Electronic Supplementary Material (ESI) for ChemComm.

Supplementary information

Enantioselective synthesis of adamantylalanine and carboranylalanine and their incorporation into the proteasome inhibitor bortezomib

Gerjan de Bruin, Elliot D. Mock, Sascha Hoogendoorn, Adrianus M. C. H. van den Nieuwendijk, Jaroslaw Mazurek, Gijsbert A. van der Marel, Bogdan I. Florea and Herman S. Overkleeft.

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1. Supplemental figures and table

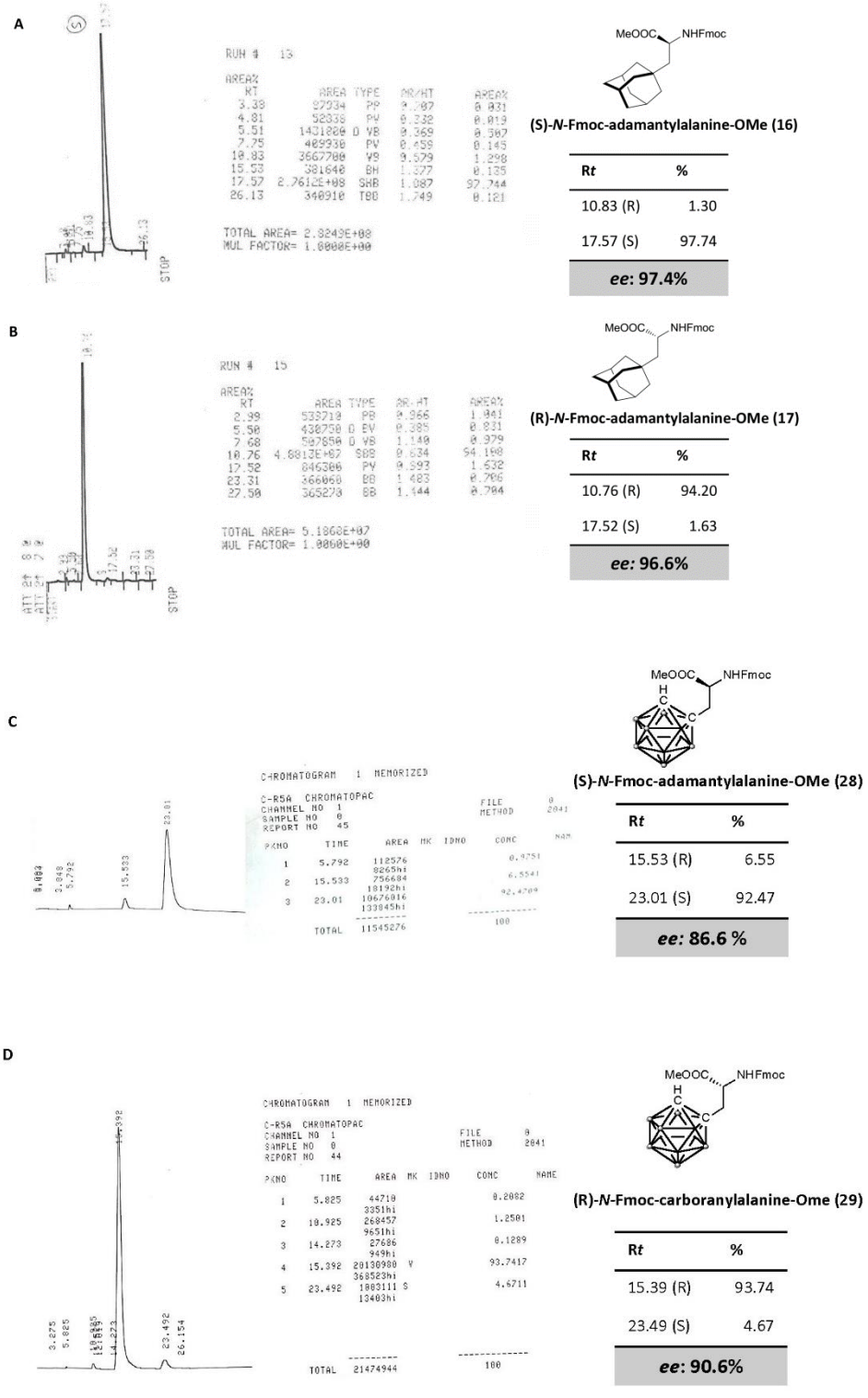


Figure S1. Chiral HPLC analysis of compounds **16** (a) and **17** (b) (Daicell Chiralcel OD (90:10 hexane/iPrOH)) and **28** (c), **29** (d) (Chiralpak AD (90:10 hexane/iPrOH)).

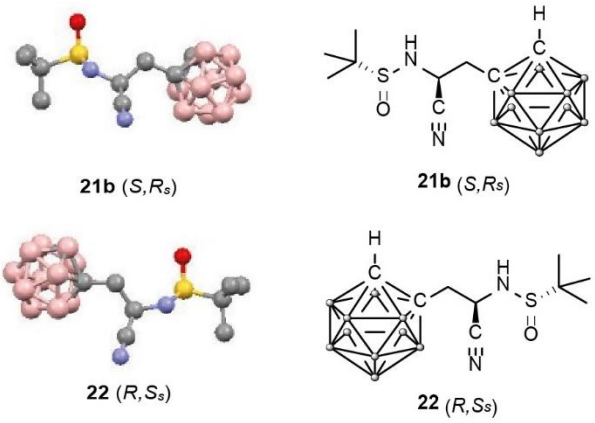


Figure S2. X-ray crystal structures of (S,Rs)-cyanosulfinamide 21b and (R,Ss)-cyanosulfinamide 22. Grey = carbon, red = oxygen, blue = nitrogen, yellow = sulfur, pink = boron.

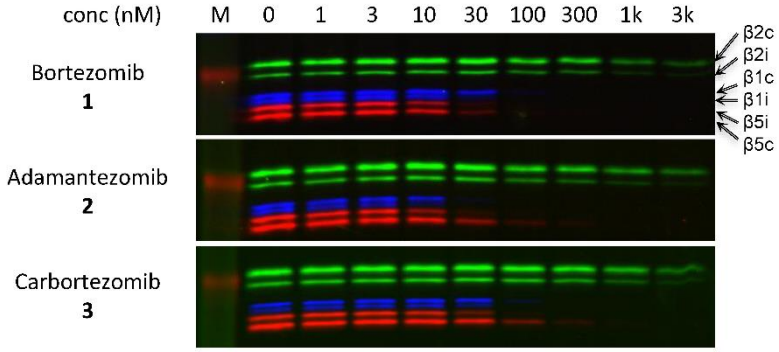


Figure S3. Inhibitory profiles of bortezomib, adamantezomib and carbortezomib in Raji cell lysate after 1 h treatment.

Raji cell-lysate	**β1c**	**β1i**	**β2c**	**β2i**	**β5c**	**β5i**
Bortezomib **1**	7.48 ± 0.04	7.85 ± 0.03	5.84 ± 0.07	6.18 ± 0.08	7.84 ± 0.04	8.03 ± 0.03
Adamantezomib **2**	7.81 ± 0.04	8.21 ± 0.03	5.87 ± 0.08	6.52 ± 0.08	7.73 ± 0.05	8.22 ± 0.03
Carbortezomib **3**	7.03 ± 0.03	7.44 ± 0.02	5.88 ± 0.04	6.28 ± 0.05	7.27 ± 0.03	7.78 ± 0.02
RPMI-8226 (intact cells)						
Bortezomib **1**	7.19 ± 0.04	7.62 ± 0.04	<6.00	<6.00	7.87 ± 0.03	7.94 ± 0.03
Adamantezomib **2**	7.70 ± 0.03	8.19 ± 0.02	<6.00	<6.00	7.30 ± 0.06	8.04 ± 0.04
Carbortezomib **3**	6.86 ± 0.03	7.22 ± 0.03	<6.00	<6.00	7.35 ± 0.03	7.98 ± 0.03

Table S1. pIC_{50} values ± standard deviation as determined in Raji cell lysates and RPMI-8226 intact cells.

2. Crystal data and structure refinement

Table 1: Crystal data and structure refinement for compound **21b** (EDM345)

Identification code	compound **21b (**EDM345)
Empirical formula	$C_9H_{24}B_{10}N_2O$ S
Formula weight	316.46
Temperature	296(2) K
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	C2
Unit cell dimensions	
a [Å]	26.2252(15)
b [Å]	6.7734(3)
c [Å]	11.5919(6)
Volume [$Å^3$]	1853.38(17)
Z	4
D_c [g/cm^3]	1.134
μ [mm^{-1}]	0.170
F(000)	664
Crystal size [mm^3]	0.420 x 0.280 x 0.150
θ range for data collection [°]	3.1 to 32.6
Index ranges	-39<=h<=39, -10<=k<=10, -17<=l<=17
Reflections collected	13344
Independent reflections	6764 [R_{int} = 0.0261]
Completeness to θ = 25.24° [%]	99.5
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	6764 / 1 / 268
Goodness-of-fit on F^2	1.079
Final R indices [I>2σ(I)]	R1 = 0.0470, wR2 = 0.1033
R indices (all data)	R1 = 0.0637, wR2 = 0.1098
Absolute structure parameter	0.01(2)
Extinction coefficient	n/a
Largest diff. peak and hole [$e/Å^3$]	0.253 and -0.167

Table 2. Hydrogen bonds for EDM345

D-H...A		D-H [Å]	H...A [Å]	D...A [Å]	D-H...A [°]
N(7)-H(7)...O(6)^{i}	0.82(3)	2.25(3)	2.946(3)	143(3)	

Symmetry transformations: (i) 0.5-x, y-0.5, -z

Table 3: Crystal data and structure refinement for compound **22** (EDM371)

Identification code	EDM371
Empirical formula	$C_9H_{24}B_{10}N_2OS \bullet H_2O$
Formula weight	334.48
Temperature	296(2)
Wavelength	0.71073
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	
a [Å]	6.7451(3)
b [Å]	11.1419(6)
c [Å]	26.4844(18)
Volume [$Å^3$]	1990.39(19)
Z	4
D_c [g/cm^3]	1.116
μ [mm^{-1}]	0.165
F(000)	704
Crystal size [mm^3]	0.35 x 0.15 x 0.08
θ range for data collection [°]	2.9 to 27.5
Index ranges	-8<=h<=7, -14<=k<=13, -34<=l<=31
Reflections collected	11885
Independent reflections	4524 [R_{int} = 0.0466]
Completeness to θ = 25.24° [%]	98.3
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4524 / 2 / 285
Goodness-of-fit on F^2	1.056
Final R indices [I>2σ(I)]	R1 = 0.0552, wR2 = 0.1418
R indices (all data)	R1 = 0.0621, wR2 = 0.1468
Absolute structure parameter	0.00(3)
Extinction coefficient	n/a
Largest diff. peak and hole [$e/Å^3$]	0.365 and -0.217

Table 4. Hydrogen bonds for compounds **22** (EDM371)

D-H...A	D-H [Å]	H...A [Å]	D...A [Å]	D-H...A [°]
N(7)-H(7)...O(24)^{i}	0.79(4)	2.08(4)	2.831(4)	160(4)
O(24)-H(24A)...O(6)	0.89(2)	1.93(3)	2.749(4)	151(6)
O(24)-H(24B)...O(6)^{ii}	0.89(2)	1.91(2)	2.771(4)	162(6)

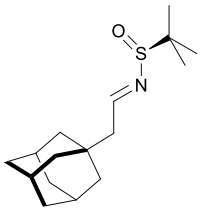
Symmetry transformations: (i) x-1, y, z; (ii) x+0.5, 1.5-y, 1-z

3. Synthetic procedures

General

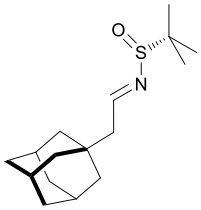
Acetonitrile (ACN), dichloromethane (DCM), N,N-dimethylformamide (DMF), methanol (MeOH), diisopropylethylamine (DiPEA) and trifluoroacetic acid (TFA) were of peptide synthesis grade, purchased at Biosolve, and used as received. All general chemicals (Fluka, Acros, Merck, Aldrich, Sigma, Iris Biotech) were used as received. Traces of water were removed from reagents used in reactions that require anhydrous conditions by co-evaporation with toluene. Column chromatography was performed on Screening Devices b.v. Silica Gel, with a particle size of 40-63 µm and pore diameter of 60 Å. TLC analysis was conducted on Merck aluminium sheets (Silica gel 60 F254). Compounds were visualized by UV absorption (254 nm), by spraying with a solution of $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid, a solution of $KMnO_4$ (20 g/L) and K_2CO_3 (10 g/L) in water, or ninhydrin (0.75 g/L) and acetic acid (12.5 mL/L) in ethanol, where appropriate, followed by charring at ca. 150 ºC. ^1H and ^{13}C-NMR spectra were recorded on a Bruker AV-400 (400 MHz) or AV-600 (600 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane, CD_3OD or $CDCl_3$ as internal standard. High resolution mass spectra were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile 50/50 (v/v) and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60,000 at m/z 400 (mass range m/z = 150-2,000) and dioctylpthalate (m/z = 391.28428) as a “lock mass”. The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). LC-MS analysis was performed on a Finnigan Surveyor HPLC system with a Gemini C_{18} 50 × 4.60 mm column (detection at 200−600 nm) coupled to a Finnigan LCQ Advantage Max mass spectrometer with ESI. The applied buffers were H_2O, MeCN and 1.0% TFA in H_2O (0.1% TFA end concentration). Methods used are: 10→90% MeCN, 15.0 min (0→0.5 min: 10% MeCN; 0.5→10.5 min: gradient time; 10.5→12.5 min: 90% MeCN; 12.5→15.0 min: 90% → 10% MeCN), HPLC purification was performed on a Gilson HPLC system coupled to a C_4 Phenomenex Gemini 5µm 250×10 mm column and a GX281 fraction collector. Chiral HPLC analysis was performed using a Daicell Chiralcel OD column (250 x 5.4 mm) or a Chiralpak AD (250 x 5.4 mm), using hexane/isopropanol solvent mixtures, flowrate: 1 mL/min. All tested compounds are >95% pure on the basis of LC-MS and NMR. (1R)-4-(1-chloro-3-methyl(butyl)-2,9,9-trimethyl-3,5-dioxa-4-bora-tricyclo[6.1.1.02,6]decane[1], boronoleucine pinanediol ester[2] and bortezomib[1] were synthesized according to literature procedures.

Synthesis of (Fmoc/Boc-adamantylalanine(-OMe)



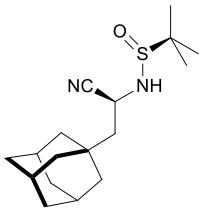
(S,E)-*N*-(2-(adamantan-1-yl)ethylidene)-*tert*-butyl-sulfinamide (7)

A solution of adamantylacetaldehyde (1.42 g, 8 mmol, 1 equiv) and (*S*)-*tert*-butylsulfinamide (1.06 g, 8.8 mmol, 1.1 equiv) in toluene (50 mL) was rotated overnight under continuous removal of water using a rotary evaporator (50°C, 100 mbar). After concentration, the crude product was purified by column chromatography (0→10% EtOAc:toluene) providing the title compound (2.02 g, 7.1 mmol, 90%). $[\alpha]_D^{21}$ = +253.8 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.09 (t, *J* = 5.9 Hz, 1H), 2.34 – 2.14 (m, 2H), 1.93 (s, 3H), 1.72 – 1.46 (m, 12H), 1.16 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.37, 56.42, 50.17, 42.56, 42.18, 36.60, 33.50, 28.50, 28.42, 22.36. HRMS: calcd. for $C_{16}H_{28}NOS$ 282.18861 $[M+H]^+$; found 282.18854.



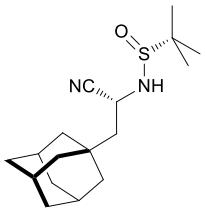
(R,E)-*N*-(2-(adamantan-1-yl)ethylidene)- *tert*-butyl-sulfinamide (8)

A solution of adamantylacetaldehyde (1.78 g, 10 mmol, 1 equiv) and (*R*)-*tert*-butylsulfinamide (1.33 g, 11 mmol, 1.1 equiv) in toluene (50 mL) was rotated overnight under continuous removal of water using a rotary evaporator (50°C, 100 mbar). After concentration, the crude product was purified by column chromatography (0→10% EtOAc:toluene) providing the title compound (2.50 g, 8.9 mmol, 89%). $[\alpha]_D^{21}$ = -242.6 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 8.09 (t, *J* = 5.9 Hz, 1H), 2.24 (m, 2H), 1.93 (s, 3H), 1.72 – 1.50 (m, 12H), 1.16 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 168.34, 56.41, 50.17, 42.57, 42.19, 36.64, 36.60, 33.50, 28.51, 28.42, 22.36. HRMS: calcd. for $C_{16}H_{28}NOS$ 282.18861 $[M+H]^+$; found 282.18849.



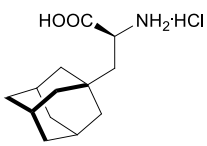
(S)-*N*-((S)-2-(adamantan-1-yl)-1-cyanoethyl)- *tert*-butyl-sulfinamide (9)

Et_2AlCN (1M in toluene, 28.3 mmol, 28.3 mL, 1.5 equiv) was added to THF (55 mL), followed by the addition of iPrOH (57 mmol, 4.3 mL, 3 equiv) resulting in some discolouration of the mixture, from red to slightly yellow. After stirring for 15 min, the Et_2AlCN/iPrOH solution was added in 25 min to a solution of sulfinamide **7** (5.32 g, 18.9 mmol, 1 equiv) in THF (120 mL) at -78°C. After stirring for 30 min at -78°C, the reaction mixture was let to warm up to RT. After stirring for 3 hour at RT, TLC showed full consumption of starting material. The reaction mixture was cooled to -78°C and quenched by the addition of 10% $NaHCO_3$ (40 mL). After warming up to RT, the mixture was diluted by $NaHCO_3$ and extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography (10→40% EtOAc:PE) provided the title compound (4.97 g, 16.1 mmol, 85%) as a 96:4 mixture of diastereomers. Recrystallization from DCM:n-hexane provided enantiomerially pure product (3.85 g, 12.5 mmol, 66%). $[\alpha]_D^{21}$ = +25.8 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 4.17 (td, *J* = 8.3, 5.2 Hz, 1H), 3.84 (d, *J* = 8.2 Hz, 1H), 2.01 – 1.93 (m, 3H), 1.84 (dd, *J* = 14.3, 8.3 Hz, 1H), 1.76 – 1.51 (m, 13H), 1.22 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.80, 57.03, 49.44, 42.33, 41.74, 36.74, 32.41, 28.45, 22.57. HRMS: calcd. for $C_{17}H_{29}N_2OS$ 309.19951 $[M+H]^+$; found 309.19955



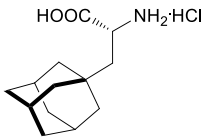
(R)-*N*-((R)-2-(adamantan-1-yl)-1-cyanoethyl)- *tert*-butyl-sulfinamide (10)

Et_2AlCN (1M in toluene, 11.85 mmol, 11.85 mL, 1.5 equiv)was added to THF (24 mL), followed by the addition of iPrOH (23.7 mmol, 1.61 mL, 3 equiv) resulting in some discolouration of the mixture, from red to slightly yellow. After stirring for 15 min, the Et_2AlCN/iPrOH solution was added in 25 min to a solution of sulfinamide **8** (2.29 g, 7.9 mmol, 1 equiv) in THF (55 mL) at -78°C. After stirring for 30 min at -78°C, the reaction mixture was let to warm up to RT. After stirring for 1 hour at RT, TLC showed full consumption of starting material. The reaction mixture was cooled to -78°C and quenched by the addition of 10% $NaHCO_3$ (20 mL). After warming up to RT, the mixture was diluted by $NaHCO_3$ and extracted with EtOAc (3x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. Column chromatography (10→30% EtOAc:PE) provided the title compound (1.86 g, 6.0 mmol, 77%) as a 96:4 mixture of diastereomers. Recrystallization from DCM:n-hexane provided enantiomerially pure product (1.51 g, 4.9 mmol, 62%). $[\alpha]_D^{21}$ = -25.8 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 4.15 (td, *J* = 8.3, 5.2 Hz, 1H), 4.04 (d, *J* = 8.4 Hz, 1H), 1.95 (m, 3H), 1.82 (dd, *J* = 14.3, 8.2 Hz, 1H), 1.73 – 1.49 (m, 13H), 1.20 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.86, 56.97, 49.33, 42.25, 41.75, 36.69, 32.33, 28.39, 22.55. HRMS: calcd. for $C_{17}H_{29}N_2OS$ 309.19951 $[M+H]^+$; found 309.19958



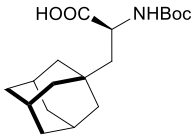
(S)-3-(adamantan-1-yl)-2-aminopropanoic acid hydrochloride (11)

Compound **9** (3.85 g, 12.5 mmol) was dissolved in 6N HCl (400 mL) and refluxed at 130°C overnight. The reaction mixture was cooled on ice, resulting in precipitation of the product. The precipitate was collected by filtration, washed with ice-cold water and dried under vacuum yielding the title product as a white solid (3.17 g, 12.2 mmol, 98%). $[\alpha]_D^{21}$ = +16.8 (C=1, MeOH). ^{1}H NMR (400 MHz, Methanol-d4) δ 3.98 (t, *J* = 5.6 Hz, 1H), 2.00 (s, 3H), 1.89 – 1.38 (m, 14H). ^{13}C NMR (101 MHz, MeOD) δ 172.97, 49.85, 46.40, 42.93, 37.71, 33.31, 29.91. HRMS: calcd. for $C_{13}H_{22}NO_2$ $[M+H]^+$ 224.16451; found 224.16451.



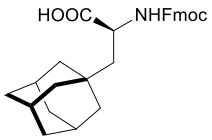
(R)-3-(adamantan-1-yl)-2-aminopropanoic acid (12)

Compound **10** (1.40 g, 4.5 mmol) was dissolved in 6N HCl (140 mL) and refluxed at 130°C overnight. The reaction mixture was cooled on ice, resulting in precipitation of the product. The precipitate was collected by filtration, washed with ice-cold water and dried under vacuum yielding the title product as a white solid (1.13 g, 4.4 mmol, 96%). $[\alpha]_D^{21}$ = -16.6 (C=1, MeOH). ^{1}H NMR (400 MHz, Methanol-d4) δ 3.98 (t, *J* = 5.5 Hz, 1H), 2.00 (s, 3H), 1.89 – 1.37 (m, 14H). ^{13}C NMR (101 MHz, MeOD) δ 172.97, 49.87, 46.41, 42.94, 37.71, 33.32, 29.92. HRMS: calcd. for $C_{13}H_{22}NO_2$ $[M+H]^+$ 224.16451; found 224.16454.



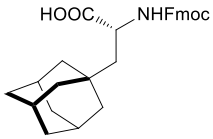
(S)-*N*-Boc-adamantylalanine-OMe (13)

To a solution of adamantyl-alanine **11** (1.0 g, 3.85 mmol, 1 equiv) in H_2O (4.5 mL) at 0°C was added Na_2CO_3 (466 mg, 8.01 mmol, 2.1 equiv). After 5 min, Boc_2O (1.68 g, 7.7 mmol, 2.0 equiv) in dioxane (13.2 mL) was added. After stirring overnight, LC-MS analysis showed incomplete conversion. Therefore, another 2 equivalent Boc_2O (1.68 g, 7.7 mmol) in dioxane (9 mL) was added and after 2 h, LC-MS showed complete consumption of the starting material. The reaction mixture was diluted with H_2O and acidified to pH=4 using 0.5N HCl, followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4 and concentrated. Purification by column chromatography (0→5% MeOH/DCM) yielded the title compound (998 mg, 3.08 mmol, 80%). $[\alpha]_D^{21}$ = -4.2 (C=0.1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.66 (s, 1H), 6.02 (s, 0.3H), 4.89 (d, *J* = 8.4 Hz, 0.7H), 4.38 (t, *J* = 7.7 Hz, 0.7H), 4.18 (s, 0.3H), 1.98 (s, 3H), 1.91 – 1.10 (m, 23H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.98, 155.28, 80.05, 49.75, 46.78, 42.24, 36.78, 32.59, 28.50, 28.31. HRMS: calcd. for $C_{18}H_{30}NO_4$ 324.21693 $[M+H]^+$; found 324.21695



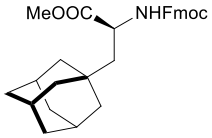
(S)-*N*-Fmoc-adamantylalanine (14)

To a solution of *S*-adamantylalanine **11** (260 mg, 1 mmol, 1 equiv) in H_2O (4.5 mL) and dioxane (3.3 mL) were added Fmoc-OSu (371 mg, 1.1 mmol, 1.1 equiv) and Na_2CO_3 (222 mg, 2.1 mmol, 2.1 equiv). After stirring overnight, the reaction mixture was diluted with H_2O and acidified to pH=2 using 1N HCl, followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4 and concentrated. Purification by column chromatography (0→2% MeOH/DCM) yielded the title compound (350 mg, 0.89 mmol, 89%). $[\alpha]_D^{21}$ = -5.6 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.83 (bs, 1H), 7.76 (d, *J* = 7.4 Hz, 2H), 7.60 (t, *J* = 8.1 Hz, 2H), 7.39 (t, *J* = 7.0 Hz, 2H), 7.30 (t, *J* = 7.3 Hz, 2H), 5.19 (d, *J* = 8.8 Hz, 1H), 4.58 – 4.33 (m, 3H), 4.24 (t, *J* = 7.0 Hz, 1H), 1.98 (s, 3H), 1.80 – 1.22 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.87, 155.93, 143.99, 143.79, 141.40, 127.80, 127.15, 125.22, 125.14, 120.07, 67.19, 50.28, 47.27, 46.66, 42.43, 36.88, 32.78, 28.62. HRMS: calcd. for $C_{28}H_{32}NO_4$ $[M+H]^+$ 446.23258; found 446.23254.



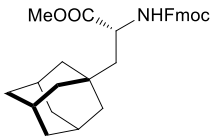
(R)-*N*-Fmoc-adamantylalanine (15)

To a solution of *R*-adamantylalanine **12** (260 mg, 1 mmol, 1 equiv) H_2O (4.5 mL) and dioxane (3.3 mL) were added Fmoc-OSu (371 mg, 1.1 mmol, 1.1 equiv) and Na_2CO_3 (222 mg, 2.1 mmol, 2.1 equiv). After stirring overnight, the reaction mixture was diluted with H_2O and acidified to pH=2 using 1N HCl, followed by extraction with EtOAc (3x). The combined organic layers were washed with brine (1x), dried over Na_2SO_4 and concentrated. Purification by column chromatography (0→2% MeOH/DCM) yielded the title compound (350 mg, 0.89 mmol, 89%) $[\alpha]_D^{21}$ = +5.6 (C=1, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.16 (bs, 1H), 7.78 (d, *J* = 7.5 Hz, 2H), 7.66 – 7.58 (m, 2H), 7.41 (t, *J* = 7.5 Hz, 2H), 7.31 (dd, *J* = 14.8, 7.4 Hz, 2H), 5.28 – 5.01 (m, 1H), 4.58 – 4.39 (m, 3H), 4.27 (t, *J* = 6.8 Hz, 1H), 2.01 (s, 3H), 1.82 – 1.28 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.11, 156.11, 144.10, 143.99, 141.57, 127.85, 127.21, 125.17 125.13, 120.10, 67.43, 50.54, 47.53, 46.87, 42.63, 37.05, 32.86, 28.84. HRMS: calcd. for $C_{28}H_{32}NO_4$ $[M+H]^+$ 446.23258; found 446.23257.



(S)-*N*-Fmoc-adamantylalanine-OMe (16)

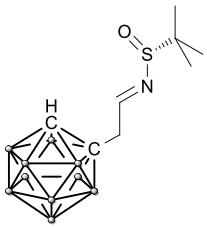
To a solution of Fmoc protected adamantyl-alanine **14** (50 mg, 0.11 mmol, 1 equiv) in MeOH (1 mL) was added slowly added $TMSCH_2N_2$ (2M in hexanes, added until solution stayed clear, 7 equiv in total). After concentration of the reaction mixture, the crude product was purified by column chromatography (0→10% EtOAc/PE) providing the title product (48 mg, 0.10 mmol, 93%). *ee*: 97.4% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralcell OD). $[\alpha]_D^{21}$ = -5.8 (C=0.5, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.77 (d, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 7.8 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.35 – 7.28 (m, 2H), 5.11 (d, *J* = 8.9 Hz, 1H), 4.53 – 4.33 (m, 3H), 4.24 (t, *J* = 7.1 Hz, 1H), 3.73 (s, 3H), 1.97 (s, 3H), 1.77 – 1.29 (m, 14H). HRMS: calcd. for $C_{29}H_{34}NO_4$ 460.24824 $[M+H]^+$; found 460.24820.



(R)-*N*-Fmoc-adamantylalanine-OMe (17)

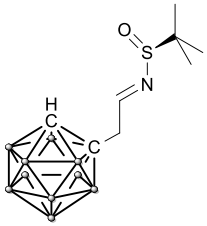
To a solution of Fmoc protected adamantylalanine **15** (50 mg, 0.11 mmol, 1 equiv) in MeOH (1 mL) was added slowly added $TMSCH_2N_2$ (2M in hexanes, added until solution stayed clear, 9 equiv in total). After concentration of the reaction mixture, the crude product was purified by column chromatography (0→10% EtOAc/PE) providing the title product (50 mg, 0.11 mmol, 99%). *ee*: 96.6% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralcell OD). $[\alpha]_D^{21}$ = +5.6 (C=0.5, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (t, *J* = 7.6 Hz, 2H), 7.40 (t, *J* = 7.5 Hz, 2H), 7.31 (td, *J* = 7.5, 1.0 Hz, 2H), 5.10 (d, *J* = 8.9 Hz, 1H), 4.55 – 4.32 (m, 3H), 4.24 (t, *J* = 7.1 Hz, 1H), 3.73 (s, 3H), 1.97 (s, 3H), 1.76 – 1.14 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.21, 155.60, 143.97, 143.78, 141.32, 127.69, 127.05, 125.12, 125.06, 119.98, 67.00, 52.36, 50.29, 47.22, 46.91, 42.39, 36.82, 32.64, 28.56. HRMS: calcd. for $C_{29}H_{34}NO_4$ 460.24824 $[M+H]^+$; found 460.24823).

Synthesis of (Fmoc/Boc-carboranylalanine(-OMe)



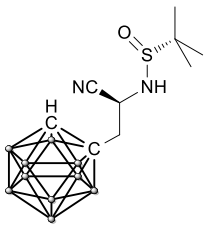
(*R*,E)- *N* -[2-(1',2'-Dicarba-*closo*-dodecaboranyl)ethylidene]-*tert*-butyl-sulfinamide (19).

To a solution of aldehyde **18** (0.96 g, 5.18 mmol, 1 equiv) in dry DCM (25 mL) was added (*R*)-*tert*-butylsulfinamide (0.69 g, 5.7 mmol, 1.1 equiv) and anhydrous $CuSO_4$ (2.65 g, 16.6 mmol, 3.2 equiv) at rt under an argon atmosphere. TLC showed complete conversion of starting material after stirring overnight. The suspension was vacuum filtrated over a Whatman glass microfiber filter, washed with DCM and concentrated under reduced pressure. Flash column chromatography (5% → 30% EtOAc in pentane) afforded the sulfinimine **19** as a white powder (1.41 g, 4.88 mmol, 94%). $[\alpha]_D^{20}$ = -231.4 (*c* = 1.0, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (t, *J* = 5.3 Hz, 1H), 3.84 (s, 1H), 3.54 – 3.29 (m, 2H,), 3.16 – 1.48 (m, 10H), 1.21 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.29, 69.85, 60.55, 57.73, 42.69, 22.55. ^{11}B NMR (128 MHz, $CDCl_3$) δ -1.82, -4.92, -8.87, -11.31, -12.69. HRMS (m/z): calcd. for $C_8H_{24}B_{10}NOS$ 290.25777 $[M+H]^+$, found 290.25791.



(*S,* E)-*N*-[2-(1',2'-Dicarba-*closo*-dodecaboranyl)ethylidene]-*tert*-butyl-sulfinamide (20).

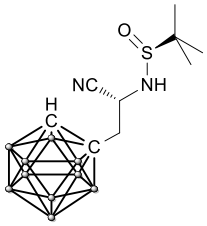
To a solution of aldehyde **18** (2.65 g, 14.2 mmol, 1 equiv) in dry DCM (70 mL) was added (*S*)-*tert*-butylsulfinamide (1.89 g, 15.6 mmol, 1.1 equiv) and anhydrous $CuSO_4$ (7.25 g, 45.4 mmol, 3.2 equiv) at rt under an argon atmosphere. TLC showed complete conversion of starting material after stirring overnight. The suspension was vacuum filtrated over a Whatman glass microfiber filter, washed with DCM and concentrated under reduced pressure. Flash column chromatography (10% → 50% EtOAc in pentane) afforded the sulfinimine **19** as a white powder (3.86 g, 13.3 mmol, 94%). $[\alpha]_D^{20}$ = +239.0° (*c* = 1.0, DCM). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (t, *J* = 5.3 Hz, 1H), 3.82 (s, 1H), 3.51 – 3.28 (m, 2H), 3.21 – 1.50 (m, 10H), 1.21 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.29, 69.84, 60.52, 57.76, 42.72, 22.58. ^{11}B NMR (128 MHz, $CDCl_3$) δ -1.87, -4.94, -8.91, -11.36, -12.78. HRMS (m/z): calcd. for $C_8H_{24}B_{10}NOS$ $[M+H]^+$ 290.25777, found 290.25797.



(S_s)-(+)-*N*-[(*R*)-1-Cyano-2-(1',2'-dicarba-*closo*-dodecaboranyl)ethyl]-*tert*-butylsulfinamide (21).

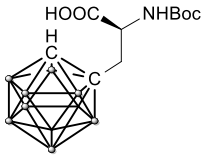
Sulfinamide **19** (1.74 g, 6.00 mmol, 1 equiv) was dissolved in dry DMF (30 mL) and cooled to -50 °C under an argon atmosphere. CsF (hygroscopic!) (1.00 g, 6.60 mmol, 1.1 equiv) was added, followed by the dropwise addition of TMSCN (0.83 mL, 6.60 mmol, 1.1 equiv). The mixture turned bright yellow and stirring was kept at -50 °C. After 24 h additional CsF (0.27 g, 1.80 mmol, 0.3 equiv) was added as well as TMSCN (0.23 mL, 1.80 mmol, 0.3 equiv). TMSCN (0.3 equiv) was added two times more after 43 h and 67 h until TLC showed complete conversion of the starting material after 71 h. The reaction was quenched with a sat. aq. NH_4Cl solution (50 mL) and water (100 mL) was added. The aqueous layer was extracted with EtOAc (3 x 150 mL) and the combined organic layers were washed with brine (1 x 400 mL), dried over $MgSO_4$, filtrated and concentrated by rotary evaporation. The product was co-evaporated three times with toluene to remove leftover DMF and purified by flash column chromatography (30% →60% EtOAc in pentane) to yield cyanosulfinamide **21** (1.77 g, 5.58 mmol, 93%) as a pale yellow

powder in a diastereomeric ratio of 93:7 (anti/syn, determined by ^{1}H NMR). Recrystallization from EtOH/pentane at -20 °C afforded cyanosulfinamide **21** as white crystals (1.03 g, 3.24 mmol, 54%) as a single diastereomer (de ≥ 99%). $[\alpha]_D^{20}$ = +62.1° (*c* = 2.2, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 4.71 (s, 1H), 4.51 (dd, *J* = 9.1, 5.0 Hz, 1H), 3.00 (dd, *J* = 15.4, 9.2 Hz, 1H), 2.90 (dd, *J* = 15.4, 5.0 Hz, 1H), 3.21 – 1.43 (m, 10H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 119.19, 72.20 $_b$), 63.72, 58.24, 48.08, 42.22, 22.68. ^{11}B NMR (128 MHz, MeOD) δ -2.38, -5.01, -9.35, -11.70, -12.75. HRMS (m/z): calcd. for $C_9H_{24}B_{10}N_2OS$ $[M+H]^+$ 317.26863, found 317.26900.



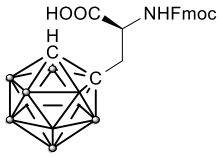
(*R*$_s$)-(+)-*N*-[(*S*)-1-Cyano-2-(1',2'-dicarba-*closo*-dodecaboranyl)ethyl]-*tert*-butylsulfinamide (22).

Sulfinamide **20** (1.01 g, 3.50 mmol, 1 equiv) was dissolved in dry DMF (18 mL) and cooled to -50 °C under an argon atmosphere. CsF (hygroscopic!) (0.69 g, 4.60 mmol, 1.3 equiv) was added, followed by the dropwise addition of TMSCN (0.57 mL, 4.60 mmol, 1.3 equiv). The mixture turned bright yellow and stirring was kept at -50 °C. After 24 h additional TMSCN (0.13 mL, 1.10 mmol, 0.3 equiv) was added and stirring was maintained at -50 °C for two days. TMSCN (0.3 equiv) was added two times more after 92 h and 97 h until TLC showed complete conversion of the starting material after 99 h. The reaction was quenched with a sat. aq. NH_4Cl solution (30 mL) and water (60 mL) was added. The aqueous layer was extracted with EtOAc (3 x 100 mL) and the combined organic layers were washed with brine (1 x 300 mL), dried over $MgSO_4$, filtrated and concentrated by rotary evaporation, which gave cyanosulfinamide **22** (1.09 g, 3.43 mmol, 98%) as an orange/yellow solid which was pure according to ^{1}H-NMR-analysis in a diastereomeric ratio of 93:7 (anti/syn, determined by ^{1}H NMR). Recrystallization from EtOH/pentane at -20 °C afforded cyanosulfinamide **22** as yellow crystals (0.54 g, 1.69 mmol, 48%) as a single diastereomer (de ≥ 99%). $[\alpha]_D^{20}$ = -59.4° (*c* = 2.2, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 4.70 (s, 1H), 4.50 (dd, *J* = 9.1, 5.0 Hz, 1H), 3.00 (dd, *J* = 15.4, 9.2 Hz, 1H), 2.89 (dd, *J* = 15.4, 5.0 Hz, 1H), 3.20 – 1.47 (m, 10H), 1.25 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 119.17, 72.15, 63.68, 58.21, 48.03, 42.18, 22.68. ^{11}B NMR (128 MHz, MeOD) δ -2.36, -4.97, -9.33, -11.71, -12.71. HRMS (m/z): calcd. for $C_9H_{25}B_{10}N_2OS$ $[M+ H]^+$ 317.26863, found 317.26905.



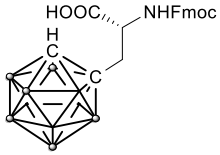
(*S*)-(-)-*N*-Boc-*o*-carboranylalanine (25).

Cyanosulfinamide **21** (520 mg, 1.64 mmol, 1 equiv) was dissolved in 6N HCl (aq., 10 mL) at rt and refluxed overnight. The mixture was co-evaporated three times with toluene to remove all solvent to afford unprotected amino acid as the HCl salt **23**. Subsequently, the amino acid was redissolved in THF/water (8 mL, 1:1) at rt under an argon atmosphere and Boc_2O (537 mg, 2.46 mmol, 1.5 equiv) and Et_3N (0.69 mL, 4.92 mmol, 3 equiv) were added and the mixture was stirred overnight. The solvent was removed under reduced pressure and co-evaporated with toluene (3x). Flash column chromatography (100% DCM → 20% MeOH in DCM) gave the Boc protected amino acid **25** as an off-white powder (462 mg, 1.39, 85%). $[\alpha]_D^{20}$ = -19.7° (*c* = 2.3, MeOH). ^{1}H NMR (400 MHz, MeOD) δ 4.52 (s, 1H), 4.15 (d, *J* = 8.8 Hz, 1H), 2.92 (d, *J* = 15.2 Hz, 1H), 2.64 (dd, *J* = 15.0, 10.6 Hz, 1H), 3.14 – 1.56 (m, 10H), 1.46 (s, 9H). ^{13}C NMR (101 MHz, MeOD) δ 157.67, 80.90, 74.51, 63.18, 54.60, 39.55, 28.71. ^{11}B NMR (128 MHz, MeOD) δ -2.64, -5.55, -9.57, -11.40, -13.00. HRMS (m/z): calcd. for $C_{10}H_{26}B_{10}NO_4$ + CH_3CN $[M+CH_3CN+H]^+$ 373.31270, found 373.31299.



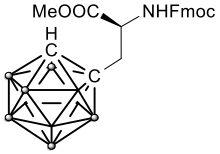
(*S*)-(-)-*N*-Fmoc-*o*-carboranylalanine (26)

Boc-carboranylalanine **25** (166 mg, 0.50 mmol, 1 equiv) was dissolved in 4M HCl in dioxane (2.5 mL, 10 mmol, 20 equiv) at rt and stirred for 1.5 h. The mixture was co-evaporated three times with toluene to remove all solvents to afford the unprotected amino acid as the HCl salt. Subsequently, the amino acid (80 mg, 0.30 mmol, 1 equiv) was redissolved in THF/water (3 mL, 1:1) at 0°C under an argon atmosphere and FmocOSu (121 mg, 0.36 mmol, 1.2 eq) and Et_3N (125 µL, 0.90 mmol, 3 eq) were added. After 1h at 0°C the mixture was stirred overnight at rt. The reaction was acidified with 0.1M HCl (30 mL) and extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (5% -> 20% MeOH in DCM) gave the Fmoc protected amino acid **26** as a clear oil (128 mg, 0.28 mmol, 94%). $[\alpha]_D^{20}$ = -9.6° (*c* = 2.3, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.75 (s, 1H), 7.74 (d, J = 7.5 Hz, 2H), 7.61 – 7.43 (m, 2H), 7.38 (t, J = 7.4 Hz, 2H), 7.28 (t, J = 7.3 Hz, 2H), 5.59 – 5.31 (m, 1H), 4.53 – 4.43 (m, 1H), 4.43 – 4.33 (m, 1H), 4.33 – 4.21 (m, 1H), 4.17 (t, J = 5.6 Hz, 1H), 3.68 (s, 1H), 2.99 (s, 12H), 2.89 (d, J = 13.6 Hz, 1H), 2.66 – 2.53 (m, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 174.17, 156.21, 143.47, 143.32, 141.39, 128.05, 127.29, 125.00, 120.23, 77.48, 77.16, 76.84, 71.69, 67.59, 61.26, 53.67, 47.04, 38.58. ^{11}B NMR (128 MHz, CDCl3) δ -2.01, -5.15, -9.15, -11.57. HRMS (m/z): calcd. for $C_{20}H_{28}B_{10}NO_4$ $[M+H]^+$ 454.30261, found 454.30215.



(*R*)-(+)-*N*-Fmoc-*o*-carboranylalanine (27)

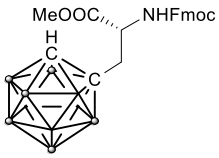
Cyanosulfinamide **22** (250 mg, 0.80 mmol, 1 eq) was dissolved in 6M HCl (aq., 8 mL) at rt and refluxed overnight. The mixture was co-evaporated three times with toluene to remove all solvents to afford the unprotected amino acid as the HCl salt. Subsequently, the amino acid (106 mg, 0.40 mmol, 1 equiv) was redissolved in THF/water (4 mL, 1:1) at 0°C under an argon atmosphere and FmocOSu (160 mg, 0.47 mmol, 1.2 eq) and Et_3N (167 µL, 1.2 mmol, 3 eq) were added. After 1h at 0°C the mixture was stirred overnight at rt. The reaction was acidified with 0.1M HCl (40 mL) and extracted with EtOAc (3 x 40 mL). The combined organic layers were washed with brine (1 x 100 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (5% -> 20% MeOH in DCM) gave the Fmoc protected amino acid **27** as a clear oil (147 mg, 0.32 mmol, 81%). $[\alpha]_D^{20}$ = +9.5° (*c* = 2.0, $CHCl_3$). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.93 (s, 1H), 7.73 (d, J = 7.5 Hz, 2H), 7.51 (d, J = 6.8 Hz, 2H), 7.37 (t, J = 7.5 Hz, 2H), 7.27 (t, J = 7.4 Hz, 2H), 5.63 – 5.46 (m, 1H), 4.52 – 4.40 (m, 1H), 4.40 – 4.28 (m, 1H), 4.28 – 4.19 (m, 1H), 4.19 – 4.09 (m, 1H), 3.67 (s, 1H), 3.10 – 1.42 (m, 10H), 2.86 (d, J = 14.5 Hz, 1H), 2.55 (dd, J = 15.3, 8.9 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 173.75, 156.34, 143.49, 143.31, 141.39, 128.07, 127.30, 125.00, 120.24, 77.48, 77.16, 76.84, 71.80, 67.60, 61.28, 53.66, 47.02, 38.54. ^{11}B NMR (128 MHz, CDCl3) δ -2.02, -5.06, -9.13, -11.49. ESI-HRMS (m/z): calcd. for $C_{20}H_{28}B_{10}NO_4$ $[M+H]^+$ 454.30261, found 454.30206.



(*S*)-(-)-*N*-Fmoc-*o*-carboranylalanine methyl ester (28)

Fmoc-carboranylalanine **26** (114 mg, 0.25 mmol, 1 equiv) was dissolved in DCM (2 mL) at 0°C under an argon atmosphere. HOBt (46 mg, 0.33 mmol, 1.3 equiv), EDC·HCl (62 mg, 0.33 mmol, 1.3 equiv) and MeOH (0.5 mL) were added and the reaction was stirred at rt overnight. The mixture was diluted with EtOAc (25 mL) and washed with 0.1M HCl (2 x 25 mL), sat. aq. $NaHCO_3$ (2 x 25 mL) and brine (1 x 25 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (10% -> 40% EtOAc in pentane) gave the fully protected amino acid **28** as a clear oil (92 mg, 0.20 mmol, 79%). $[\alpha]_D^{20}$ = -10.0° (*c* = 1.6, $CHCl_3$). *ee*: 90.6% (as

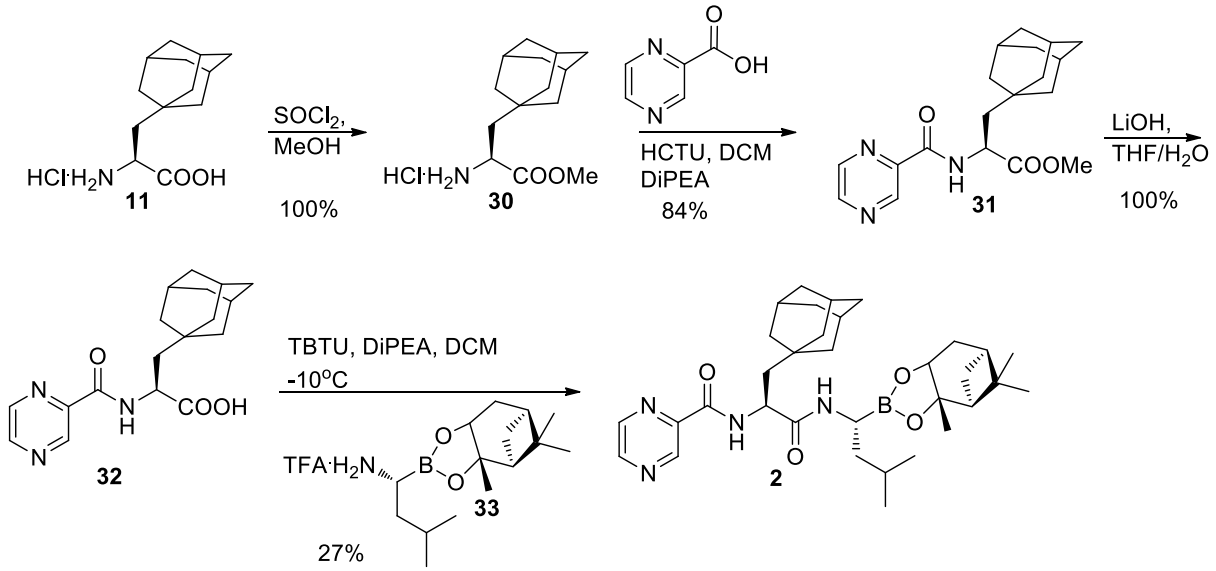
determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralpak AD). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 6.3 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.37 (d, J = 8.2 Hz, 1H), 4.57 – 4.46 (m, 1H), 4.46 – 4.37 (m, 1H), 4.31 (s, 1H), 4.20 (t, J = 6.3 Hz, 1H), 3.77 (s, 1H), 3.73 (s, 3H), 3.03 – 1.46 (m, 10H), 2.91 (d, J = 14.6 Hz, 1H), 2.61 (dd, J = 14.9, 7.9 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 170.54, 155.79, 143.61, 143.49, 141.44, 127.97, 127.25, 125.00, 120.19, 77.48, 77.16, 76.84, 71.72, 67.31, 60.96, 53.56, 53.31, 47.15, 39.07. ^{11}B NMR (128 MHz, CDCl3) δ -2.09, -5.01, -9.14, -11.41, -12.71. HRMS (m/z): calcd. for $C_{21}H_{30}B_{10}NO_4$ $[M+H]^+$ 468.31830, found 468.31741.



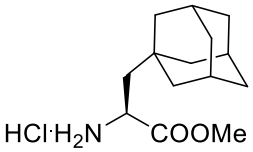
(*R*)-(+)-*N*-Fmoc-*o*-carboranylalanine methyl ester (29)

Fmoc-carboranylalanine **27** (102 mg, 0.22 mmol, 1 equiv) was dissolved in DCM (2 mL) at 0°C under an argon atmosphere. HOBt (40 mg, 0.29 mmol, 1.3 equiv), EDC·HCl (56 mg, 0.29 mmol, 1.3 equiv) and MeOH (0.5 mL) were added and the reaction was stirred at rt overnight. The mixture was diluted with EtOAc (25 mL) and washed with 0.1M HCl (2 x 25 mL), sat. aq. $NaHCO_3$ (2 x 25 mL) and brine (1 x 25 mL), dried over $MgSO_4$ and concentrated. Flash column chromatography (10% -> 40% EtOAc in pentane) gave the fully protected amino acid **29** as a clear oil (80 mg, 0.17 mmol, 78%). $[\alpha]_D^{20}$ = +10.6° (*c* = 1.6, $CHCl_3$). *ee*: 86.6% (as determined by chiral HPLC using 90:10 hexane/isopropanol, Chiralpak AD). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.76 (d, J = 7.5 Hz, 2H), 7.56 (d, J = 6.5 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J = 7.4 Hz, 2H), 5.38 (d, J = 8.3 Hz, 1H), 4.56 – 4.46 (m, 1H), 4.45 – 4.37 (m, 1H), 4.36 – 4.26 (m, 1H), 4.20 (t, J = 6.4 Hz, 1H), 3.77 (s, 1H), 3.74 (s, 3H), 3.13 – 1.36 (m, 10H), 2.91 (d, J = 13.2 Hz, 1H), 2.61 (dd, J = 15.4, 8.0 Hz, 1H). ^{13}C NMR (101 MHz, CDCl3) δ 170.58, 155.78, 143.60, 143.48, 141.43, 127.97, 127.25, 125.00, 120.19, 77.48, 77.16, 76.84, 71.70, 67.31, 60.96, 53.54, 53.32, 47.13, 39.06. ^{11}B NMR (128 MHz, CDCl3) δ -2.08, -4.98, -9.15, -11.40, -12.64. HRMS (m/z): calcd. for $C_{21}H_{30}B_{10}NO_4$ $[M+H]^+$ 468.31830, found 468.31784.

Synthesis of adamantezomib

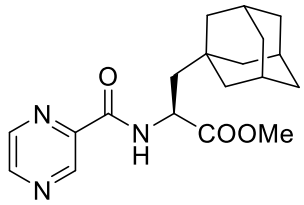


Scheme 1. Syntesis of adamantezomib 2 starting from enantiopure S-adamantylalanine



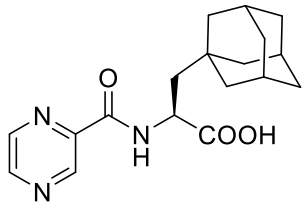
(S)-methyl 3-(adamantan-1-yl)-2-aminopropanoate hydrochloride (30)

To a solution of *S*-adamantylalanine **11** (519 mg, 2 mmol, 1 equiv) in MeOH (10 mL) was added $SOCl_2$ (435 µL, 6 mmol, 3 equiv). After refluxing for 3 hours, the solvent was removed by evaporation providing the product in a quantitative yield. ^{1}H NMR (400 MHz, Methanol-d4) δ 4.07 (t, *J* = 5.0 Hz, 1H), 3.84 (s, 3H), 1.99 (s, 3H), 1.89 – 1.65 (m, 7H), 1.63 – 1.49 (m, 7H). ^{13}C NMR (101 MHz, MeOD) δ 171.89, 53.79, 49.95, 46.16, 42.78, 37.64, 33.16, 29.80.



(S)-methyl 3-(adamantan-1-yl)-2-(pyrazine-2-carboxamido)propanoate (31)

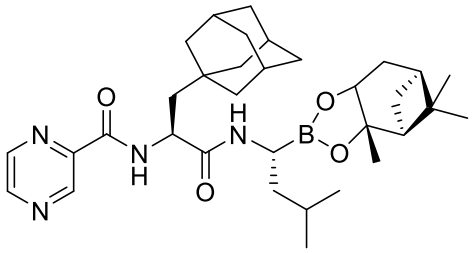
To a solution of pyrazinecarboxylic acid (74 mg, 0.6 mmol, 1.2 equiv) and HCTU (248 mg, 0.6 mmol, 1.2 equiv) in DCM was added DiPEA (304 µL, 1.75 mmol, 3.5 equiv). After stirring for 5 min, methylester **30** (137 mg, 0.5 mmol, 1 equiv) was added and the resulting mixture was stirred for 3 hours. The reaction mixture was evaporated and dissolved in EtOAc and washed with 0.1N HCl (2x) and sat. $NaHCO_3$ (2x), dried over Na_2SO_4 and concentrated. Column chromatography (30→50% EtOAc:PE) provided the title compound (144 mg, 0.42 mmol, 84%). ^{1}H NMR (400 MHz, Chloroform-d) δ 9.36 (d, *J* = 1.1 Hz, 1H), 8.73 (d, *J* = 2.4 Hz, 1H), 8.59 – 8.47 (m, 1H), 8.01 (d, *J* = 8.5 Hz, 1H), 4.82 (td, *J* = 8.8, 3.3 Hz, 1H), 3.71 (s, 3H), 1.91 (s, 3H), 1.74 (dd, *J* = 14.6, 3.3 Hz, 1H), 1.58 (dt, *J* = 31.6, 11.9 Hz, 13H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.48, 162.48, 147.53, 144.54, 144.05, 142.79, 52.56, 48.50, 46.83, 42.31, 36.78, 32.73, 28.51. HRMS (m/z): calcd. for $C_{18}H_{26}N_3O_3$ $[M+H]^+$ 330.18122, found 330.18118



(S)-3-(adamantan-1-yl)-2-(pyrazine-2-carboxamido)propanoic acid 32

To a solution of methyl ester **31** (144 mg, 0.42 mmol) in THF (5 mL) was added LiOH (11 mg, 0.46 mmol, 1.1 equiv) in H_2O (1 mL). After 1.5 hours, 4 mg LiOH was added since TLC-analysis showed remaining starting

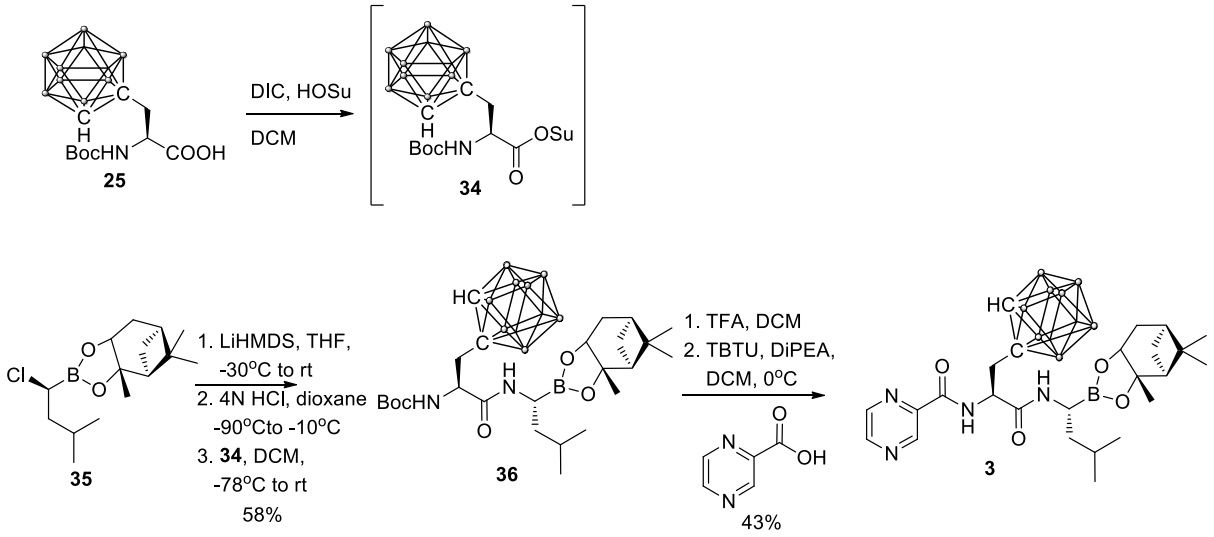
material. After 15 min, TLC-analysis showed complete conversion of starting material and the reaction mixture was diluted by the addition of EtOAc. 1N HCl was added and the mixture was extracted with EtOAc (2x). The combined organic layers were dried over Na_2SO_4, filtered and concentrated, providing the title compound in a quantitative yield. ^{1}H NMR (400 MHz, Chloroform-d) δ 10.47 (bs, 1H), 9.36 (s, 1H), 8.76 (s, 1H), 8.56 (s, 1H), 8.06 (d, *J* = 8.2 Hz, 1H), 4.83 (s, 1H), 1.91 (s, 3H), 1.83 (d, *J* = 14.0 Hz, 1H), 1.69 – 1.45 (m, 13H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.70, 162.44, 146.97, 144.08, 143.96, 142.95, 48.36, 46.42, 42.11, 36.59, 32.61, 29.57, 28.33.



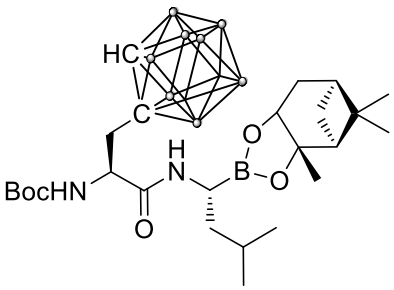
(1*S*, 2*S*, 3*R*, 5*S*) - Pinanediol - *N* - pyrazinoyl - L - adamantylalanine - L - boronoleucine 'Adamantezomib' (2)

To a solution of TBTU (35.3 mg, 0.11 mmol, 1.1 equiv), boronoleucine **33** (33.7 mg, 0.1 mmol, 1 equiv) and dipeptide **32** in DCM at -10°C was added DiPEA (52.3 µL, 0.3 mmol, 3 equiv). After stirring for 2 hours at -10°C, TLC analysis (5% MeOH:DCM) indicated complete conversion of starting material. The reaction mixture was concentrated and the residue was dissolved in EtOAc, washed with 0.1N HCl (2x), sat. $NaHCO_3$ (2x) and brine, dried over Na_2SO_4, filtered and concentrated. Column chromography (0→2% MeOH:DCM) followed by HPLC purification (C4, 50-90% MeCN, 0.1 % TFA, 10 min gradient) provided the title compound (15.67 mg, 0.027 mmol, 27%). ^{1}H NMR (600 MHz, Chloroform-d) δ 9.40 (d, *J* = 1.3 Hz, 1H), 8.79 (d, *J* = 2.4 Hz, 1H), 8.56 (dd, *J* = 2.3, 1.6 Hz, 1H), 8.05 (d, *J* = 8.6 Hz, 1H), 6.44 (d, *J* = 4.7 Hz, 1H), 4.71 (td, *J* = 8.0, 4.9 Hz, 1H), 4.31 (dd, *J* = 8.8, 2.0 Hz, 1H), 3.21 (dt, *J* = 9.1, 5.9 Hz, 1H), 2.33 (ddt, *J* = 13.9, 8.8, 2.3 Hz, 1H), 2.22 – 2.15 (m, 1H), 2.02 – 1.94 (m, 5H), 1.92 (tt, *J* = 5.8, 3.1 Hz, 1H), 1.85 (dt, *J* = 14.5, 2.6 Hz, 1H), 1.73 – 1.60 (m, 7H), 1.59 – 1.56 (m, 6H), 1.56 – 1.43 (m, 3H), 1.40 (s, 3H), 1.30 (s, 3H), 1.27 (d, *J* = 10.8 Hz, 1H), 0.89 (t, *J* = 6.4 Hz, 6H), 0.86 (s, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.68, 162.78, 147.62, 144.54, 144.15, 142.89, 85.74, 77.84, 51.58, 48.62, 45.93, 42.55, 40.17, 39.78, 38.32, 36.96, 35.75, 32.41, 28.74, 28.67, 27.29, 26.51, 25.75, 24.21, 23.14, 22.26. LC-MS (linear gradient 50 → 90% MeCN, 0.1% TFA, 15 min): R_t (min): 9.30 (ESI-MS (m/z): 577.20. HRMS (m/z): calcd. for $C_{33}H_{50}BN_4O_4$ $[M+H]^+$ 577.39196, found 577.39203

Synthesis of carbortezomib

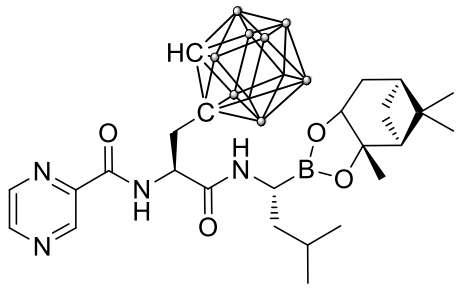


*Scheme 2. Synthesis of carbortezomib **3** starting from enantiopure L-N-Boc-carboranylalanine*



(1*S*,2*S*,3*R*,5*S*)-Pinanediol -*N*- Boc-L-carboranylalanine-L-borono-leucine (36)

N-Boc carboranylalanine (83 mg, 0.25 mmol, 1.25 equiv) in dry CH_2Cl_2 (0.2M) under an argon atmosphere at rt, was treated with *N*-hydroxysuccinimide (52 mg, 0.45 mmol, 2.25 equiv) and *N*,*N*'-diisopropylcarbodiimide (57 mg, 0.45 mmol, 2.25 equiv). The mixture was stirred until TLC showed complete conversion of the starting material, after 6 h, yielding the crude OSu ester. Separately, chloroboronate **35** (57 mg, 0.2 mmol, 1 equiv) was dissolved in dry THF (0.2M) at -30 °C under an argon atmosphere and treated with LiHMDS (0.26 mL, 1M in THF, 1.3 equiv). The mixture was slowly warmed to rt and re-cooled to -90 °C when TLC indicated complete conversion of the starting material typically after 5 h. HCl (0.23 mL, 4N in 1,4-dioxane, 4.5 equiv) was added and the reaction was allowed to warm to -10°C. The mixture was cooled again to -80 °C and DiPEA (12 equiv) was added, followed by the crude OSu ester solution. The reaction was stirred overnight and allowed to warm up to rt. The mixture was filtrated over a Whatmann glass microfiber filter and concentrated by rotary evaporation. Column chromatography (10% → 30% EtOAc in pentane) afforded dipeptide **36** as a colourless oil (67 mg, 0.12 mmol, 58%). ^{1}H NMR (400 MHz, MeOD) δ 4.55 (s, 1H, $C_{Carb}H$), 4.34 (dd, J = 9.6, 3.5 Hz, 1H), 4.21 (dd, J = 8.5, 1.7 Hz, 1H), 3.03 – 1.58 (m, 10H), 2.87 – 2.76 (m, 2H), 2.62 (dd, J = 15.6, 9.7 Hz, 1H), 2.40 – 2.30 (m, 1H), 2.19 – 2.11 (m, 1H), 1.96 (t, J = 5.5 Hz, 1H), 1.91 – 1.85 (m, 1H), 1.79 (dt, J = 14.3, 2.6 Hz, 1H), 1.75 – 1.67 (m, 1H), 1.46 (s, 9H), 1.42 – 1.32 (m, 6H), 1.29 (s, 3H), 0.92 (dd, J = 6.5, 3.6 Hz, 6H), 0.88 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 175.22, 156.99, 85.16, 81.27, 77.82, 73.91, 63.32, 53.23, 52.88, 41.16), 41.12, 40.97 (HSQC confirmed), 39.56, 39.21, 37.22, 29.49, 28.68, 27.68, 27.33, 26.61, 24.54, 23.43, 22.48. ^{11}B NMR (128 MHz, MeOD) δ 21.56, -2.51, -5.57, -9.57, -11.81, -13.11. HRMS (m/z): calcd. for $C_{25}H_{51}B_{11}N_2O_5$ $[M+H]^+$ 579.49725, found 579.49817.



(1*S*, 2*S*, 3*R*, 5*S*) - Pinanediol - *N* - pyrazinoyl - L - carboranylalanine - L - boronoleucine 'carbortezomib' (3). Dipeptide **52** (23 mg, 40 µmol, 1 equiv) was dissolved in dry DCM/TFA (1:1, 1.5 mL) at rt under an argon atmosphere. After 40 min the solvents were removed by co-evaporation with toluene (3x) and the deprotected dipeptide re-dissolved in dry DCM (1.5 mL) and cooled to 0 °C. 2-Pyrazinecarboxylic acid (8 mg, 60 µmol, 1.5 equiv), TBTU (20 mg, 60 µmol, 1.5 equiv) and DiPEA (20 µL, 120 µmol, 3 equiv) were added and the mixture was stirred for 1 h at 0 °C. The DCM was removed by rotary evaporation and redissolved in EtOAc (20 mL). The organic layer was washed with 0.1M aq. HCl (2 x 20 mL), 2% aq. $NaCO_3$ (2 x 20 mL) and brine (1 x 20 mL). The EtOAc layer was then dried over $MgSO_4$, filtrated and concentrated by rotary evaporation. Flash column chromatography (20 → 50% EtOAc in pentane) followed by HPLC purification (C_{18}, 80→86% MeCN, 0.1 % TFA, 12 min gradient) and lyophilisation, afforded the title compound **3** as a white powder (10.08 mg, 17.24 µmol, 43%). ^{1}H NMR at 303 K (600 MHz, MeOD) δ 9.26 (d, *J* = 1.3 Hz, 1H), 8.82 (d, *J* = 2.4 Hz, 1H), 8.76 – 8.68 (m, 1H), 4.86 (dd, *J* = 8.8, 4.3 Hz, 1H), 4.59 (s, 1H), 4.25 (dd, *J* = 8.7, 1.9 Hz, 1H), 3.04 (dd, *J* = 15.8, 4.3 Hz, 1H), 2.97 – 2.88 (m, 2H), 2.78 – 1.57 (m, 10H), 2.38 – 2.32 (m, 1H), 2.19 – 2.13 (m, 1H), 1.95 (t, *J* = 5.5 Hz, 1H), 1.90 – 1.85 (m, 1H), 1.79 (dt, *J* = 14.3, 2.5 Hz, 1H), 1.69 (dtt, *J* = 20.5, 13.3, 6.6 Hz, 1H), 1.47 – 1.36 (m, 2H), 1.35 (s, 3H), 1.35 – 1.32 (m, 1H), 1.29 (s, 3H, $C_qC\mathit{H}_3CH_3$), 0.91 – 0.84 (m, 9H). ^{13}C NMR (151 MHz, MeOD) δ 173.52, 165.38, 148.92, 145.71, 144.99, 144.86, 85.72, 78.23, 73.97, 63.65, 53.15, 52.35, 41.13, 40.94, 40.28, 39.30, 39.24, 37.03, 29.35, 27.63, 27.30, 26.60, 24.47, 23.28, 22.52. ^{11}B NMR (128 MHz, MeOD) δ 22.40, -2.51, -5.47, -9.54, -11.59, -12.94. . LC-MS (linear gradient 50 → 90% MeCN, 0.1% TFA, 15 min): R_t (min): 9.81 (ESI-MS (m/z): 585.20. HRMS : calcd. for $C_{25}H_{46}B_{11}N_4O_4$ $[M+H]^+$ 585.46237, found 585.46251.

4. Biochemical methods

General methods

Lysates of cells were prepared by treating cell pellets with 4 volumes of lysis buffer containing 50 mM Tris pH 7.5, 2 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 2 mM ATP, and 0.05% digitonin for 15-60 min. Protein concentration was determined using Qubit® protein assay kit (Thermofisher). All cell lysate labelling experiments were performed in assay buffer containing 50 mM Tris pH 7.5, 2 mM DTT, 5 mM $MgCl_2$, 10% glycerol, 2 mM ATP. Cell lysate labelling and competition experiments were performed at 37°C. The 10x concentrated ABP cocktail is composed of: 1 µM Cy5-NC-001, 0.3 µM BODIPY(FL)-LU-112, 1 µM BODIPY(TMR)-NC-005-VS, mixed in DMSO. Prior to fractionation on 12.5% SDS-PAGE (TRIS/glycine), samples were boiled for 3 min in a reducing gel loading buffer. The 7.5x10 cm (L x W) gels were run for 15 min at 80V followed by 120 min at 130V. In-gel fluorescence in the wet gel slabs was directly detected on a ChemiDoc™ MP System using Cy2 setting to detect BODIPY(FL)-LU-112, Cy3 settings to detect BODIPY(TMR)-NC-005-VS and Cy5 settings to detect Cy5-NC-001.

Competition experiments in cell lysate

Cell lysates (diluted to 10-15 µg total protein in 9 µL buffer) were exposed to the inhibitors (10x stock in DMSO) at indicated concentrations for 1 h at 37 °C, followed by addition of probe cocktail (1.1 µL) and SDS-PAGE as described in general methods. Intensities of bands were measured by fluorescent densitometry and divided by the intensity of bands in mock-treated extracts. Average values of three independent experiments were plotted against inhibitor concentrations. IC_{50} (inhibitor concentrations giving 50% inhibition) values were calculated using GraphPad Prism software

Competition experiments in living RPMI-8226 cells

RPMI-8226 were cultured in RPMI-1640 media supplemented with 10% fetal calf serum, GlutaMAX™, penicillin, streptomycin in a 5% CO_2 humidified incubator. 5-8 × 10^5 cells/mL were exposed to inhibitors for 1 h at 37 °C. Cells were harvested and washed twice with PBS. Cell pellets were treated with lysis buffer on ice for 15 min, followed by centrifugation at 14000 rpm for 10 min. Proteasome inhibition in the obtained cell lysates was determined using the method described above (60 min incubation with ABP cocktail). Intensities of bands were measured by fluorescent densitometry and divided by the intensity of bands in mock-treated extracts. Average values of three independent experiments were plotted against inhibitor concentrations. IC_{50} (inhibitor concentrations giving 50% inhibition) values were calculated using GraphPad Prism software.

Inhibitor washout experiments

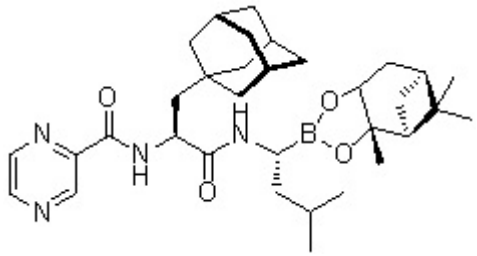
5x 10^5 RPMI-8226 cells were treated with 1 µM of inhibitor (1% DMSO end concentration) at 37°C. After 1 h, the cells were washed with medium (2x) and incubated at 37°C for 0, 2 or 4 hours. The cells were harvested and washed with PBS, lysed in standard lysis buffer for 15 min, followed by centrifugation at 14000 rpm for 5 min. Proteasome inhibition in the obtained cell lysates was determined using the method described above (30 min incubation with ABP cocktail). Intensities of bands were measured by fluorescent densitometry and divided by the intensity of bands in mock-treated extracts and corrected for gel loading using Coomassie staining. Average values of three independent experiments are reported.

5. References

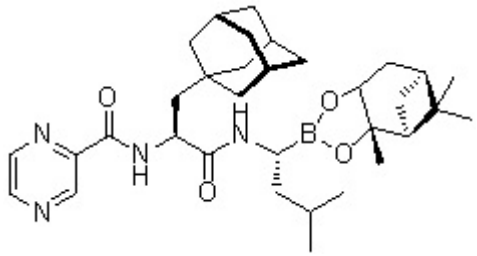
1. M. Verdoes, B. I. Florea, V. Menendez-Benito, C. J. Maynard, M. D. Witte, W. A. van der Linden, A. M. C. H. van den Nieuwendijk, T. Hofmann, C. R. Berkers, F. W. B. van Leeuwen, T. A. Groothuis, M. A. Leeuwenburgh, H. Ovaa, J. J. Neefjes, D. V. Filippov, G. A. van der Marel, N. P. Dantuma and H. S. Overkleeft, *Chem. Biol.*, 2006, **13**, 1217-1226.
2. A. S. Ivanov, A. A. Zhalnina and S. V. Shishkov, *Tetrahedron*, 2009, **65**, 7105-7108.

6. NMR and LC/MS spectra

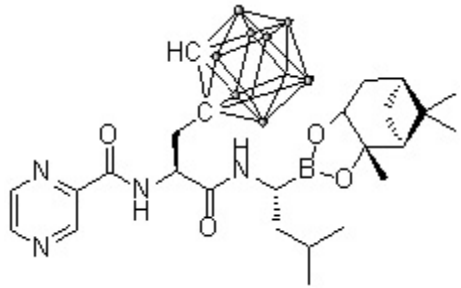
2 ^{1}H NMR (600 MHz, Chloroform-d)



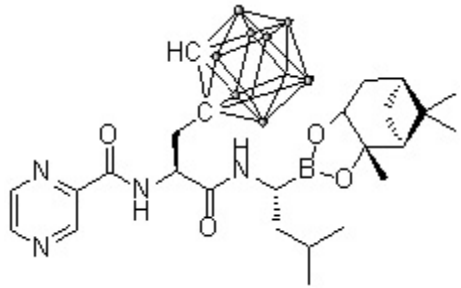
2 ^{13}C NMR (150 MHz, Chloroform-d)



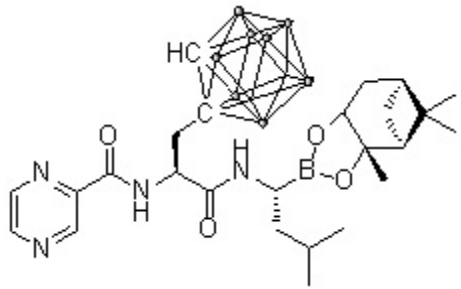
3 ^{1}H NMR (400 MHz, Methanol-d)



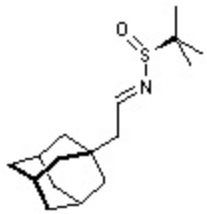
3 ^{11}B NMR (128 MHz, Methanol-d)



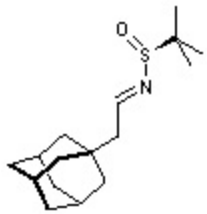
3 ^{13}C NMR (100 MHz, Methanol-d)



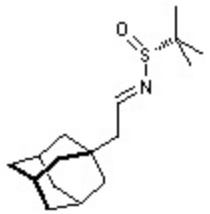
7 ^{1}H NMR (400 MHz, Chloroform-d)



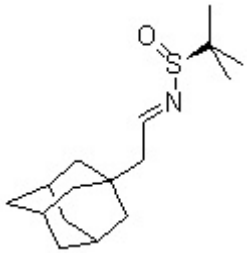
7 ^{13}C NMR (100 MHz, Chloroform-d)



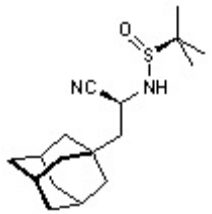
8 ^{1}H NMR (400 MHz, Chloroform-d)



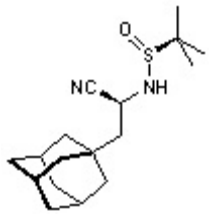
8 ^{13}C NMR (100 MHz, Chloroform-d)



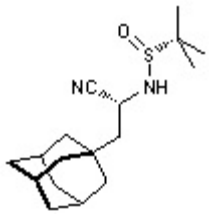
9 ^{1}H NMR (400 MHz, Chloroform-d)



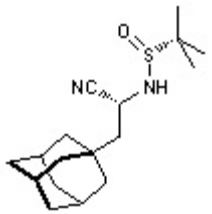
9 ^{13}C NMR (100 MHz, Chloroform-d)



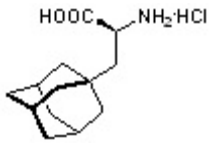
10 ^{1}H NMR (400 MHz, Chloroform-d)



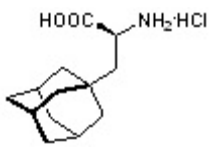
10 ^{13}C NMR (100 MHz, Chloroform-d)



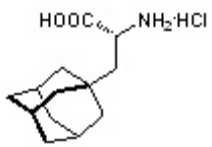
11 ^{1}H NMR (400 MHz, Methanol-d)



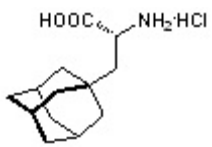
11 ^{13}C NMR (100 MHz, Methanol-d)



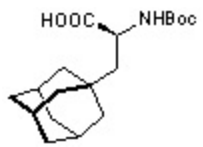
12 ^{1}H NMR (400 MHz, Methanol-d)



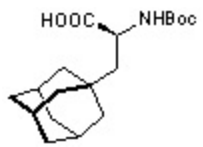
12 ^{13}C NMR (100 MHz, Methanol-d)



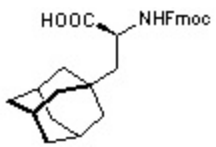
13 ^{1}H NMR (400 MHz, Chloroform-d)



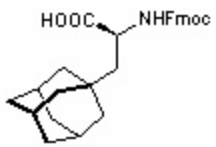
13 ^{13}C NMR (100 MHz, Chloroform-d)



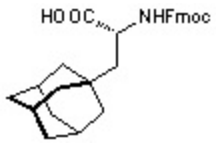
14 ^{1}H NMR (400 MHz, Chloroform-d)



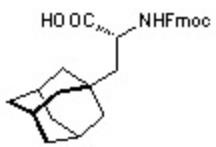
14 ^{13}C NMR (100 MHz, Chloroform-d)



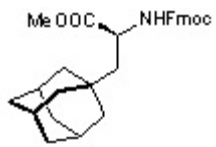
15 ^{1}H NMR (400 MHz, Chloroform-d)



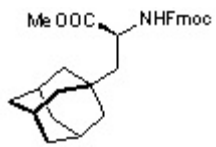
15 ^{13}C NMR (100 MHz, Chloroform-d)



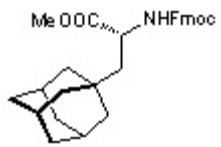
16 ^{1}H NMR (400 MHz, Chloroform-d)



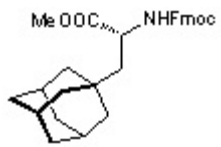
16 ^{13}C NMR (100 MHz, Chloroform-d)



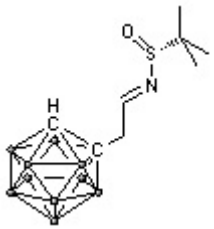
17 ^{1}H NMR (400 MHz, Chloroform-d)



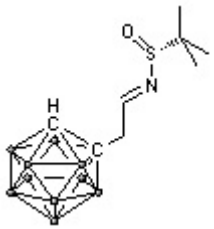
17 ^{13}C NMR (100 MHz, Chloroform-d)



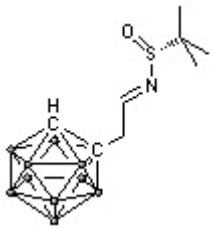
19 ^{1}H NMR (400 MHz, Chloroform-d)



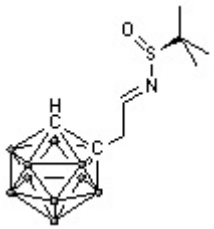
19 ^{11}B NMR (128 MHz, Chloroform-d)



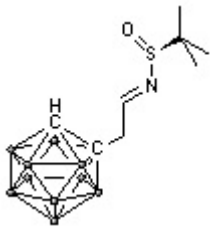
19 ^{13}C NMR (100 MHz, Chloroform-d)



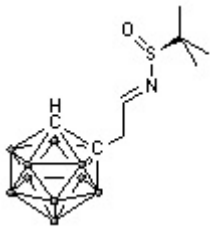
20 ^{11}B NMR (128 MHz, Chloroform-d)



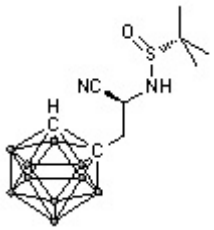
20 ^{1}H NMR (400 MHz, Chloroform-d)



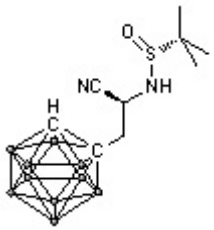
20 ^{13}C NMR (100 MHz, Chloroform-d)



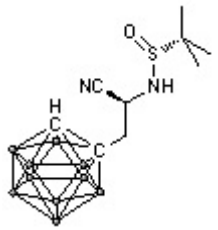
21 ^{1}H NMR (400 MHz, Methanol-d)



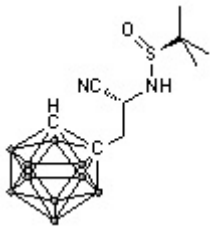
21 ^{11}B NMR (128 MHz, Methanol-d)



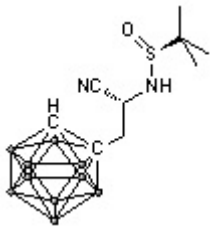
21 ^{13}C NMR (100 MHz, Methanol-d)



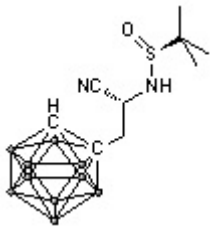
22 ^{1}H NMR (400 MHz, Methanol-d)



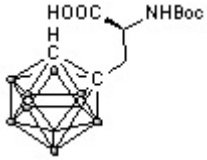
22 ^{11}B NMR (128 MHz, Methanol-d)



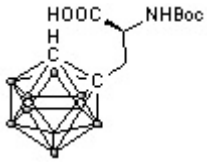
22 ^{13}C NMR (100 MHz, Methanol-d)



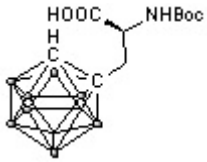
25 ^{1}H NMR (400 MHz, Methanol-d)



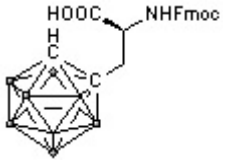
25 ^{11}B NMR (128 MHz, Methanol-d)



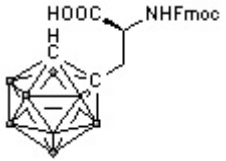
25 ^{13}C NMR (100 MHz, Methanol-d)

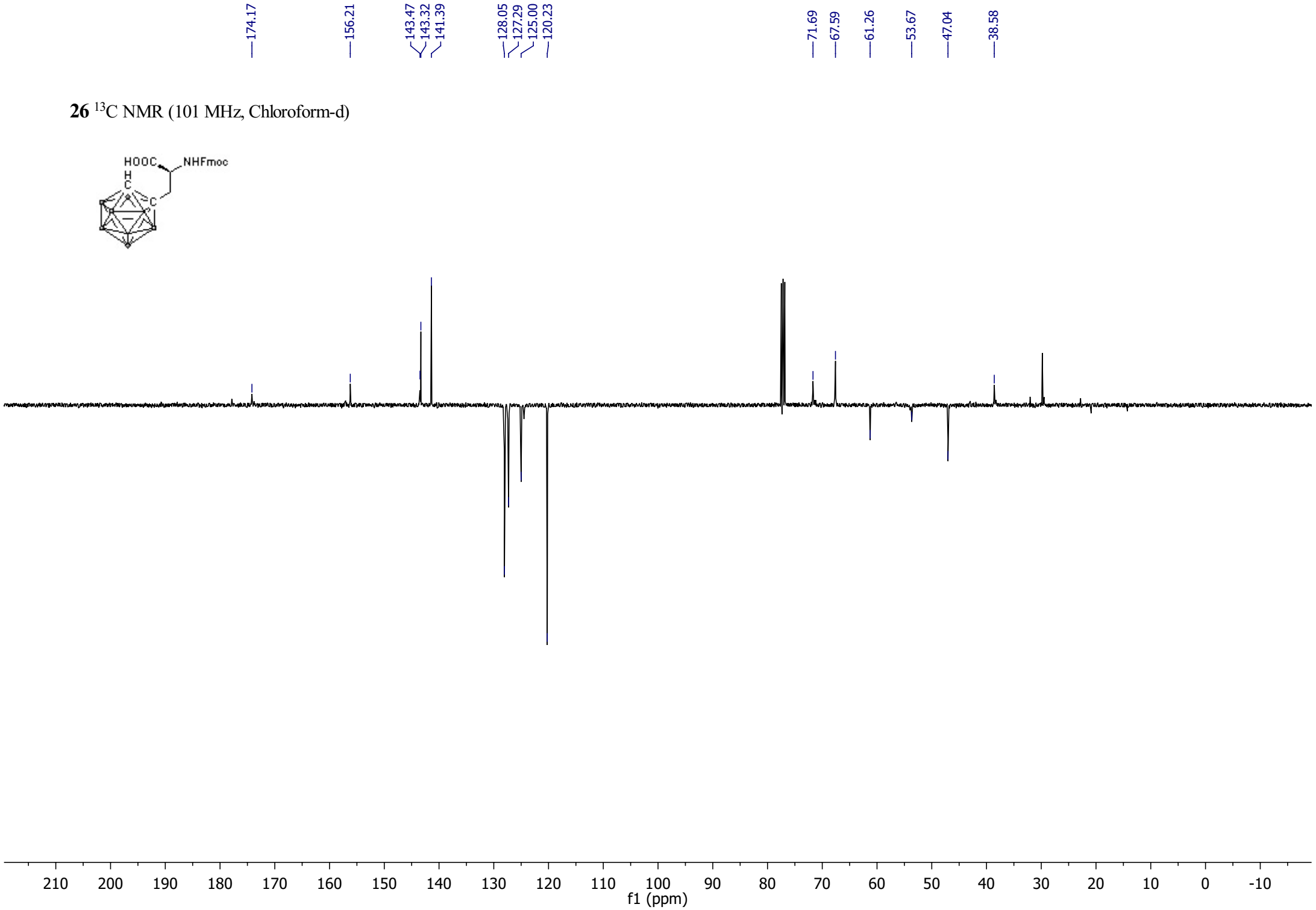


26 ^{1}H NMR (400 MHz, Chloroform-d)



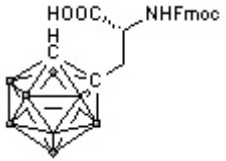
26 ^{11}B NMR (128 MHz, Chloroform-d)



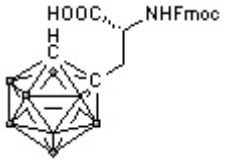


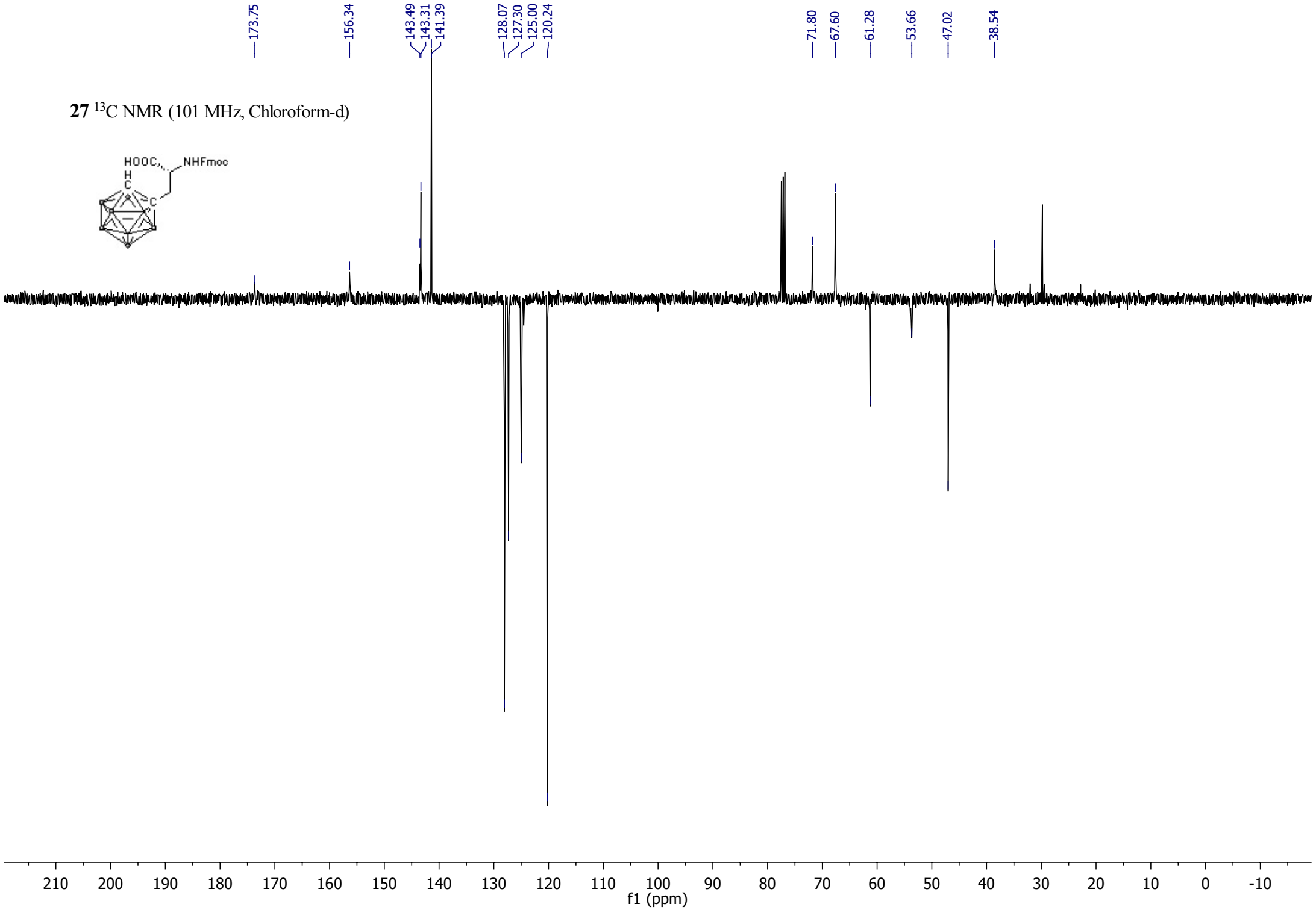
26 ^{13}C NMR (101 MHz, Chloroform-d)

27 ^{1}H NMR (400 MHz, Chloroform-d)

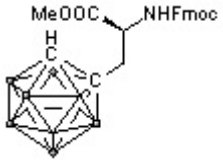


27 ^{11}B NMR (128 MHz, Chloroform-d)

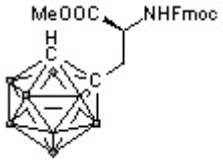


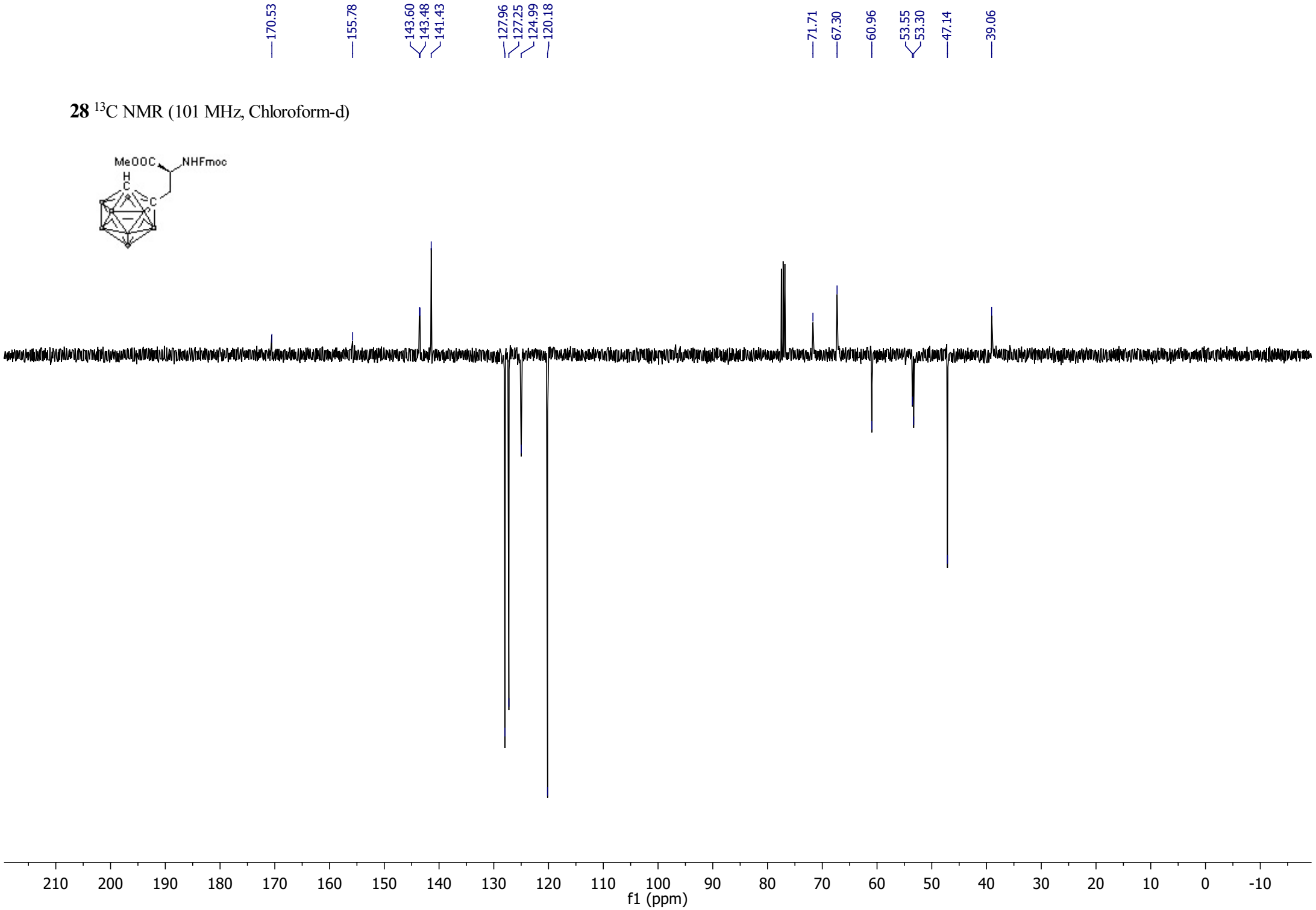

173.75
156.34
143.49
143.31
141.39
128.07
127.30
125.00
120.24
71.80
67.60
61.28
53.66
47.02
38.54
27 13C NMR (101 MHz, Chloroform-d)
HOOC
NHFmoc
H
C
C
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

28 ^{1}H NMR (400 MHz, Chloroform-d)



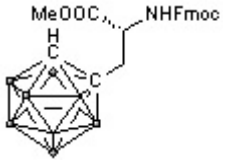
28 ^{11}B NMR (128 MHz, Chloroform-d)



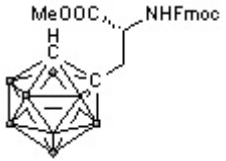


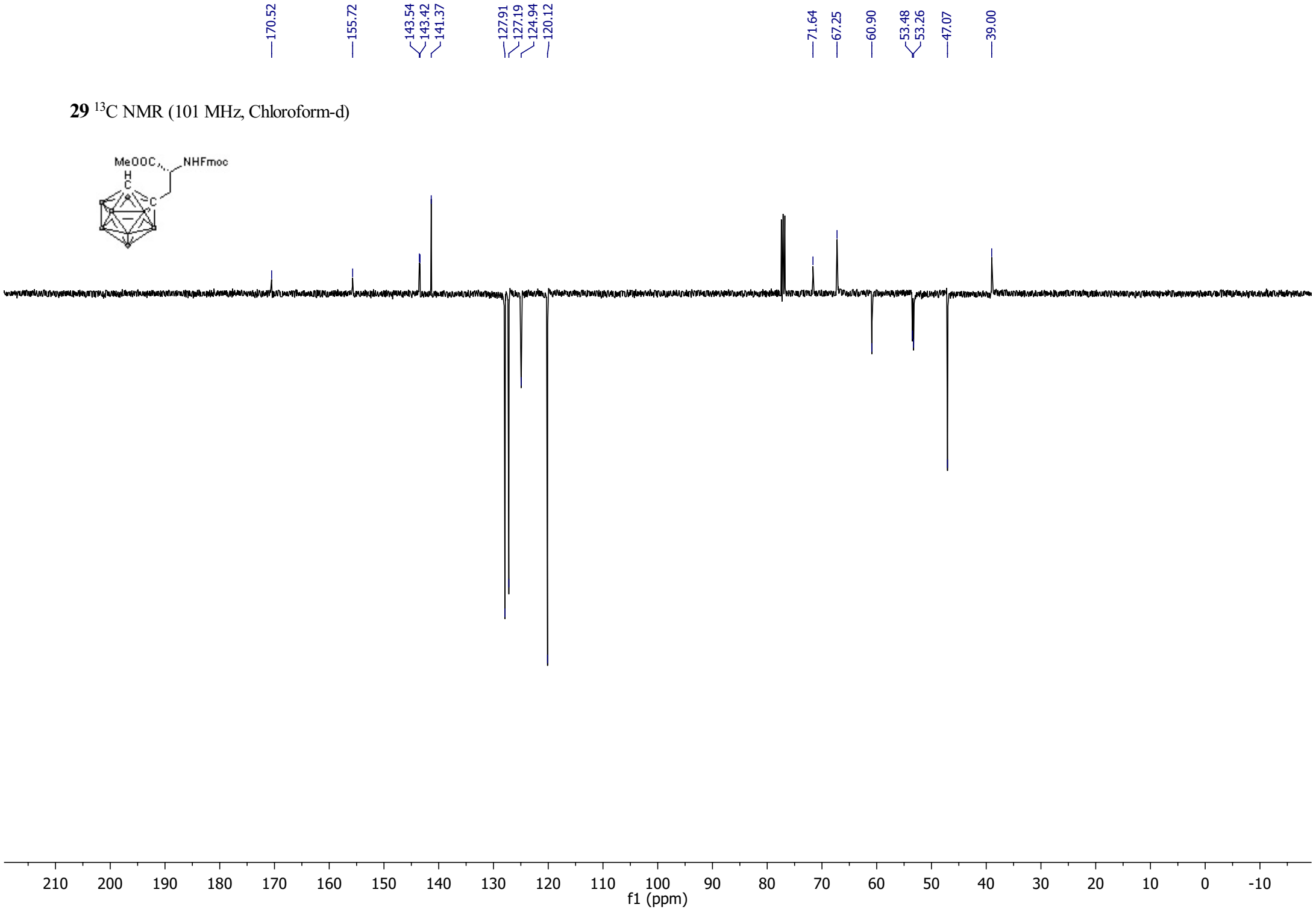
28 ^{13}C NMR (101 MHz, Chloroform-d)

29 ^{1}H NMR (400 MHz, Chloroform-d)



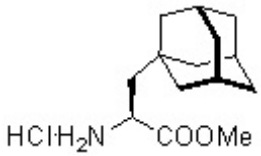
29 ^{11}B NMR (128 MHz, Chloroform-d)



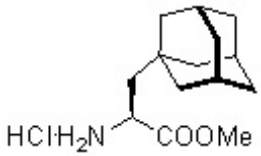


29 ^{13}C NMR (101 MHz, Chloroform-d)

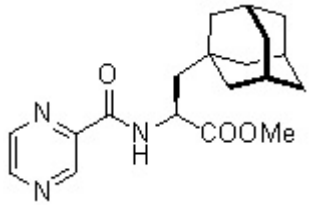
30 ^{1}H NMR (400 MHz, Methanol-d)



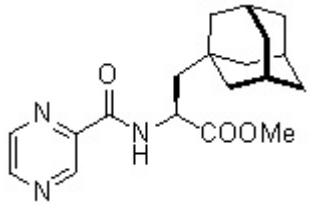
30 ^{13}C NMR (100 MHz, Methanol-d)



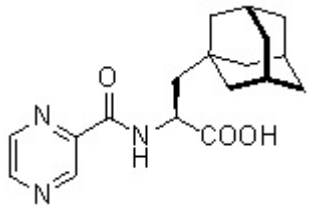
31 ^{1}H NMR (400 MHz, Chloroform-d)



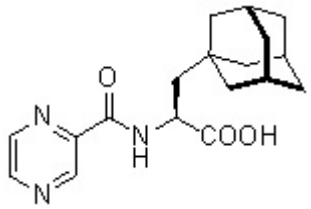
31 ^{13}C NMR (100 MHz, Chloroform-d)



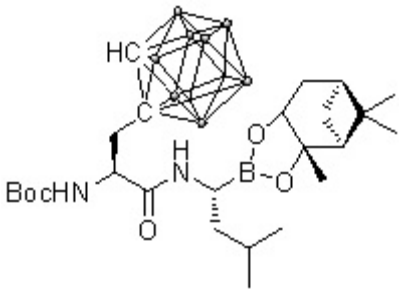
32 ^{1}H NMR (400 MHz, Chloroform-d)



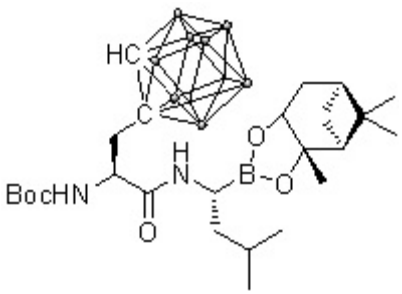
32 ^{13}C NMR (00 MHz, Chloroform-d)



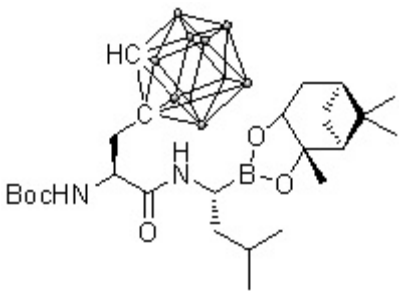
36 ^{1}H NMR (400 MHz, Methanol-d)



36 ^{11}B NMR (128 MHz, Methanol-d)

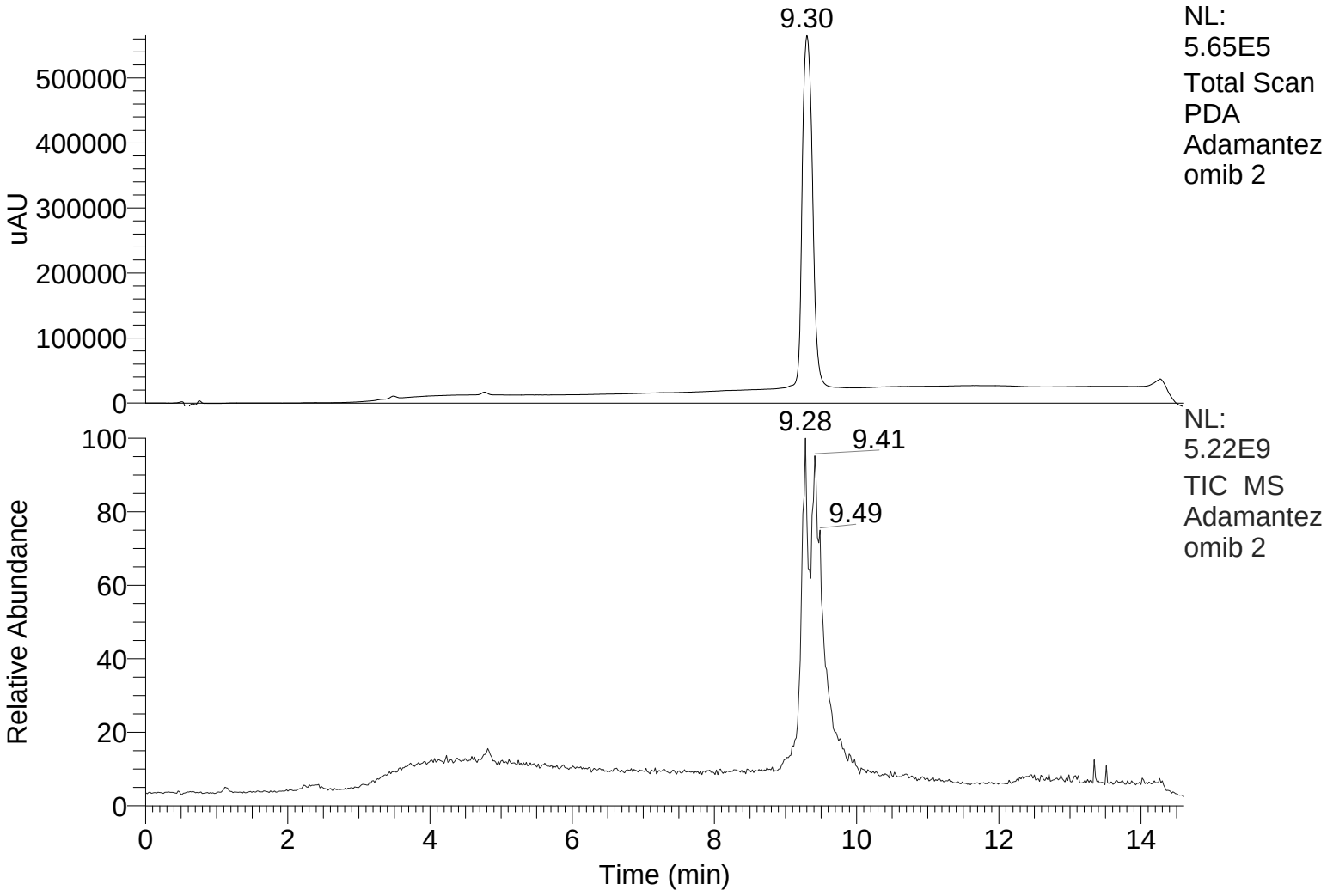


36 ^{13}C NMR (100 MHz, Methanol-d)



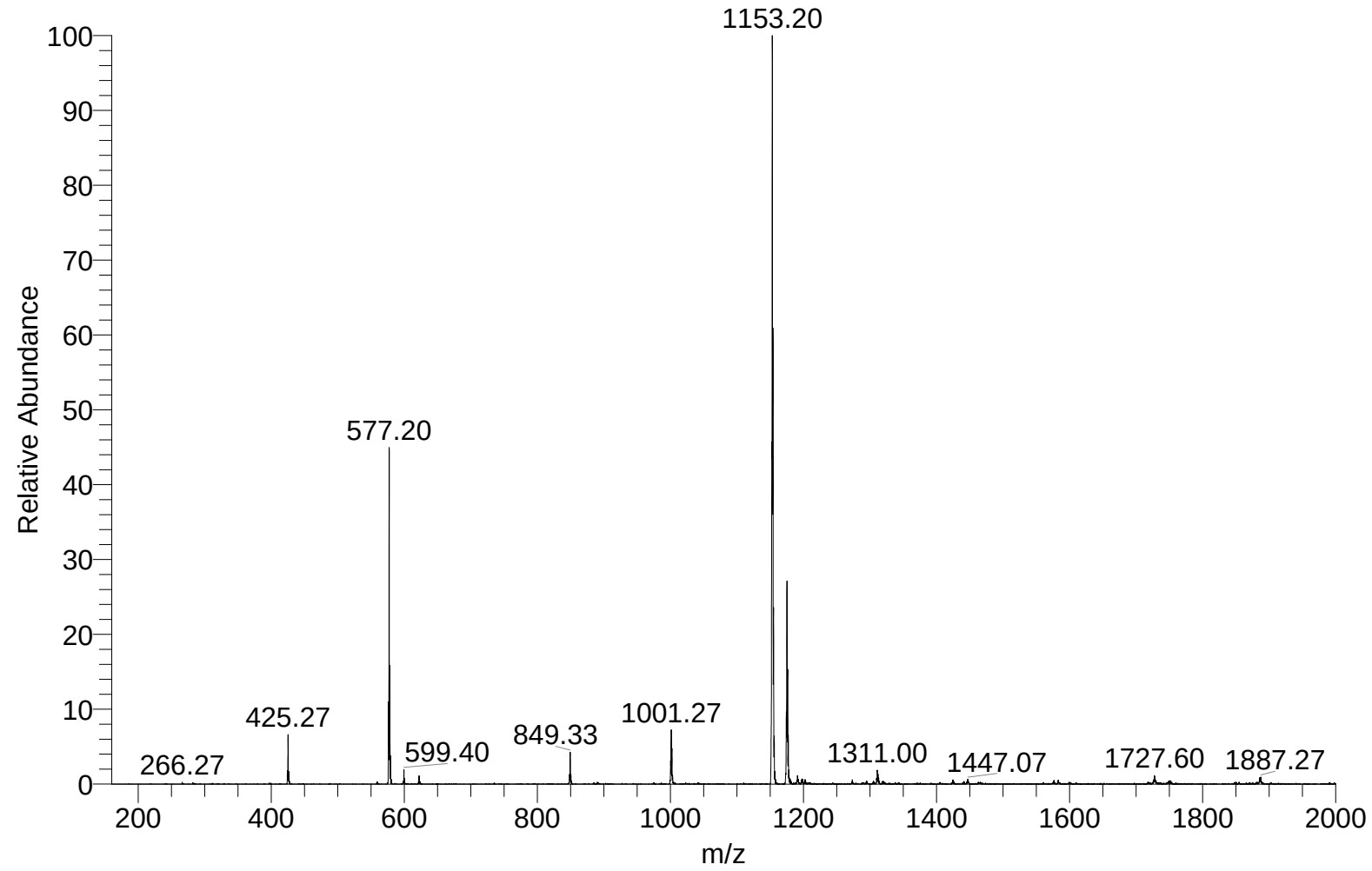
Adamantezomib 2 Linear gradient 50-90% MeCN, 15 min

RT: 0.00 - 14.60

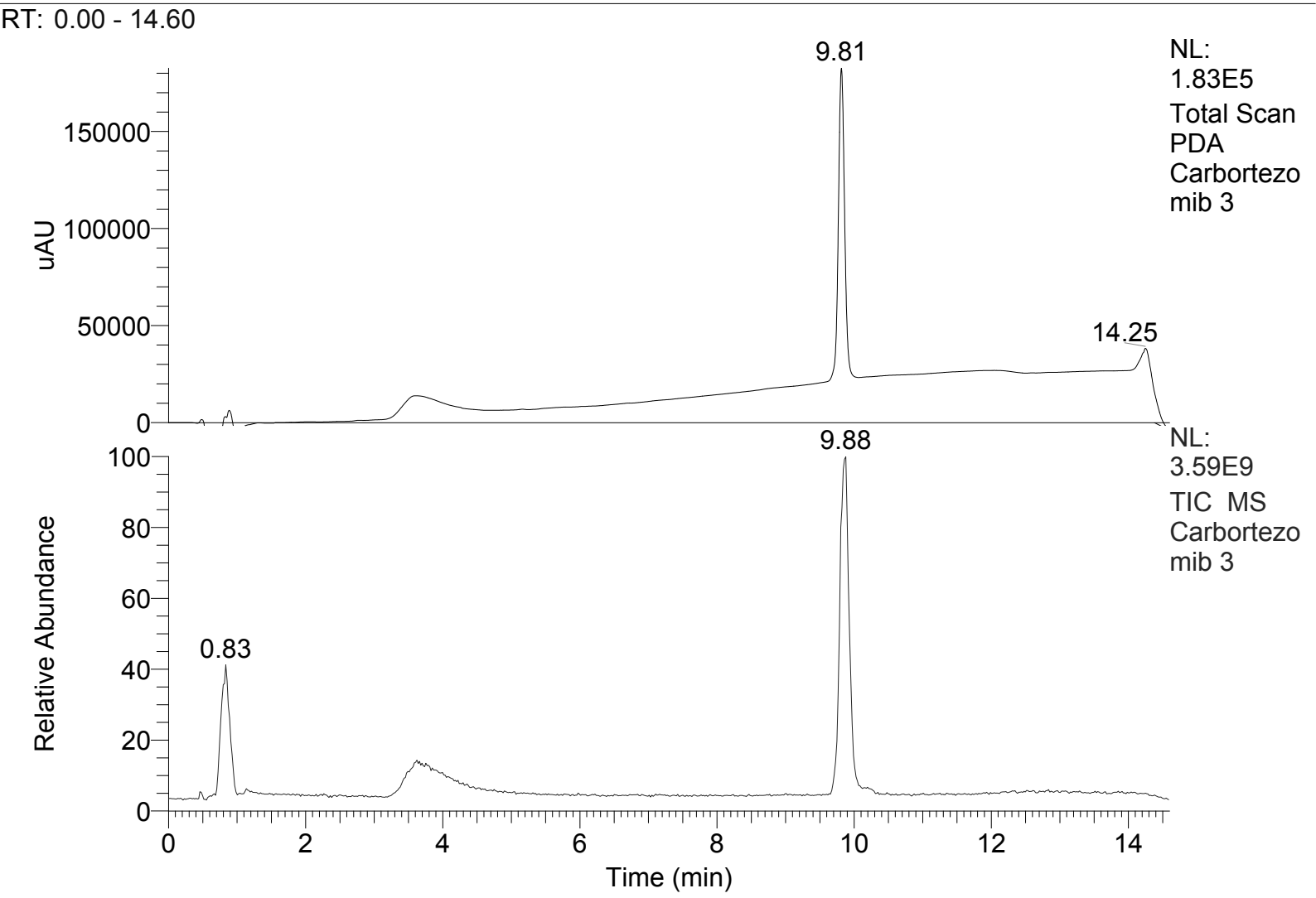


Adamantezomib 2 #491-513 RT: 9.19-9.60 AV: 23 NL: 7.24E7

T: + p ESI Full ms [160.00-2000.00]



Carbortezomib 3 Linear gradient 50 → 90% MeCN, 0.1% TFA, 15 min



Carbortezomib 3 #516-533 RT: 9.71-10.03 AV: 18 NL: 3.76E7
T: + p ESI Full ms [160.00-2000.00]