Non-peptidic lysosomal modulators derived from Z-Phe-Ala-diazomethylketone

for treating protein accumulation diseases

Supporting Information

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Contents

Synthesis.

The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance -500 and 300 instruments. Chemical shifts are reported in ppm and are referenced to residual $CHCl_3$ solvent; 7.26 and 77.0 ppm for 1H and 13C, respectively. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t= triplet, q = quartet, m = multiplet, b = broad. High-resolution mass spectra (HRMS) were obtained from the University of Connecticut Mass Spectrometry Laboratory using AccuTOF mass spectrometer using DART source. Reactions were monitored using thin layer chromatography (TLC) carried out on Whatman Partisil K6F silica gel 60 plates. All glasswares were flame-dried and allowed to cool under an argon atmosphere. Anhydrous dichlormethane, ether, and tetrahydrofuran were used directly from Baker cycletainers. All reagents were used directly from commercial sources unless otherwise stated.

Methods

Sprague-Dawley rats (Charles River Laboratories) were used at 12 days postnatal to prepare hippocampal slice cultures as previously described[1-3]. All studies were carried out in strict accordance with the recommendations from the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. Animal use and analyses were conducted in accordance with protocols approved by Animal Care and Use Committees.

Hippocampal homogenates were prepared and protein content determined with the Pierce BCA assay (Thermo Scientific, Rockford, IL). The resulting material was used to measure cathepsin B proteolytic activity in the absence or presence of added compounds, using the Z-Arg-Arg AMC substrate and the fluorogenic Calbiochem assay kit (EMD Chemicals).

To measure cathepsin protein levels in harvested tissue, equal protein aliquots of hippocampal slice samples were subjected to SDS-PAGE and transferred to nitrocellulose for staining with antibodies against cathepsin B (1:200; Millipore) and actin (1:1000; Sigma). Immunoreactive bands were assessed for integrated optical density with BIOQUANT software.

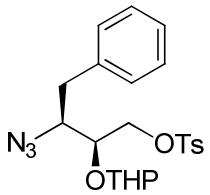
References:

1. Bendiske, J.; Bahr, B. A. Lysosomal activation is a compensatory response against protein accumulation and associated synaptopathogenesis - An approach for slowing Alzheimer disease?. *J. Neuropathol. Exp. Neurol.* **2003**, *62,* 451-463.

2. Butler, D.; Brown, Q. B.; Chin, D. J.; Batey, L.; Karim, S.; Mutneja, M. S.; Karanian, D. A.; Bahr, B. A. Cellular Responses to Protein Accumulation Involve Autophagy and Lysosomal Enzyme Activation. *Rejuvenation Res.* **2005**, *8,* 227-237.

3. Butler, D.; Hwang, J.; Estick, C.; Nishiyama, A.; Kumar, S. S.; Baveghems, C.; Young-Oxendine, H. B.; Wisniewski, M. L.; Charalambides, A.; Bahr, B. A. Protective effects of positive lysosomal modulation in Alzheimer's disease transgenic mouse models. *PLoS One* **2011**, *6,* e20501.

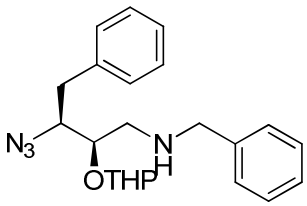
Toluene-4-sulfonic acid 3-azido-4-phenyl-2-(tetrahydro-pyran-2-yloxy)-butyl ester



3

To a solution of 2(0.70g, 3.38 mmol) in 6 ml pyridine was added tosyl chloride (0.966g, 5.07 mmol) at 0°C. The reaction mixture was then stirred at RT for 24 hrs and quenched by adding 2N HCl. The organic layer was extracted with (2x 15ml) EtOAc and washed with $NaHCO_3$, brine and dried over Na_2SO_4. The dried organic layer was then filtered and concentrated. The product was then purified by column chromatography and was obtained as oil (1.09g, 90%). To a solution of tosyl alcohol (900 mg, 2.49 mmol) in 20 ml CH2Cl2 was added dihydropyran (0.310g, 3.73 mmol) was added dropwise. To this pyridinium p-toluene sulfonate(0.093g, 10 mol %) was added . The reaction mixture was stirred for 24 hrs and was washed with satuarated $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure to give light brown colored oil. It was then subjected to silica gel chromatography with hexane/ ethyl acetate (3:1) as eluent to give isomers of THP protected alcohol **3** as an oil (1.042g, 94%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.86 – 7.80 (m, 2H), 7.37 (dd, *J* = 8.2, 3.4 Hz, 2H), 7.33 (ddd, *J* = 7.5, 6.5, 1.3 Hz, 2H), 7.30 – 7.26 (m, 1H), 7.25 – 7.21 (m, 2H), 4.70 (ddd, *J* = 23.4, 4.5, 2.9 Hz, 1H), 4.30 (ddd, *J* = 10.9, 4.1, 2.0 Hz, 1H), 4.20 (ddd, *J* = 44.7, 10.7, 5.3 Hz, 1H), 3.96 – 3.76 (m, 3H), 3.56 – 3.40 (m, 1H), 2.97 (ddd, *J* = 30.4, 14.1, 4.0 Hz, 1H), 2.69 (ddd, *J* = 26.2, 14.0, 9.8 Hz, 1H), 2.46 (d, *J* = 4.2 Hz, 3H), 1.89 – 1.42 (m, 7H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 145.18, 145.12, 137.39, 137.35, 132.76, 132.69, 130.07, 130.04, 129.97, 129.27, 129.25, 128.78, 128.72, 128.70, 128.12, 128.00, 126.98, 126.96, 99.76, 98.68, 75.59, 68.97, 68.36, 65.15, 63.39, 63.21, 62.50, 37.53, 36.11, 30.63, 30.53, 25.28, 25.26, 21.71, 19.57, 19.18; HRMS (DART, MN_2^-) *m/z* 418.1690 (calculated for $C_{22}H_{28}NO_5S$, 418.1688).

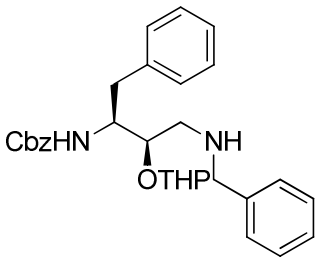
[3-Azido-4-phenyl-2-(tetrahydro-pyran-2-yloxy)-butyl]-benzyl-amine



4

To a solution of tosyl protected alcohol **3** (0.842g, 1.89 mmol) in anhydrous THF (10 ml) benzylamine (1.05 ml, 9.46 mmol) was added. The reaction mixture was refluxed at 90˚C for 56 hrs, cooled and then concentrated. It was then purified using column chromatography (SiO2, 1:1 Hex/ EtOAc) to give the product as two isomers (0.485g, 67%). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.41 – 7.25 (m, 10H), 4.81 (dd, *J* = 3.11, 4.67 Hz, 1H), 4.10 (m, 1H), 4.00 (m, 1H), 3.92 (q, *J* = 4.51 Hz, 1H), 3.90 – 3.83 (m, 2H), 3.56 (m, 1H), 3.04 (dd, *J* = 4.32, 14.22 Hz, 1H), 2.98 (m, 1H), 2.91 (m, 1H), 2.77 (m, 1H), 1.99 – 1.52 (m, 7H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 140.59, 138.50, 129.31, 128.63, 128.51, 128.16, 127.08, 126.71, 99.11, 78.76, 66.23, 63.25, 54.23, 48.48, 37.57, 31.25, 25.53, 19.98; HRMS (DART, MH^+) *m/z* 381.2310 (calculated for $C_{22}H_{29}N_4O_2$, 381.2291).

[1-Benzyl-3-benzylamino-2-(tetrahydro-pyran-2-yloxy)-propyl]-carbamic acid benzyl ester

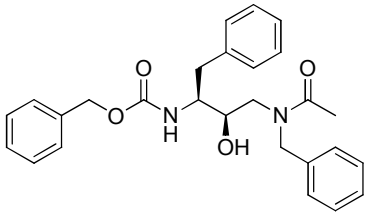


5

The THP protected benzyl amine **4**(0.485 g, 1.28 mmol) was dissolved in 8 ml of absolute methanol and Pd/C (0.014g,10mol%) was added.The reaction mixture was then stirred in a hydrogen atmosphere for 12 hrs and then filtered through celite. The solvent was removed under reduced pressure and the residue was concentrated to give the reduced amine as oil (0.365g, 81%). The reduced primary amine (0.365g, 1.03 mmol) as taken in 6 ml of ether along with 3 ml of saturated sodium bicarbonate solution and cooled to 0˚C. To this benzyl chloroformate (0.265g, 1.54mmol) was added dropwise and stirred for 5 hours. The aqueous layer was then extracted with (2x10ml) EtOAc and the organic layer was dried over Na_2SO_4. It was then filtered and concentrated under reduced pressure. It was then subjected to column chromatography (SiO_2 , 2:1 hexane/ EtOAc) to give Cbz preotected amine as an oil (0.490g , 97%) .^{1}H NMR (500 MHz, Chloroform-d) δ 7.47 – 7.13 (m, 15H), 5.23 (s, 2H), 5.01 (s, 2H), 4.84 (d, *J* = 15.70 Hz, 1H), 4.69 (s, 1H), 4.49 (d, *J* = 15.37 Hz, 1H), 4.27 – 3.68 (m, 3H), 3.39 (s, 2H), 2.74 (m, 1H), 1.75

(d, *J* = 31.19 Hz, 2H), 1.9bs, 4H), 1.40 – 1.20 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 156.12, 137.71, 129.29, 129.04, 128.70, 128.64, 128.57, 128.15, 128.02, 127.87, 127.73, 127.52, 126.45, 76.32, 67.63, 63.39, 51.35, 35.96, 31.75, 30.91, 29.87, 25.43, 22.82, 20.25, 14.30.

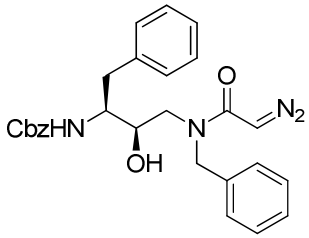
[3-(Acetyl-benzyl-amino)-1-benzyl-2-hydroxy-propyl]-carbamic acid benzyl ester



SD1002

To the Cbz protected amine **5** (0.495g, 1.02 mmol) was dissolved in 10 ml of ethanol and to this PPTS (0.026g, 10mol %) was added. The reaction mixture was stirred at 60˚C for 5 hrs and it was then quenched with saturated sodium bicarbonate solution. The organic layer was extracted with (2x10ml) EtOAc, separated, dried over Na2SO4 and concentrated under reduced pressure to get the alcohol. To the Cbz protected amino alcohol (0.035g, 0.086 mmol) in 1 ml of anhydrous THF, Et_3N (0.011g, 0.11 mmol) was added dropwise. After 10 minutes acetic anhydride (0.012g, 0.11mmol) was added and 1 mg of DMAP was added. The reaction mixture was then stirred for 6 hrs and was quenched with water. The organic layer was extracted with (2x5ml) EtOAc, separated, dried over Na_2SO_4 and concentrated under reduced pressure. It was then subjected to column chromatography (SiO_2 2:1 hexane/ ethyl acetate) to give the **SD1002** as oil (0.029 g, 75%). ^{1}H NMR (500 MHz, Chloroform-d) δ 7.37 – 7.21 (m, 15H), 5.24 (s, 2H), 5.03 (q, *J* = 12.47 Hz, 3H), 4.80 – 4.35 (m, 2H), 4.20 (bs, 1H), 3.87 – 3.55 (m, 1H), 3.43 (dd, *J* = 7.79, 14.73 Hz, 1H), 2.96 (s, 1H), 2.79 – 2.53 (m, 1H), 2.10 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.45, 156.02, 137.51, 136.78, 129.31, 128.81, 128.69, 128.67, 128.60, 128.25, 128.21, 128.11, 127.99, 127.66, 126.79, 67.88, 66.87, 51.28, 47.11, 36.87, 20.88; HRMS (DART, MH^+) *m/z* 447.2288 (calculated for $C_{27}H_{31}N_2O_4$, 447.2284).

{1-Benzyl-3-[benzyl-(2-diazo-acetyl)-amino]-2-hydroxy-propyl}-carbamic acid benzyl ester



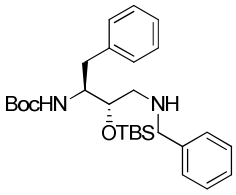
SD1003

The Cbz protected amine **5** (0.080g, 0.19 mmol) was dissolved in 2 ml of ethanol and to this diketene (23 µL, 0.29 mmol) was added dropwise. The reaction mixture was then stirred for 1 hour and diluted with 4 ml dichloromethane. It was then poured in to water and the organic layer was separated, dried over

Na_2SO_4. It was then subjected to silica gel chromatography with 2:1 hexane/ ethyl acetate as an eluent to give the keto-amide as oil(90mg (89% yield). The keto amide (0.040g, 0.08 mmol) was dissolved in 0.5 ml of acetonitrile and cooled to 0˚C. To this tosyl azide(0.024g, 0.12mmmol) was added followed by the addition of DBU(18µL, 0.12 mmmol) dropwise. The reaction mixture was stirred for 1 hr at 0˚C and then 3 hrs at room temperature. It was then concentrated under reduced pressure to give dark red oil. The diazo ketamide (0.020g, 0.038 mmol) was dissolved in 0.5 ml of 1:1 THF/ H_2O and to this LiOH (5 mg , 0.19 mmol) dissolved in 0.2 ml of water was added. The reaction mixture was stirred for 5 hrs and diluted with 2 ml of dichloromethane. The organic layer was separated, dried over Na_2SO_4 and concentrated. The product was then isolated by passing through a plug of silica as oil (0.015g, 81% yield).

The crude product was taken up in 2 ml EtOH and to this PPTS (0.003g, 10mol %) was added. The reaction mixture was heated at 40°C for 5 hrs and was then quenched with saturated sodium bicarbonate solution. The organic layer was extracted with (2x5ml) EtOAc, separated, dried over Na2SO4 and concentrated under reduced pressure. The crude product was then compound was eluted with 2:1 hexane/ ethyl acetate to give **SD1003** as yellow oil (0.008 g, 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.61 – 6.82 (m, 15H), 5.23 (s, 2H), 5.03 (s, 2H), 4.55 (d, *J* = 15.27 Hz, 2H), 4.18 (d, *J* = 18.39 Hz, 1H), 3.94 – 3.70 (m, 1H), 3.63 – 3.31 (m, 1H), 3.04 – 2.77 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.21, 137.32, 128.76, 128.68, 128.60, 128.56, 128.50, 128.35, 128.27, 128.02, 127.86, 127.36, 68.14, 67.45, 51.01, 50.50, 41.57, 36.70, 31.94, 29.71, 29.37;

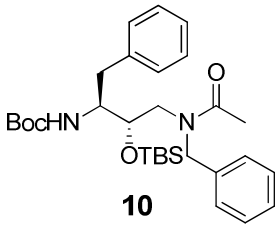
[1-Benzyl-3-benzylamino-2-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-carbamic acid *tert*-butyl ester



9

To a solution of the amine **7**(0.795g, 2.015mmol) in 8 ml MeOH was added 3A°molecular sieves (400mg), glacial acetic acid (0.6ml), $NaCNBH_3$ (0.225g, 3.4 mmol) and benzaldehyde (0.375g, 3.22mmol) was added in a sequential manner. The reaction mixture was stirred until the disappearance of the starting material by TLC. The reaction mixture was filtered through celite, diluted with EtOAc, washed with $NaHCO_3$ and dried over Na_2SO_4. The crude product is then purified using column chromatography to get the pure product as oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.29 (m, 10H), 4.88 (d, *J* = 9.81 Hz, 1H), 4.19 (q, *J* = 8.42 Hz, 1H), 3.94 – 3.87 (m, 1H), 2.86 (m 3H), 2.76 (dd, *J* = 8.48, 12.09 Hz, 1H), 2.64 (dd, *J* = 5.09, 12.14 Hz, 1H), 1.35 (s, 9H), 1.01 (s, 9H), 0.14 (d, *J* = 24.14 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 155.92, 140.22, 138.71, 129.21, 128.39, 128.33, 128.15, 126.91, 126.21, 79.02, 72.45, 54.10, 53.28, 51.92, 38.66, 28.40, , 26.07, 18.21, -3.95, -4.59; HRMS (DART, MH^+) *m/z* 485.3218 (calculated for $C_{28}H_{45}N_2O_3Si$, 485.3199).

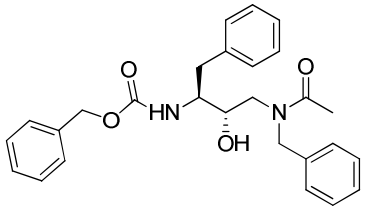
[3-(Acetyl-benzyl-amino)-1-benzyl-2-(*tert*-butyl-dimethyl-silanyloxy)-propyl]-carbamic acid *tert*-butyl ester



10

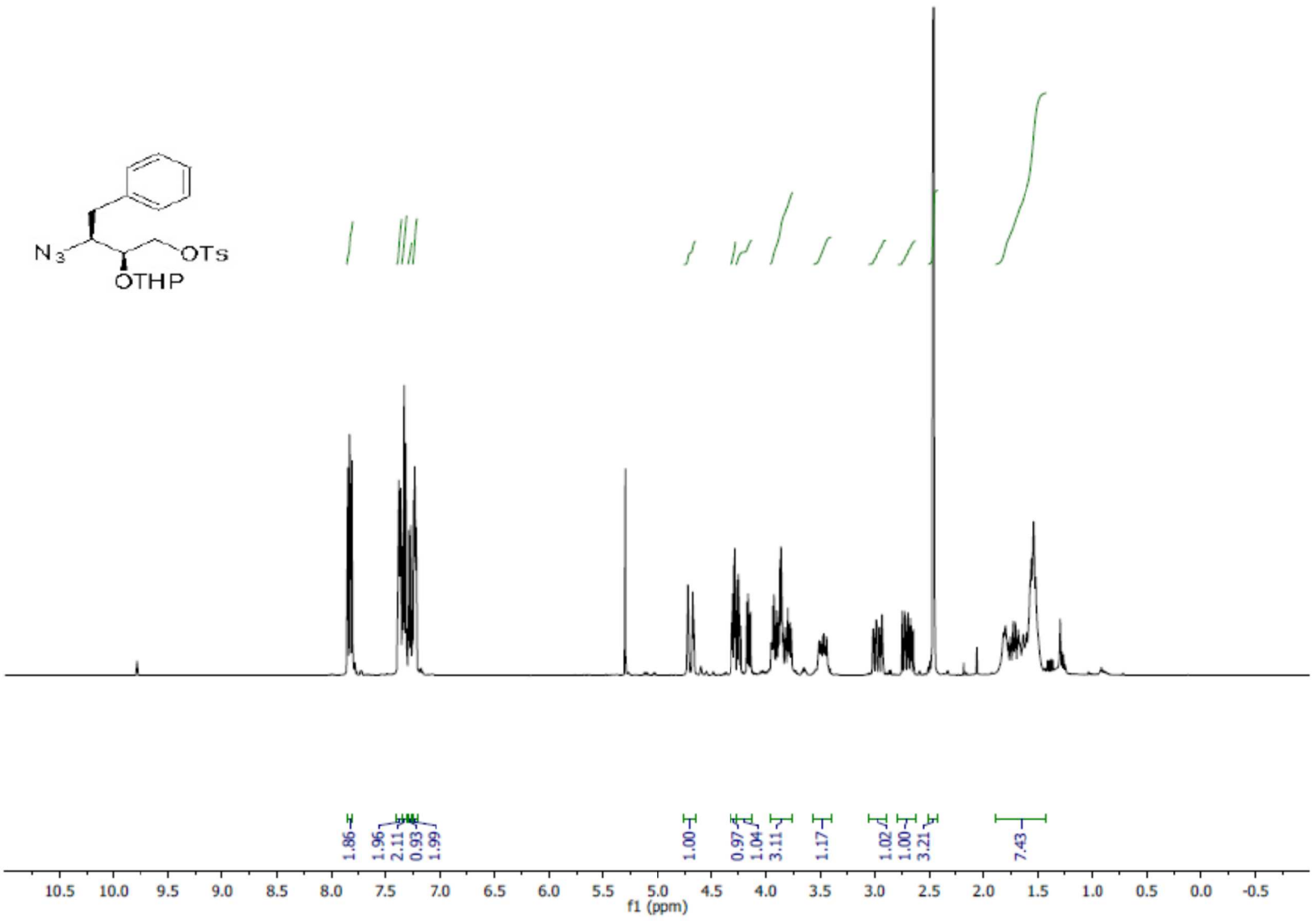
To an ice-cooled solution of **9** (0.475 g, 0.98mmol and Et_3N (0.152g, 1.5mmol) in 4ml CH_2Cl_2 was added acylchloride (0.088g, 1.13mmol) dropwise. The reaction mixture was stirred at RT for 15 min and 5ml 10% NaHCO3 added to quench the reaction. The reaction mixture was then extracted with 30 ml EtOAc, washed with brine and dried over Na_2SO_4. The organic extract was then concentrated and the crude product was used for the next reaction. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 – 6.98 (m, 10H), 4.87 – 4.72 (m, 1H), 4.70 (d, *J* = 8.15 Hz, 1H), 4.61 – 4.45 (m, 1H), 4.24 – 4.08 (m, 2H), 4.07 – 3.81 (m, 2H), 3.67 (dd, *J* = 3.95, 13.30 Hz, 1H), 2.14 (d, *J* = 8.87 Hz, 3H), 1.31 (s, 9H), 0.96 (s, 9H), 0.20 (d, *J* = 6.00 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.90, 155.54, 138.41, 136.97, 129.27, 128.81, 128.57, 128.26, 128.12, 127.38, 126.22, 126.16, 79.19, 70.23, 53.67, 53.09, 49.50, 39.83, 28.26, 26.00, 21.72, 18.04, -4.07, -4.82.

[3-(Acetyl-benzyl-amino)-1-benzyl-2-hydroxy-propyl]-carbamic acid benzyl ester



SD1006

The Boc protected amine **10** (0.163g, 0.31 mmol) was taken in 0.5ml of 4N HCl-dioxane and stirred for 15 min. The reaction was then concentrated and to the crude amine salt was added 2ml CH_2Cl_2, Et_3N (0.042g, 0.416mmol) and Cbz-Cl(0.056g, 0.311mmol). The reaction mixture was stirred for 1 hour and quenched by adding 1 ml 1N NaOH. EtOAc was added to the crude mixture and the product was extracted in the organic layer. The organic layer was washed with 2x5ml 1N HCl, brine and dried over Na_2SO_4. The dried organic layer was then filtered, concentrated and the crude protected alcohol was then stirred with in 1 ml MeCN along with HF/pyridine. The reaction mixture was then stirred for 4 hrs and poured into 10ml EtOAc. The organic layer was washed with 2N NaOH, brine and dried over Na_2SO_4. The organic layer was then filtered, concentrated and purified using column chromatography to get the **SD 1006** as on oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.03 (m, 15H), 5.38 (d, *J* = 9.55 Hz, 1H), 5.11 – 5.02 (m, 2H), 4.91 – 4.76 (m, 1H), 4.47 (q, *J* = 16.79 Hz, 2H), 3.81 – 3.65 (m, 2H), 2.97 (m, 1H), 2.88 (d, *J* = 7.53 Hz, 1H), 2.13 (d, *J* = 14.21 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.22, 156.33, 137.94, 135.65, 129.40, 129.22, 129.03, 128.74, 128.49, 128.44, 128.04, 127.94, 127.91, 126.33, 70.92, 66.66, 55.67, 54.21, 53.30, 38.90, 21.61; HRMS (DART, MH^+) *m/z* 447.2310 (calculated for $C_{27}H_{31}N_2O_4$, 447.2284).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1.00
3.21
7.43
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
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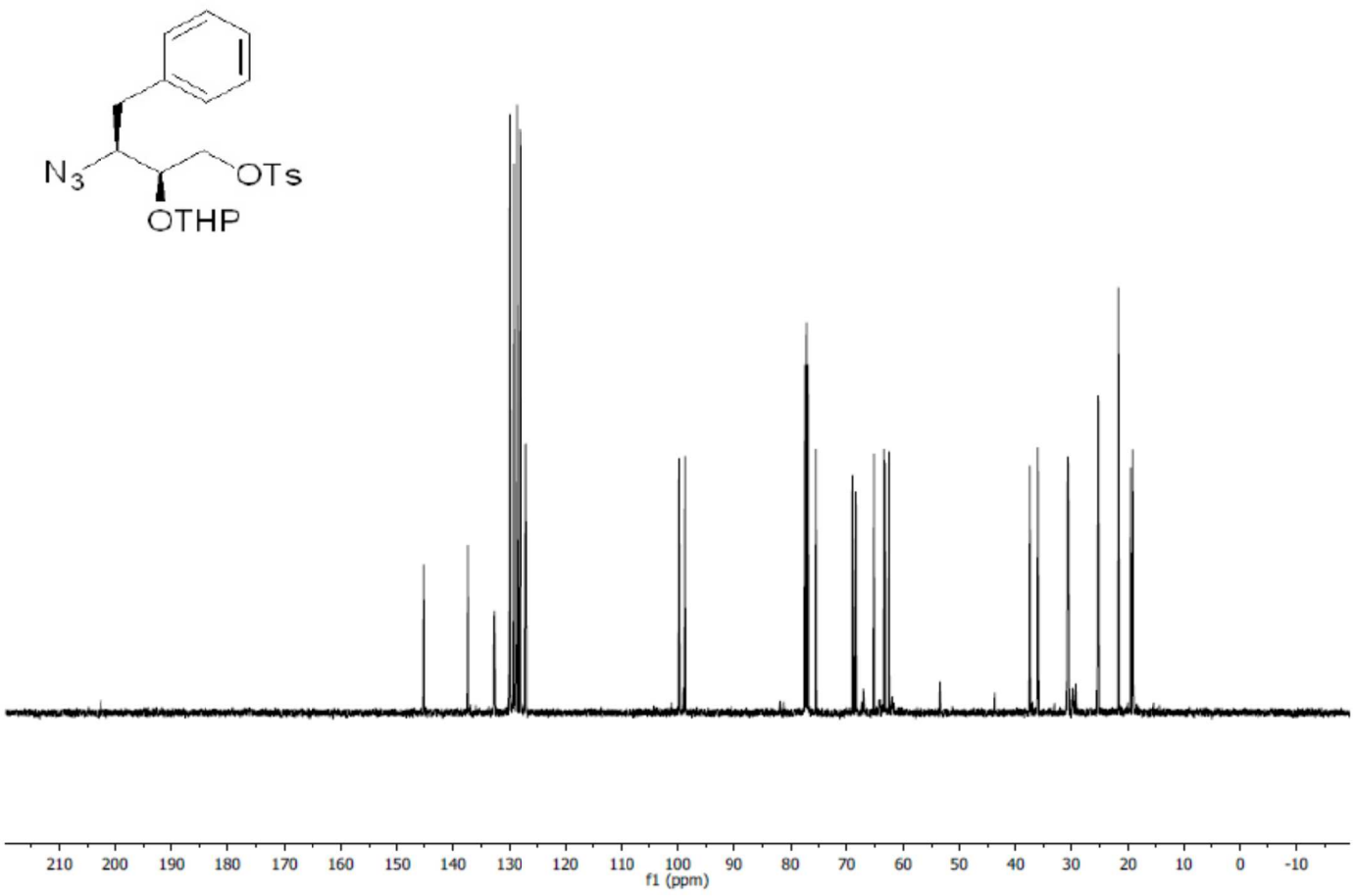
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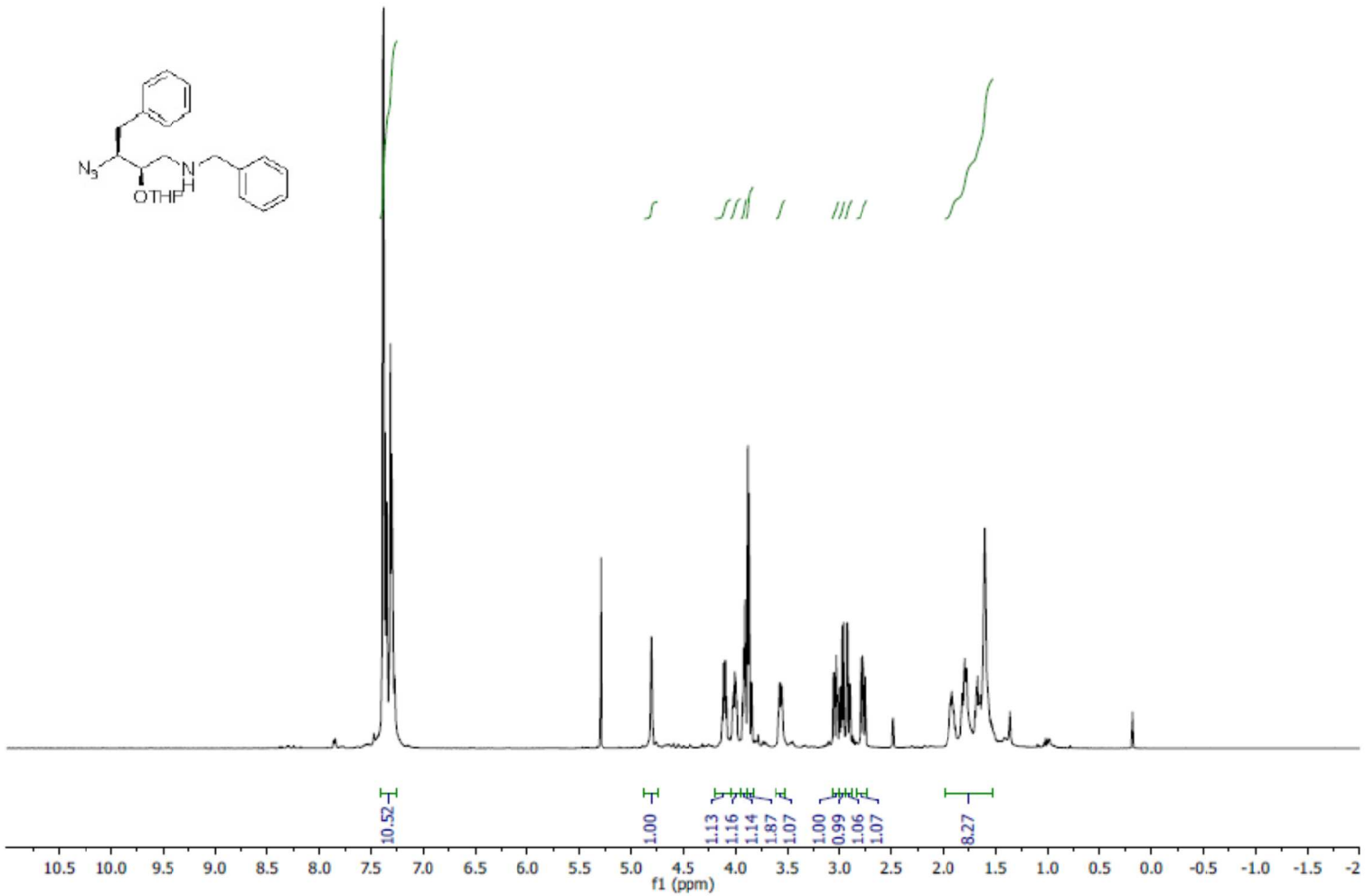
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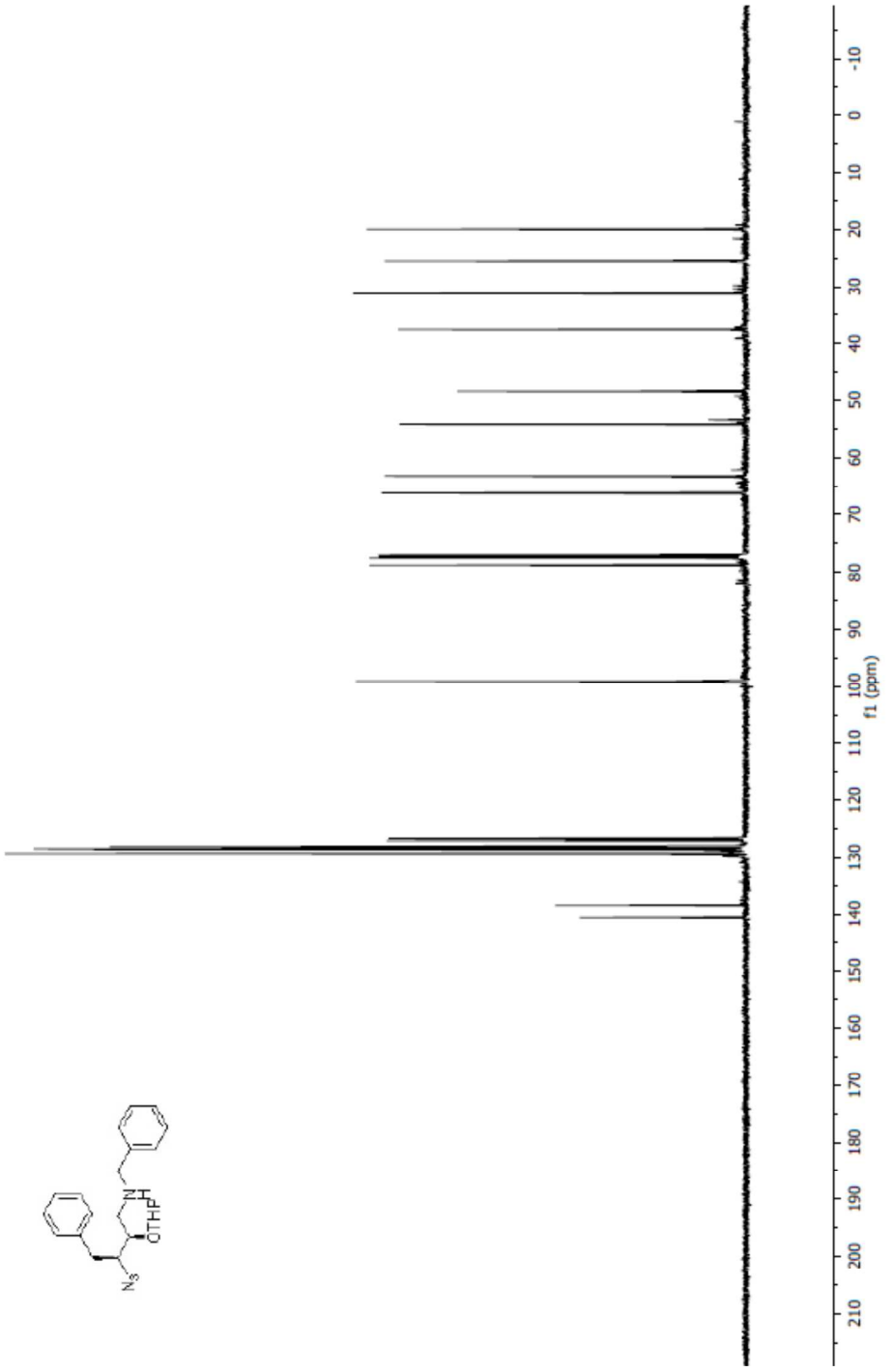
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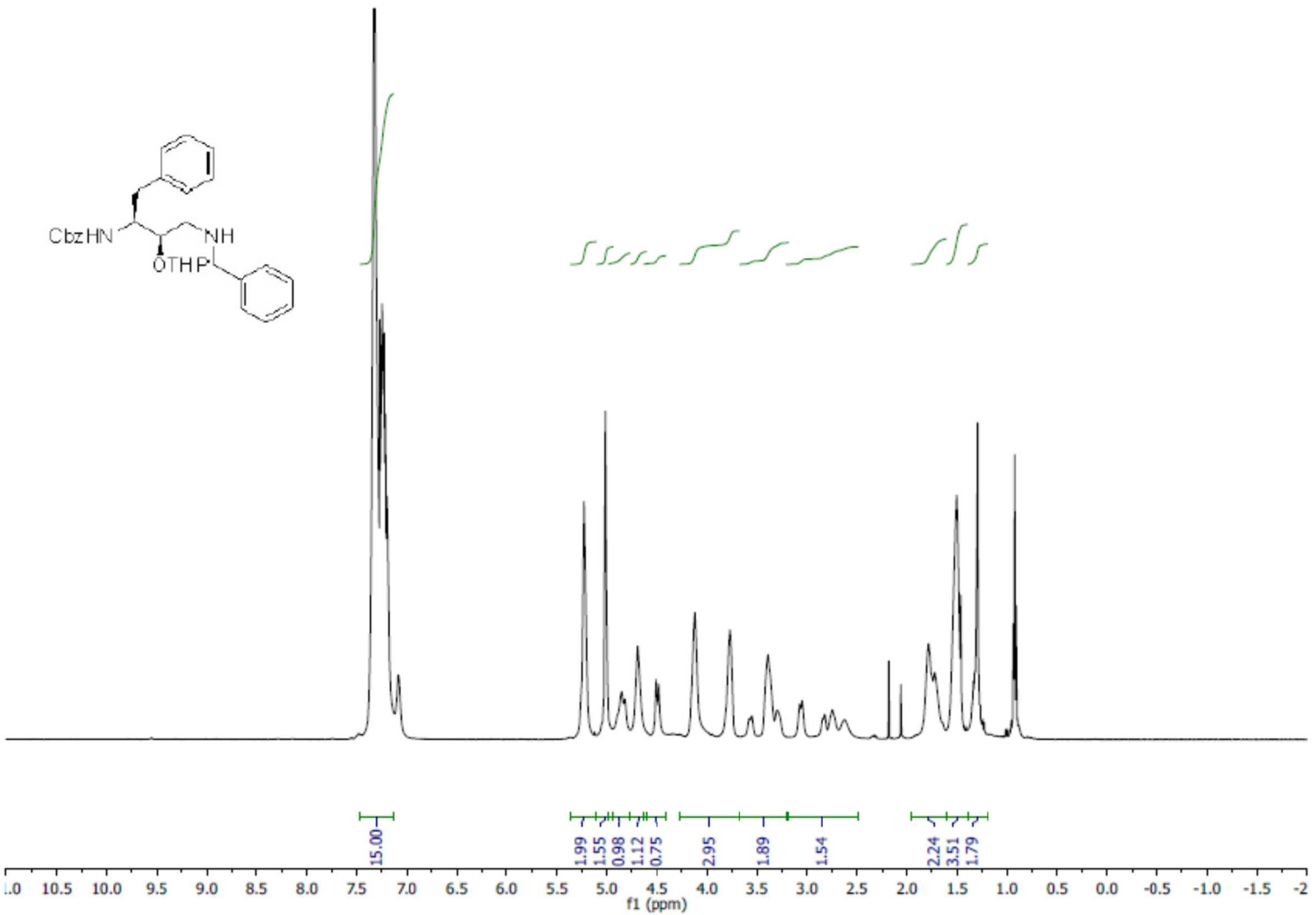
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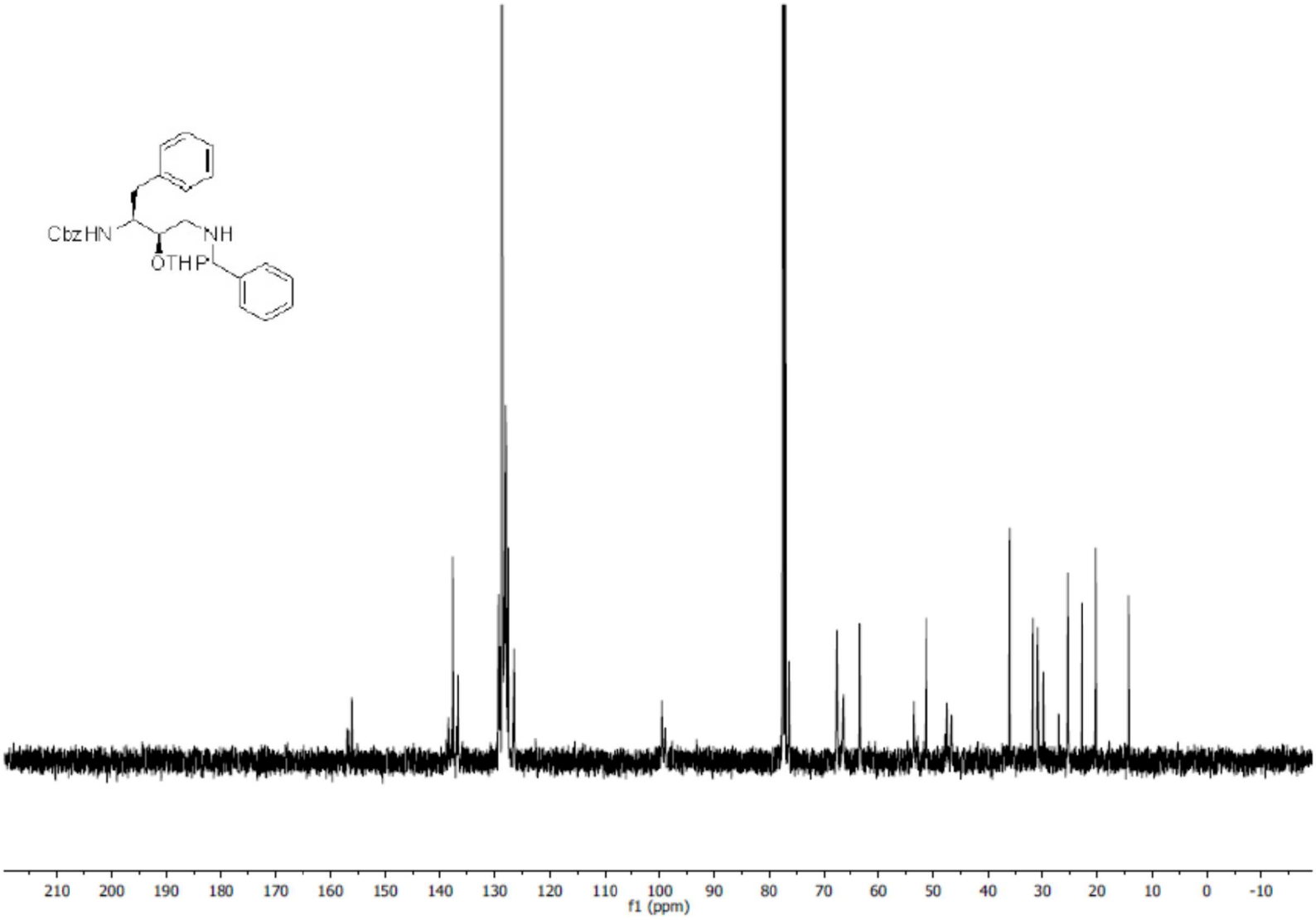
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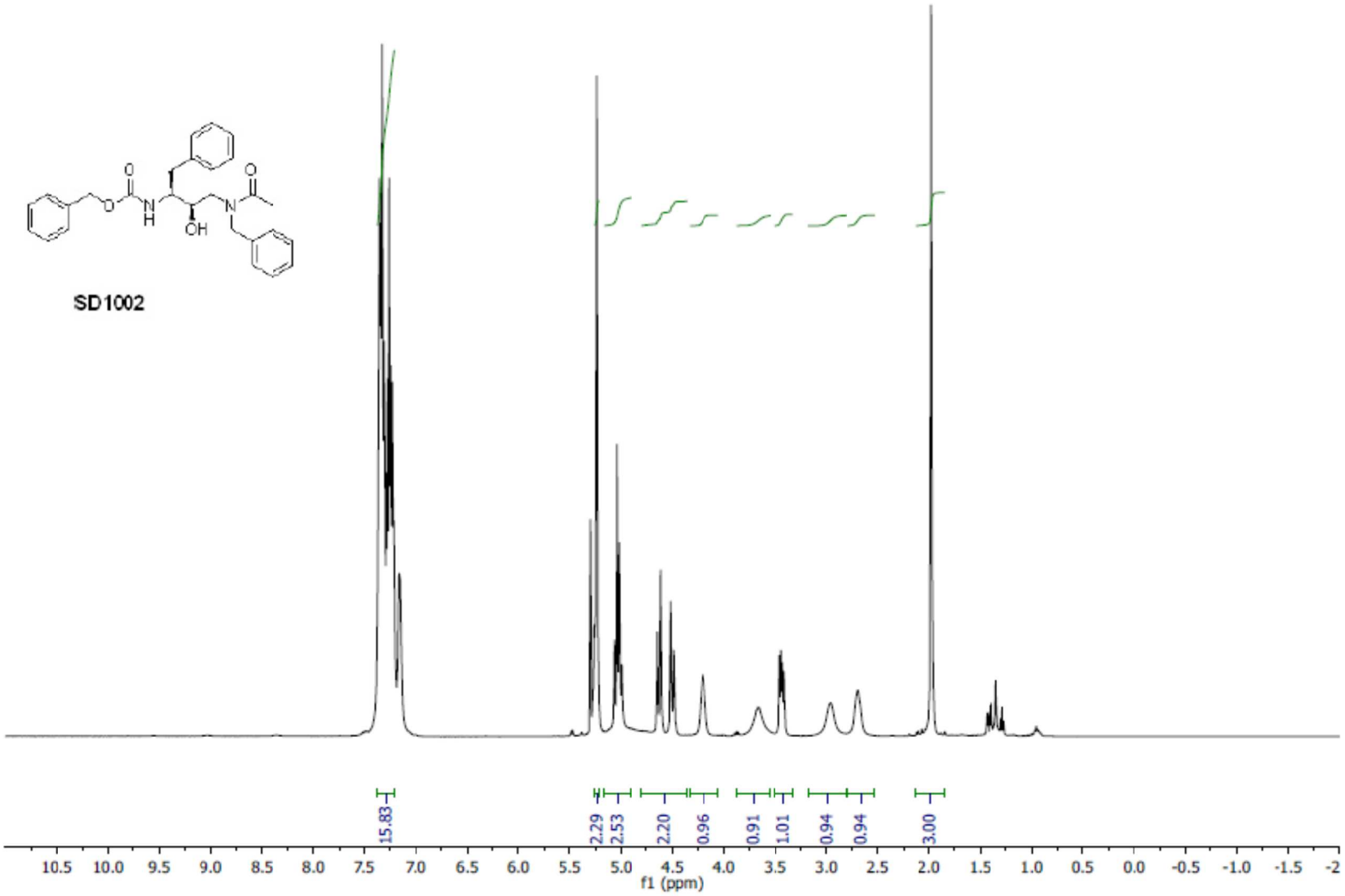
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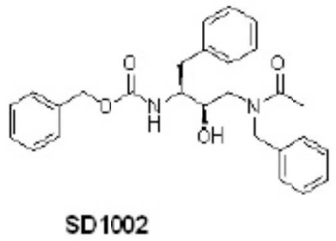
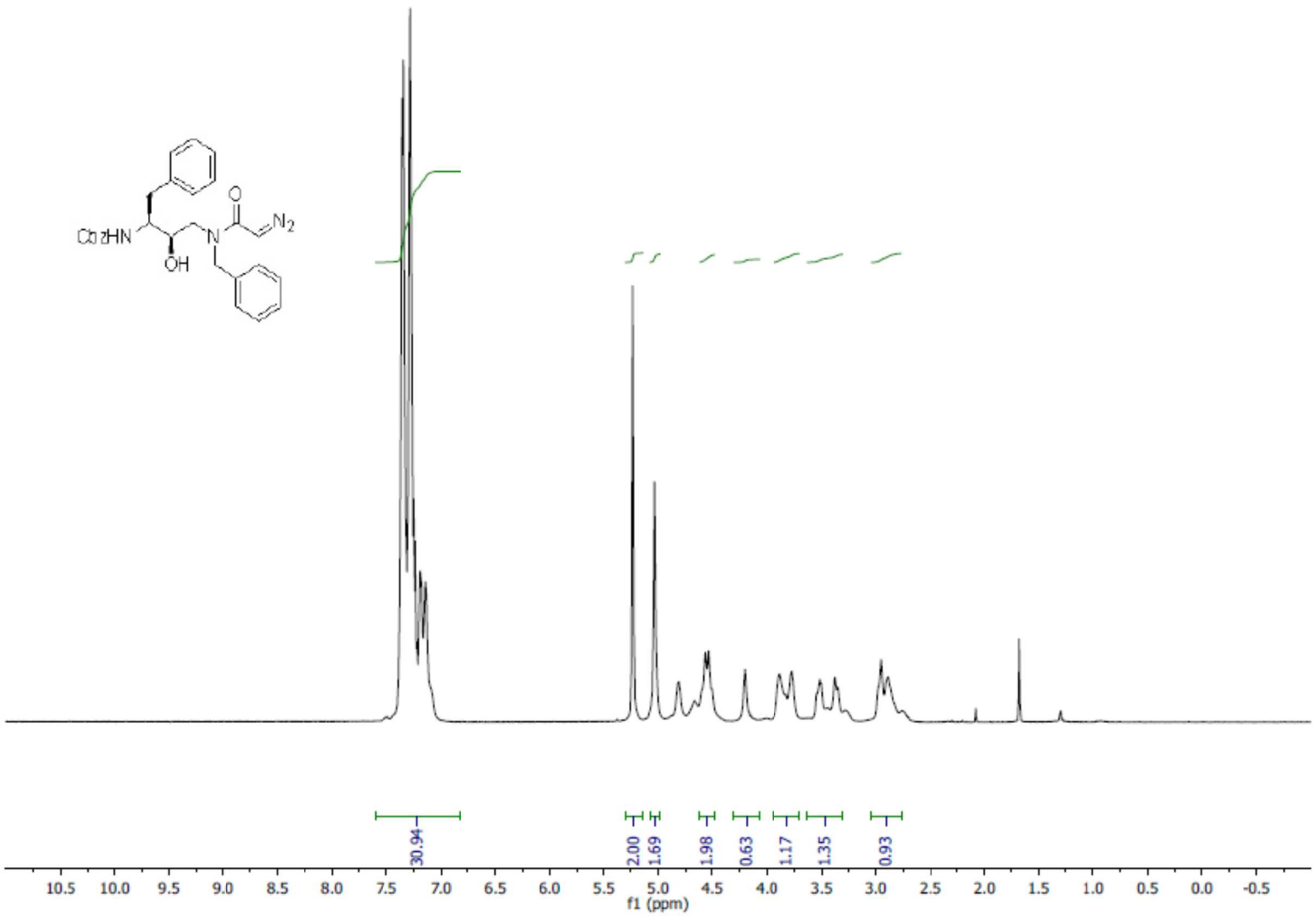
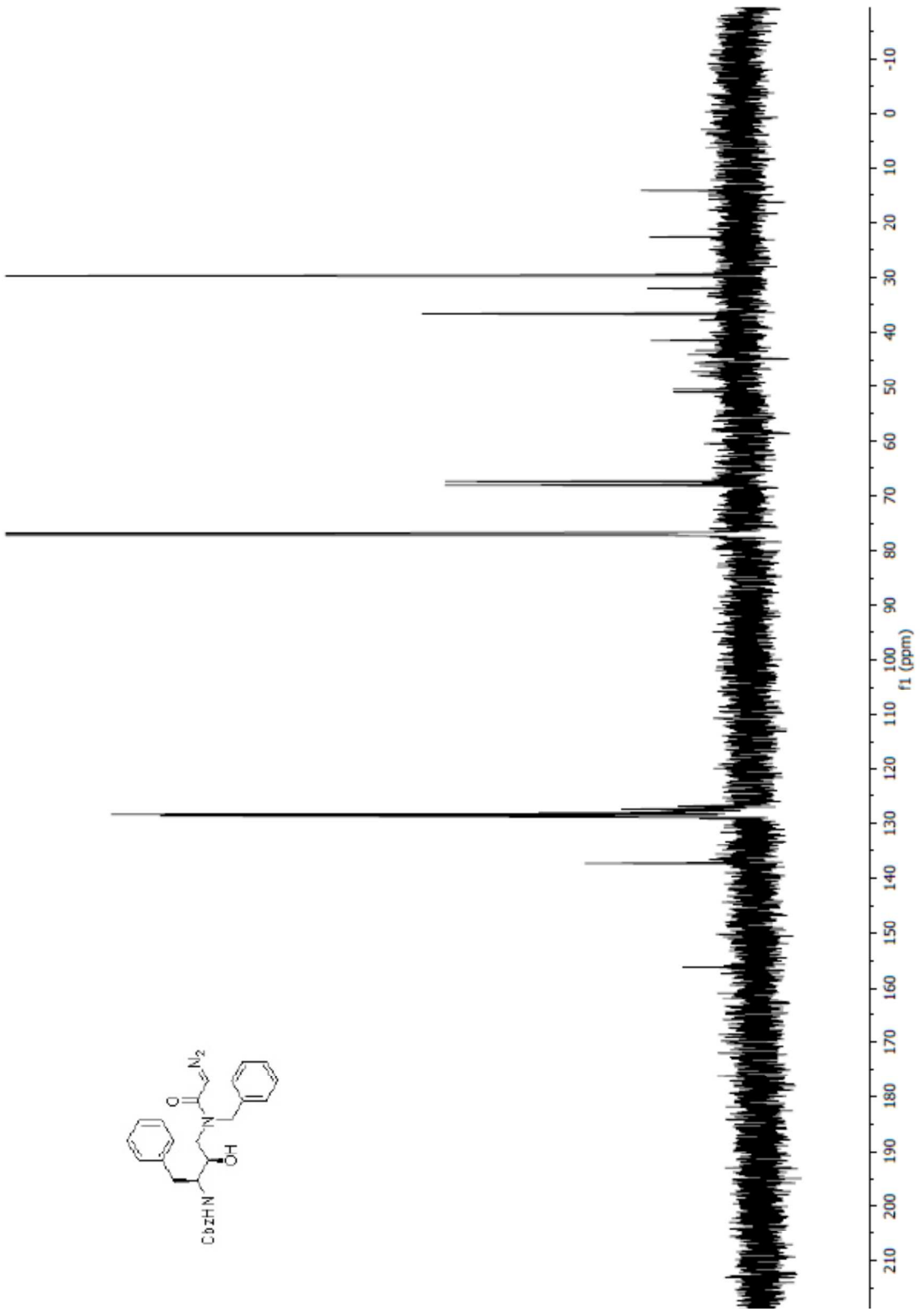
2.20
0.96
0.91
1.01
0.94
0.94
3.00
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5 -2
f1 (ppm)

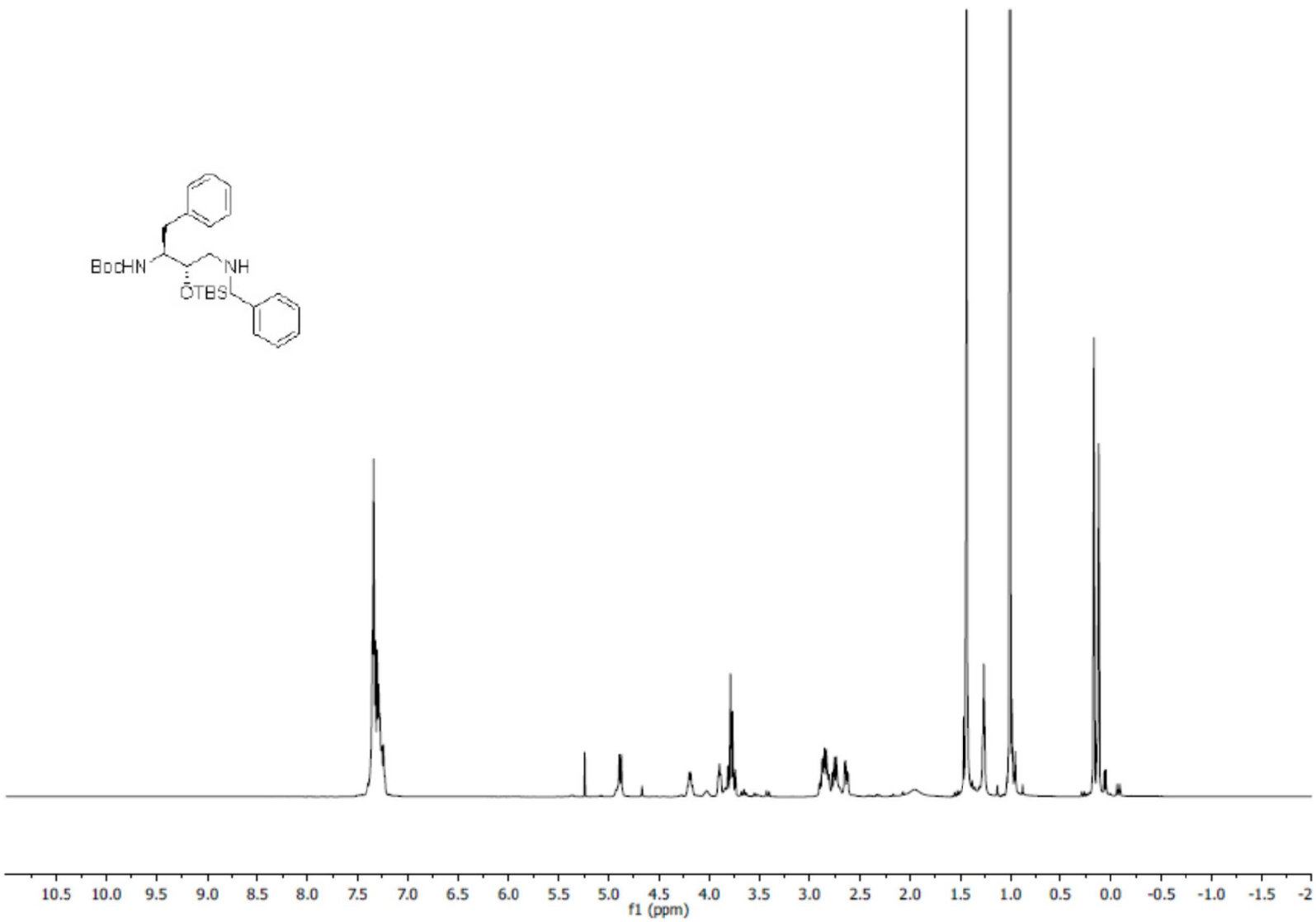

O
O
N
H
OH
N
O

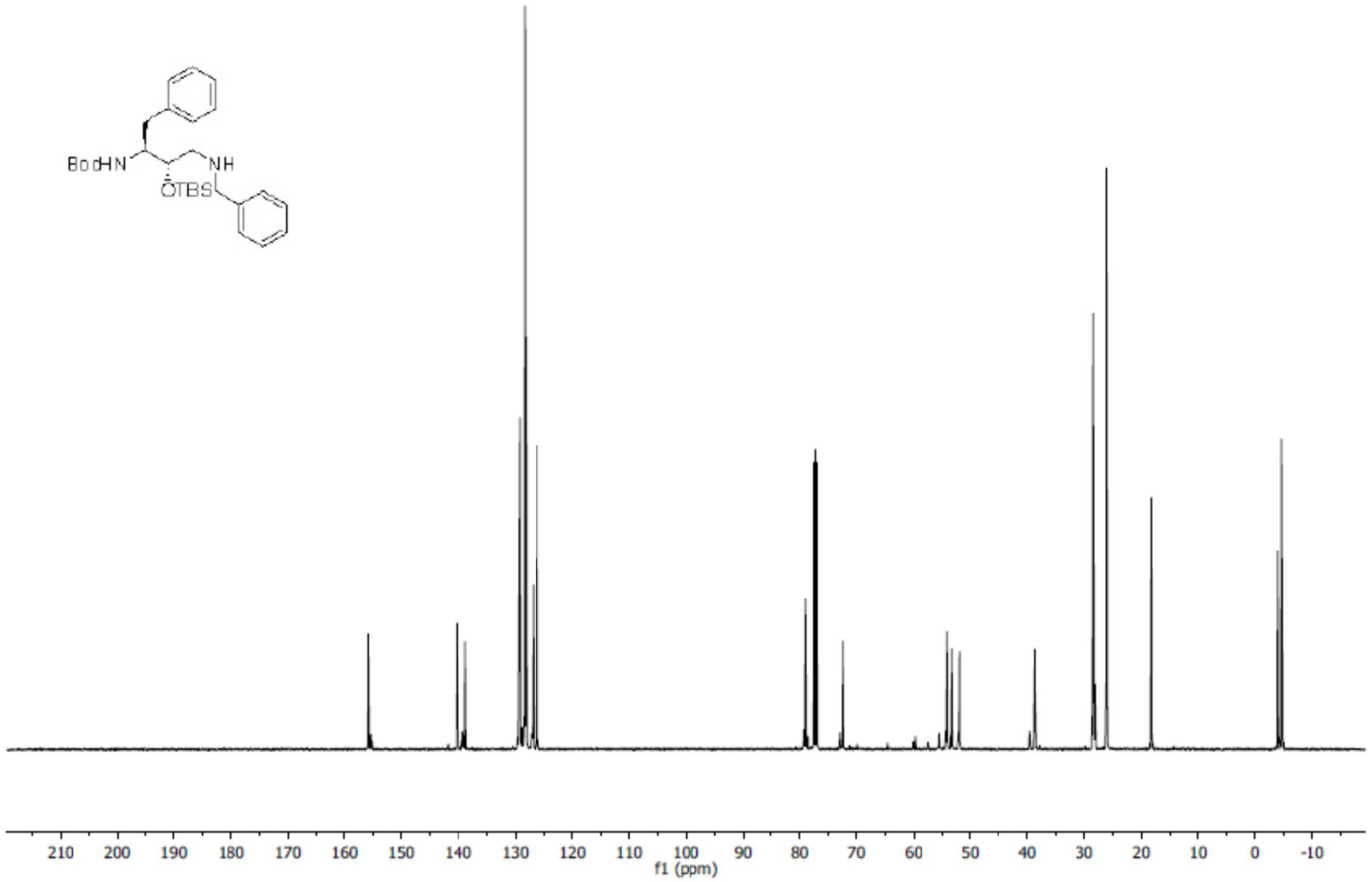
SD1002

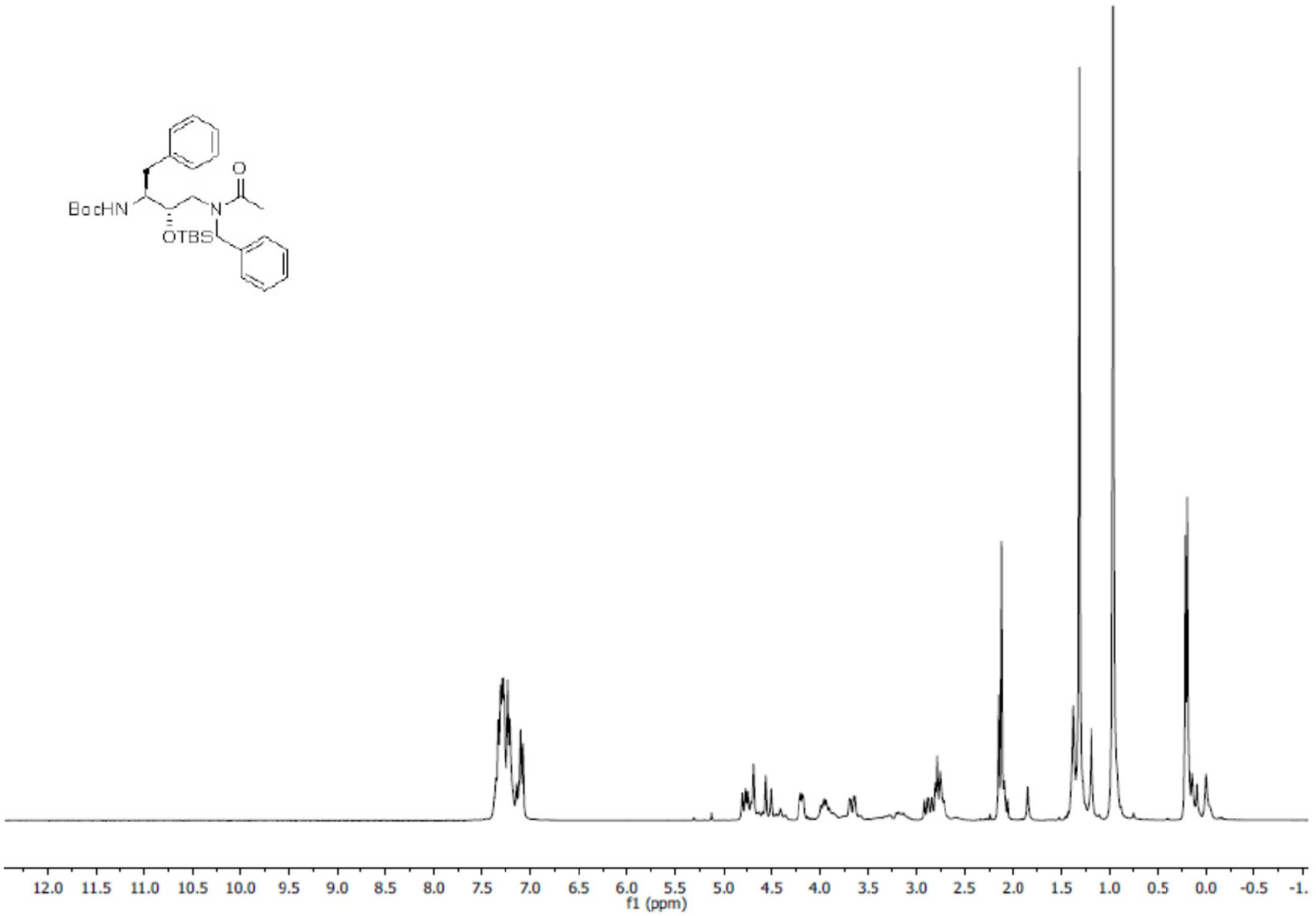
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

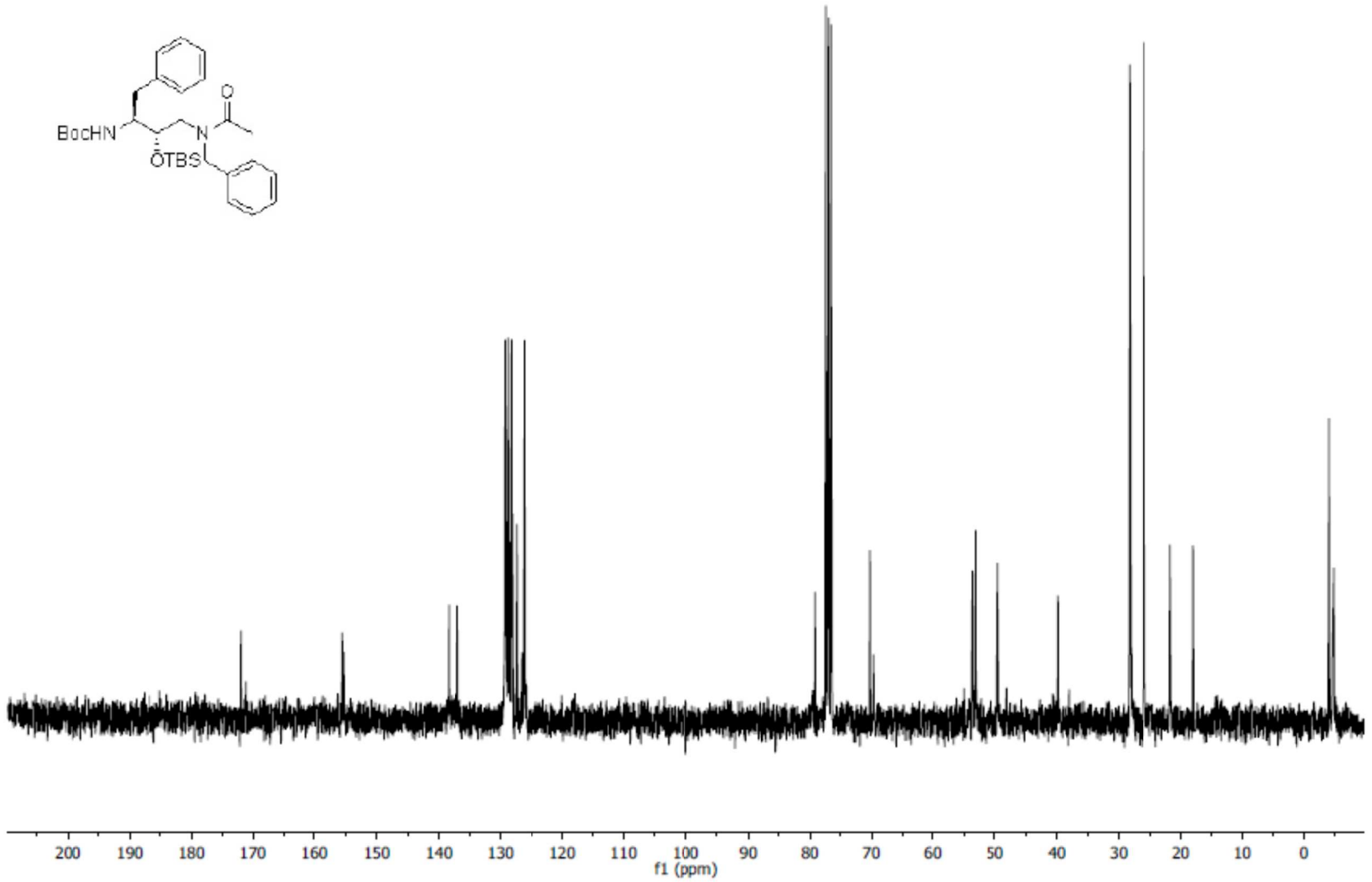

CbzHN
OH
O
N
N2
30.94
2.00
1.69
1.98
0.63
1.17
1.35
0.93
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

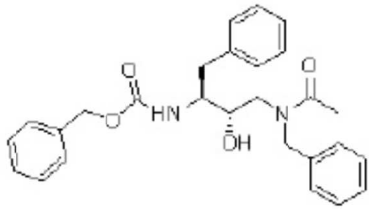

f1 (ppm)


BocHN
NH
OTBS
10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5 -2
f1 (ppm)


BocHN
NH
OTBS
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)

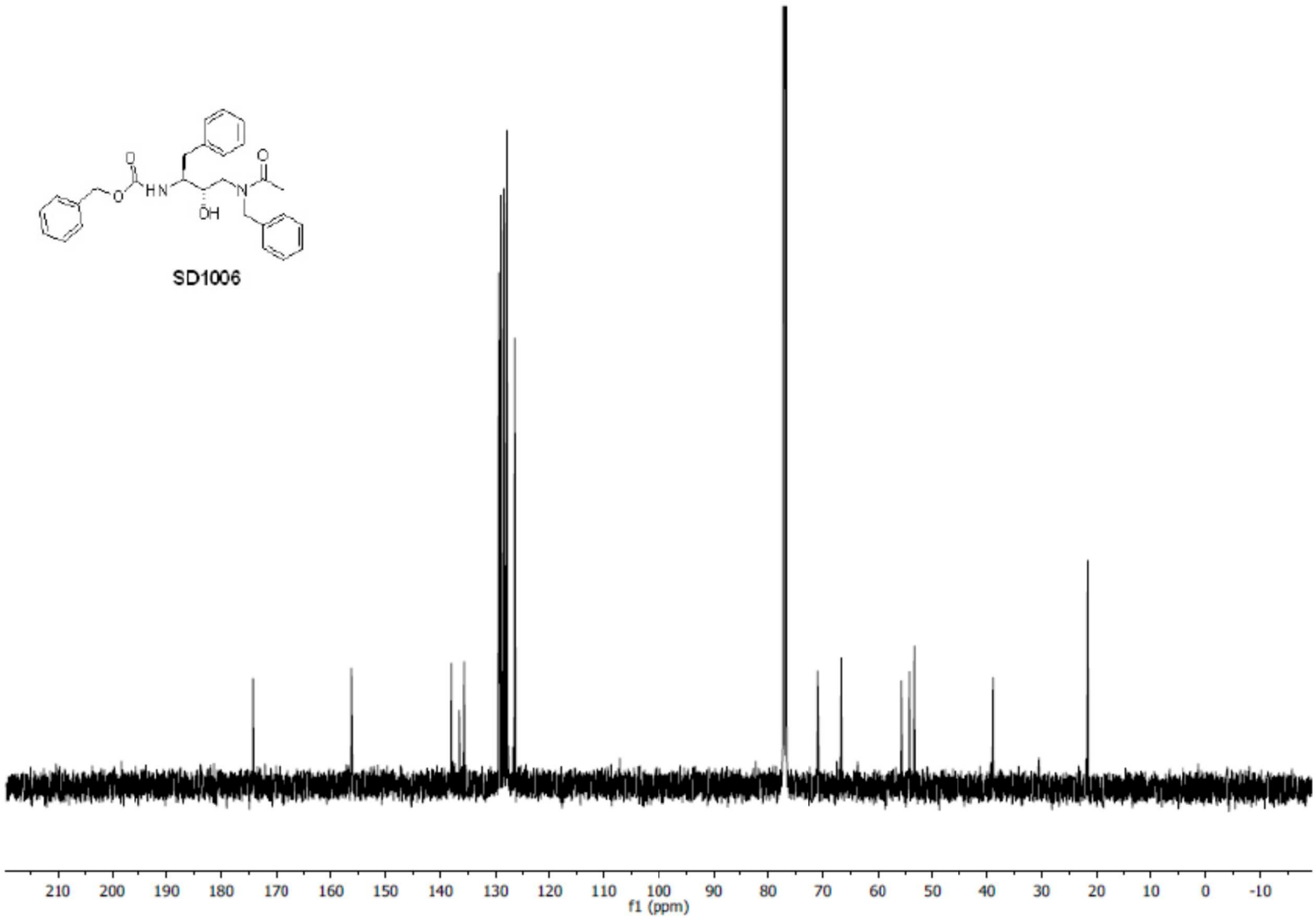

BocHN
O
N
OTBS
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.
f1 (ppm)

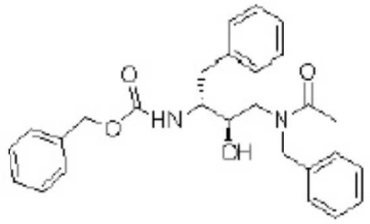

BocHN
N
O
OTBS
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)


O
HN
O
OH
N

SD1006

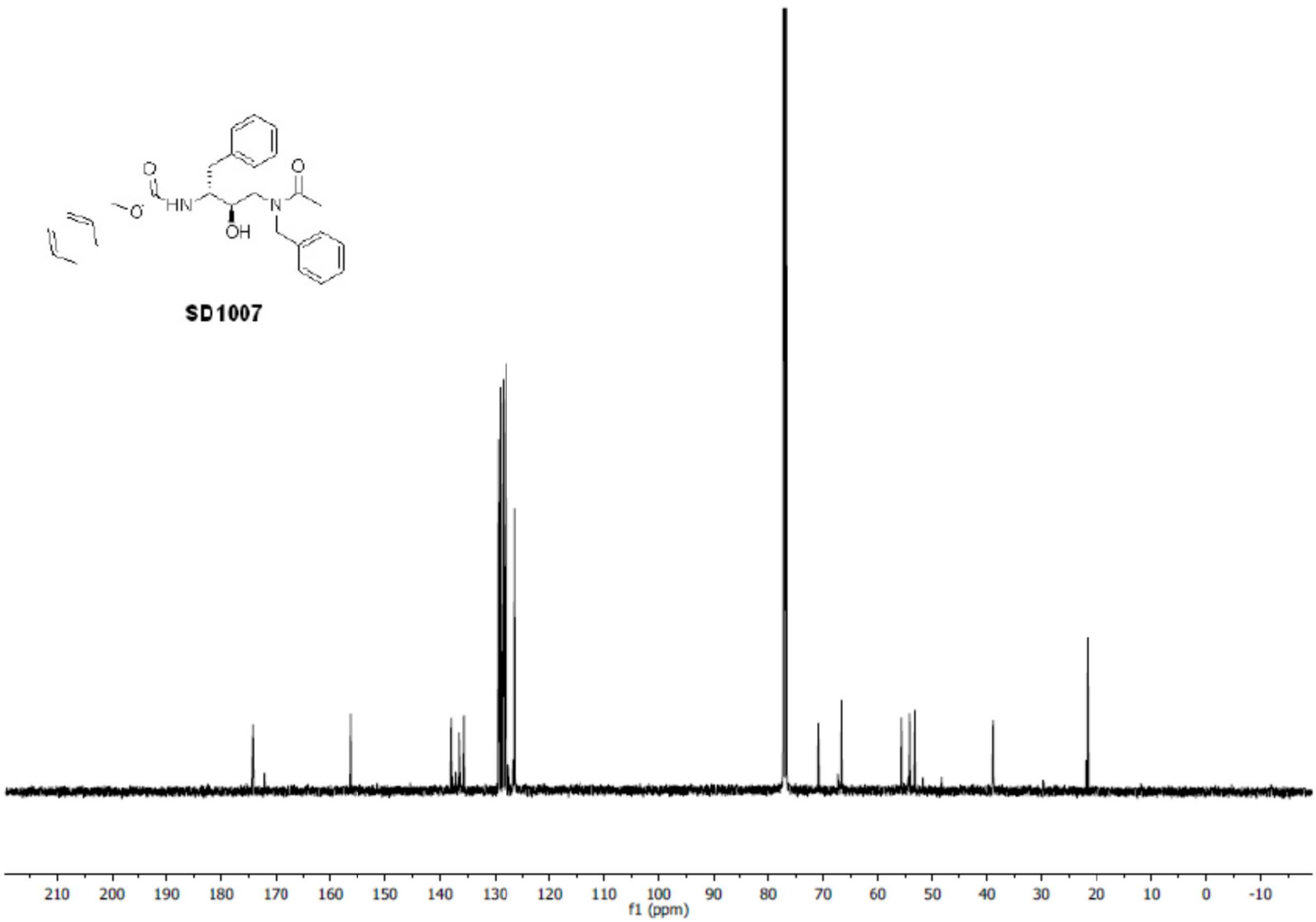
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5 -2
f1 (ppm)


SD1006
O
HN
O
OH
N
O
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)


O
HN
O
N
OH

SD1007

1.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5 -2
f1 (ppm)


SD1007
O
HN
O
OH
N
O
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)