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Facile Synthesis of SAM-Peptide Conjugates through Alkyl Linkers Targeting Protein *N*-terminal Methyltransferase 1

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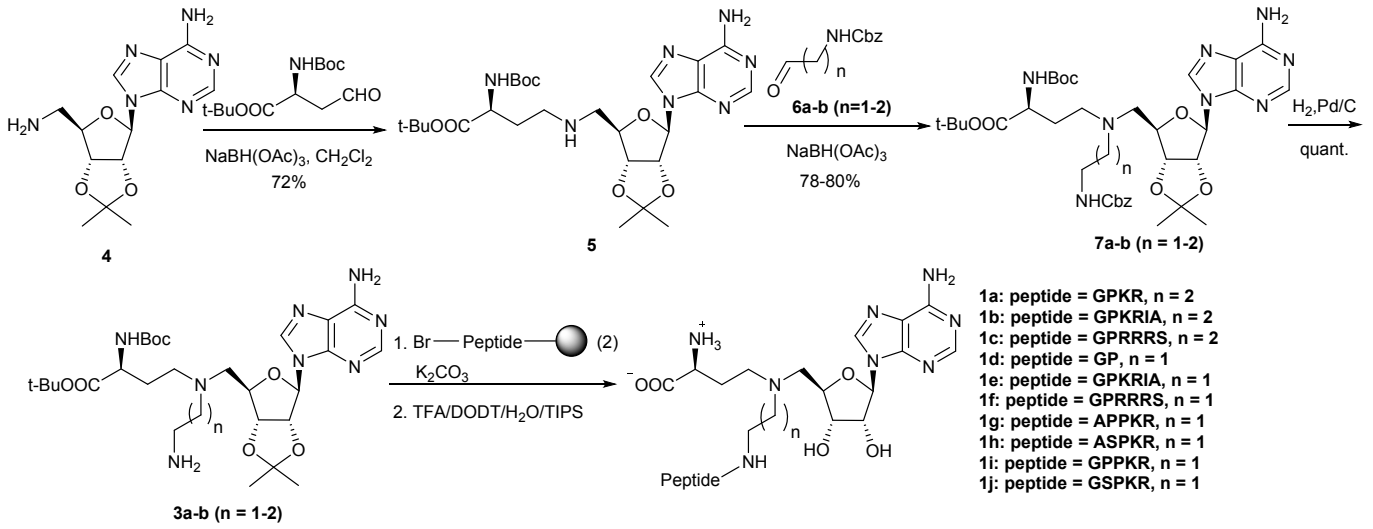
Table of Contents

General Information

Starting materials, reagents, and solvents were obtained from commercial sources and used as received. Progress of the reactions was monitored by thin-layer chromatography (TLC) analysis (Analtech, 0.25 mm silica gel GF254 on glass plates) or by analytical LC-MS using an PelkinElmer AxION 2 TOF MS with UV detection at 214 and 254 nm and an electrospray mode (ESI) coupled with a Flexar/s275/s200EP PDA detector. Purification of intermediates and final products was carried out on normal phase using an ISCO CombiFlash system and prepacked SiO_2 cartridges eluted with optimized gradients of either hexane-ethyl acetate mixture or dichloromethane-methanol as described. Preparative high pressure liquid chromatography (RP-HPLC) was performed on Waters instruments. Systems were run with 5-95% acetonitrile/water gradient with a 0.05% TFA modifier. NMR spectra were recorded on a BrukerAV400 (Avance 400 MHz) instrument. Chemical shifts (δ) are reported in parts per million (ppm) relative to deuterated solvent as the internal standard ($CDCl_3$ 7.26 ppm, DMSO-d_6 2.50 ppm, CD_3OD 3.31), and coupling constants (J) are in hertz (Hz). Peak multiplicities are expressed as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), and broad singlet (br s). All final products were characterized by ^1H NMR, ^{13}C NMR and MS analyses. Except for the known compounds, all new compounds were also characterized and confirmed by HRMS or MALDI-MS. MALDI-MS data was collected using a Micromass MALDI-R or Voyager DE-Pro operating in reflectron mode.

Synthesis of SAM-Peptide conjugates



Scheme S1 Synthesis of SAM-Peptide conjugates

Compounds **4** and **5** were synthesized according to the procedures we previously reported.[1]

Synthesis of compound 6

2-[(Benzyloxycarbonyl)amino]acetaldehyde (**6a**)[2,3]

To a solution of ethanolamine (2.0 g, 32.8mmol) in dry CH_2Cl_2 (30 mL) at 0℃ was added a solution CbzCl (3.7 mL, 26.2 mmol) in dry CH_2Cl_2 (20 mL) dropwise. The mixture was stirred for 2 h at 0℃ and then at room temperature for 18 h. The resulting mixture was washed with a saturated solution of $NaHCO_3$ and the aqueous phase was extracted with CH_2Cl_2 (3 X 50 mL). The organic phase was dried over anhydrous $MgSO_4$, filtered, and concentrated *in vacuo* to give the crude benzyl 2-hydroxyethylcarbamate as pale yellow oil (5.1 g, in quantitative yield), which was used in the next step without purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (m, 5 H), 5.10 (s, 2 H), 3.69 (t, 2 H), 3.34 (t, 2 H), 2.34 (brs, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$): 157.1, 136.4, 128.5, 128.2, 128.1, 66.9, 62.2, 43.6. HRMS (ESI$^+$) calcd for $C_{10}H_{14}NO_3$ $[M + H]^+$ m/z 196.0968, found m/z 196.0967.

To a solution of Benzyl 2-hydroxyethylcarbamate (200 mg, 1.02 mmol, 1.0 eq.) in DCM (10 mL) was added a solution of Dess-Martin periodinane (456 mg, 1.08mmol, 1.05 eq.) in DCM (5 mL) dropwise. After the mixture was stirred at room temperature for 1.5 h, a mixture of 2.1 M aqueous $Na_2S_2O_3$ (6 mL) and saturated aqueous $NaHCO_3$ (6 mL) were added. The organic phase was separated and the aqueous phase was extracted with diethyl ether (3 X 10 mL). After drying over anhydrous $MgSO_4$, the volatiles were removed *in vacuo* to afford the crude aldehyde **6a** (198 mg) in quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.63 (s, 1 H), 7.34 (m, 5 H), 5.49 (brs, 1 H), 5.13 (s, 2 H), 4.11 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): 196.4, 156.2, 136.1, 128.6, 128.3, 128.1, 67.2, 51.7. HRMS (ESI$^+$) calcd for $C_{10}H_{11}NO_3Na$ $[M + Na]^+$ m/z 216.0631, found m/z 216.0634.

3-[(Benzyloxycarbonyl)amino]propionaldehyde (**6b**)

Compound benzyl 2-hydroxypropylcarbamate was prepared from 3-amino-1-propanol following the same method as described above. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (m, 5 H), 5.10 (s, 2 H), 3.66 (t, 2 H), 3.33 (t, 2 H), 1.69 (m, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): 157.2, 136.5, 128.5, 128.1, 128.0, 66.8, 59.8, 38.0, 32.5. HRMS (ESI$^+$) calcd for $C_{11}H_{15}NO_3Na$ $(M + Na)^+$ m/z 232.0944, found m/z 232.0935.

Compound **6b** was prepared from benzyl 2-hydroxypropylcarbamate following the same method as described for **6a**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.74 (s, 1 H), 7.33 (m, 5 H), 5.20 (brs, 1 H), 5.08 (s, 2 H), 3.47 (t, 2 H), 2.70 (s, 2 H). ^{13}C NMR (100 MHz, $CDCl_3$): 201.0, 156.3, 136.4, 128.5, 128.1, 128.0, 66.7, 44.1, 34.5. HRMS (ESI$^+$) calcd for $C_{11}H_{13}NO_3Na$ $[M + Na]^+$ m/z 230.0788, found m/z 230.0785.

Synthesis of compound 7

5′-N-benzyloxycarbonylaminoethyl-N′-[4-{(2S)-2-(N-tert-butoxycarbonyl)amino-butyric acid}] tert-butyl ester-5′-deoxy-2′,3′-O,O-(1-methylethylidene)adenosine (**7a**)

To a stirred solution of compound **5** (233 mg, 0.41 mmol, 1.0 eq.) in CH_2Cl_2 (5 mL) was added into a solution of aldehyde **6a** (88 mg, 0.45 mmol, 1.1 eq.) in CH_2Cl_2 (5 mL) at room temperature. The solution was stirred for 20 min at room temperature. Then sodium triacetoxyborohydride (105 mg, 0.50 mmol, 1.2 eq.) was added in portions and the mixture was stirred at room temperature for 3 h. Saturated aqueous Na_2CO_3 (10 mL) was added to quench the reaction. The mixture was extracted with CH_2Cl_2 (2 X 10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by ISCO

CombiFlash system (CH_2Cl_2/MeOH, 30:1) to give **7a** as a white solid (237 mg, 0.32 mmol, 78%).^{1}H NMR (400 MHz, CD_3OD): δ 8.22 (s, 1 H), 8.21 (s, 1 H), 7.32 (m, 5 H), 7.29 (m, 1 H), 7.27 (m, 1 H), 6.15 (m, 1 H), 5.42 (m, 1 H), 5.09 (s, 2 H), 5.06 (m, 1 H), 4.28 (m, 1 H), 4.11 (m, 1 H), 3.13 (m, 2 H), 2.85 (m, 1 H), 2.64 (m, 3 H), 2.49 (m, 2 H), 1.98 (m, 1 H), 1.64 (m, 1 H), 1.54 (s, 3 H), 1.44 (s, 9 H), 1.42 (s, 9 H), 1.32 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): 173.9, 158.7, 158.1, 157.4, 154.0, 150.2, 142.1, 138.4, 129.5, 128.9, 128.8, 120.8, 115.8, 91.6, 86.5, 85.1, 84.7, 82.6, 80.5, 67.5, 57.4, 55.4, 54.0, 52.1, 40.1, 30.2, 28.8, 28.4, 27.6, 25.8. HRMS (ESI$^+$) calcd for $C_{36}H_{53}N_8O_9$ [M + H]$^+$ m/z 741.3930, found m/z 741.3954.

5′-N-benzyloxycarbonylaminopropyl-N′-[4-{(2S)-2-(N-tert-butoxycarbonyl)amino-butyric acid}] tert-butyl ester-5′-deoxy-2′,3′-O,O-(1-methylethylidene)adenosine (**7b**)

Compound **7b** was prepared from **6b** as described above. ^{1}H NMR (400 MHz, CD_3OD): δ 8.22 (s, 1 H), 8.21 (s, 1 H), 7.30 (m, 5 H), 6.16 (m, 1 H), 5.47 (m, 1 H), 5.05 (m, 3 H), 4.34 (m, 1 H), 4.08 (m, 1 H), 3.13 (m, 2 H), 2.85 (m, 1 H), 2.62 (m, 3 H), 2.43 (m, 2 H), 1.94 (m, 1 H), 1.66 (m, 1 H), 1.58 (m, 3 H), 1.44 (s, 9 H), 1.42 (s, 9 H), 1.38 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): 173.7, 158.8, 158.0, 157.5, 154.0, 150.2, 142.1, 138.5, 129.5, 129.0, 128.8, 120.8, 115.6, 91.8, 86.7, 85.2, 84.8, 82.6, 80.5, 67.4, 57.4, 54.2, 53.1, 52.0, 39.8, 29.8, 28.8, 28.7, 28.4, 28.1, 27.5. HRMS (ESI$^+$) calcd for $C_{37}H_{55}N_8O_9$ [M + H]$^+$ m/z 755.4087, found m/z 755.4065.

Synthesis of compound 3

5′-N-aminoethyl-N′-[4-{(2S)-2-(N-tert-butoxycarbonyl)amino-butyric acid}] tert-butyl ester-5′-deoxy-2′,3′-O,O-(1-methylethylidene)adenosine (**3a**)

To a solution of compound **7a** (420 mg, 0.57 mmol, 1.0 eq.) in methanol (20 mL) was added Palladium on activated charcoal (42 mg, 0.1 eq. w/w). The reaction mixture was degassed under vacuum and then under an atmosphere of H_2 overnight. The reaction mixture was filtered through a short pad of celite. The volatiles were removed to afford 3a (344 mg, 100%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.26 (s, 1 H), 8.23 (s, 1 H), 6.18 (m, 1 H), 5.43 (m, 1 H), 5.06 (m, 1 H), 4.31 (m, 1 H), 4.14 (m, 1 H), 2.89 (m, 1 H), 2.71 (m, 3 H), 2.61 (m, 3 H), 2.54 (m, 1 H), 1.98 (m, 1 H), 1.66 (m, 1 H), 1.60 (s, 3 H), 1.46 (s, 9 H), 1.44 (s, 9 H), 1.39 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): 173.6, 158.4, 157.6, 154.2, 150.3, 142.0, 120.9, 116.3, 92.3, 91.1, 86.3, 85.1, 84.2, 83.0, 80.8, 57.2, 53.5, 53.1, 52.3, 38.8, 30.7, 28.8, 28.4, 27.5, 25.6. HRMS (ESI$^+$) calcd for $C_{28}H_{47}N_8O_7$ [M + H]$^+$ m/z 607.3562, found m/z 607.3560.

5′-N-aminopropyl-N′-[4-{(2S)-2-(N-tert-butoxycarbonyl)amino-butyric acid}] tert-butyl ester-5′-deoxy-2′,3′-O,O-(1-methylethylidene)adenosine (**3b**)

Compound **3b** was prepared from **7b** as described above. ^{1}H NMR (400 MHz, CD_3OD): δ 8.26 (s, 1 H), 8.23 (s, 1 H), 6.18 (m, 1 H), 5.50 (m, 1 H), 5.06 (m, 1 H), 4.33 (m, 1 H), 4.07 (m, 1 H), 2.82 (m, 1 H), 2.68 (m, 2 H), 2.60 (m, 3 H), 2.43 (m, 2 H), 1.94 (m, 1 H), 1.65 (m, 1 H), 1.60 (s, 3 H), 1.55 (m, 2 H), 1.45 (s, 9 H), 1.44 (s, 9 H), 1.39 (s, 3 H). ^{13}C NMR (100 MHz, CD_3OD): 173.4, 158.3, 157.5, 154.0, 150.3, 142.0, 120.8, 115.9, 93.8, 91.5, 86.1, 85.1, 84.5, 82.9, 80.8, 57.4, 53.9, 53.4, 52.1, 39.8, 29.8, 28.8, 28.3, 27.5, 25.7, 9.3. HRMS (ESI$^+$) calcd for $C_{29}H_{49}N_8O_7$ [M + H]$^+$ m/z 621.3719, found m/z 621.3739.

Synthesis of compound 1

Peptide synthesis:

The peptides (G*P, G*PKR, G*PPKR, A*PPKR, G*SPKR, A*SPKR, G*PKRIA and G*PRRRS) were synthesized using a Liberty Automated Microwave Peptide Synthesizer (CEM) with the manufacturers standard coupling cycles at 0.1mmol scale. The peptides were synthesized on Rink Amide MBHA resin (Chem Impex) using N-α-Fmoc–protected amino acids (Chem Impex) or α-bromo carboxylic acids (Alfa Aesar).

NAM-C3-GPKR (**1a**)

To a suspension of G*PKR on resin (0.04 mmol, 1.0 eq.) in DMF (3 mL) was added compound **3b** (50 mg, 0.08 mmol, 2.0 eq.) and anhydrous K_2CO_3 (11 mg, 0.08 mmol, 2.0 eq.). The mixture was shaken at room temperature overnight. The suspension was filtered, and the resin was washed alternatively with H_2O (2 mL x 3), MeOH (2 mL x 3), and CH_2Cl_2 (2 mL x 3). The peptide conjugates were cleaved from the resin in the presence of a cleavage cocktail containing trifluoroacetic acid (TFA) (Sigma Aldrich)/2,2'-(Ethylenedioxy)-diethanethiol (Sigma Aldrich)/triisopropylsilane (TIPS) (Acros Organics)/water (94:2.5:1:2.5 v/v) at room temperature for 4-5 h and resin was rinsed with a small amount of TFA. Volatiles of the filtrate were removed under N_2 and the residue was precipitated with 10 vol. cold anhydrous ether, and collected by centrifugation. The supernatant was discarded and the pellet was washed with chilled ether and air-dried. The white precipitate was dissolved in ddH_2O and purified by reverse phase HPLC using a waters system with a XBridge (BEH C18, 5µm, 10 X 250 mm) column with 0.05% TFA in water (A) and 0.05% TFA in CH_3CN (B) as the mobile phase with monitoring at 214 nm and 254 nm. MALDI-TOF (positive) *m/z*: calcd for $C_{36}H_{63}N_{16}O_9$ $[M + H]^+$ m/z 863.5, found m/z 862.1.

NAM-C3-GPKRIA (**1b**)

Compound **1b** was prepared from **3b** and G*PKRIA as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{45}H_{79}N_{18}O_{11}$ $[M + H]^+$ m/z 1047.6, found m/z 1047.7.

NAM-C3-GPRRRS (**1c**)

Compound **1c** was prepared from **3b** and G*PRRRS as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{45}H_{80}N_{23}O_{12}$ $[M + H]^+$ m/z 1134.6, found m/z 1135.1.

NAM-C2-GP (**1d**)

Compound **1d** was prepared from **3a** and G*P as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{23}H_{37}N_{10}O_7$ $[M + H]^+$ m/z 565.3, found m/z 567.5.

NAM-C2-GPKRIA (**1e**)

Compound **1e** was prepared from **3a** and G*PKRIA as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{44}H_{77}N_{18}O_{11}$ $[M + H]^+$ m/z 1033.6, found m/z 1033.9.

NAM-C2-GPRRRS (**1f**)

Compound **1f** was prepared from **3a** and G*PRRRS as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{44}H_{78}N_{23}O_{12}$ $[M + H]^+$ m/z 1120.6, found m/z 1120.8.

NAM-C2-APPKR (**1g**)

Compound **1g** was prepared from **3a** and A*PPKR as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{41}H_{70}N_{17}O_{10}$ $[M + H]^+$ m/z 960.5, found m/z 960.3.

NAM-C2-ASPKR (**1h**)

Compound **1h** was prepared from **3a** and A*SPKR as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{39}H_{68}N_{17}O_{11}$ $[M + H]^+$ m/z 950.5, found m/z 950.5.

NAM-C2-GPPKR (**1i**)

Compound **1i** was prepared from **3a** and G*PPKR as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{40}H_{68}N_{17}O_{10}$ $[M + H]^+$ m/z 946.5, found m/z 946.2.

NAM-C2-GSPKR (**1j**)

Compound **1j** was prepared from **3a** and G*SPKR as described above. MALDI-TOF (positive) *m/z*: calcd for $C_{38}H_{66}N_{17}O_{11}$ $[M + H]^+$ m/z 936.5, found m/z 936.4.

NTMT1, PRMT1 and G9a inhibition assays

Human NTMT1, PRMT1, and G9a were expressed in *E. Coli* BL21 (DE3) codon plus RIL cells in LB medium in the presence of 50 μg/mL of kanamycin, respectively.[4-6] All inhibitory activities were determined with both substrates at their K_m values at 37 ℃ (For NTMT1, SAM at 8 μM, RCC1-10 at 0.9 μM; for PRMT1, SAM at 20 μM, H4-12 at 25 μM; for G9a, SAM at 2 μM, H3-15 at 20 μM). NTMT1 assay was performed under the following condition: pH7.4, 25 mM Tris, pH7.4, 50 mM KCl, 0.2 μM His-NTMT1, 10 μM SsSAH hydrolyase, 8 μM SAM, 15 μM ThioGlo 1, various concentration of inhibitor.[4] The assay was initiated by the addition of the peptide substrate RCC1-10 which was the first 10 residues of RCC1. Fluorescence was then measured in a FlexStation 3 Muti-Mode Microplate Reader using 370 nm excitation and 500 nm emission. The IC_{50} values were determined by fitting the activity data with GraphPad. Similar experiments were carried out for inhibitory effects on PRMT1 and G9a.[5,6]

K_i values calculation

Except compound 1c, K_i values of all other compounds were calculated by the Cheng-Prusoff equation.[7] This equation is:

$$K_i=(IC_{50}/(1+(S/K_m)))$$

Where K_i is the inhibition constant, IC_{50} is the functional strength of the inhibitor, [S] is fixed substrate concentration and Km is the Michaelis-Menten constant.

For compound 1c, its K_i value was fitted with GraphPad using Morrison equation.[8]

$Q=(K_i*(1+(S/K_m)))$

$Y=Vo*(1-((((Et+X+Q)-(((Et+X+Q)^2)-4*Et*X)^0.5))/(2*Et)))$

Where Et is the concentration of enzyme used in the experiment. S is fixed substrate concentration, K_m is the Michaelis-Menten constant, V_0 is the enzyme velocity with no inhibitor, expressed in the same units as Y. K_i is the inhibition constant.

Docking study of NAM-C3-GPRRRS 1c

The X-ray crystal structure of human protein N-terminal RCC1 methyltransferase (pdb:2EX4) complexed with *S*-adenosyl homocysteine (SAH) was obtained from pdb.org. The protonation state of the protein and the ligand were calculated using the default settings. The active site was defined by a sphere of 6.0 Å from the native ligand SAH in the crystal structure. Molecules used for the docking experiments were constructed in ChemBio3D Ultra 13.0 and minimized using the MMFF94x force field. The ligand for docking was prepared using SYBYL X2.1, and the energy was minimized using the external Tripos force field. The docked poses were scored using CHEMPLP scoring function. The best docked pose of the ligand was visualized using Pymol Version 1.3 and its score was shown in the table below.

Supplementary Table 1 The score of the best docked pose of NAM-C3-GPRRRS

Score	S (PLP)	S (hbond)	S (cho)	S (metal)	DE (clash)	DE (tors)	intcor
109.01	-98.57	8.56	0.00	0.00	1.27	17.95	21.02

Supporting References

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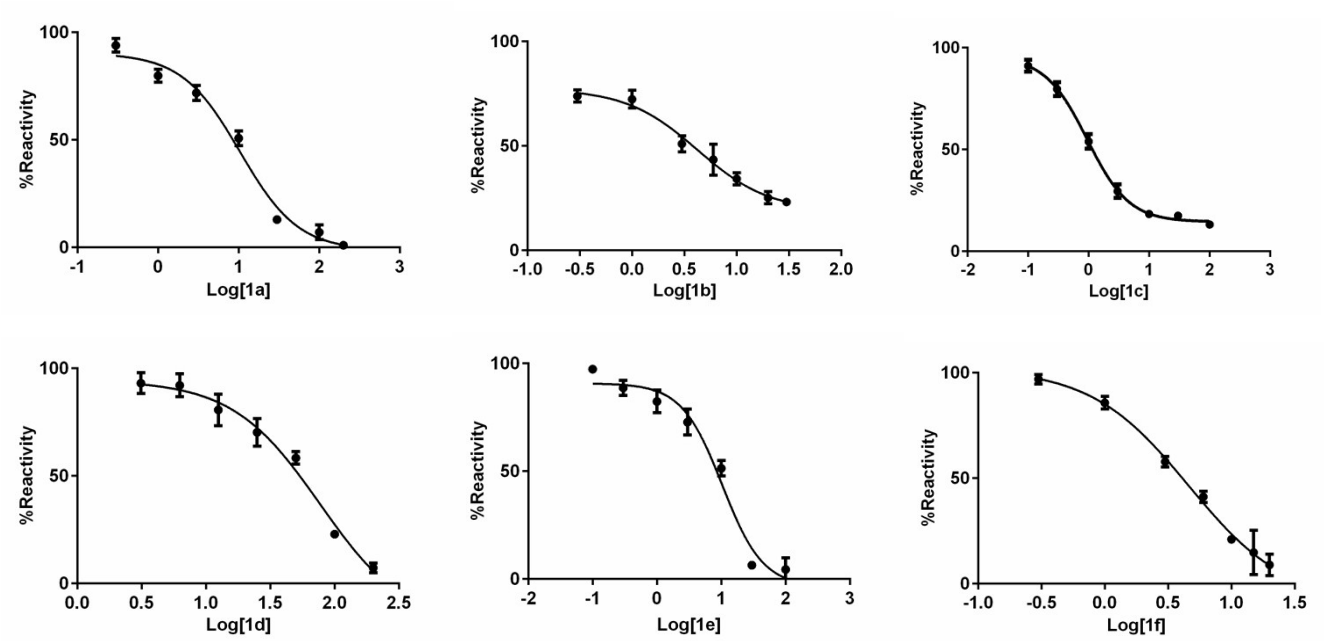
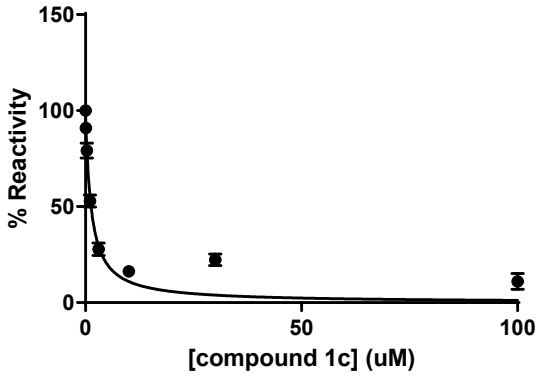
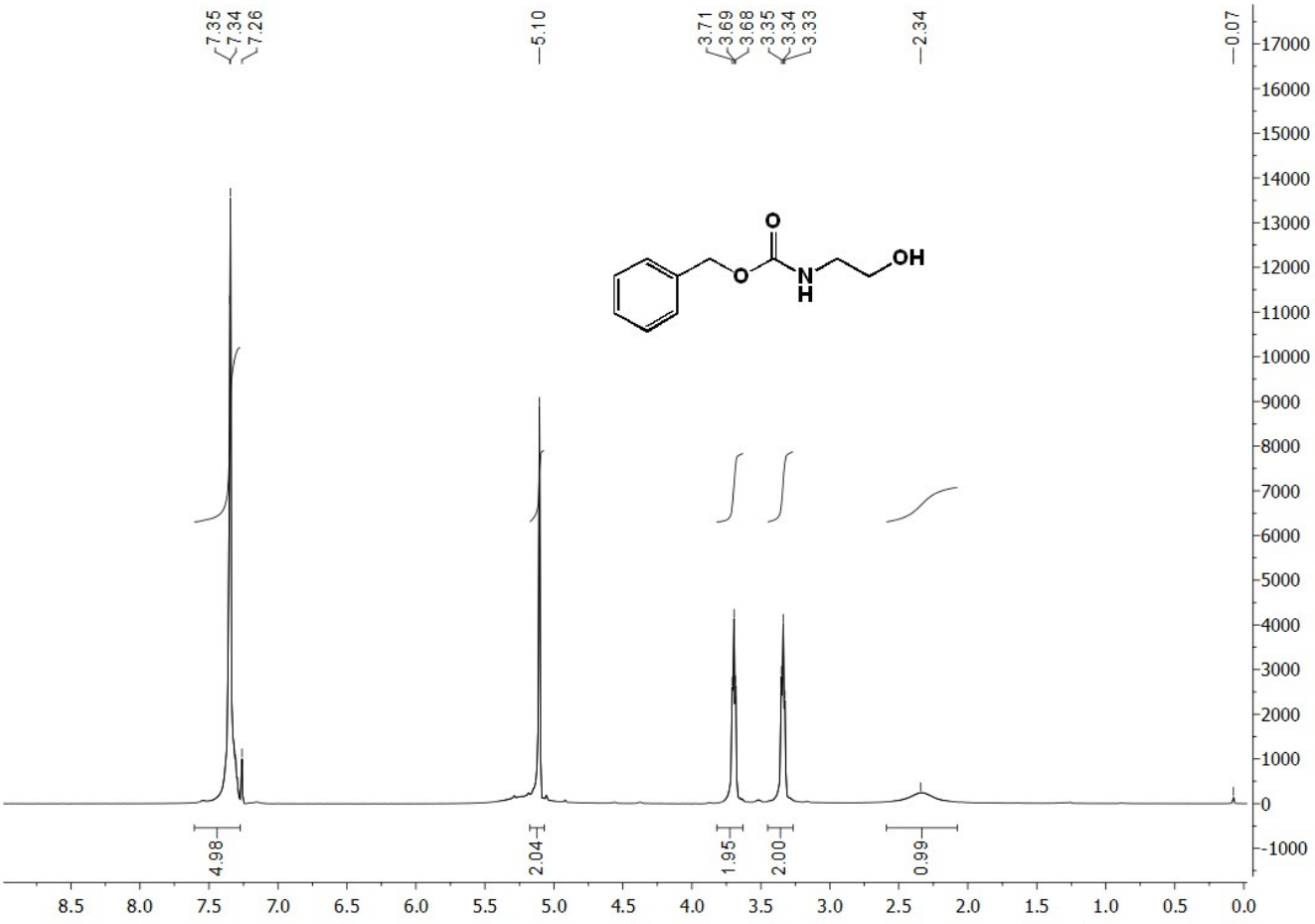
IC_{50} curves for Compounds 1a, 1b, 1c, 1d, 1e and 1f

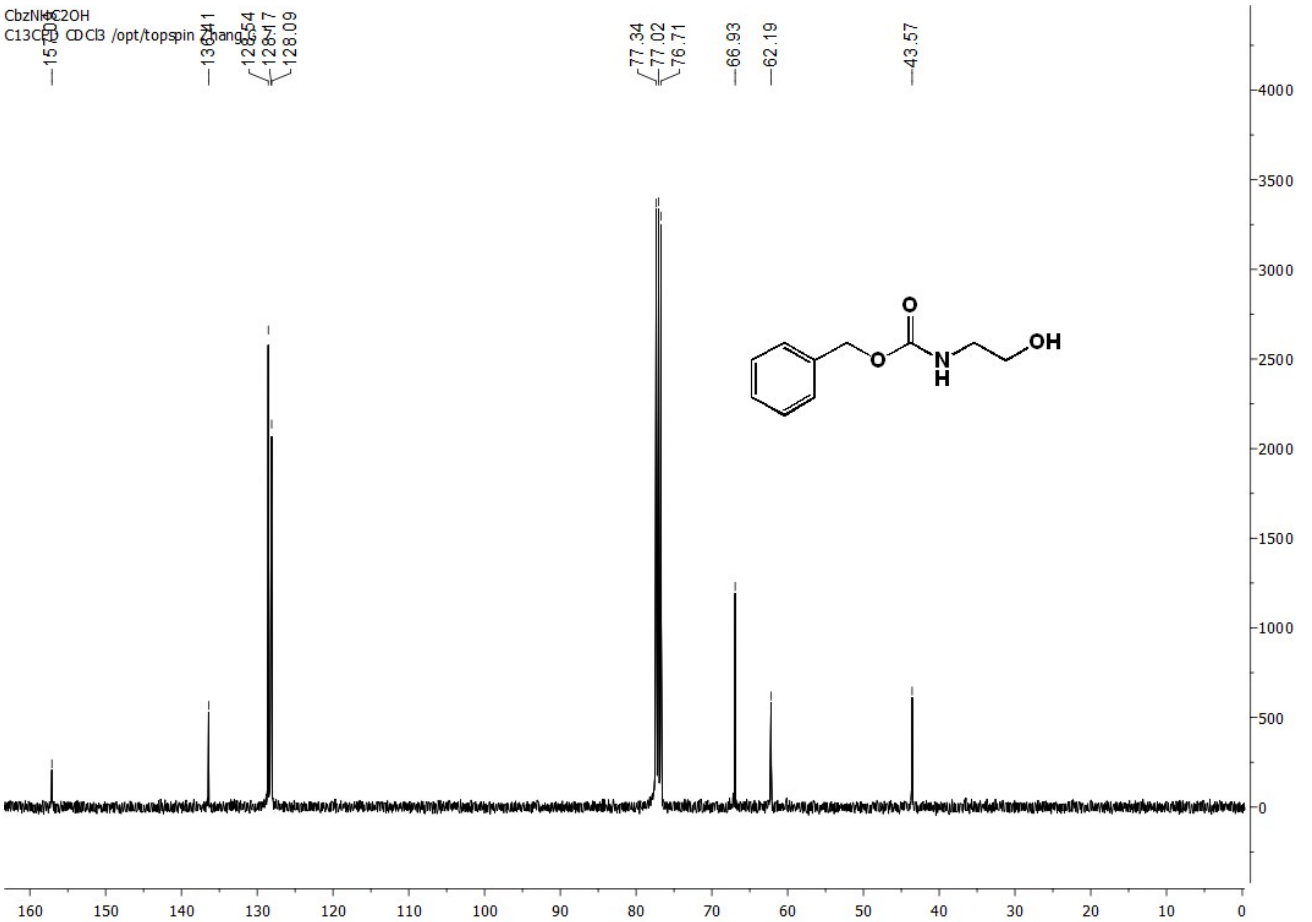


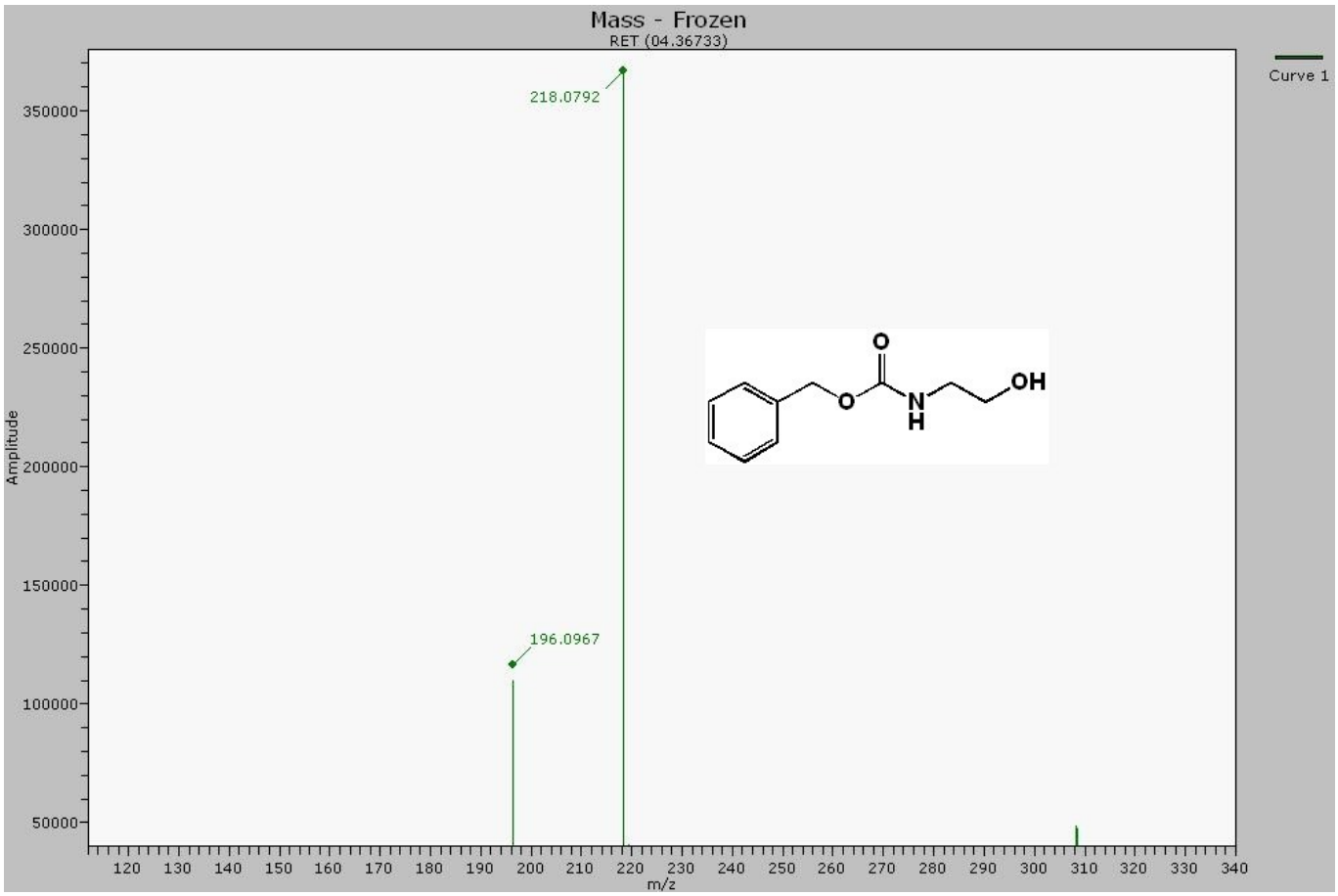
K_i curve for Compound 1c.



Characterization of ^{1}HNMR, ^{13}CNMR, HRMS and MALDI-MS



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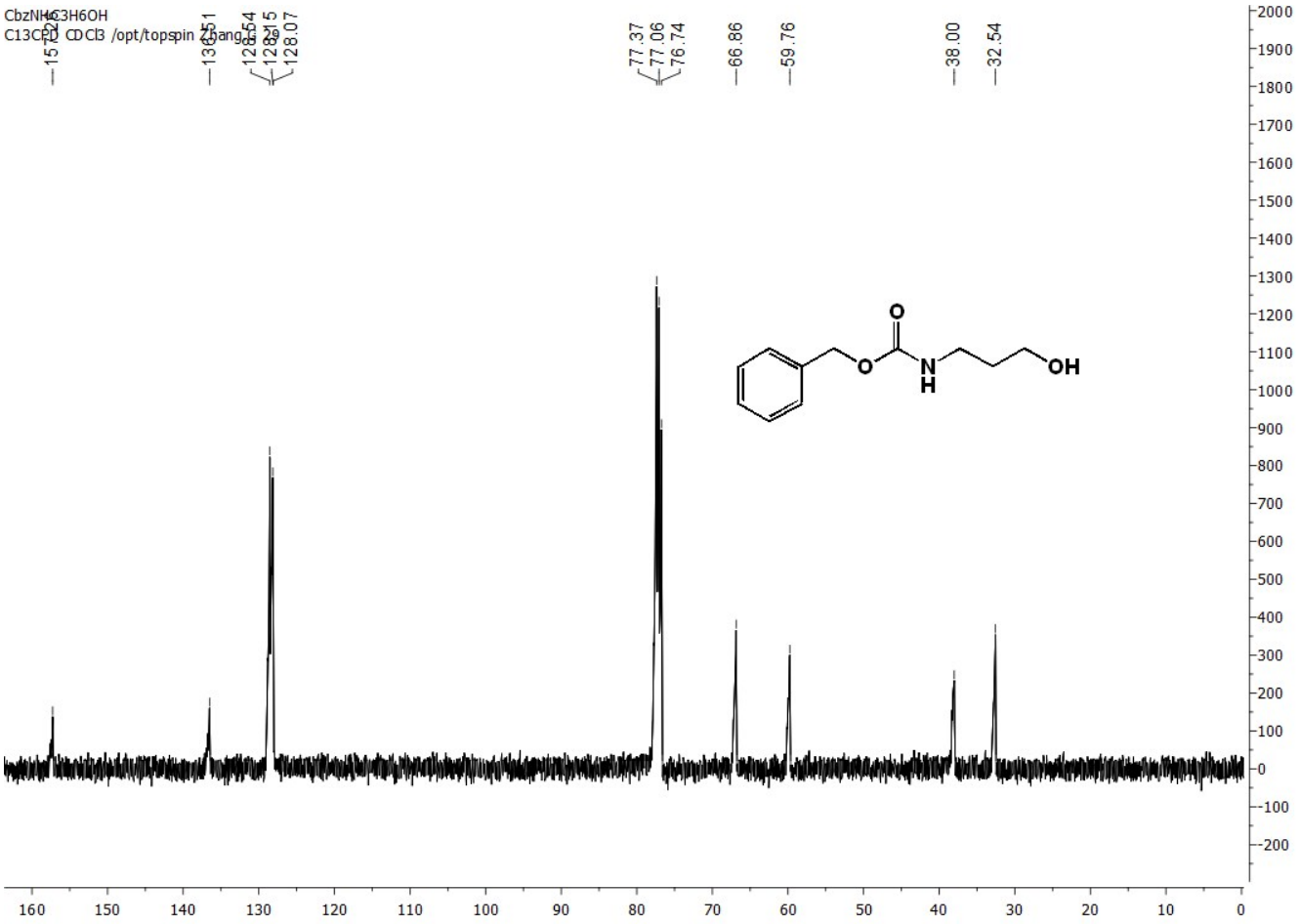
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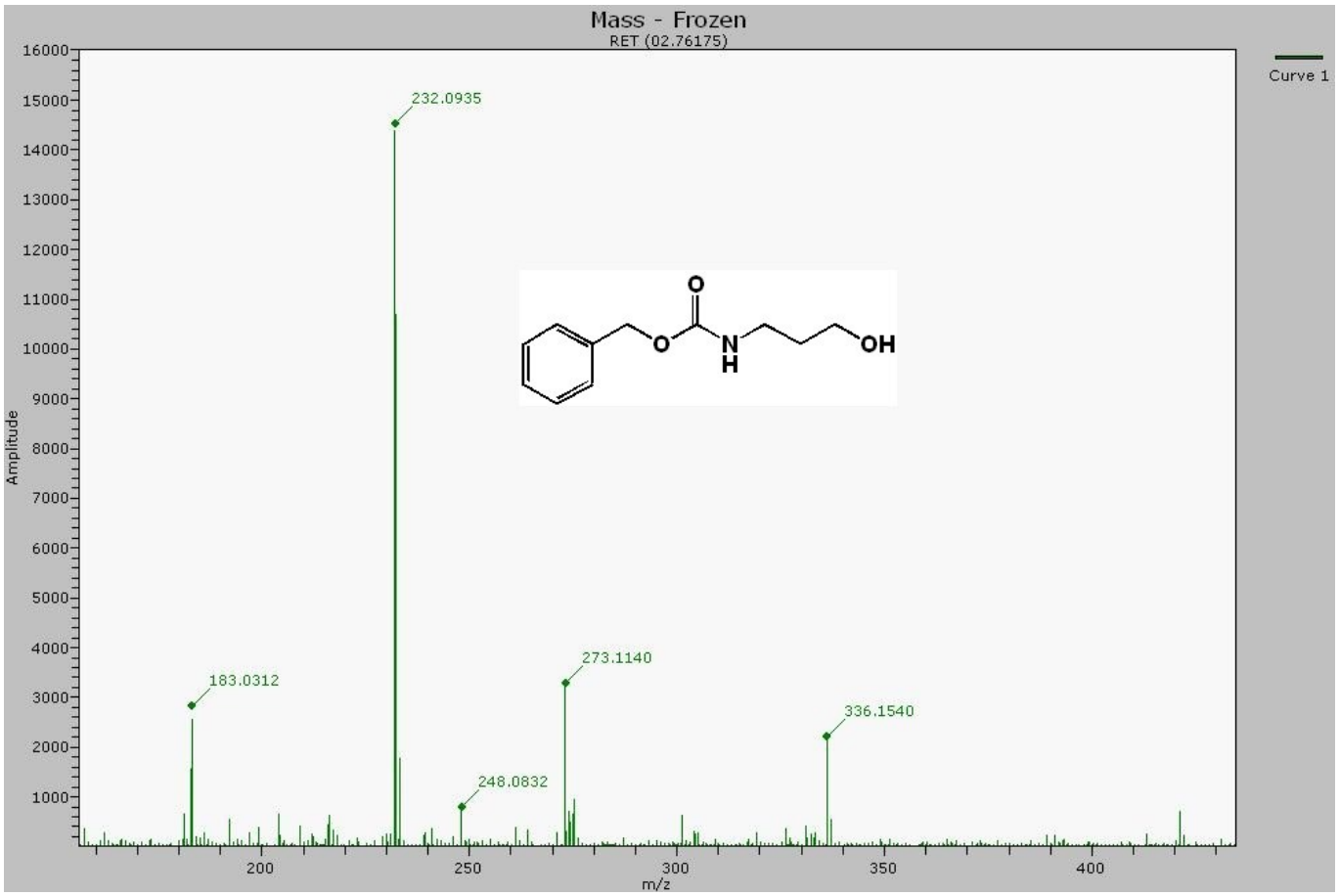
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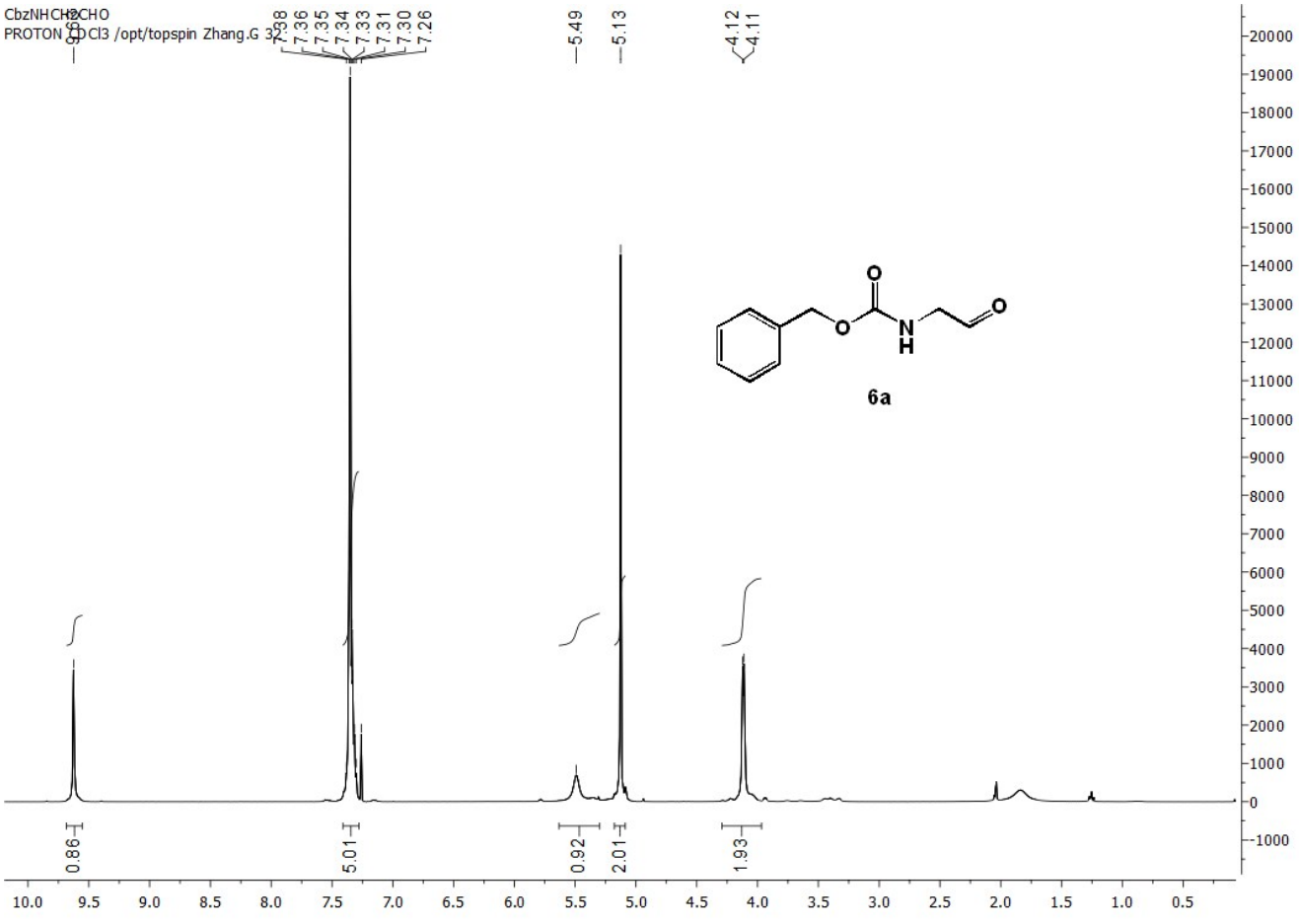
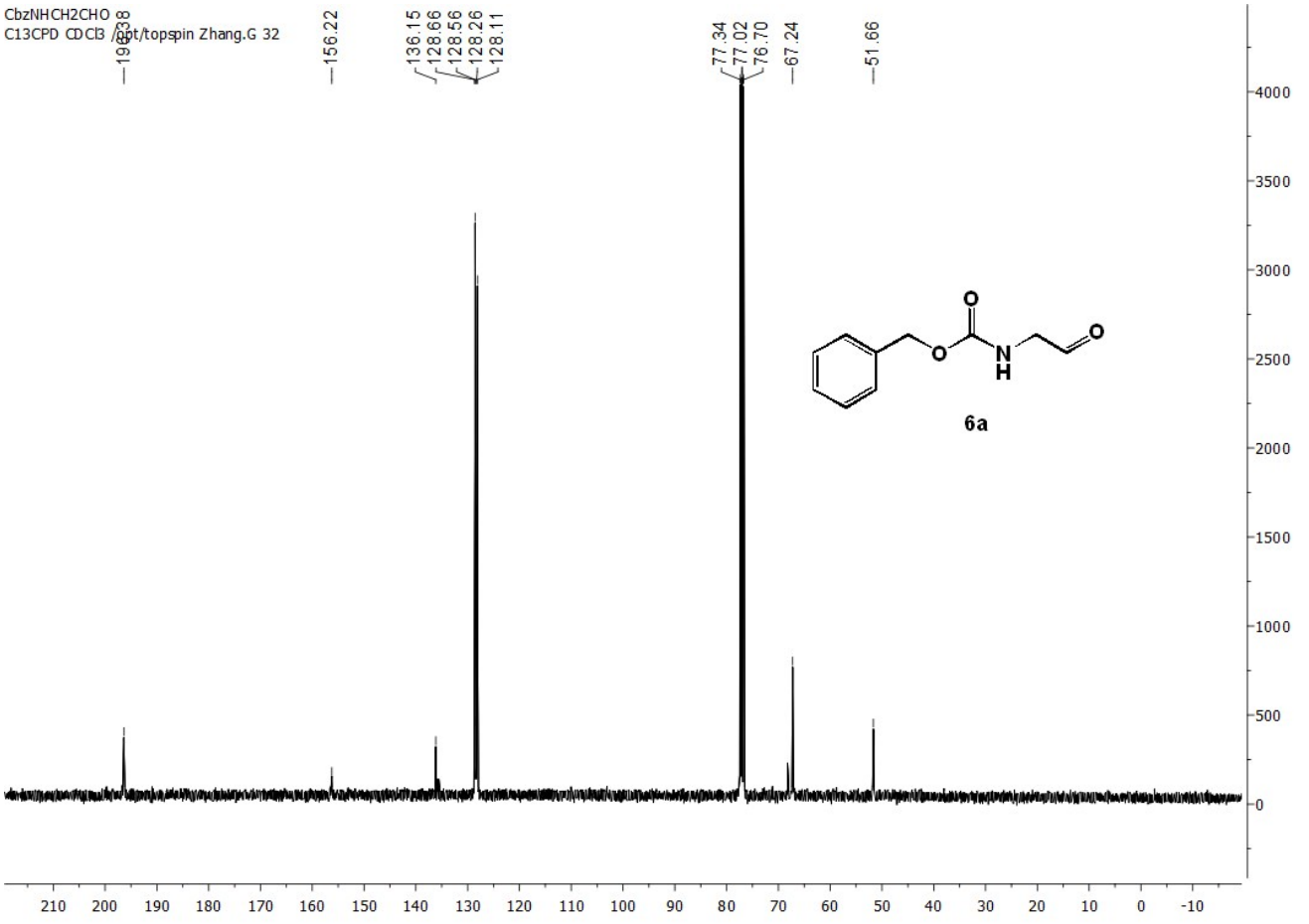
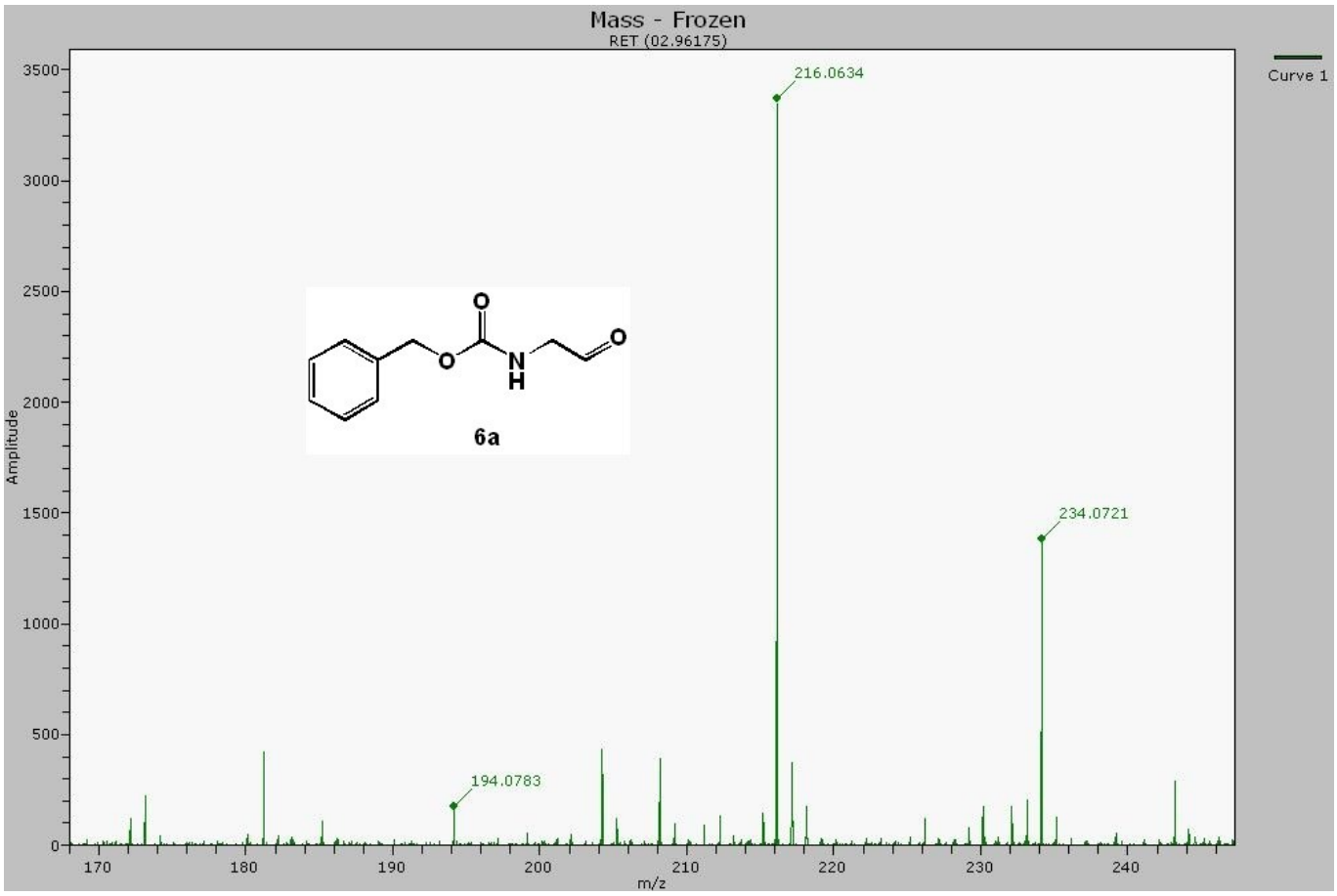
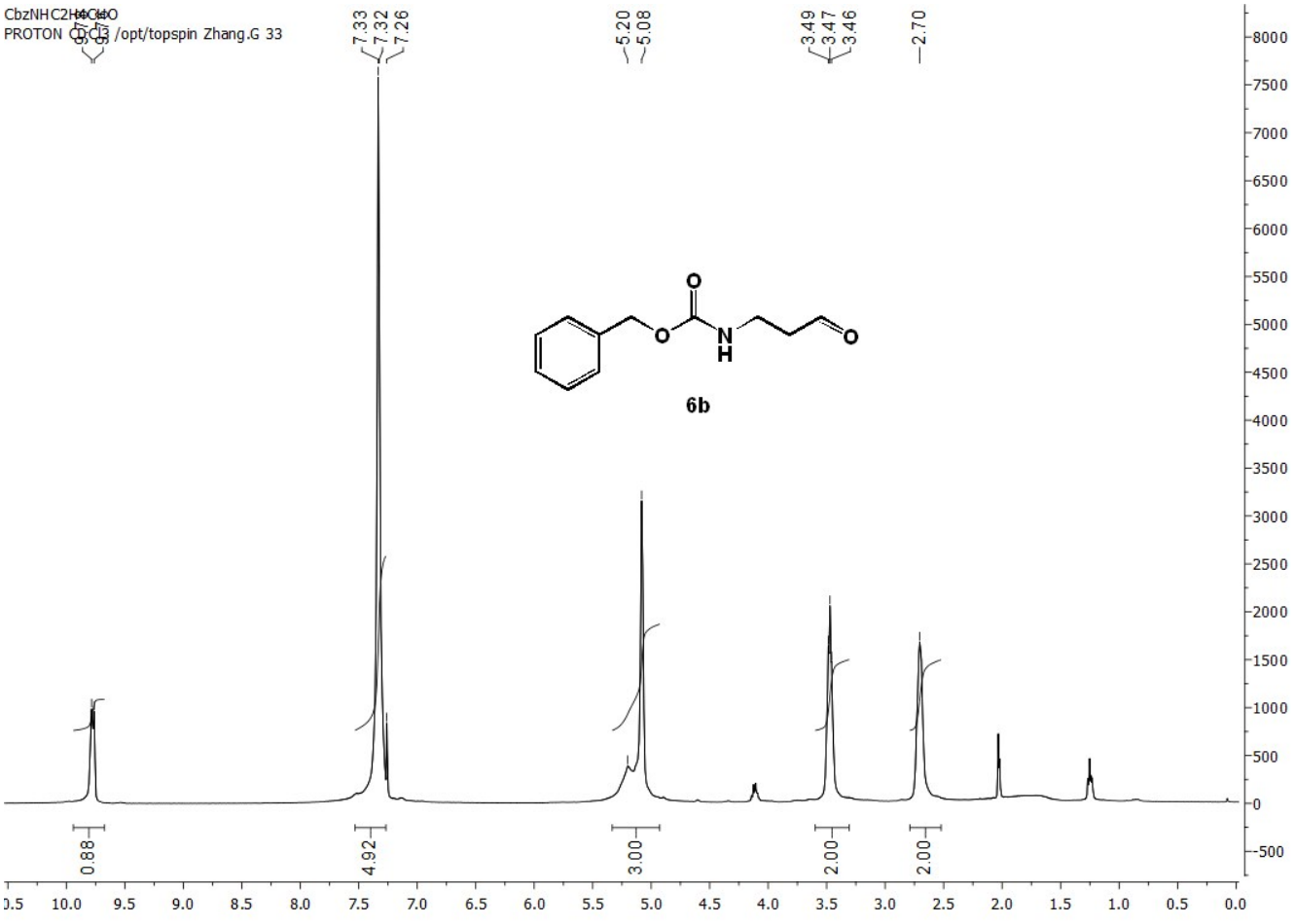
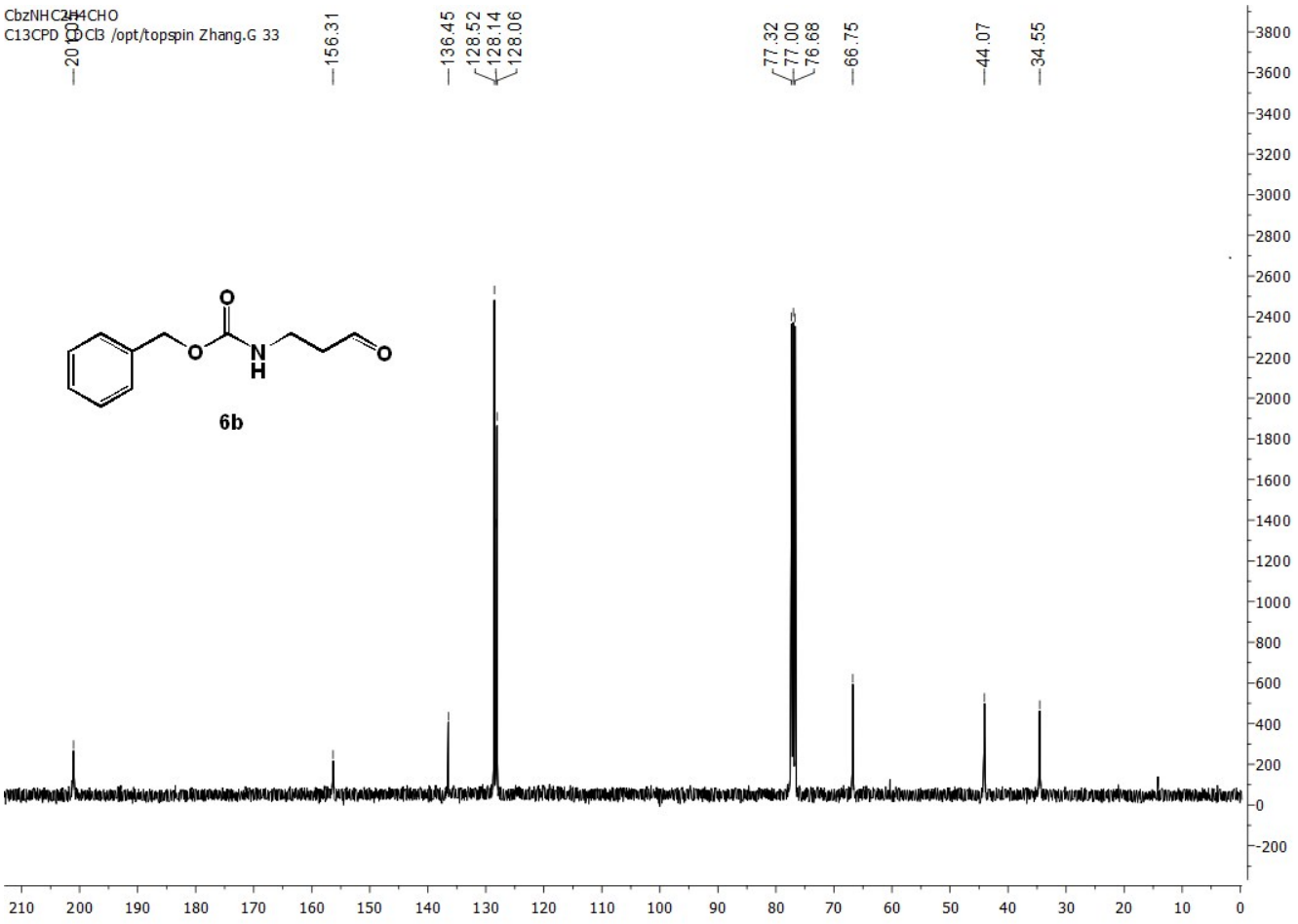
OH

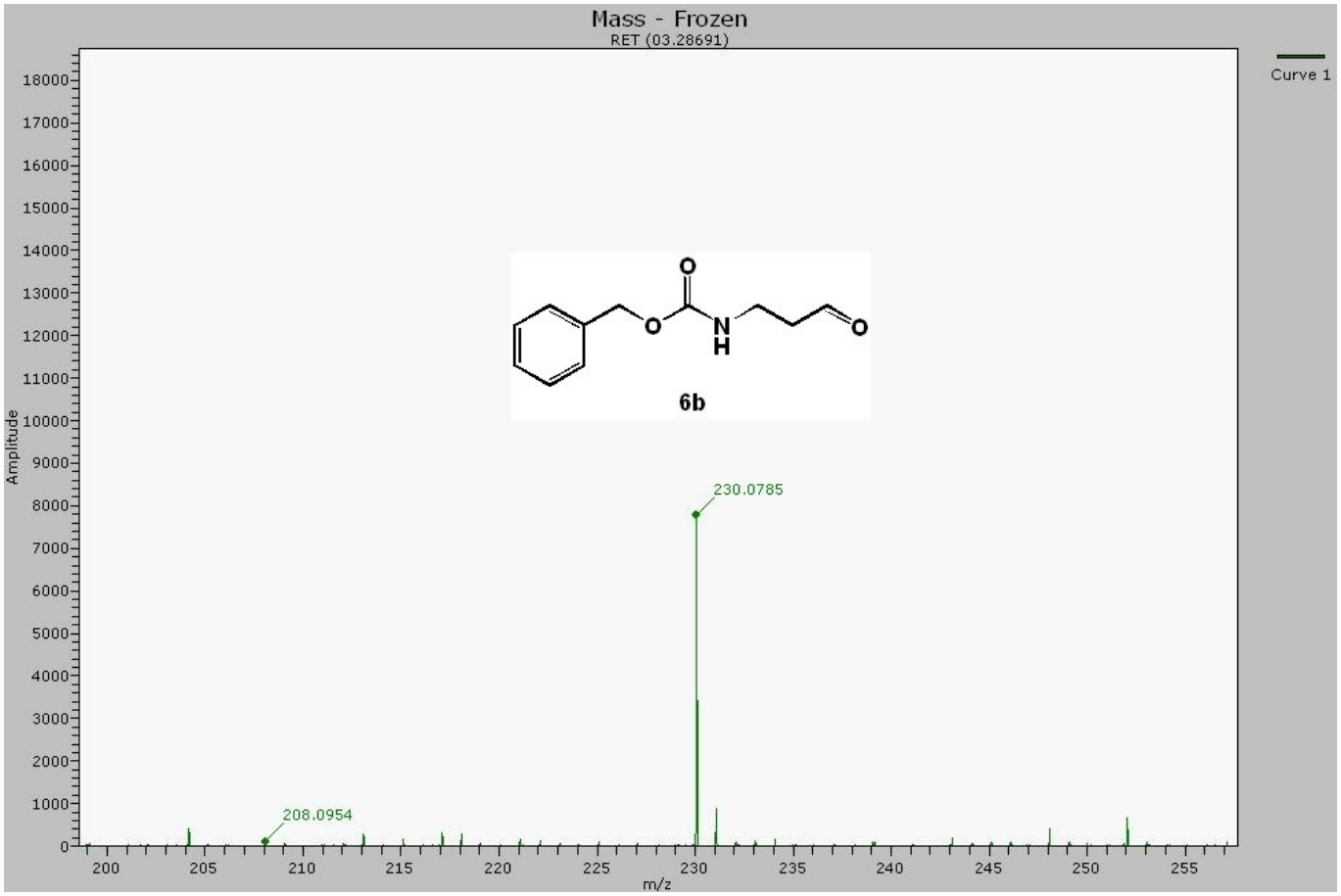

CbzNHCH2CHO
PROTON CDCl3 /opt/topspin Zhang.G 32
9.66
7.38
7.36
7.35
7.34
7.33
7.31
7.30
7.26
5.49
5.13
4.12
4.11
0.86
5.01
0.92
2.01
1.93
6a

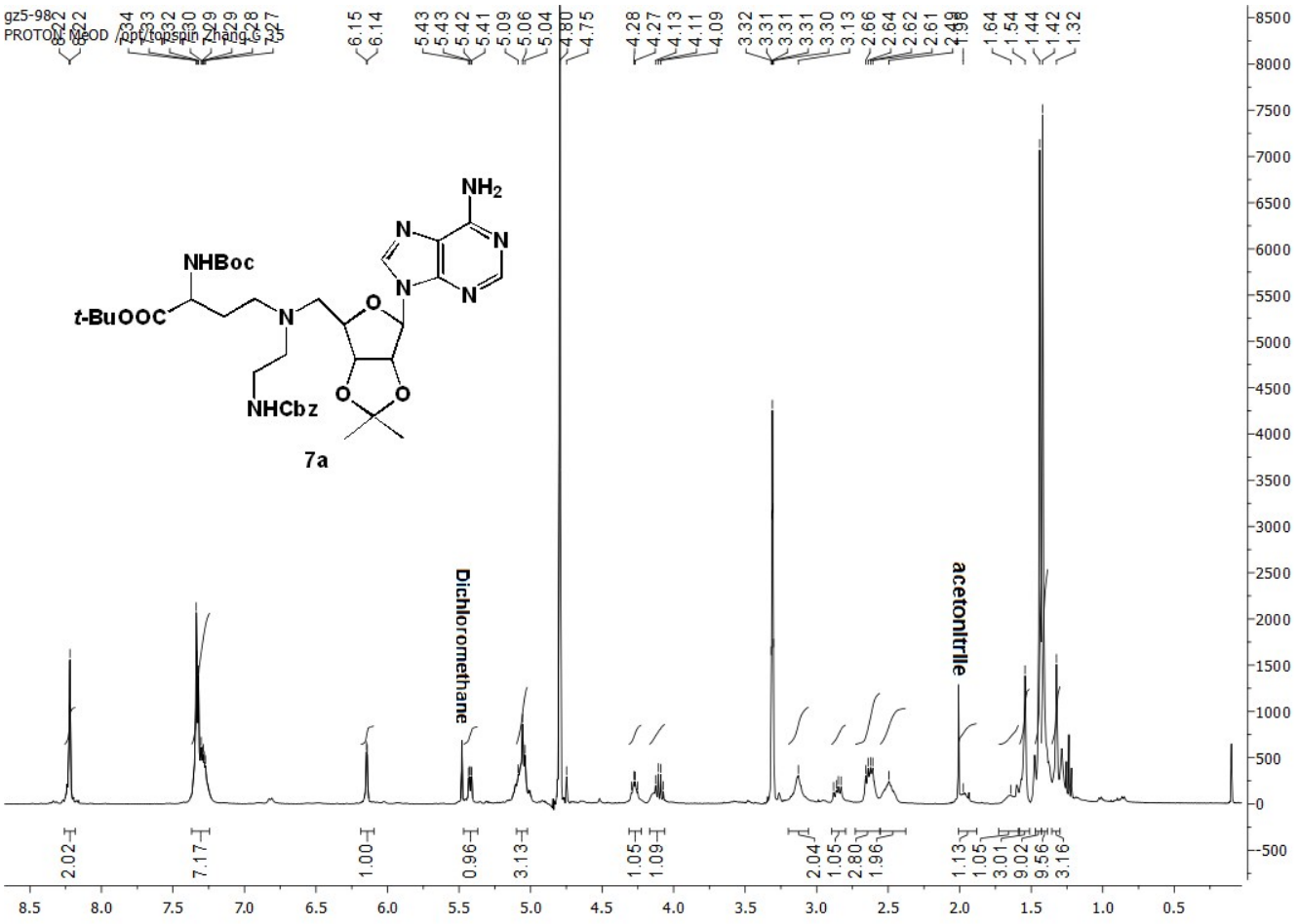

CbzNHCH2CHO
C13CPD CDCl3 /opt/topspin Zhang.G 32
156.22
136.15
128.66
128.56
128.26
128.11
77.34
77.02
76.70
67.24
51.66
6a

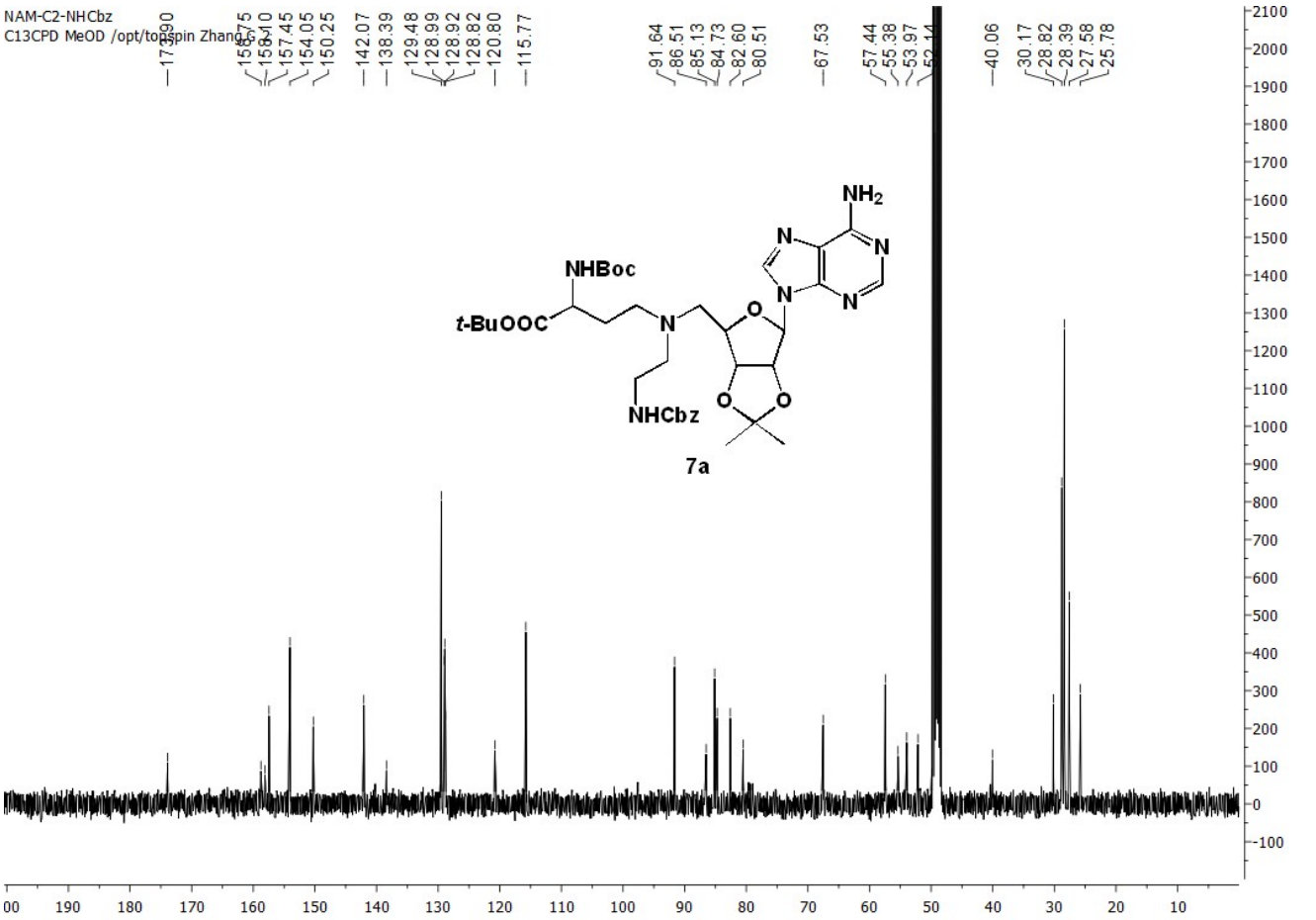

Mass - Frozen
RET (02.96175)
Curve 1
216.0634
234.0721
194.0783
Amplitude
m/z
6a

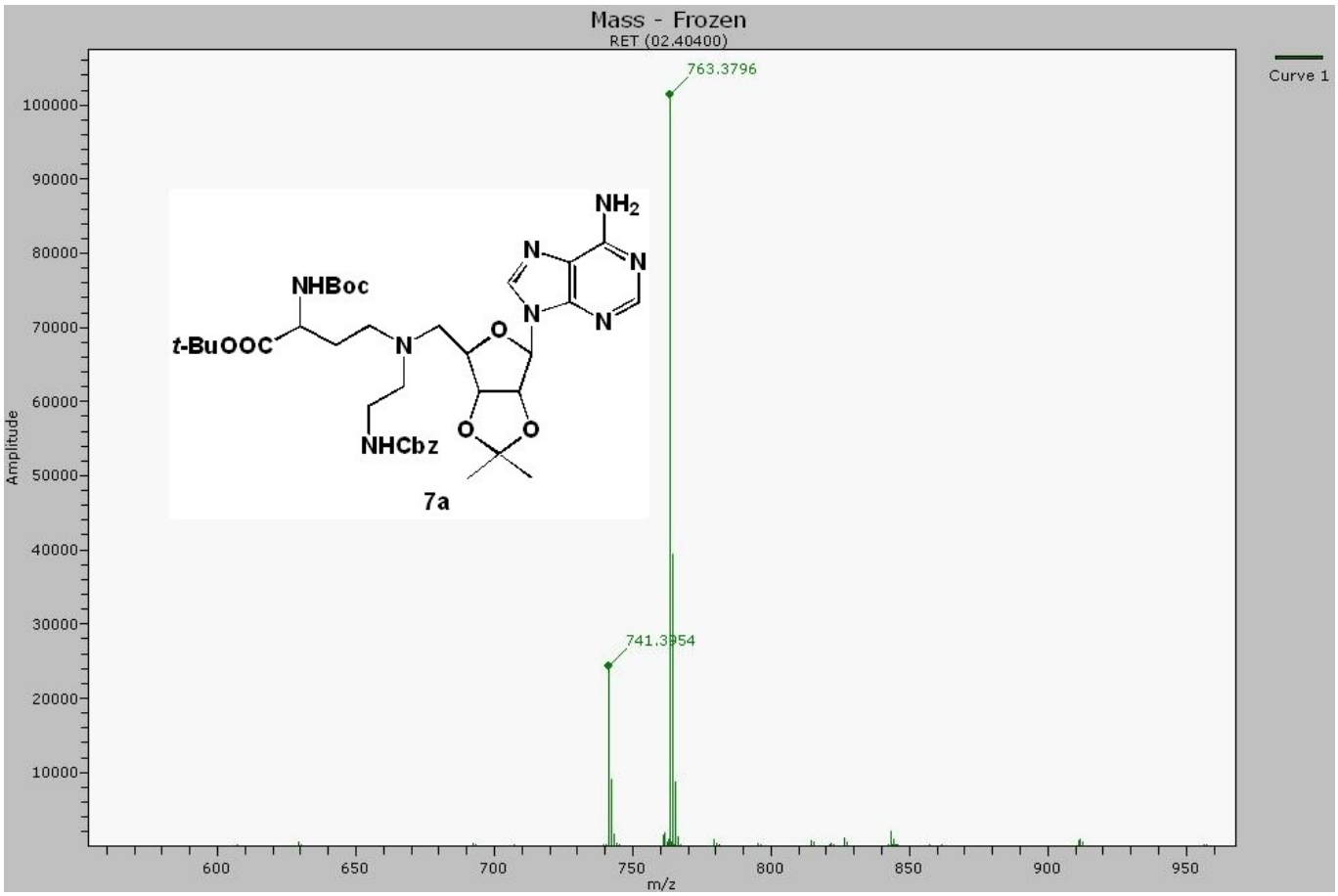

PROTON CDCl3 /opt/topspin Zhang.G 33
7.33
7.32
7.26
5.20
5.08
3.49
3.47
3.46
2.70
0.88
4.92
3.00
2.00
2.00
6b

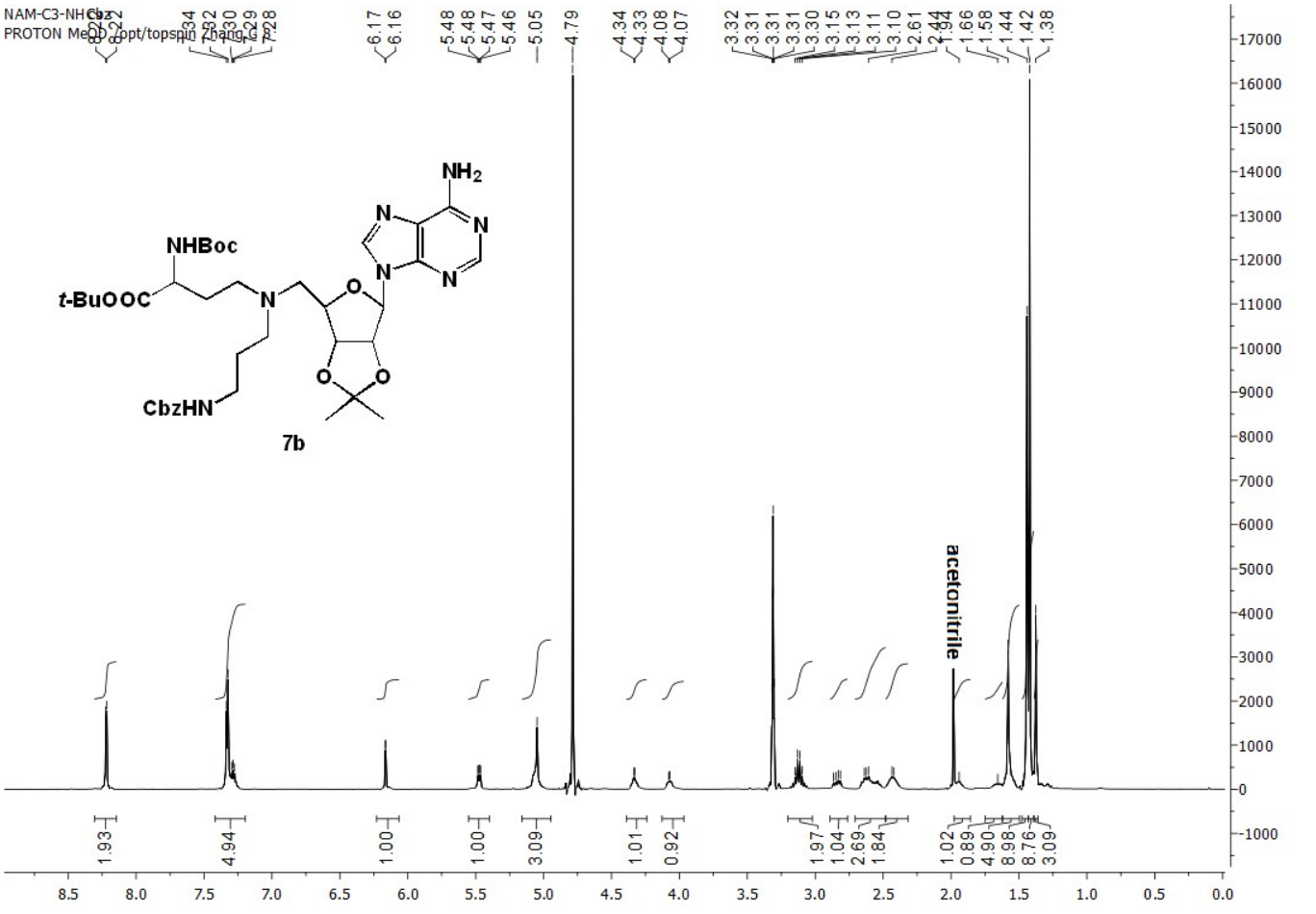

CbzNHC2H4CHO
C13CPD CDCl3 /opt/topspin Zhang.G 33
156.31
136.45
128.52
128.14
128.06
77.32
77.00
76.68
66.75
44.07
34.55
6b

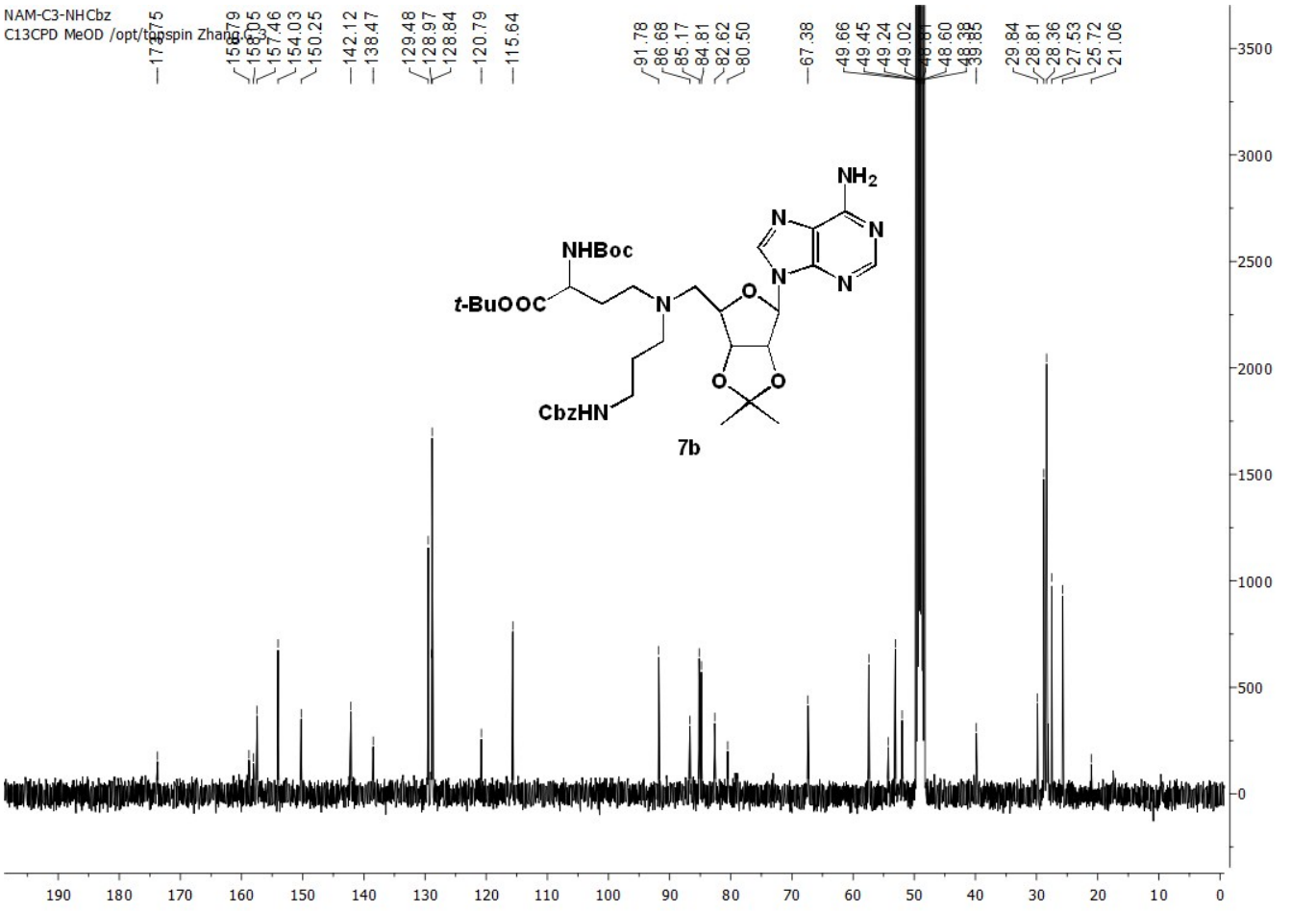

Mass - Frozen
RET (03.28691)
Curve 1
Amplitude
m/z
230.0785
208.0954
6b

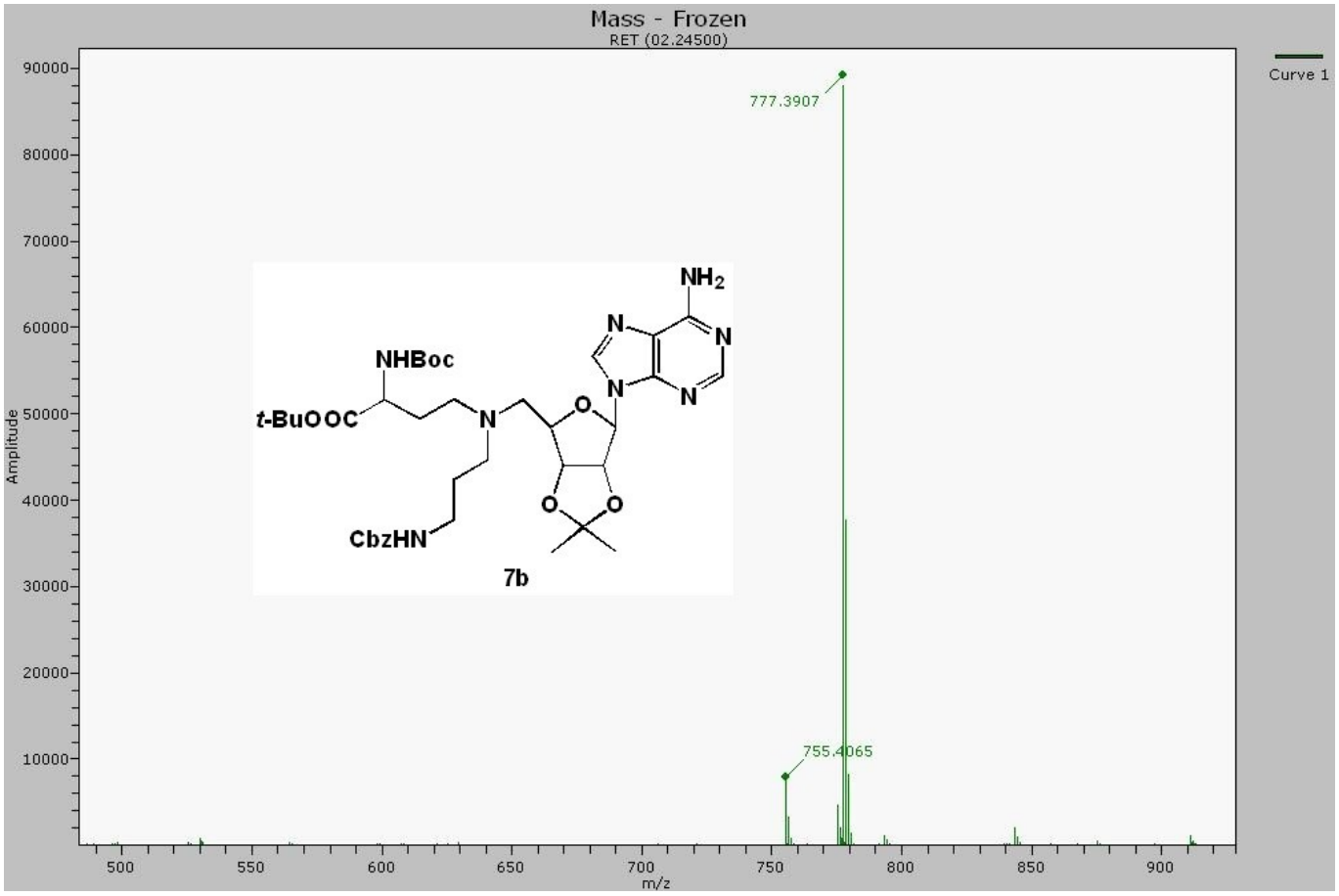

gz5-98
PROTON MeOD /opt/topspin Zhang G 5
NH2
NHBoc
t-BuOOC
NHCbz
7a
Dichloromethane
acetonitrile

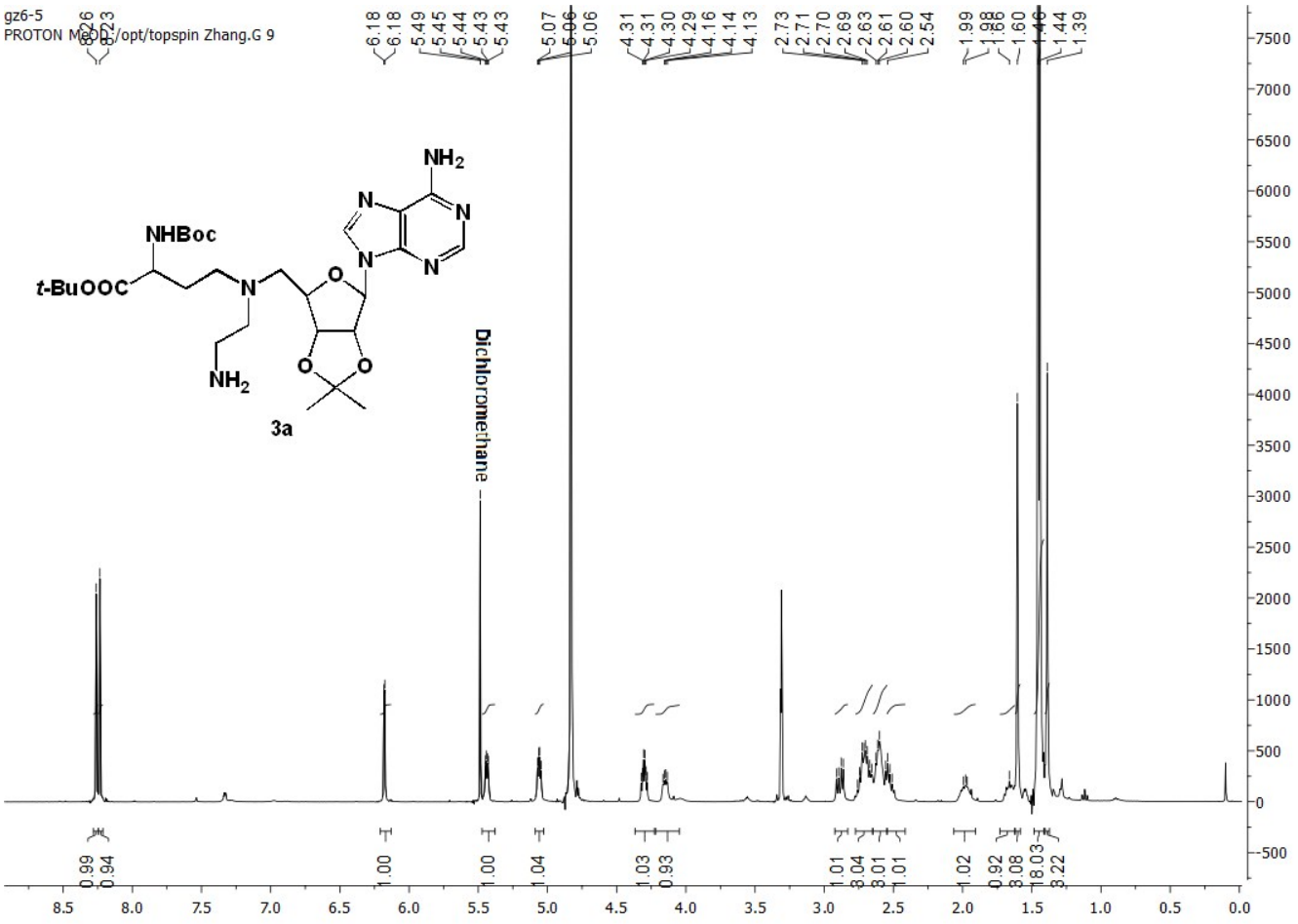

NAM-C2-NHCbz
C13CPD MeOD /opt/topspin Zhang G
NH2
NHBoc
t-BuOOC
NHCbz
7a

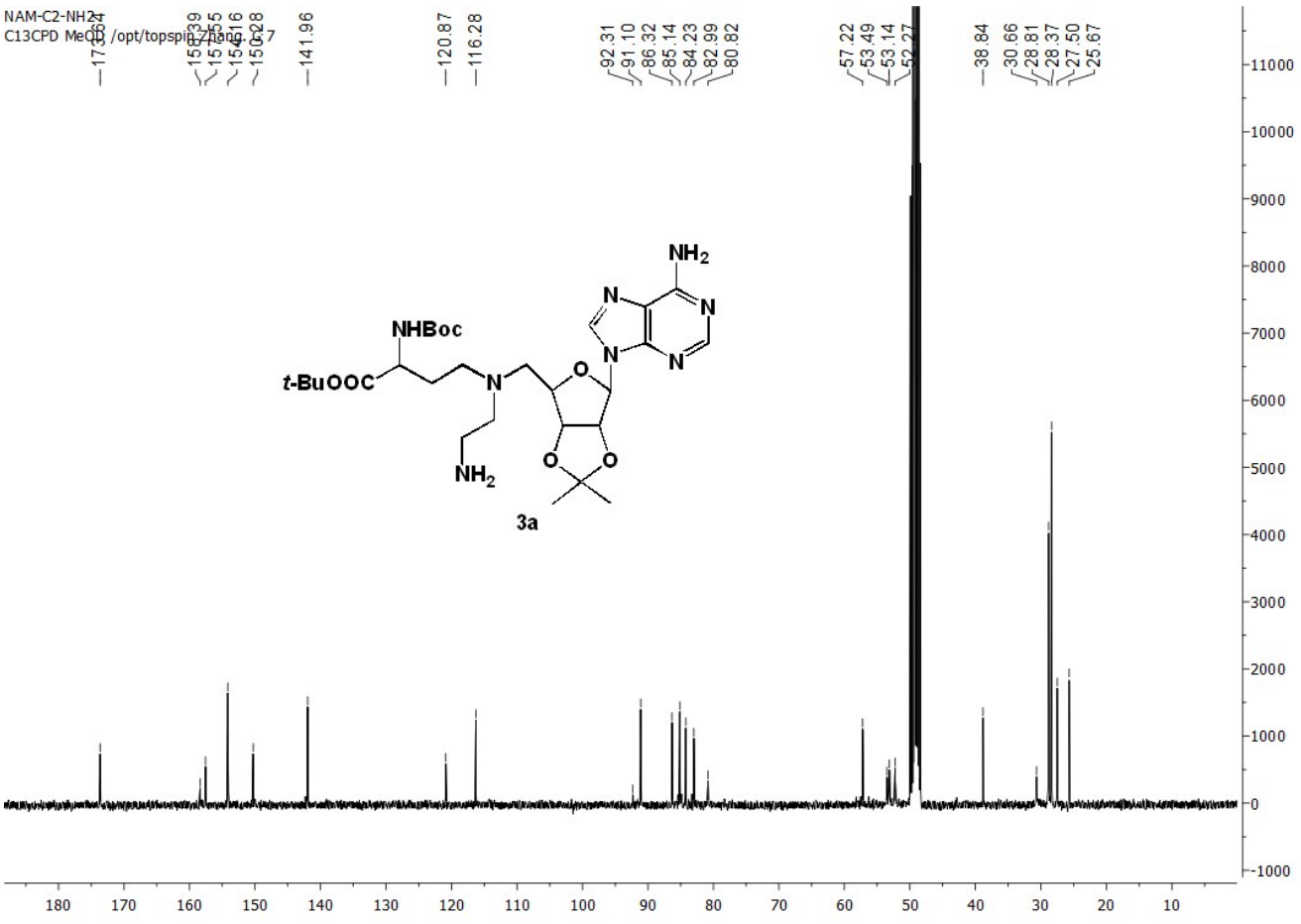

Mass - Frozen
RET (02.40400)
Curve 1
763.3796
741.3954
Amplitude
m/z
NHBoc
t-BuOOC
NHCbz
NH2
7a

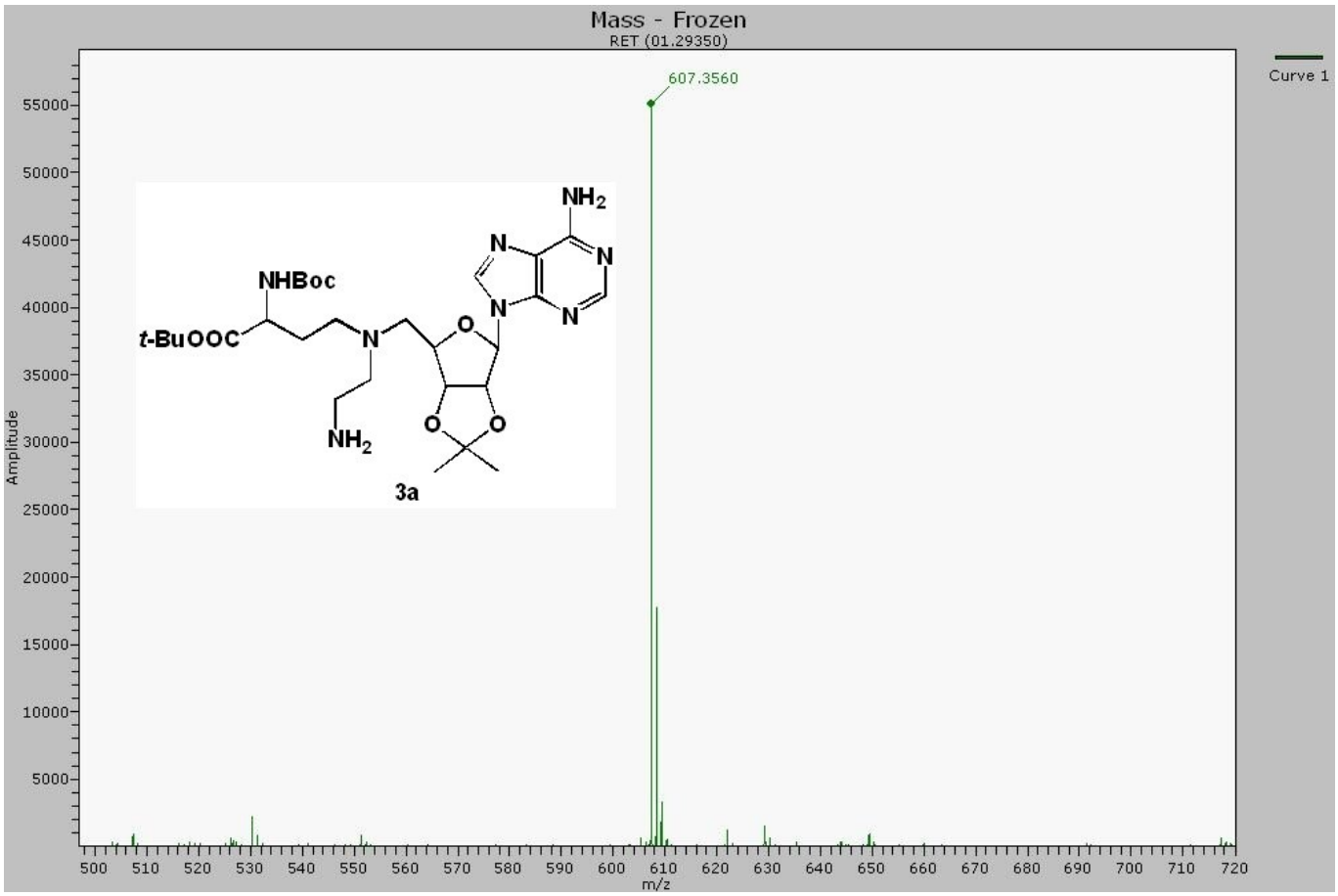

NAM-C3-NHCbz
PROTON MeOD /opt/topspin zhang.c 8
acetonitrile
NHBoc
t-BuOOC
CbzHN
NH2
7b

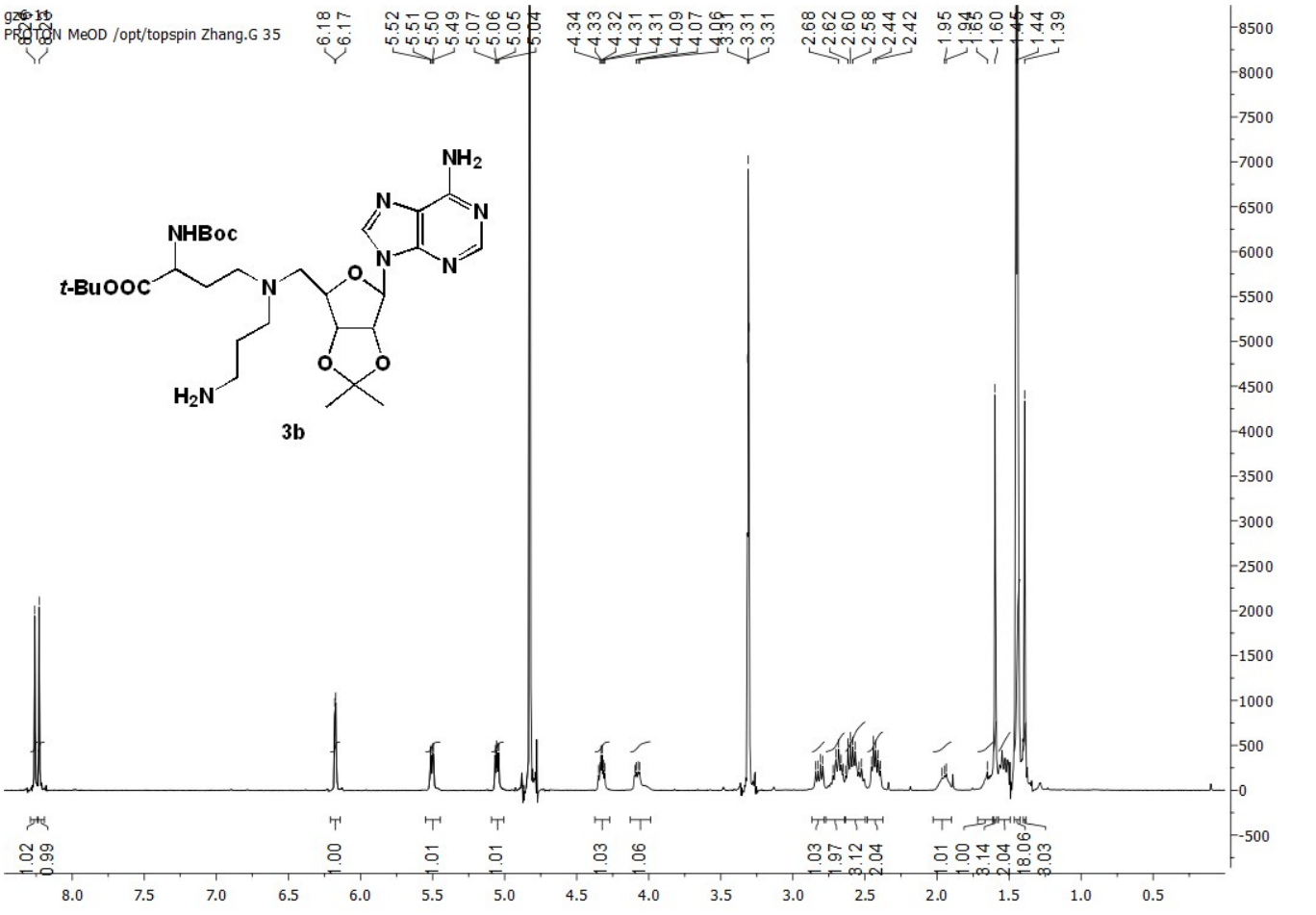

NAM-C3-NHCbz
C13CPD MeOD /opt/topspin Zhang C3
7b

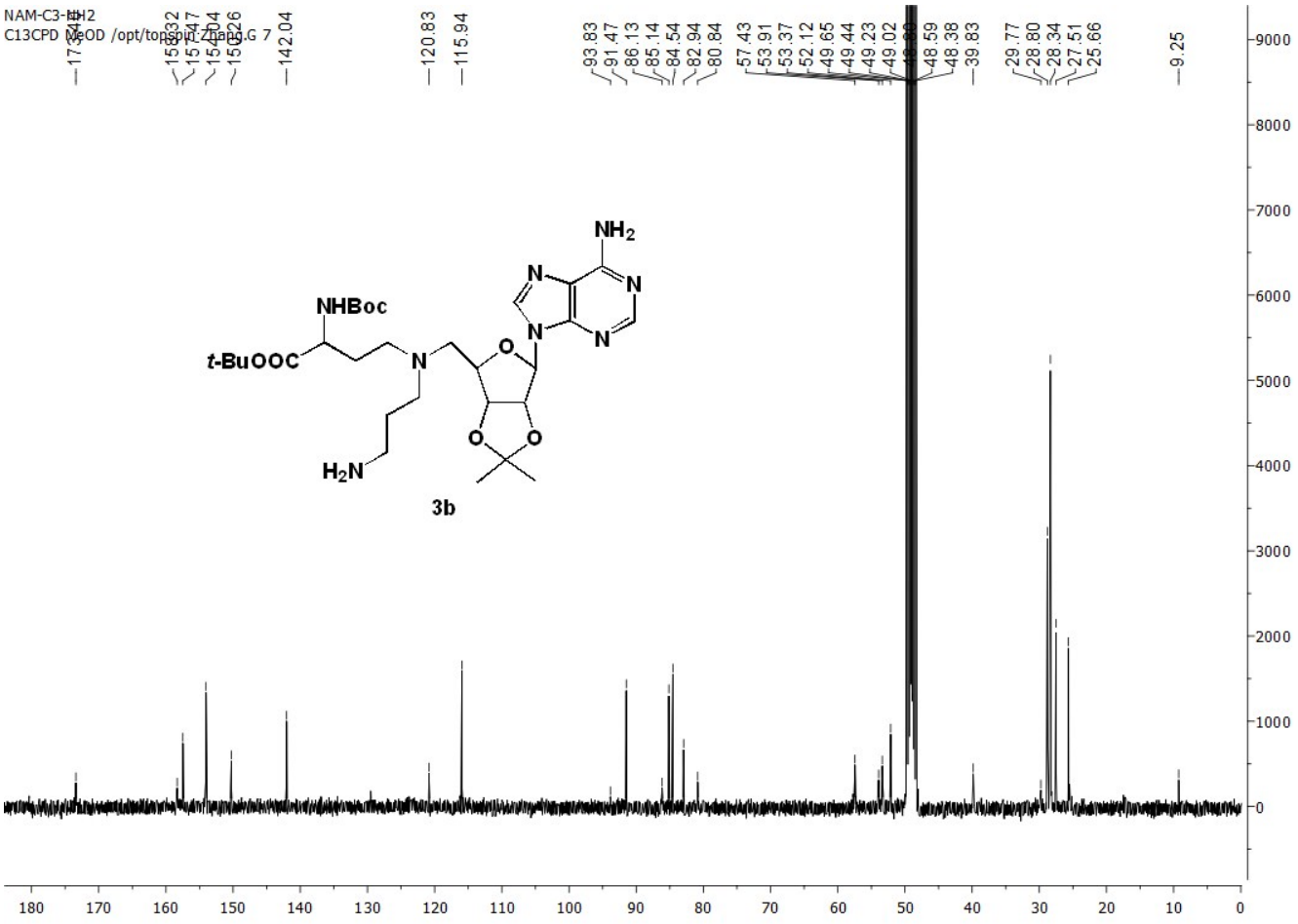

Mass - Frozen
RET (02.24500)
Curve 1
777.3907
755.4065
Amplitude
m/z
7b

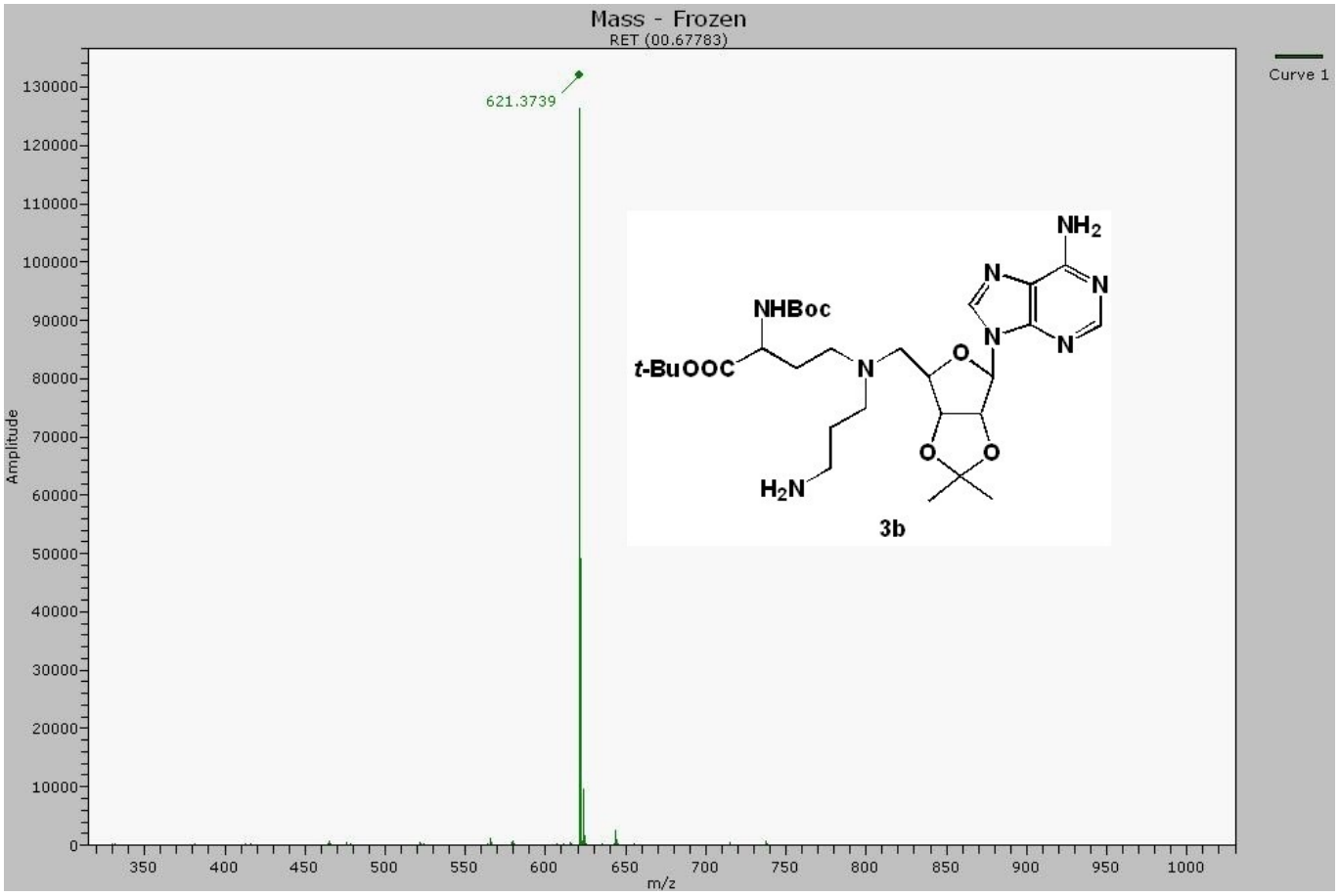

gz6-5
PROTON Me
/opt/topspin Zhang.G 9
Dichloromethane
NHBoc
t-BuOOC
NH2
3a


NAM-C2-NH2
C13CPD Me
/opt/topspin Zhang.G 7
NHBoc
t-BuOOC
NH2
3a

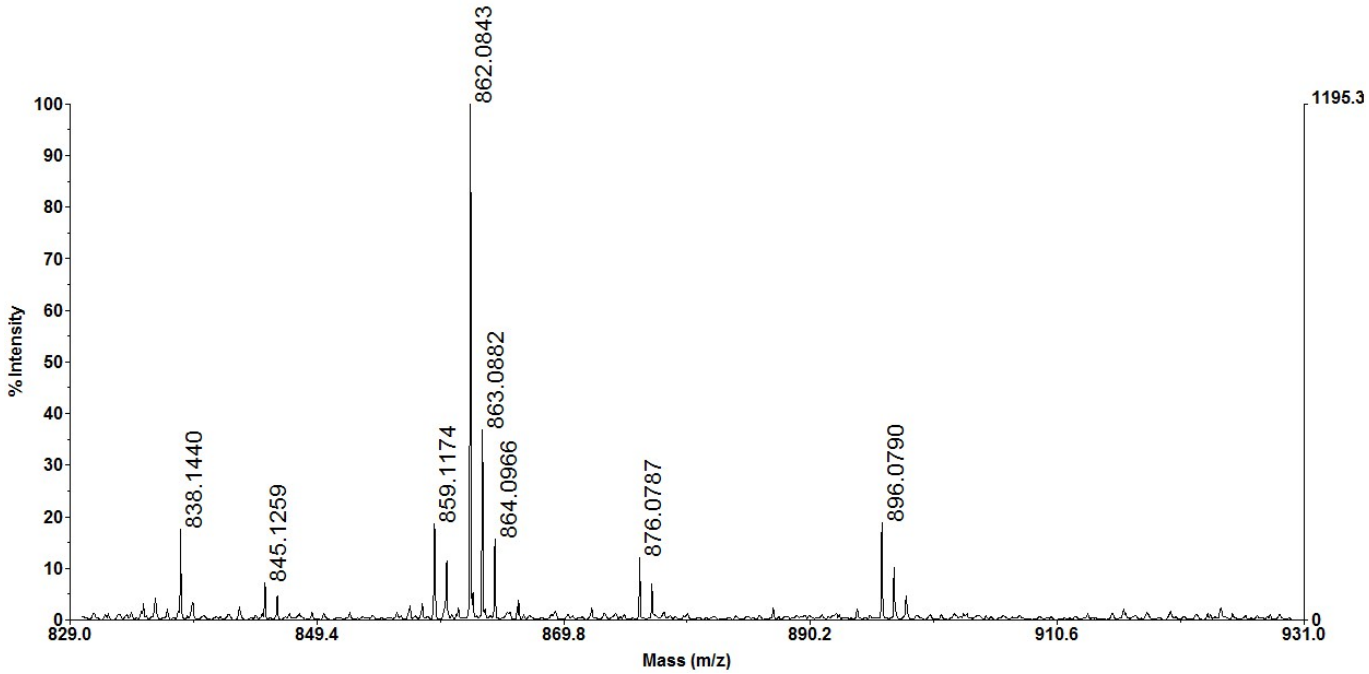

Mass - Frozen
RET (01.29350)
Curve 1
607.3560
Amplitude
m/z
NHBoc
t-BuOOC
NH2
3a


PROTON MeOD /opt/topspin Zhang.G 35
NHBoc
t-BuOOC
NH2
H2N
3b

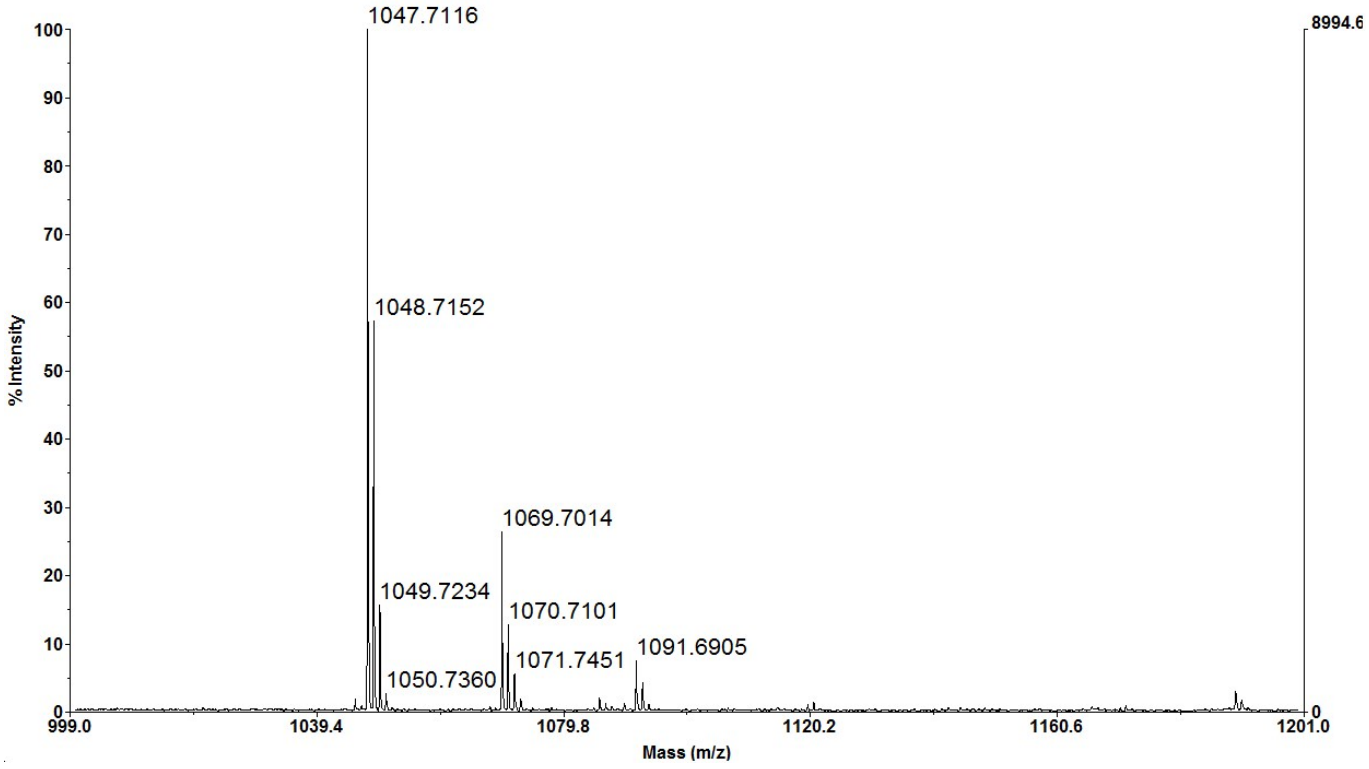

NAM-C3-EH2
C13CPD MeOD /opt/topspin/zg30 G 7
158.32
157.47
157.04
156.26
142.04
120.83
115.94
93.83
91.47
86.13
85.14
84.54
82.94
80.84
57.43
53.91
53.37
52.12
49.65
49.44
49.23
49.02
48.59
48.38
39.83
29.77
28.80
28.34
27.51
25.66
9.25
NHBoc
t-BuOOC
NH2
H2N
3b


Mass - Frozen
RET (00.67783)
Curve 1
621.3739
Amplitude
m/z
NHBoc
t-BuOOC
NH2
H2N
3b

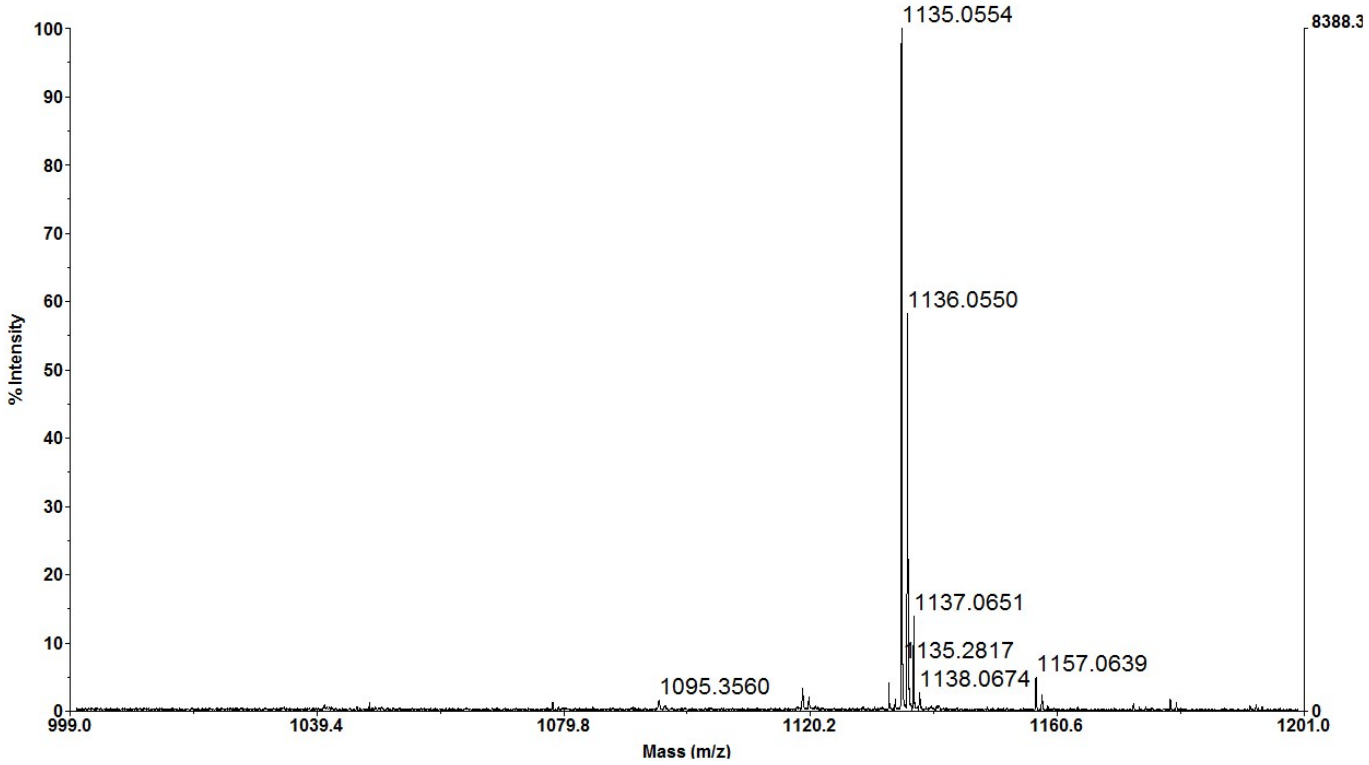
1a: NAM-C3-GPKR



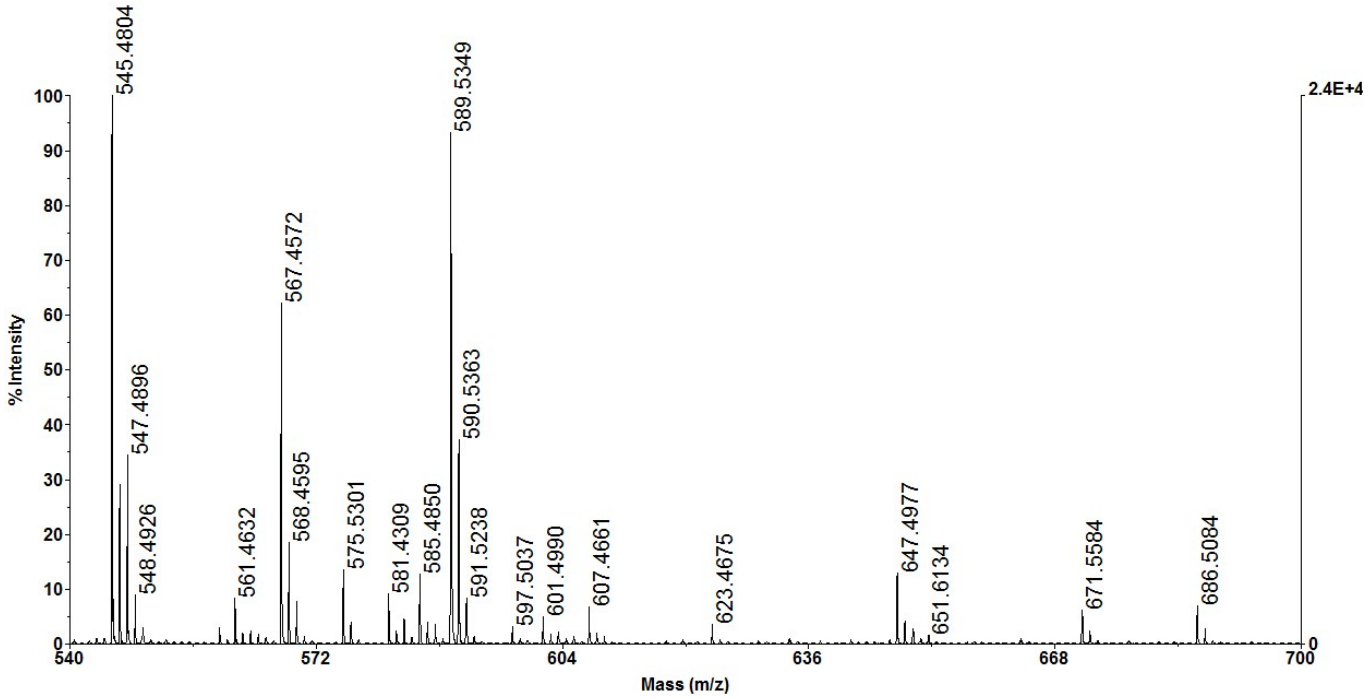
1b: NAM-C3-GPKRIA

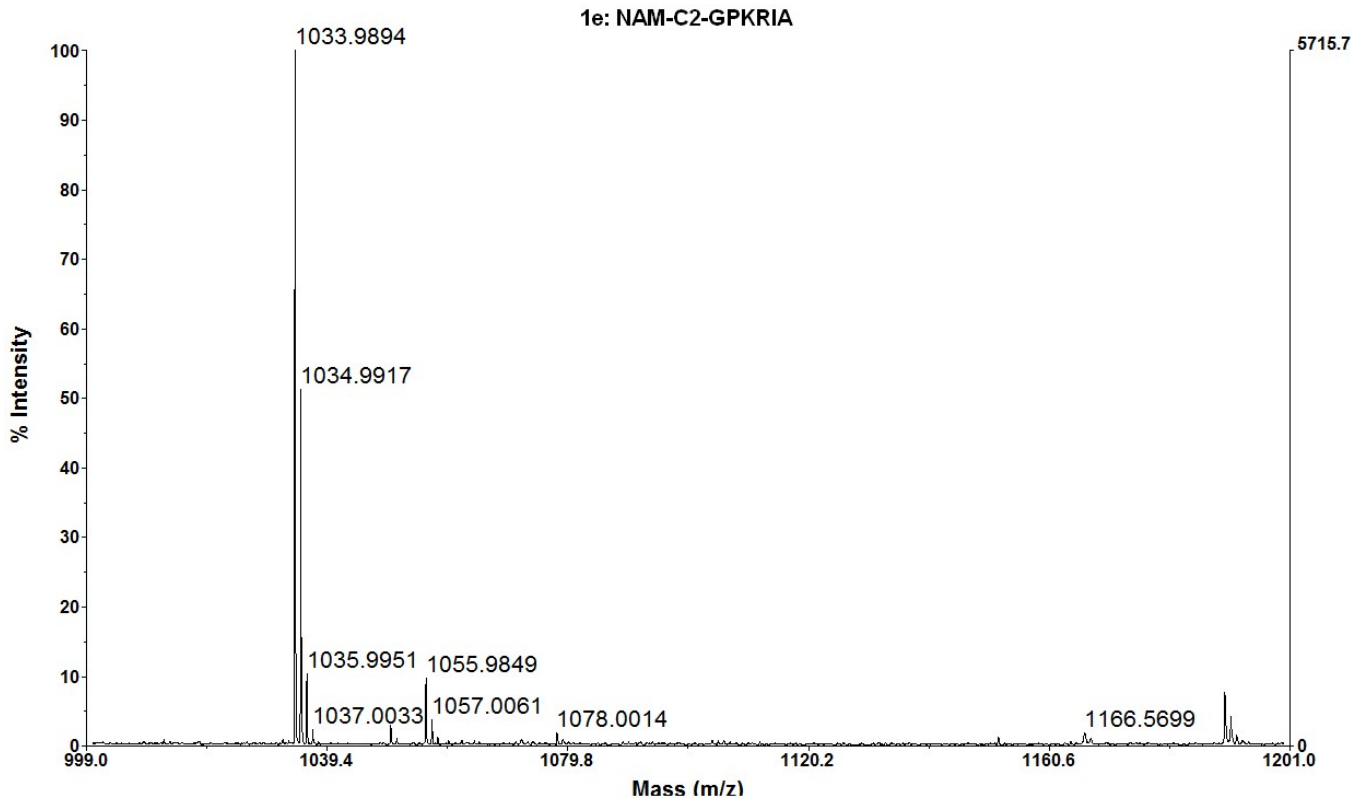


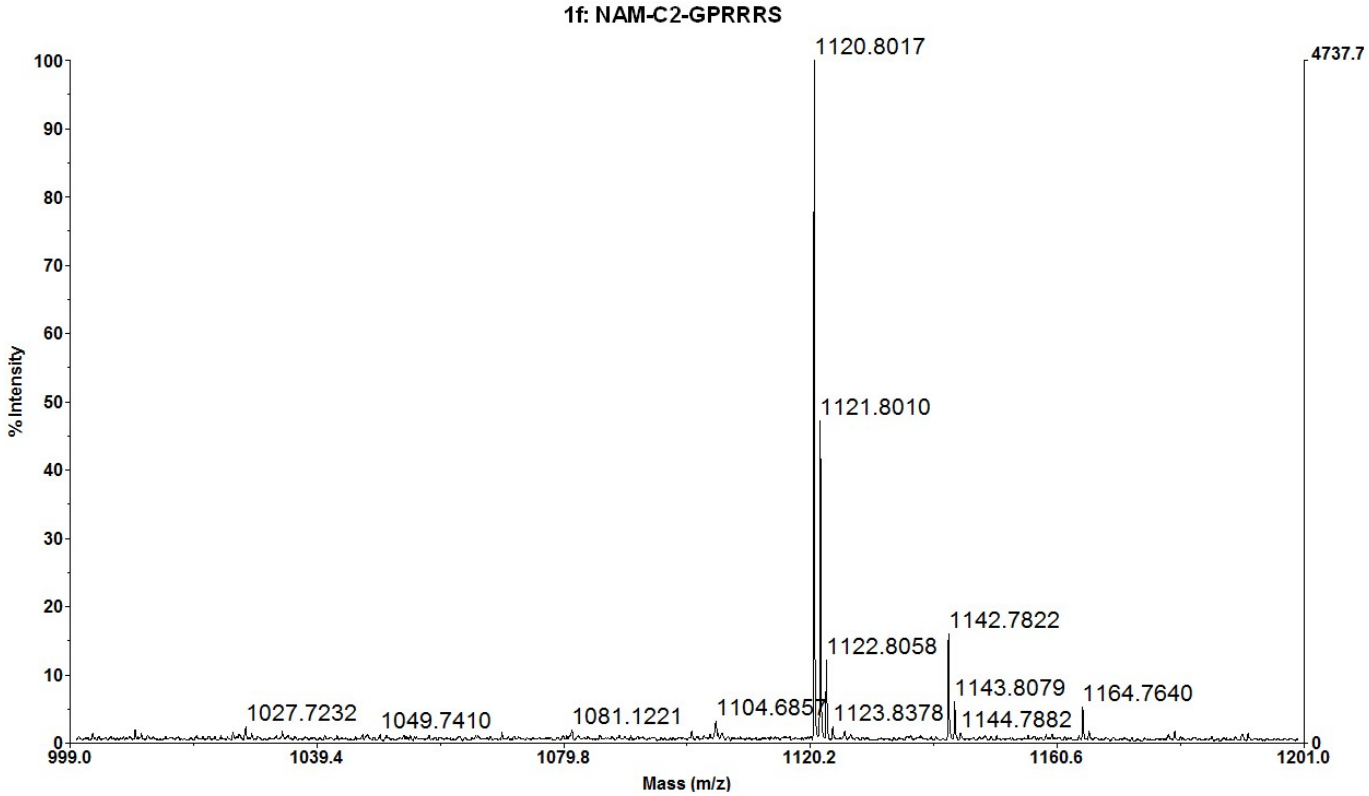
1c: NAM-C3-GPRRRS

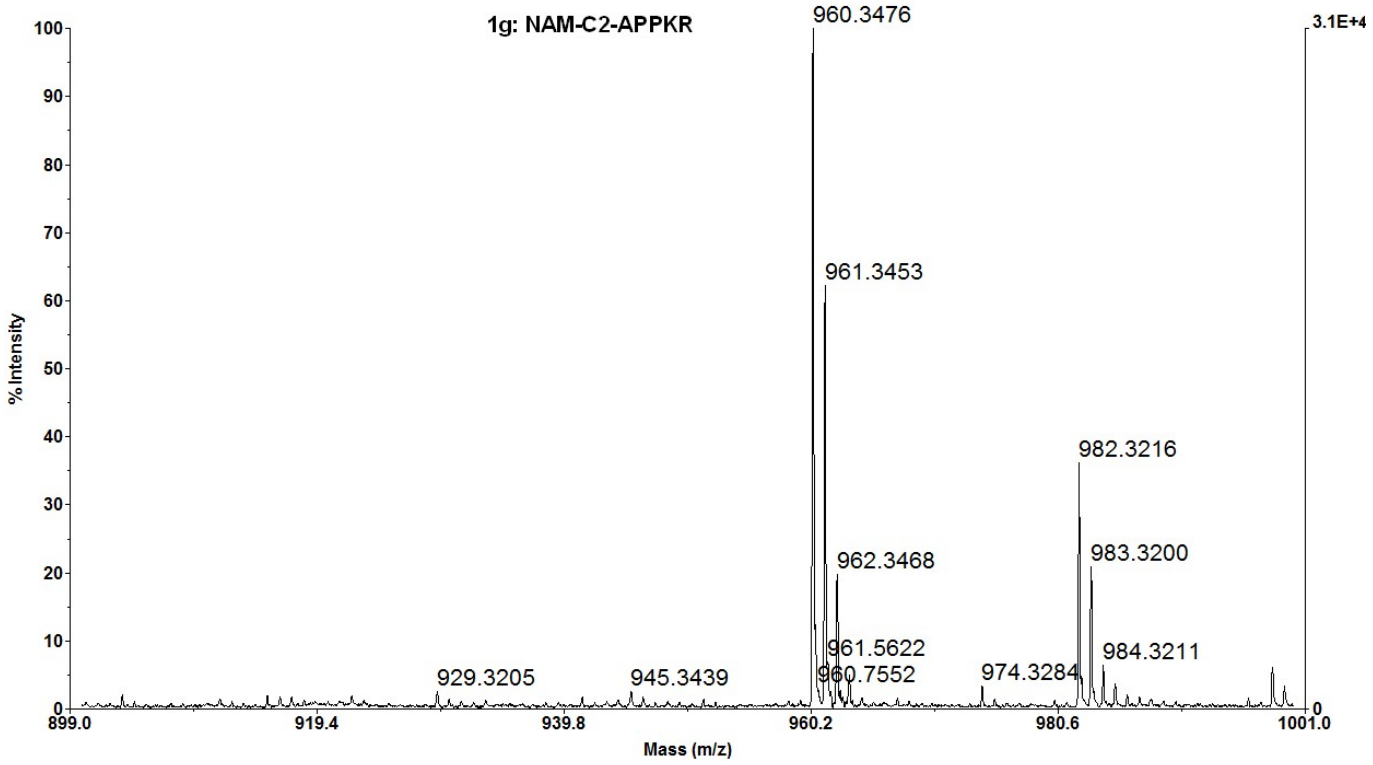


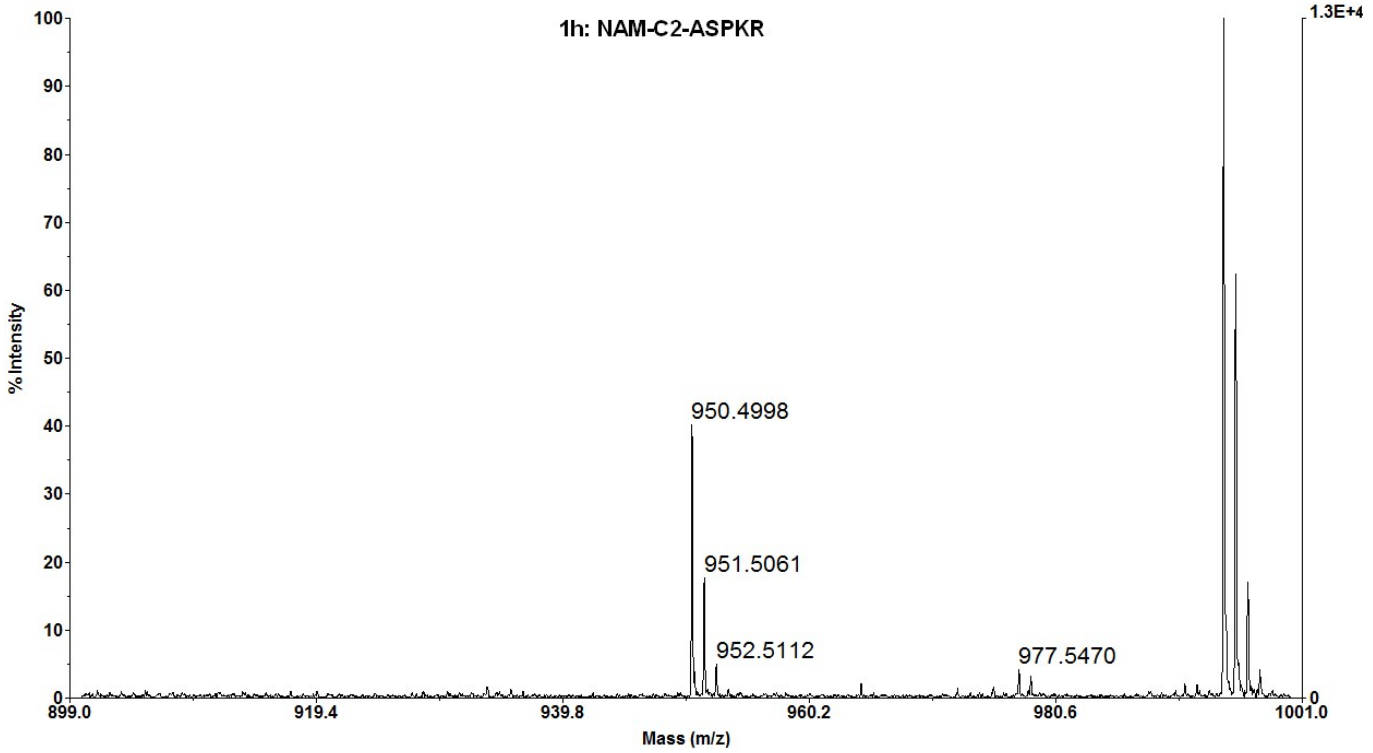
1d: NAM-C2-GP



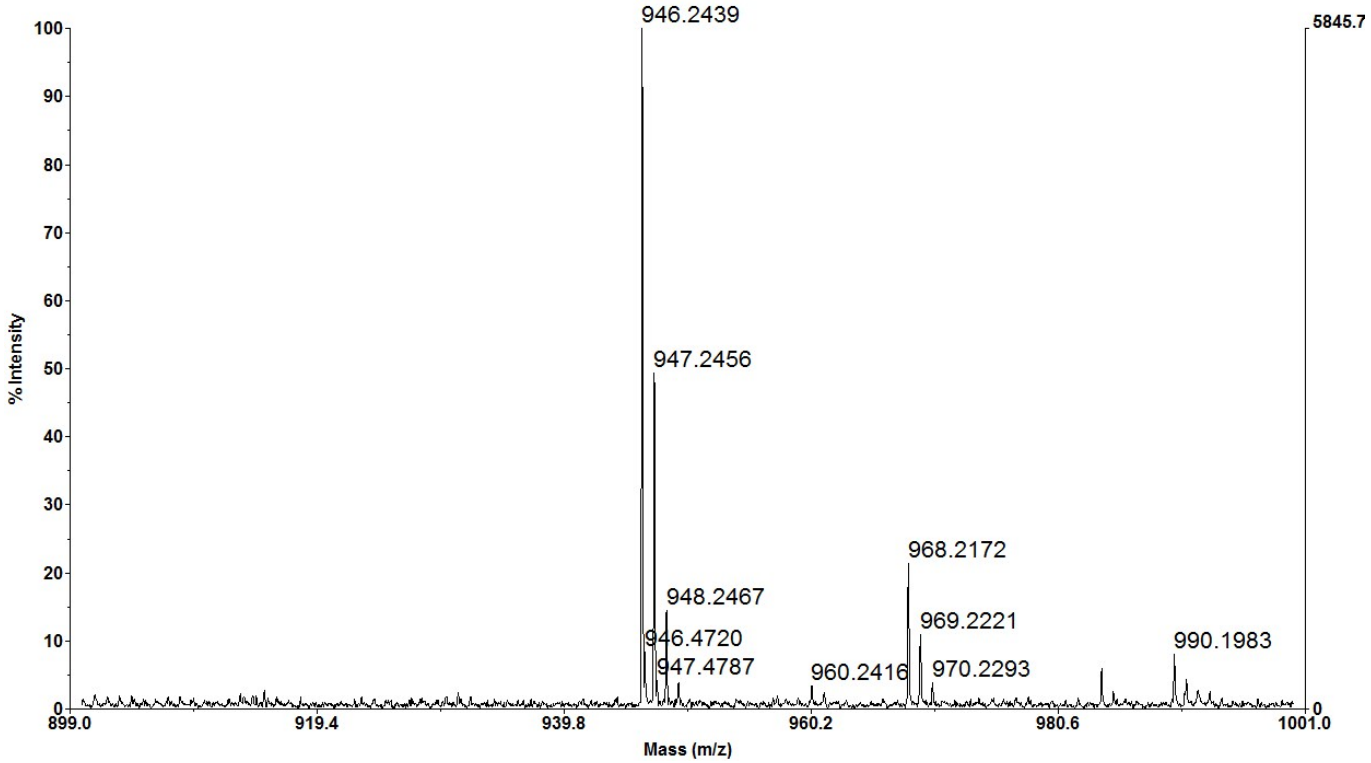

1e: NAM-C2-GPKRIA
1033.9894
5715.7
1034.9917
1035.9951
1055.9849
1037.0033
1057.0061
1078.0014
1166.5699
% Intensity
Mass (m/z)
999.0
1039.4
1079.8
1120.2
1160.6
1201.0


1f: NAM-C2-GPRRRS
1120.8017
4737.7
1121.8010
1142.7822
1122.8058
1143.8079
1164.7640
1027.7232
1049.7410
1081.1221
1104.6857
1123.8378
1144.7882
% Intensity
Mass (m/z)
999.0
1039.4
1079.8
1120.2
1160.6
1201.0

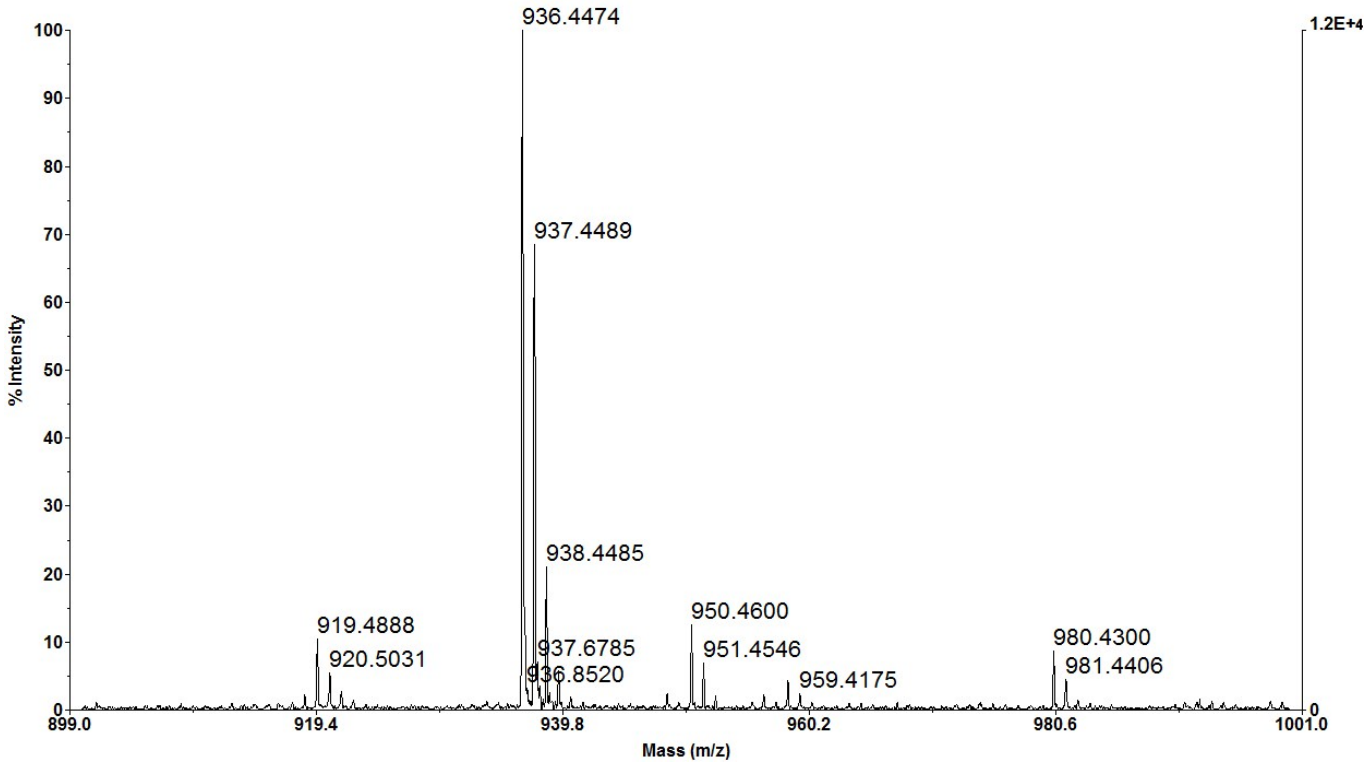

1g: NAM-C2-APPKR
960.3476
3.1E+4
961.3453
982.3216
962.3468
983.3200
961.5622
984.3211
929.3205
945.3439
960.7552
974.3284
% Intensity
Mass (m/z)
899.0
919.4
939.8
960.2
980.6
1001.0


1h: NAM-C2-ASPKR
1.3E+4
950.4998
951.5061
952.5112
977.5470
% Intensity
Mass (m/z)
899.0
919.4
939.8
960.2
980.6
1001.0

1i: NAM-C2-GPPKR



1j: NAM-C2-GSPKR



HPLC-UV spectrum of 1c (NAM-C3-GPRRRS)