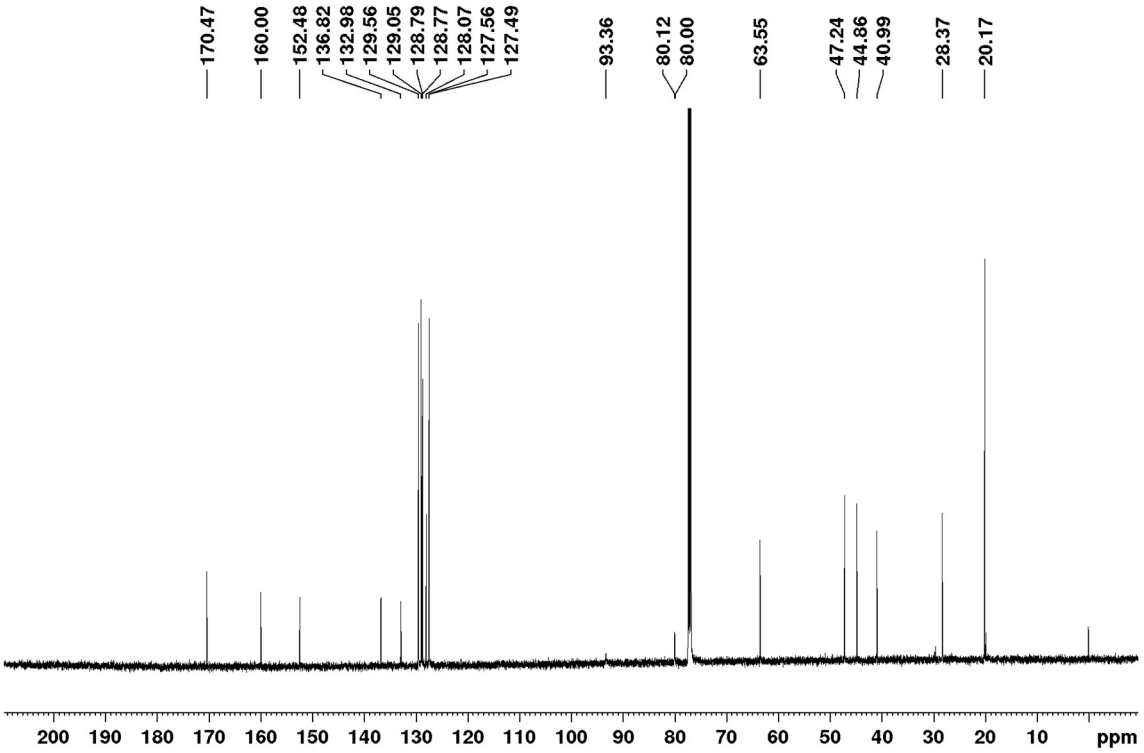


1-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vc)

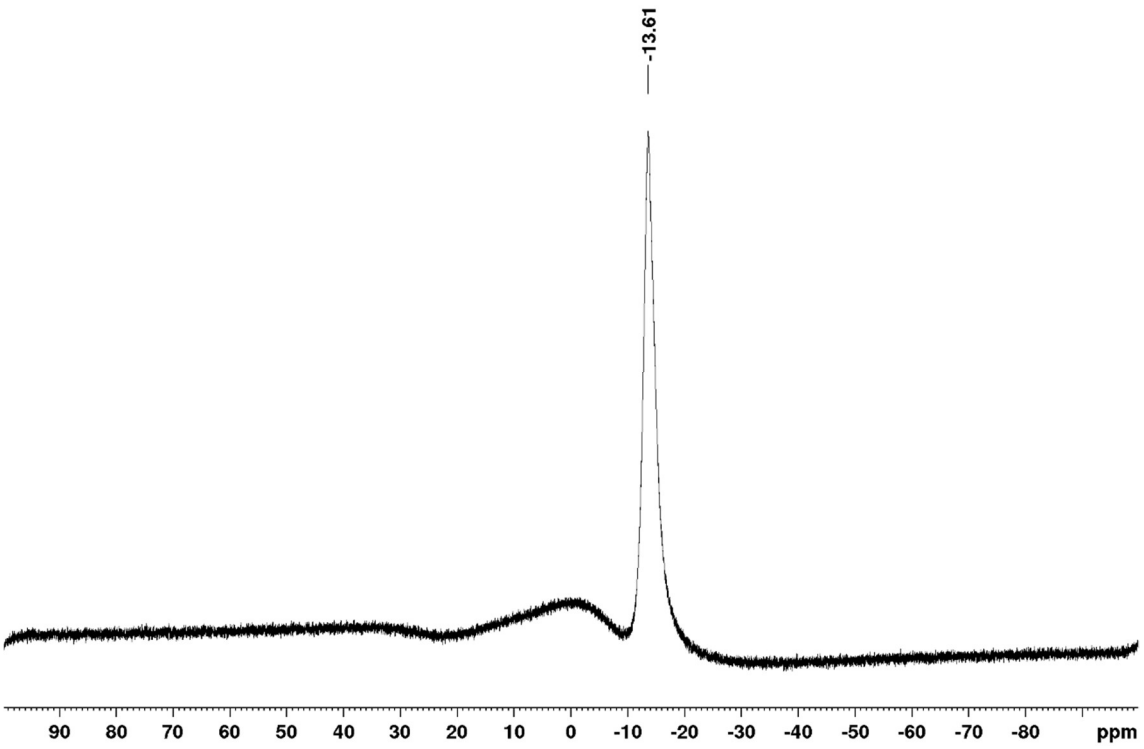
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

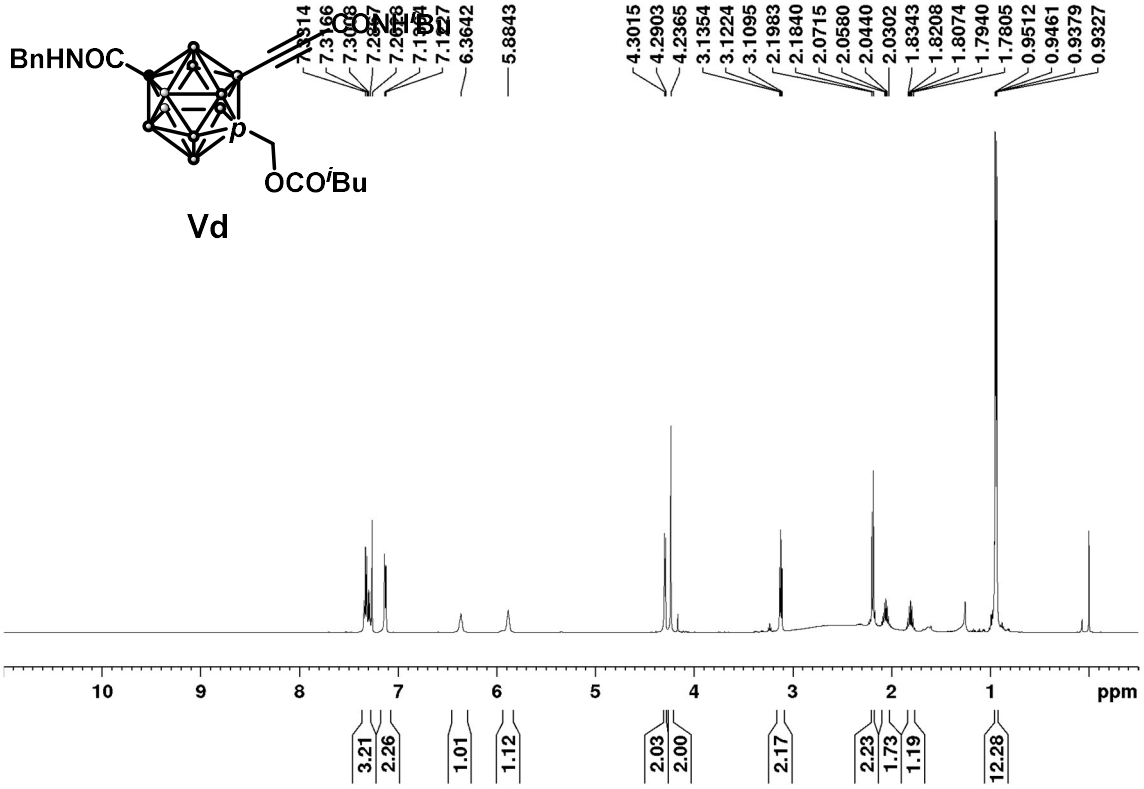


^{11}B NMR (160 MHz; $CDCl_3$)

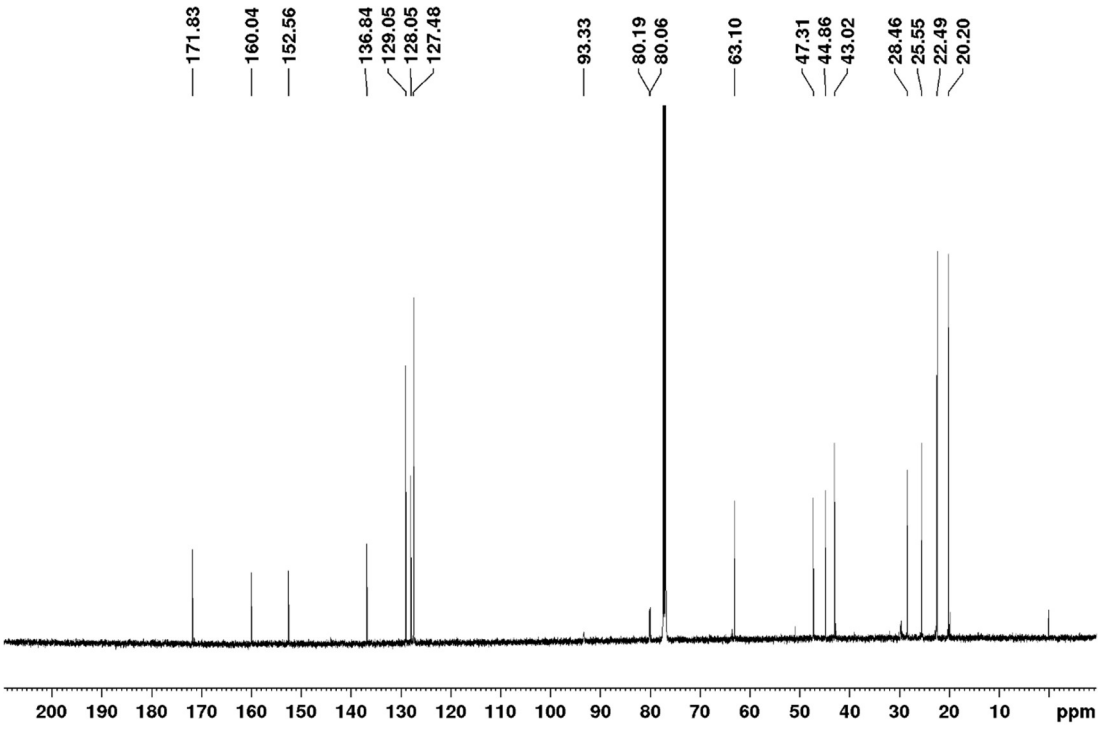


1-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vd)

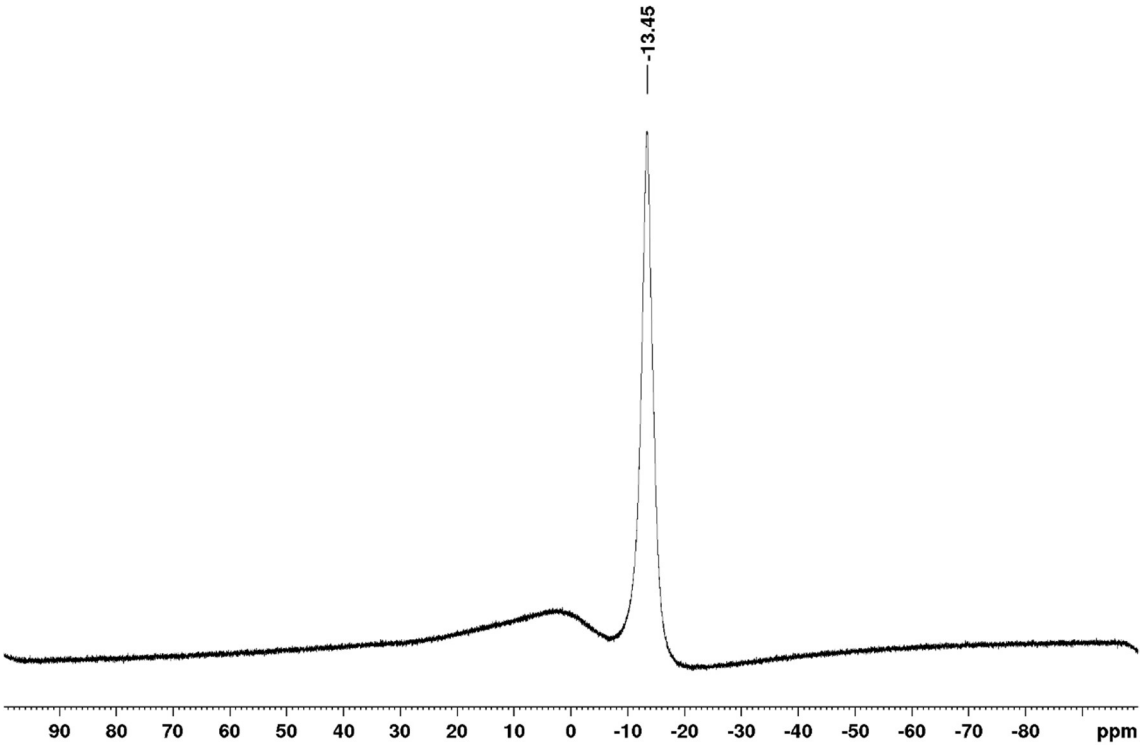
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

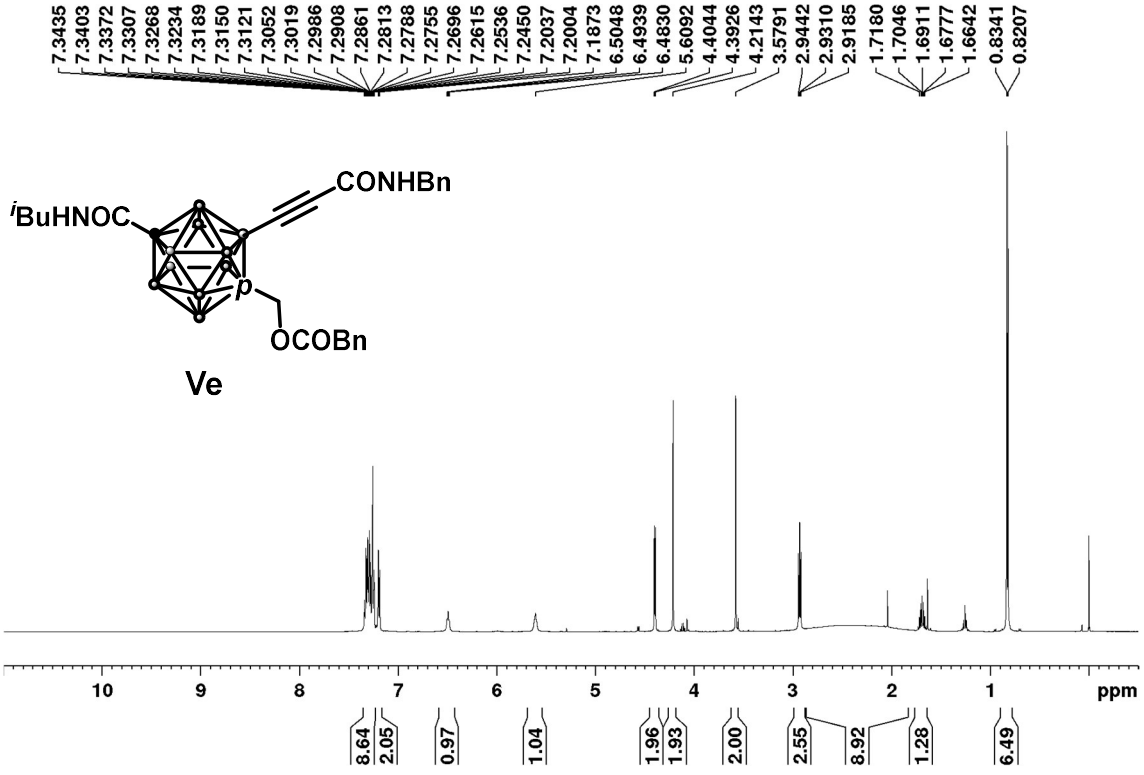


^{11}B NMR (160 MHz; $CDCl_3$)

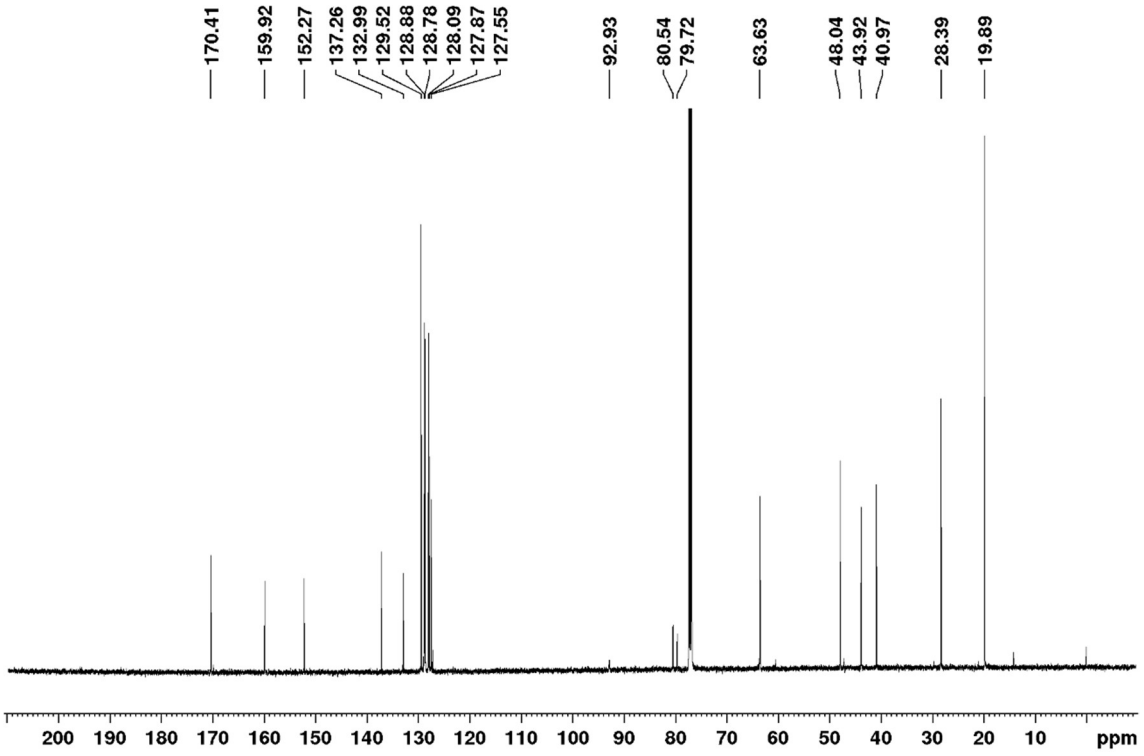


Synthesis of 1-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Ve)

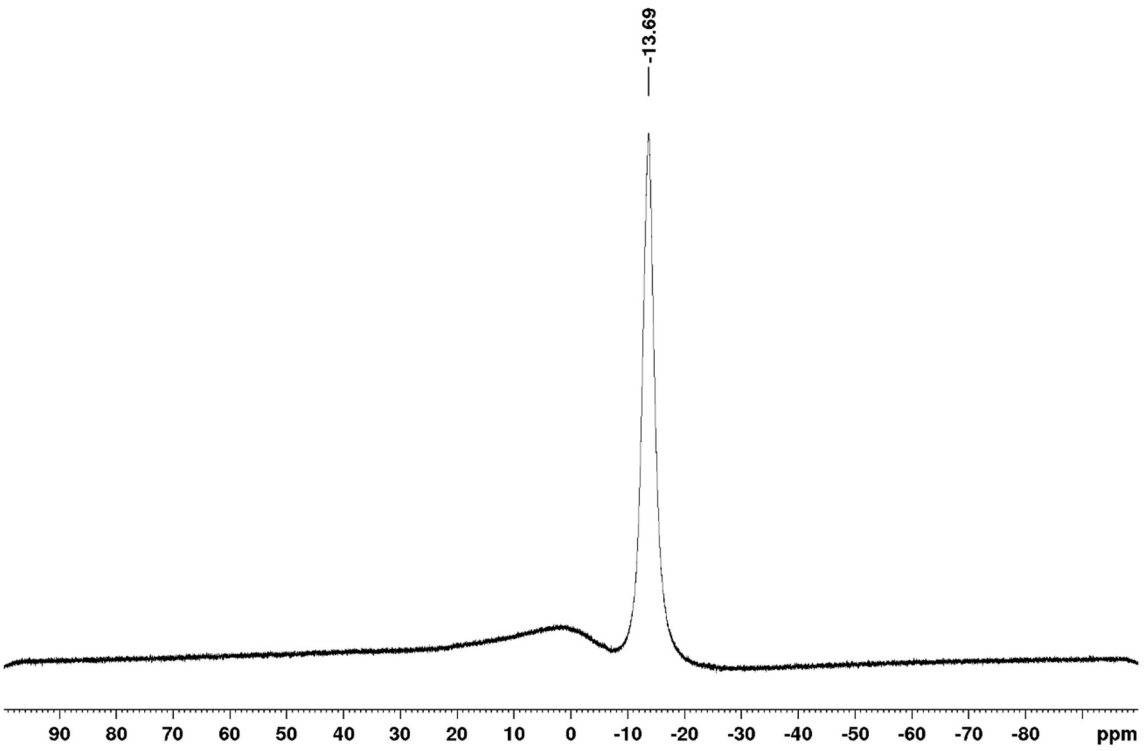
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

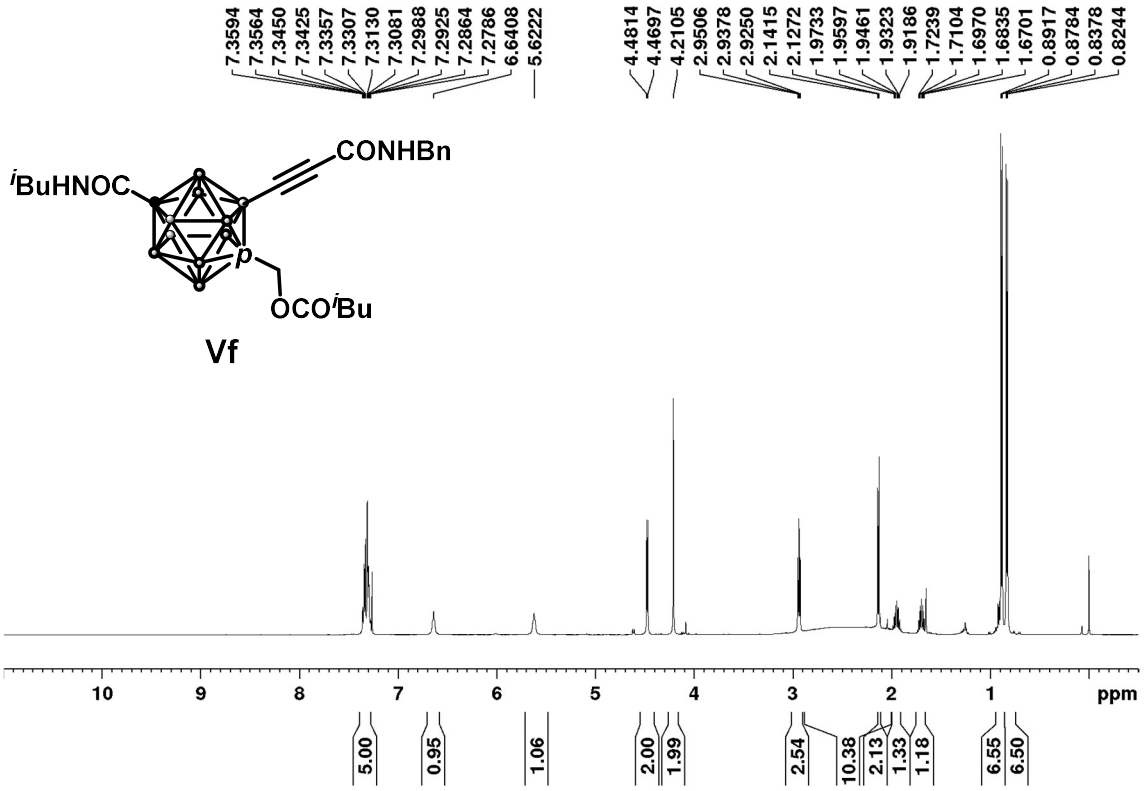


^{11}B NMR (160 MHz; $CDCl_3$)

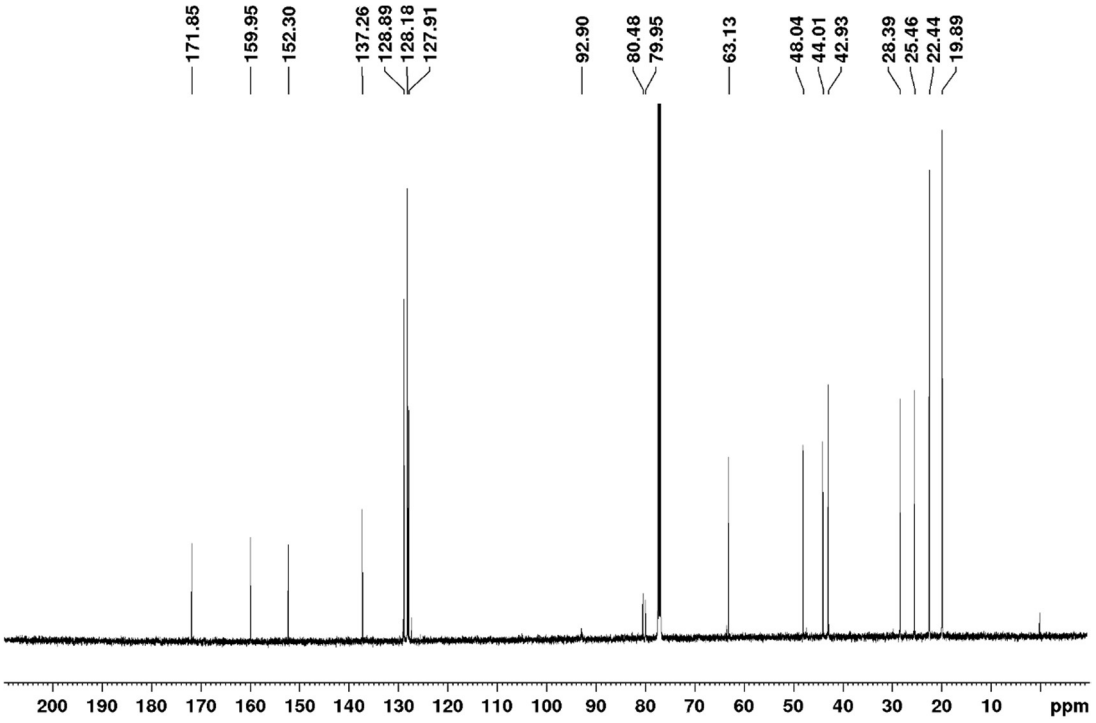


1-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vf)

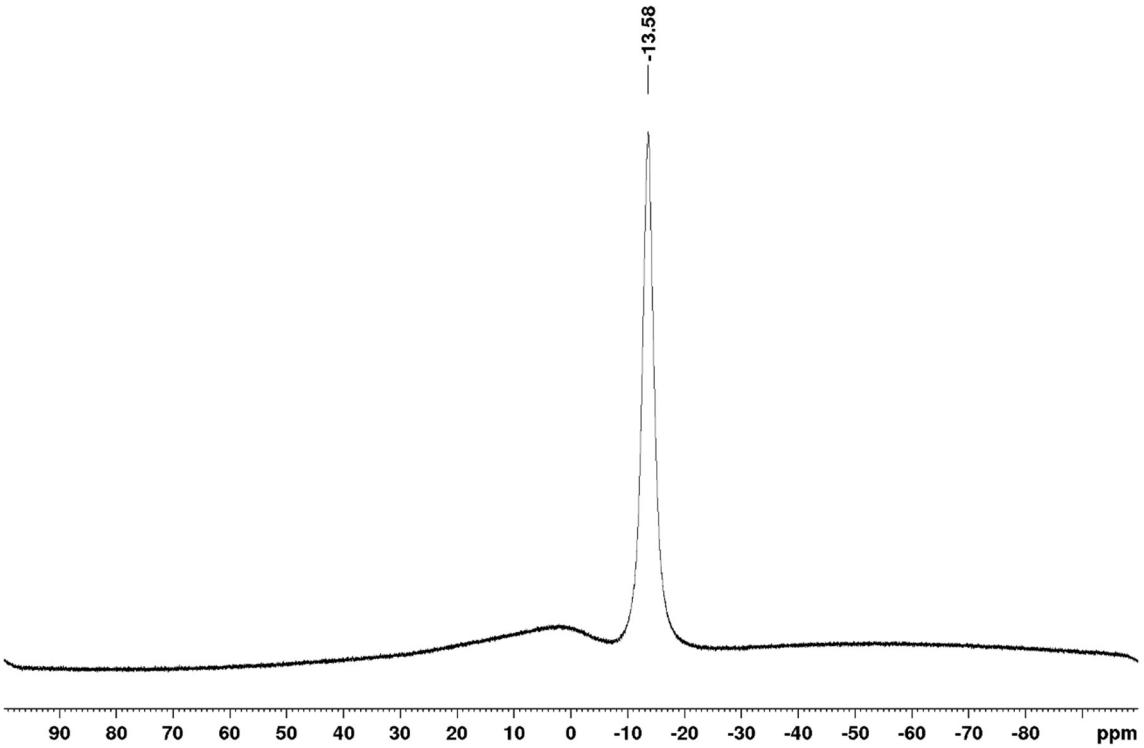
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

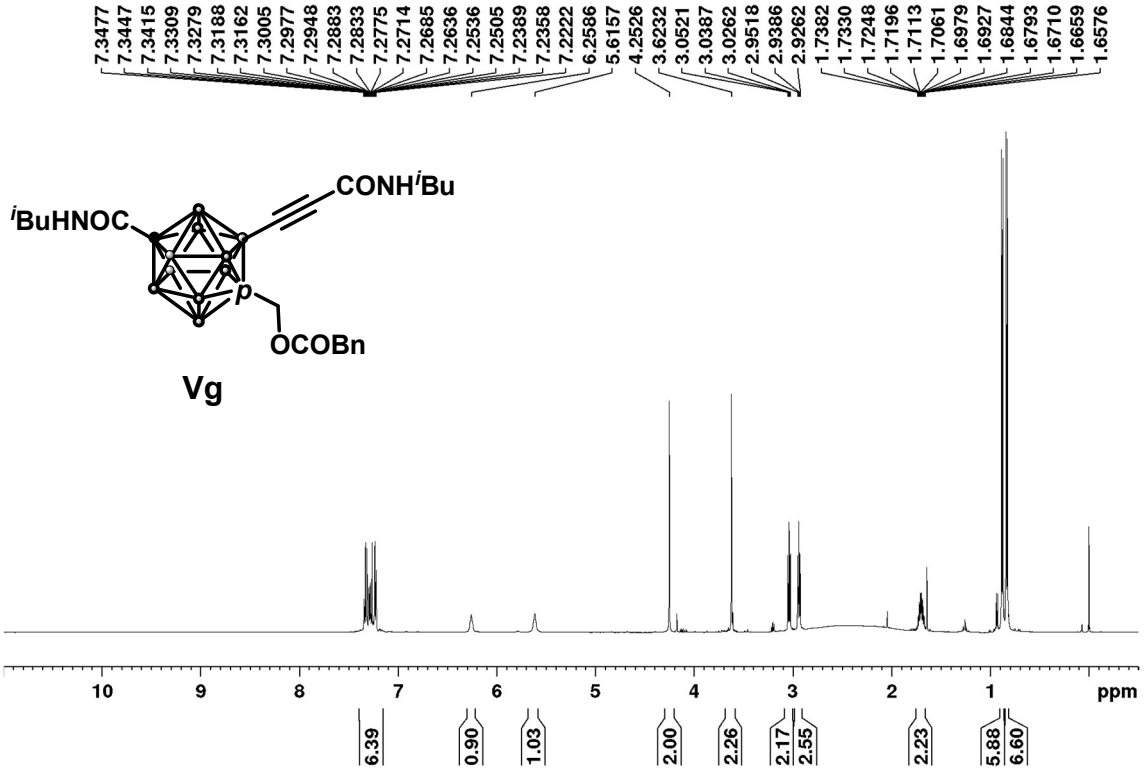


^{11}B NMR (160 MHz; $CDCl_3$)

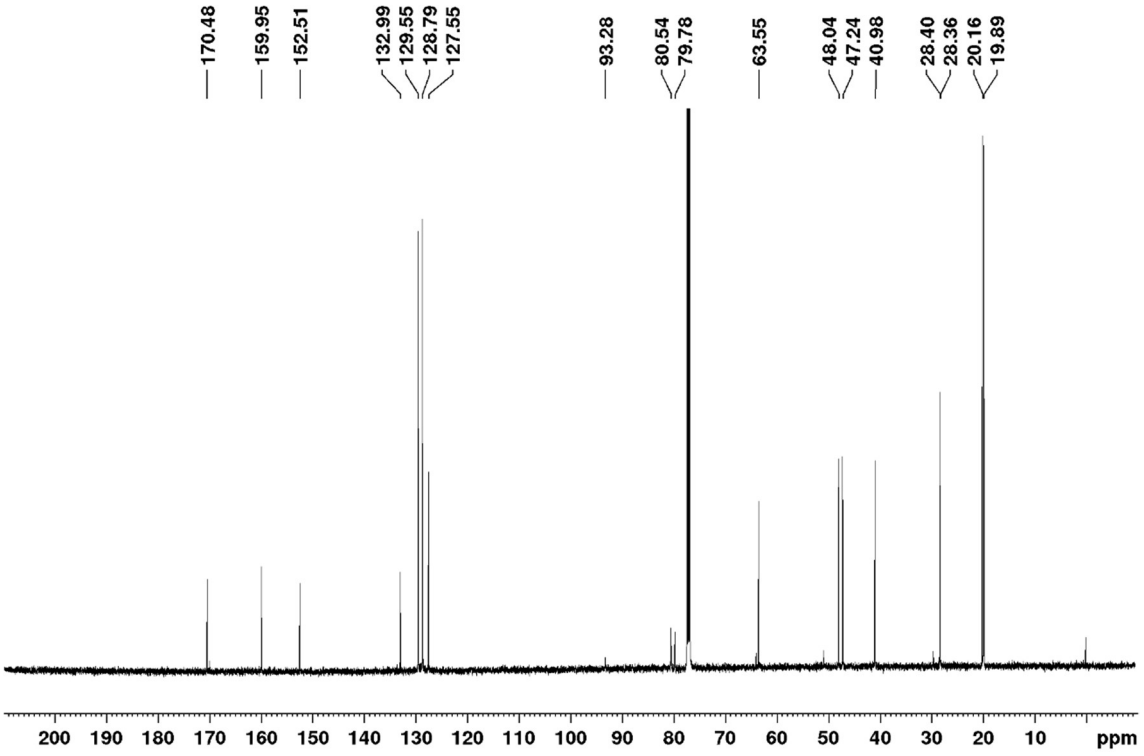


1-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vg)

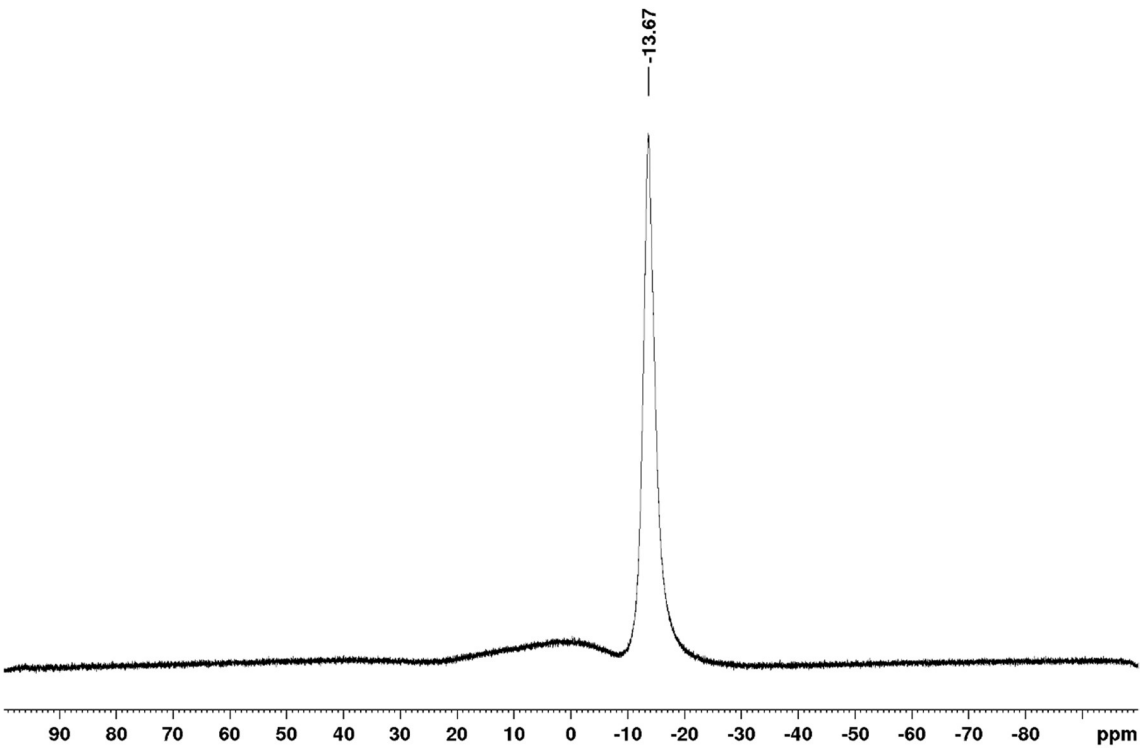
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

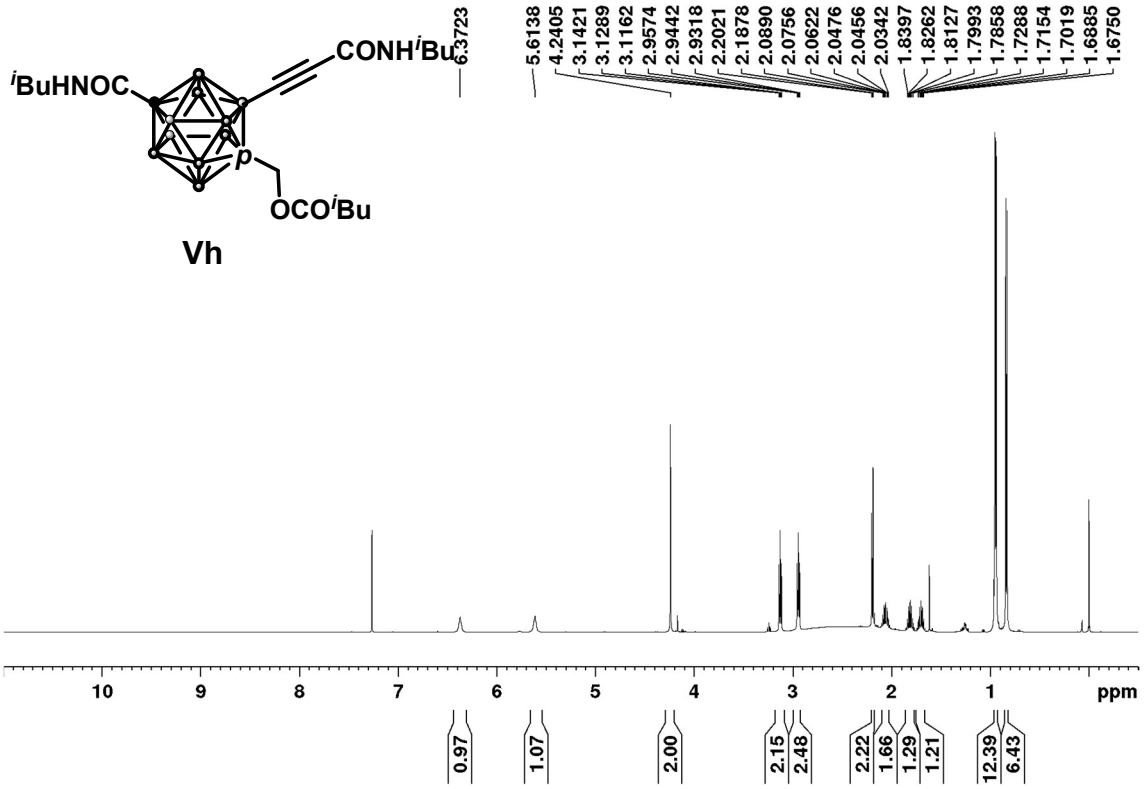


^{11}B NMR (160 MHz; $CDCl_3$)

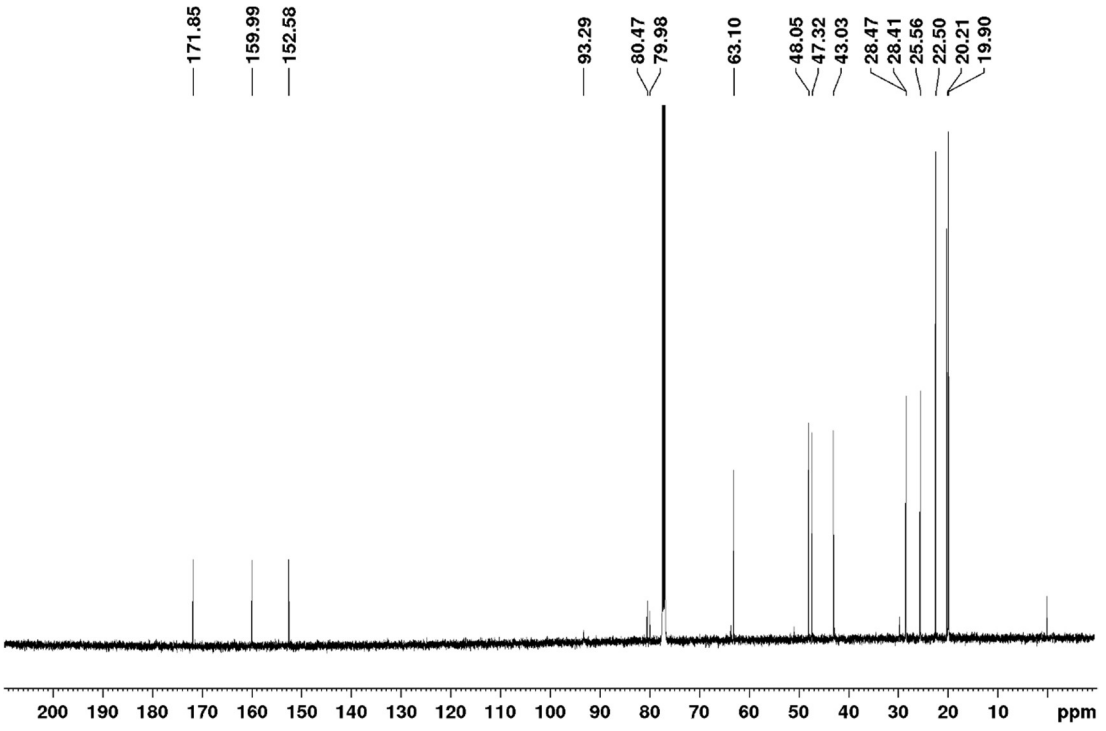


((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-12-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vh)

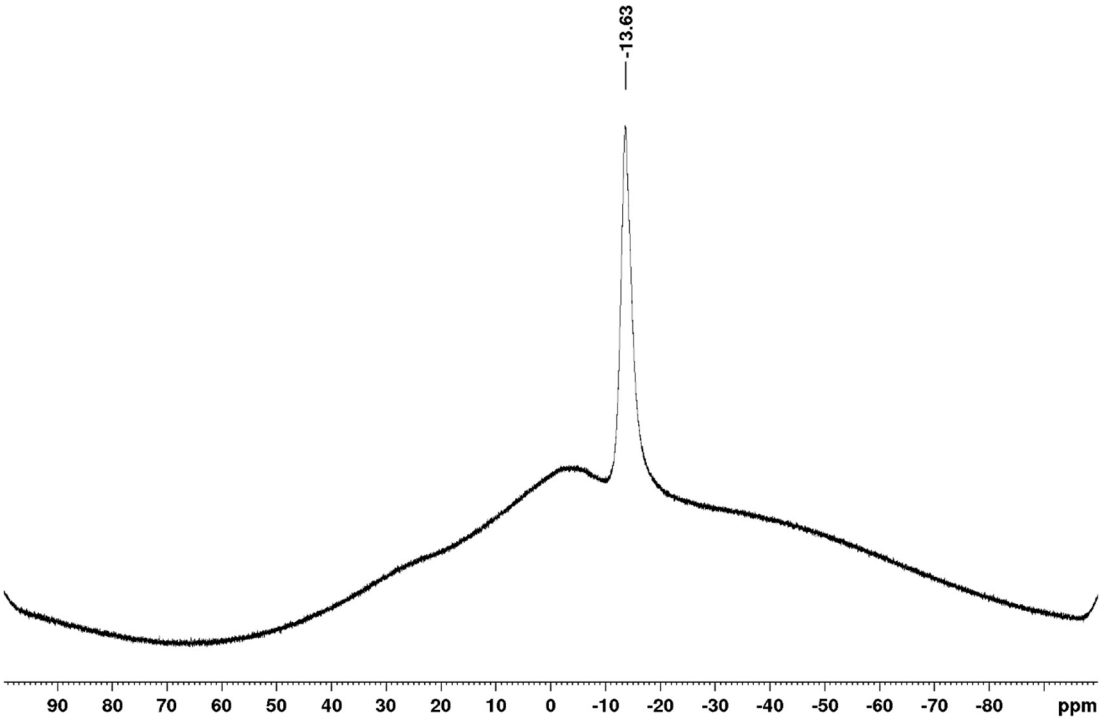
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

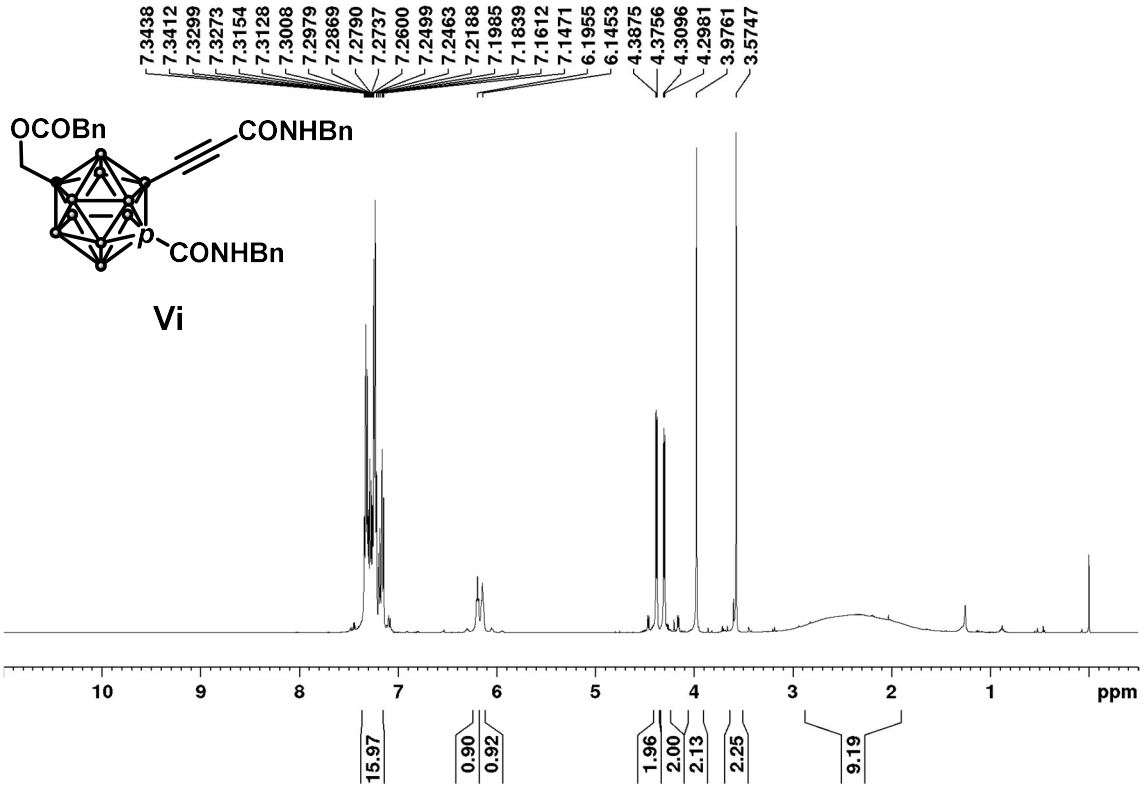


^{11}B NMR (160 MHz; $CDCl_3$)

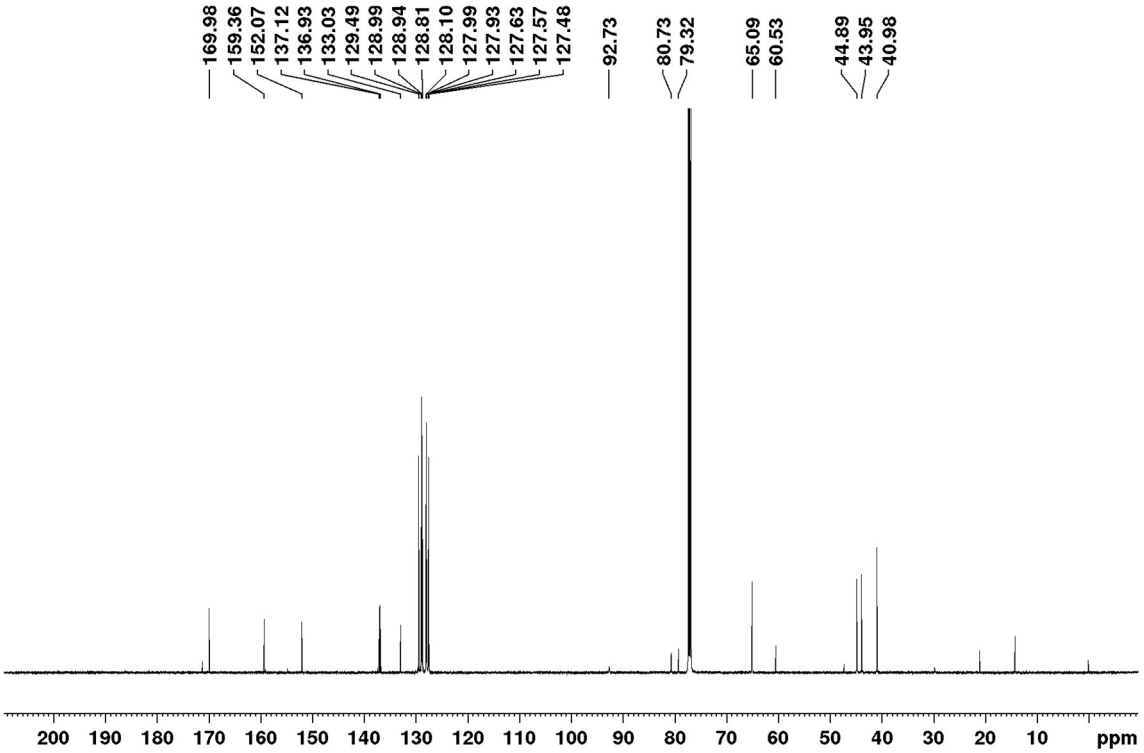


12-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vi)

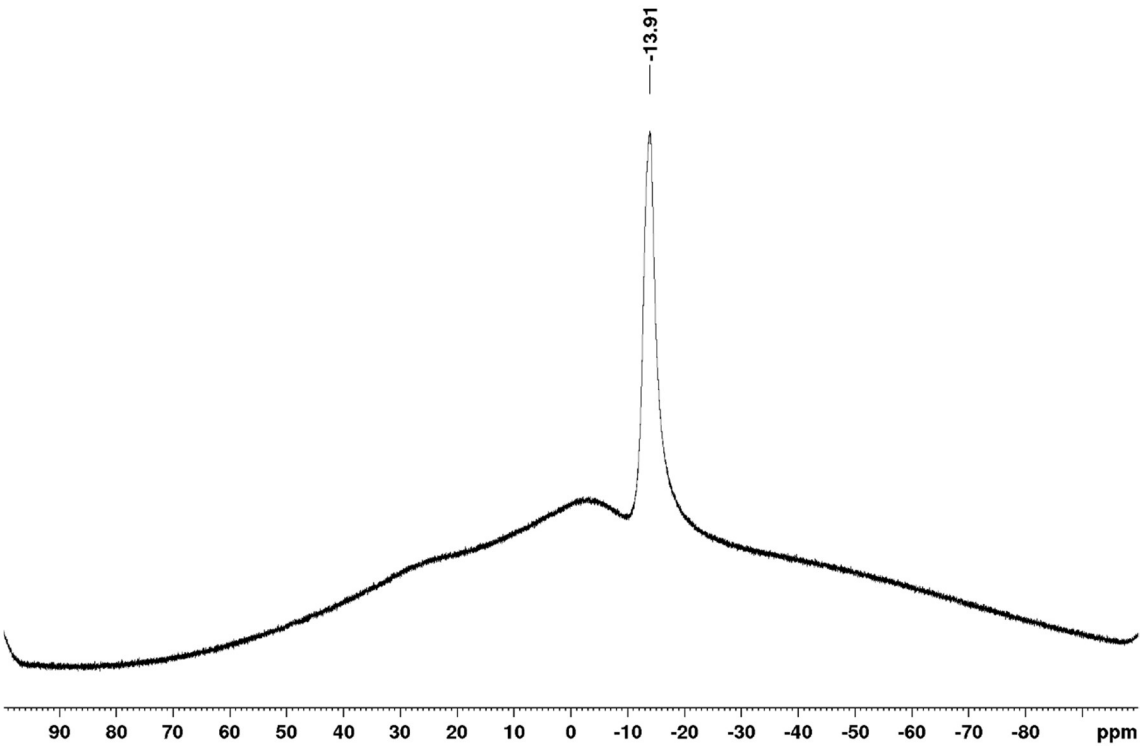
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

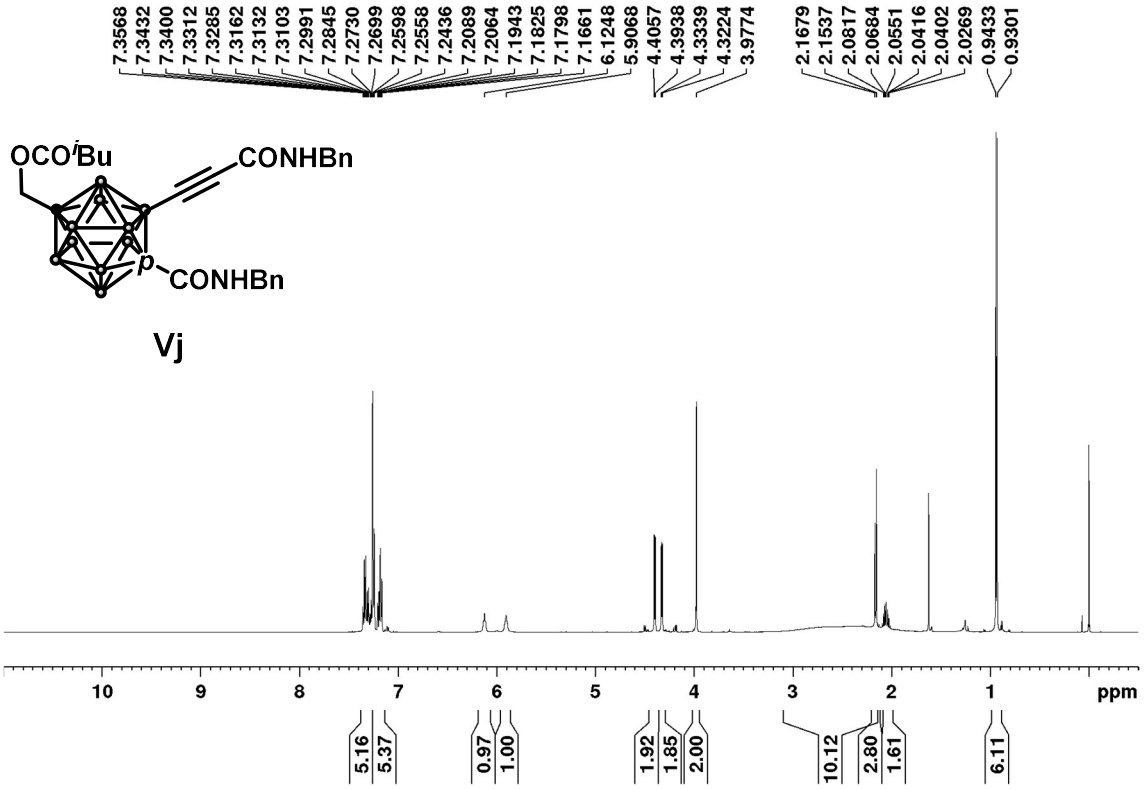


^{11}B NMR (160 MHz; $CDCl_3$)

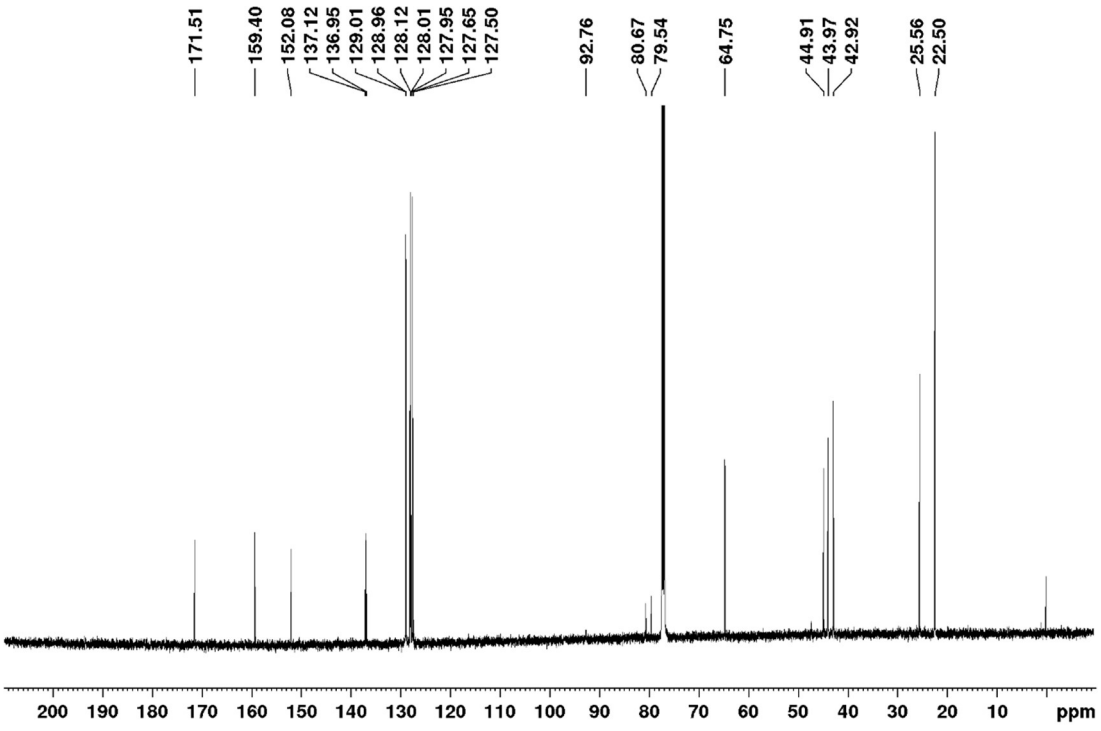


12-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vj)

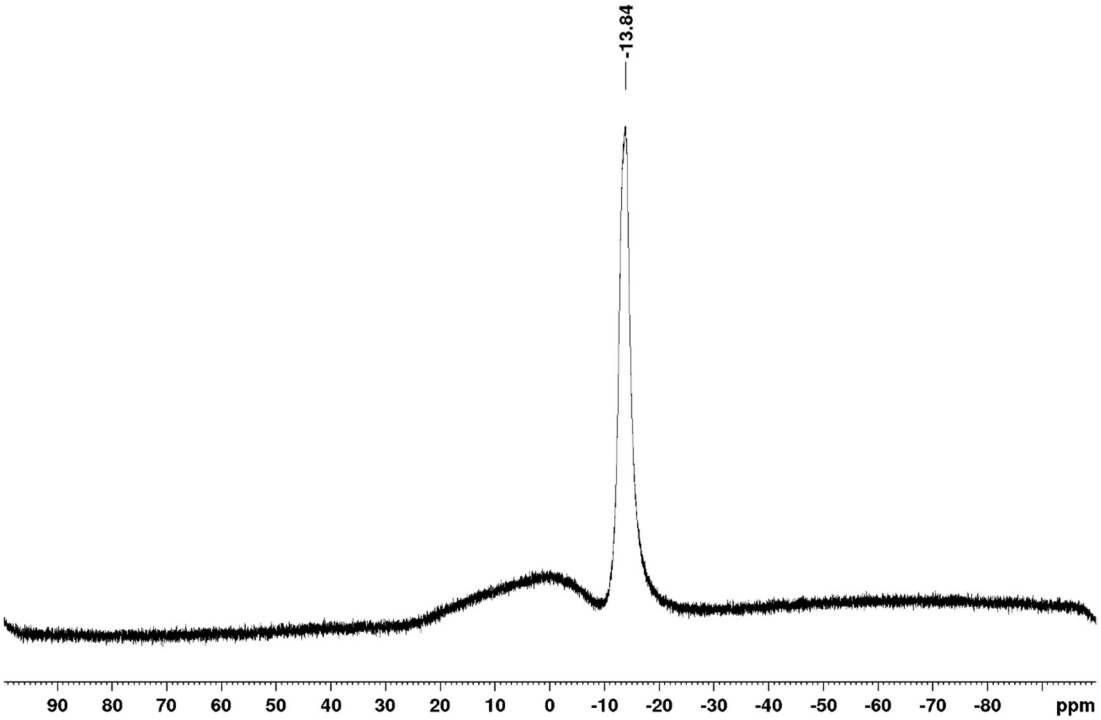
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

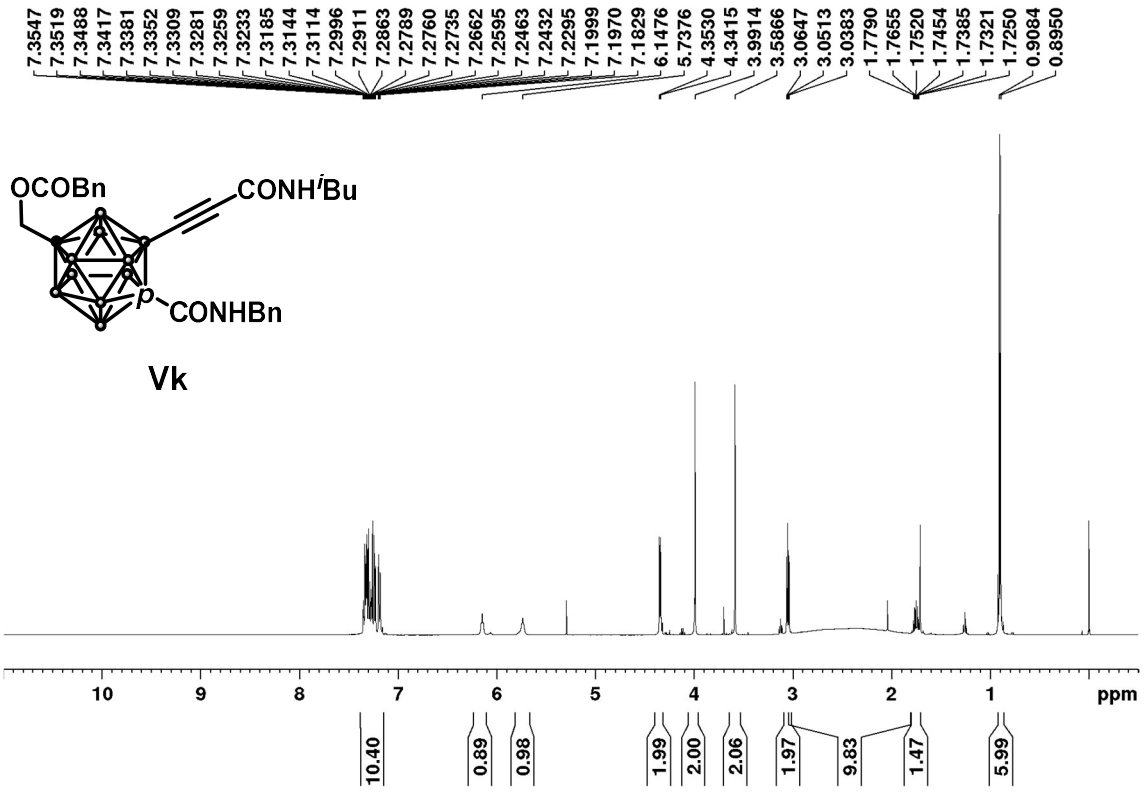


^{11}B NMR (160 MHz; $CDCl_3$)

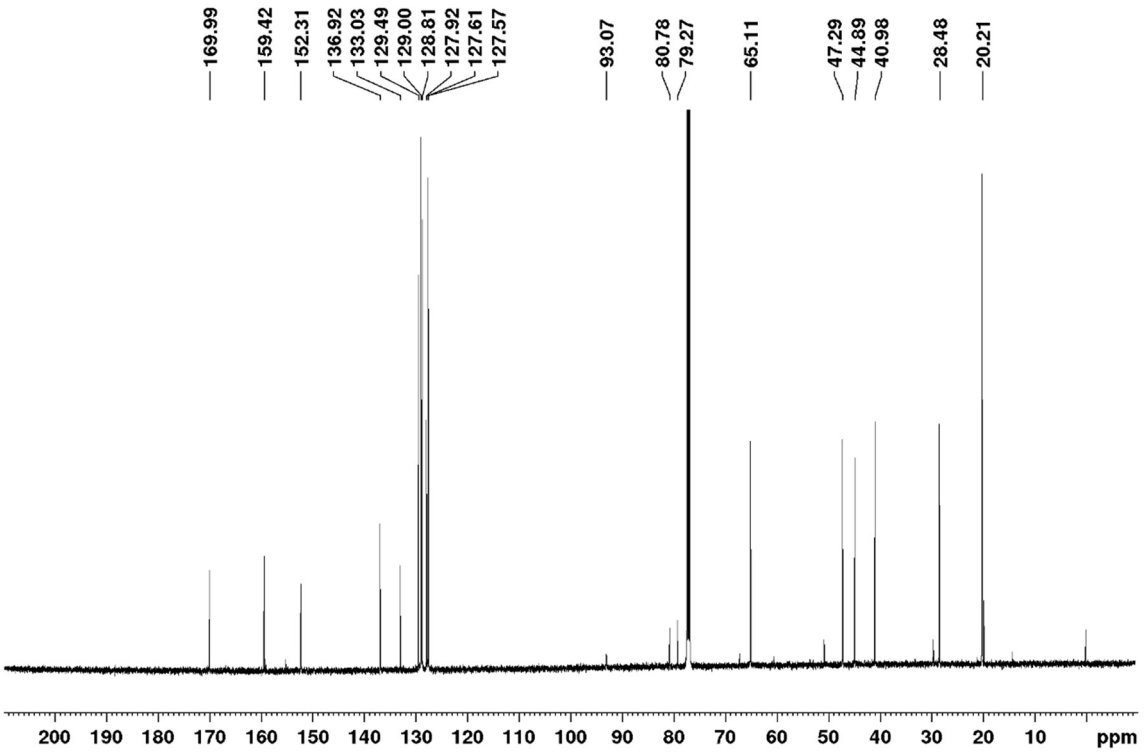


12-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vk)

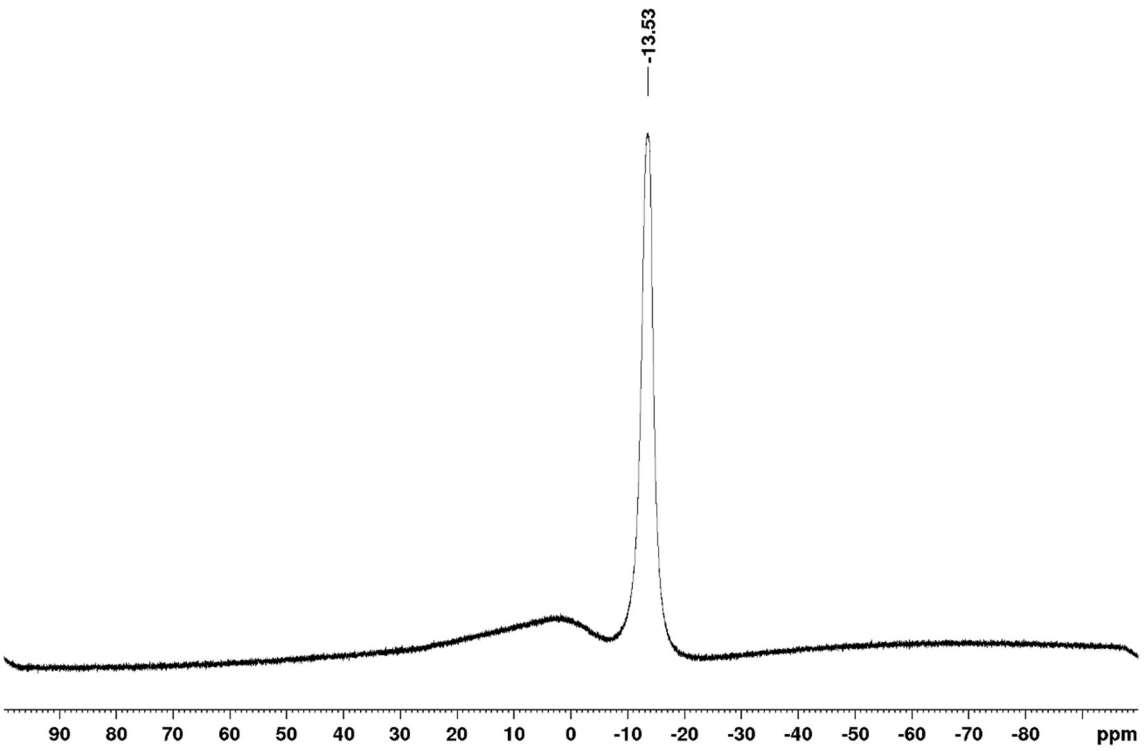
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

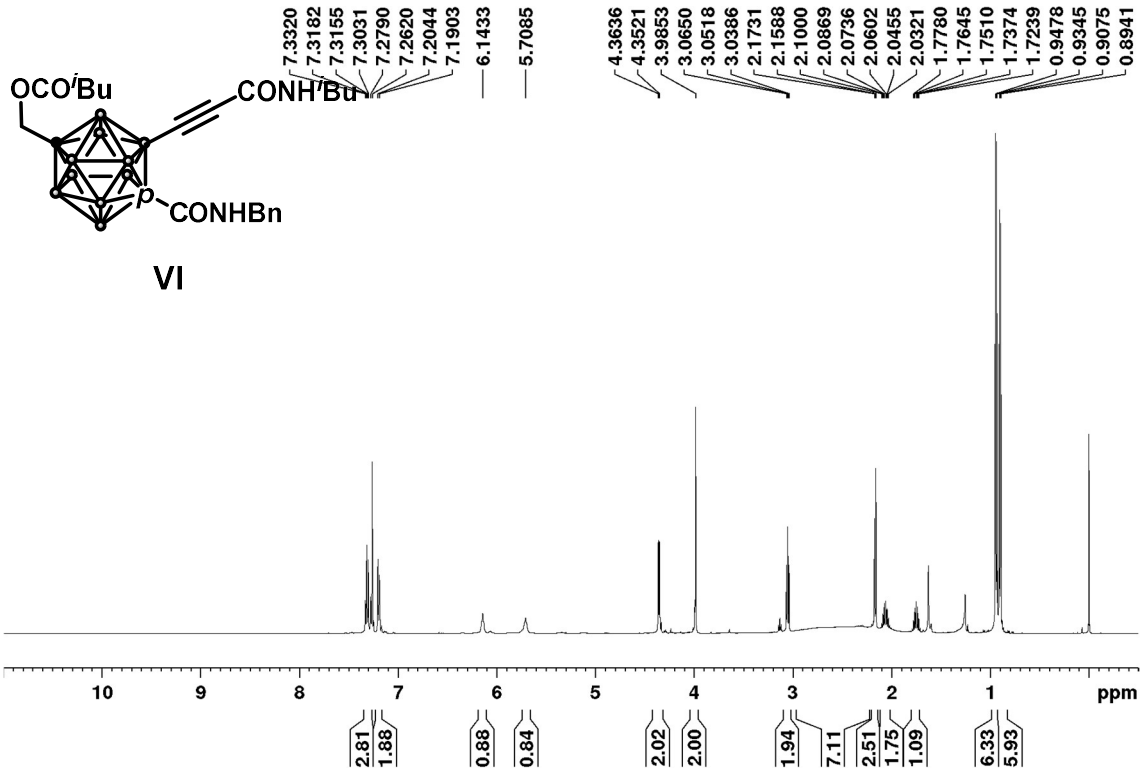


^{11}B NMR (160 MHz; $CDCl_3$)

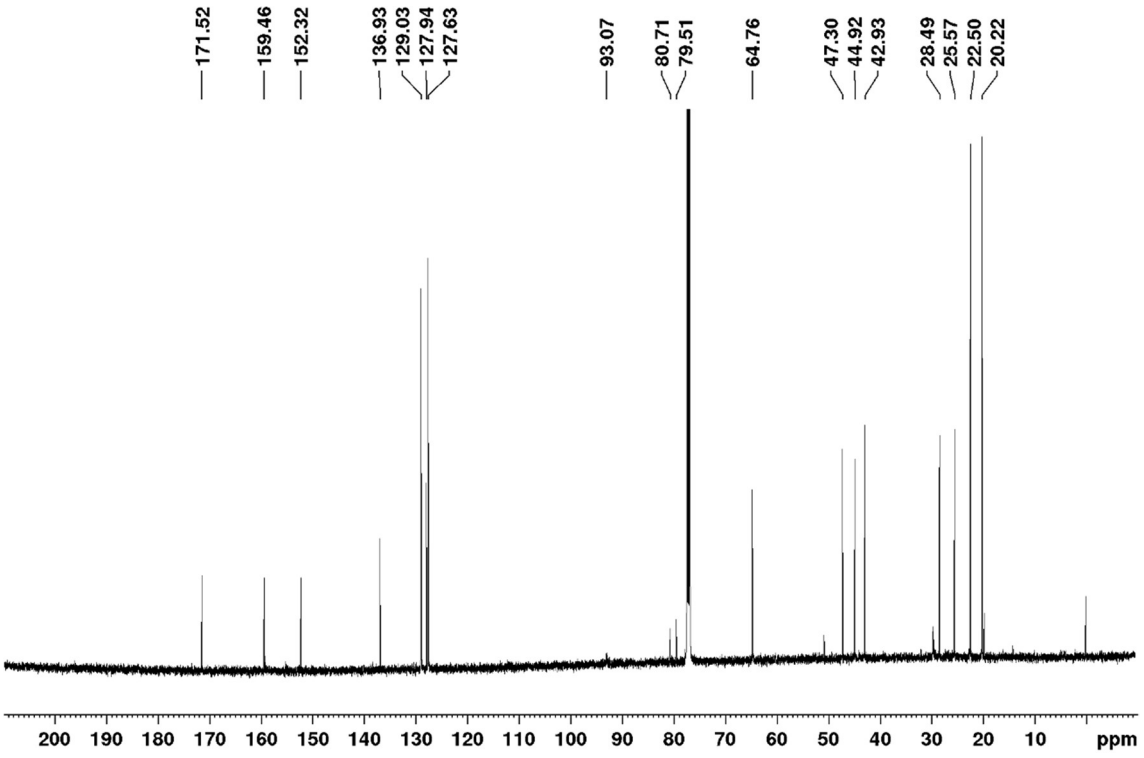


12-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vl)

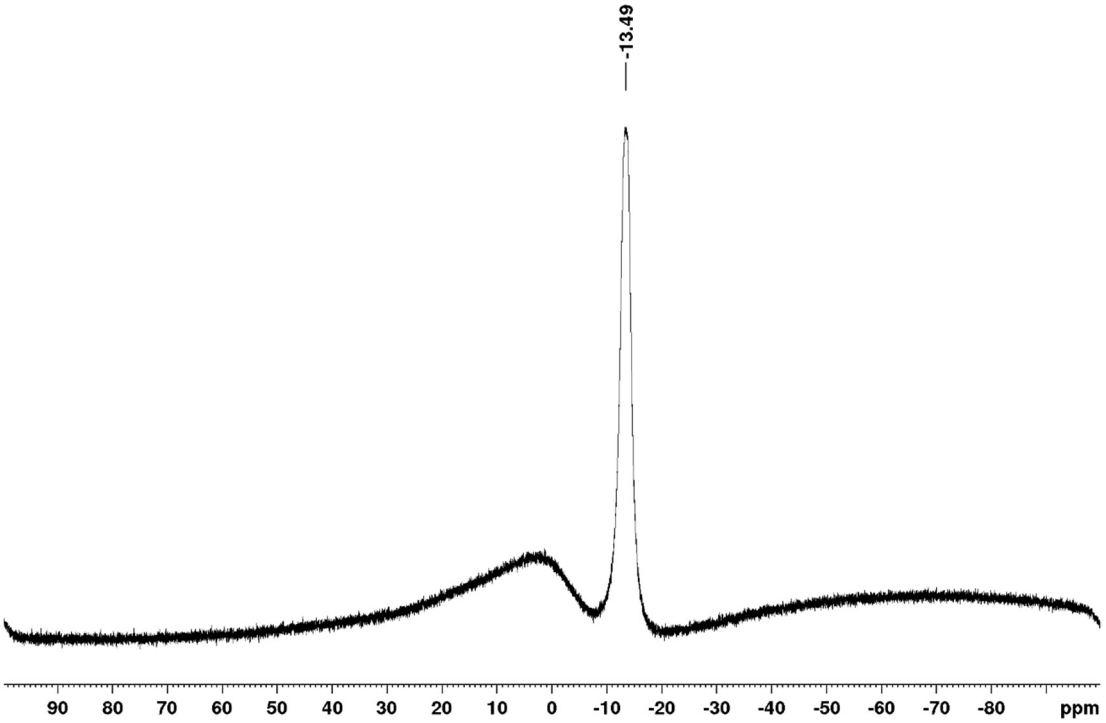
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

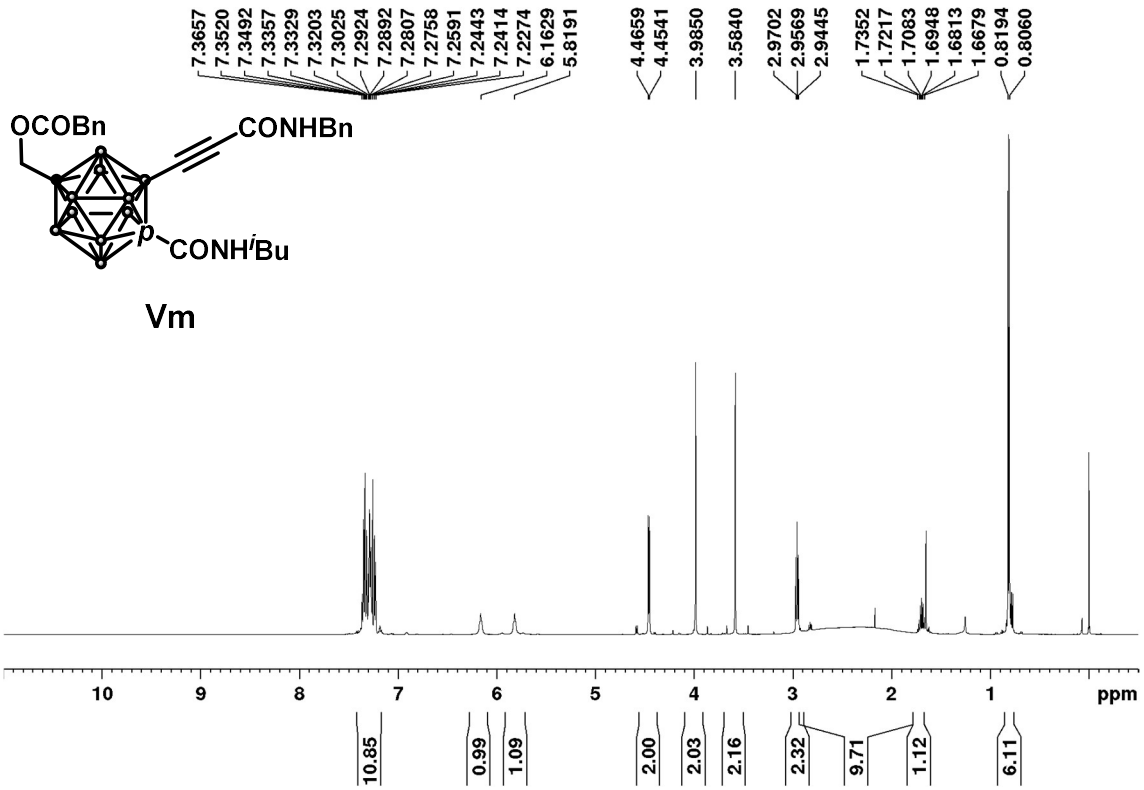


^{11}B NMR (160 MHz; $CDCl_3$)

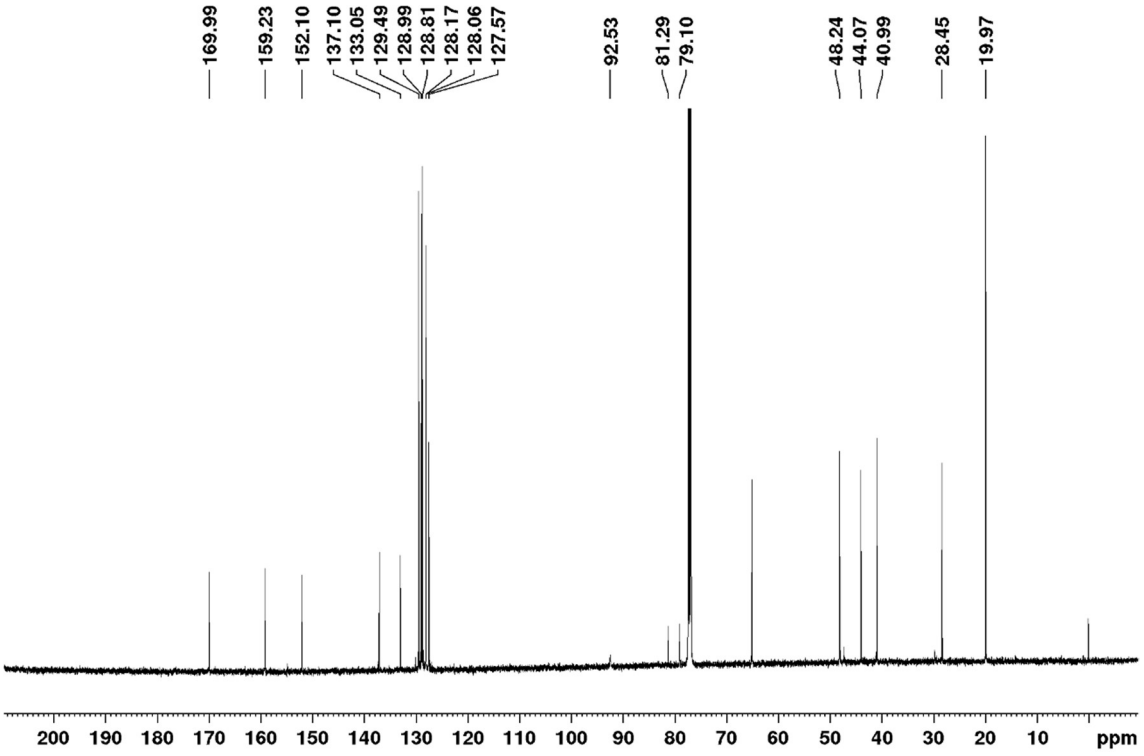


Synthesis of 12-phenylacetoxymethyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vm)

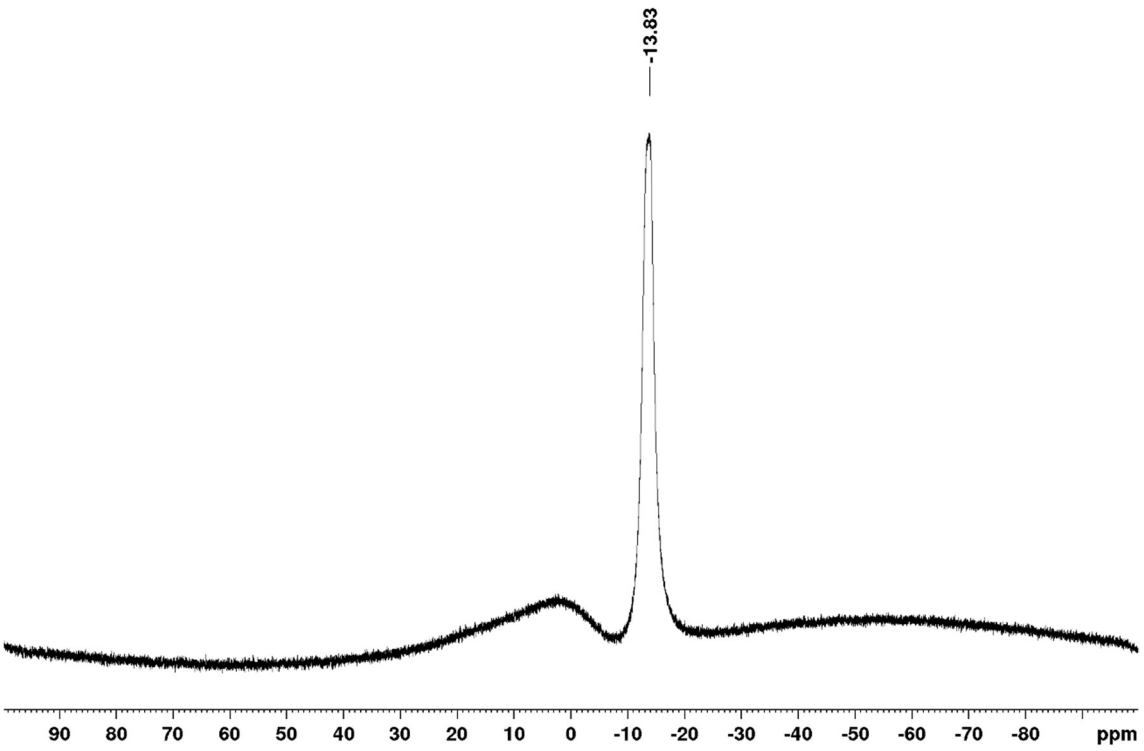
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

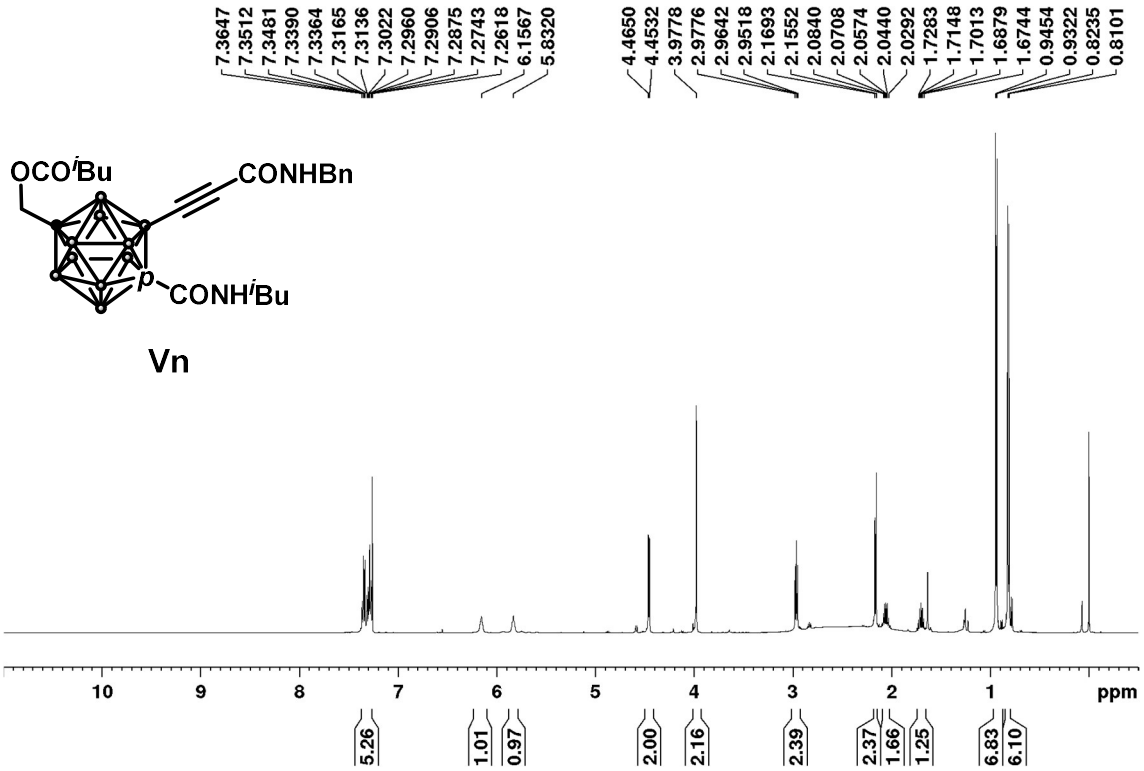


^{11}B NMR (160 MHz; $CDCl_3$)

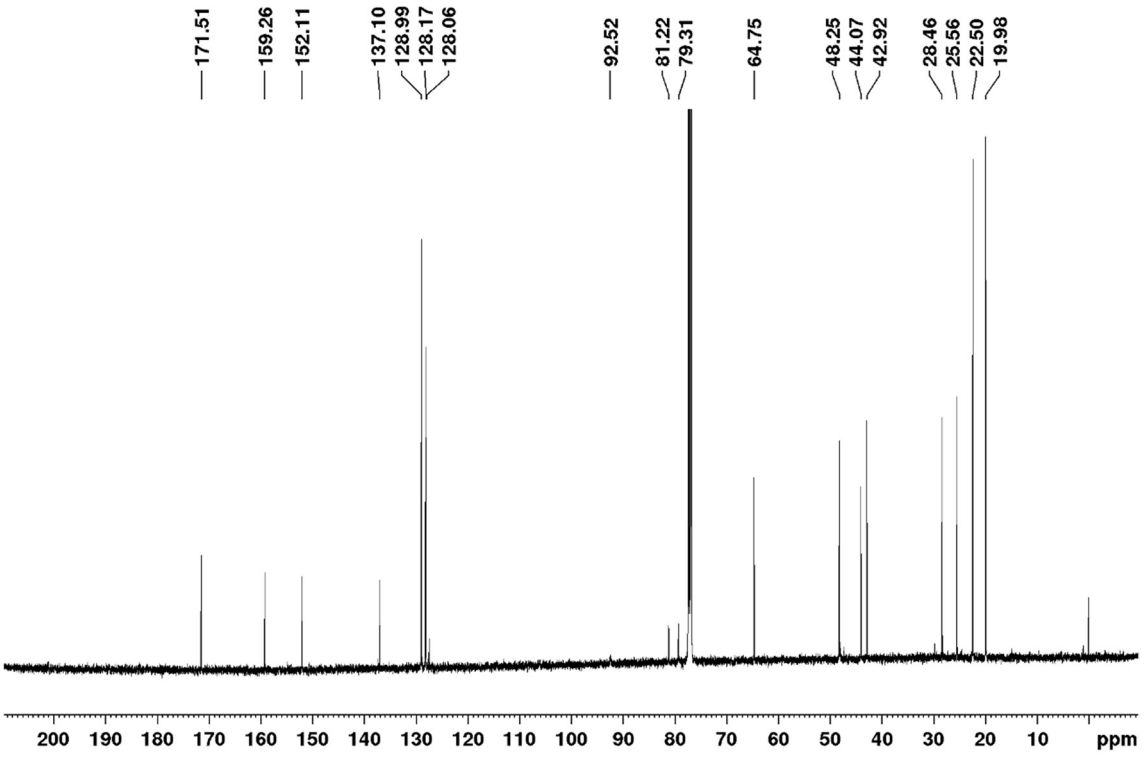


12-((3-methylbutanoyl)oxy)methyl-2-(3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vn)

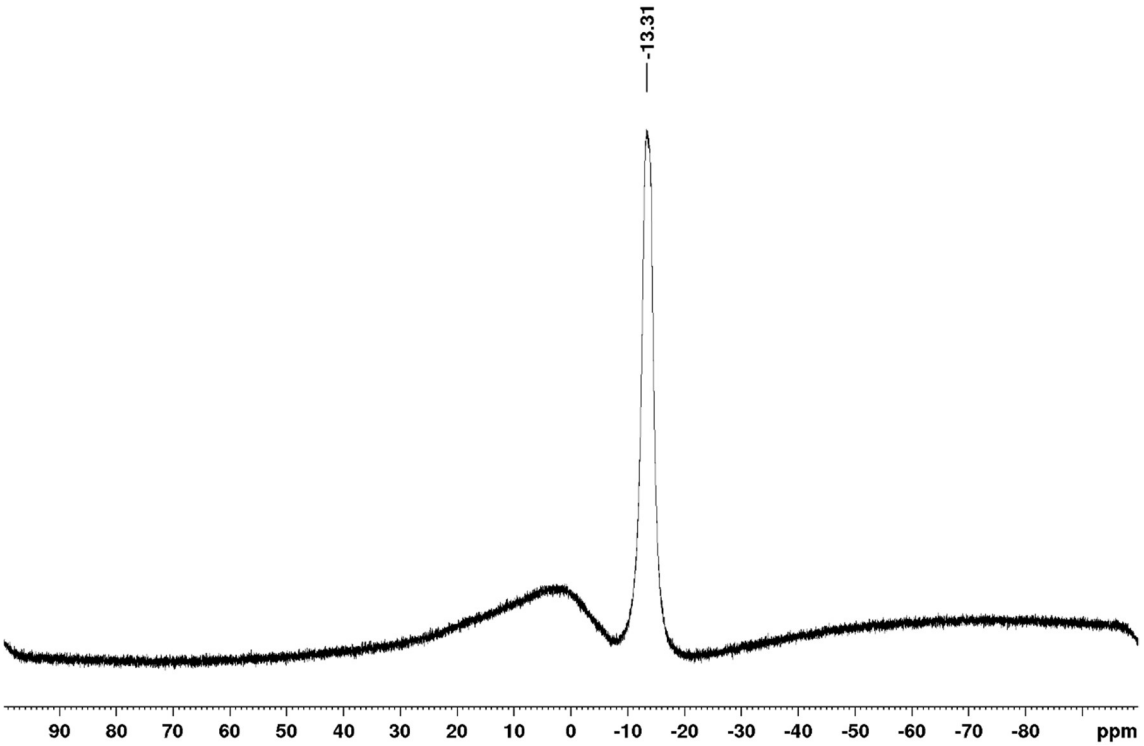
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

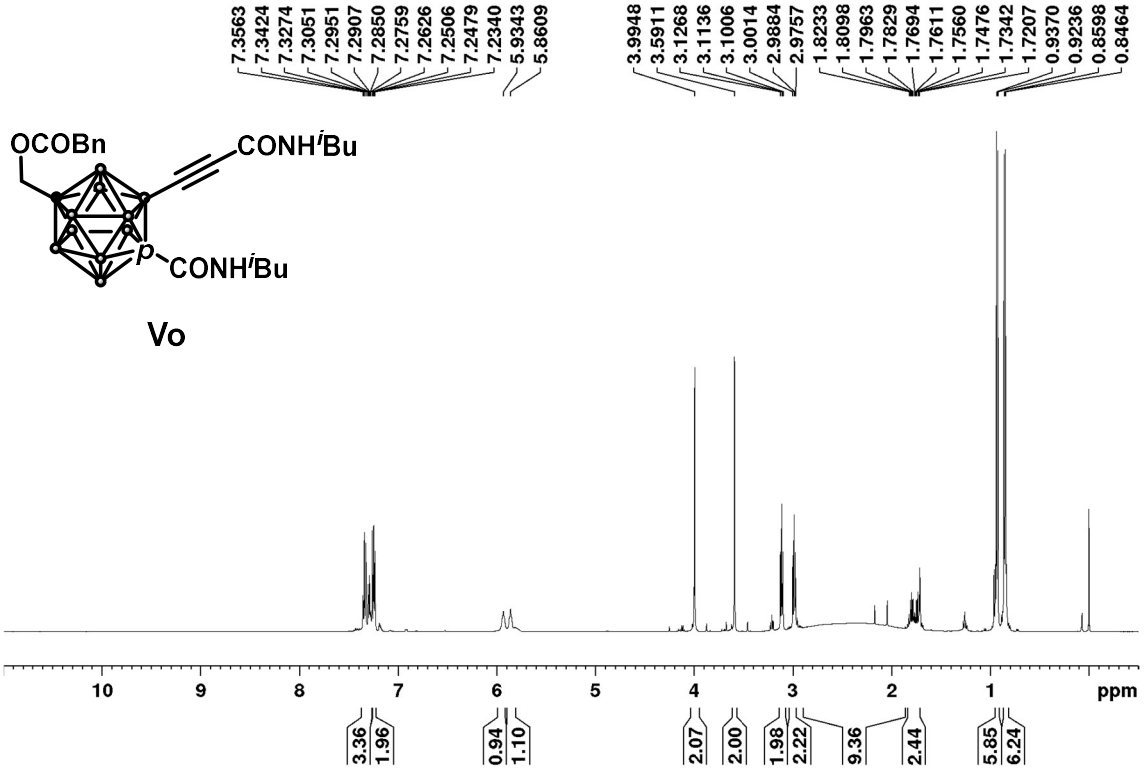


^{11}B NMR (160 MHz; $CDCl_3$)

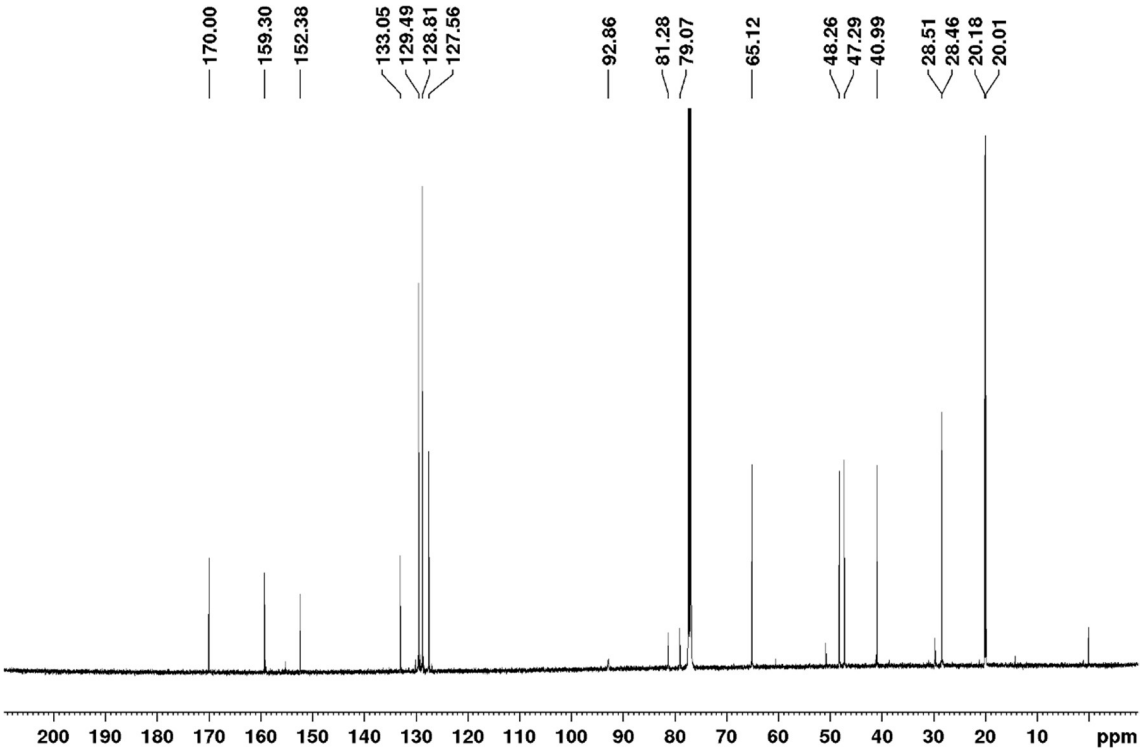


12-phenylacetoxymethyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vo)

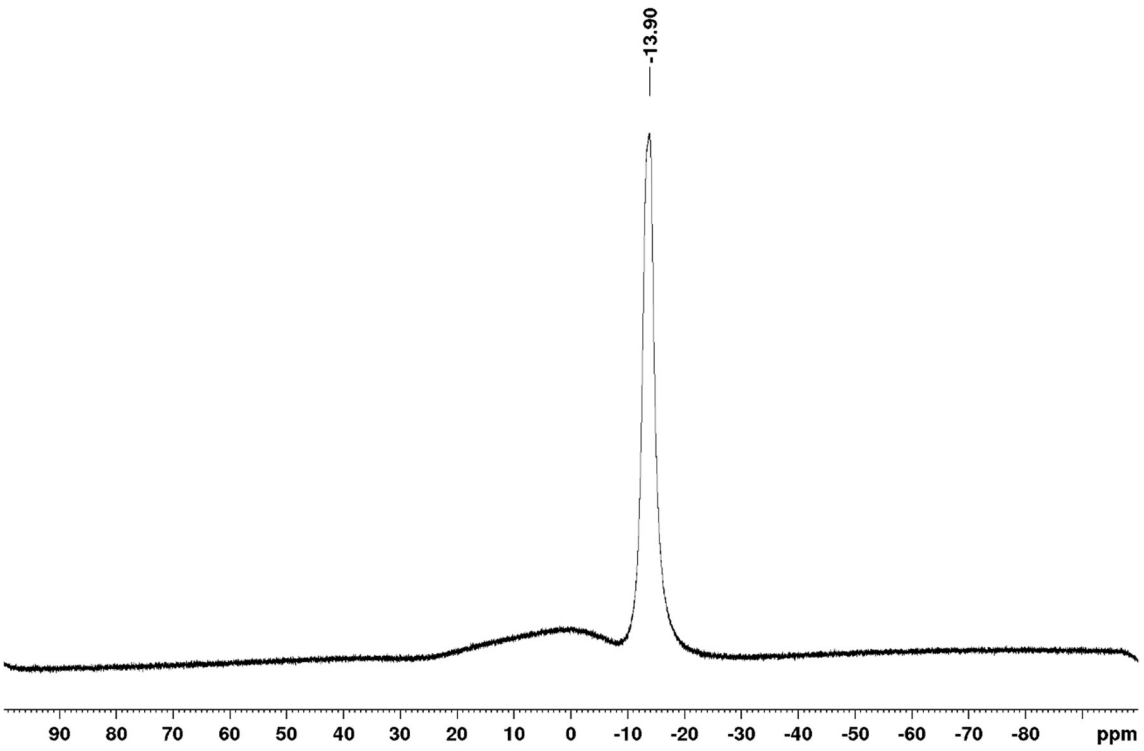
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

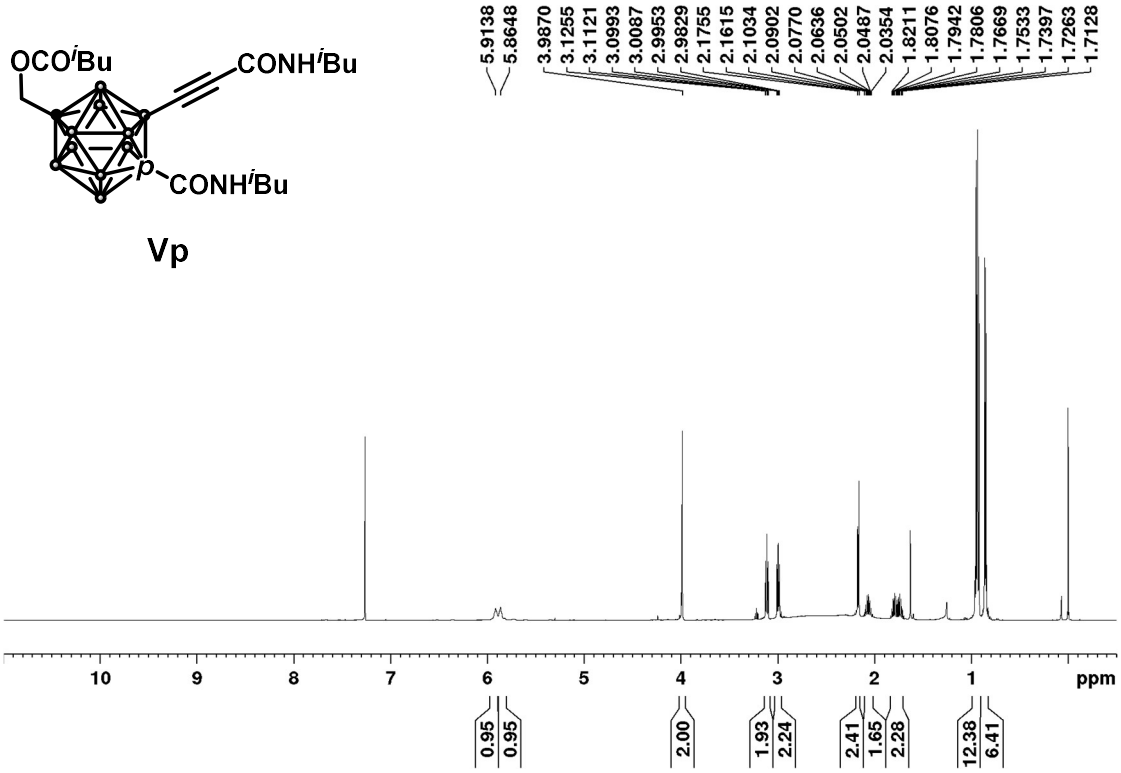


^{11}B NMR (160 MHz; $CDCl_3$)

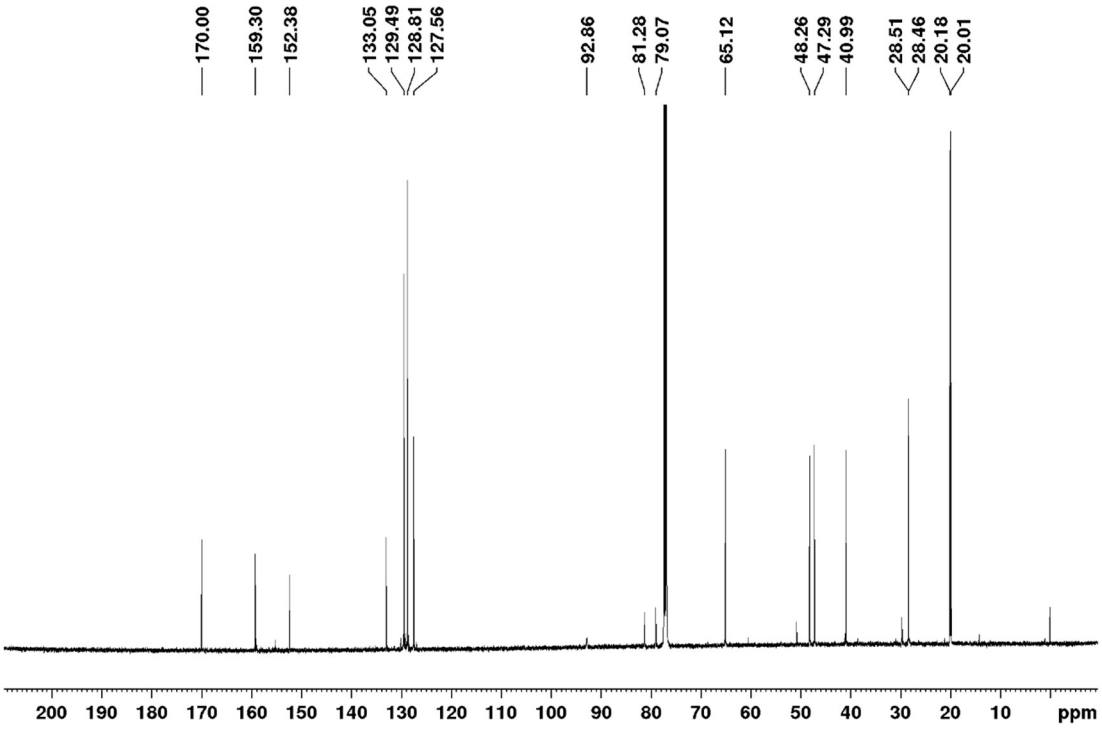


12-((3-methylbutanoyl)oxy)methyl-2-(3-(isobutylamino)-3-oxoprop-1-yn-1-yl)-1-isobutylcarbamoyl-1,12-dicarba-*closo*-dodecaborane (Vp)

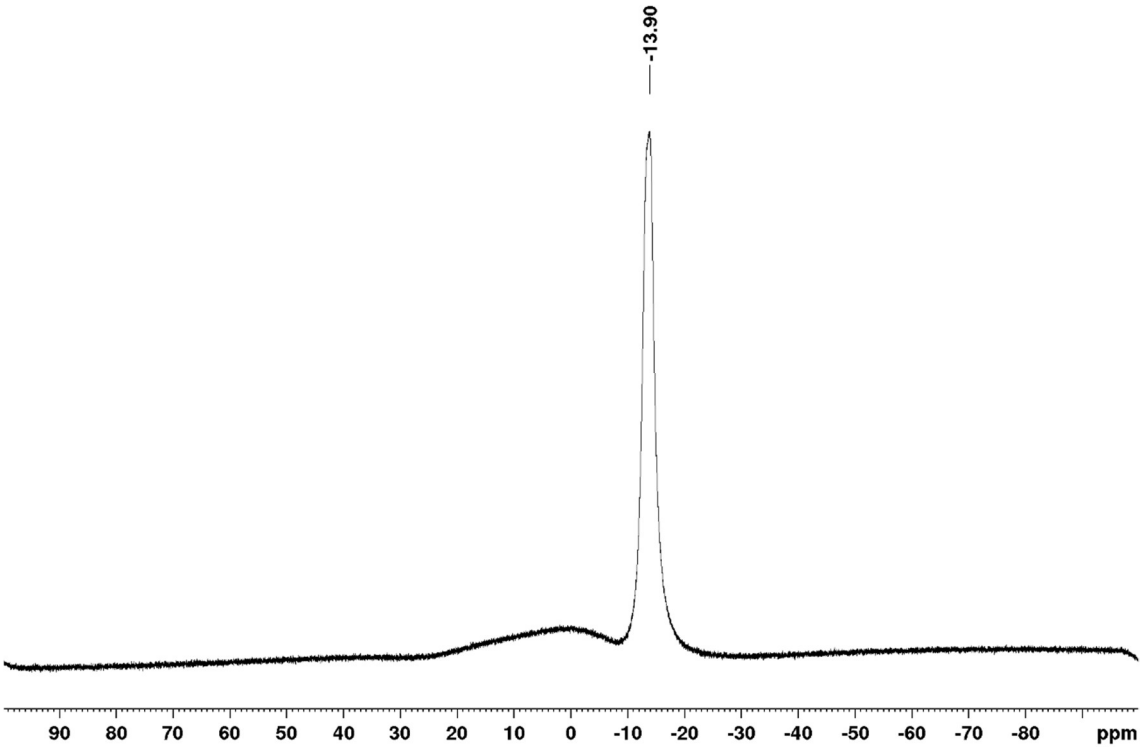
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

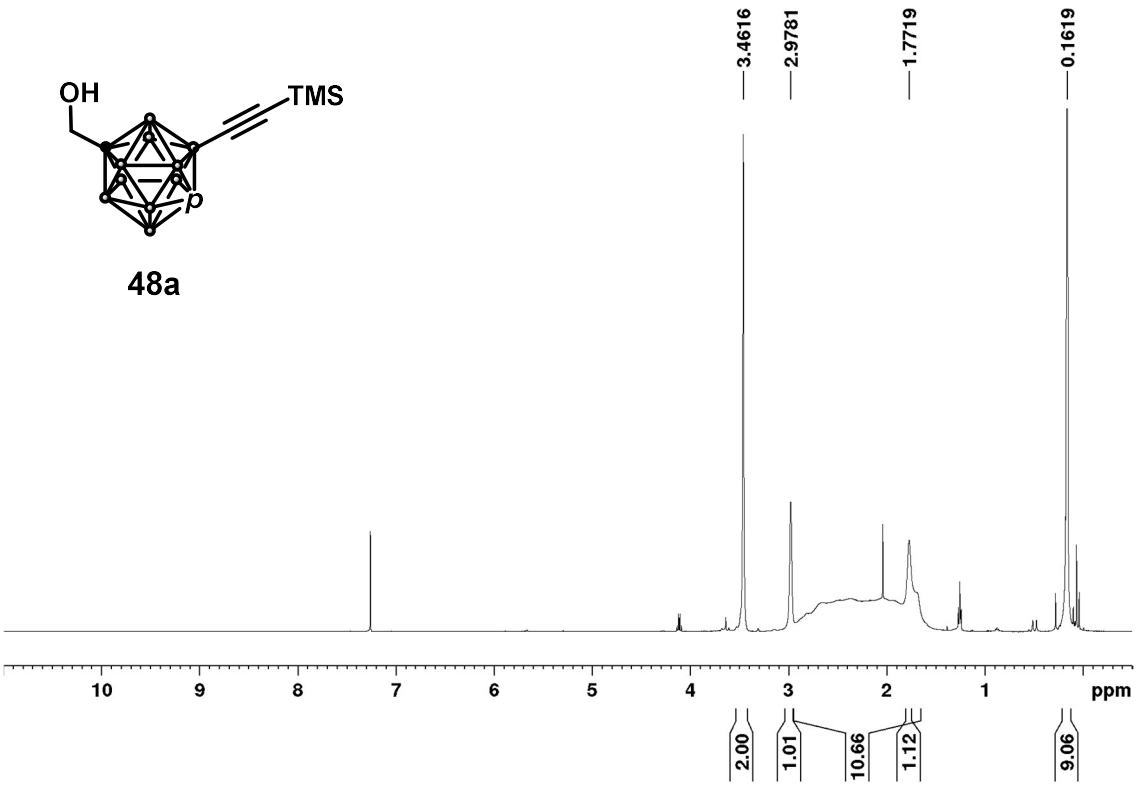


^{11}B NMR (160 MHz; $CDCl_3$)

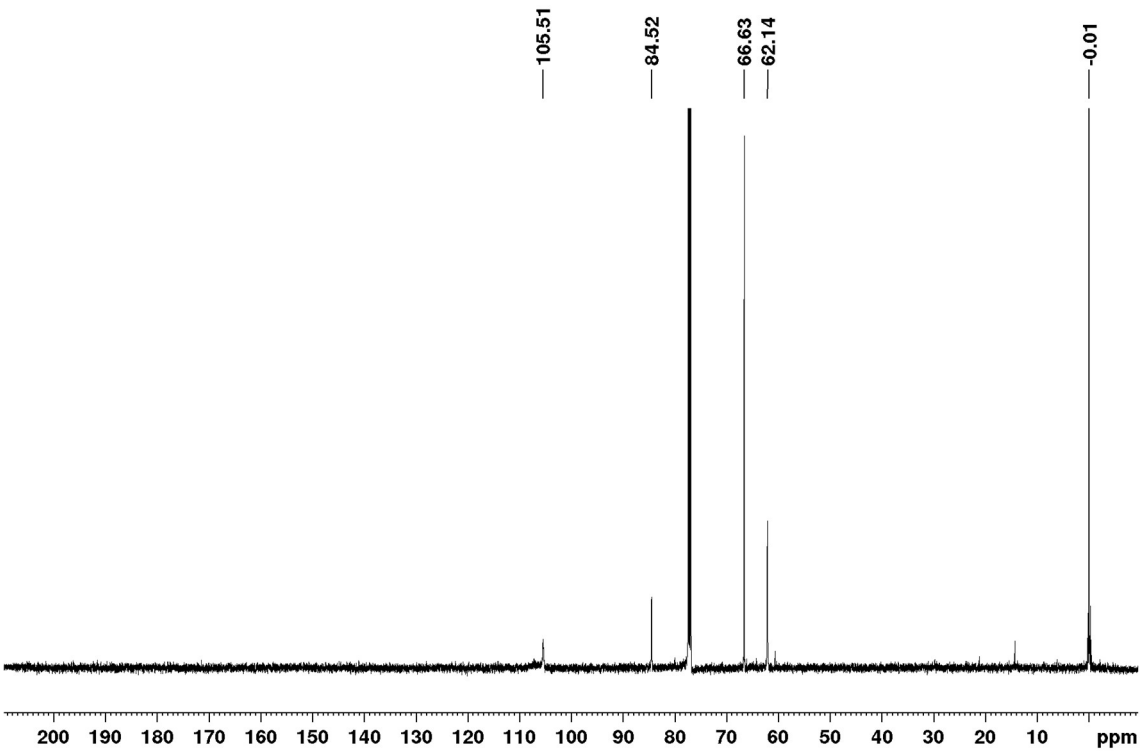


1-(hydroxymethyl)-7-((trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (48a)

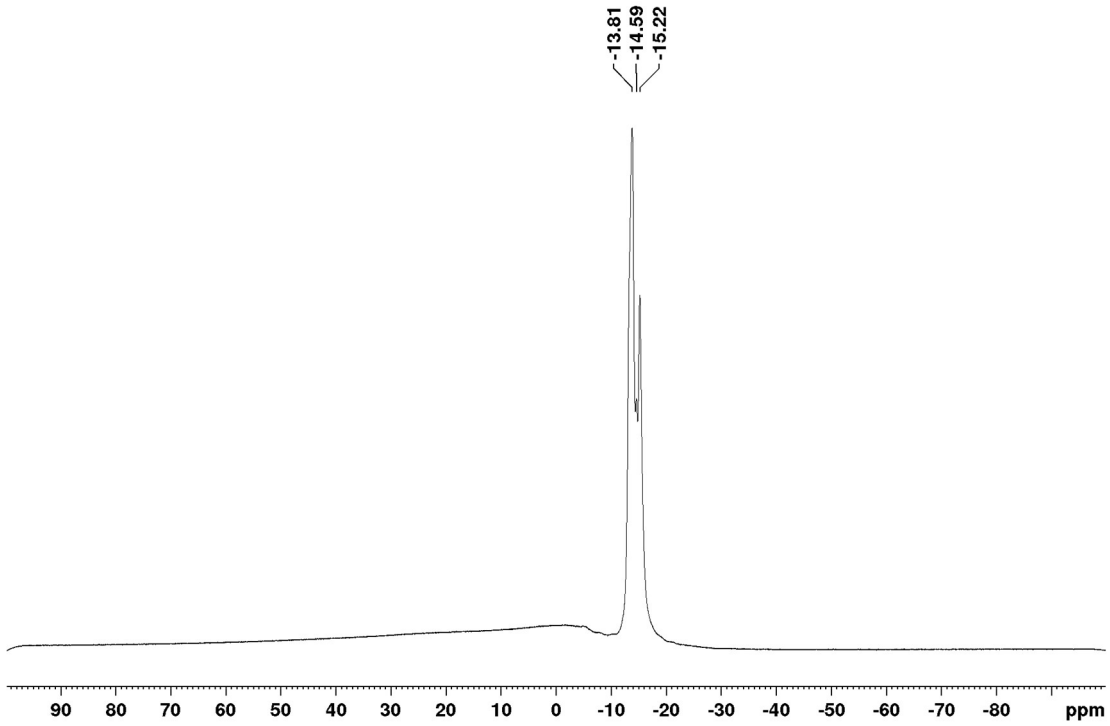
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

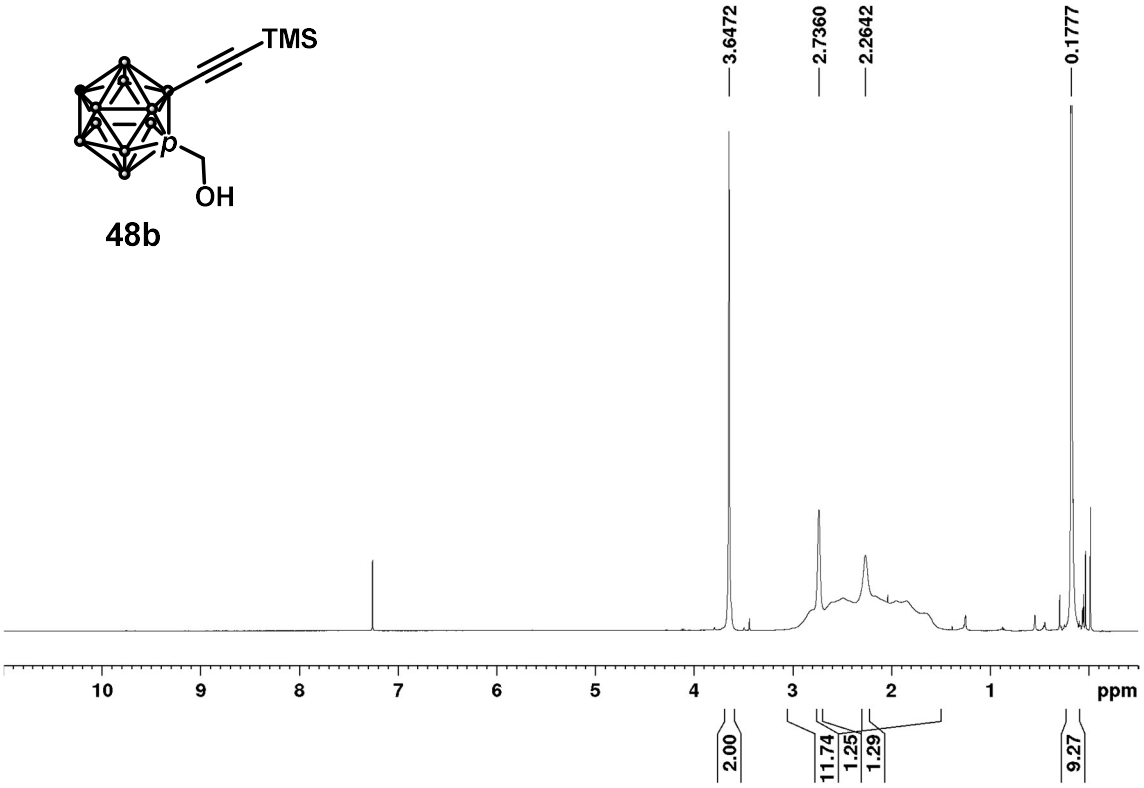


^{11}B NMR (160 MHz; $CDCl_3$)

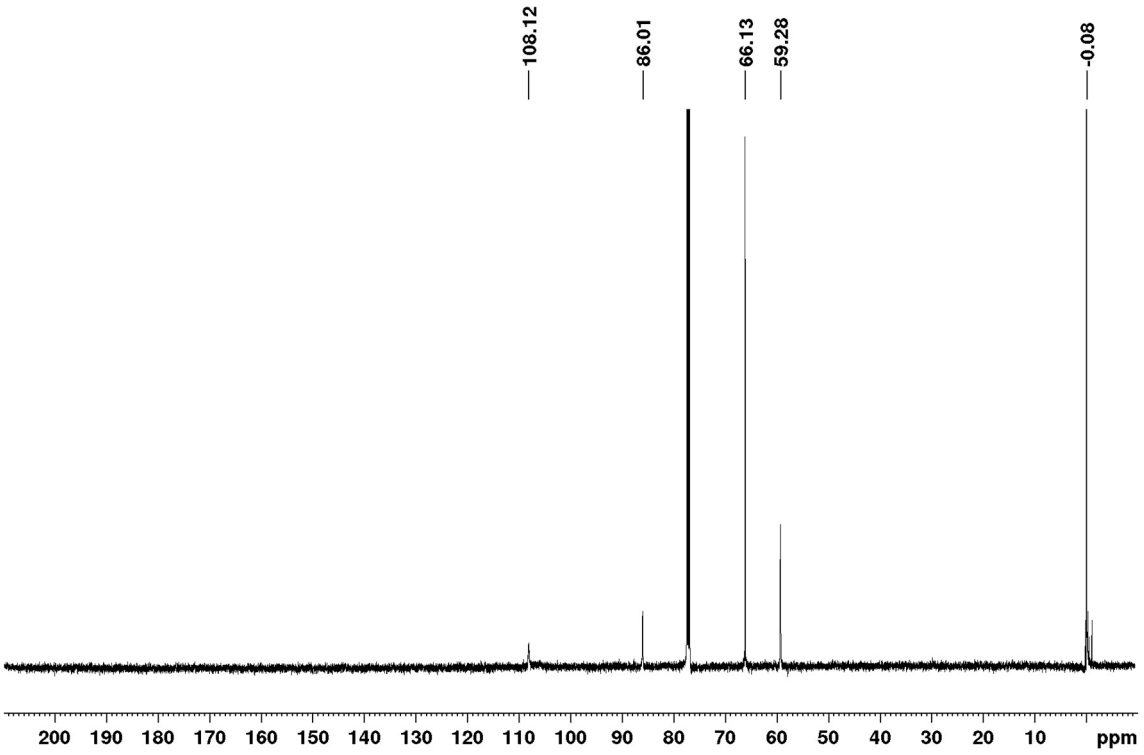


1-(hydroxymethyl)-2-((trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (48b)

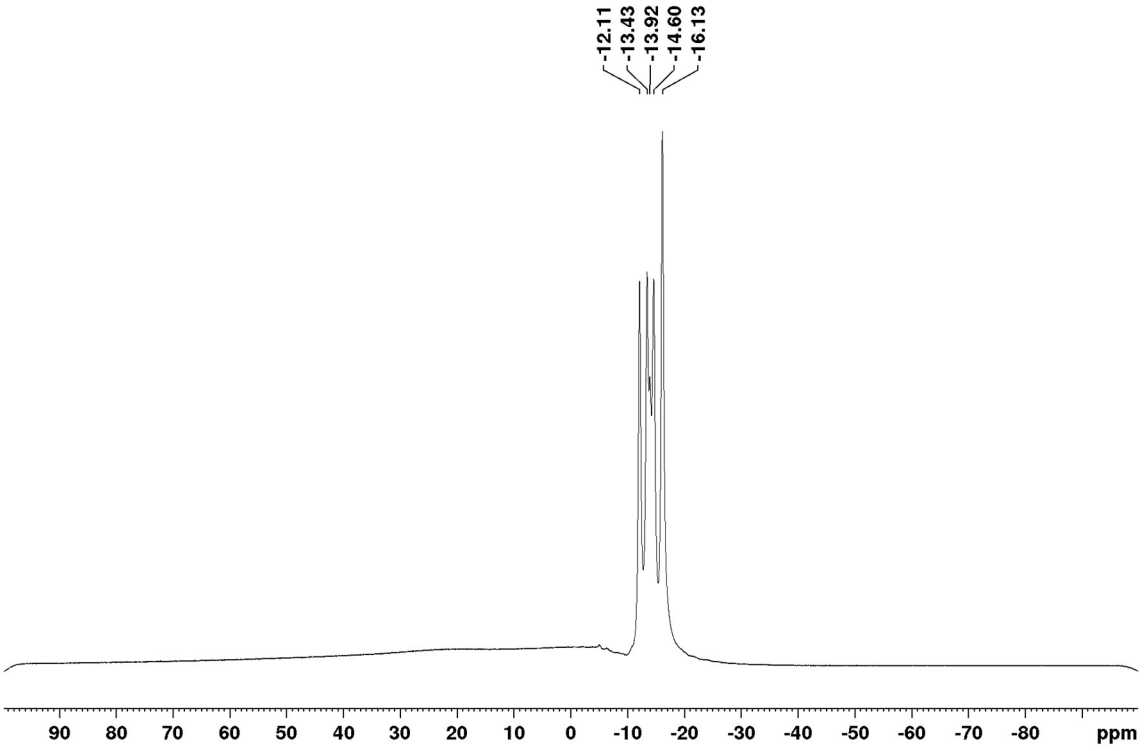
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)



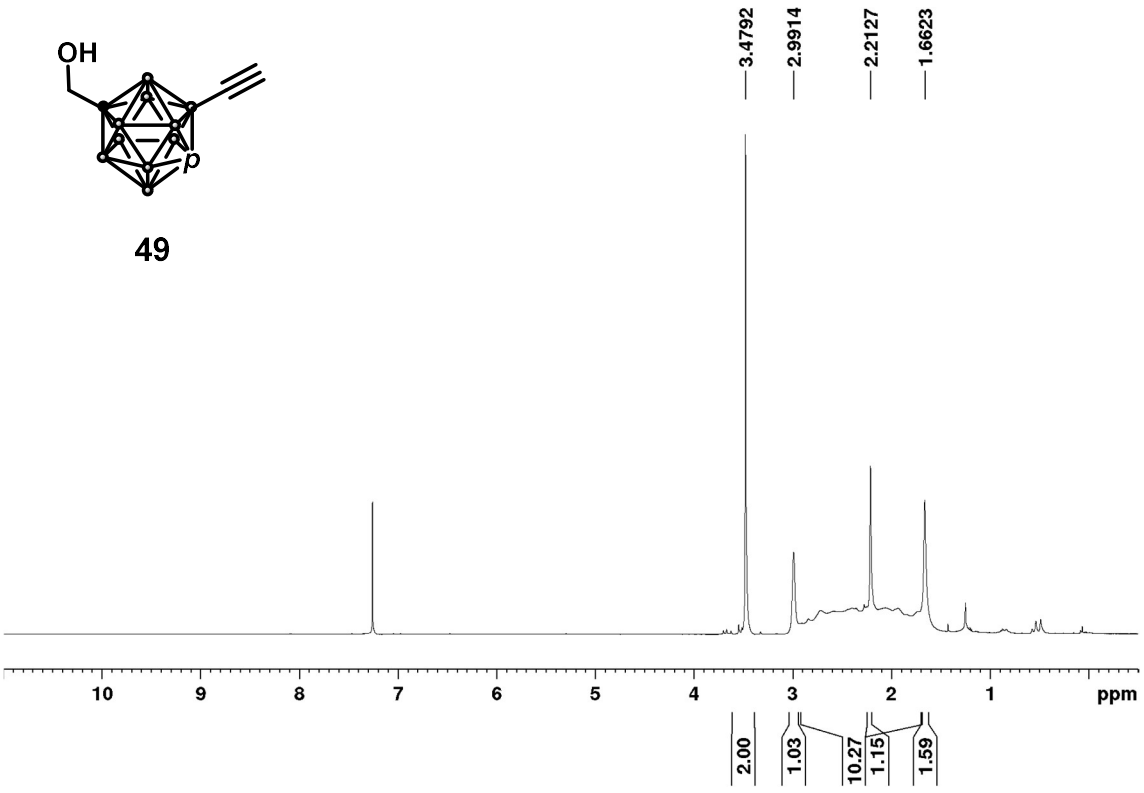
^{11}B NMR (160 MHz; $CDCl_3$)



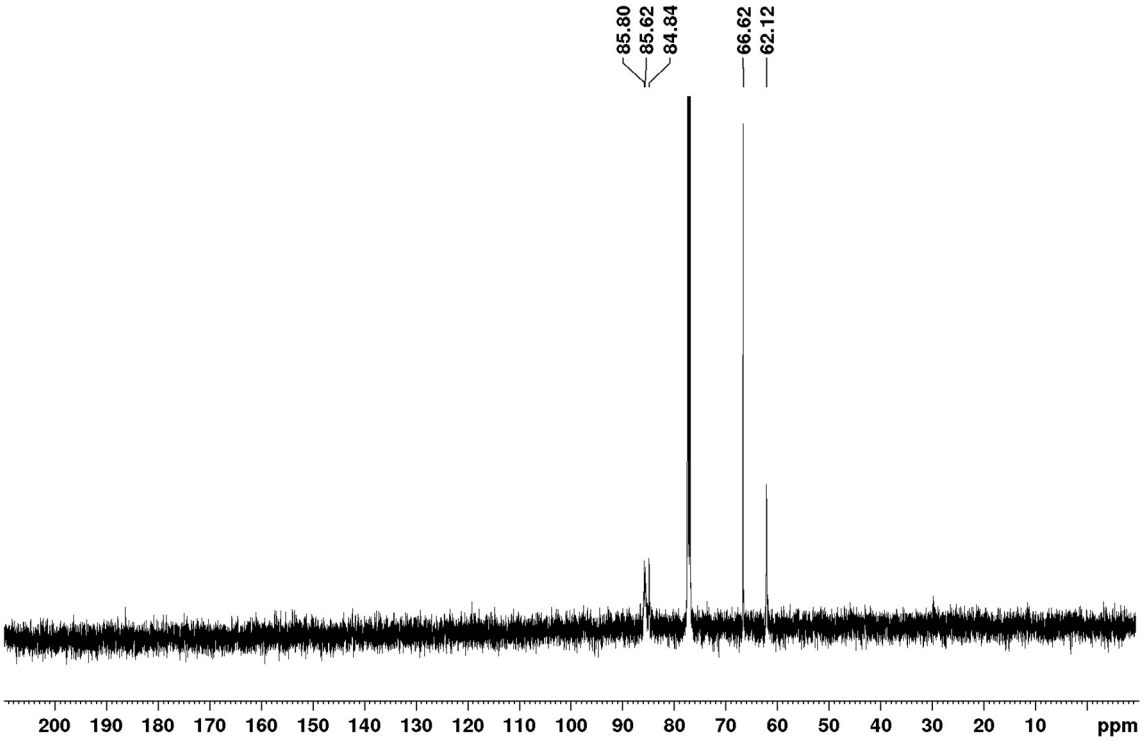
1-(hydroxymethyl)-7-ethynyl-1,12-dicarba-*closo*-dodecaborane (49)

^{1}H NMR (500 MHz; $CDCl_3$)

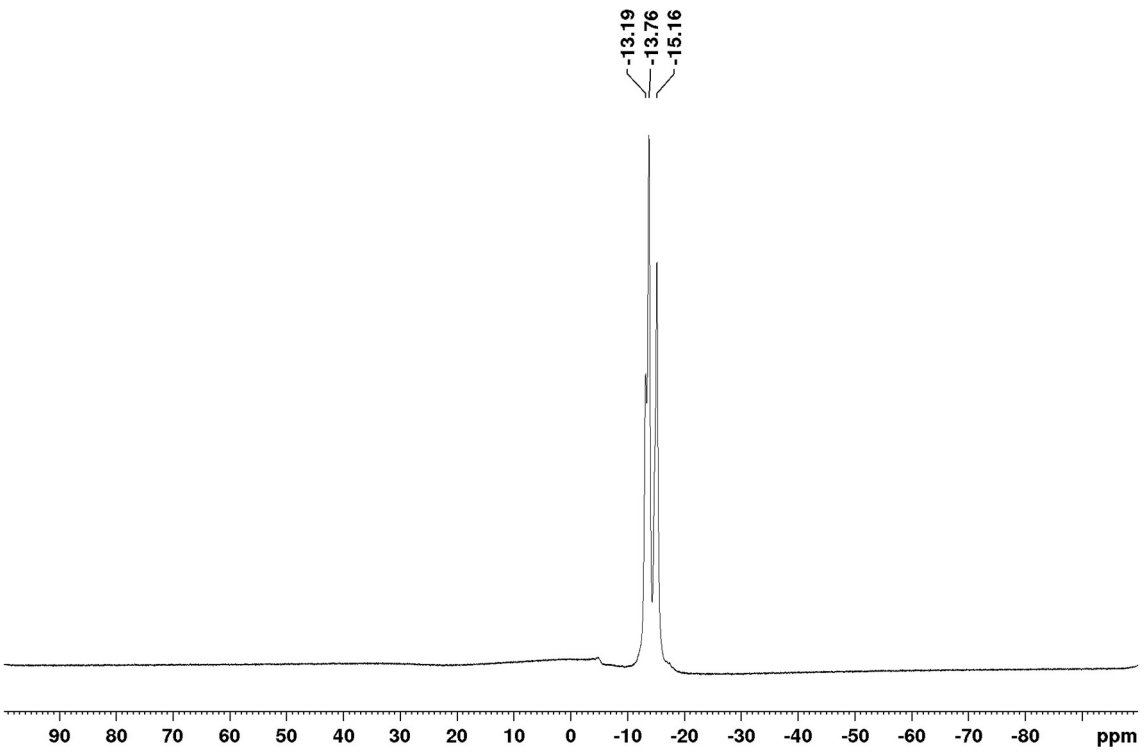
49



^{13}C NMR (125 MHz; $CDCl_3$)

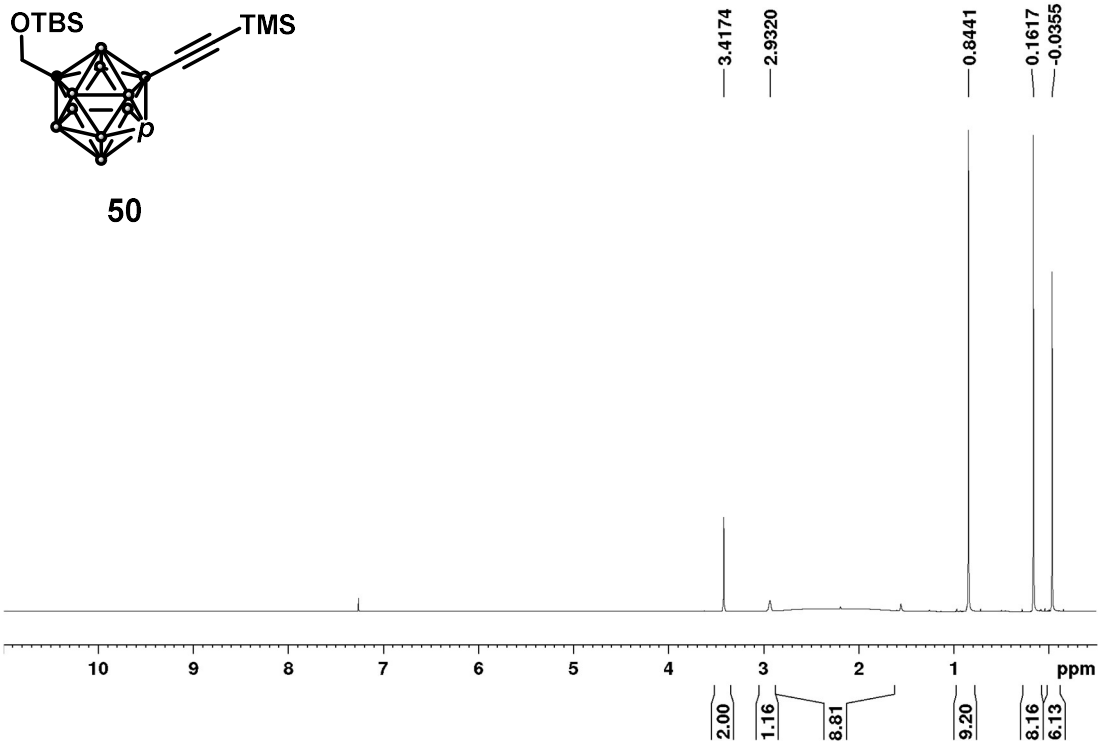


^{11}B NMR (160 MHz; $CDCl_3$)

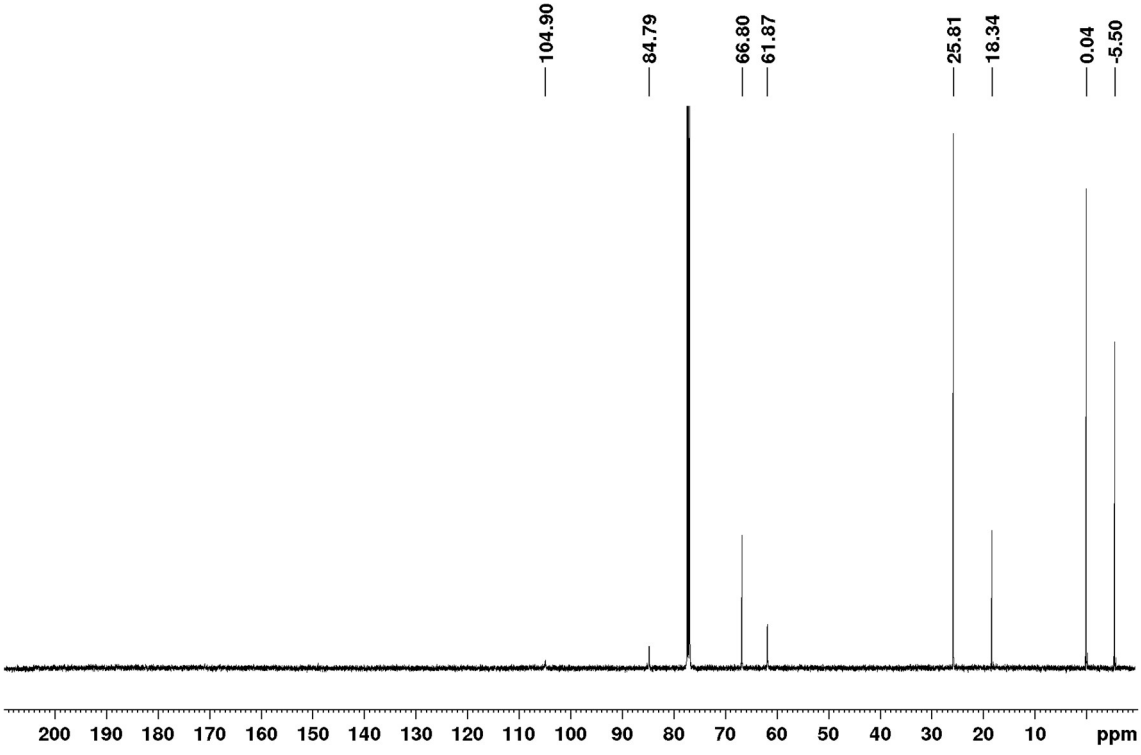


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-((trimethylsilyl)ethynyl)-1,12-dicarba-*closo*-dodecaborane (50)

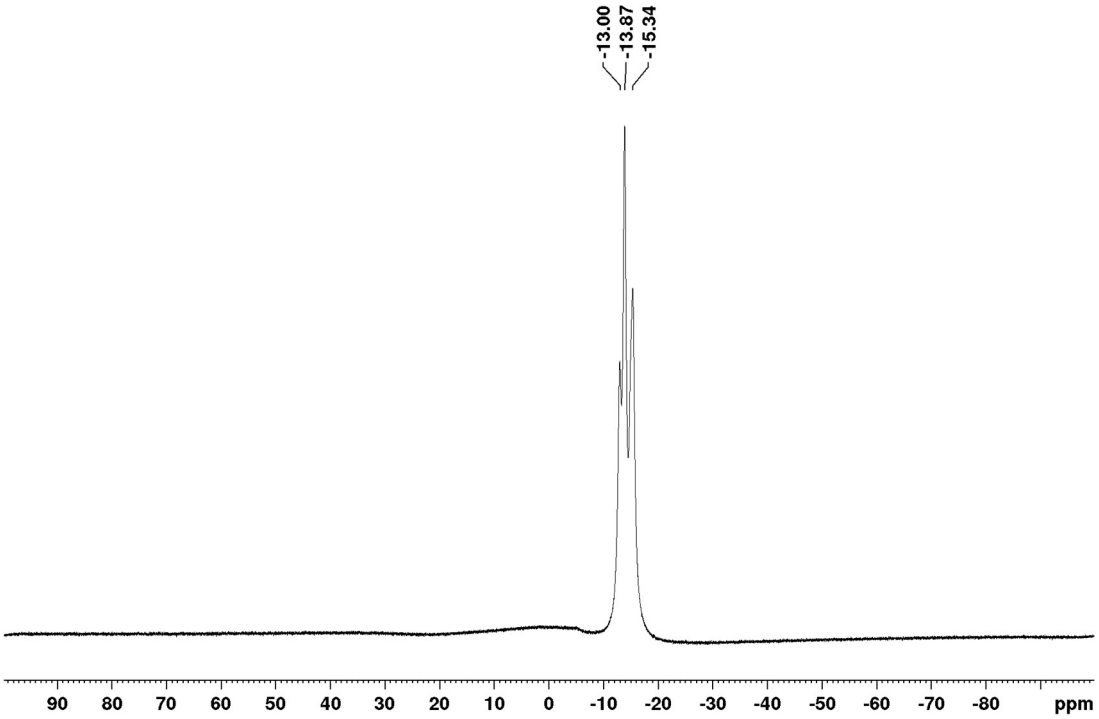
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

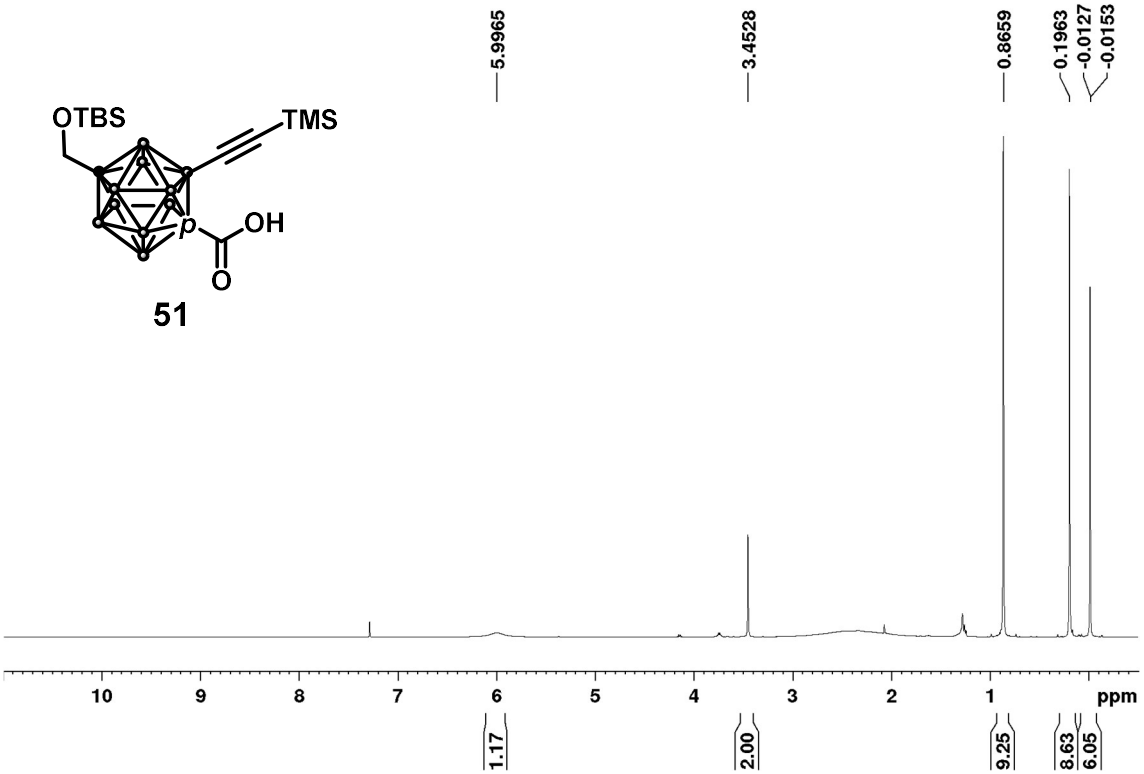


^{11}B NMR (160 MHz; $CDCl_3$)

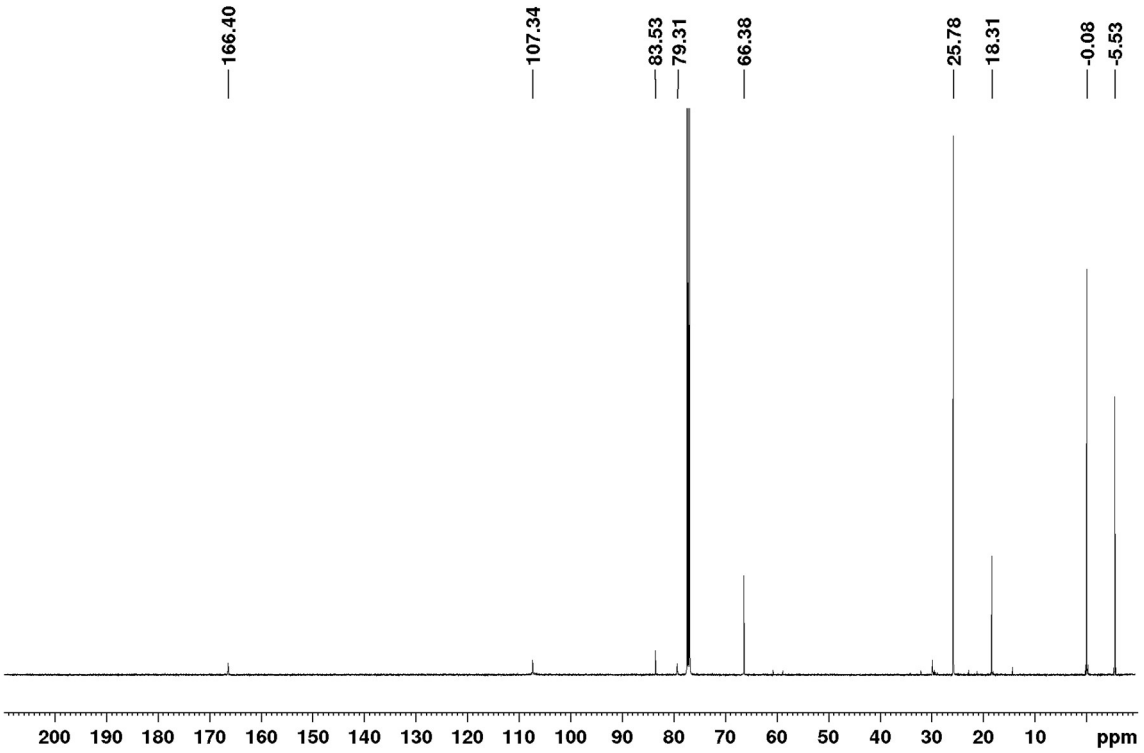


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-2-((trimethylsilyl)ethynyl)-12-carboxyl-1,12-dicarba-*closo*-dodecaborane (51)

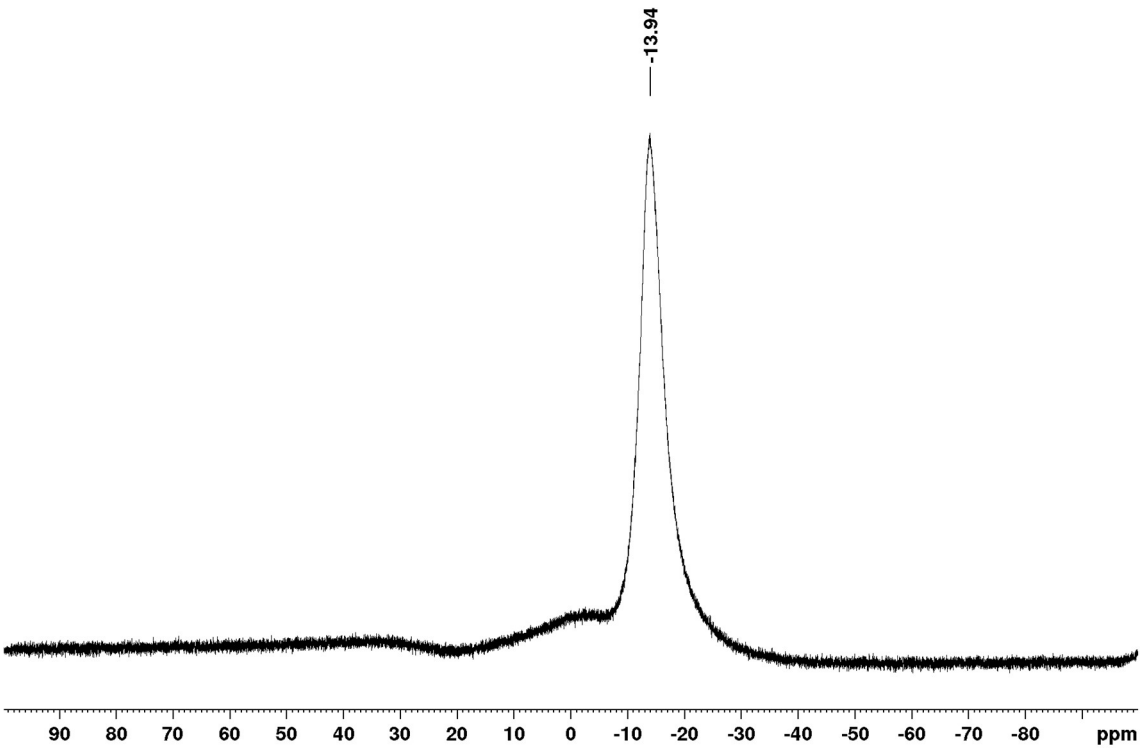
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

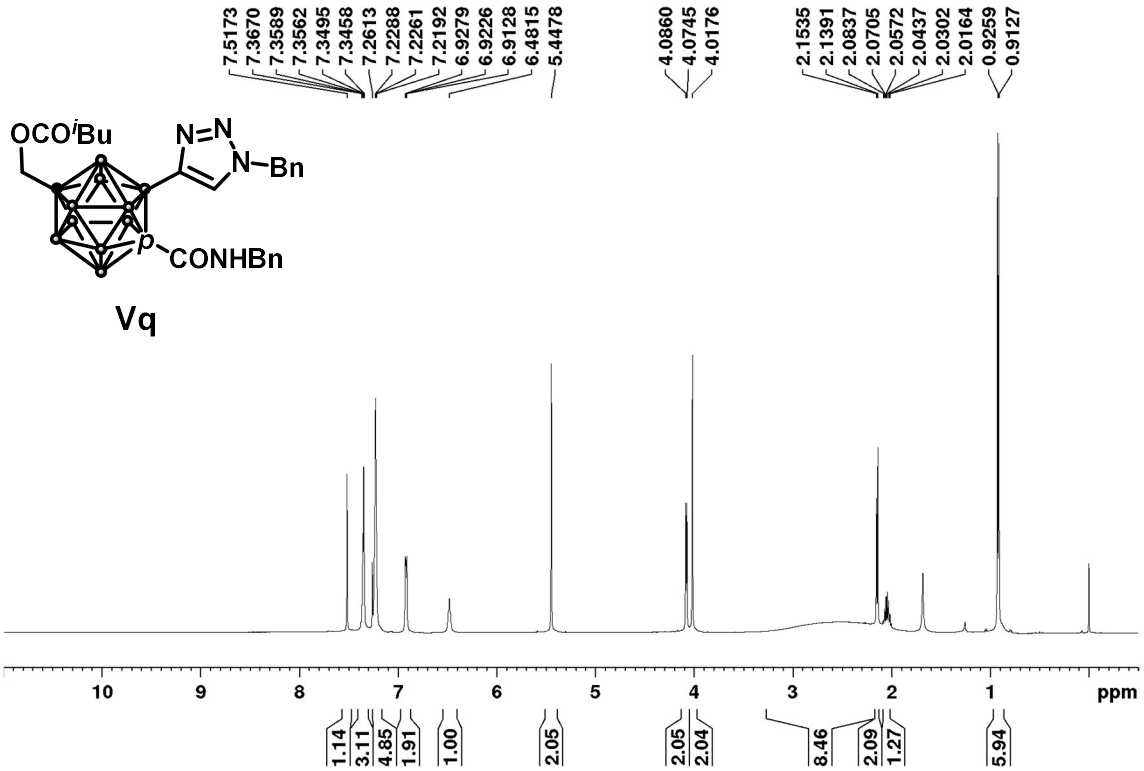


^{11}B NMR (160 MHz; $CDCl_3$)

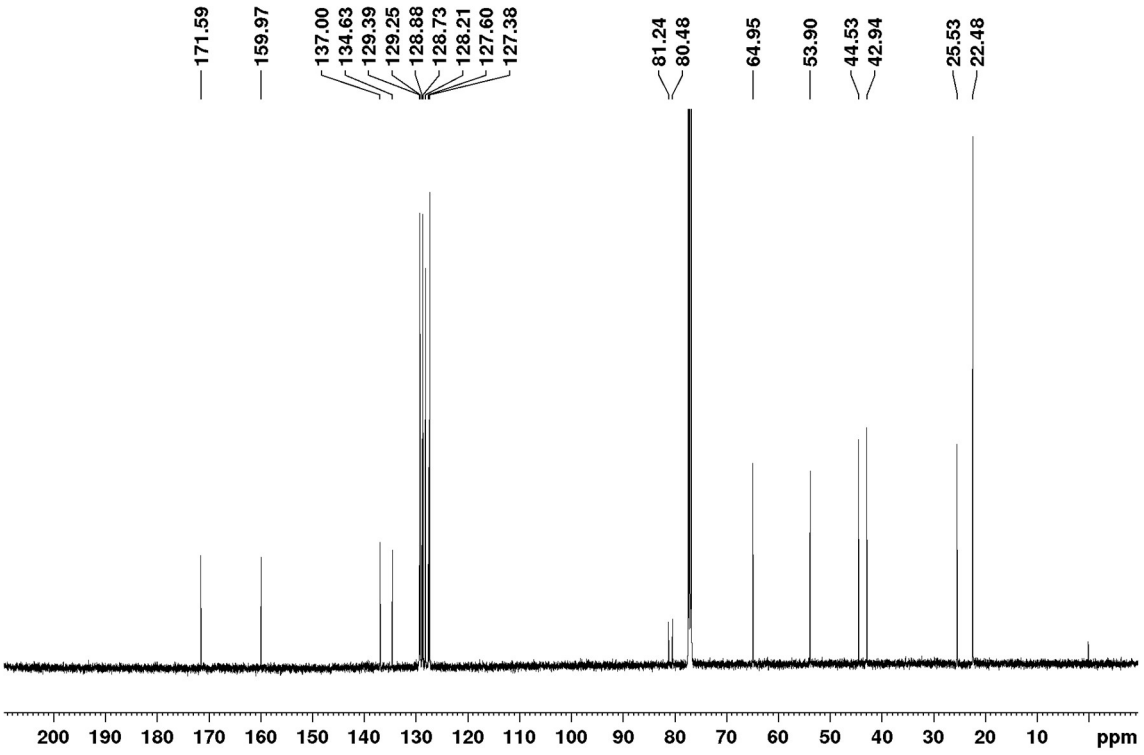


1-(3-methylbutanoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-12-benzylaminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vq)

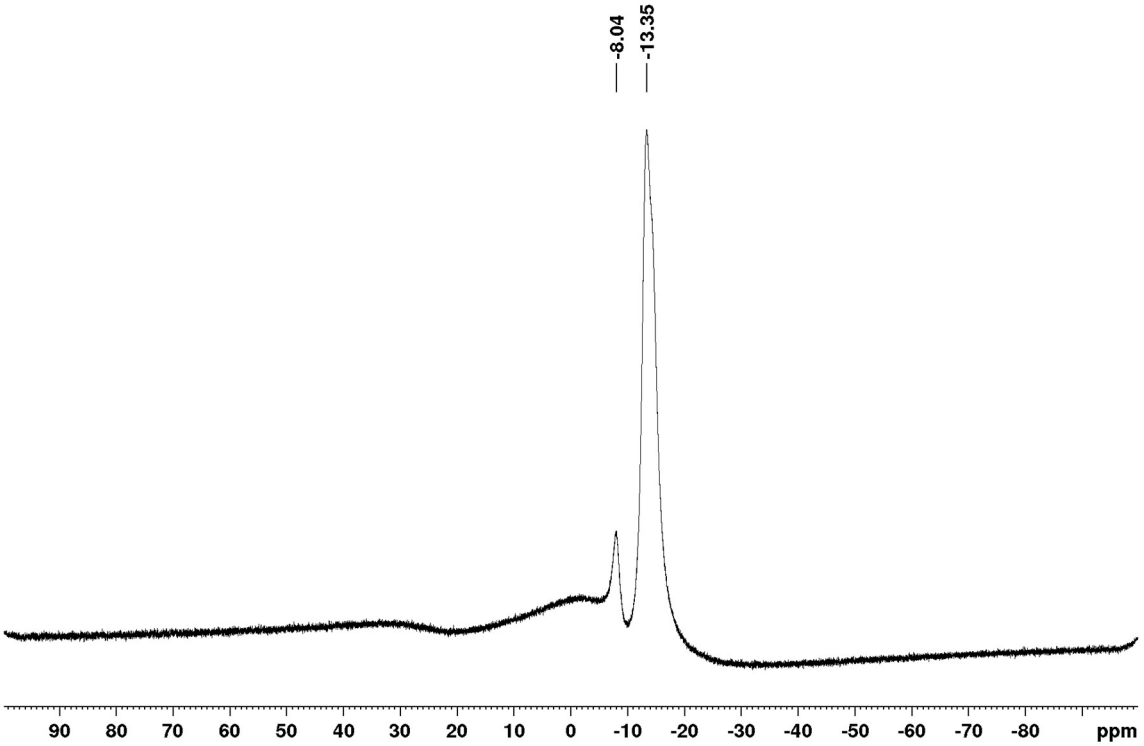
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

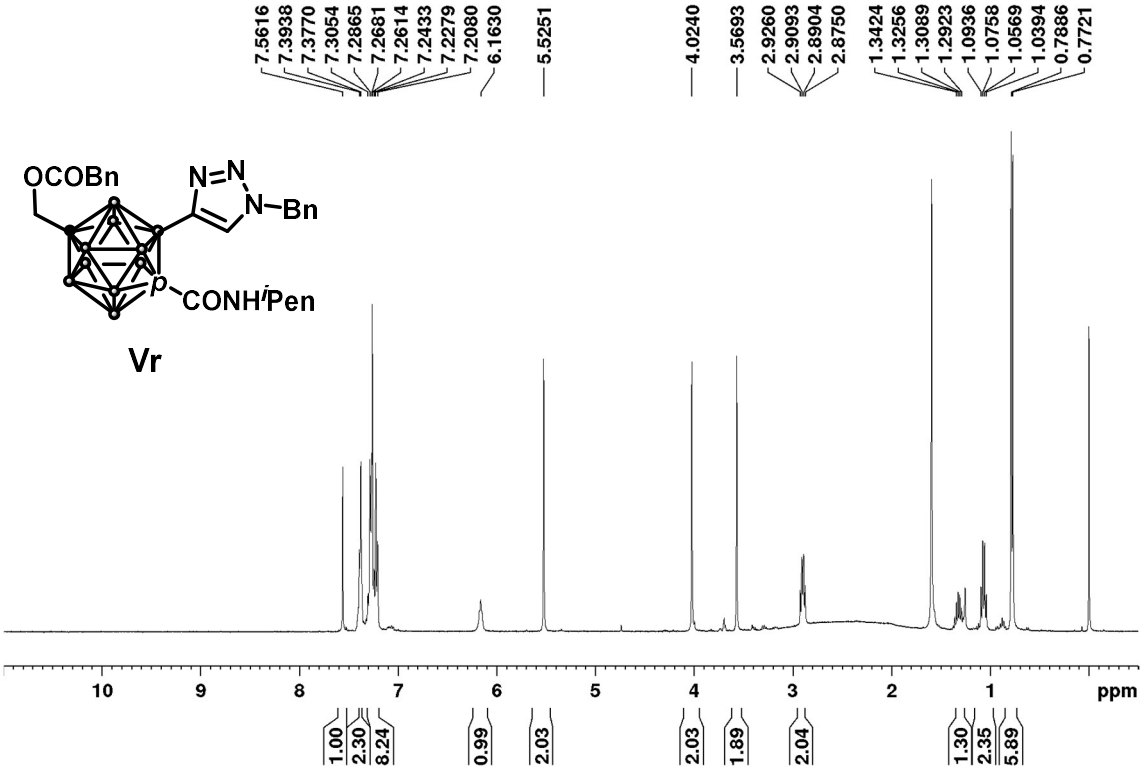


^{11}B NMR (160 MHz; $CDCl_3$)

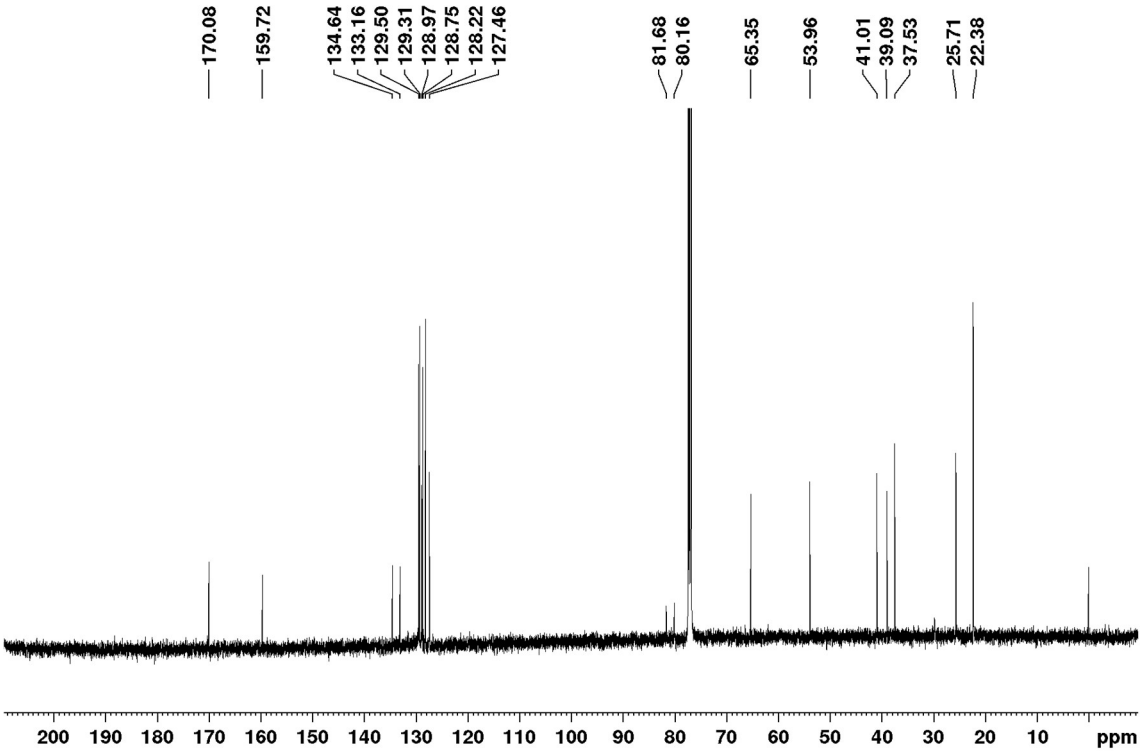


1-(phenylacetoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-12-(3-methylbutyl)aminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vr)

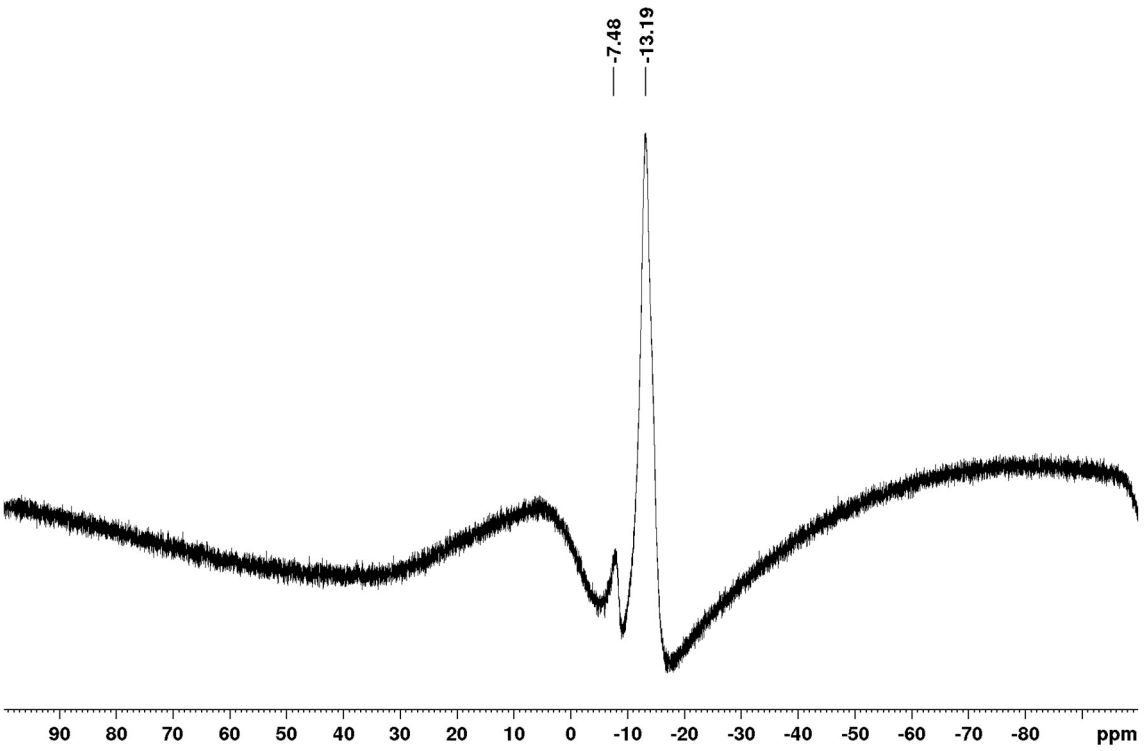
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

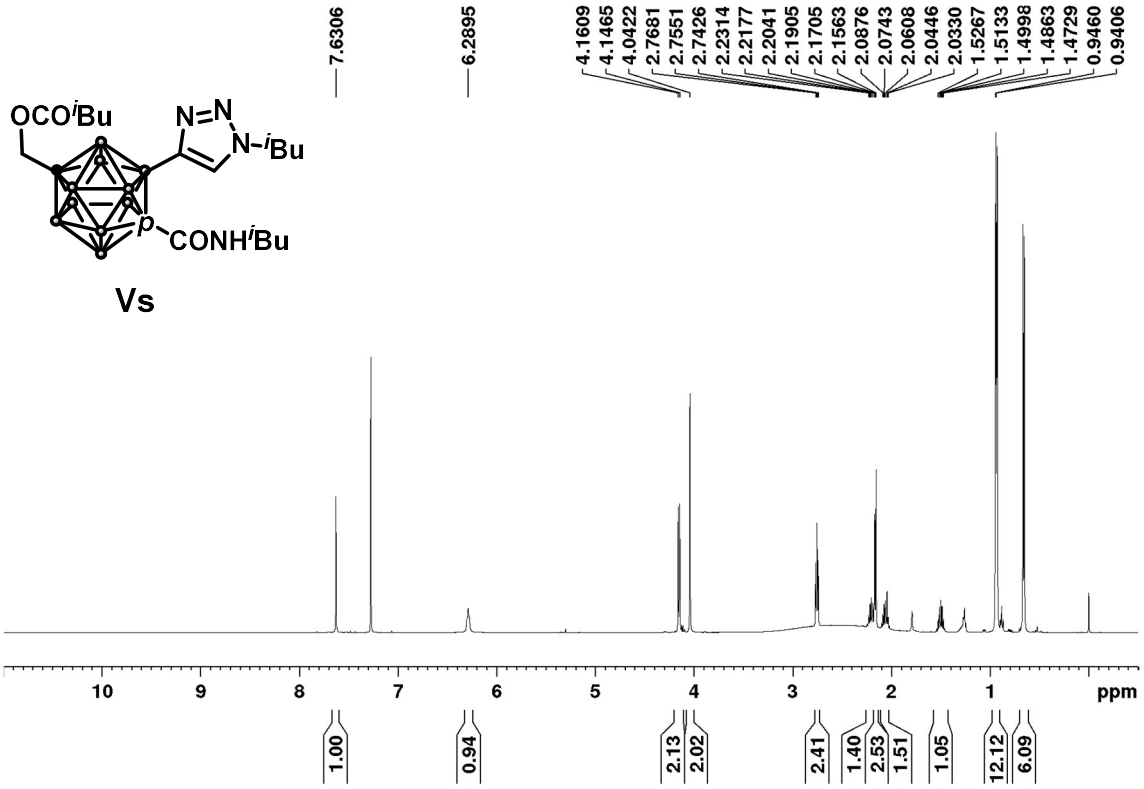


^{11}B NMR (160 MHz; $CDCl_3$)

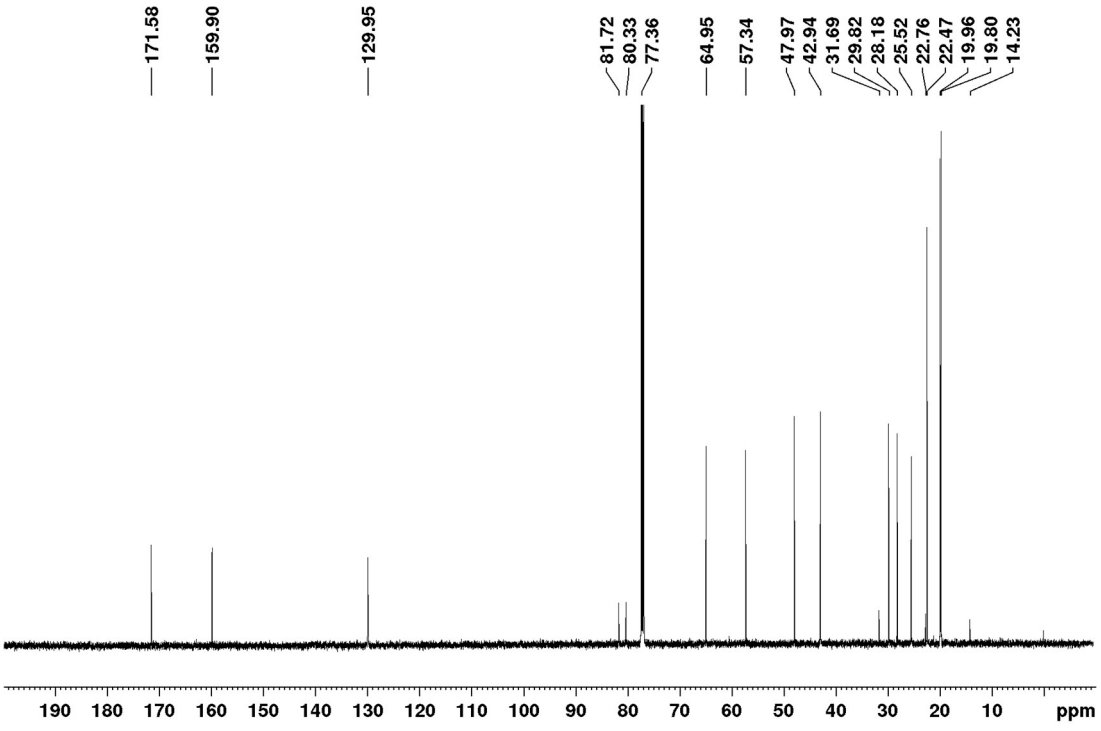


1-(3-methylbutanoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-12-(3-methylbutyl)aminocarboxyl-1,12-dicarba-*closo*-dodecaborane (Vs)

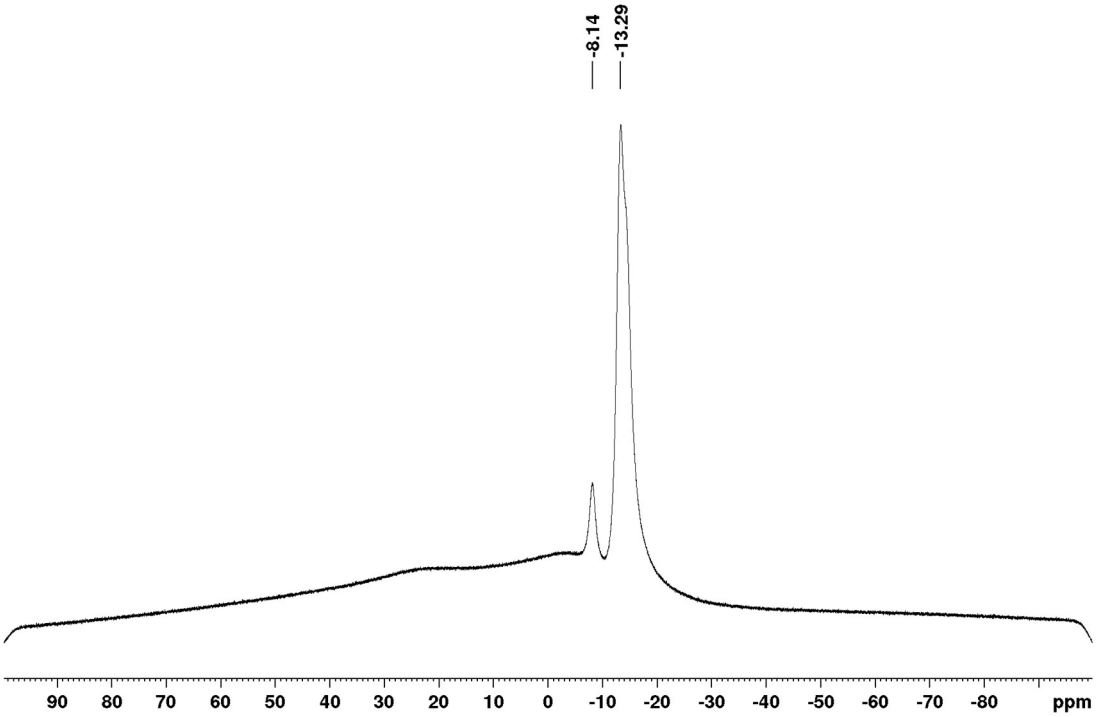
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

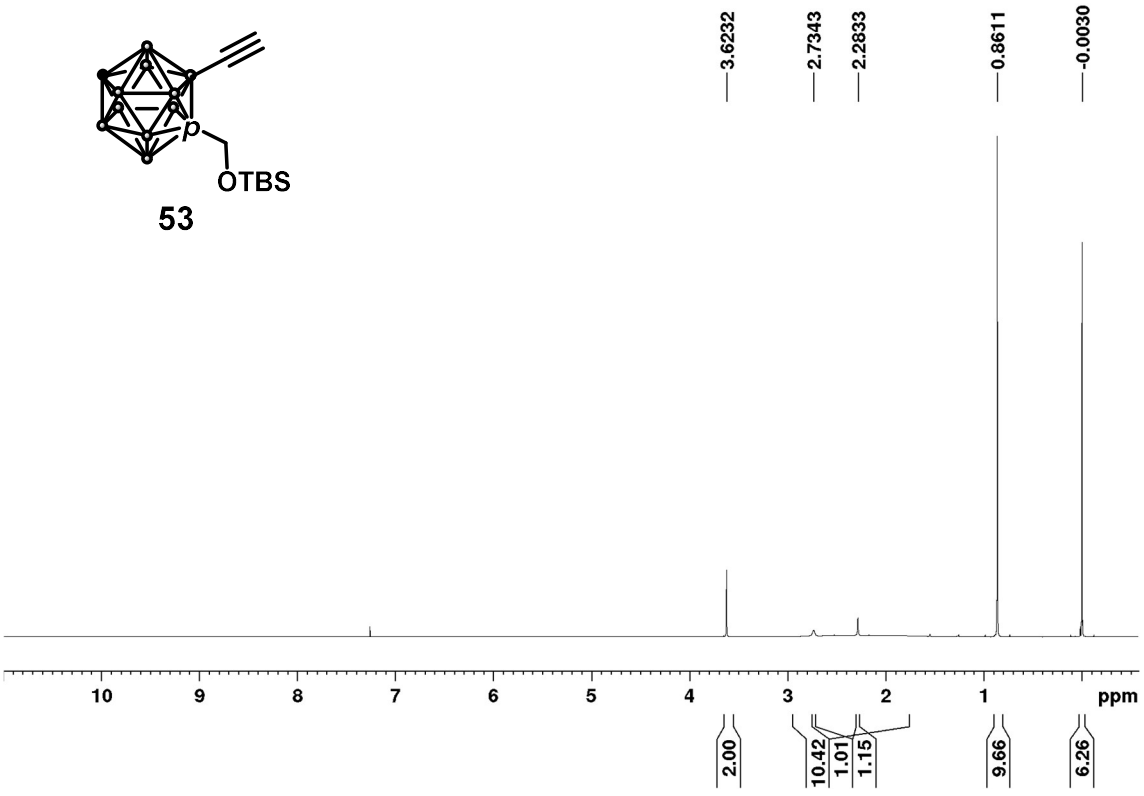


^{11}B NMR (160 MHz; $CDCl_3$)

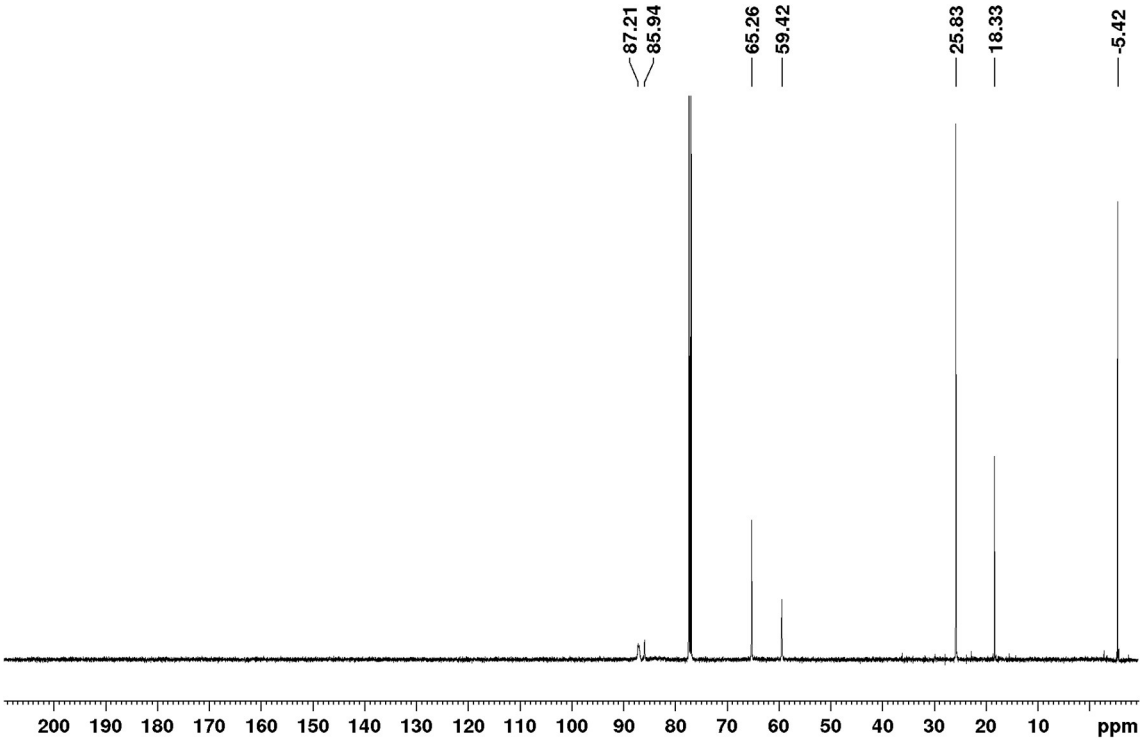


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-ethynyl-1,12-dicarba-*closo*-dodecaborane (53)

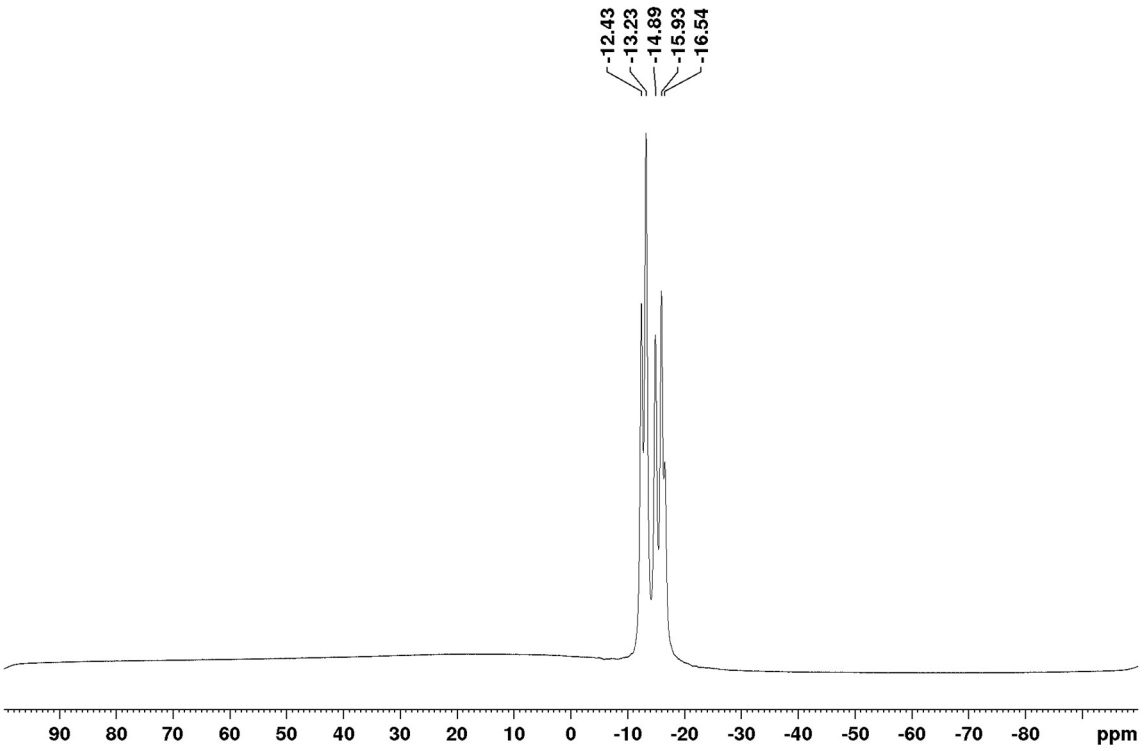
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

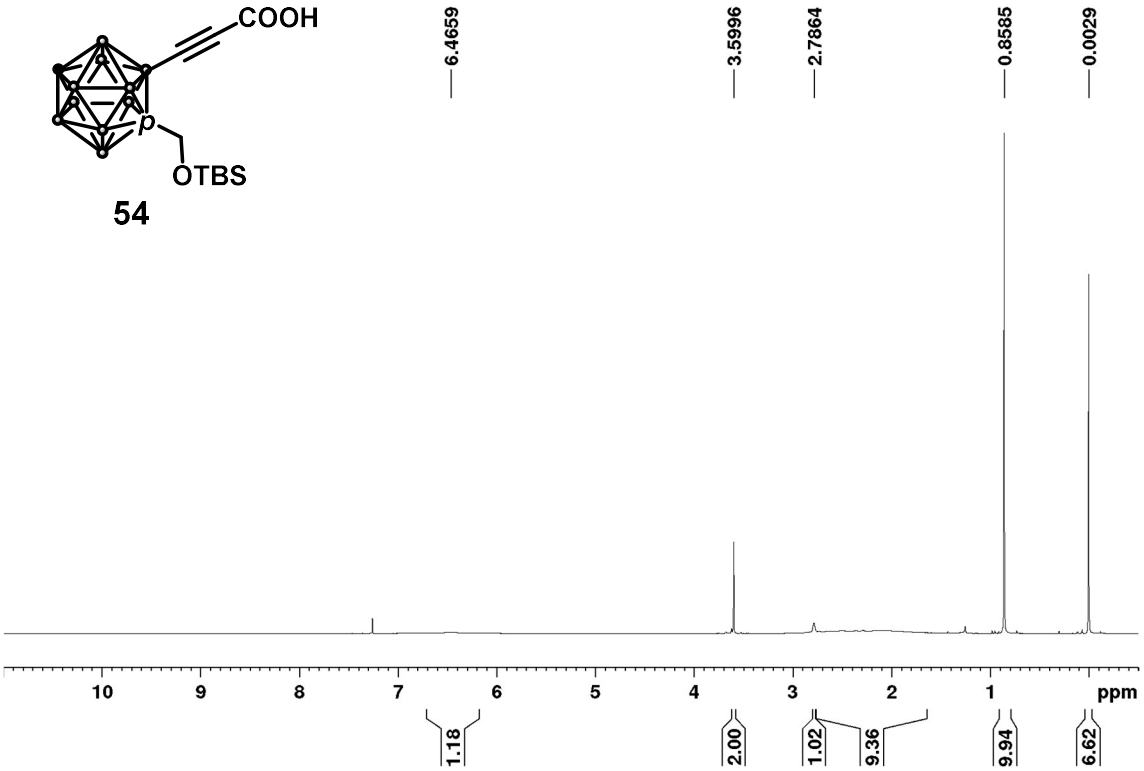


^{11}B NMR (160 MHz; $CDCl_3$)

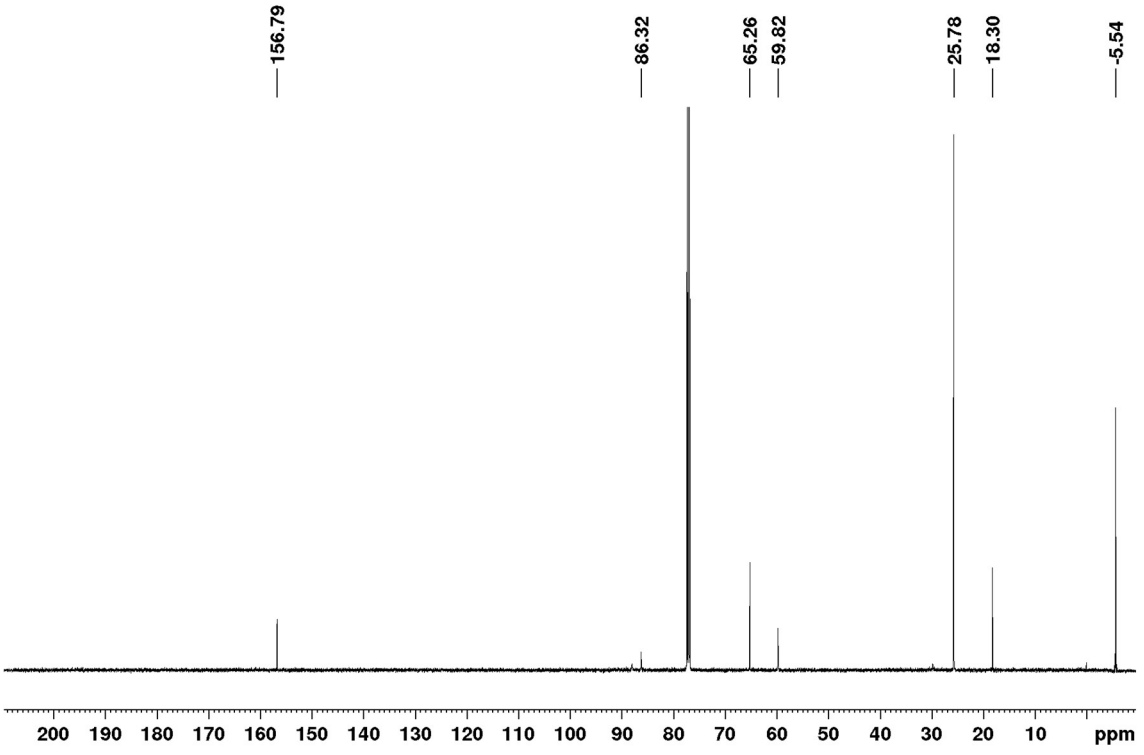


1-((*tert*-butyl)dimethylsilyl)hydroxymethyl)-7-carboxyethynyl-1,12-dicarba-*closo*-dodecaborane (54)

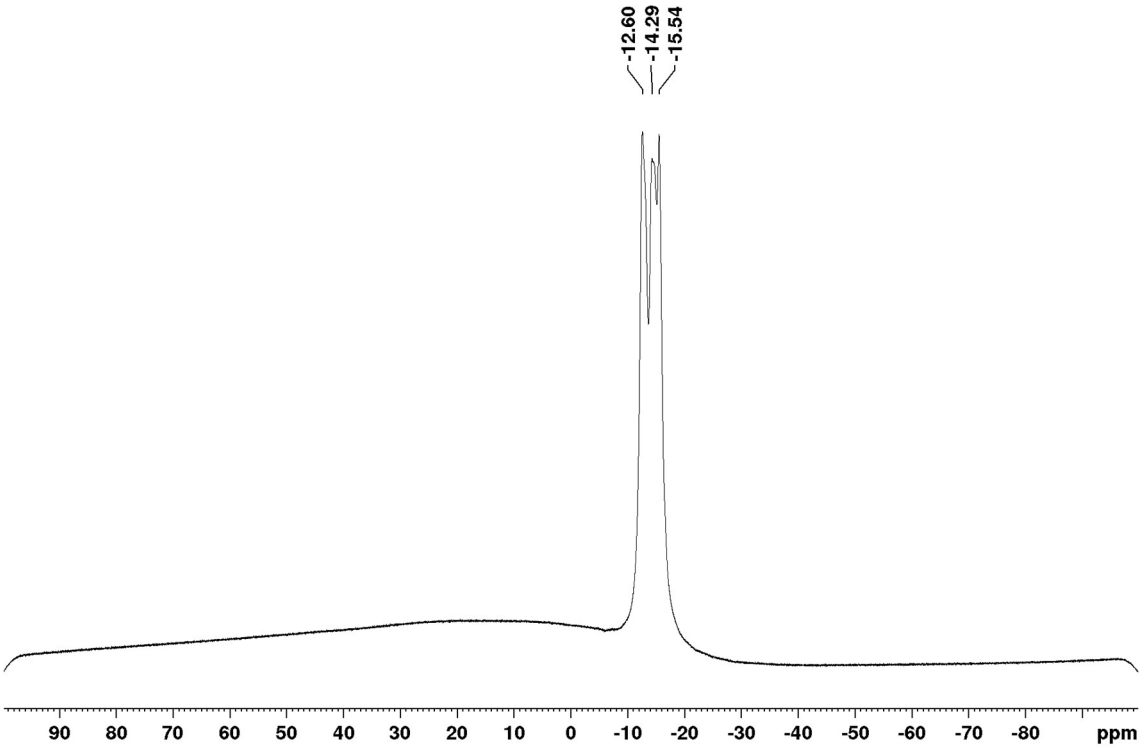
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

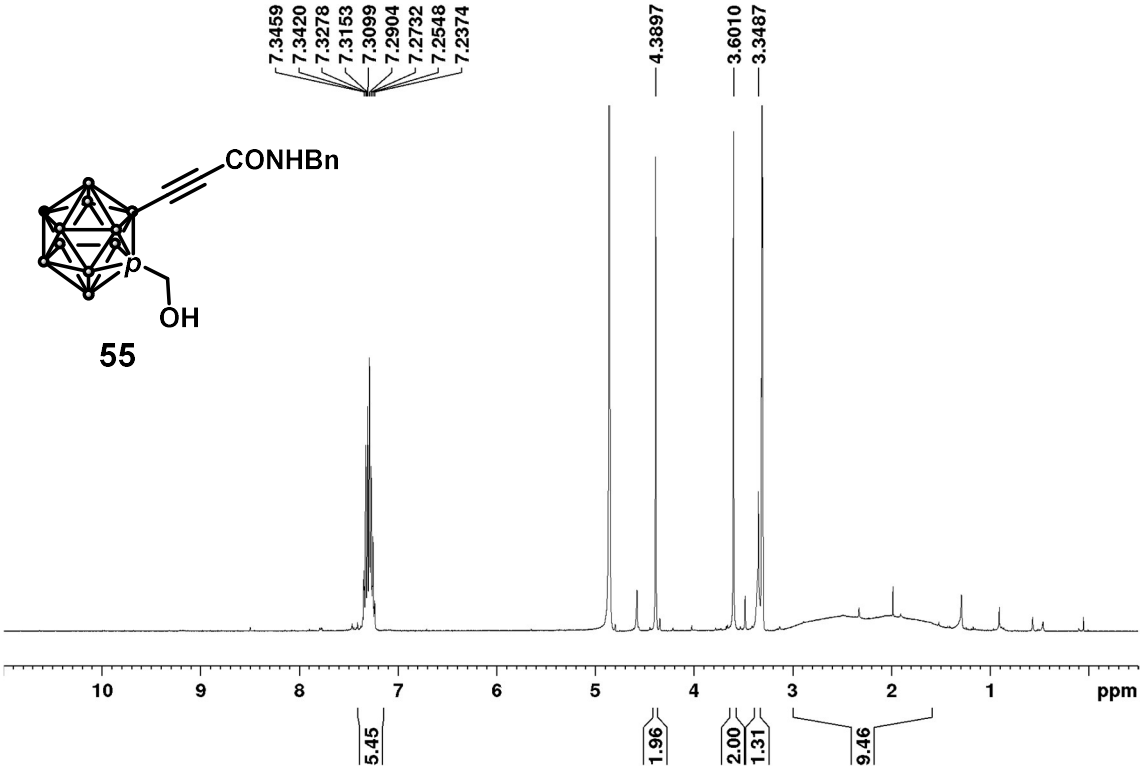


^{11}B NMR (160 MHz; $CDCl_3$)

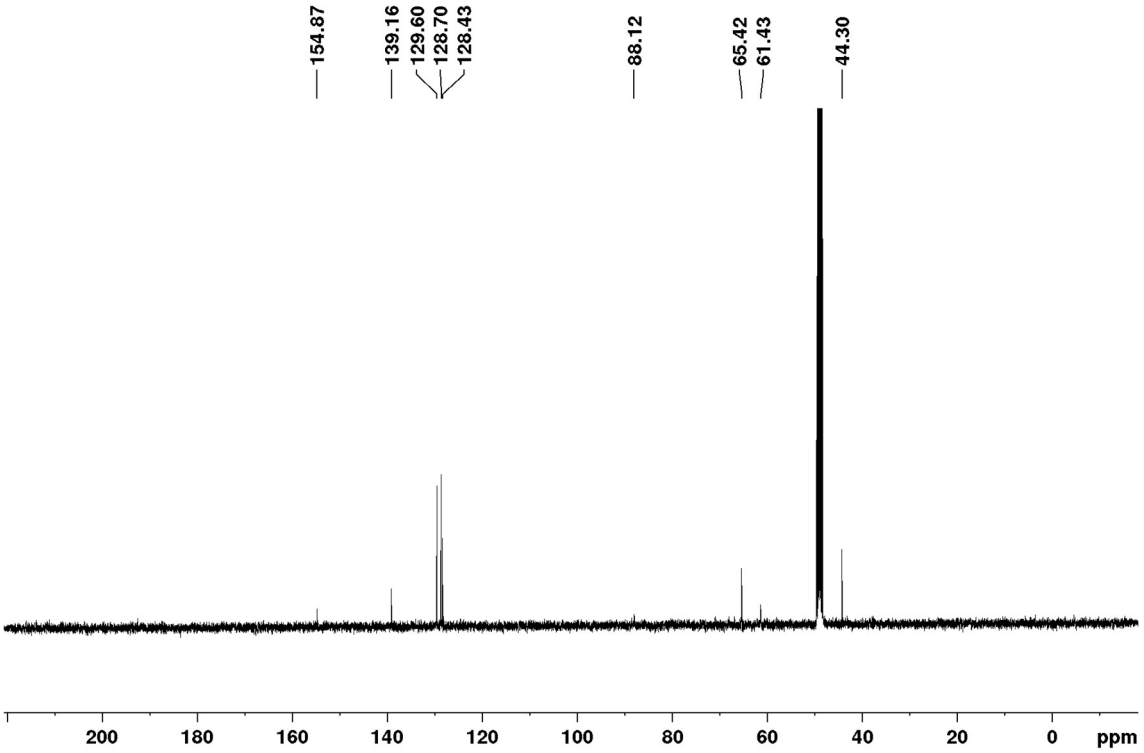


1-hydroxymethyl-7-(3-3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaborane (55)

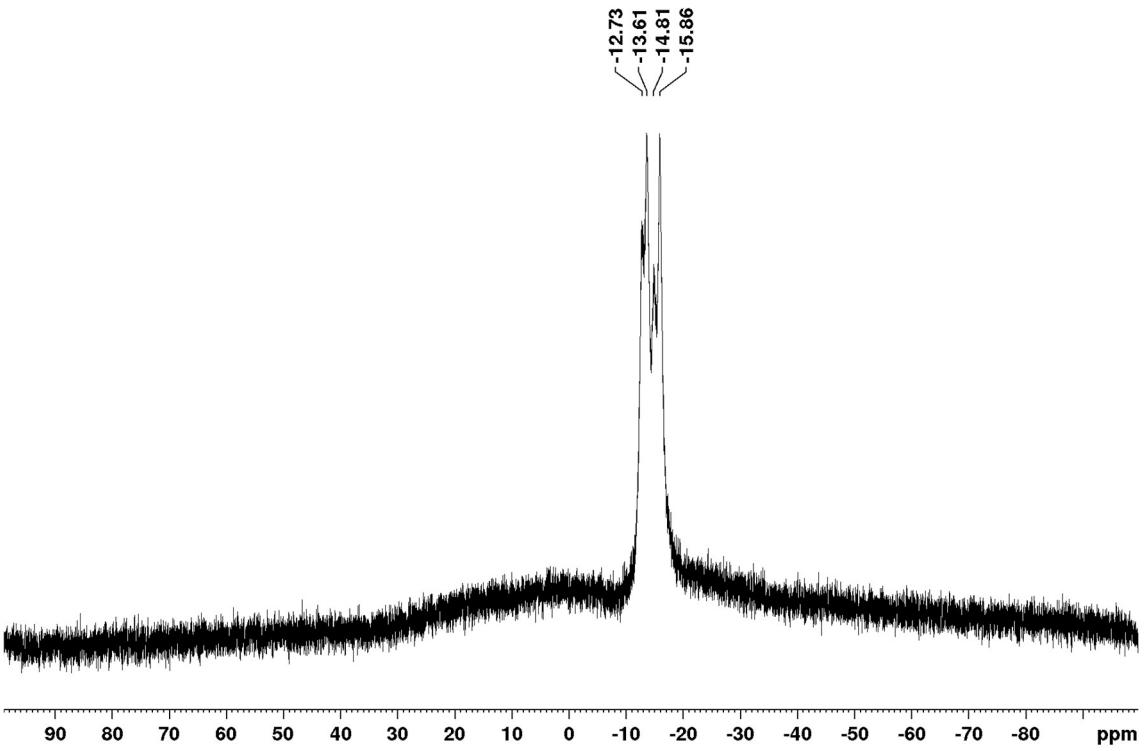
^{1}H NMR (400 MHz; CD_3OD)



^{13}C NMR (125 MHz; CD_3OD)

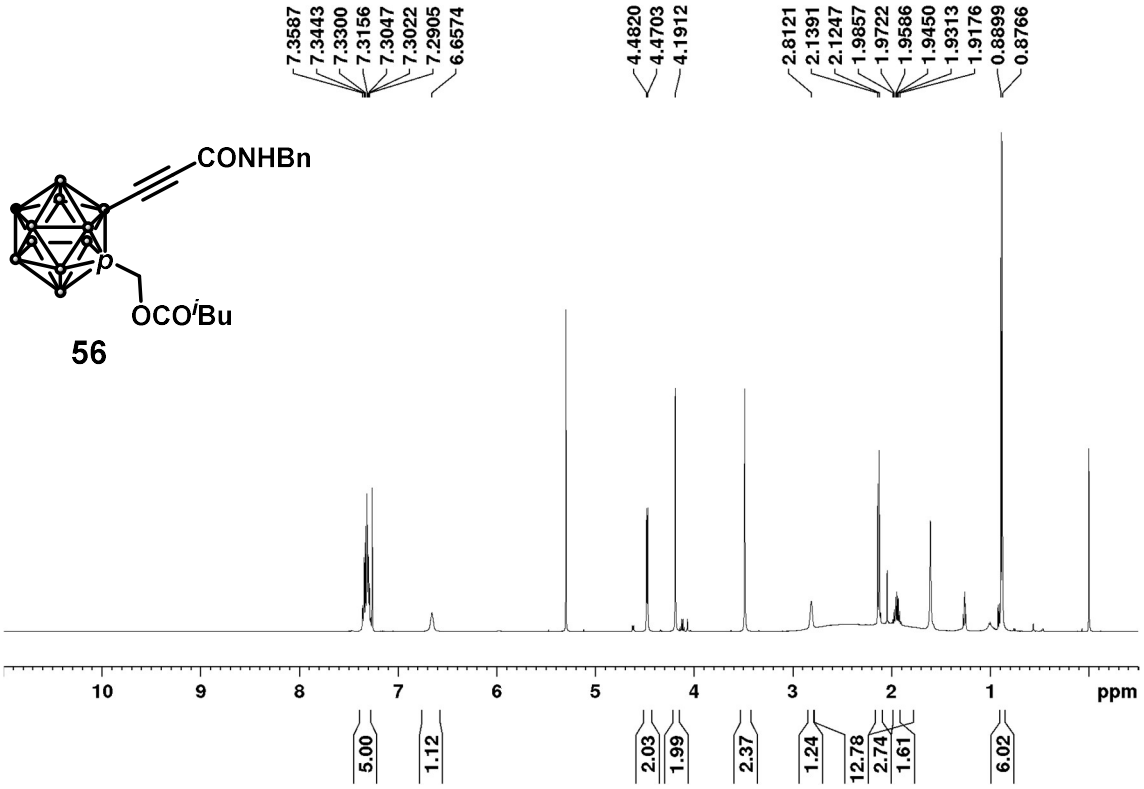


^{11}B NMR (160 MHz; CD_3OD)

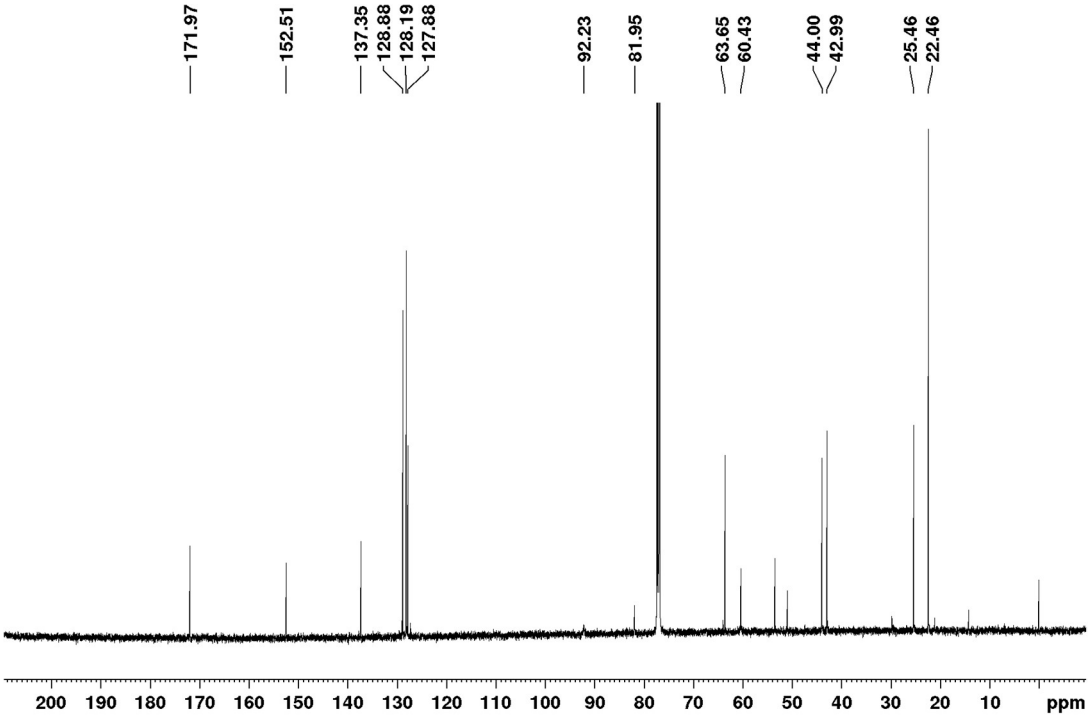


1-(3-methylbutanoxymethyl)-7-(3-3-(benzylamino)-3-oxoprop-1-yn-1-yl)-1,12-dicarba-*closo*-dodecaborane (56)

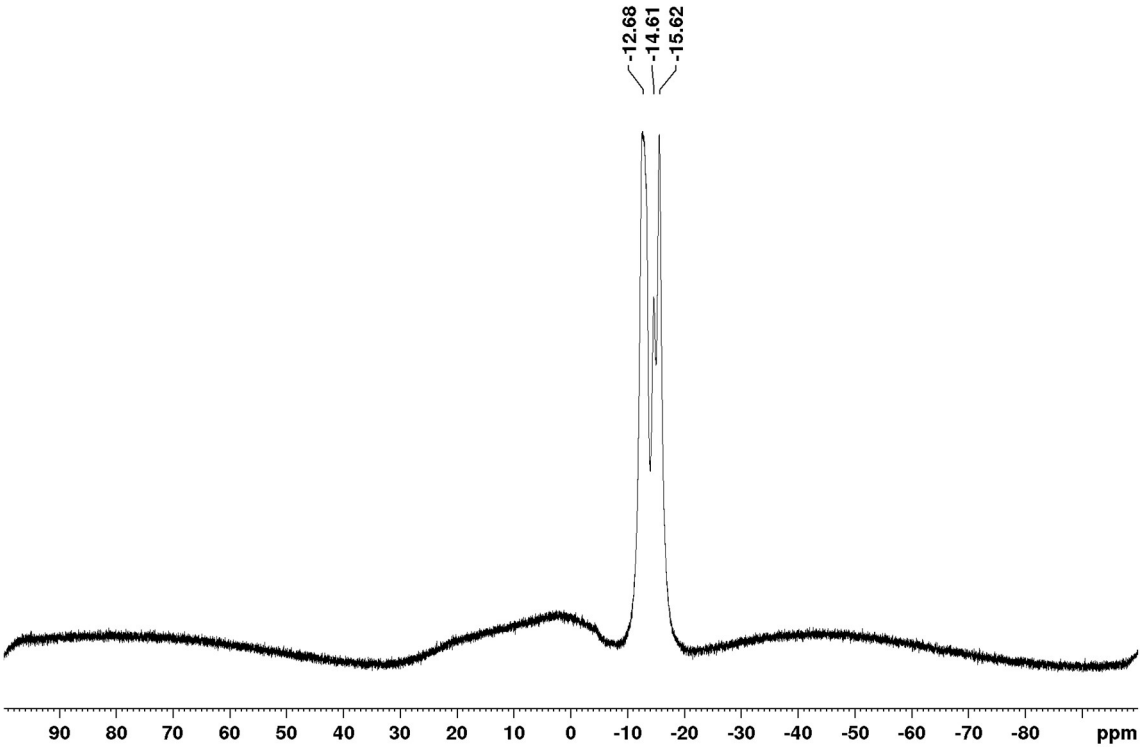
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

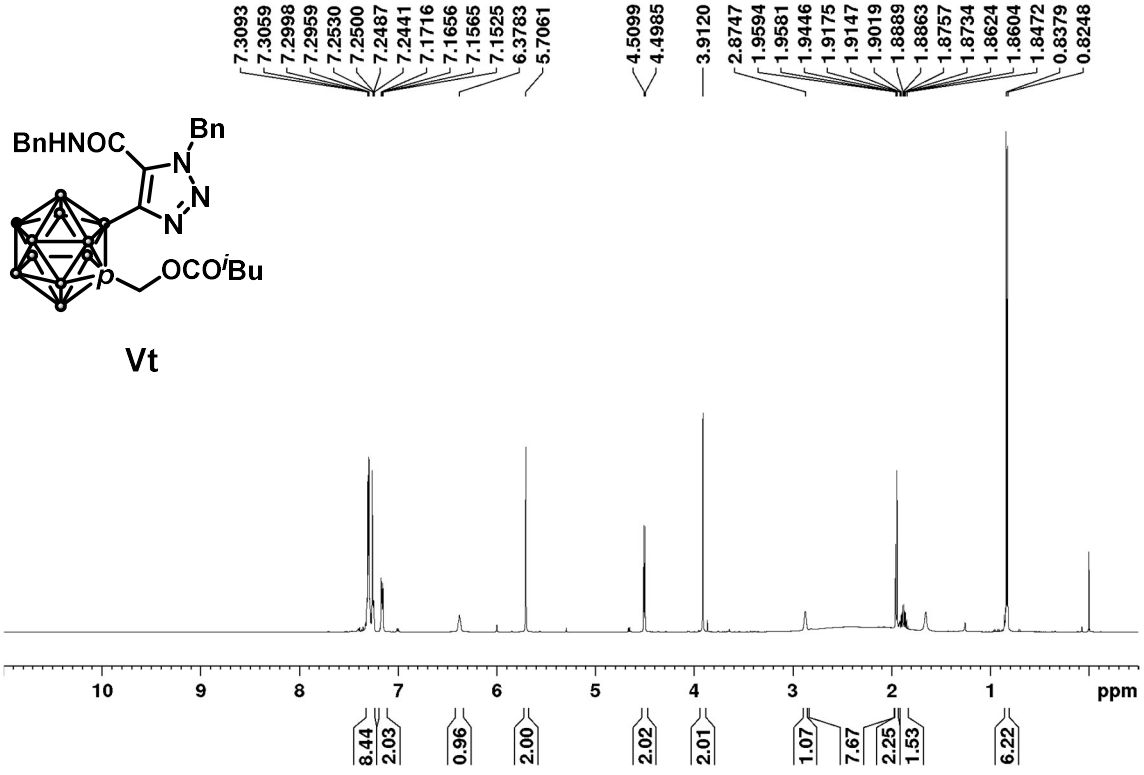


^{11}B NMR (160 MHz; $CDCl_3$)

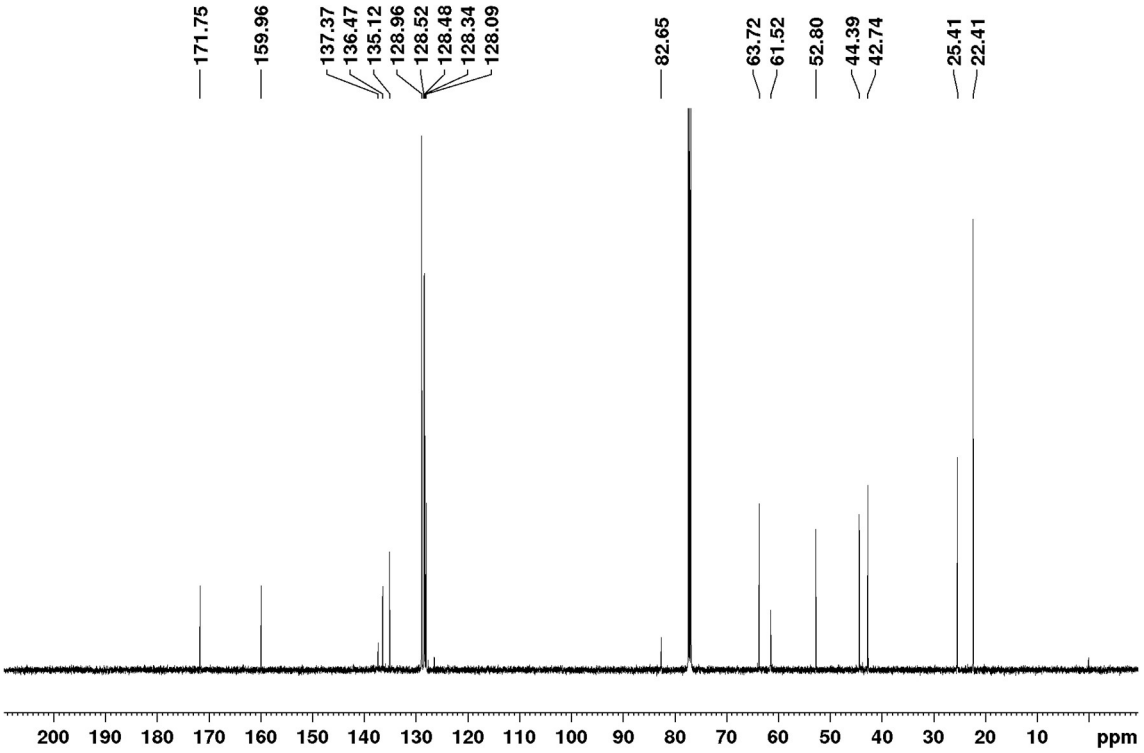


1-(3-methylbutanoxymethyl)-2-((1*H*-1,2,3-triazol-4-yl)benzylcarbamoyl)-7-(1-benzyl-5--1,12-dicarba-*closo*-dodecaborane (Vt)

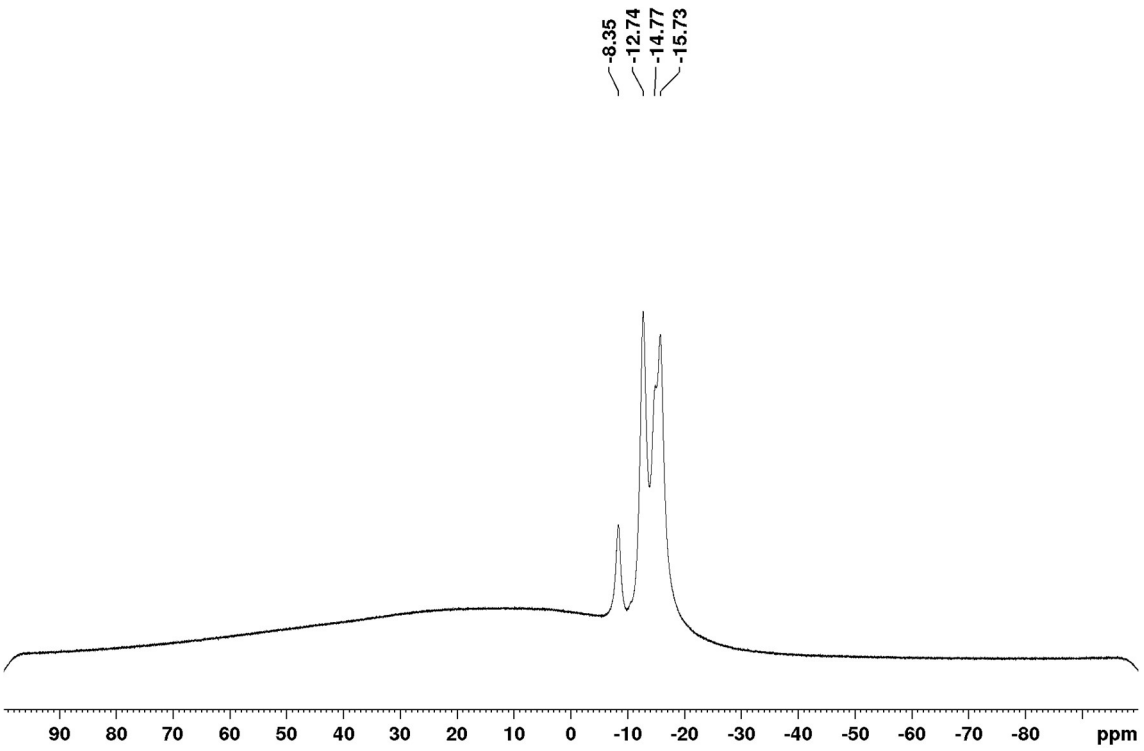
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

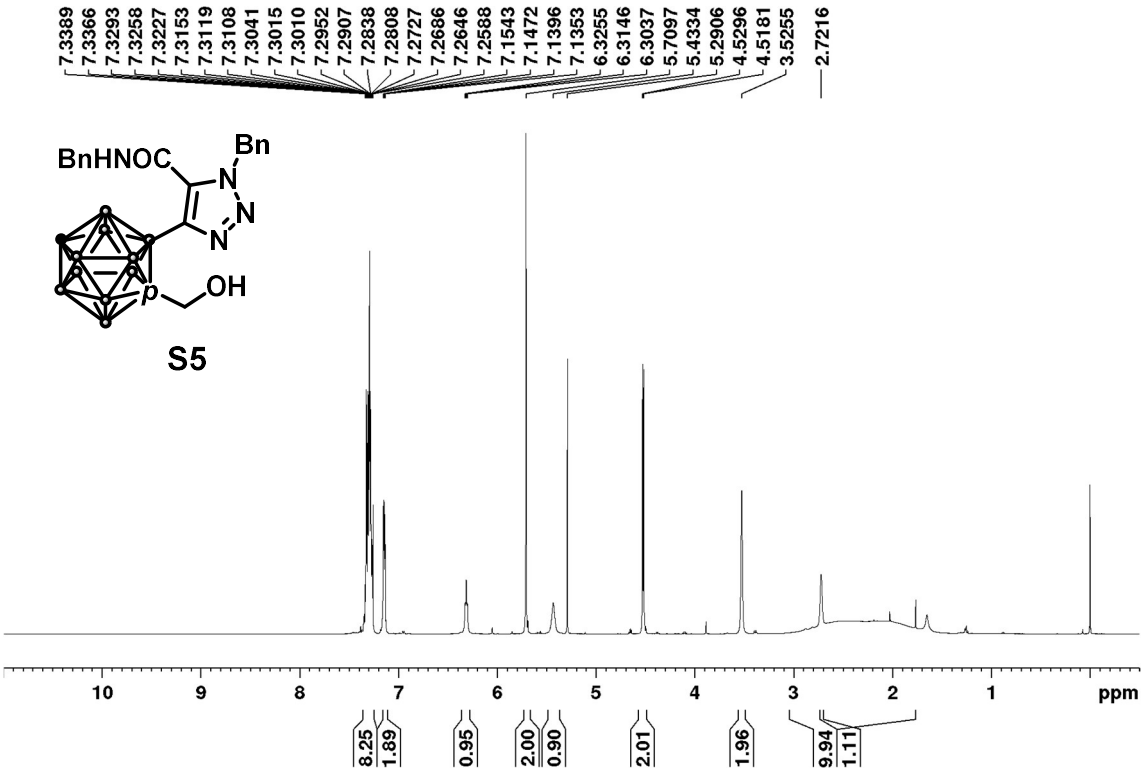


^{11}B NMR (160 MHz; $CDCl_3$)

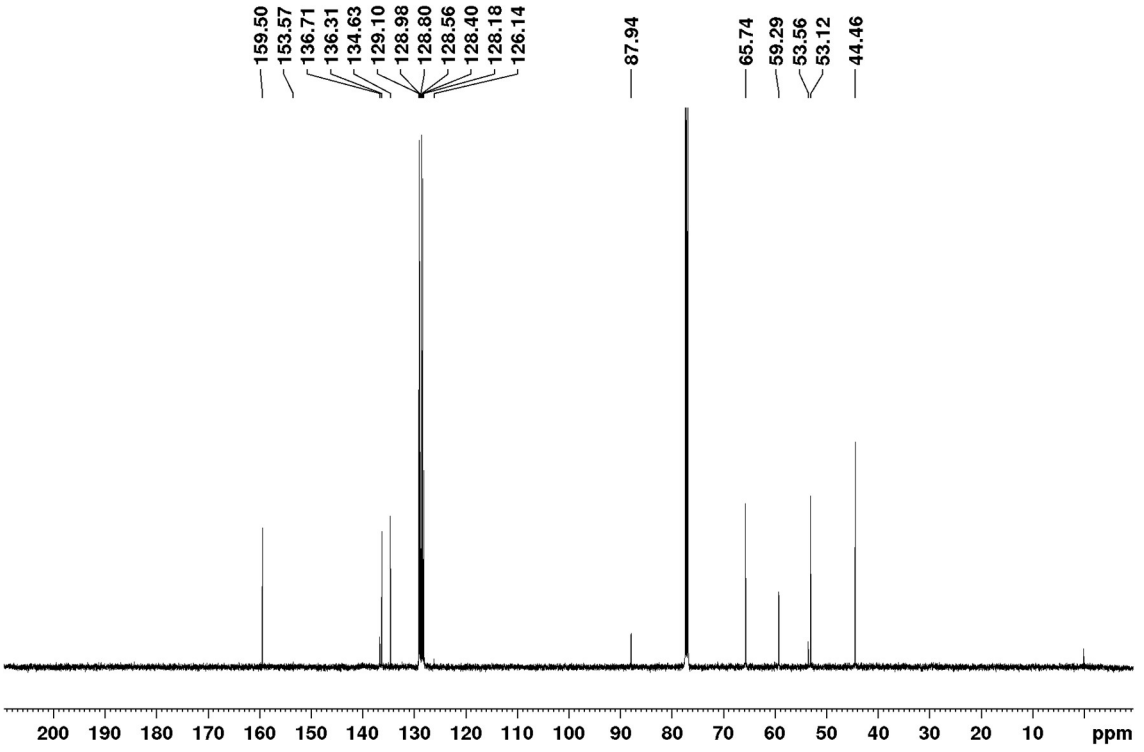


1-hydroxymethyl-2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaborane (S5)

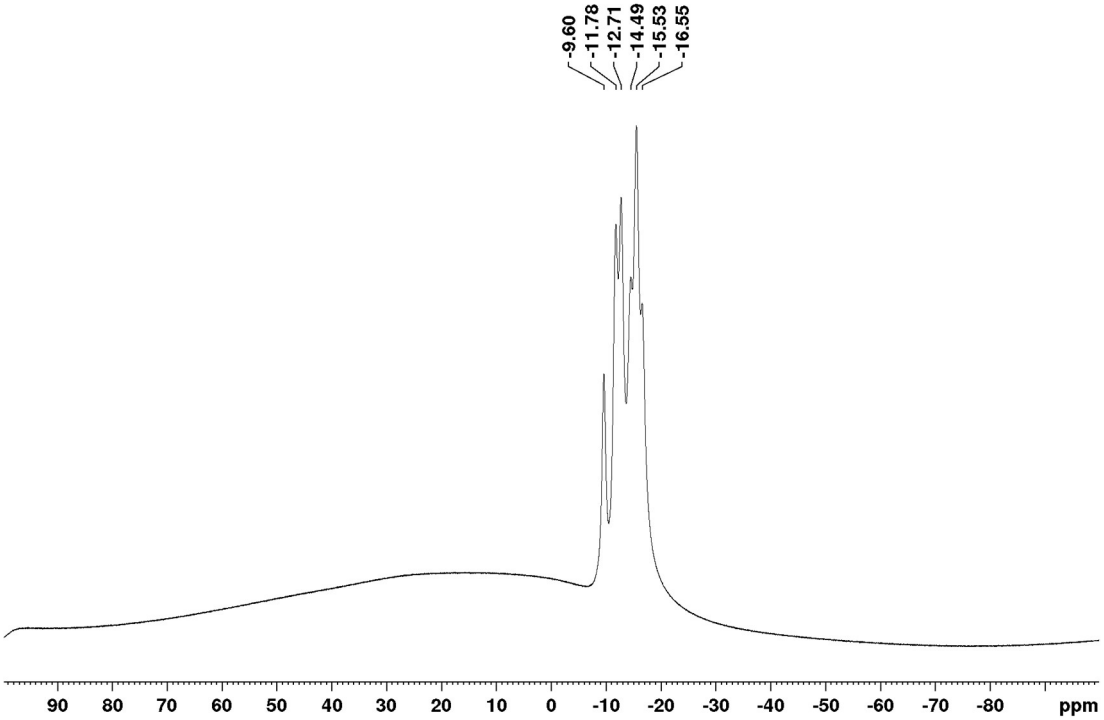
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

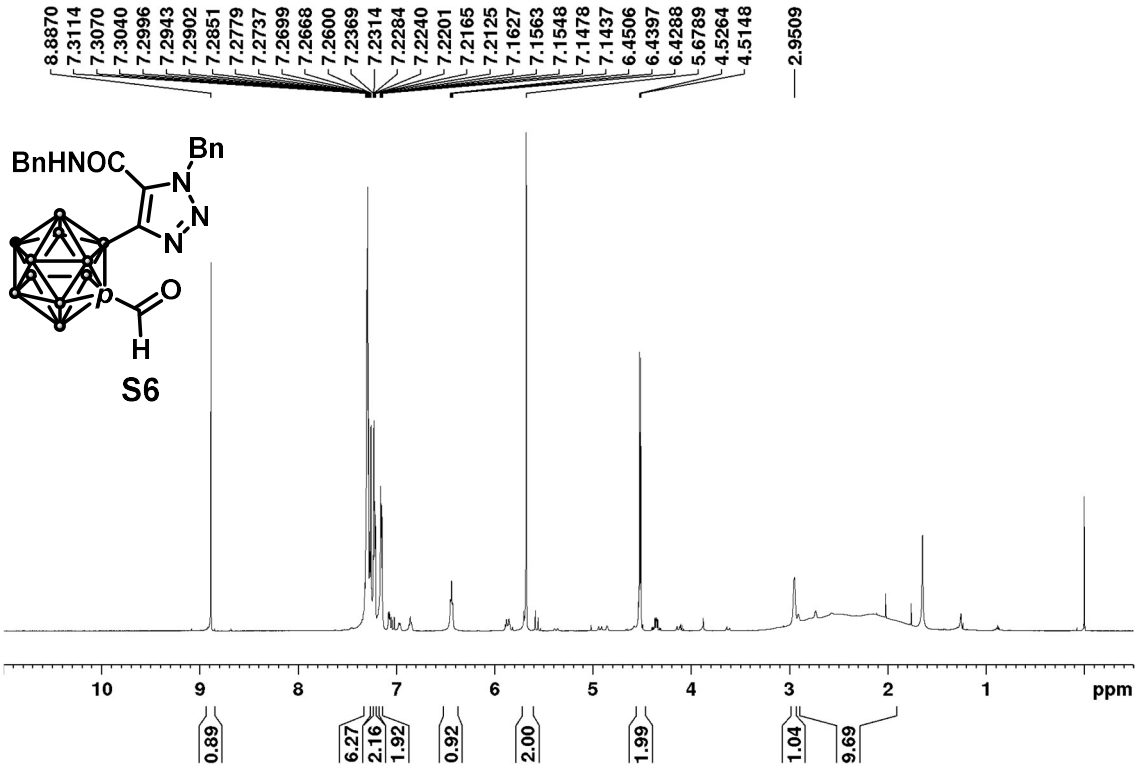


^{11}B NMR (160 MHz; $CDCl_3$)

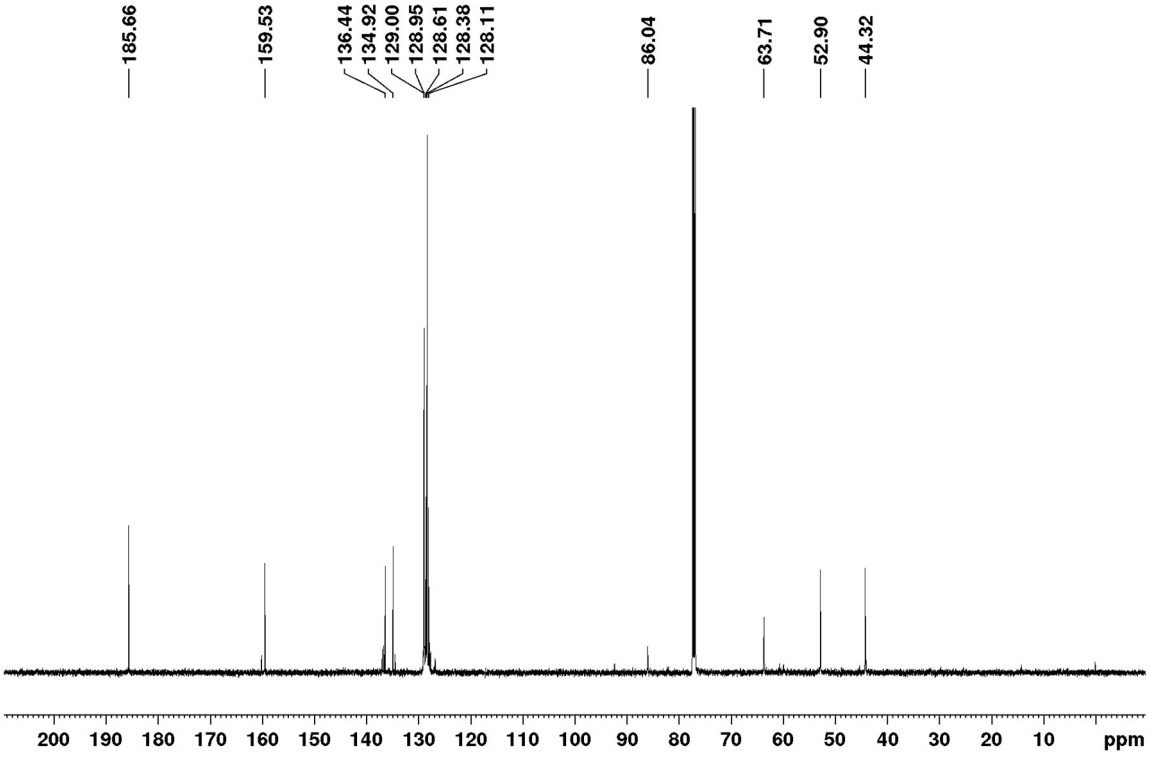


1-formyl-2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaborane (S5)

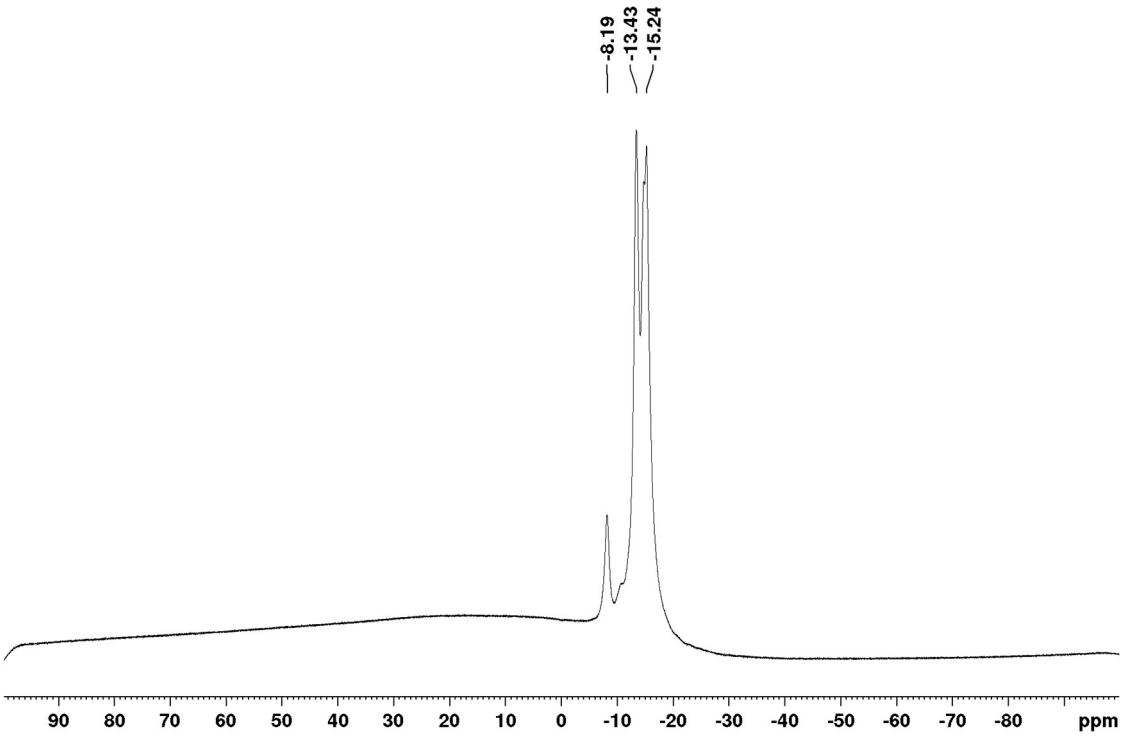
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

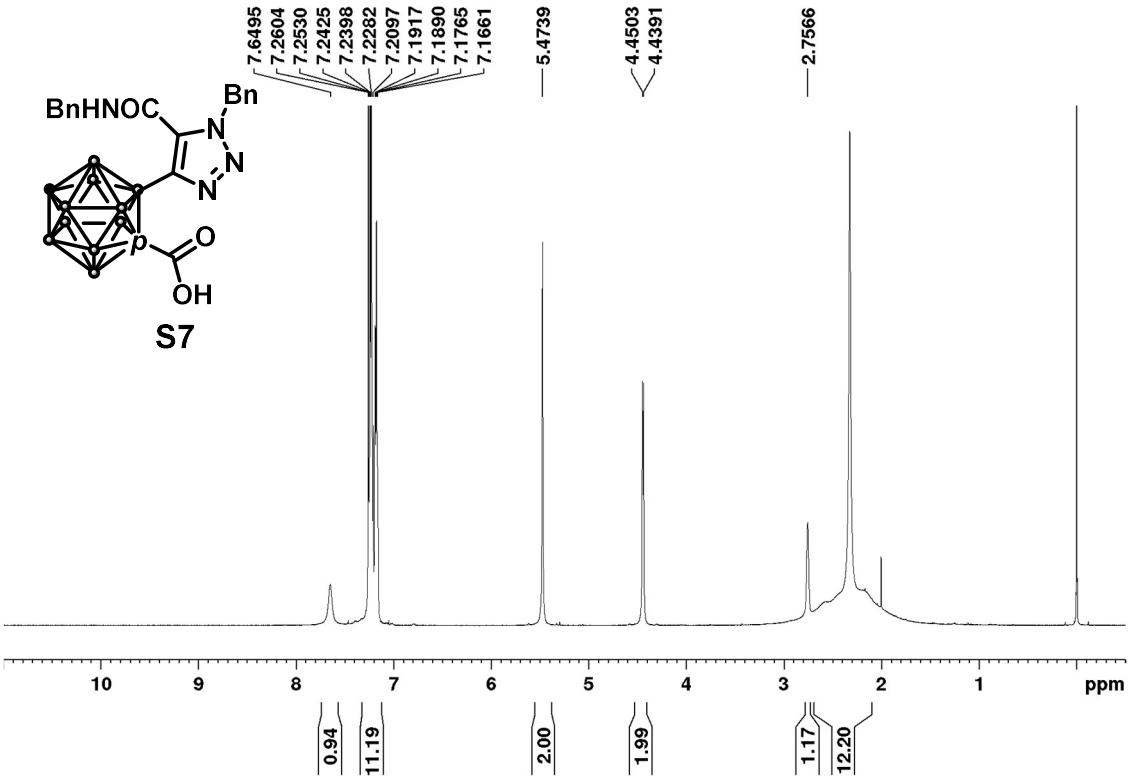


^{11}B NMR (160 MHz; $CDCl_3$)

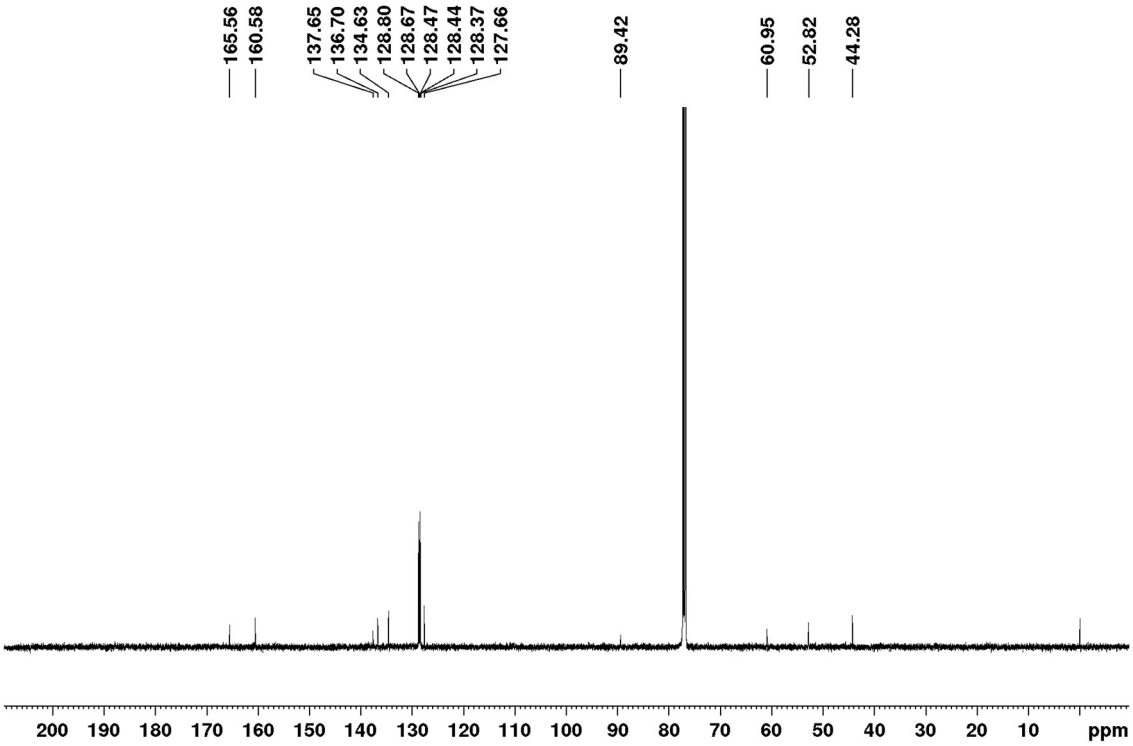


2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaboranyl-1-carboxylic acid (S7)

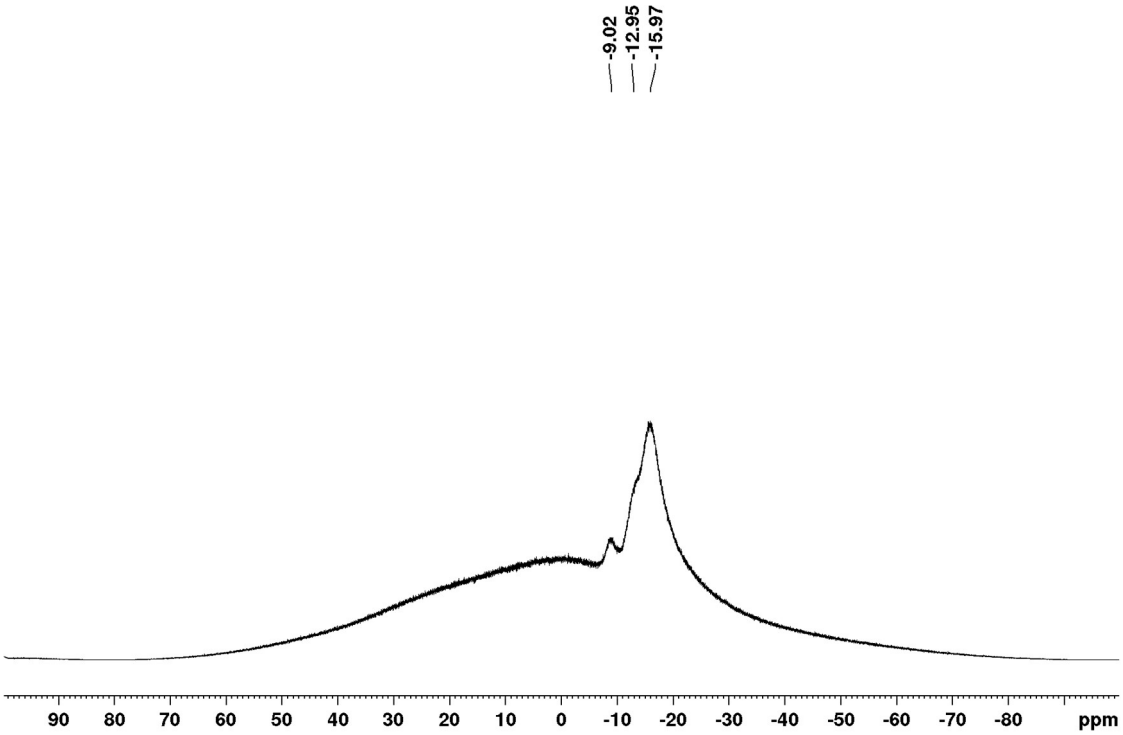
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)

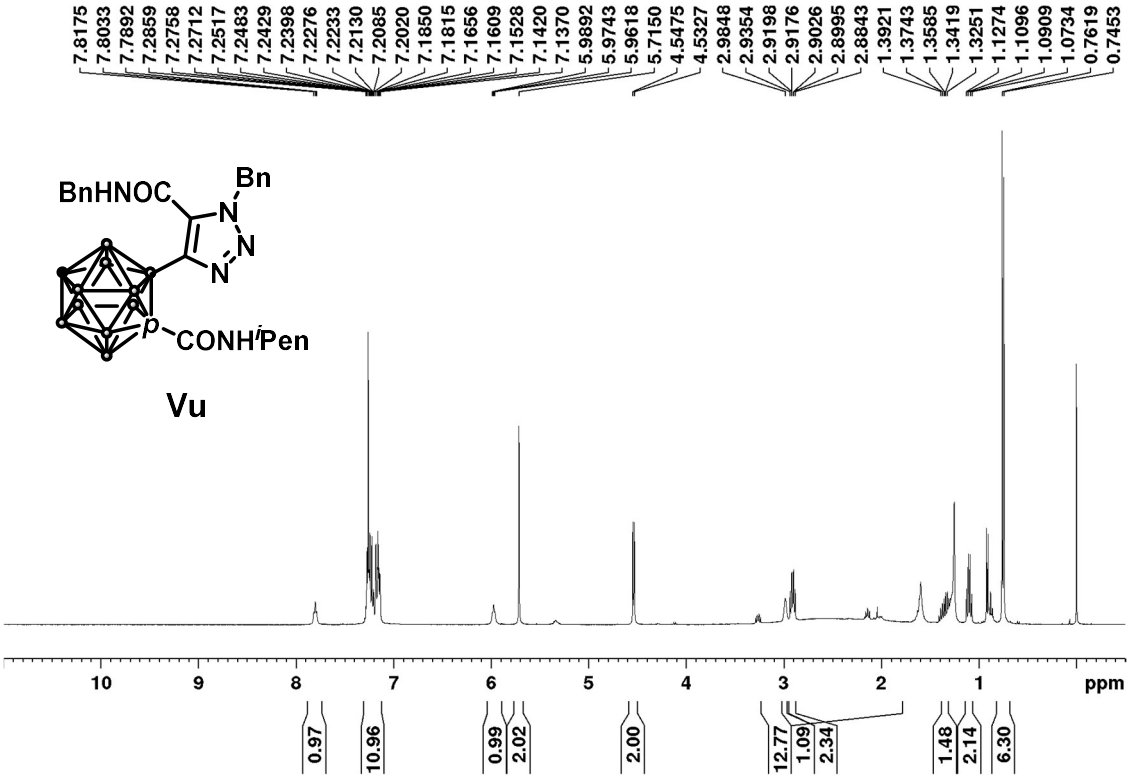


^{11}B NMR (160 MHz; $CDCl_3$)

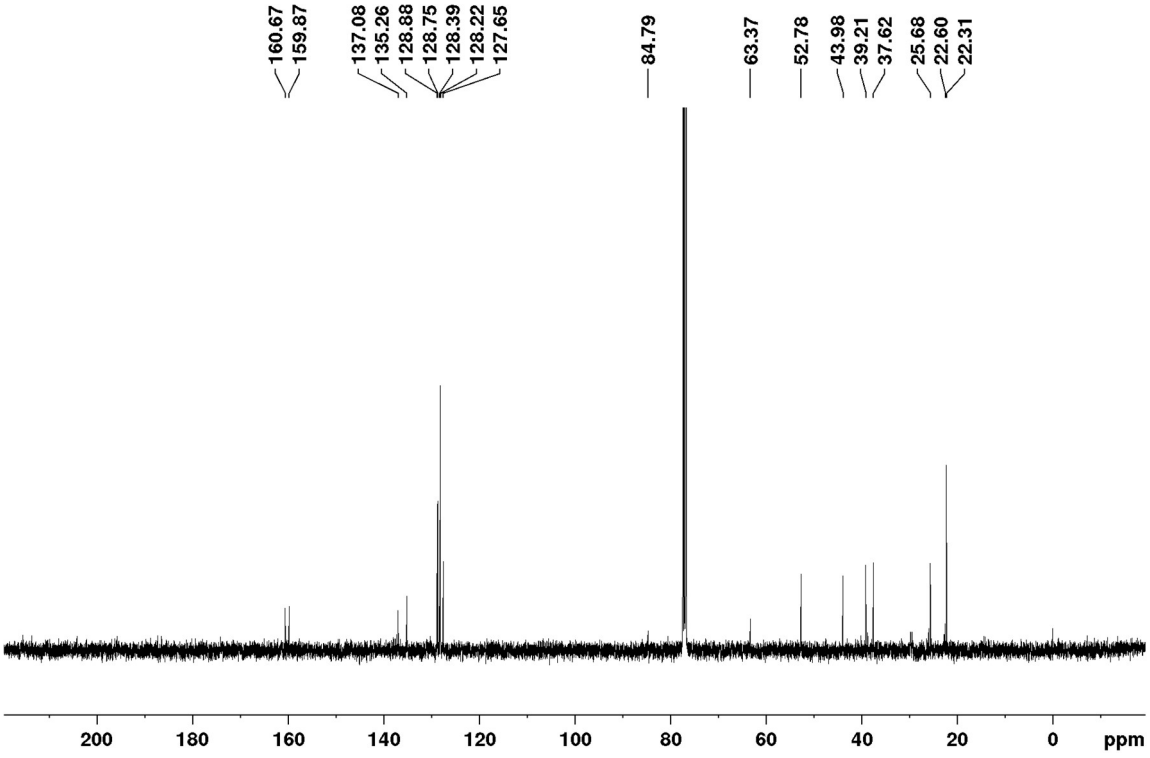


1-isopentylcarbamoyl-2-(1-benzyl-5-(benzylcarbamoyl)-1*H*-1,2,3-triazol-4-yl)-1,12-dicarba-*closo*-dodecaborane (Vu)

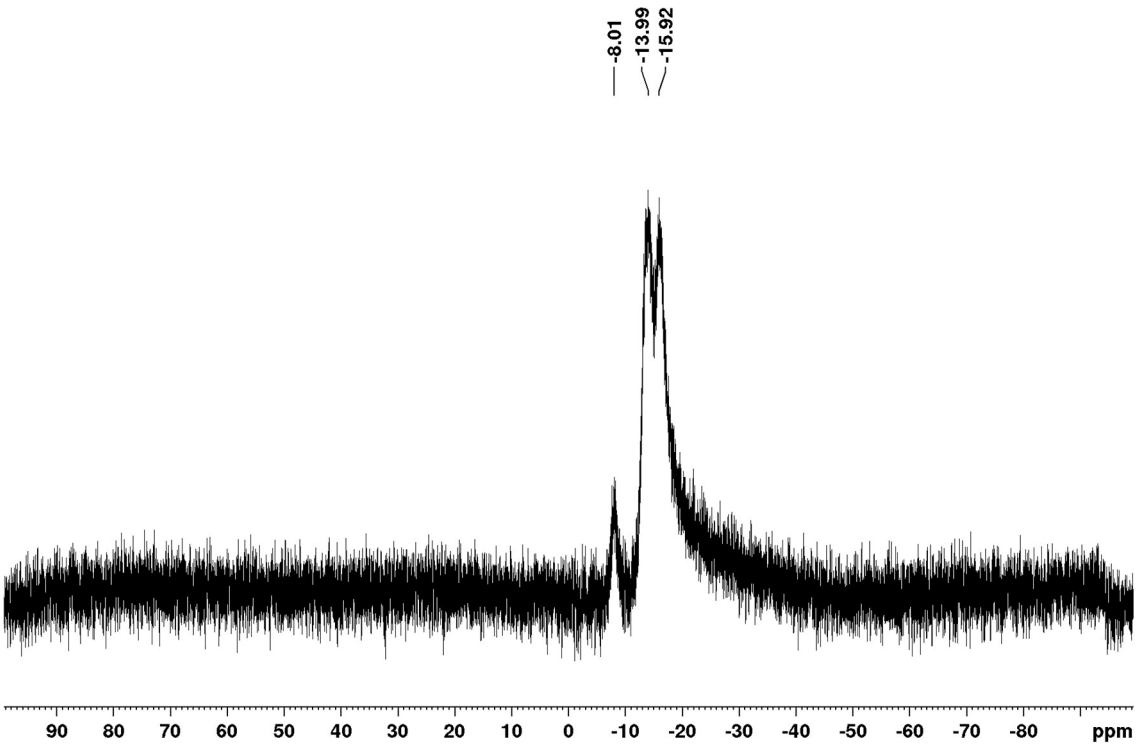
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)

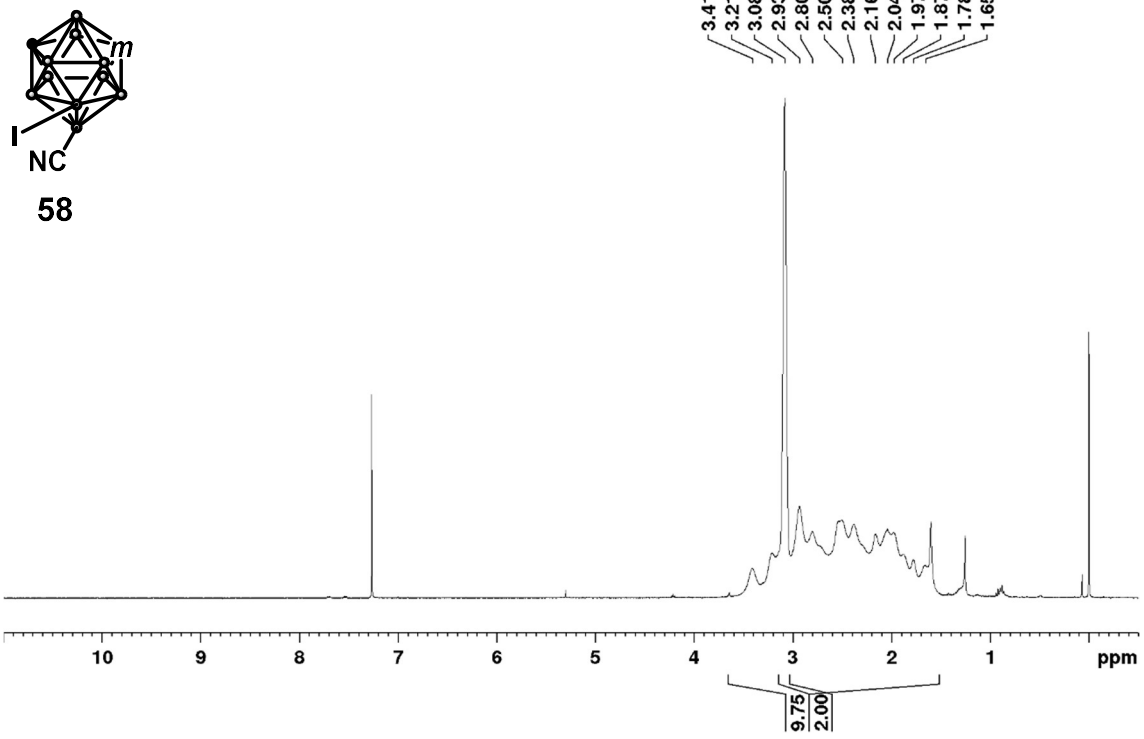


^{11}B NMR (128 MHz; $CDCl_3$)

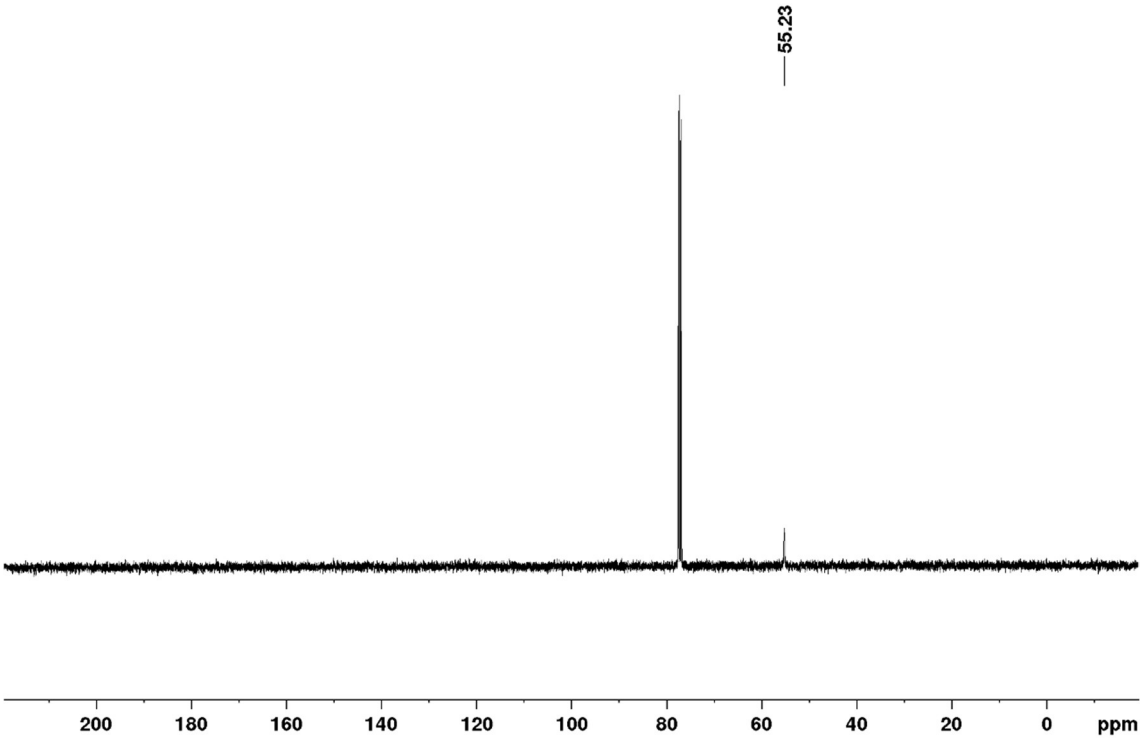


9-cyano-10-iodo-1,7-dicarba-*closo*-dodecaborane (58)

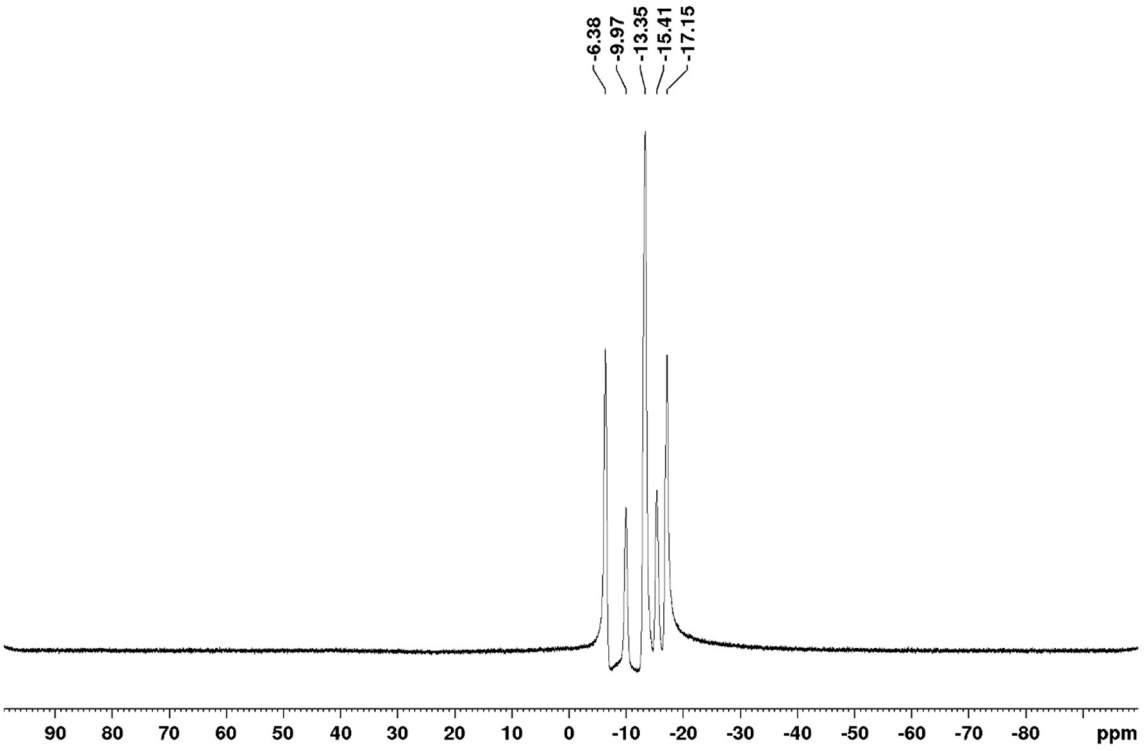
^{1}H NMR (400 MHz; $CDCl_3$)



^{13}C NMR (100 MHz; $CDCl_3$)



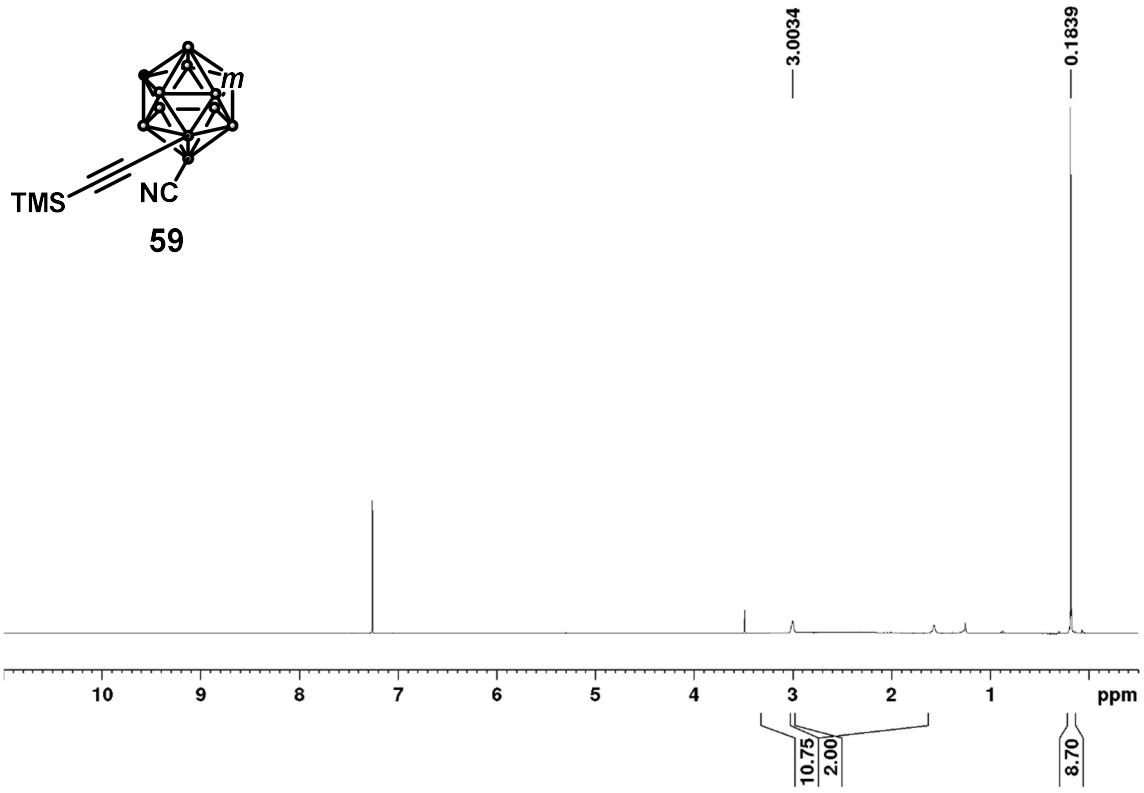
^{11}B NMR (128 MHz; $CDCl_3$)



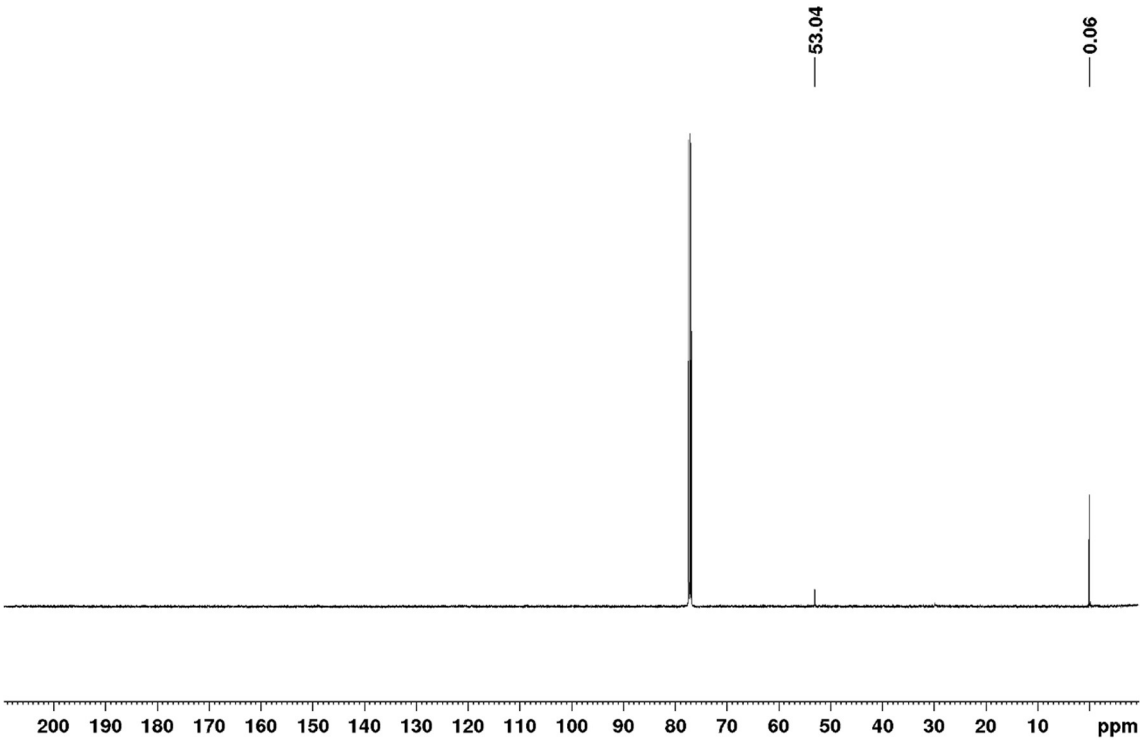
9-cyano-10-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (59)

^{1}H NMR (500 MHz; $CDCl_3$)

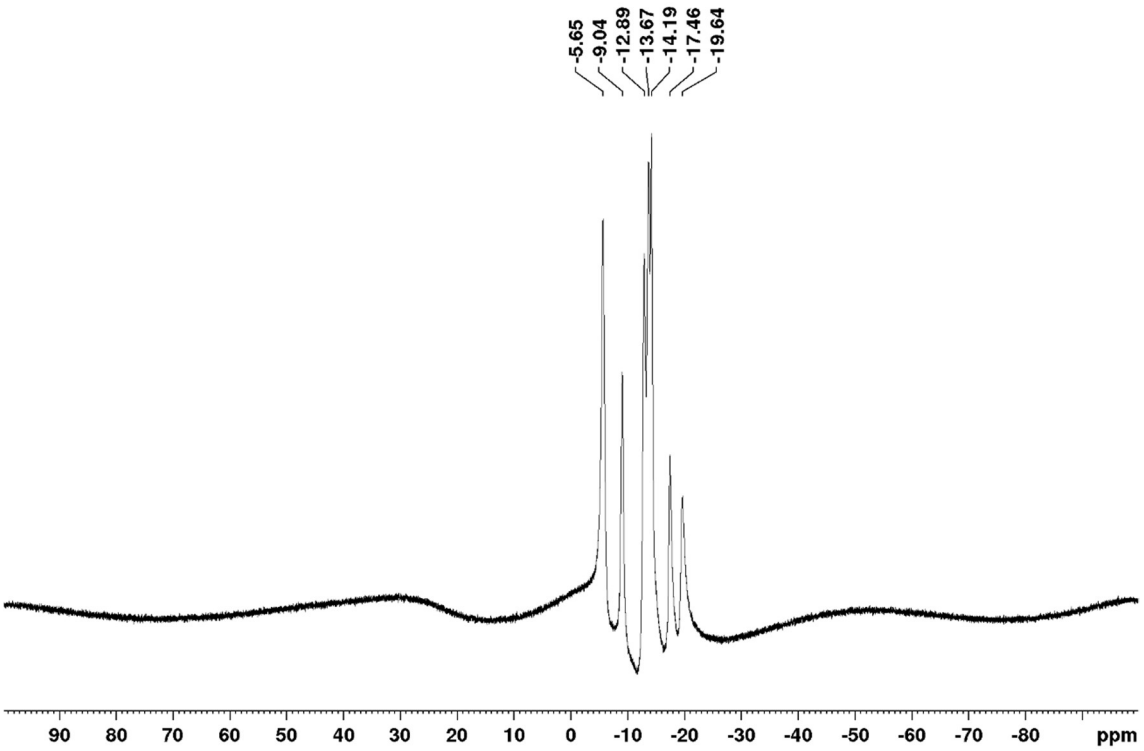
59



^{13}C NMR (125 MHz; $CDCl_3$)

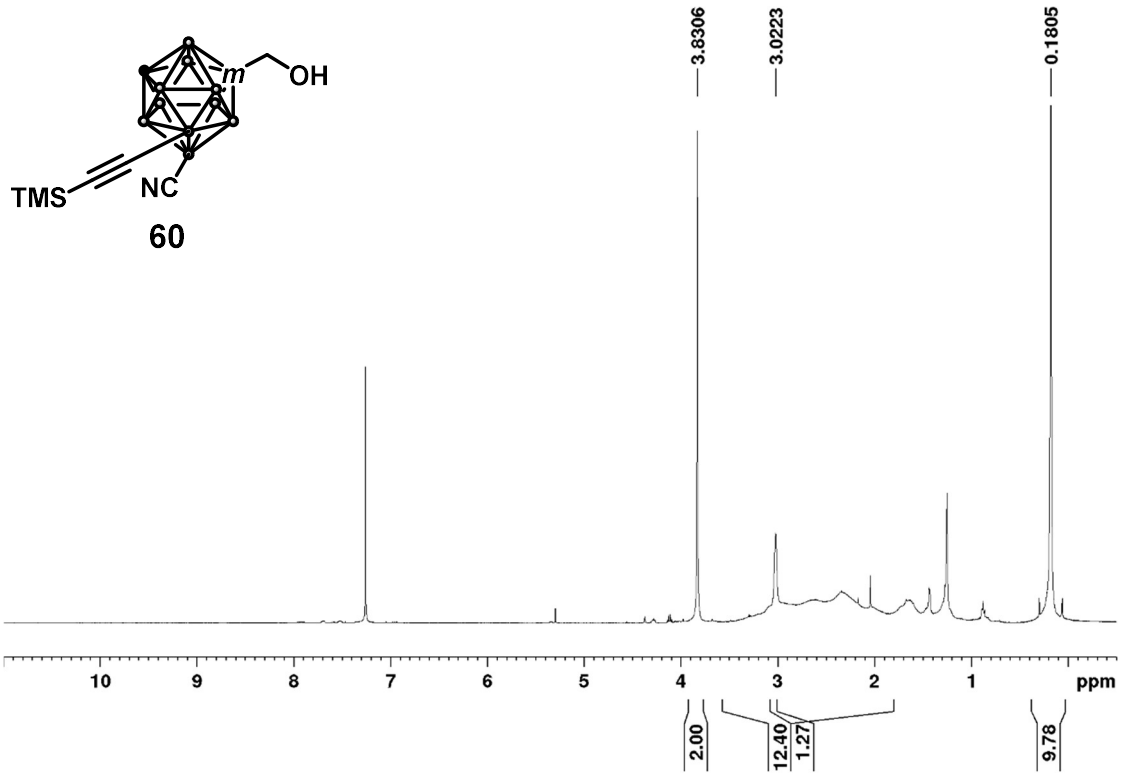


^{11}B NMR (160 MHz; $CDCl_3$)

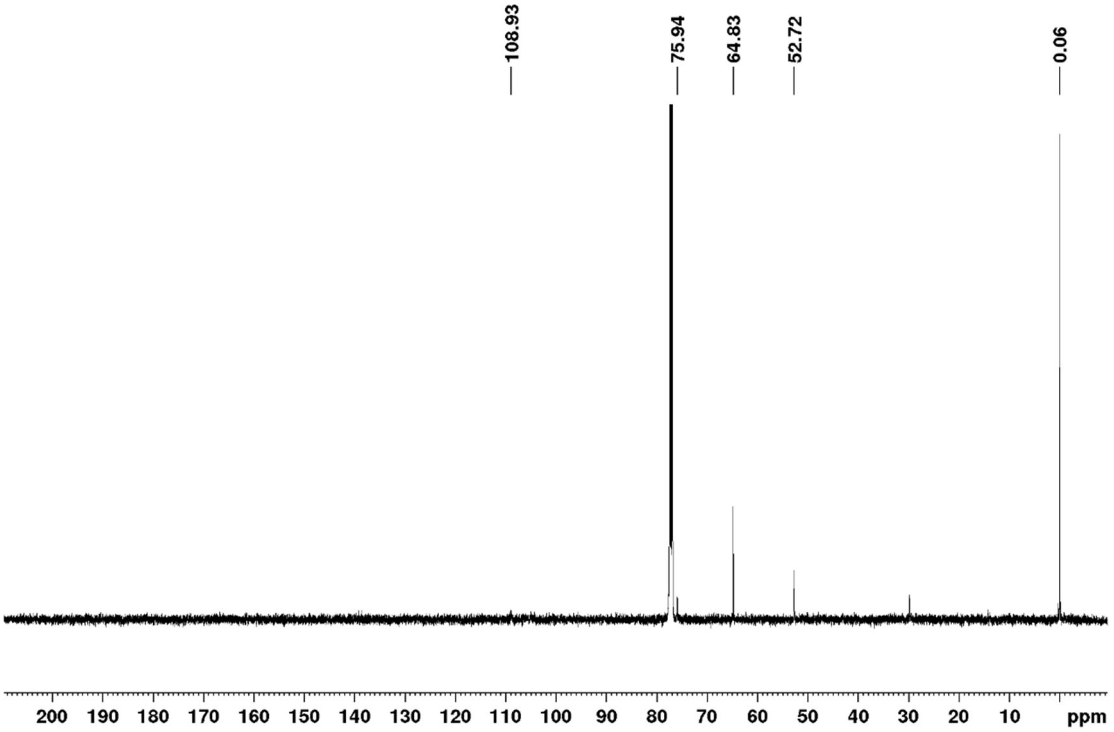


1-hydroxymethyl-9-cyano-10-((trimethylsilyl)ethynyl)-1,7-dicarba-*closo*-dodecaborane (60)

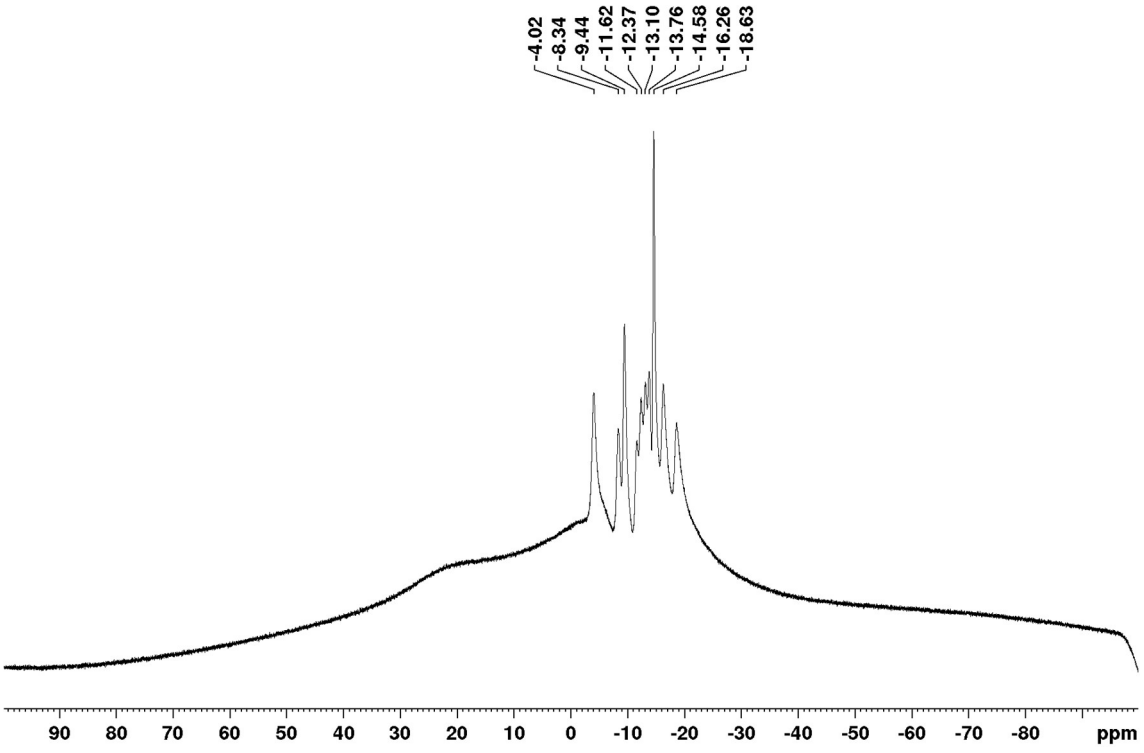
^{1}H NMR (500 MHz; $CDCl_3$)



^{13}C NMR (125 MHz; $CDCl_3$)



^{11}B NMR (160 MHz; $CDCl_3$)



第七章

結論

7-1　本論文のまとめ

本論文は、低分子医薬品の標的分子拡充を目指してカルボランを基盤とした分子設計による新たな創薬戦略の提唱し、有望な生物活性分子を創出することで、その概念実証を行った。

第一章「序論」では、低分子の創薬標的が従来の酵素などからタンパク質-タンパク質相互作用（PPI）にシフトしたことで新たな分子設計戦略が期待されており、近年までに開発されてきた戦略を紹介した。一方で、カルボランのユニークな構造と疎水性から、疎水性ファーマコフォアとして新たな創薬のビルディングブロックとして利用されてきていることを述べた。

第二章「カルボランのドッキングシミュレーションの確立と疎水性ファーマコフォアとして利用した NAMPT 阻害剤の創出」では、Autodock Vina を用いたカルボランのドッキングシミュレーションを駆使して設計した、新たな NAMPT 阻害剤の構造活性相関（SAR）を明らかにした。さらに、得られた SAR とドッキングシミュレーションの結果を比較したところ、2 つのカルボランが配置されうる疎水性ポケットの存在に気付いた。そこで、1 分子に 2 つのカルボランを有するジカルボラン化合物を合成、評価した結果、この仮説を実証しただけでなく、<u>カルボランの異性体を利用することで、厳密な置換基配置制御が可能である</u>という新たな気付きを得た（Figure 7-1）。

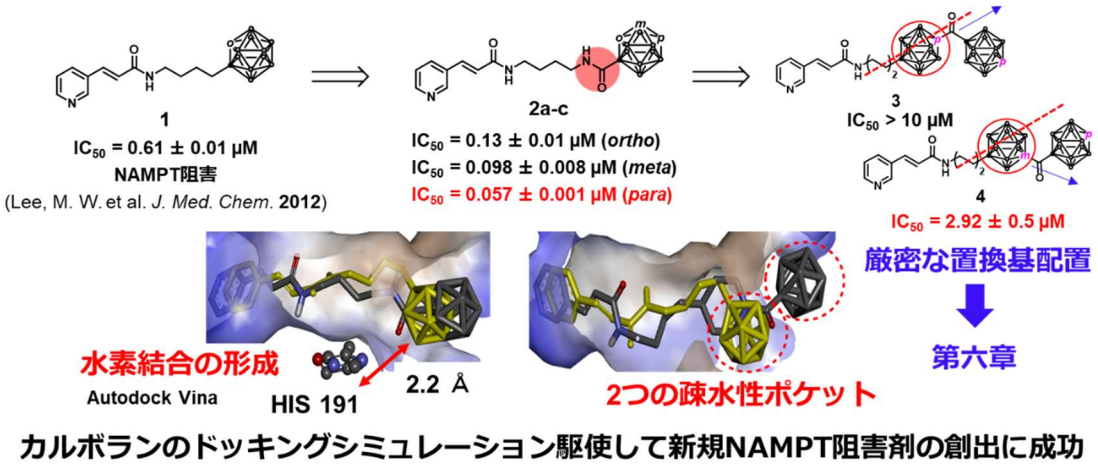


Figure 7-1. カルボラン骨格を有する NAMPT 阻害剤の創成

第三章「疎水性タグとしてカルボランを利用したタンパク質分解誘導剤の創成と機能解明」では、近年注目されている疎水性タグ（HyT）システムによる標的タンパク質の分解戦略に基づいて、カルボランの疎水性を HyT に利用することを着想し、これまで一般的に用いられてきたアダマンチル部位に比べてカルボランが高いタンパク質分解効率を示すことを見出した。さらに、HyT 結合タンパク質モデルとして MIC-BSA を合成し、タンパク質の構造変化を追跡した結果、HyT によってリジン残基が露出することを発見した。最後に MIC-BSA が細胞内でユビキチンプロテアソーム系によって分解されることを示した（Figure 7-2）。

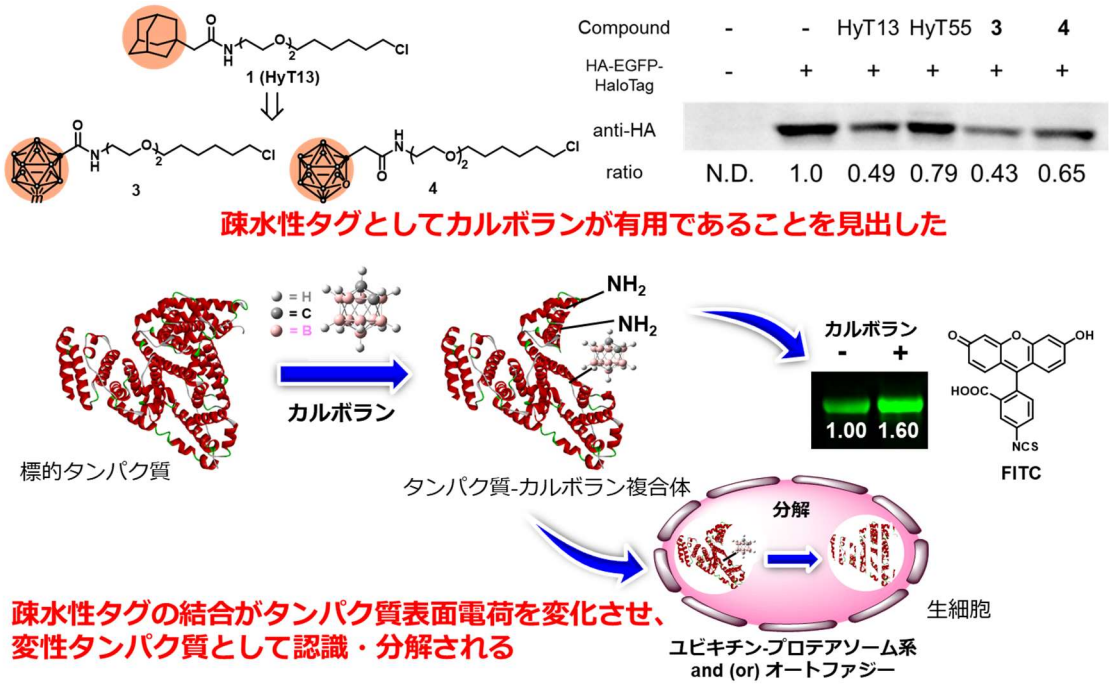


Figure 7-2. カルボランを HyT としてタンパク質分解誘導剤に活用、HyT システムにおけるタンパク質の構造変化を追跡

第四章「磁気共鳴イメージングを指向した Gd メタラカルボラン錯体の合成研究」では、2 つのカルボランをアミド基リンカーでつなげることで安定な錯体の合成を試みたが、予想外の錯体が確認された。この結果を基に新たな Gd メタラカルボラン錯体モデルを提唱し、その量子力学計算による解析を行った（Figure 7-3）。

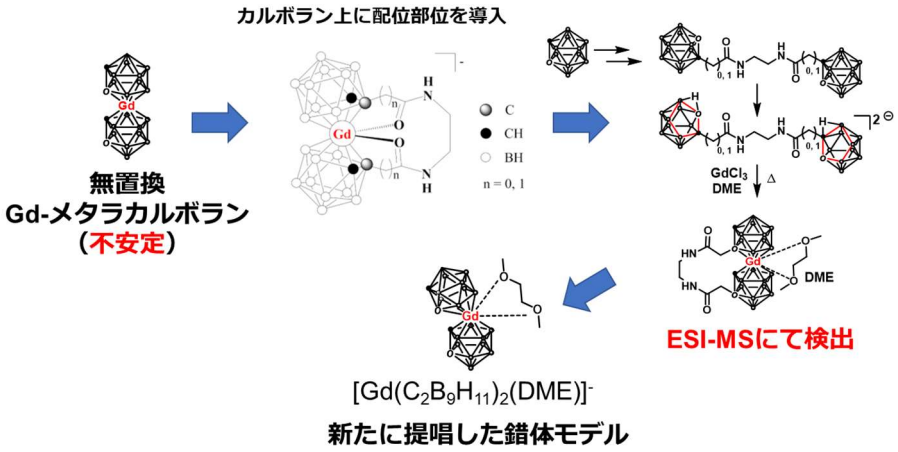


Figure 7-3. Gd-メタラカルボランの合成検討

第五章「公共データベースの網羅的な SAR 解析による Activity cliff の予測とその実験的検証」では、大規模な SAR の公共データベースである ChEMBL から得た、SAR 情報の網羅的解析を SAR matrix (SARM)を用いて可能とし、新たな Activity cliff の予測、その実験的検証を行った。この研究から、**SARM を用いた SAR データの網羅的解析によって、主観的介入なく、体系的にスクリーニングできる**ことが重要であることを示した一方で、**データベース内に含まれる多様な母骨格の必要性**に気づいた（Figure 7-4）。

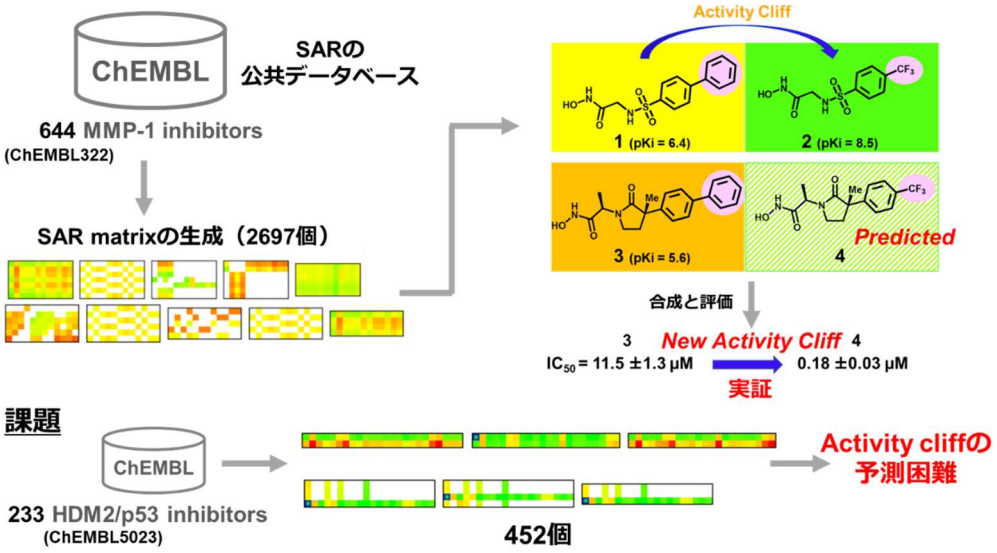


Figure 7-4. SARM を用いた SAR の網羅的解析による魅力的な活性を有する化合物の設計

第六章「カルボランの置換基配置を駆使した 3 次元ケミカルスペースの網羅的探索」では、第二章で気づいたカルボランの異性体を用いた厳密な置換基配置と、第五章で得られた網羅的な SAR の有用性と多様な母骨格の必要性を融合し、着想した「カルボランの 12 頂点から任意の 3 か所に置換基を導入することで、ケミカルスペースにおけるすべての空間を網羅する」という全く新しい分子設計戦略の概念実証を行った（Figure 7-5）。

第一に実現性は、これまでに合成例のない医薬品候補化合物の開発を志向した 3 置換カルボランを、すべての空間を網羅する 5 パターンのカルボラン骨格で合成を達成したことにより実証した。

第二に実用性は、系統的に合成した 42 化合物の PMI 解析により、5 パターンの骨格は、それぞれで異なる多様な分子形状をもつことが明らかにするとともに、新たな創薬標的である PPI を狙った生物活性評価を行い、新発見の活性を有する化合物を含めて、27 の活性化合物を見出したことで実証した。

最後に将来性について、すべての空間を探索できる3置換カルボラン化合物を用いたスクリーニングによる網羅的なSAR解析は、標的分子が未知であることが多い医薬品開発の初期段階においてを行うことで、早期に有望な分子を発見することに役立つと期待される。

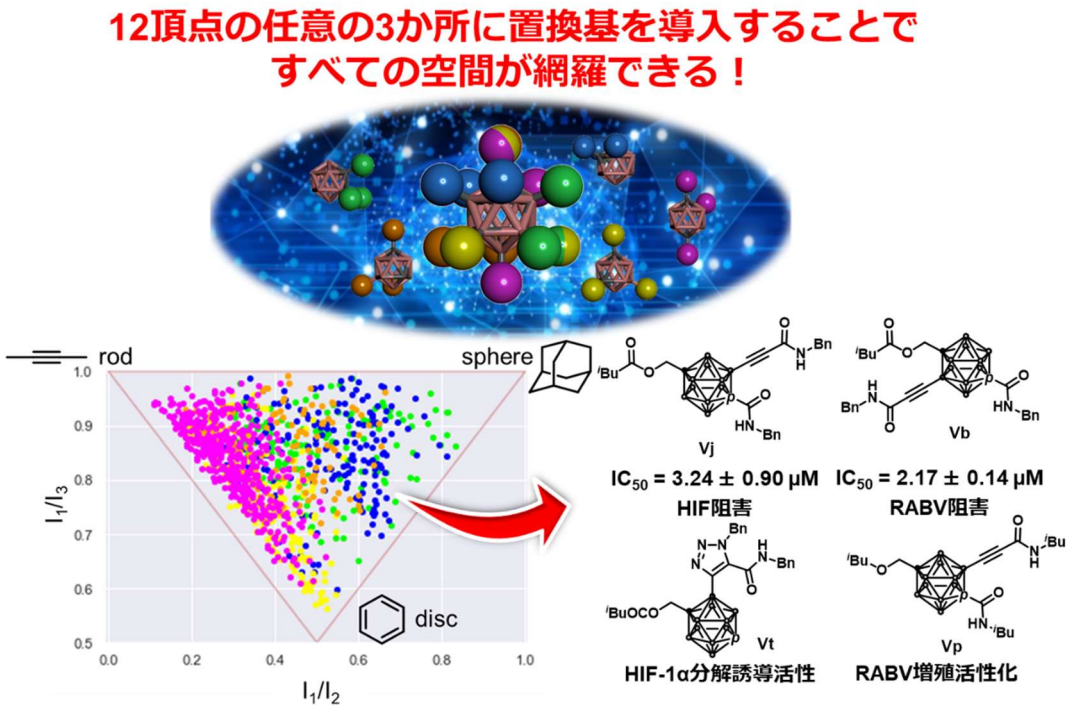


Figure 7-5. ジカルボラン骨格を有する NAMPT 阻害剤の設計（**Figure 4-2** 参照）

7-2　今後の展望

第六章で骨格 V をもつ RABV 阻害活性化合物のファーマコフォアを作成し、他の骨格の活性化合物でフィッティングさせ、その検証を行った（Figure 6-8）。このファーマコフォアには、RABV 阻害活性化合物の置換基の座標と相互作用の特徴が含まれるが、「カルボランが母骨格である」という情報は含まれていない。つまり、カルボランは置換基を 3 次元的配置させる足場（Scaffold）であり、側鎖情報だけがファーマコフォアに抽出されている。さらに、これまでにない 3 次元分子のファーマコフォアであるため、新たな新薬創成が期待できるものである。

そこで、多置換カルボランライブラリを用いた医薬品化合物スクリーニングで得られたヒット化合物に対し、Scaffold hopping[1,a]を実施することで、そのヒット化合物の多様化を図れると考えた。これを「**脱カルボラン計画**」とし、その実現方法を述べる。

1 つ目に、ファーマコフォアを満たす分子を、出来る限り多様な骨格で効率よく探索することに主眼を置いた場合、深層強化学習を用いた構造発生を利用する方法を考えた。理論上医薬品候補となりうる化合物数は 10^{60} 以上であり、到底すべてを網羅的に検証することはできない[2]。一方で、新しい 3 次元ケミカルスペースを探索するにあたり、既存の化合物ライブラリでは不十分であるため、考えられうるバーチャル化合物でスクリーニングするよりも、深層強化学習を用いた推論ベースで新規構造を探索する方が、計算コストの低減が見込めると考えた。そこで、吉森博士らが開発したファーマコフォアのフィッティングスコアを評価値として、2 つの Recurrent Neural Network（RNN）[b]を学習させる *de novo* 設計法に着目した[3]。すでに本手法は、Discoidin Domain Receptor 1（DDR1）を標的分子にして、ponatinib との共結晶構造（PDB:3ZOS）からファーマコフォアを作成し、ponatinib と異なる骨格を有する 7 化合物の活性化合物を見出すことで実証されている[4]。なお、本研究には筆者も関わらせていただいた。

Figure 7-6 にシステムの概要を示す。まず、ChEMBL に収録されている分子構造を学習させ、ドラッグライクな構造を出力する RNN（Prior Network）を構築した。続いて Prior Network を基に、もう一つの RNN（Agent Network）を、カルボラン化合物のファーマコフォアに基づくフィッティングスコアによる活性予測値が向上する分子を出力するように深層強化学習した。こうして、カルボラン化合物と同様の活性をもち、一般的でドラッグライクな分子を設計する。

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[a] Scaffold hopping：既知の活性化合物から、側鎖の構造は保持しつつ母骨格だけを変更することにより、構造的に新しい分子を発見する方法。母骨格構造に依存した毒性などを回避できる可能性があるため、医薬品開発のリスクを軽減できると期待されている。

[b] RNN：自動翻訳、自動応答など自然言語処理に使われる人工知能技術の一つ。再帰（recurrent）構造をネットワーク含むことから、予測する文字の前にある文字列を考慮した出力が可能になっており、文脈を理解したような予測を与える。化合物構造生成の場合は、構造式を文字列にした SMILES 形式（序論 **1-4** 参照）を言語として扱うことで実現できる。

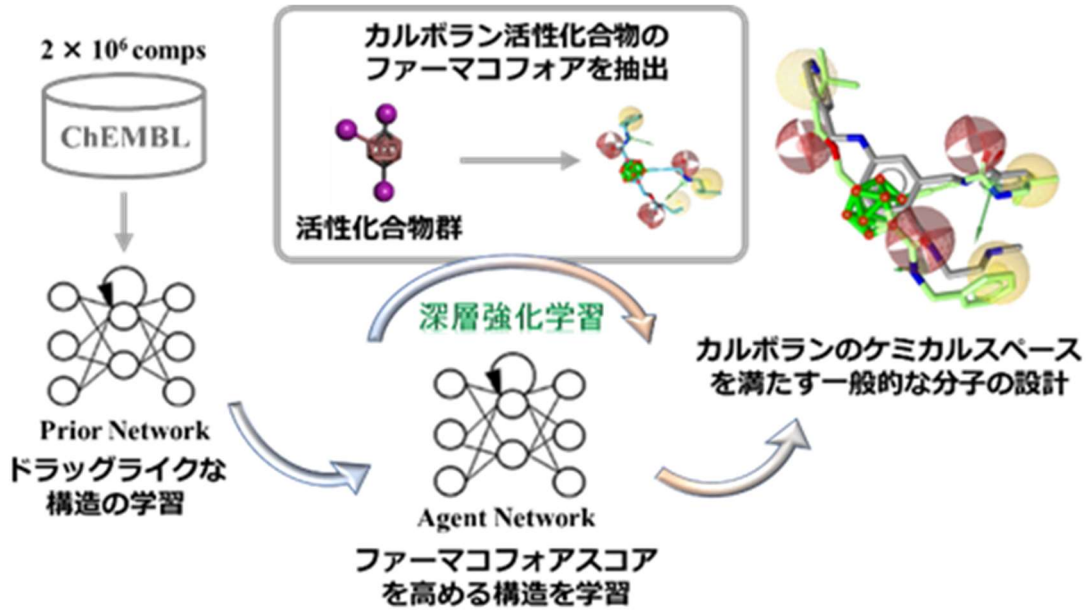


Figure 7-6. 深層強化学習を使った脱カルボラン分子の設計

2 つ目に、カルボランのファーマコフォアを満たす分子を、予め 3 次元の母骨格を固定しておき、側鎖のみをバーチャルスクリーニングで探索する方法が考えられる。これは、3 次元骨格の合成法が確立していなければ難しい方法であるが、当研究室より合成された 3 次元骨格を利用できると考えた [5,6]。Figure 7-7 に示すスキームで、バーチャルスクリーニングを実施する。①母骨格として 3 つの置換基を別々に導入できる diazatricyclododecene を利用し、②アミノ酸を模倣した 20 種類の置換基に加えて、PPI 阻害化合物に使われるの側鎖ライブラリ[c]や PPI 安定化に利用される置換基を 1000 種類選択する [7,8]。③置換基を網羅的に組み合わせて 10 億（10^9）のバーチャルライブラリを構築した後、化合物の特徴量（序論 **1-4**）でフィルタリング、④ドッキングシミュレーションやファーマコフォアフィッティングによるバーチャルスクリーニングの実施によって、合成候補化合物を絞り込む。

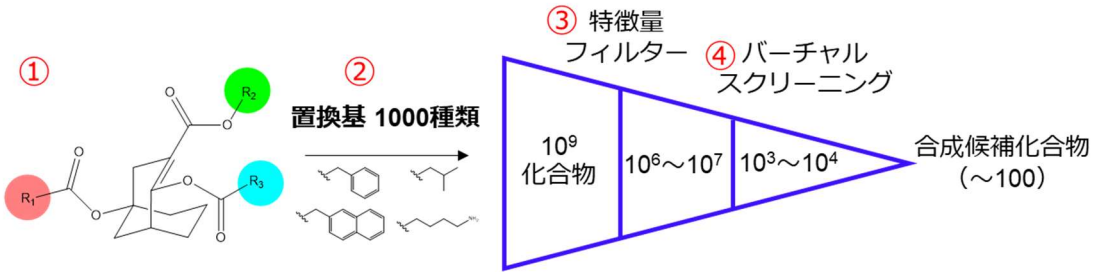


Figure 7-7. バーチャルスクリーニングを利用した脱カルボラン分子の設計

[c] LIFE CHEMICALS PPI Fragment Libarary；Lifechemicals.com

このように、本論文でカルボランという珍しい骨格をもつ分子に焦点を当てて、他の骨格では合成が困難な 3 次元空間をすべて網羅できる化合物ライブラリの構築、これまで見つからなかった生物活性化合物の発見と有用性を開拓した。しかし、その稀さゆえに、単にカルボランの応用研究という認識に留まることもある。こうしたバイアスを払拭するため、「脱カルボラン計画」のように、カルボランを使って様々な医薬分子を設計できることを実証し、多くの科学者からカルボランを認知してもらうことが、カルボラン創薬研究の展望だと確信している。

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