Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Ligations of *N*-Acyl tryptophan units to give Native Peptides *via* 7-, 10-, 11- and 12-membered Cyclic Transition states

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SUPPORTING INFORMATION

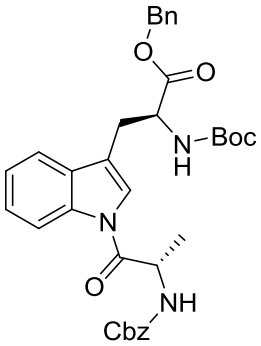
1. General Information. All commercial materials (Aldrich, Fluka) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher Solvents were dried using standard protocols kept under a dry atmosphere of nitrogen. Melting points were determined on a capillary point apparatus equipped with a digital thermometer and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$, DMSO-d_6 or CD_3OD using a 300 MHz spectrometer (with TMS as an internal standard) at ambient temperature unless otherwise stated. Chemical shifts are reported in parts per million relative to residual solvent $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.16 ppm), DMSO-d_6 (^{1}H, 2.50 ppm; ^{13}C, 39.52 ppm), CD_3OD (^{1}H, 3.31 ppm; ^{13}C, 49.00 ppm). All ^{13}C NMR spectra were recorded with complete proton decoupling. The data have been reported in order to provide the maximum amount of information regarding coupling constants, which has necessarily led to integrals reported following a group of peaks in some instances. High-resolution and high-performance liquid chromatography mass spectral analyses were performed by the University of Florida chemistry department facility staff. Reactions were carried out in oven-dried glassware under an argon or nitrogen atmosphere unless otherwise noted. All microwave assisted reactions were carried out with a single mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stirrer bar. The tube was closed with a silicon septum and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 sec.; PowerMax-cooling mode). Analytical TLC was performed on E. Merck silica gel 60 F254 plates and visualized by UV and potassium permanganate staining. Flash column chromatography was performed on E. Merck silica gel 60 (40–63 mm). Yields refer to chromatographically and spectroscopically pure compounds. HPLC-MS analyses were performed on reverse phase gradient Phenomenex Synergi Hydro-RP (2.1×150 mm; 5 um) + guard column (2×4 mm) or Thermoscientific Hypurity C8 (5um; 2.1×100 mm + guard column) using 0.2% acetic acid in H_2O/methanol as mobile phases; wavelength = 254 nm; and mass spectrometry was done with electro spray ionization (ESI).

Experimental Procedure

Procedure for preparation of isodipeptide 3

DBU (0.78 g, 5.10 mmol) was added dropwise to a stirred solution of N^{α}-Boc-L-Trp-OBn (2.00 g, 5.07 mmol) and Cbz-L-Ala-Bt (1.64 g, 5.07 mmol) in dry MeCN (30 mL) at 23 °C. The reaction mixture was stirred overnight at this temperature. After that the solvent was evaporated under reduced pressure and the residue was recrystallized from hot hexanes to give N^{α}-Boc-L-Trp(Cbz-L-Ala)-OBn **3** (2.37 g, 3.95 mmol).

N^{α}-Boc-L-Trp(Z-L-Ala)-OBn (3)



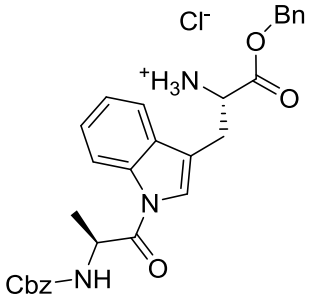
3

White microcrystals, 78% yield, mp 64–66 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 8.0 Hz, 1H), 7.52 (d, J = 7.0 Hz, 1H), 7.36–7.19 (m, 14H), 5.79 (d, J = 7.7 Hz, 1H), 5.23–4.88 (m, 5H), 4.76–4.71 (m, 1H), 3.29–3.15 (m, 2H), 1.47–1.42 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6, 170.9, 155.5, 155.2, 136.3, 136.0, 135.1, 130.7, 128.7, 128.6, 128.3, 128.2, 125.8, 124.3, 122.3, 119.1, 118.5, 116.9, 80.3, 67.3, 67.2, 53.7, 49.6, 28.4, 28.1, 19.7. Anal. Calcd for $C_{34}H_{37}N_3O_7$: C, 68.10; H, 6.22; N, 7.01. Found C, 67.71; H, 6.40; N, 7.35.

Procedure for preparation of hydrogen chloride of unprotected isodipeptide 4

N^{α}-Boc-L-Trp-OBn **3** (0.40 g, 0.67 mmol) was dissolved in 4N HCl solution in 1,4–dioxane (15 ml) at 23 °C and stirred for 4 h. Reaction mixture was evaporated and recrystallized from diethyl ether to give L-Trp(Cbz-L-Ala)-OBn hydrogen chloride **4** (0.32 g, 0.60 mmol). Yield 90%.

L-Trp(Z-L-Ala)-OBn hydrogen chloride (4)



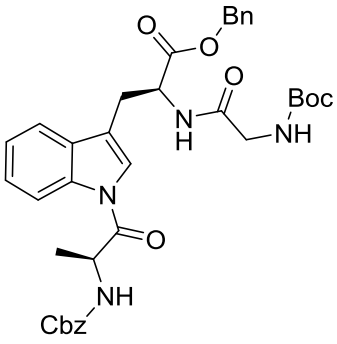
4

Light brown microcrystals, 90% yield, mp 143–145 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.43 (d, J = 7.6 Hz, 1H), 7.81 (s, 1H), 7.65–7.54 (m, 1H), 7.32–7.02 (m, 13H), 5.18–5.01 (m, 5H), 4.50 (t, J = 6.7 Hz, 1H), 3.40 (d, J = 6.5 Hz, 2H), 1.44 (d, J = 7.1 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 173.1, 169.9, 158.2, 137.9, 137.4, 135.8, 130.9, 129.5, 129.4, 129.3, 128.9, 128.7, 126.6, 125.6, 125.1, 119.6, 117.8, 116.3, 69.3, 67.7, 53.8, 50.9, 27.0, 18.1. Anal. Calcd for $C_{29}H_{30}ClN_3O_5$: C, 64.98; H, 5.64; N, 7.84. Found C, 64.60; H, 5.83; N, 7.57.

General procedure for preparation of isotripeptides 8a-c

L-Trp(Cbz-L-Ala)-OBn hydrogen chloride **4** (0.54 g, 1.0 mmol) and benzotriazolide of Boc-protected α-, β- or γ-amino acids **7a-c** (1.0 mmol) were dissolved in MeCN (15 mL) at ice-bath cooling and then DIPEA (0.52 mL, 3.0 mmol) was added to the mixture. The reaction was stirred for 2 h at 0 °C and 8 h at room temperature. After completion of the reaction solvent was concentrated under reduced pressure. The crude product was dissolved in 50 mL of EtOAc and washed with saturated solution of sodium carbonate. Organic layer was dried over magnesium sulfate. Evaporation and then recrystallization from diethyl ether gave corresponding isotripeptides **8a-c**.

Boc-Gly-L-Trp(Z-L-Ala)-OBn (8a)

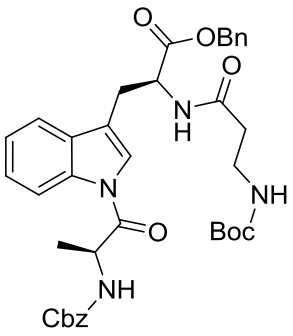


8a

White microcrystals, 76% yield, mp 75–76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (d, J = 7.7 Hz, 1H), 7.50–7.08 (m, 15H), 6.12 (d, J = 8.4 Hz, 1H), 5.18–4.88 (m, 6H), 3.99–3.55 (m, 2H), 3.28–3.13 (m, 2H), 1.44 (d, J = 6.5 Hz, 3H), 1.33 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9,

169.8, 169.7, 156.2, 155.7, 136.0, 135.8, 134.8, 130.4, 128.5, 128.4, 128.1, 128.0, 125.5, 124.0, 123.0, 118.7, 117.4, 116.8, 79.9, 67.4, 67.1, 52.0, 49.2, 44.3, 28.1, 27.1, 18.9. Anal. Calcd for $C_{36}H_{40}N_4O_8$: C, 65.84; H, 6.14; N, 8.53. Found C, 65.44; H, 6.22; N, 8.44.

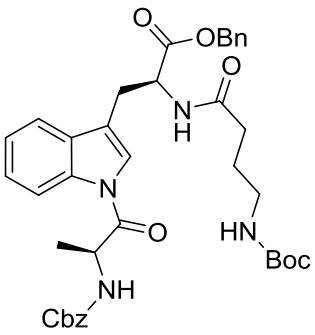
Boc-β-Ala-L-Trp(Z-L-Ala)-OBn (8b)



8b

White microcrystals, 63% yield, mp 126–128 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.39 (d, J = 8.3 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.36–7.08 (m, 15H), 5.21–4.95 (m, 6H), 3.39–3.11 (m, 4H), 2.41 (t, J = 6.5 Hz, 2H), 1.49–1.33 (m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ 173.8, 172.9, 172.7, 158.2, 158.1, 138.0, 137.3, 136.8, 131.6, 129.4, 129.4, 128.9, 128.8, 126.3, 124.9, 124.3, 119.8, 119.1, 117.7, 80.1, 68.1, 67.8, 53.8, 50.8, 37.9, 36.9, 28.7, 28.1, 18.3. Anal. Calcd for $C_{37}H_{42}N_4O_8$: C, 66.25; H, 6.31; N, 8.35. Found C, 66.04; H, 6.27; N, 8.10.

Boc-GABA-L-Trp(Cbz-L-Ala)-OBn (8c)



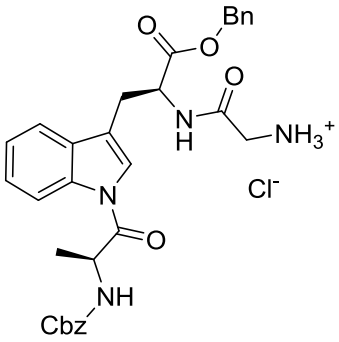
8c

White microcrystals, 67% yield, mp 134–136 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.38 (d, J = 8.0 Hz, 1H), 7.54 (d, J = 7.0 Hz, 1H), 7.35–7.10 (m, 14H), 5.16–4.91 (m, 6H), 3.39–3.13 (m, 2H), 2.97 (t, J = 6.9 Hz, 2H), 2.20 (q, J = 7.4 Hz, 2H), 1.73–1.59 (m, 2H), 1.43–1.39 (m, 12H). ^{13}C NMR (75 MHz, CD_3OD) δ 175.6, 172.9, 172.8, 158.4, 158.2, 138.1, 137.4, 136.8, 131.7, 129.5, 129.4, 129.0, 128.8, 126.3, 124.9, 124.2, 119.8, 119.3, 117.7, 79.9, 68.1, 67.8, 53.9, 50.9, 40.7, 34.0, 28.8, 28.0, 27.2, 18.2. Anal. Calcd for $C_{38}H_{44}N_4O_8$: C, 66.65; H, 6.48; N, 8.18. Found C, 66.36; H, 6.37; N, 8.02.

General procedure for preparation of hydrogen chlorides of unprotected isodipeptides 9a-c

Boc-protected isotripeptides **8a-c** (1.00 mmol) was dissolved in 4N HCl solution in 1,4–dioxane (15 ml) at 23 °C and stirred for 4 h. Reaction mixture was evaporated and recrystallized from diethyl ether to give corresponding hydrogen chloride salts of unprotected isodipeptides **9a-c**.

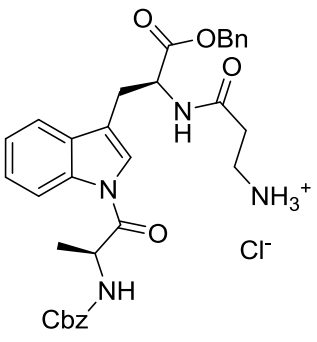
Gly-L-Trp(Z-L-Ala)-OBn hydrogen chloride (9a)



9a

White microcrystals, 94% yield, mp 64–66 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.39 (d, J = 7.6 Hz, 1H), 7.65 (s, 1H), 7.55 (d, J = 7.4 Hz, 1H), 7.36–7.10 (m, 12H), 5.15–4.96 (m, 6H), 3.72 (s, 2H), 3.33–3.15 (m, 2H), 1.44 (d, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 173.0, 172.3, 167.2, 158.3, 138.0, 137.4, 136.7, 131.6, 129.5, 129.4, 129.0, 128.7, 126.4, 125.0, 124.5, 119.8, 118.8, 117.7, 68.3, 67.8, 53.8, 41.5, 28.7, 28.2, 18.2. HRMS (ESI) calcd for $C_{31}H_{33}ClN_4O_6$ $[M - HCl + H]^+$ 557.2395, found 557.2410.

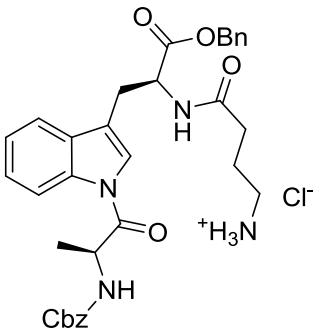
β-Ala-L-Trp(Cbz-L-Ala)-OBn hydrogen chloride (9b)



9b

White microcrystals, 92% yield, mp 130–133 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.39 (d, J = 7.9 Hz, 1H), 7.65 (s, 1H), 7.55 (d, J = 6.6 Hz, 1H), 7.36–7.22 (m, 10H), 7.18–7.12 (m, 2H), 5.23–4.87 (m, 6H), 3.42–3.16 (m, 2H), 3.12 (t, J = 6.2 Hz, 2H), 2.64 (t, J = 6.8 Hz, 2H), 1.43 (d, J = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 173.0, 172.7, 172.2, 158.2, 138.0, 137.4, 136.8, 131.7, 129.5, 129.4, 129.0, 128.7, 126.3, 124.9, 124.4, 119.8, 119.1, 117.7, 68.2, 67.8, 53.8, 50.8, 36.9, 32.5, 28.1, 18.2. HRMS (ESI) calcd for $C_{32}H_{35}ClN_4O_6$ $[M - HCl + H]^+$ 571.2551, found 571.2547.

GABA-L-Trp(Z-L-Ala)-OBn hydrogen chloride (9c)

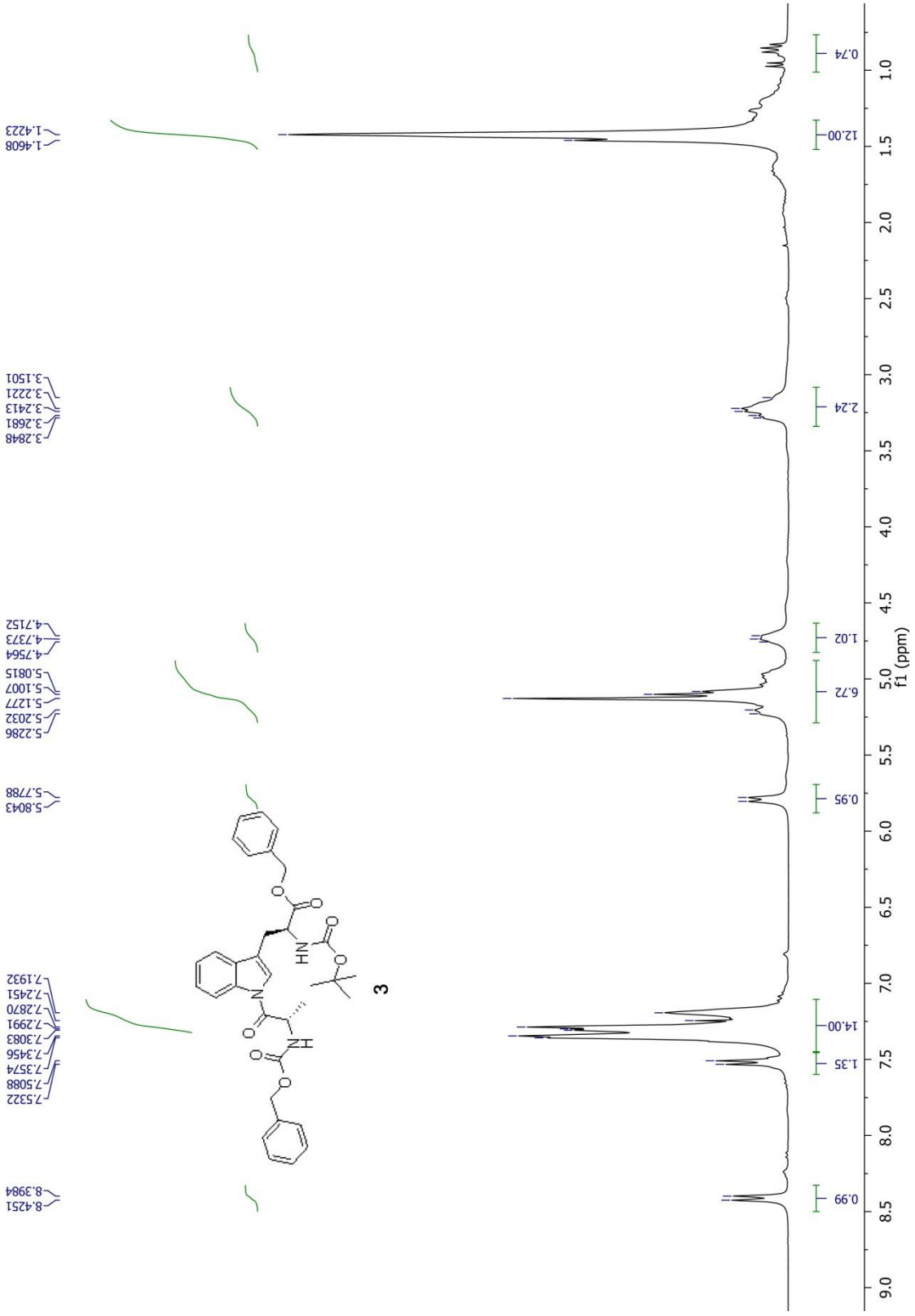


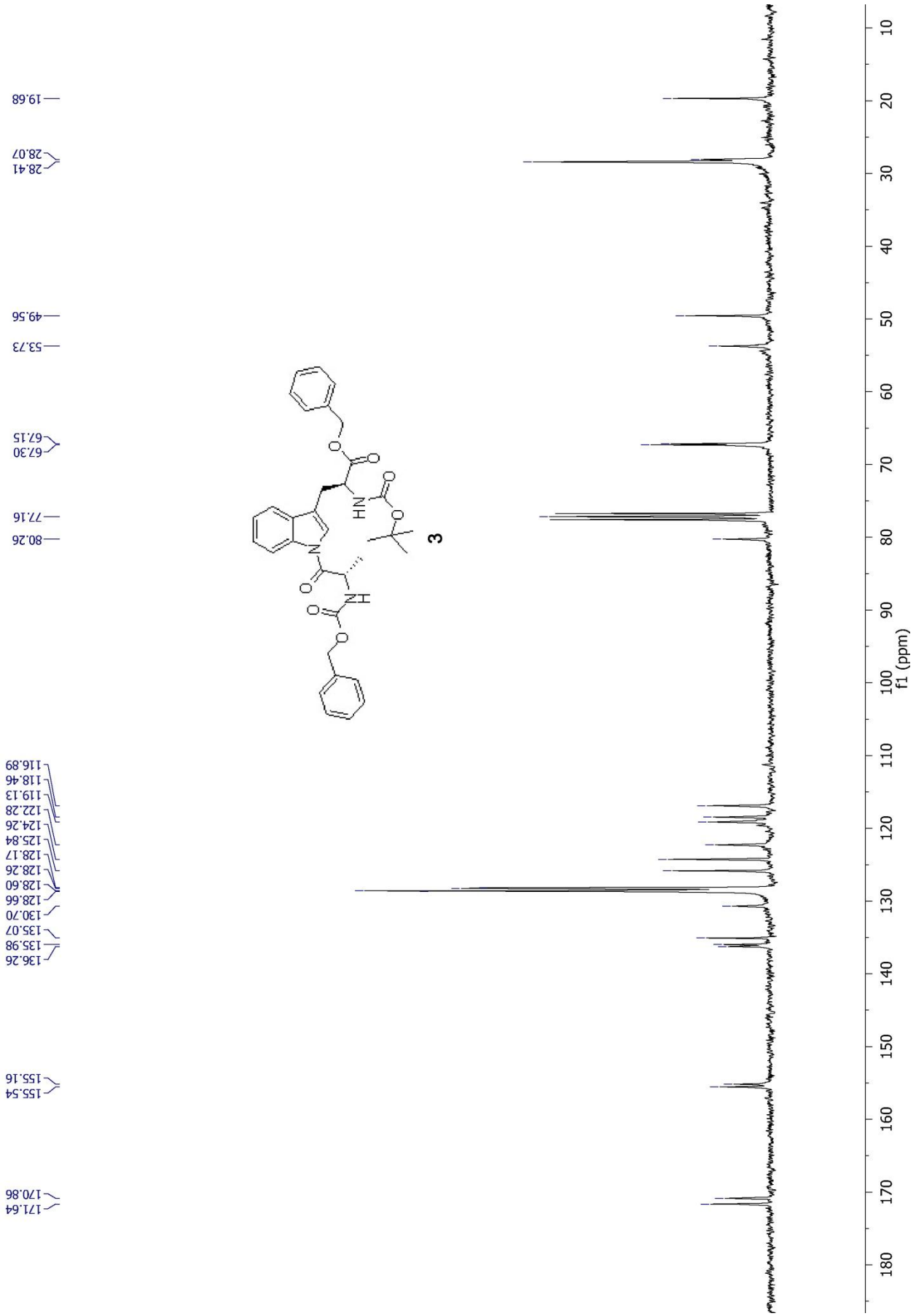
9c

White microcrystals, 94% yield, mp 85–88 °C; ^{1}H NMR (300 MHz, CD_3OD) δ 8.38 (d, J = 7.9 Hz, 1H), 7.65 (s, 1H), 7.54 (d, J = 7.5 Hz, 1H), 7.37–7.11 (m, 12H), 5.18–4.94 (m, 5H), 3.41–3.13 (m, 2H), 2.93–2.79 (m, 2H), 2.43–2.30 (m, 2H), 1.95–1.79 (m, 2H), 1.43 (d, J = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, CD_3OD) δ 174.5, 172.9, 172.7, 158.2, 138.0, 137.3, 136.8, 131.6, 129.5, 129.4, 129.0, 128.7, 126.3, 124.9, 124.2, 119.8, 119.3, 117.6, 68.1, 67.8, 53.8, 50.9, 40.2, 33.3, 28.0, 24.3, 18.2. HRMS (ESI) calcd for $C_{33}H_{37}ClN_4O_6$ $[M - HCl + H]^+$ 585.2708, found 585.2711.

SPECTRA (^{1}H, ^{13}C, CHN or MS)

N$^{\alpha}$-Boc-L-Trp(Cbz-L-Ala)-OBn (3)





EAGER 200 Stripchart

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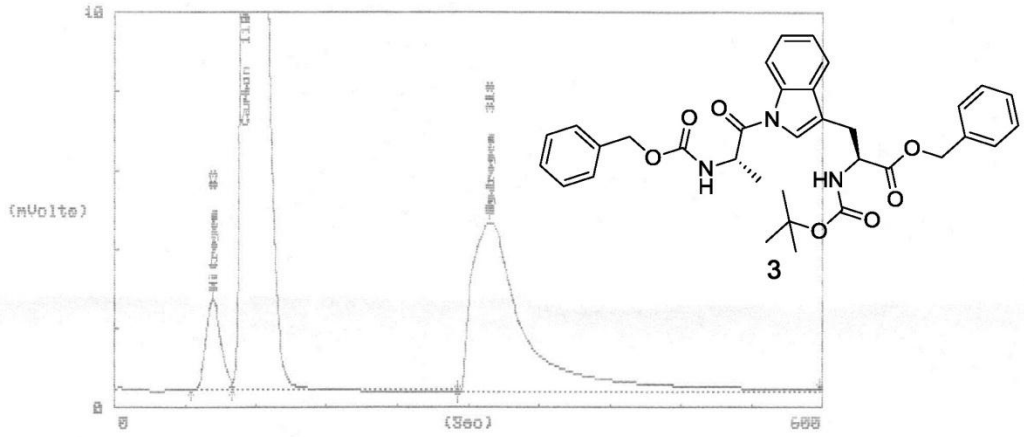

EAGER 200 Peak Integration Report

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Analysed : 08-03-12 06:00:58 Printed : 08-03-2012 08:40:04
Sample Ident. : 11 po-1135 Filename : 272211
Sample Weight : 2.139 Calc.method: using 'K. Factors'

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2	FU	100	291	110	52543.2	831939	77.01	Carbon
3	RS	291	598	318	4225.0	216167	20.01	Hydrogen
						1080325	100.00	

EAGER 200 Unk Report

Instrument name : Instrument #1 Bline drift (fV):-2.2
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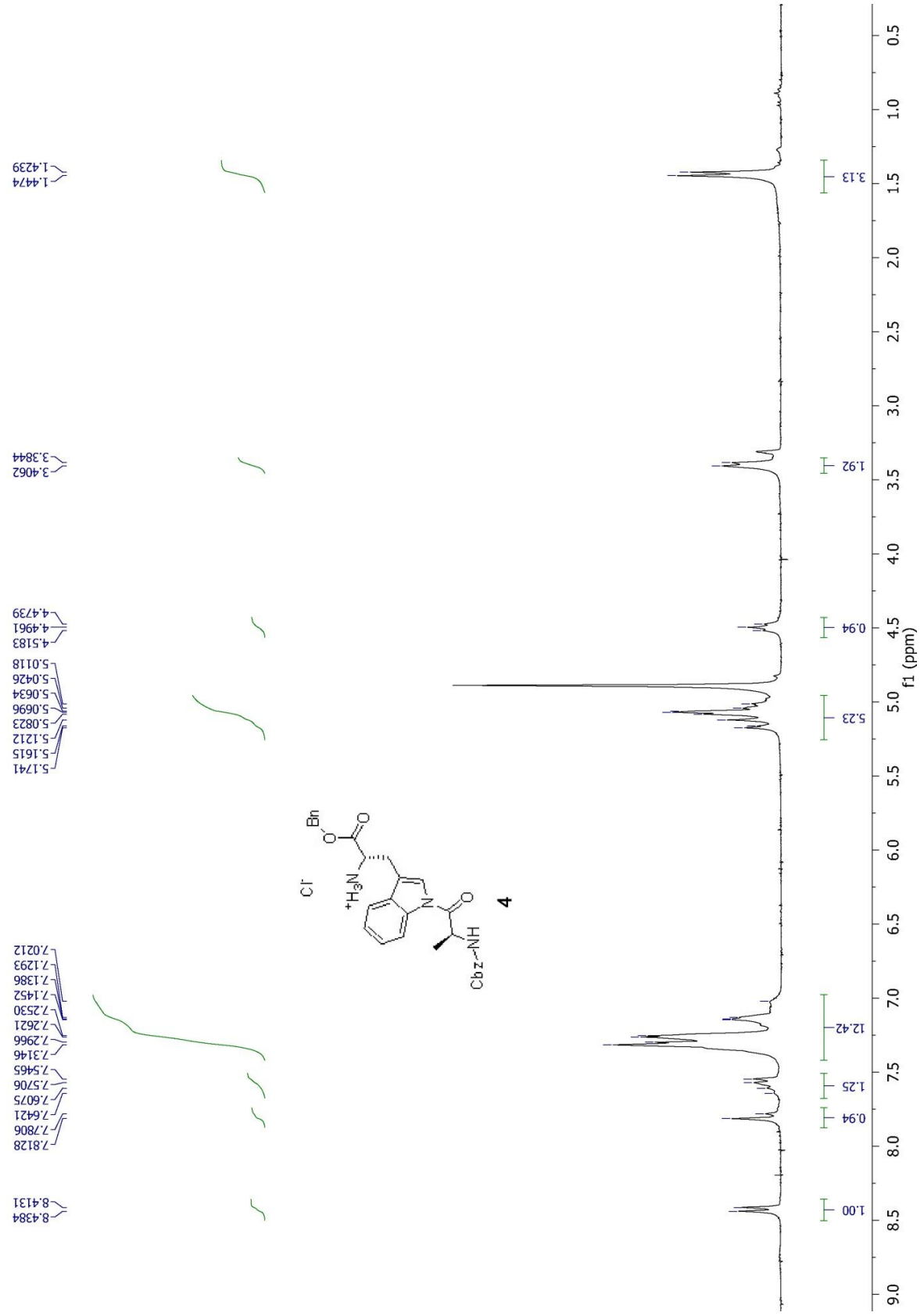
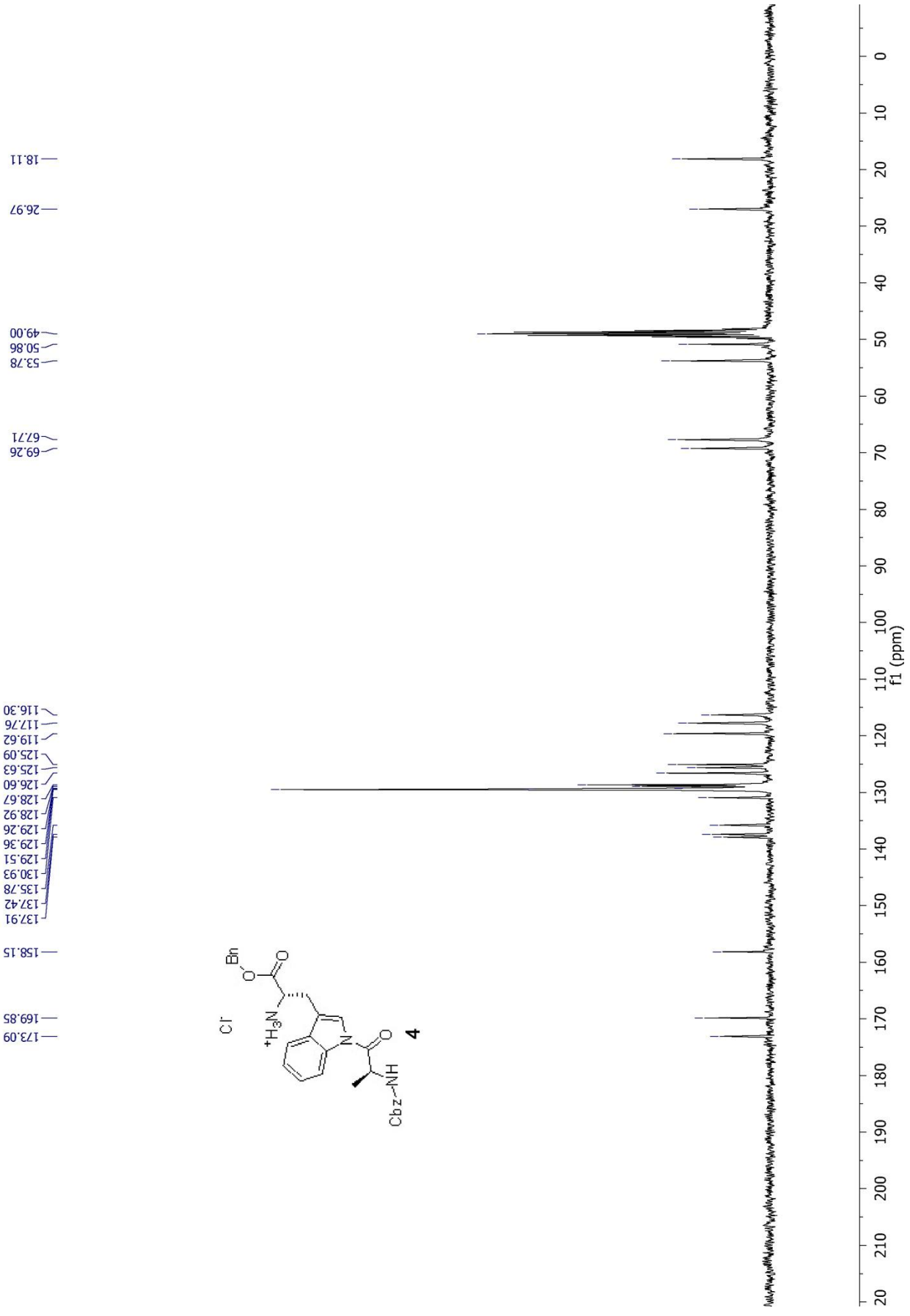
Sample Ident. : 11 po-1135 Filename : 272211
Sample Weight : 2.139 Calc.method: using 'K. Factors'

Pk. (#)	Ret Time (Sec)	Area (fV*Sec)	Element % (%)	Area Ratio	Name
1	83	32219	7.345	.258213E+02	Nitrogen
2	110	831939	67.706	.100000E+01	Carbon
3	318	216167	6.400	.384860E+01	Hydrogen

L-Trp(Cbz-L-Ala)-OBn hydrogen chloride (4)





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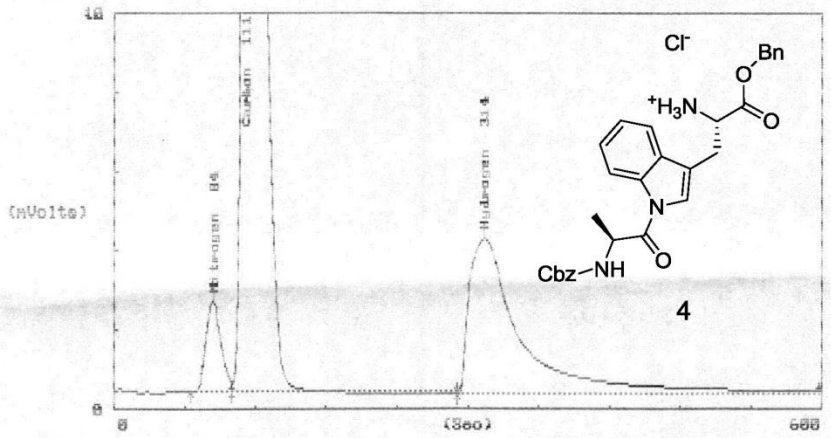

EAGER 200 Peak Integration Report

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Sample Ident. : 37 po-1150 Filename : 272937
Sample Weight : 2.131 Calc.method: using 'K. Factors'

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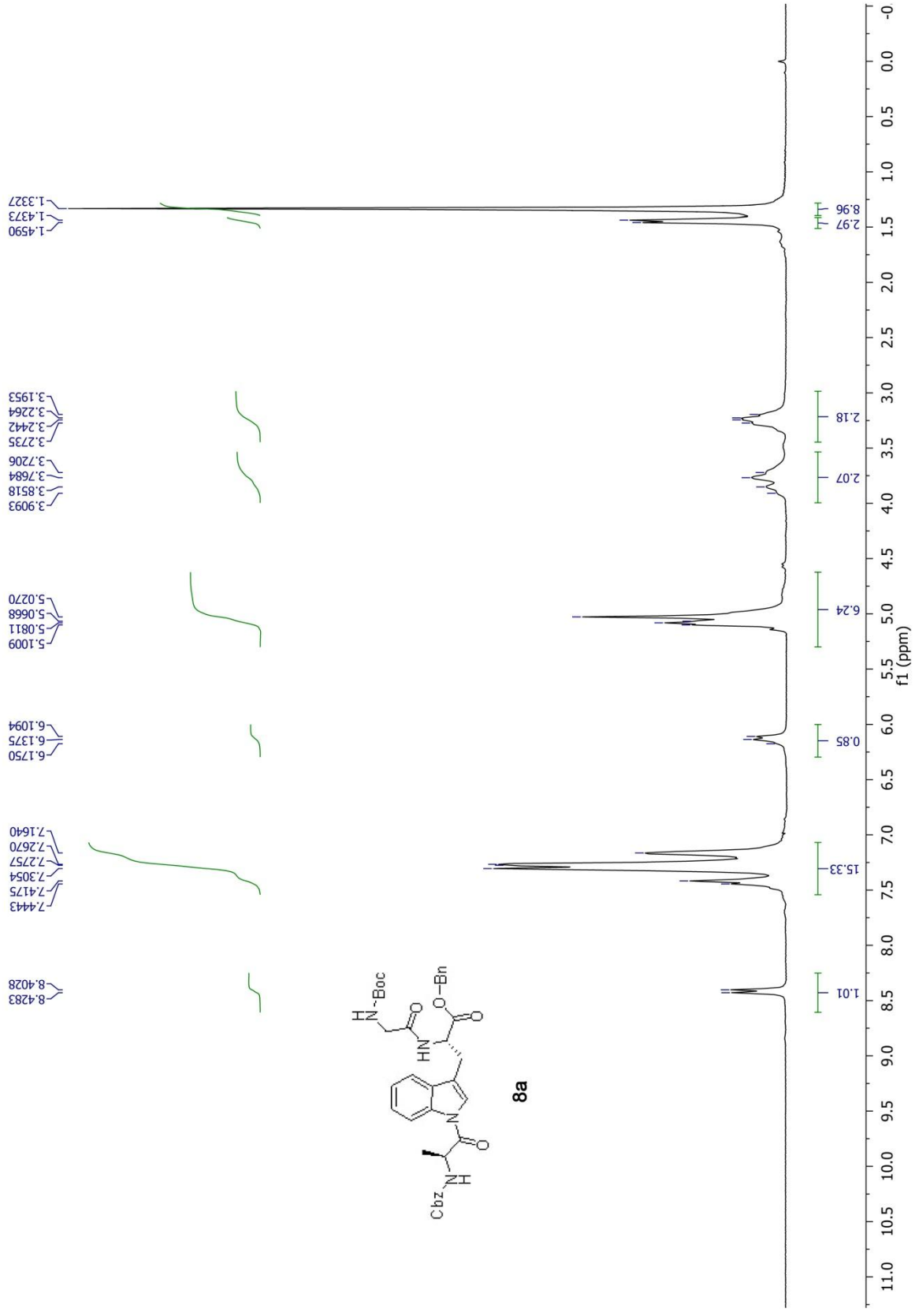
1	FU	65	100	84	2272.5	32507	3.24	Nitrogen
2	FU	100	291	111	49132.7	777695	77.51	Carbon
3	RS	291	598	314	3875.9	193081	19.24	Hydrogen
						1003283	100.00	

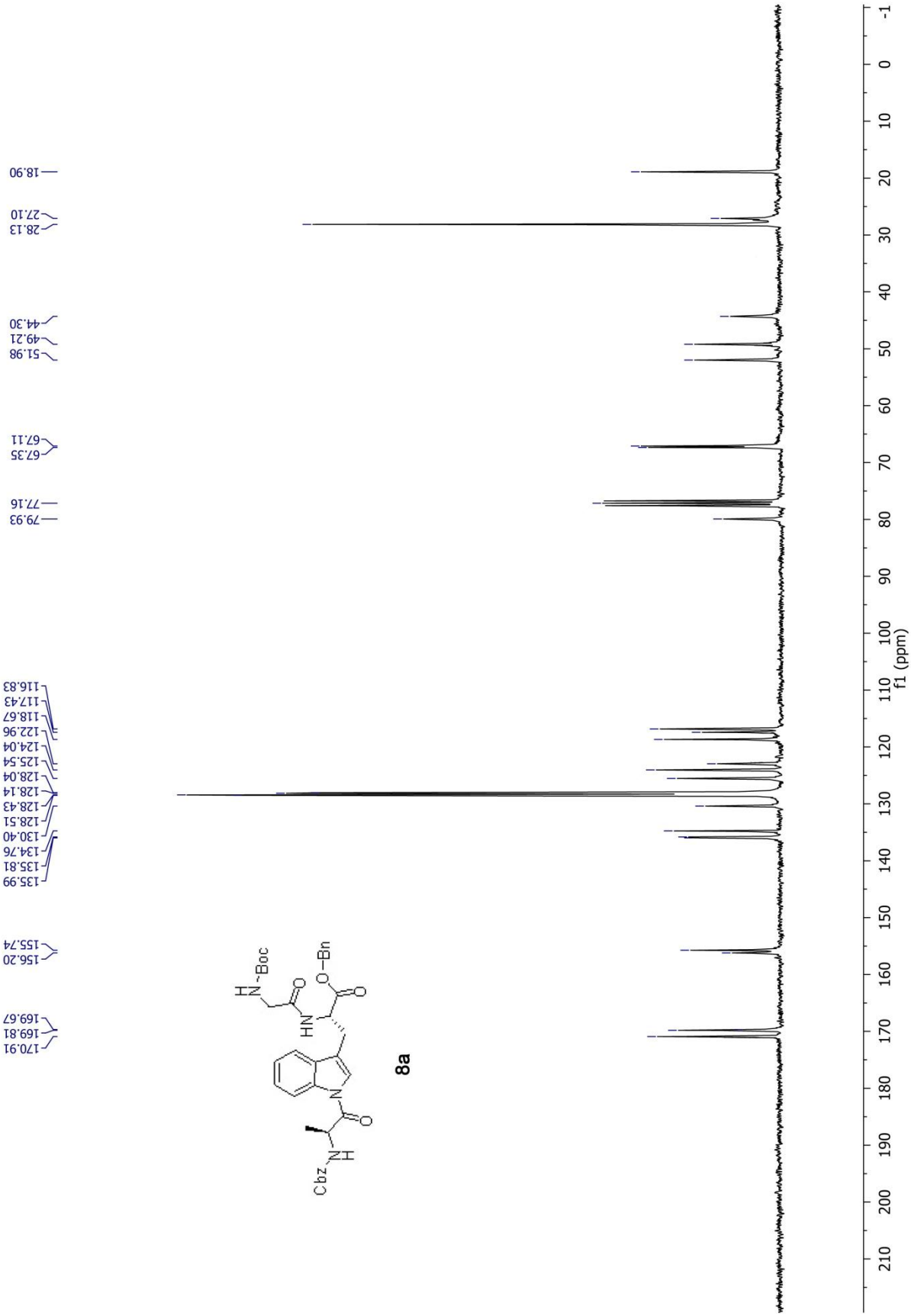
EAGER 200 Unk Report

Instrument name : Instrument #1 Bline drift (fV): 5
Company Name : U of Florida Operator Ident. : KOU
Analysed : 08-23-12 09:50:47 Printed : 08-23-2012 10:19:22
Sample Ident. : 37 po-1150 Filename : 272937
Sample Weight : 2.131 Calc.method: using 'K. Factors'

Pk. (#)	Ret Time (Sec)	Area (fV*Sec)	Element % (%)	Area Ratio	Name
1	84	32507	7.568	.239237E+02	Nitrogen
2	111	777695	64.595	.100000E+01	Carbon
3	314	193081	5.830	.402781E+01	Hydrogen

Boc-Gly-L-Trp(Cbz-L-Ala)-OBn (8a)





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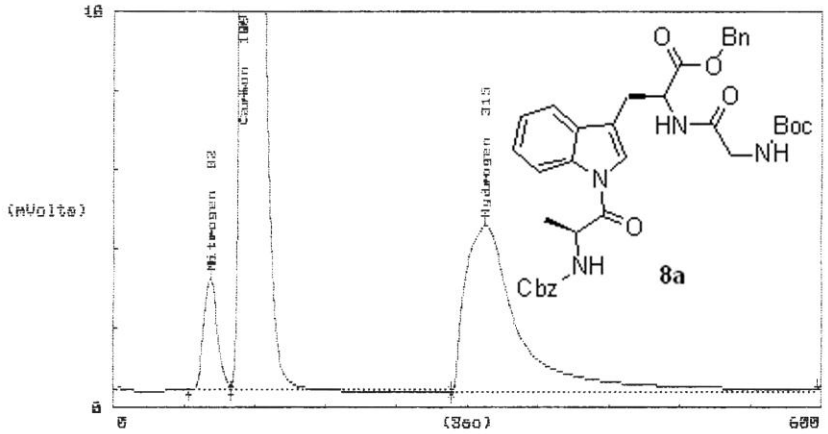

EAGER 200 Peak Integration Report

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Sample Ident. : 14 po-1165 Filename : 274314
Sample Weight : 2.186 Calc.method: using 'K. Factors'

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1	FU	64	100	82	2782.8	36336	3.35	Nitrogen
2	FU	100	286	109	56324.8	831087	76.61	Carbon
3	RS	286	597	315	4138.0	217430	20.04	Hydrogen
						1084853	100.00	

EAGER 200 Unk Report

Instrument name : Instrument #1 Bline drift (fV): 14.9
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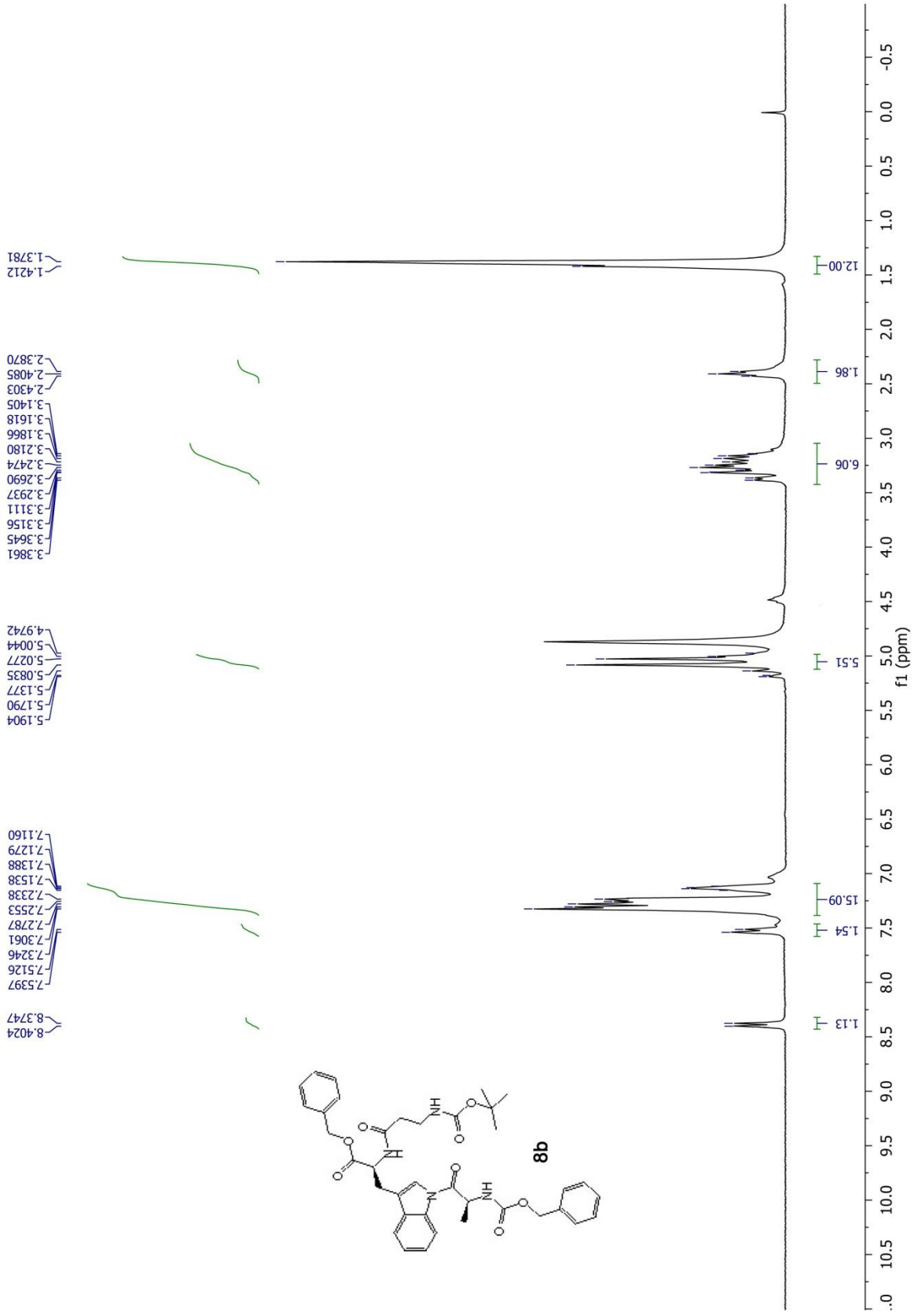
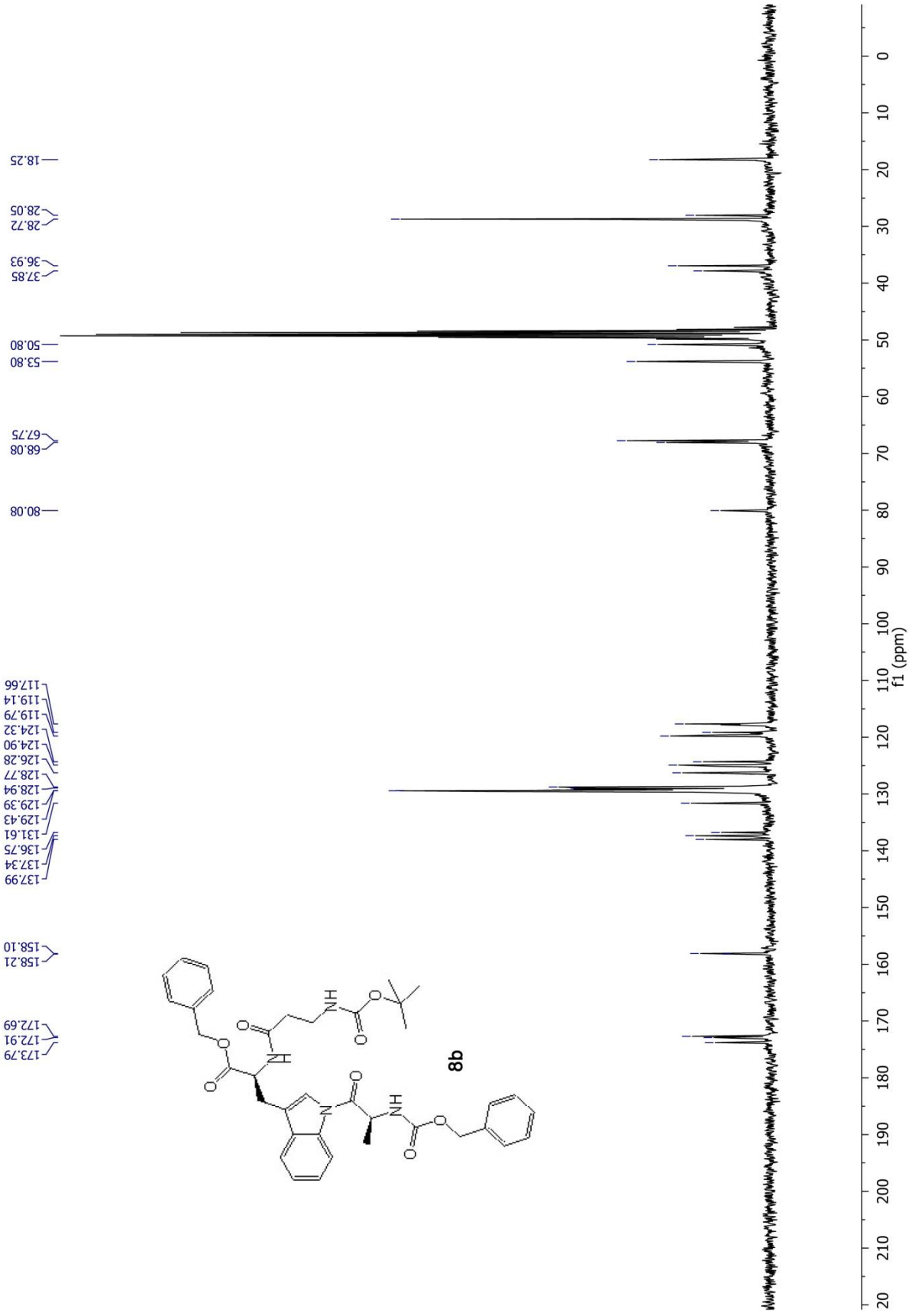
Sample Ident. : 14 po-1165 Filename : 274314
Sample Weight : 2.186 Calc.method: using 'K. Factors'

Pk. (#)	Ret Time (Sec)	Area (fV*Sec)	Element % (%)	Area Ratio	Name
1	82	36336	8.437	.228724E+02	Nitrogen
2	109	831087	65.441	.100000E+01	Carbon
3	315	217430	6.215	.382232E+01	Hydrogen

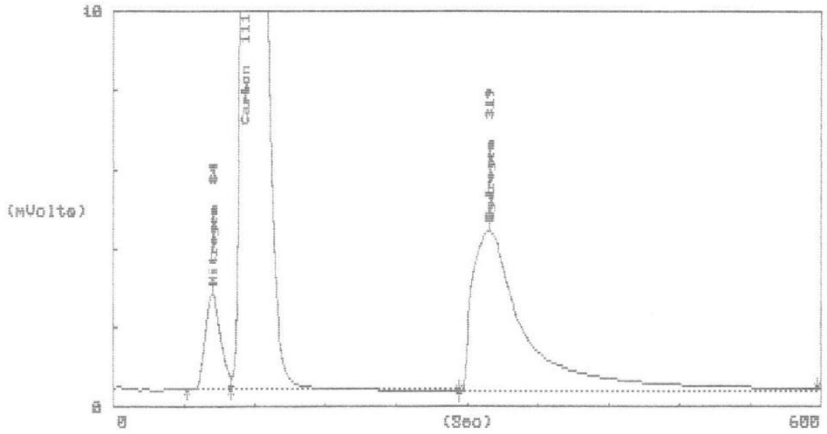
Boc-β-Ala-L-Trp(Cbz-L-Ala)-OBn (8b)

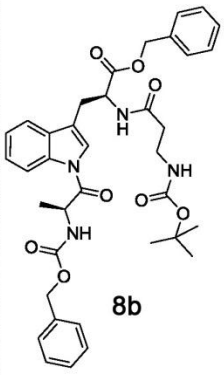




EAGER 200 Stripchart

Sample Ident. : 13 po-1156 Filename :273613
Analysed : 09-14-12 05:48:45 Printed :09-14-2012 09:02:11





EAGER 200 Peak Integration Report

Instrument name : Instrument #1 Bline drift (fV): 3.5
Company Name : U of Florida Operator Ident. : KOU
Analysed : 09-14-12 05:48:45 Printed : 09-14-2012 09:02:11
Sample Ident. : 13 po-1156 Filename : 273613
Sample Weight : 2.096 Calc.method: using 'K. Factors'

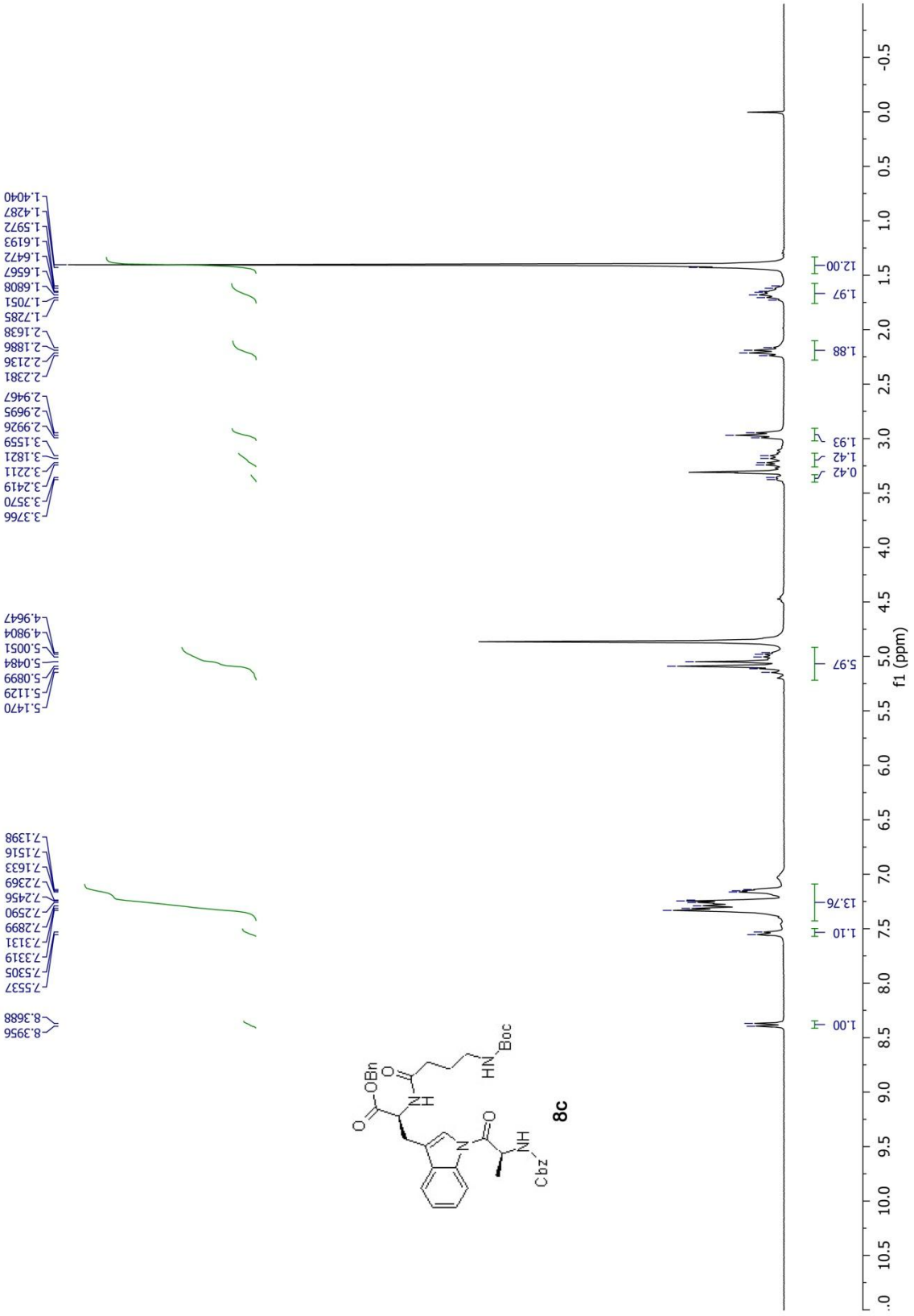
No. (#)	Type (#)	Start (Sec)	End (Sec)	Ret Time (Sec)	Height (fV)	Area (fV*Sec)	Area % (%)	Name
1	FU	63	100	84	2407.9	34636	3.35	Nitrogen
2	FU	100	293	111	49117.2	792994	76.68	Carbon
3	RS	293	597	319	4007.5	206504	19.97	Hydrogen
						1034134	100.00	

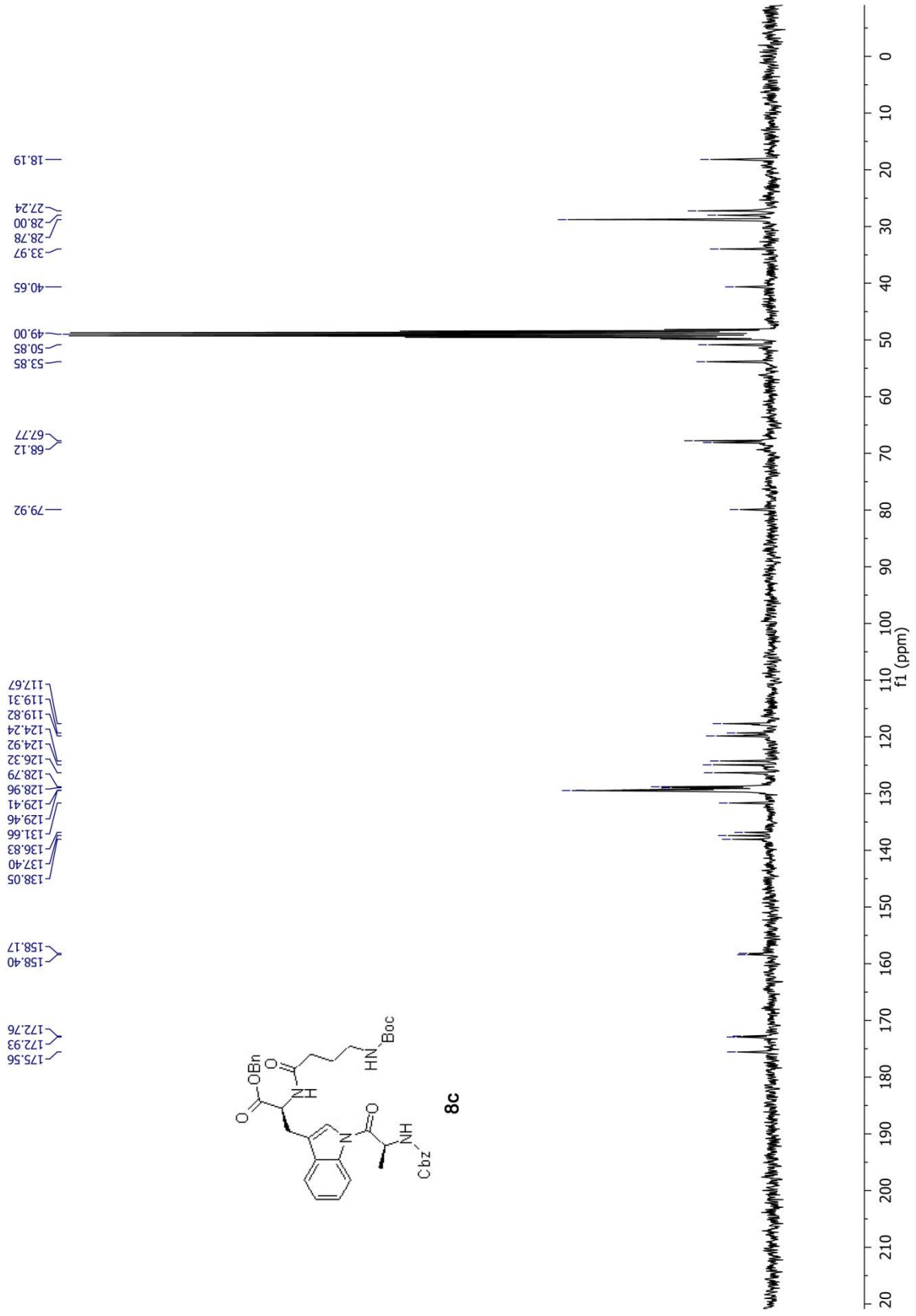
EAGER 200 Unk Report

Instrument name : Instrument #1 Bline drift (fV): 3.5
Company Name : U of Florida Operator Ident. : KOU
Analysed : 09-14-12 05:48:45 Printed : 09-14-2012 09:02:11
Sample Ident. : 13 po-1156 Filename : 273613
Sample Weight : 2.096 Calc.method: using 'K. Factors'

Pk. (#)	Ret Time (Sec)	Area (fV*Sec)	Element % (%)	Area Ratio	Name
1	84	34636	8.096	.228950E+02	Nitrogen
2	111	792994	66.042	.100000E+01	Carbon
3	319	206504	6.273	.384010E+01	Hydrogen

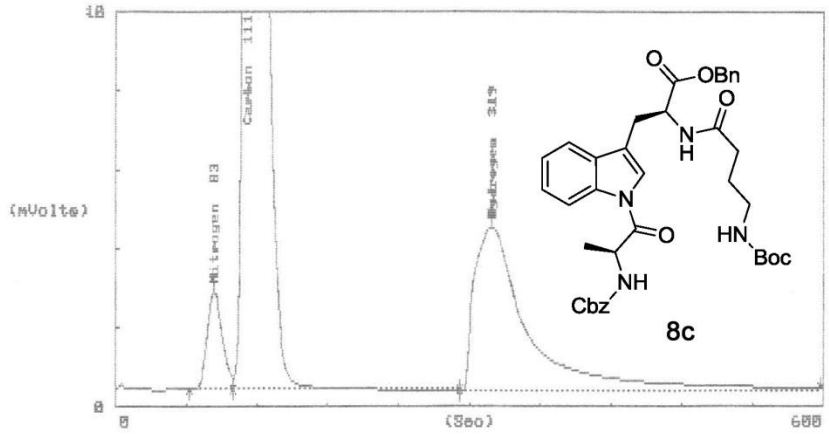
Boc-GABA-L-Trp(Cbz-L-Ala)-OBn (8c)





EAGER 200 Stripchart

Sample Ident. : 14 po-1157 Filename :273614
Analysed : 09-14-12 05:58:48 Printed :09-14-2012 06:08:51



EAGER 200 Peak Integration Report

Instrument name : Instrument #1 Bline drift (fV): 1.9
Company Name : U of Florida Operator Ident. : KOU
Analysed : 09-14-12 05:58:48 Printed : 09-14-2012 06:08:51
Sample Ident. : 14 po-1157 Filename : 273614
Sample Weight : 2.123 Calc.method: using 'K. Factors'

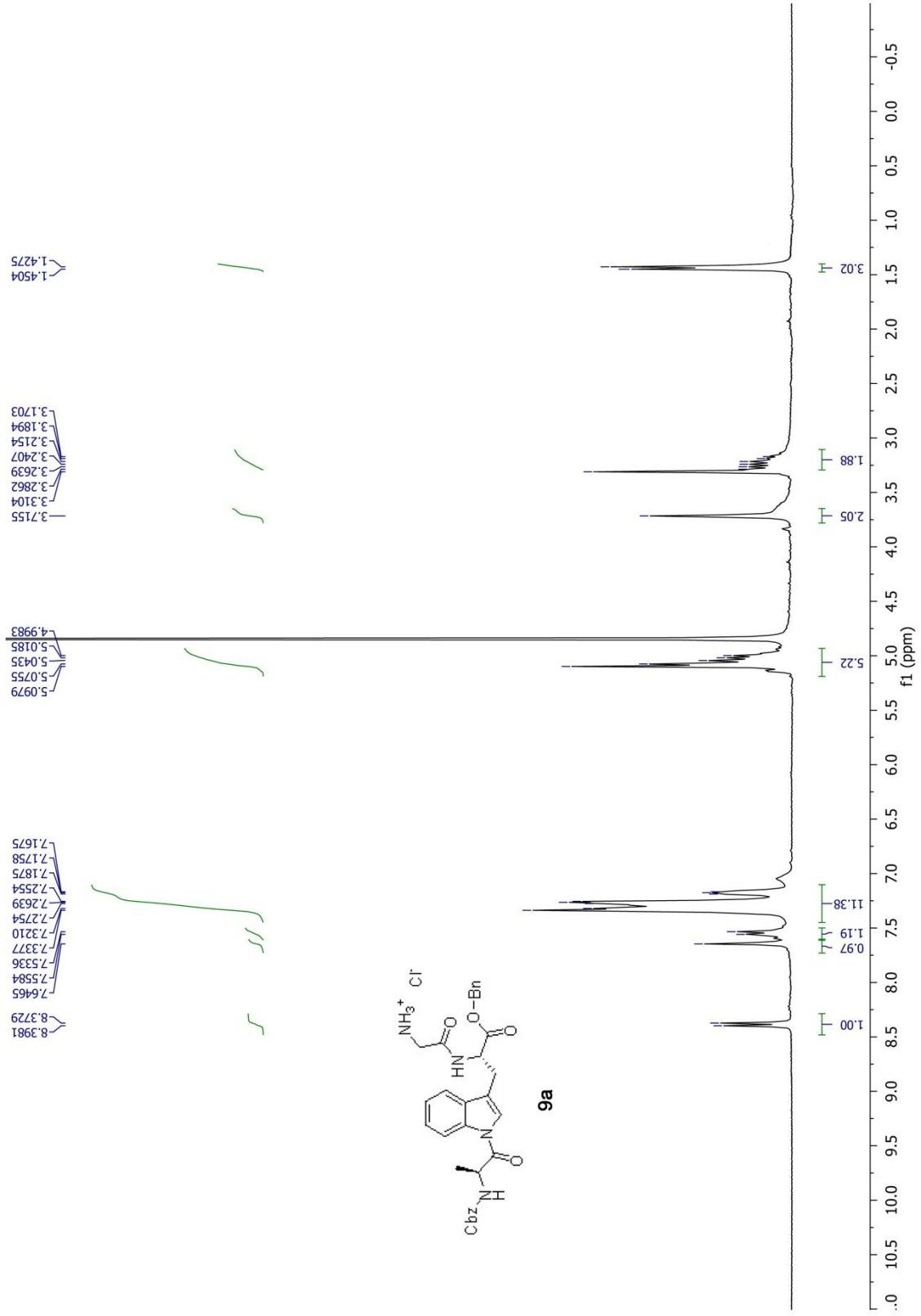
No. (#)	Type (#)	Start (Sec)	End (Sec)	Ret Time (Sec)	Height (fV)	Area (fV*Sec)	Area % (%)	Name
1	FU	63	100	83	2432.1	34765	3.30	Nitrogen
2	FU	100	292	111	50595.7	807028	76.57	Carbon
3	RS	292	598	319	4076.2	212236	20.14	Hydrogen
						1054029	100.00	

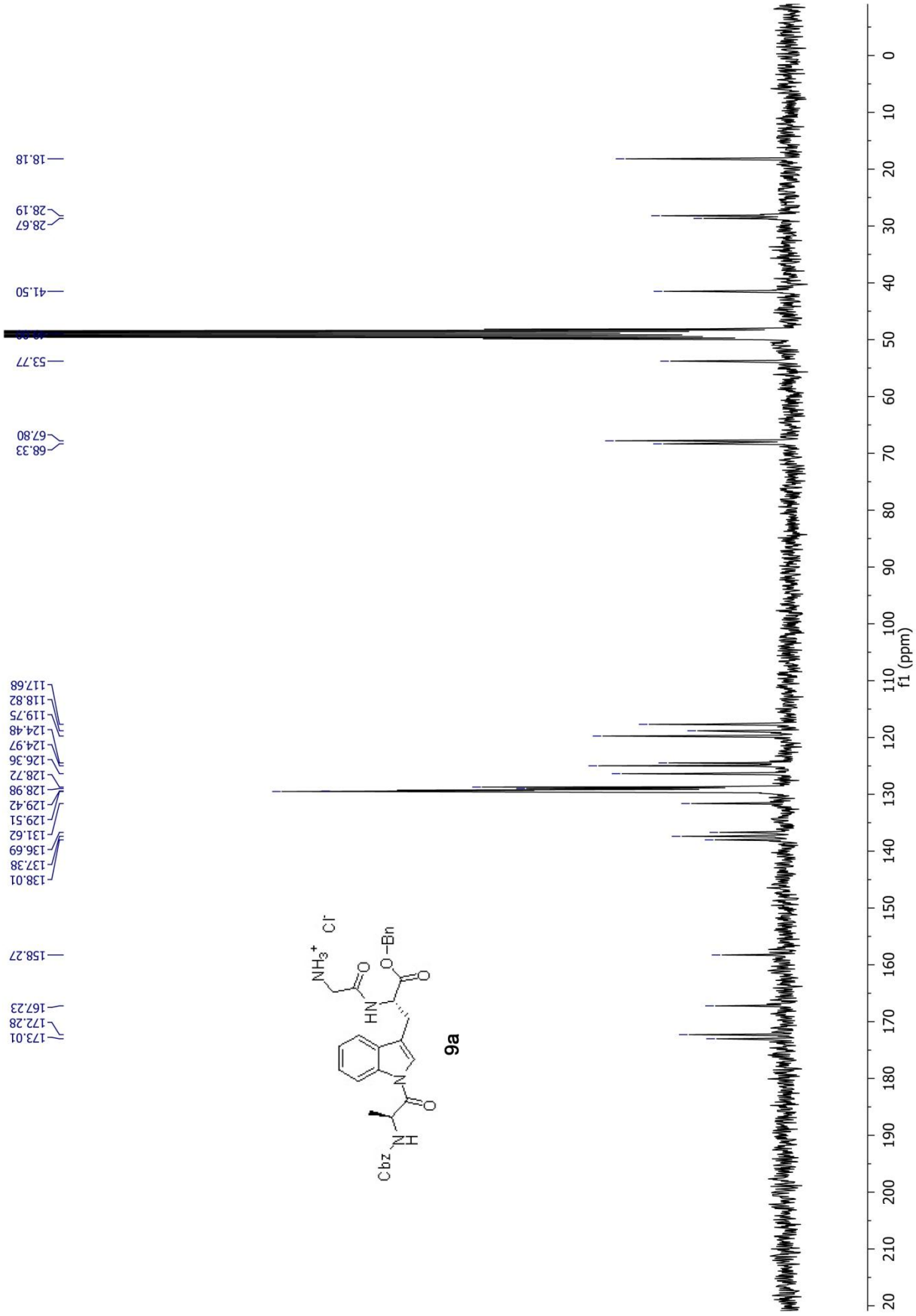
EAGER 200 Unk Report

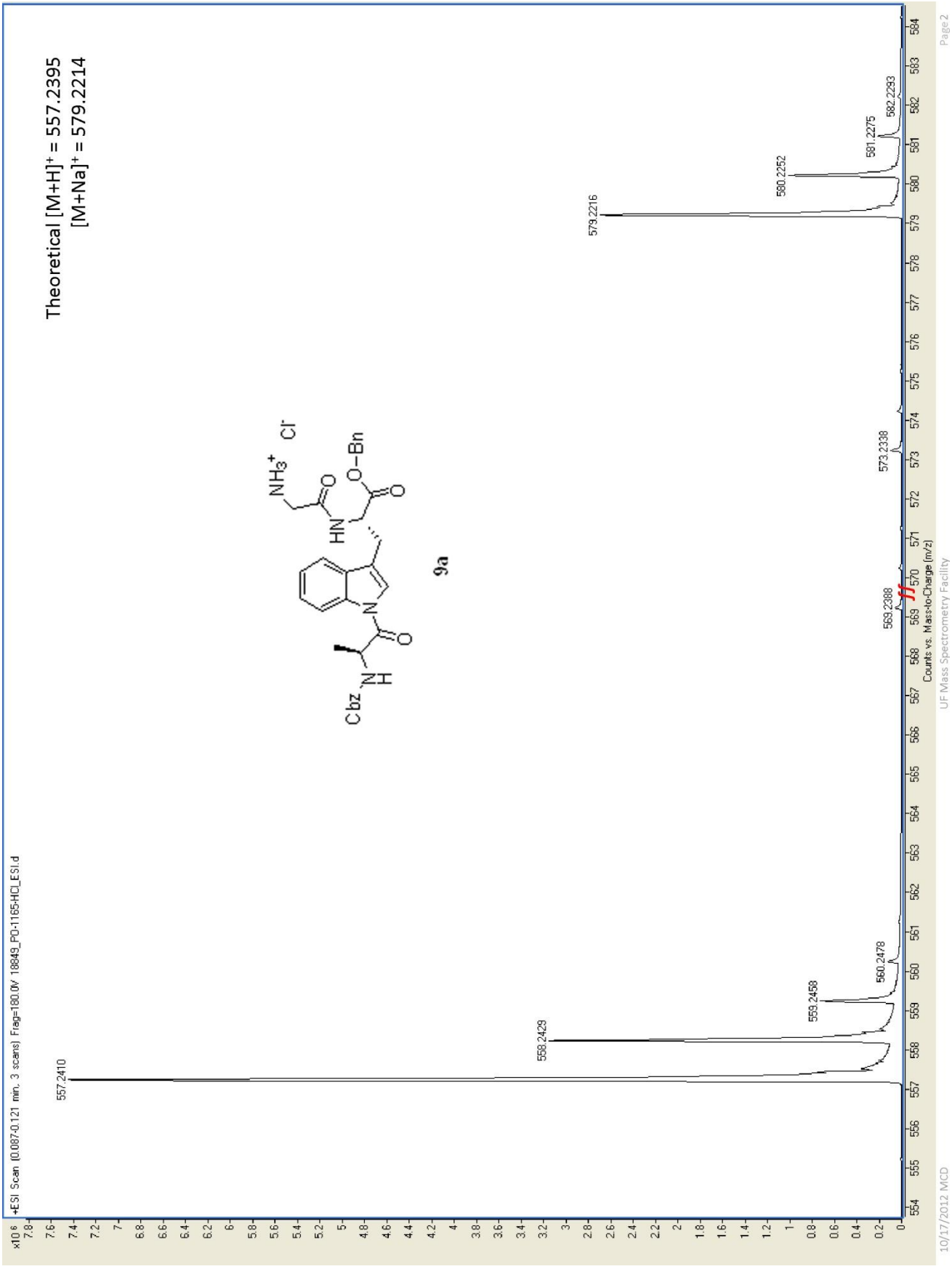
Instrument name : Instrument #1 Bline drift (fV): 1.9
Company Name : U of Florida Operator Ident. : KOU
Analysed : 09-14-12 05:58:48 Printed : 09-14-2012 06:08:51
Sample Ident. : 14 po-1157 Filename : 273614
Sample Weight : 2.123 Calc.method: using 'K. Factors'

Pk. (#)	Ret Time (Sec)	Area (fV*Sec)	Element % (%)	Area Ratio	Name
1	83	34765	8.023	.232135E+02	Nitrogen
2	111	807028	66.357	.100000E+01	Carbon
3	319	212236	6.365	.380251E+01	Hydrogen

Gly-L-Trp(Cbz-L-Ala)-OBn hydrogen chloride (9a)






Theoretical [M+H]+ = 557.2395
[M+Na]+ = 579.2214
9a
+ESI Scan (0.087-0.121 min, 3 scans) Frag=180.0V 18849_PD-1165-HCl_ESI.d
x10 6
Counts vs. Mass-to-Charge (m/z)
557.2410
558.2429
559.2458
560.2478
563.2388
573.2338
579.2216
580.2252
581.2275
582.2293
10/17/2012 MCD
UF Mass Spectrometry Facility
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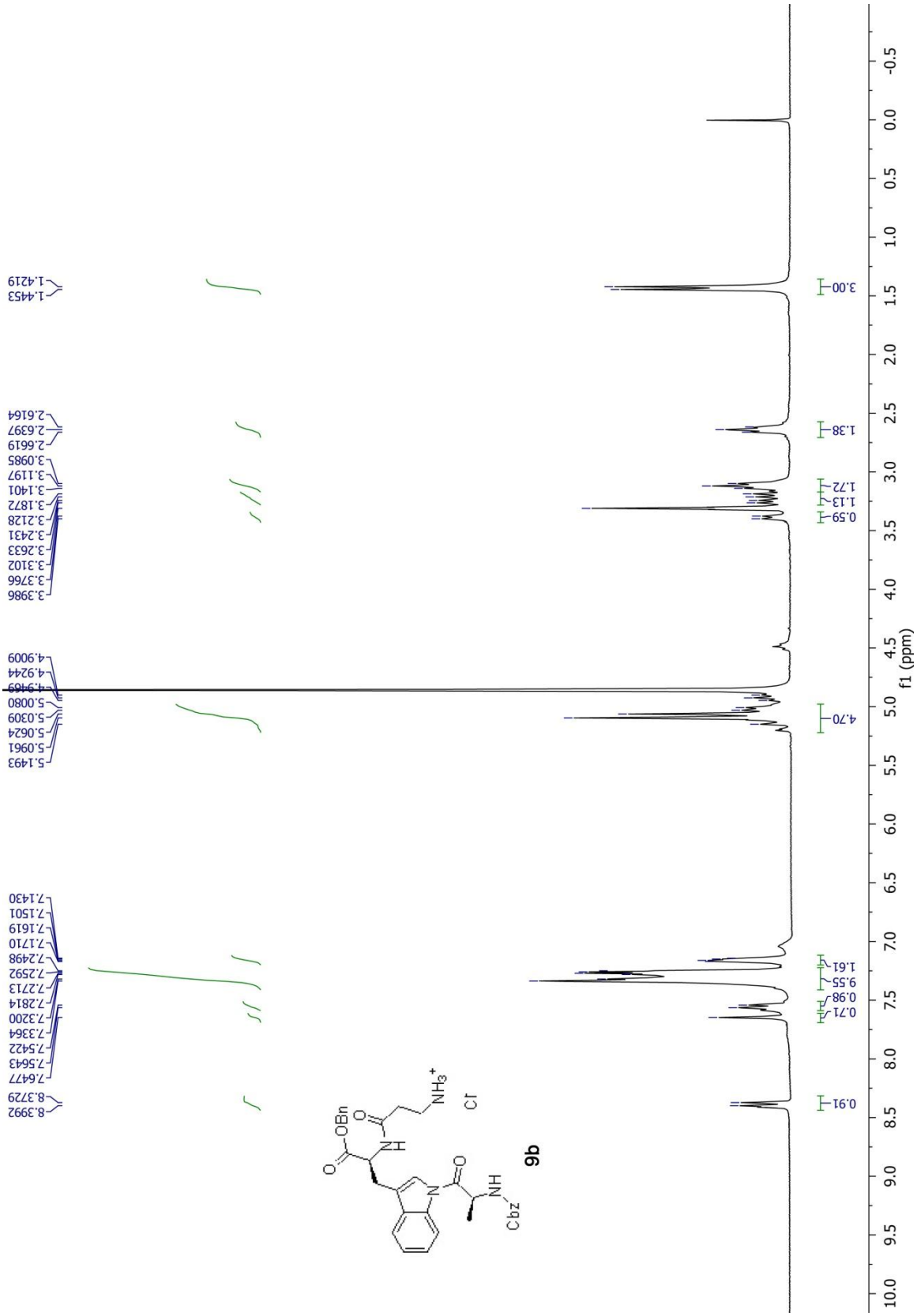
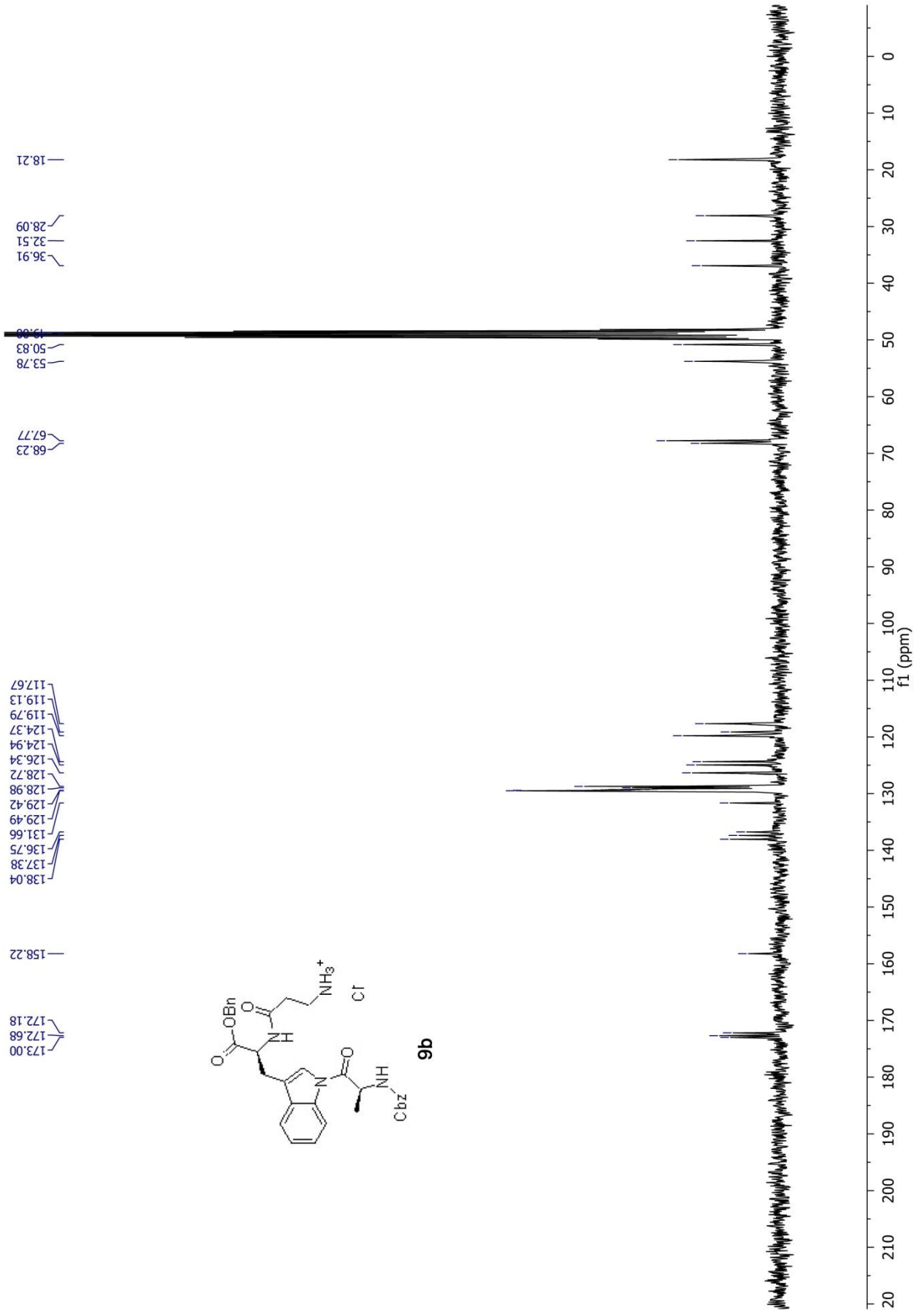
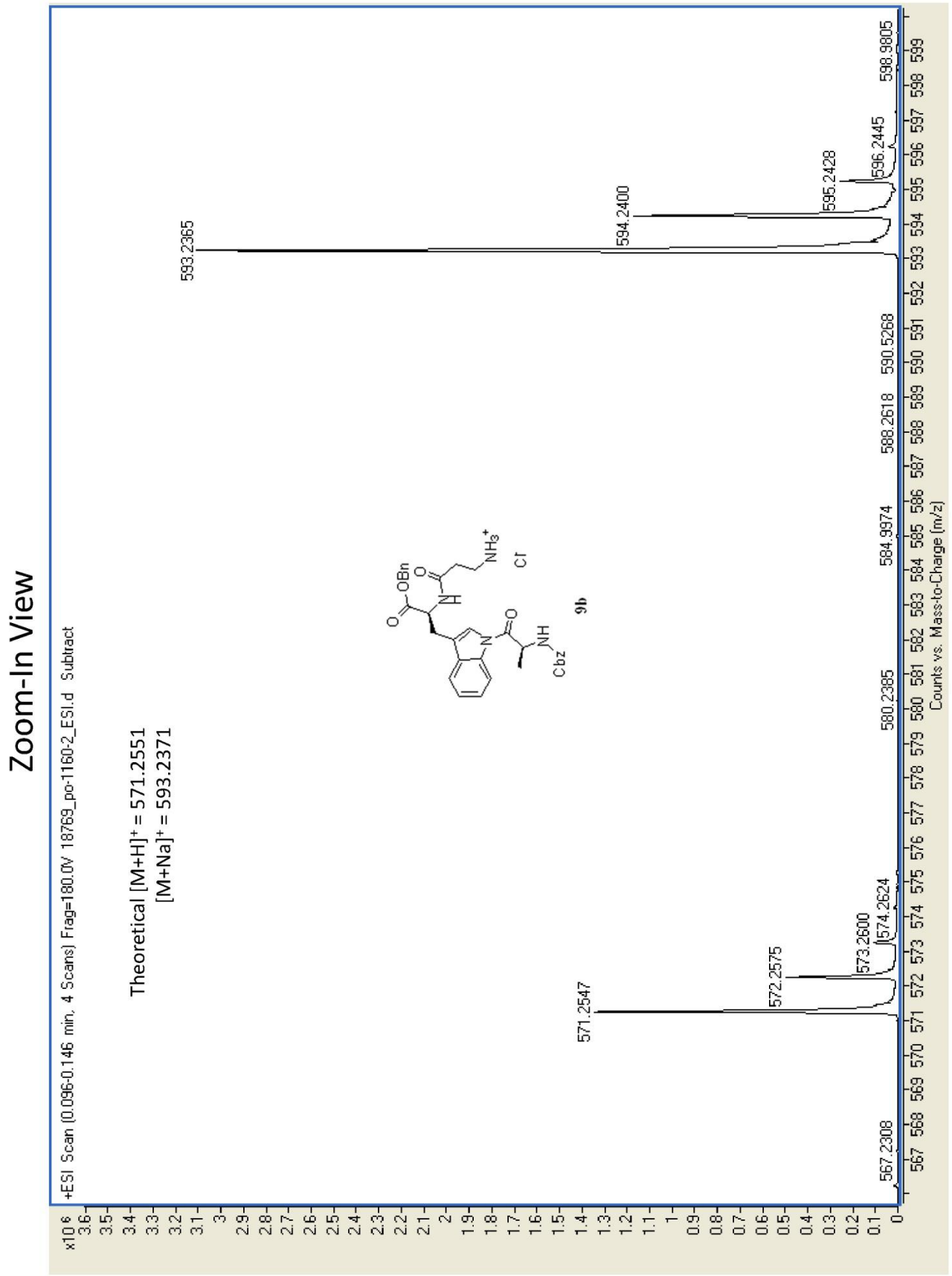
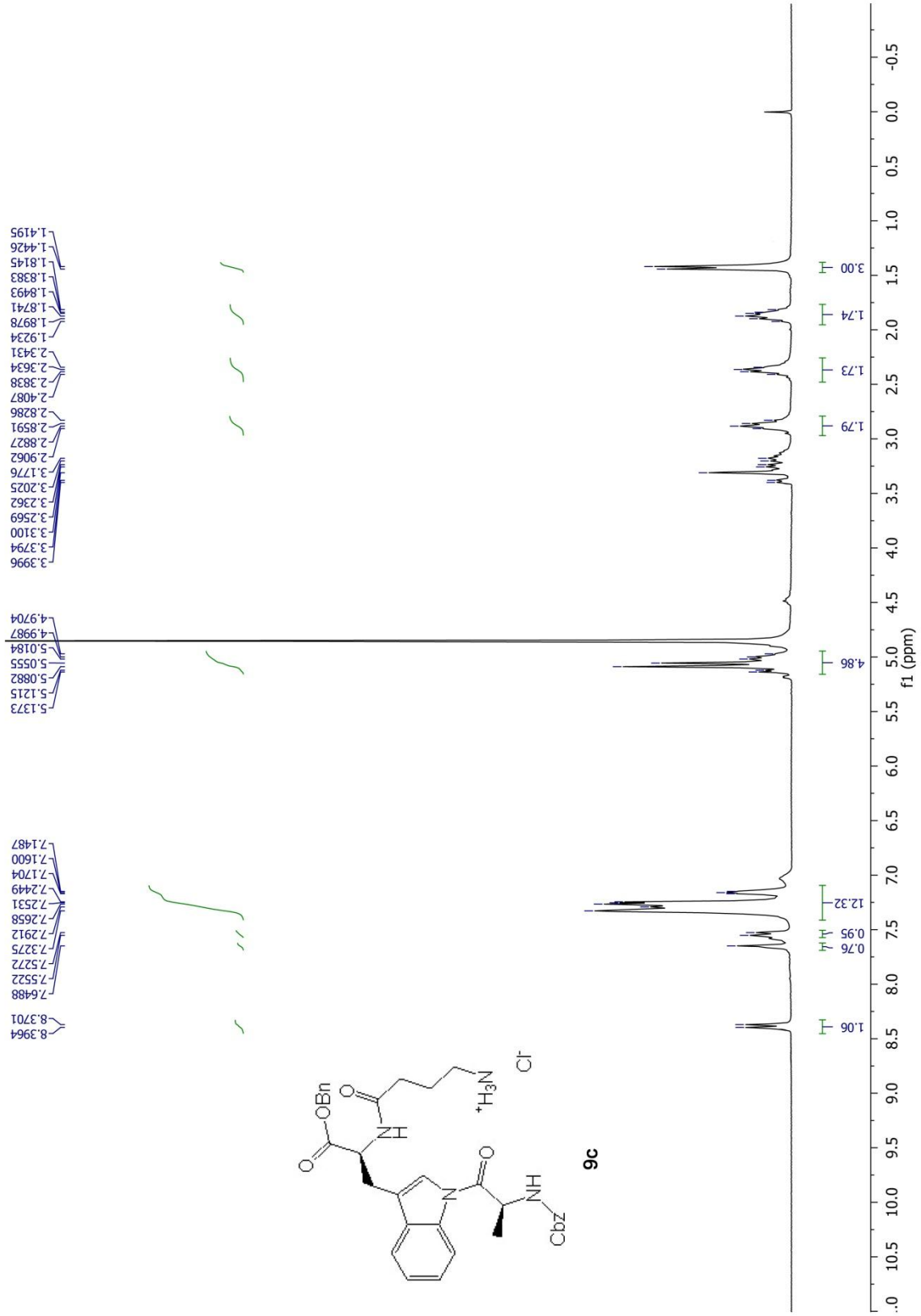
β-Ala-L-Trp(Cbz-L-Ala)-OBn hydrogen chloride (9b)

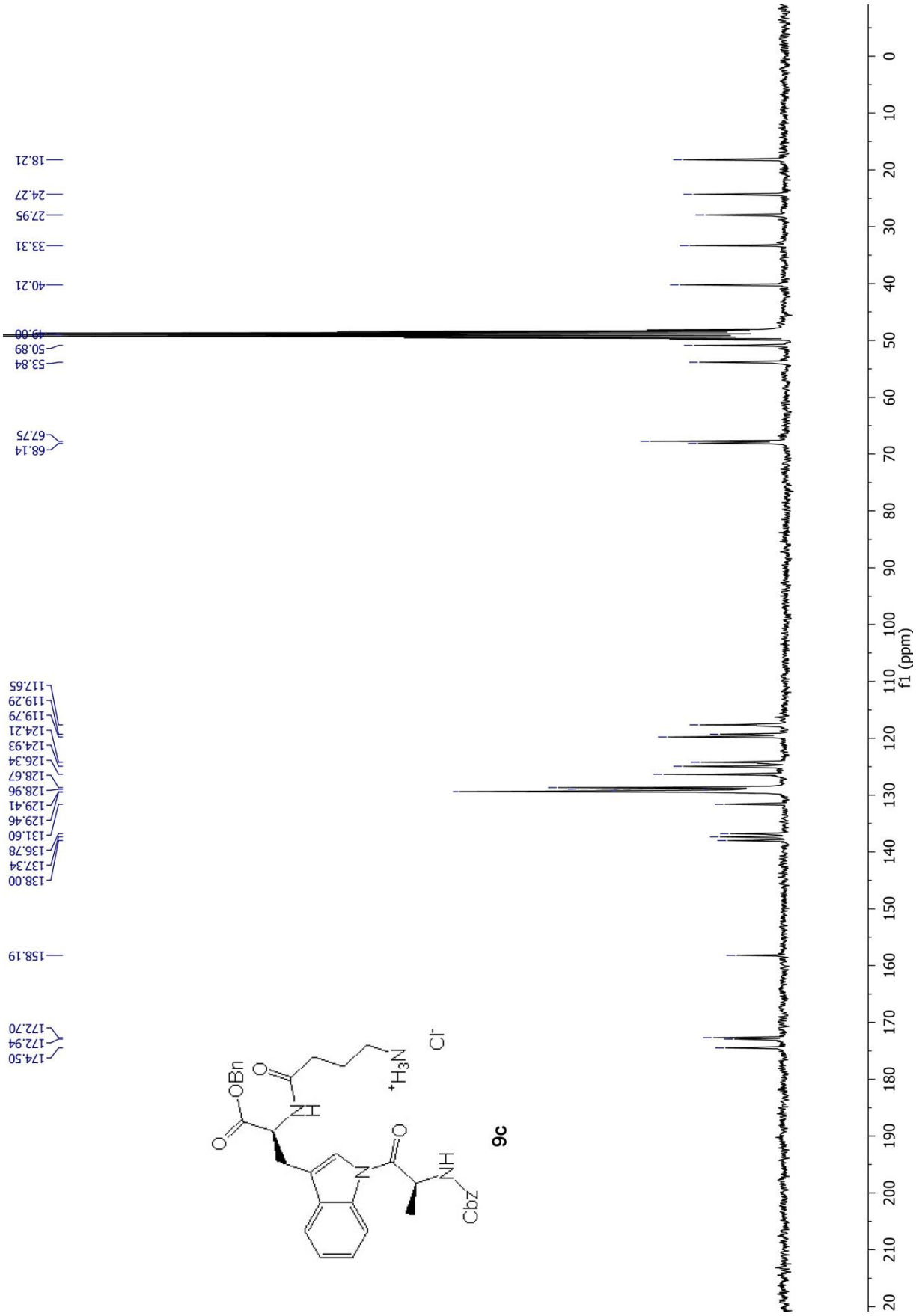


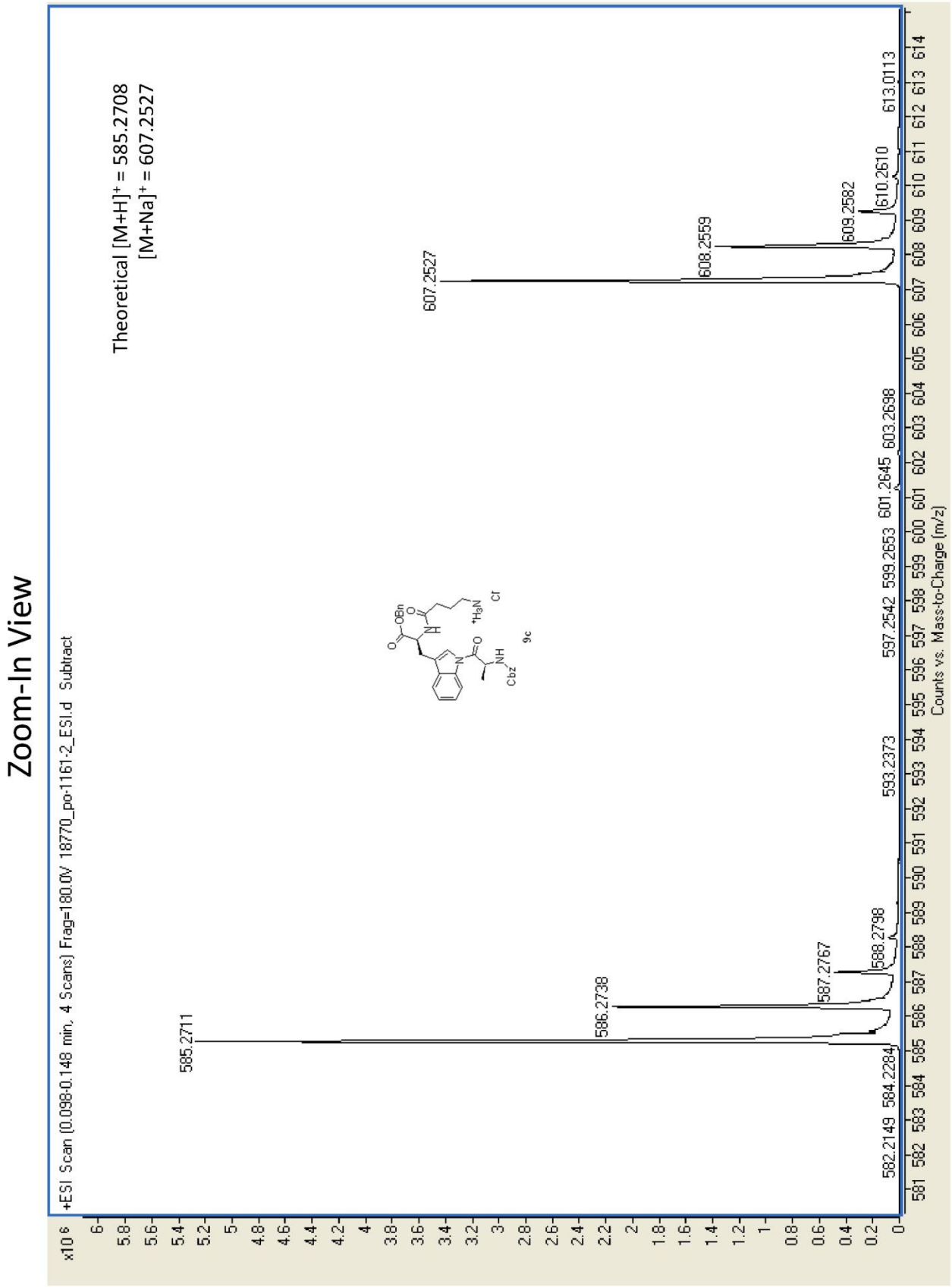




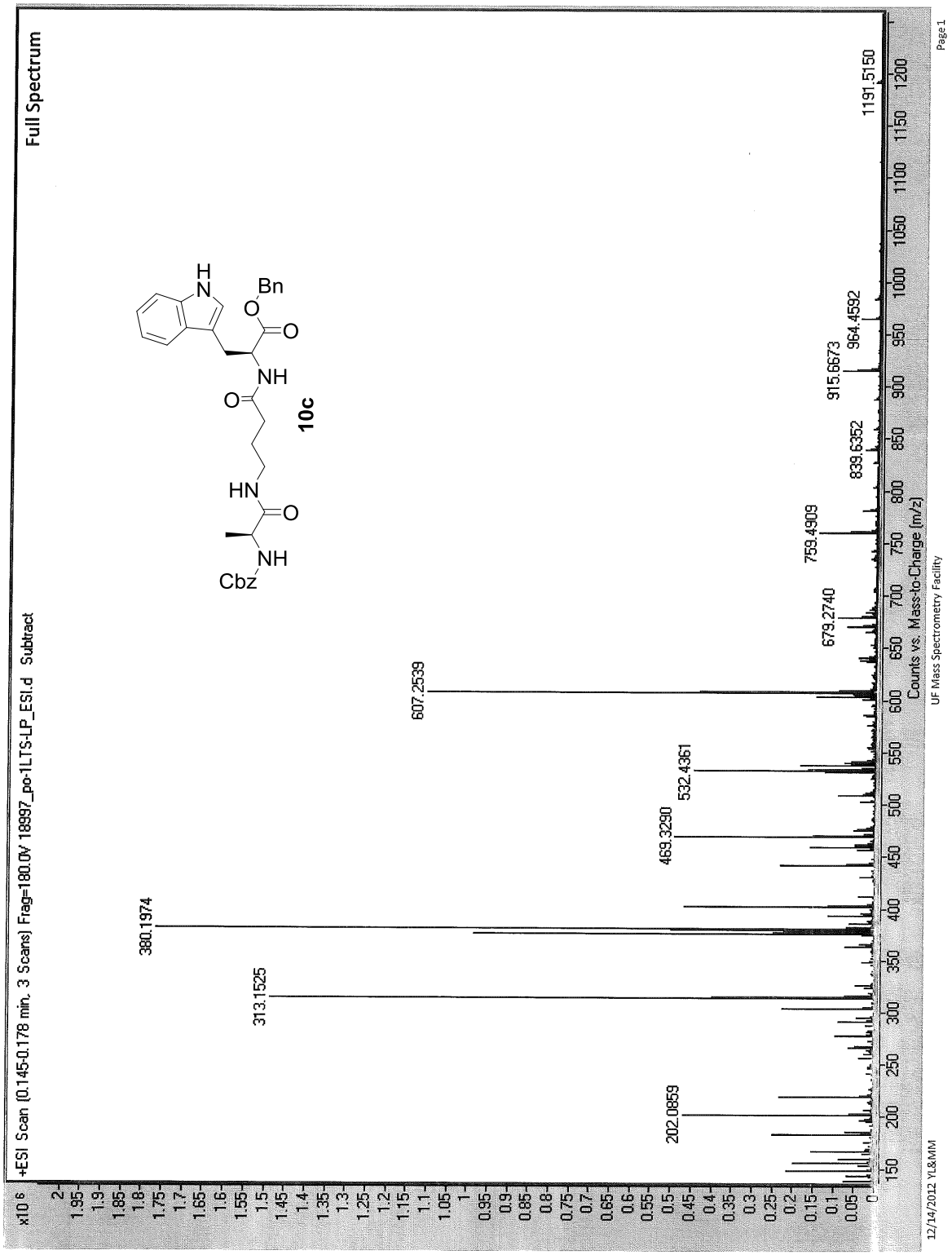
GABA-L-Trp(Cbz-L-Ala)-OBn hydrogen chloride (9c)

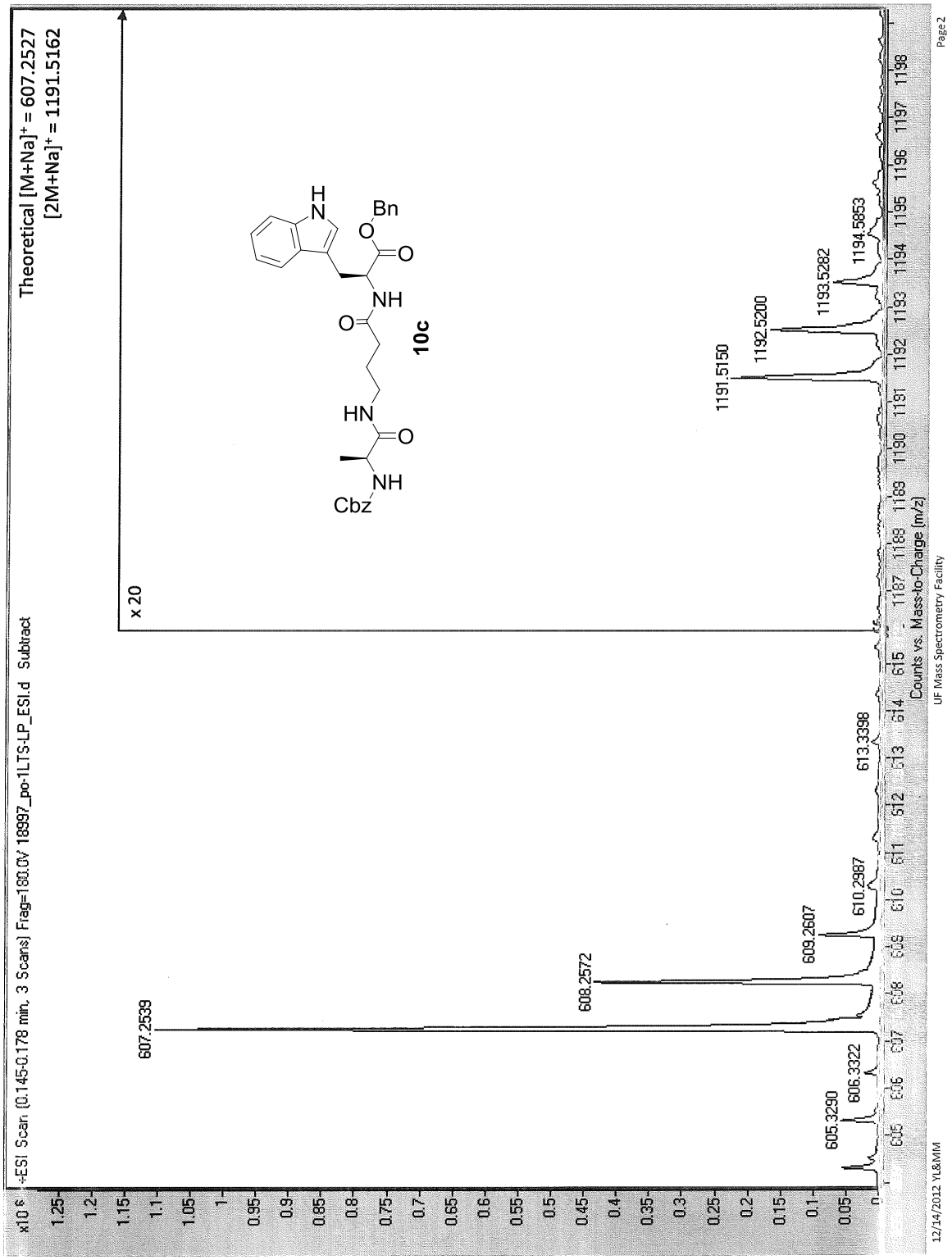






HRMS of Ligated Product 10c




Theoretical [M+Na]+ = 607.2527
[2M+Na]+ = 1191.5162
+ESI Scan (0.145-0.178 min, 3 Scans) Frag=180.0V 18997_po-1LTS-LP_ESI.d Subtract
x 20
10c
Bn
NH
HN
Cbz
607.2539
608.2572
609.2607
610.2987
613.3398
605.3290
606.3322
1191.5150
1192.5200
1193.5282
1194.5853
Counts vs. Mass-to-Charge (m/z)
12/14/2012 YL&MM
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HPLC chromatograms and ESI-MS

General procedure for chemical ligation of *N*-acyl-isotripeptide 9b,c in buffer

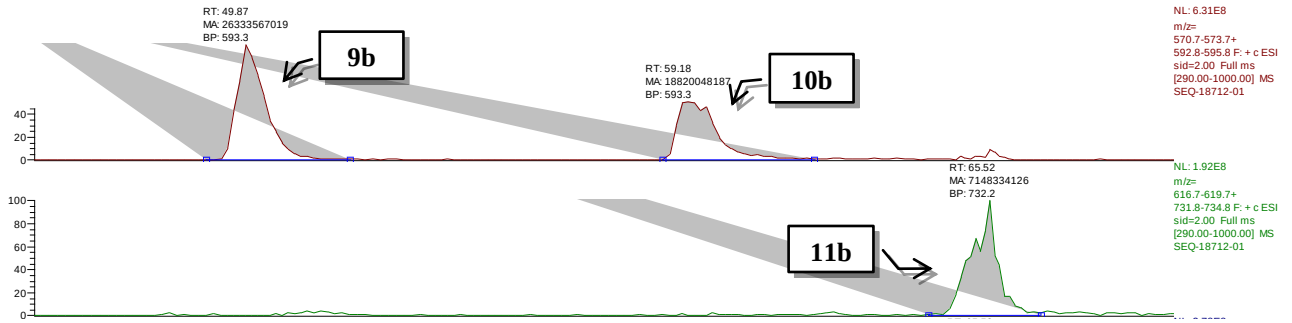
Isotripeptide (**9b,c**) (0.20 mmol) was suspended in deoxygenated phosphate buffer (NaH_2PO_4/Na_2HPO_4) (1M, pH = 7.4, 7 mL). The mixture was irradiated with microwave (50 °C, 50 W, 3 h) in a microwave tube. The reaction was allowed to cool to room temperaturet and acidified with 2N HCl to pH = 1. The mixture was extracted with ethyl acetate (3×20 mL), the combined organic extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The ligation mixture was weighed and then a solution in methanol (1 mg mL^{-1}) was analysed by HPLC-MS.

General procedure for chemical ligation of *N*-acyl-isotripeptide 9a-c in DMF-piperidine

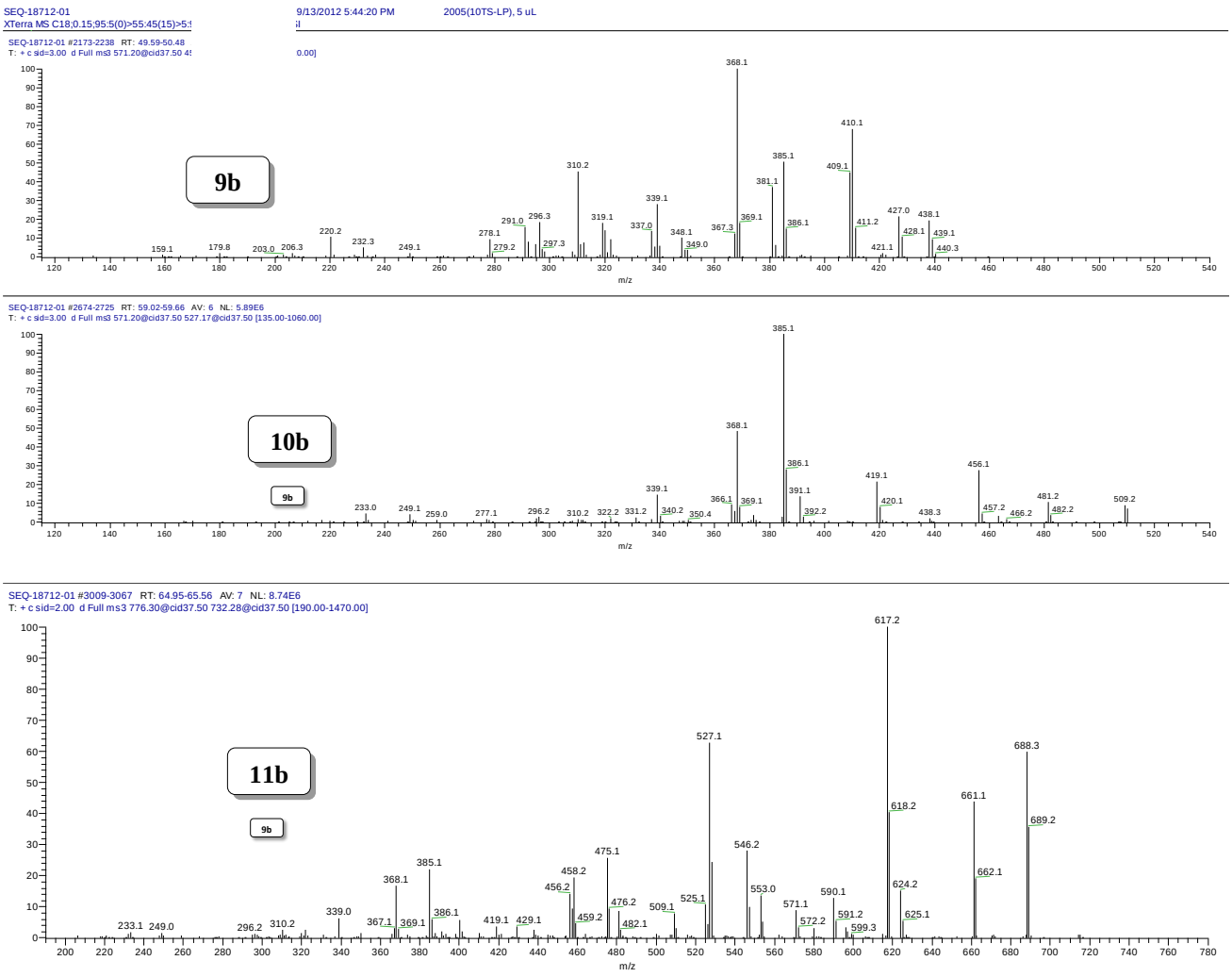
Isotripeptide (**9a-c**) (0.20 mmol) was dissolved in the mixture of DMF-piperidine (5mL/1.5mL). The mixture was irradiated with microwave (50 °C, 50 W, 3 h) in a microwave tube. The reaction was allowed to cool to room temperaturet and acidified with 2N HCl to pH = 1. The mixture was extracted with ethyl acetate (3×20 mL), the combined organic extracts were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The ligation mixture was weighed and then a solution in methanol (1 mg mL^{-1}) was analysed by HPLC-MS.

HPLC chromatograms and ESI-MS for chemical ligation of 9b,c in buffer

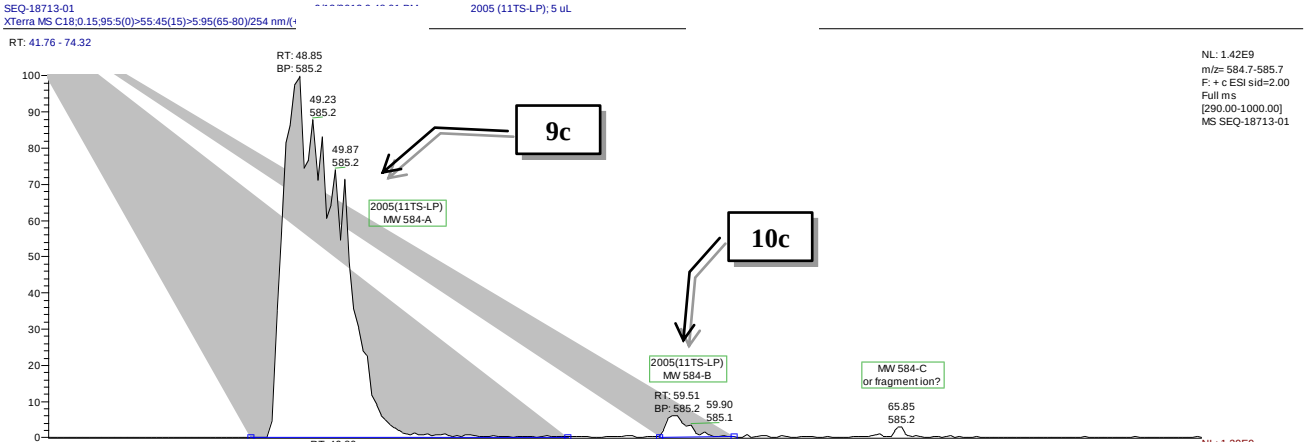
Summary Chromatogram: HPLC/(+)ESI-MS integrated ion-peaks for the **9b** (top, rt 48.9), **10b** (top, rt 59.2), and **11b** (bottom, rt 65.5).



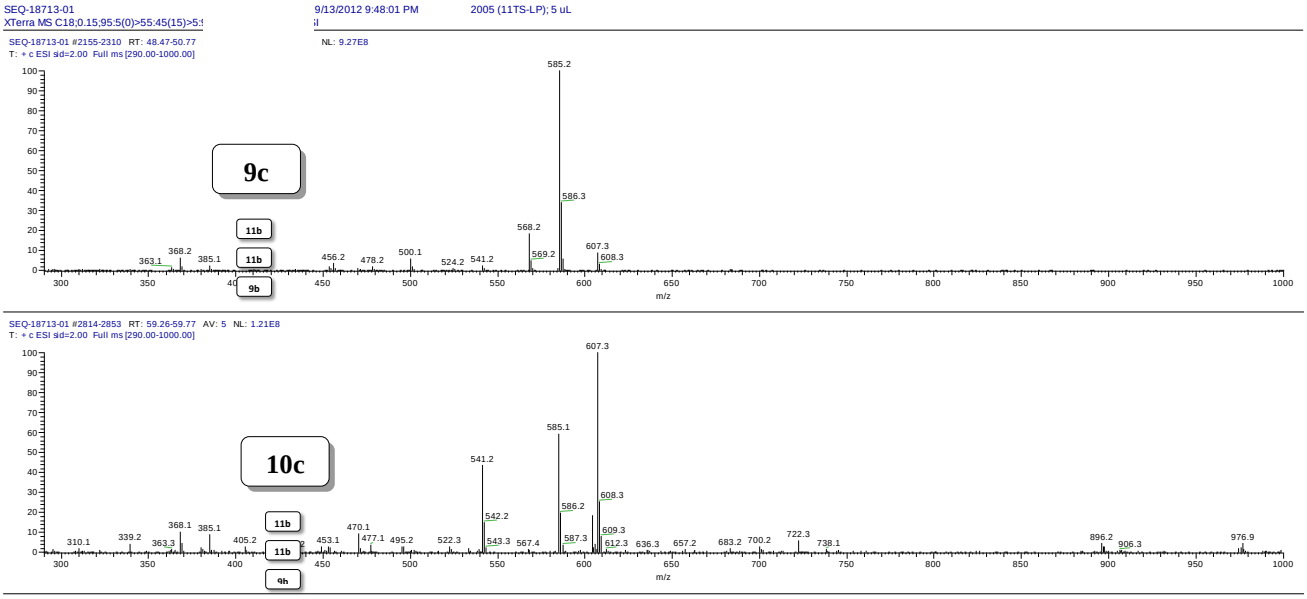
(+)ESI–MS/MS/MS of the m/z 571 [M+H]+ ions **9b** (top), **10b** (middle) and of m/z 776 [M+H]+ ion **11b** (bottom).



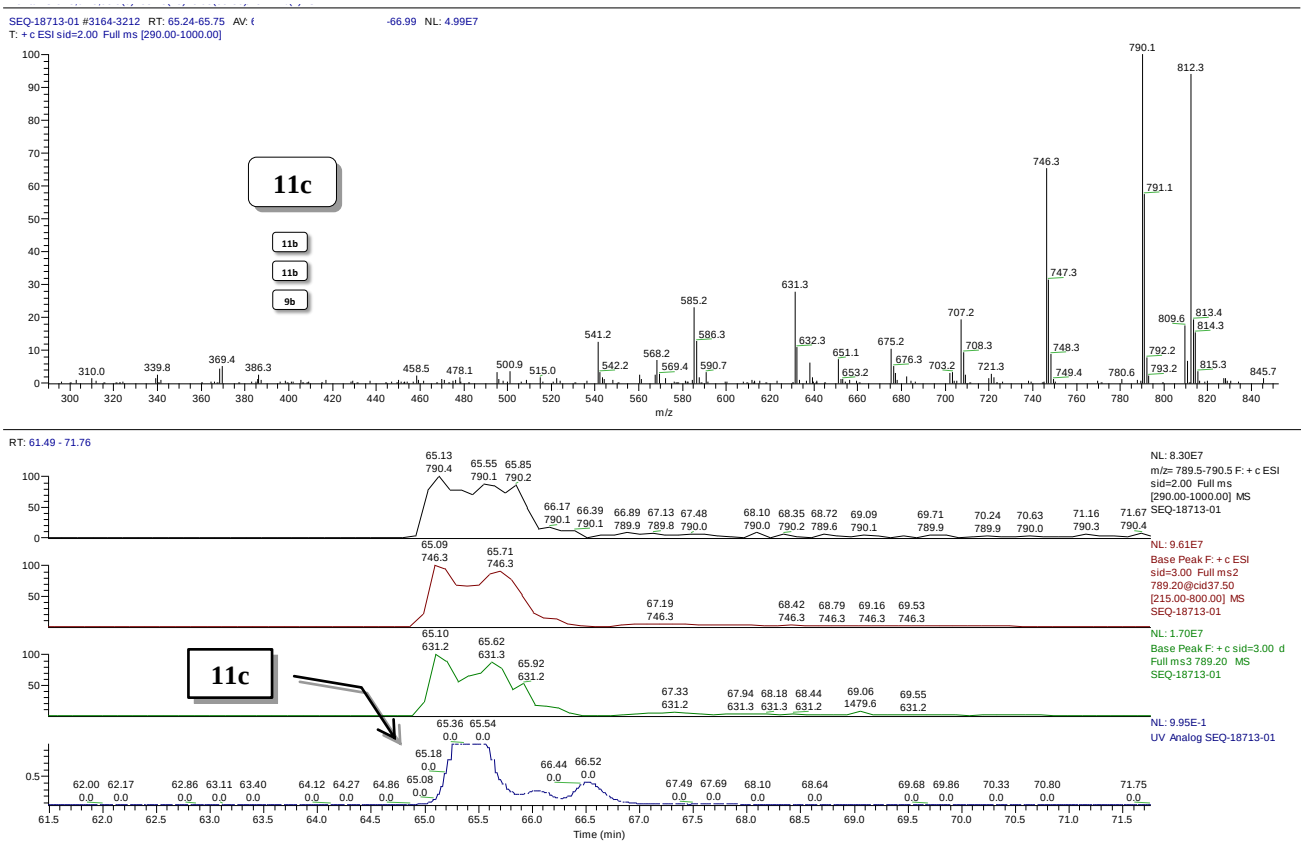
Summary Chromatogram: HPLC/(+)ESI-MS integrated ion-peaks for the **9c** (rt 48.9), **10c** (rt 59.5).



(+)ESI-MS of **9c** (top) and **10c** (bottom).

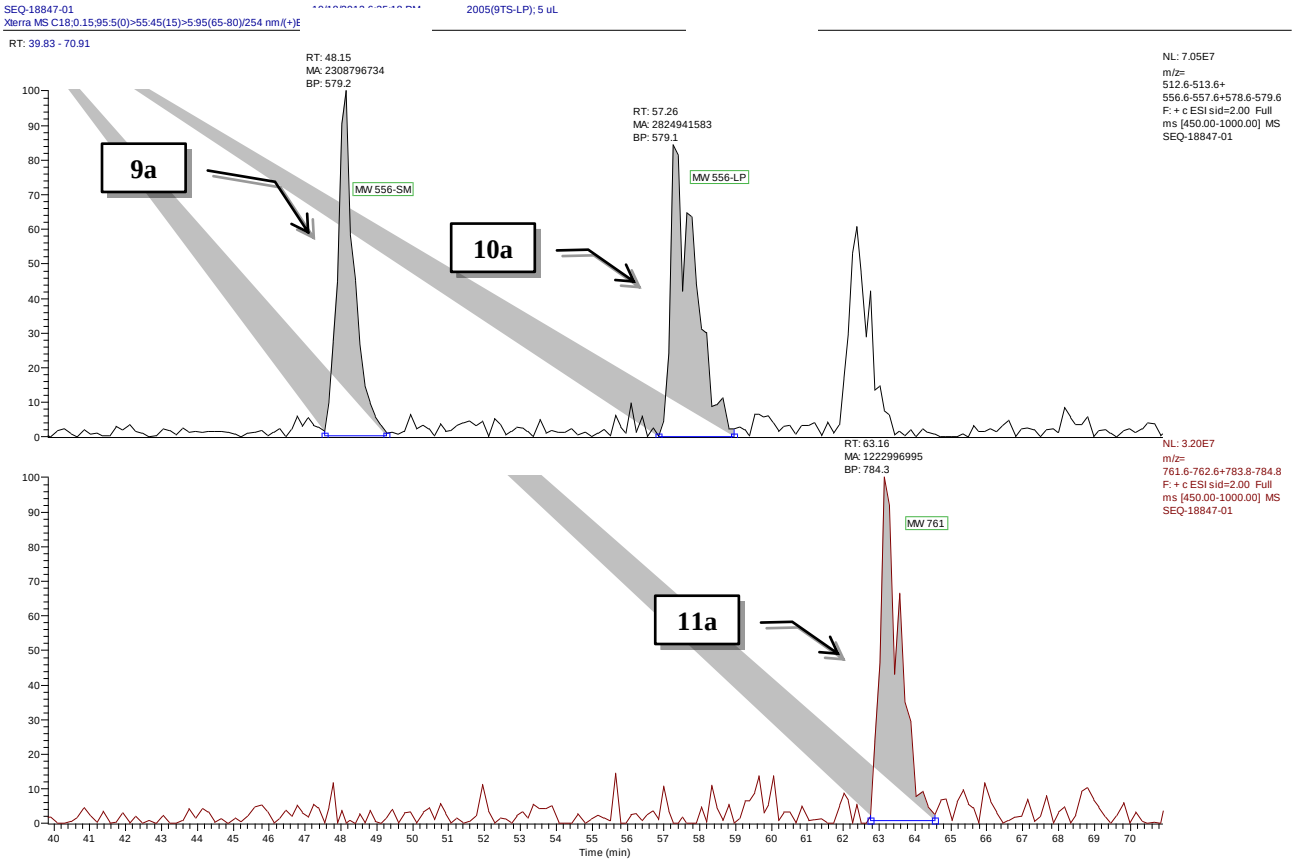


11c (MW 789) eluted at RT 65 min (bottom) and produced the (+)ESI-MS (top).

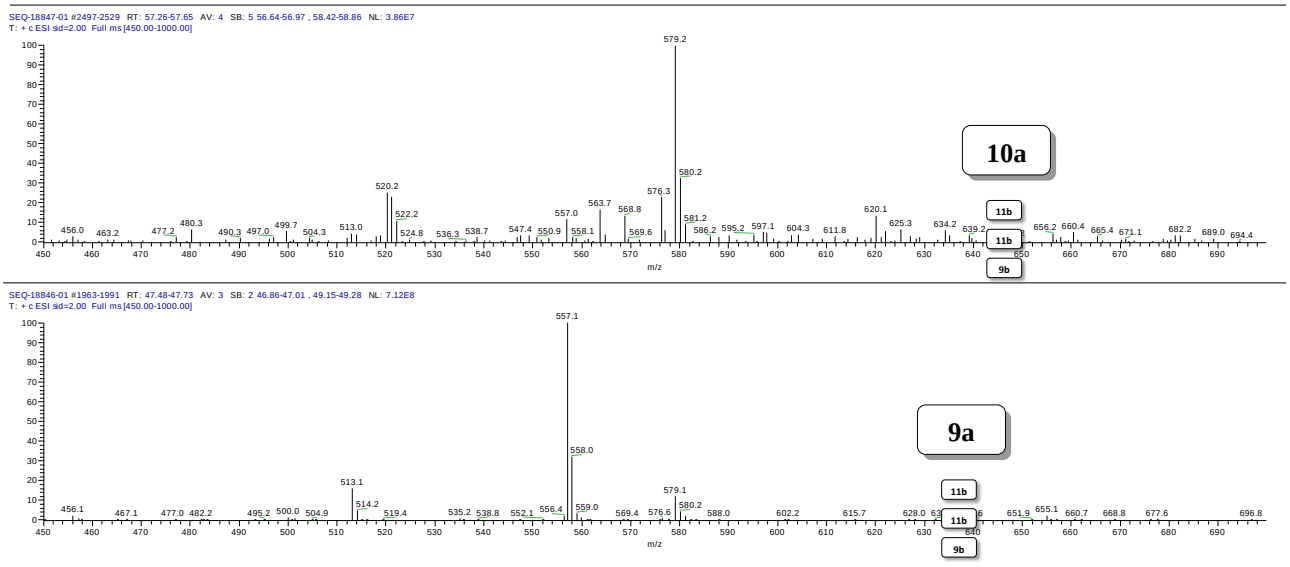


HPLC chromatograms and ESI-MS for chemical ligation of 9a-c in DMF-piperidine

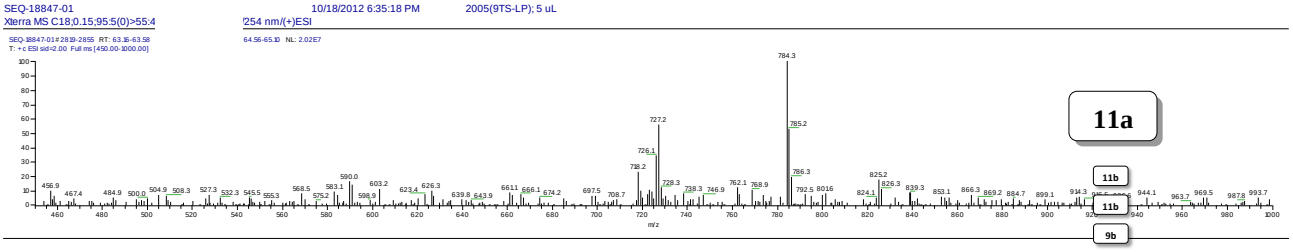
Summary Chromatogram: HPLC/(+)ESI-MS integrated ion-peaks for the **9a** (top, rt 48.2), **10a** (top, rt 57.3), and **11a** (bottom, rt 63.16).



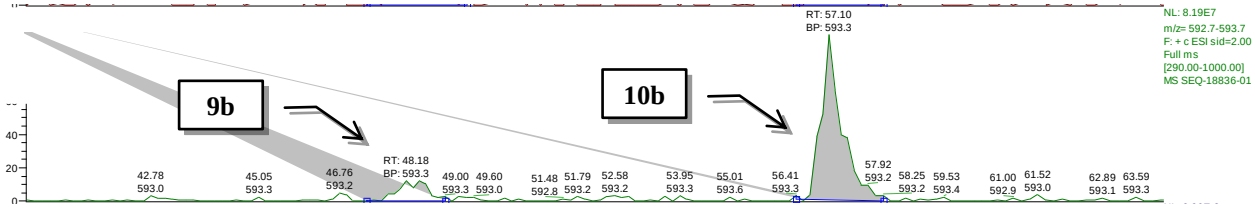
(+)ESI-MS spectra of **10a** (top) and **9a** (bottom).



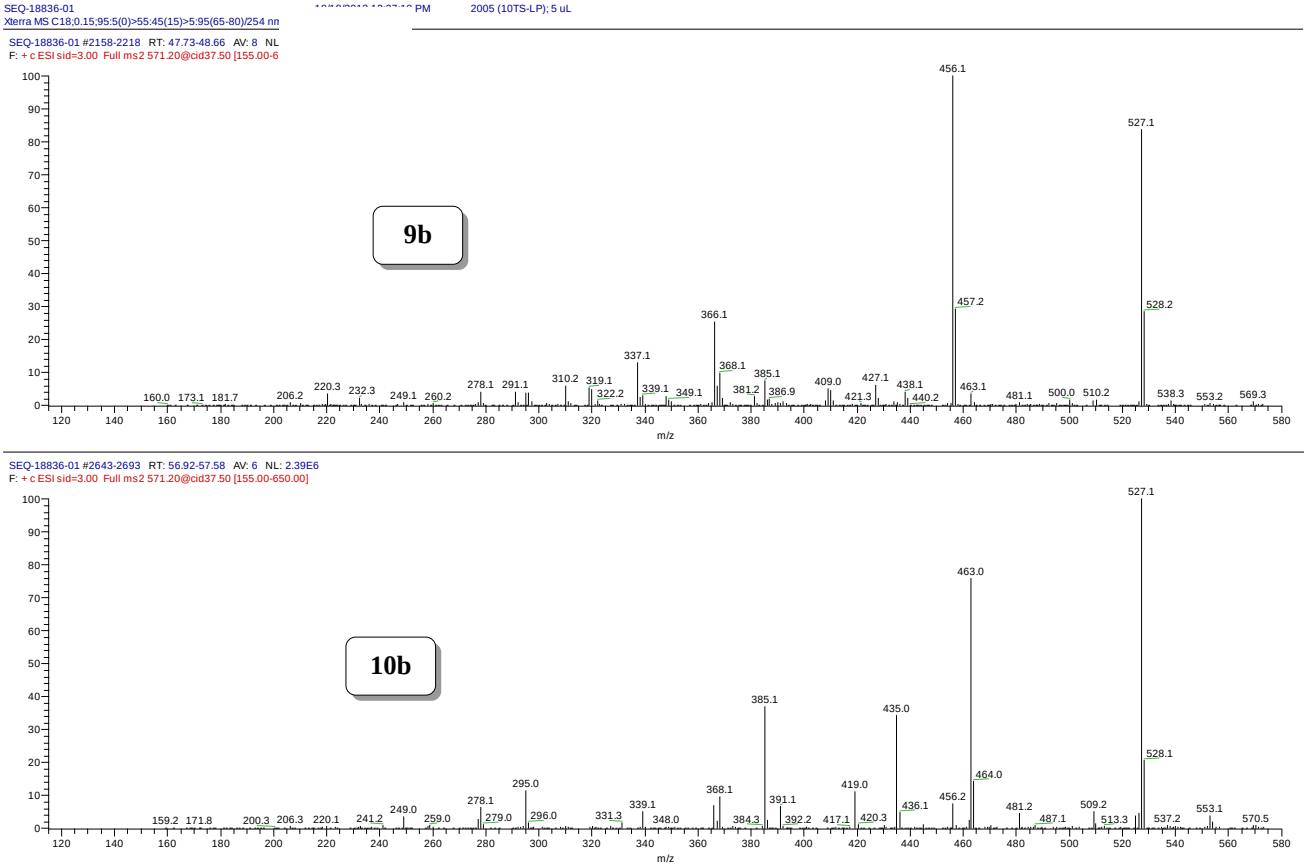
(+)ESI-MS of **11a**.



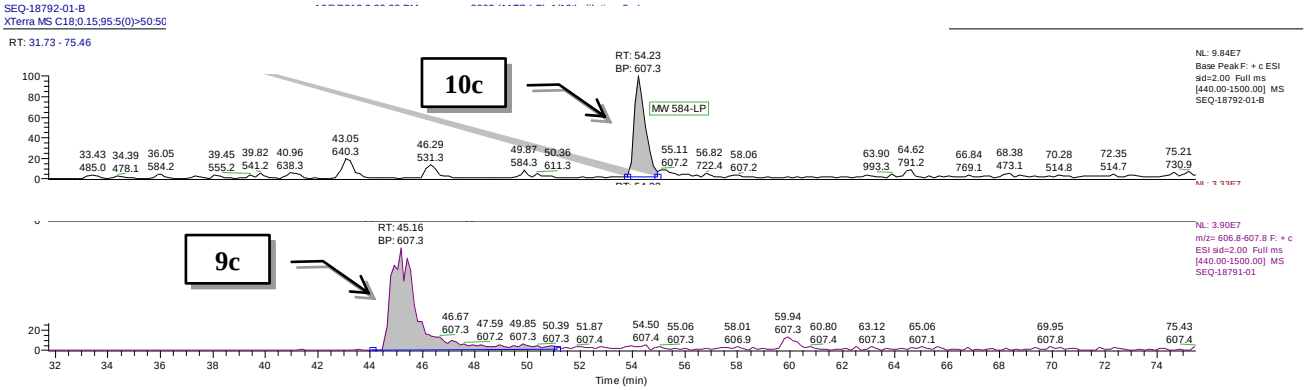
Summary Chromatogram: HPLC/(+)ESI-MS integrated ion-peaks for the **9b** (rt 48.2), **10b** (rt 57.1).



(+)ESI-MS/MS of the m/z 571 [M+H]+ ions of **9b** (top) and **10b** (bottom).



HPLC/(+)ESI-MS of **10c** (top, rt 54.24) and **9c** (bottom, rt 45.16).



(+)ESI mass spectra of **11c** (top) and **9c** (bottom).