Supporting Information

Native Chemical Ligation at Phenylalanine.

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General. Unless otherwise stated ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ solution. All solvents were dried and distilled by standard protocols. All reactions were conducted under a blanket of dry nitrogen. All organic extracts were dried over sodium sulfate and concentrated under aspirator vaccum. Chromatographic purifications were carried out over silica gel. Reverse phase HPLC was performed on a Varian Prep Star 218 HPLC system with 214-nm UV detection, using a Microsorb C-18 preparative column (250 × 21.4) at a flow rate of 10 mL/min. All runs used linear gradients of 0-100% buffer B in A (A: water containing 0.1% TFA, B: CH_3CN containing 0.1% TFA) over 60 min. Mass spectra were recorded by the Research Resources Center at the University of Illinois at Chicago. All yields refer to isolated, chromatogrphically homogeneous materials.

***N-tert*-Butoxycarbonyl-(2*S*,3*S*)-β-mercapto-L-phenylalanine methyl ester (5).**

To a solution of **4**[1] (1.03 g, 3.49 mmol) and Et_3N (730 μL, 5.24 mmol) at 0 ^{o}C in dichloromethane (15 mL) was added MsCl (326 μL, 4.19 mmol). The reaction mixture was stirred at 0 ^{o}C for 1h and then quenched with a saturated solution of NH_4Cl. The organic layer was washed with water and brine, dried, and concentrated. The concentrate was dissolved in DMF (10 mL) and treated with a preformed solution of the DBU salt of thioacetic acid [formed by the addition of thioacetic acid (1.3 mL, 17.45 mmol) to DBU (1.9 mL, 12.22 mmol) in DMF (5 mL)]. The reaction mixture was stirred at rt for 30h and concentrated. The concentrate was dissolved in ethyl acetate and washed with a saturated solution of NH_4Cl, water and brine, dried, and concentrated. The concentrate was chromatographically purified by eluting with 12% ethyl acetate in hexane to provide the acetylated thiol[2] along with various unidentified non-polar impurities (dark red color). ^{1}H

NMR (400 MHz) δ: 7.32-7.24 (m, 5H), 5.14-5.13 (d, *J* = 3.6 Hz, 1H), 5.07-5.05 (d, *J* = 8.8 Hz, 1H), 4.92-4.88 (dd, *J* = 4.8, 4.8 Hz, 1H), 3.68 (s, 3H), 2.32 (s, 3H), 1.42 (s, 9H); ^{13}C NMR (100 MHz) δ:193.6, 170.3, 155.2, 136.6, 129.7, 129.2, 128.7, 128.5, 128.3, 128.2, 80.3, 57.3, 52.4, 49.9, 29.7, 28.2.

The acetylated thiol was dissolved in methanol (20 mL) and treated with 1N NaOH solution (5 mL) for 30 min. The reaction mixture was carefully neutralized by the addition of 1N HCl (~ 6 mL) at 0 ^{o}C and concentrated. The concentrate was dissolved in ethyl acetate and washed with water and brine, dried, and concentrated. Chromatographic purification (10% ethyl acetate in hexane) provided **5** (0.65 g, 60% over three steps). ^{1}H NMR (400 MHz) δ: 7.36-7.25 (m, 5H), 5.08-5.06 (d, *J* = 8.8 Hz, 1H), 4.81-4.77 (t, *J* = 7.6 Hz, 1H), 4.48-4.45 (t, *J* = 6.4 Hz, 1H), 3.68 (s, 3H), 2.11-2.19 (d, *J* = 6.8 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz) δ: 170.7, 155.2, 138.8, 128.6, 128.1, 127.8, 80.3, 59.9, 52.3, 45.6, 28.2; ESI-HRMS Calcd for $C_{15}H_{21}NO_4SNa$ $[M + Na]^+$: 334.1084. Found 334.1080.

(2*S*,3*S*)-β-(2-ethyldisulfanyl)-L-phenylalanine methyl ester (7).

To a solution of ethyl disulfide (620 μL, 5.0 mmol) in CH_2Cl_2 (5 mL) *m*-chloroperbenzoic acid (1.12 g, 5.0 mmol) was added portionwise over a period of 30 min. at 0 ^{o}C. Then the reaction mixture was stirred at 0 ^{o}C for 4h and filtered through a silica pad and the filtrate was washed with a saturated solution of $NaHCO_3$ and brine. The organic layer was dried and concentrated. The concentrate was dissolved in CH_2Cl_2 (7 mL) and Et_3N (200 μL, 1.43 mmol) was added to the solution. A solution of **5** (0.44 g, 1.43 mmol) in CH_2Cl_2 (7 mL) was dropwise added to the above solution and stirred at rt for 30 min. The reaction mixture was concentrated to 5 mL and treated with TFA (3 mL)

for 30 min. Then the reaction mixture was concentrated and chromatographic purification (30% ethyl acetate in hexane) afforded **7** (0.29 g, 76%). ^{1}H NMR (400 MHz) δ: 7.31 (s, 5H), 4.29-4.27 (d, *J* = 6.8 Hz, 1H), 4.17-4.15 (d, *J* = 5.6 Hz, 1H), 3.70 (s, 3H), 2.50-2.45 (q, *J* = 8.0 Hz, 2H), 1.23-1.19 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz) δ: 173.4, 136.7, 128.7, 128.6, 128.3, 58.8, 57.5, 52.2, 32.4, 14.3; ESI-HRMS Calcd for $C_{12}H_{18}NO_2S_2$ [M + H]$^+$: 272.0774. Found 272.0770.

***N*-*tert*-Butoxycarbonyl-(2*S*,3*S*)-β-(2-ethyldisulfanyl)-L-phenylalanine (8).**

To a solution of ethyl disulfide (1.23 mL, 9.9 mmol) in CH_2Cl_2 (10 mL) *m*-chloroperbenzoic acid (2.22 g, 9.9 mmol) was added portionwise over a period of 30 min. at 0 ^{o}C. Then the reaction mixture was stirred at 0 ^{o}C for 4h and filtered through a silica pad and the filtrate was washed with a saturated solution of $NaHCO_3$ and brine. The organic layer was dried and concentrated. The concentrate was dissolved in CH_2Cl_2 (14 mL) and Et_3N (394 μL, 2.83 mmol) was added to the solution. A solution of **5** (0.88 g, 2.83 mmol) in CH_2Cl_2 (14 mL) was dropwise added to the above solution and stirred at rt for 30 min. before it was concentrated and subjected to chromatographic purification (6% ethyl acetate in hexane) to give the mixed disulfide (**6**) (0.84 g, 80%).^{1}H NMR (300 MHz) δ: 7.35-7.26 (m, 5H), 5.04-5.01 (m, 1H), 4.93-4.90 (d, *J* = 9.0 Hz, 1H), 4.38-4.36 (d, *J* = 6.0 Hz, 1H), 3.71 (s, 3H), 2.52-2.44 (m, 2H), 1.40 (s, 9H), 1.26-1.18 (t, *J* = 10.5 Hz, 3H).

A solution of disulfide (**6**) (0.84 g, 2.26 mmol) in THF (20 mL), was treated with a solution of lithium hydroxide (0.19 g, 4.52 mmol) in H_2O (2 mL) and stirred at rt for 12h. Then the reaction mixture was acidified with 1N HCl at 0 ^{o}C and the organic layer was extracted with ethyl acetate, washed with water and brine, dried, and concentrated.

Chromatographic purification (1% MeOH in $CHCl_3$) afforded **8** (0.40 g, 50%, 40% over two steps). ^{1}H NMR (400 MHz) δ: 10.37 (brs, 1H), 7.32-7.26 (m, 5H), 5.11-5.07 (m, 1H), 4.92-4.90 (d, *J* = 9.2 Hz, 1H), 4.47-4.46 (d, *J* = 4.8 Hz, 1H), 2.55-2.49 (m, 2H), 1.42 (s, 9H), 1.25-1.21 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz) δ: 175.6, 155.5, 136.0, 128.8, 128.6, 128.5, 80.6, 56.5, 55.9, 32.4, 28.2, 14.4; ESI-HRMS Calcd for $C_{16}H_{23}NO_4S_2Na$ [M + Na]$^+$: 380.0961. Found 380.0957.

Cbz-L-Gly-SEt (9).[3]

To a solution of Cbz-L-Gly-OH (1.00 g, 4.78 mmol), DMAP (0.060 g, 0.48 mmol), and ethanethiol (2.2 mL, 14.34 mmol) in DMF (10 mL) at 0 ^{o}C was added EDCI (1.1 mL, 5.98 mmol). Then the reaction mixture was warmed up to rt and stirred for 36h before it was concentrated. The concentrate was dissolved in ethyl acetate and washed with a solution of saturated NH_4Cl, water, and brine. The organic layer was separated, dried, and concentrated. Chromatographic purification (20% ethyl acetate in hexane) afforded **9** (0.90 g, 74%). ^{1}H NMR (400 MHz) δ: 7.36-7.26 (m, 5H), 5.45 (brs, 1H), 5.14 (s, 2H), 4.10 (d, *J* = 6.0 Hz, 2H), 2.94-2.88 (q, *J* = 8.0 Hz, 2H), 1.27-1.23 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR (100 MHz) δ: 197.7, 156.2, 136.1, 128.6, 128.3, 128.2, 67.3, 50.7, 23.1, 14.6; ESI-HRMS Calcd for $C_{12}H_{15}NO_3SNa$ [M + Na]$^+$: 276.0671. Found 276.0668.

Boc-L-Met-SEt (10).

To a solution of Boc-L-Met-OH (0.390 g, 1.47 mmol), DMAP (0.018 g, 0.15 mmol), and ethanethiol (337 μL, 4.41 mmol) in DMF (3 mL) at 0 ^{o}C was added EDCI (326 μL, 1.84 mmol). Then the reaction mixture was warmed up to rt and stirred for 36h before it was concentrated. The concentrate was dissolved in ethyl acetate and washed with a solution of saturated NH_4Cl, water, and brine. The organic layer was separated, dried, and

concentrated. Chromatographic purification (15% ethyl acetate in hexane) afforded **10** (0.280 g, 58%). ^{1}H NMR (500 MHz) δ: 5.23-5.21 (d, *J* = 9.0 Hz, 1H), 4.44-4.42 (m, 1H), 2.87-2.82 (q, *J* = 7.0 Hz, 2H), 2.54-2.49 (m, 2H), 2.13-2.10 (m, 1H), 2.07 (s, 3H), 1.87-1.83 (m, 1H), 1.42 (s, 9H), 1.22-1.19 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz) δ: 201.0, 155.1, 80.3, 59.8, 32.2, 30.0, 28.3, 23.3, 15.4, 14.4; ESI-HRMS Calcd for $C_{12}H_{13}NO_3S_2Na$ $[M + Na]^+$: 316.1012. Found 316.1008.

General Procedure 1 . Dipeptide Synthesis.

To a solution of **7** (1 equiv.) and 2-mercaptoethanesulfonate sodium salt (20 equiv.) in degassed ligation solvent (2: 1, CH_3CN: 0.1M Tris-HCl, 6.0M guanidine, pH 8), was added a solution of the thioester (1.2 equiv.) in degassed CH_3CN (such that the overall ratio with the buffer became 3: 1 and overall concentration with the external thiol became ~1.0 M). The pH of the reaction mixture was adjusted to 7.5-8.0 with 1N NaOH solution and the resulting mixture was stirred for 16h. Then the reaction mixture was concentrated and the concentrate was diluted with ethyl acetate. The organic layer was washed with water and brine, dried, concentrated. Chromatographic purification afforded the desired ligated products.

Cbz-L-Gly-(β-SH)-L-Phe-OMe (11).

Following the general procedure 1 with the thioester **9**, and eluting with 40% ethyl acetate in hexane, **11** was prepared in 75% yield. ^{1}H NMR (500 MHz) δ: 7.38-7.23 (m, 10H), 6.76-6.75 (d, *J* = 9.0 Hz, 1H), 5.49 (brs, 1H), 5.14-5.11 (m, 3H), 4.51-4.48 (t, *J* = 7.0 Hz, 1H), 3.87-3.77 (m, 2H), 3.68 (s, 3H), 2.22-2.21 (d, *J* = 7.5 Hz, 1H); ^{13}C NMR (125 MHz) δ: 170.1, 169.0, 156.6, 138.4, 136.1, 128.8, 128.7, 128.66, 128.59, 128.53,

128.49, 128.4, 128.3, 128.2, 128.1, 127.6, 67.3, 58.2, 52.7, 52.5, 45.2, 44.5; ESI-HRMS Calcd for $C_{20}H_{22}N_2O_5SNa$ $[M + Na]^+$: 425.1147. Found 425.1162.

Boc-L-Met-(β-SH)-L-Phe-OMe (12).

Following the general procedure 1 with the thioester **10**, and eluting with 25% ethyl acetate in hexane, **12** was prepared in 50% yield. ^{1}H NMR (500 MHz) δ: 7.33-7.26 (m, 5H), 6.78-6.77 (d, *J* = 8.5 Hz, 1H), 5.18-5.17 (d, *J* = 7.0 Hz, 1H), 5.12-5.09 (dd, *J* = 6.0, 6.0 Hz, 1H), 4.55-4.53 (t, *J* = 6.0 Hz, 1H), 4.27-4.25 (d, *J* = 6.5 Hz, 1H), 3.68 (s, 3H), 2.48-2.45 (m, 2H), 2.28-2.27 (d, *J* = 7.0 Hz, 1H), 2.08-1.81 (m, 5H), 1.44 (s, 9H); ^{13}C NMR (125 MHz) δ: 171.4, 169.9, 155.5, 138.4, 128.9, 128.7, 128.7, 128.6, 128.5, 128.3, 128.2, 127.7, 127.6, 80.3, 58.3, 53.5, 52.5, 45.1, 45.0, 30.9, 29.9, 28.3, 15.1; ESI-HRMS Calcd for $C_{20}H_{30}N_2O_5S_2Na$ $[M + Na]^+$: 465.1489. Found 465.1474.

General Procedure 2. Desulfurization of the ligated dipeptides.

A solution of the ligated peptide and $NiCl_2$,$6H_2O$ (3 equiv.) in methanol (0.03 M) at 0 ^{o}C, was treated with $NaBH_4$ (9 equiv.) portionwise and stirred at the same temperature for 15 min. before it was filtered through a silica pad and washed with MeOH. The filtrate was concentrated and chromatographic purification afforded the desired dipeptides.

Cbz-L-Gly-L-Phe-OMe (13).[4]

Following the general procedure 2 with **11**, and eluting with 40% ethyl acetate in hexane, **13** was prepared in 80% yield. ^{1}H NMR (400 MHz) δ: 7.34-7.19 (m, 8H), 7.09-7.07 (d, *J* = 6.8 Hz, 2H), 6.72-6.70 (d, *J* = 5.6 Hz, 1H), 5.61 (brs, 1H), 5.10 (s, 2H), 4.89-4.84 (q, *J* = 6.0 Hz, 1H), 3.85-3.77 (m, 2H), 3.69 (s, 3H), 3.12-3.03 (m, 2H); ^{13}C NMR (100 MHz) δ: 171.8, 168.8, 156.6, 136.2, 135.6, 129.2, 128.6, 128.5, 128.4, 1282, 128.1, 127.2, 67.2,

53.1, 52.4, 44.3, 37.8; ESI-HRMS Calcd for $C_{20}H_{22}N_2O_5Na$ $[M + Na]^+$: 393.1421. Found 393.1419.

Boc-L-Met-L-Phe-OMe (14).[5]

Following the general procedure 2 with **12**, and eluting with 25% ethyl acetate in hexane, **14** was prepared in 70% yield. ^{1}H NMR (400 MHz) δ: 7.29-7.20 (m, 3H), 7.11-7.09 (d, *J* = 6.8 Hz, 2H), 6.69-6.67 (d, *J* = 7.2 Hz, 1H), 5.24-5.22 (d, *J* = 8.4 Hz, 1H), 4.84-4.81 (q, *J* = 8.0 Hz, 1H), 4.26-4.25 (d, *J* = 6.8 Hz, 1H), 3.69 (s, 3H), 3.11-3.04 (m, 2H), 2.53-2.49 (t, *J* = 7.2 Hz, 2H), 2.03 (s, 3H), 2.01-1.86 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (100 MHz) δ: 171.6, 171.2, 155.3, 135.6, 129.2, 128.6, 127.2, 80.1, 53.2, 53.1, 52.4, 37.8, 31.6, 30.0, 28.3, 15.1; ESI-HRMS Calcd for $C_{20}H_{30}N_2O_5SNa$ $[M + Na]^+$: 433.1768. Found 433.1755.

Competitive Desulfurization of Boc-(β-SH)-L-Phe-OMe and Boc-S-Acm-L-Cys-OMe

A solution of Boc-(β-SH)-L-Phe-OMe (38 mg, 0.122 mmol), Boc-S-Acm-L-Cys-OMe (37 mg, 0.122 mmol) and $NiCl_2$,$6H_2O$ (58 mg, 0.244 mmol) in methanol (6 mL) at 0 ^oC, was treated with $NaBH_4$ (28 mg, 0.732 mmol) portion wise and stirred at the same temperature for 10 min. before it was filtered through a silica pad and washed with ethyl acetate. The filtrate was concentrated and chromatographic purification afforded Boc-L-Phe-OMe (26 mg, 76%) and recovered Boc-S-Acm-L-Cys-OMe (31 mg, 83%).

β-(EtSS)FRANK (15).

Pentapeptide **15** was prepared using **8** and other Fmoc-amino acids in the Protein Research Laboratories at UIC following Fmoc-SPPS on Wang resin. Subsequently, the peptide cleavage/deprotections were performed using the reagent K (82.5% TFA, 5% Phenol, 5% H_2O, 5% Thioanisole, and 2.5% Ethanedithiol). The crude peptide was

precipitated with cold Et_2O and centrifuged (4000 RPM) for 1h. The precipitate was dissolved in 50% aqueous CH_3CN, lyophilized and purified by reverse phase HPLC. Retention time 22.75 min.; ESI-HRMS Calcd for $C_{30}H_{51}N_{10}O_7S_2$ $[M + H]^+$: 727.3378. Found 727.3373.

General Procedure 3. Thioesterification of Pentapeptides 16 and 17.[6]

The protected C-terminal pentapeptide carboxylic acids (**16** and **17**) were prepared in the Protein Research Laboratories at UIC on chlorotrityl resin and used without further purification. To a solution of peptide (~0.03 mmol) in DMF (1 mL), was added 4Å molecular sieves (~ 0.03 g) and benzylthiol (30 equiv.) and the mixture was stirred at -20 ^oC. After 15 min., PyBOP (5 equiv.) and DIEA (5 equiv.) were added and the reaction mixture was stirred at -20 ^oC for 4h, before it was filtered, quenched with a satured solution of NH_4Cl, and diluted with CH_2Cl_2. The organic layer was washed with water and brine, dried and concentrated. Chromatographic purification (3% MeOH in CH_2Cl_2) provided the protected peptide-thioesters. The deprotection was performed using the reagent K (82.5% TFA, 5% Phenol, 5% H_2O, 5% Thioanisole, and 2.5% Ethanedithiol) (~ 2 mL) at rt for 2h. The crude peptide was precipitated with cold Et_2O and centrifuged (4000 RPM) for 1h. The precipitate was dissolved in 50% aqueous CH_3CN, lyophilized and purified by reverse phase HPLC to provide desired peptide thioesters.

General Procedure 4. Native Chemical Ligation of Pentapeptides.

The N-terminal pentapeptide **15** (~ 0.023 mmol, 1 equiv.), the C-terminal thioester (~ 0.025 mmol, 1.1 equiv.), 2-mercaptoethanesulfonate sodium salt (15 equiv.), and TCEP-HCl (15 equiv.) were dissolved in degassed ligation buffer (0.1M Tris-HCl, 6.0M guanidine, pH 8, ~1.5 mL). The pH of the reaction mixture was adjusted to 8.0 with 1N

NaOH solution and the resulting mixture was stirred for 16h. Purification by reverse phase HPLC provided the desired ligated peptides.

General Procedure 5. Desulfurization of Decapeptides 20 and 21.

A solution of the ligated peptide (~ 0.015 mmol) and $NiCl_2$,$6H_2O$ (5 equiv.) in degassed 0.1M phosphate buffer containing 6.0 M guanidine (pH 7, 2 mL) at 0 ^oC, was treated with $NaBH_4$ (15 equiv.) portionwise and stirred at the same temperature for 30 min. The reaction mixture was diluted with deionized water and centrifuged (4000 RPM) for 15 min. The supernatant was collected, lyophilized, and purified by reverse phase HPLC to provide the desired decapeptides.

LYRMG-SBn (18).

Following the general procedure 3, **18** was prepared from **16** in 78% yield. Retention time 30.89 min.; ESI-HRMS Calcd for $C_{35}H_{53}N_8O_6S_2$ $[M + H]^+$: 745.3524. Found 745.3525.

LYRAM-SBn (19).

Following the general procedure 3, **19** was prepared from **17** in 71% yield. Retention time 32.25 min.; ESI-HRMS Calcd for $C_{36}H_{55}N_8O_6S_2$ $[M + H]^+$: 759.3681. Found 759.3651.

LYRMG-(β-SH)FRANK (20).

Following the general procedure 4, **20** was prepared using C-terminal thioester **18** in 72% yield. Retention time 23.91 min.; ESI-HRMS Calcd for $C_{56}H_{91}N_{18}O_{13}S_2$ $[M + H]^+$: 1287.6449. Found 1287.6467.

LYRAM-(β-SH)FRANK (21).

Following the general procedure 4, **21** was prepared using C-terminal thioester **19** in 74% yield. Retention time 24.04 min.; ESI-HRMS Calcd for $C_{57}H_{93}N_{18}O_{13}S$ $[M + H]^+$: 1255.6728. Found 1255.6714.

LYRMGFRANK (22).

Following the general procedure 5, **22** was obtained from **20** in 60% yield. Retention time 22.79 min.; ESI-HRMS Calcd for $C_{56}H_{91}N_{18}O_{13}S_2$ $[M + H]^+$: 1301.6605. Found 1301.6589.

LYRAMFRANK (23).

Following the general procedure 5, **23** was obtained from **21** in 57% yield. Retention time 23.48 min.; ESI-HRMS Calcd for $C_{57}H_{93}N_{18}O_{13}S$ $[M + H]^+$: 1269.6885. Found 1269.6853.

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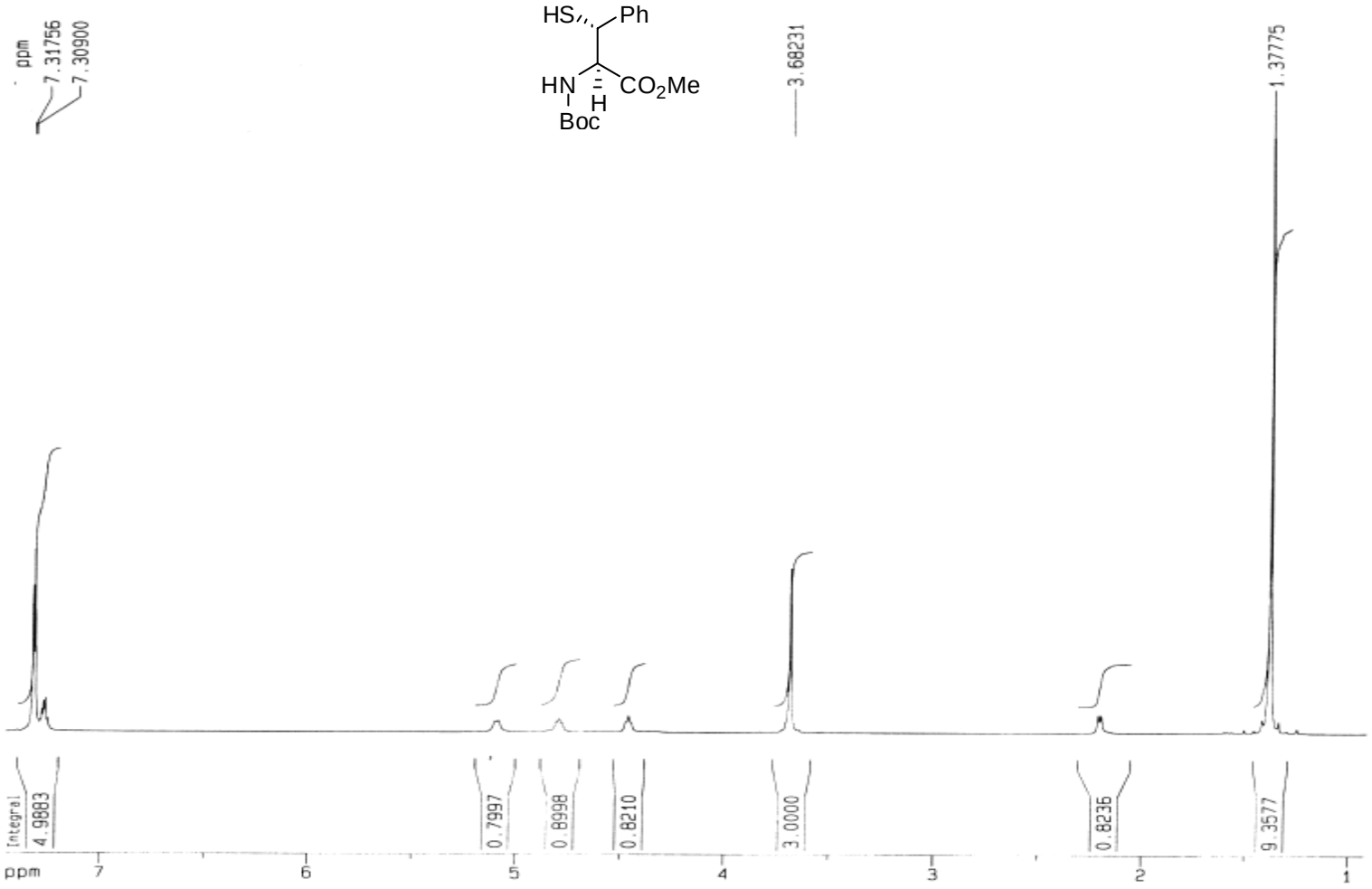
N-tert-Butoxycarbonyl-(2_S_,3_S_)-β-mercapto-L-phenylalanine methyl ester (5)



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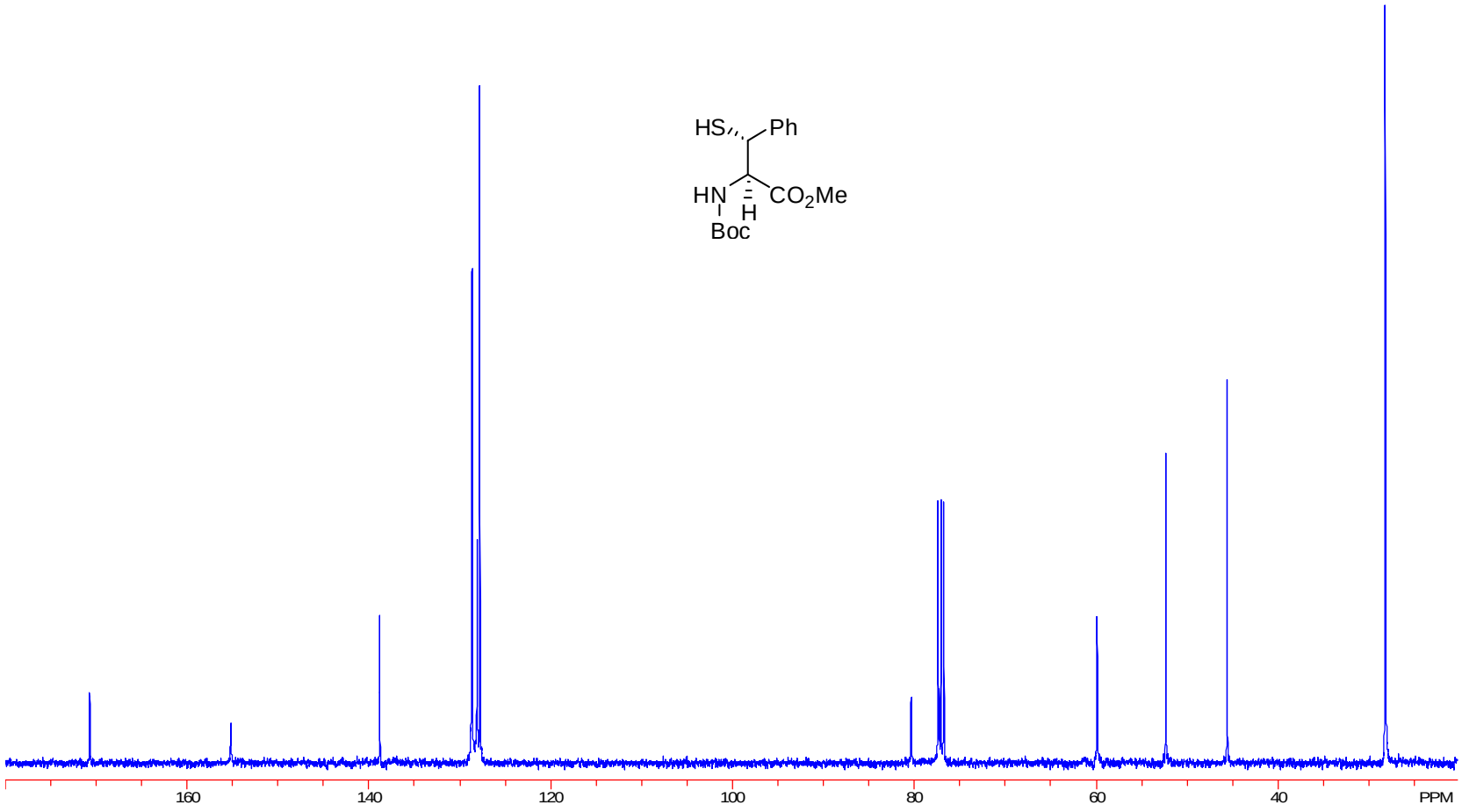

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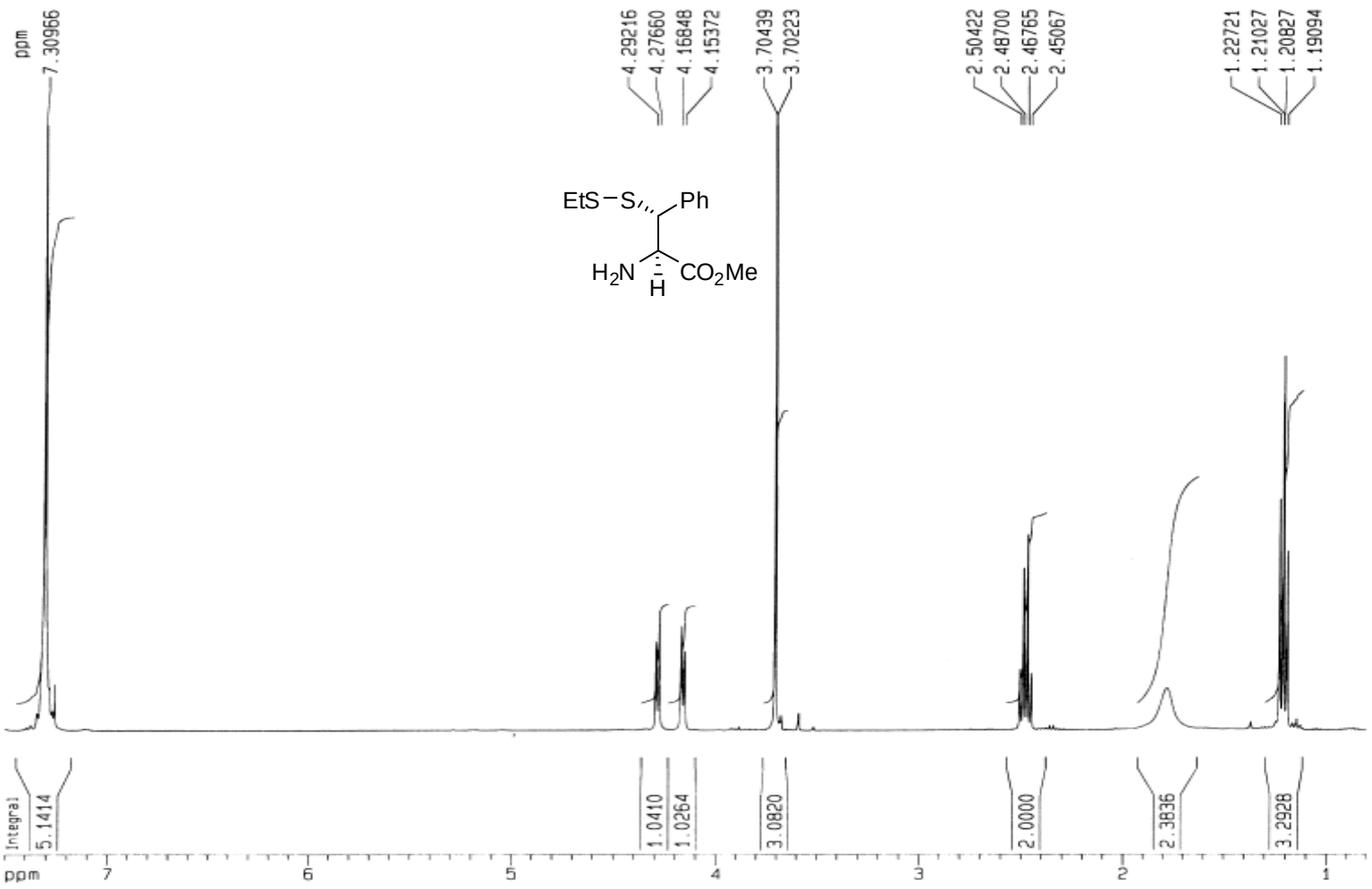

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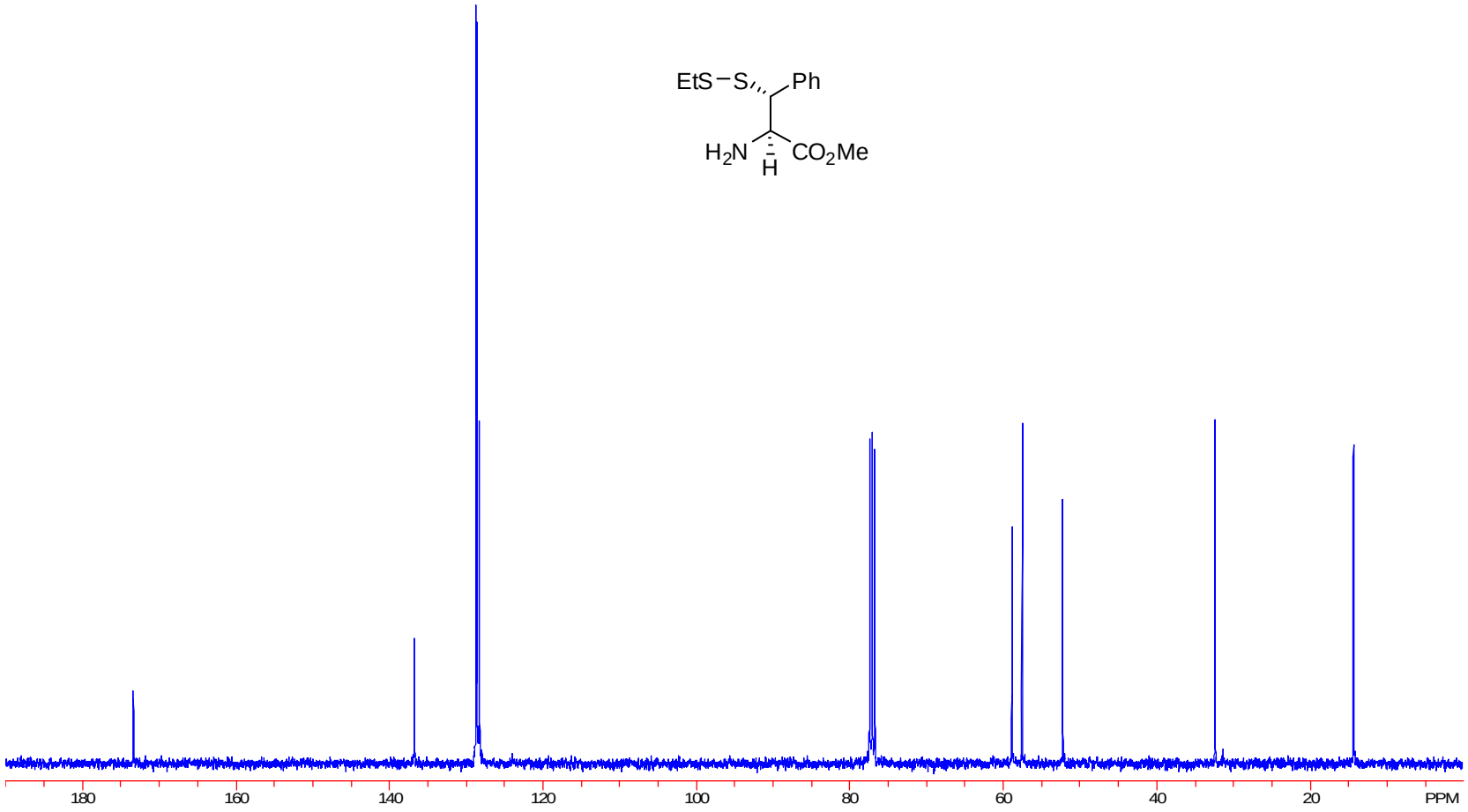
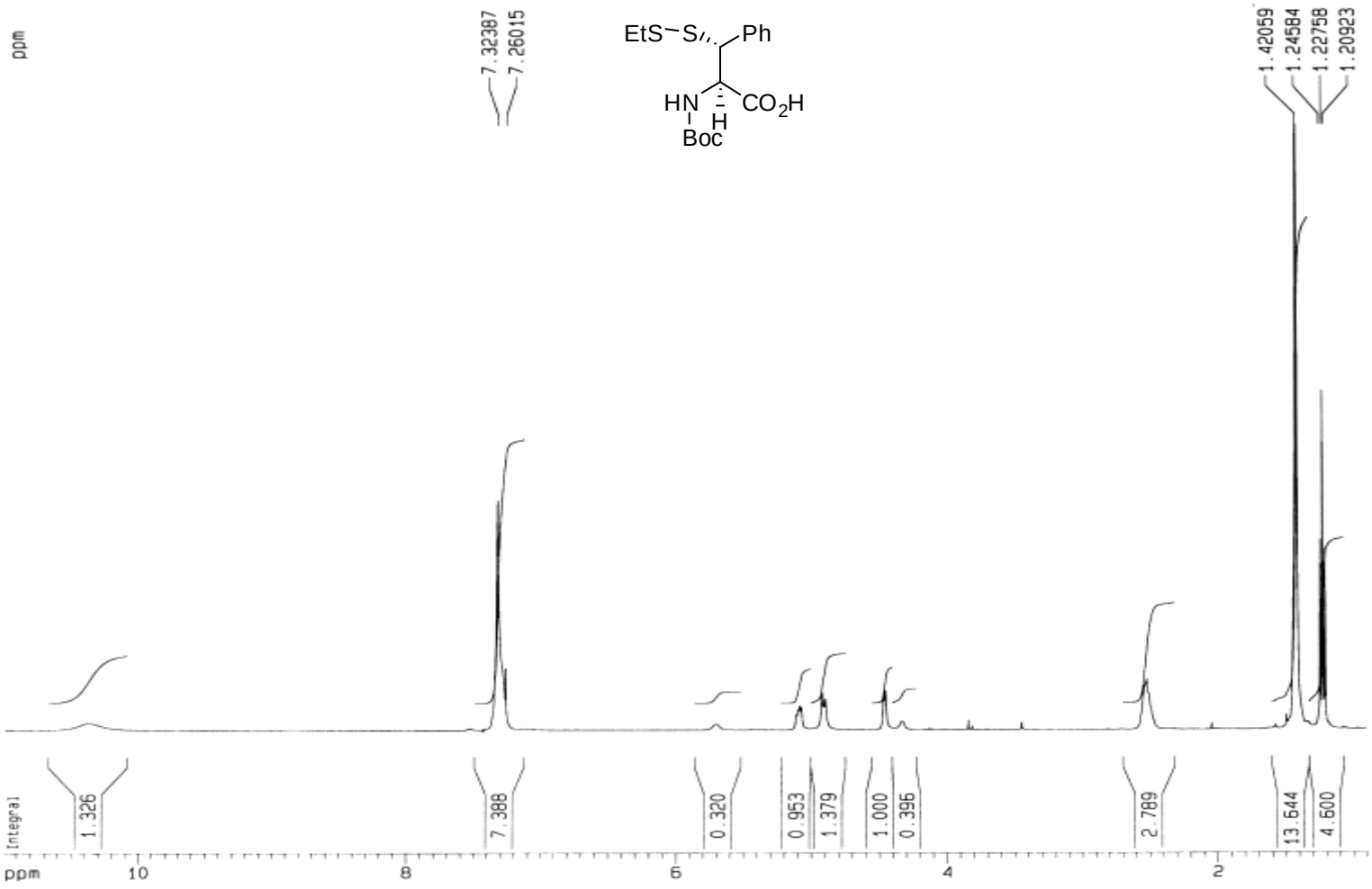


_N_-_tert_-Butoxycarbonyl-(2_S_,3_S_)-β-(2-ethyldisulfanyl)-L-phenylalanine (8)



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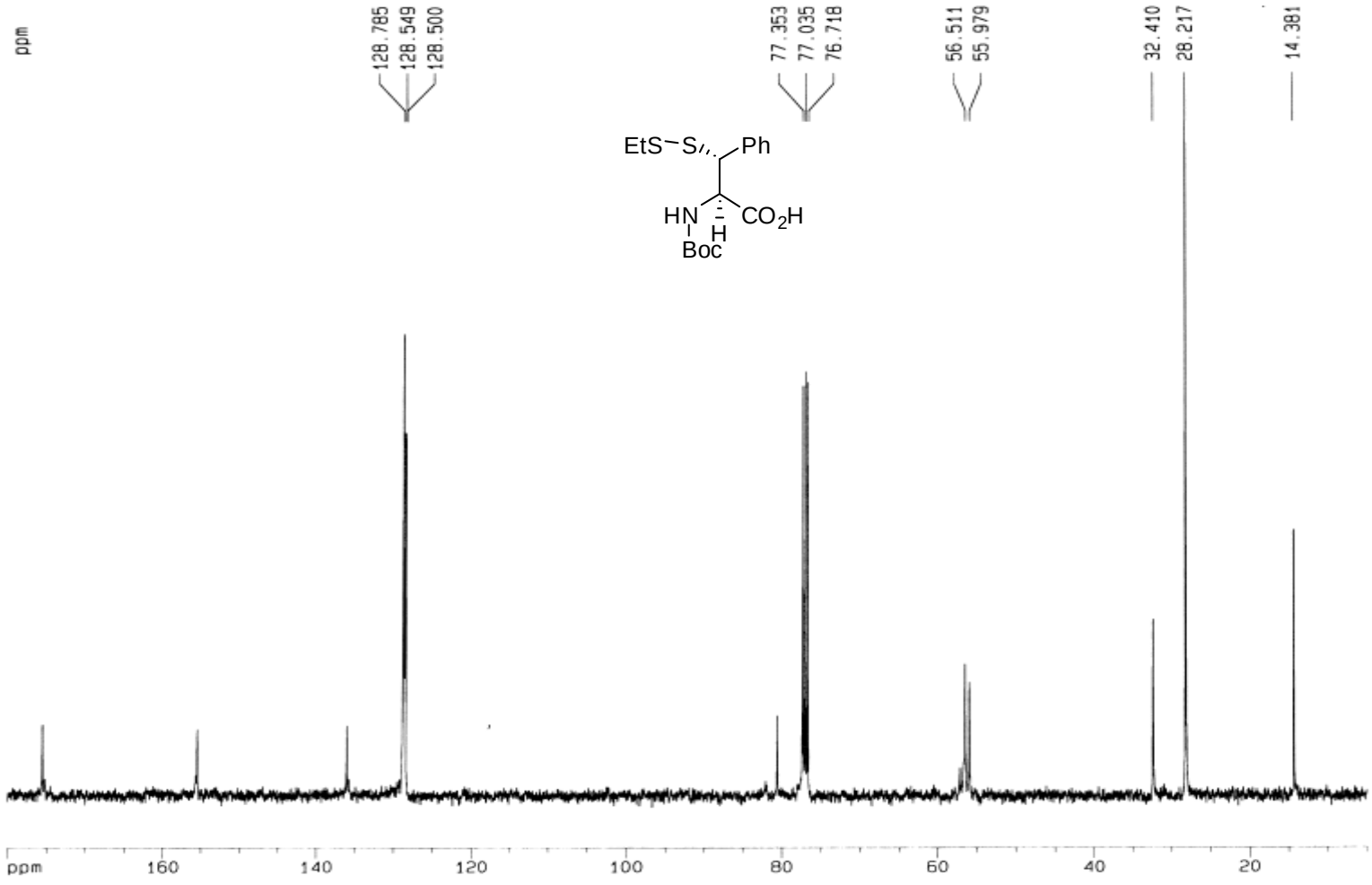

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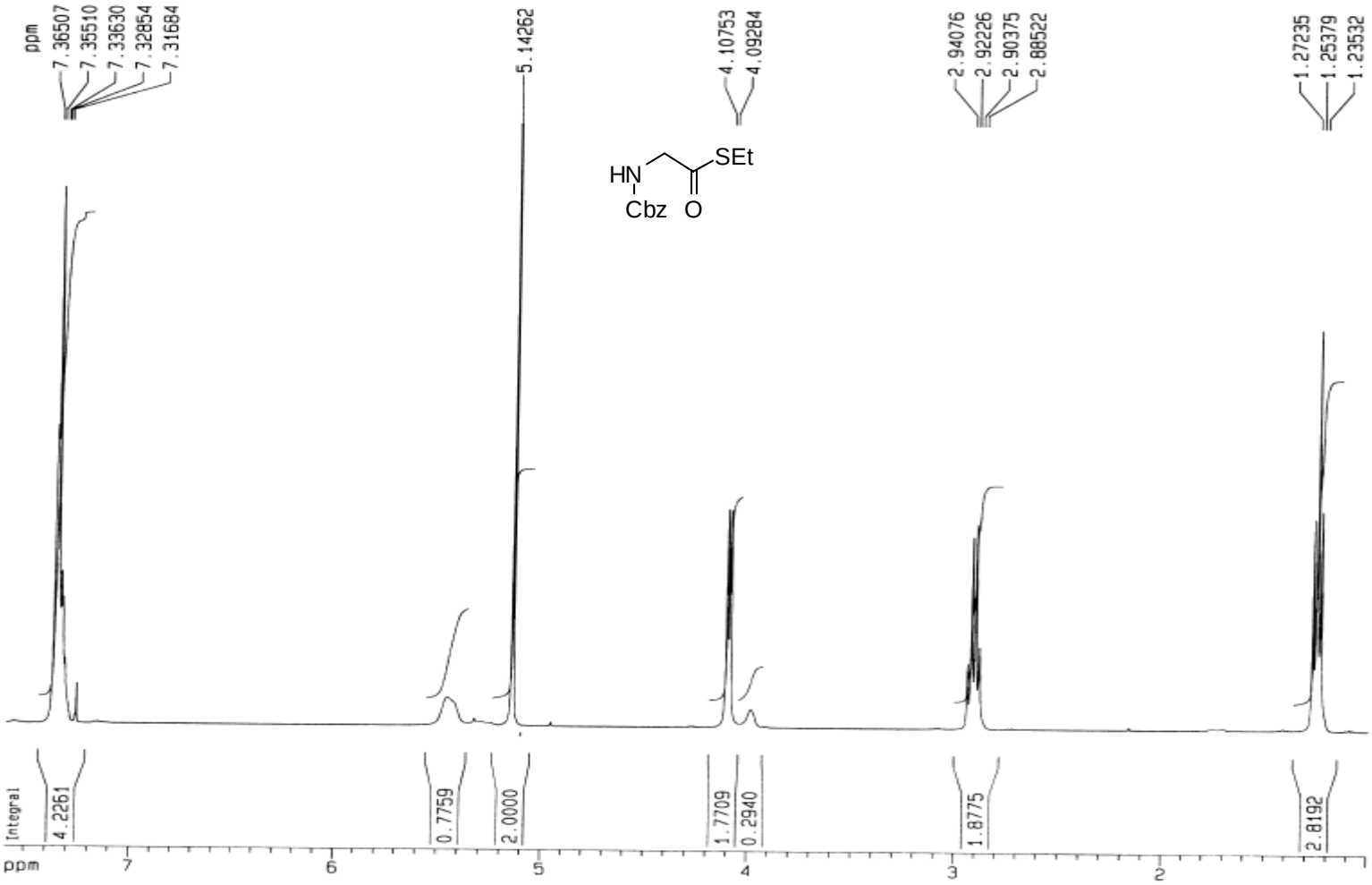

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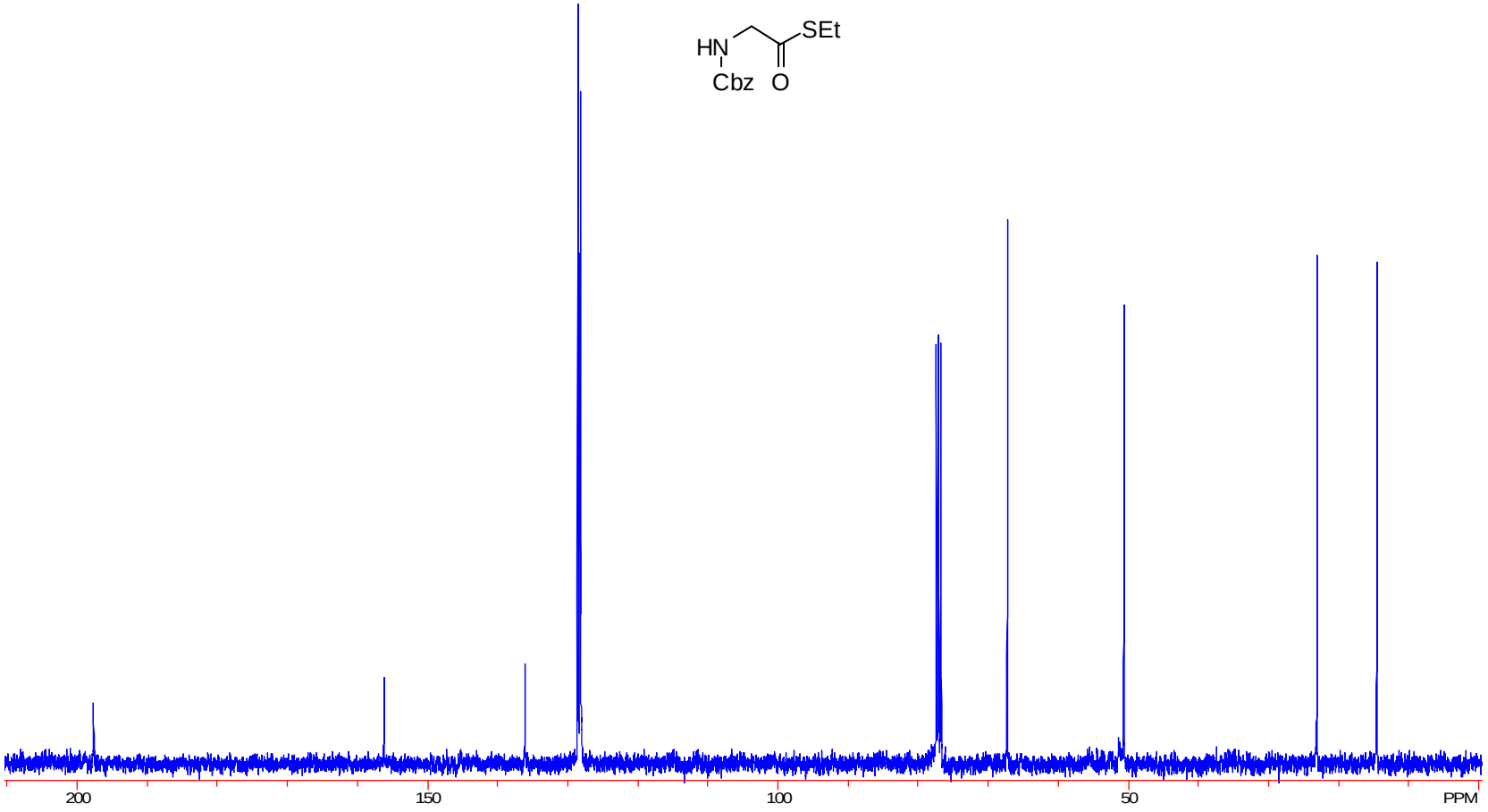
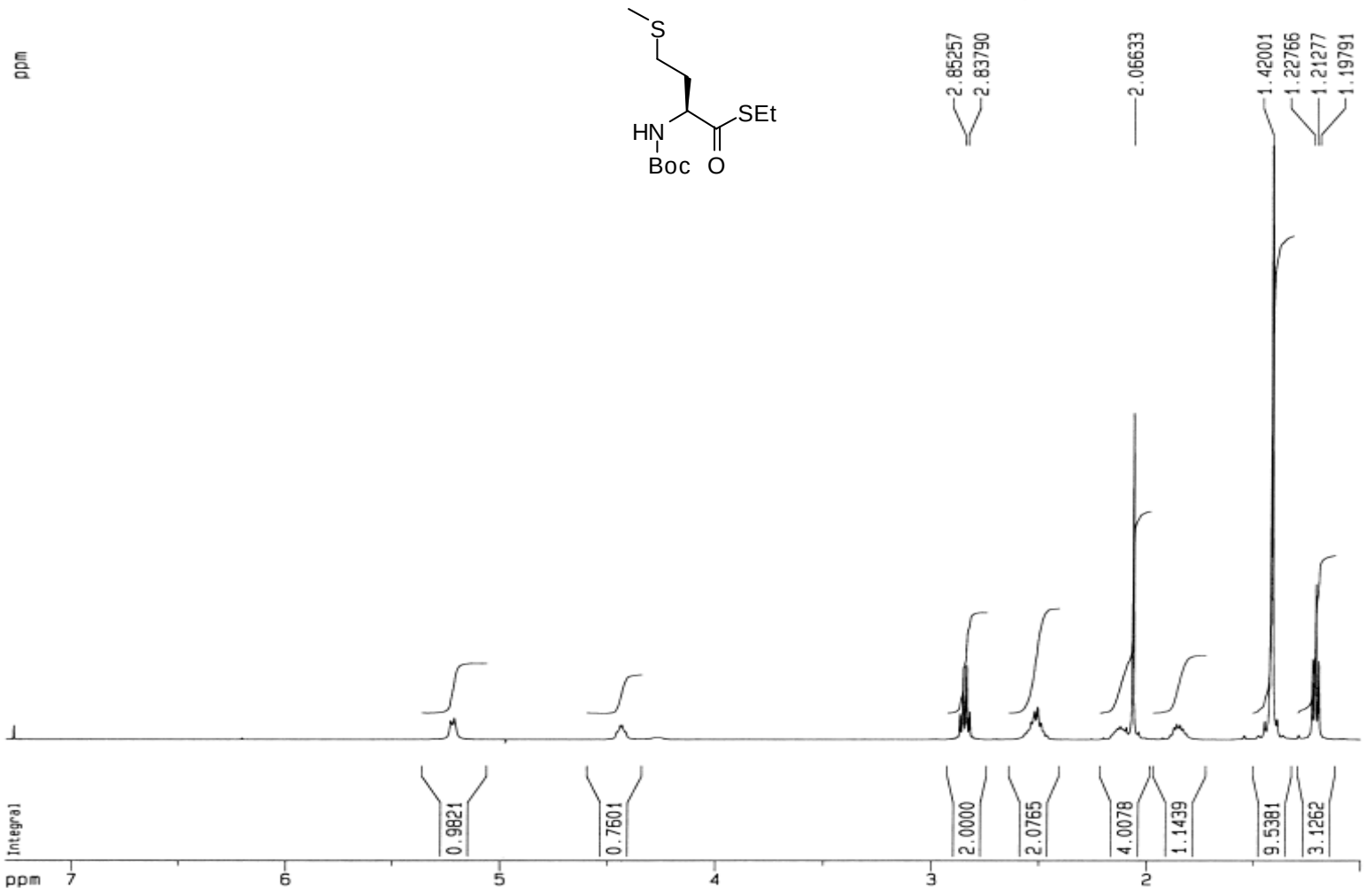
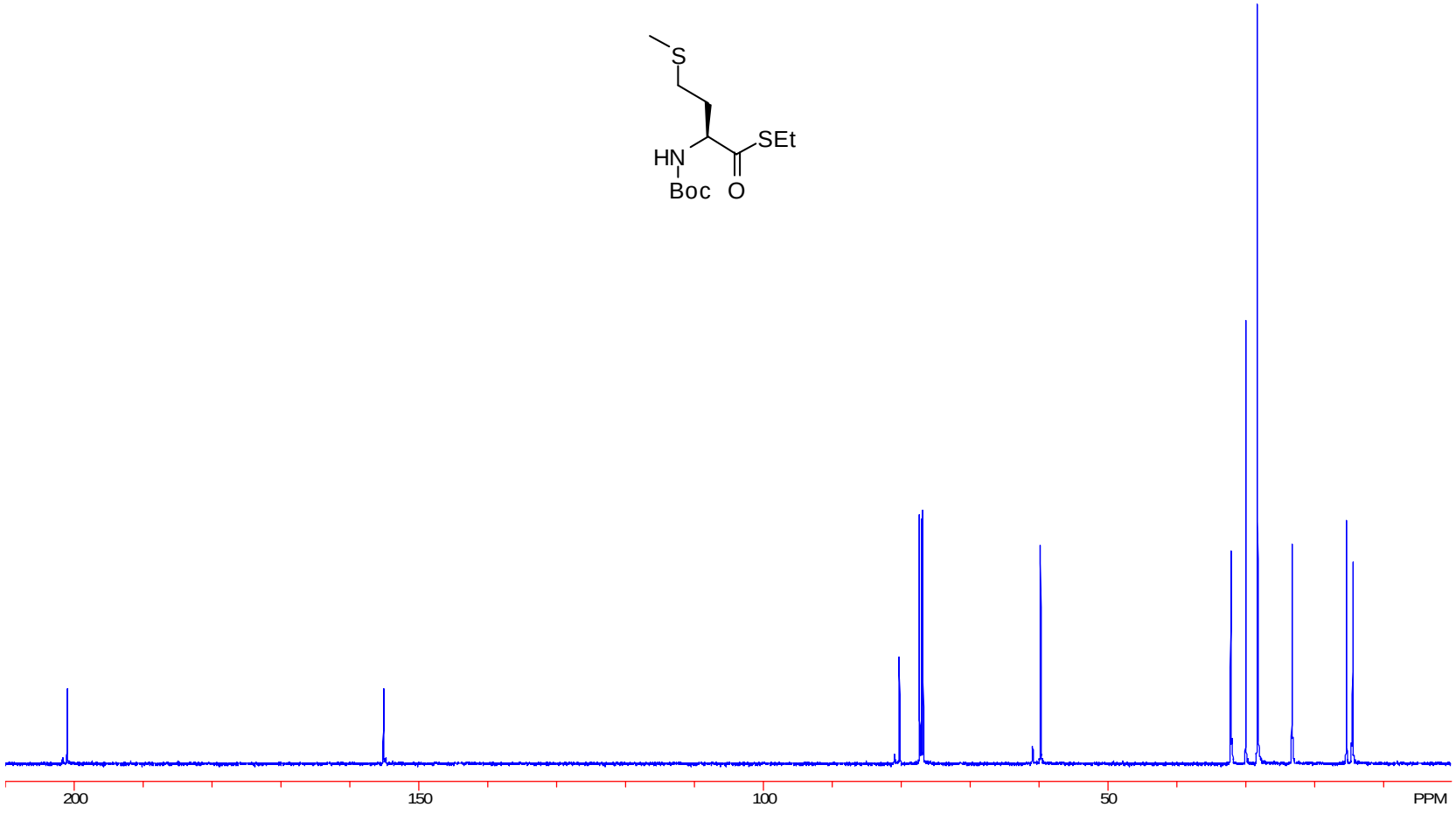


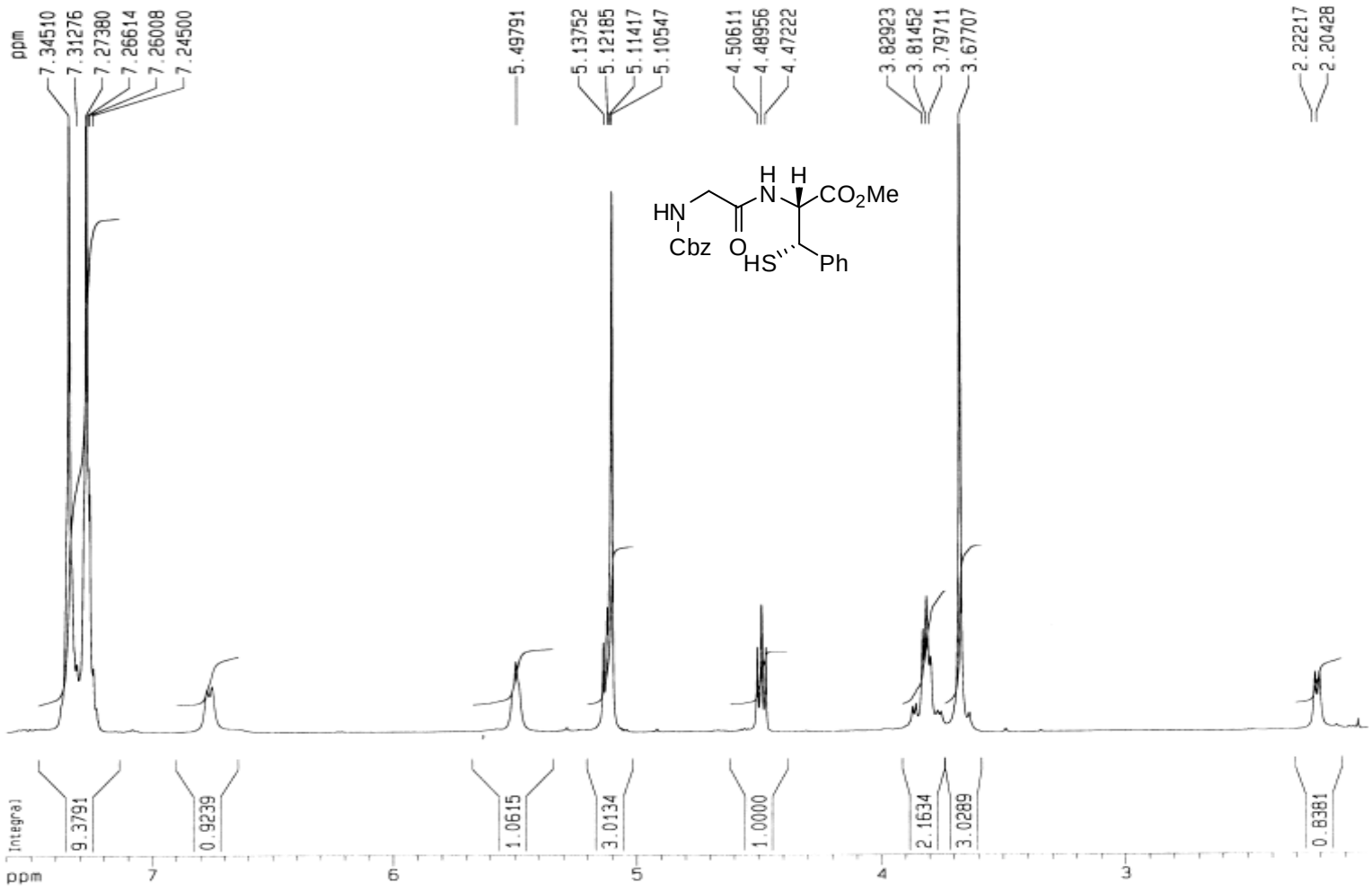
Boc-L-Met-SEt (10)



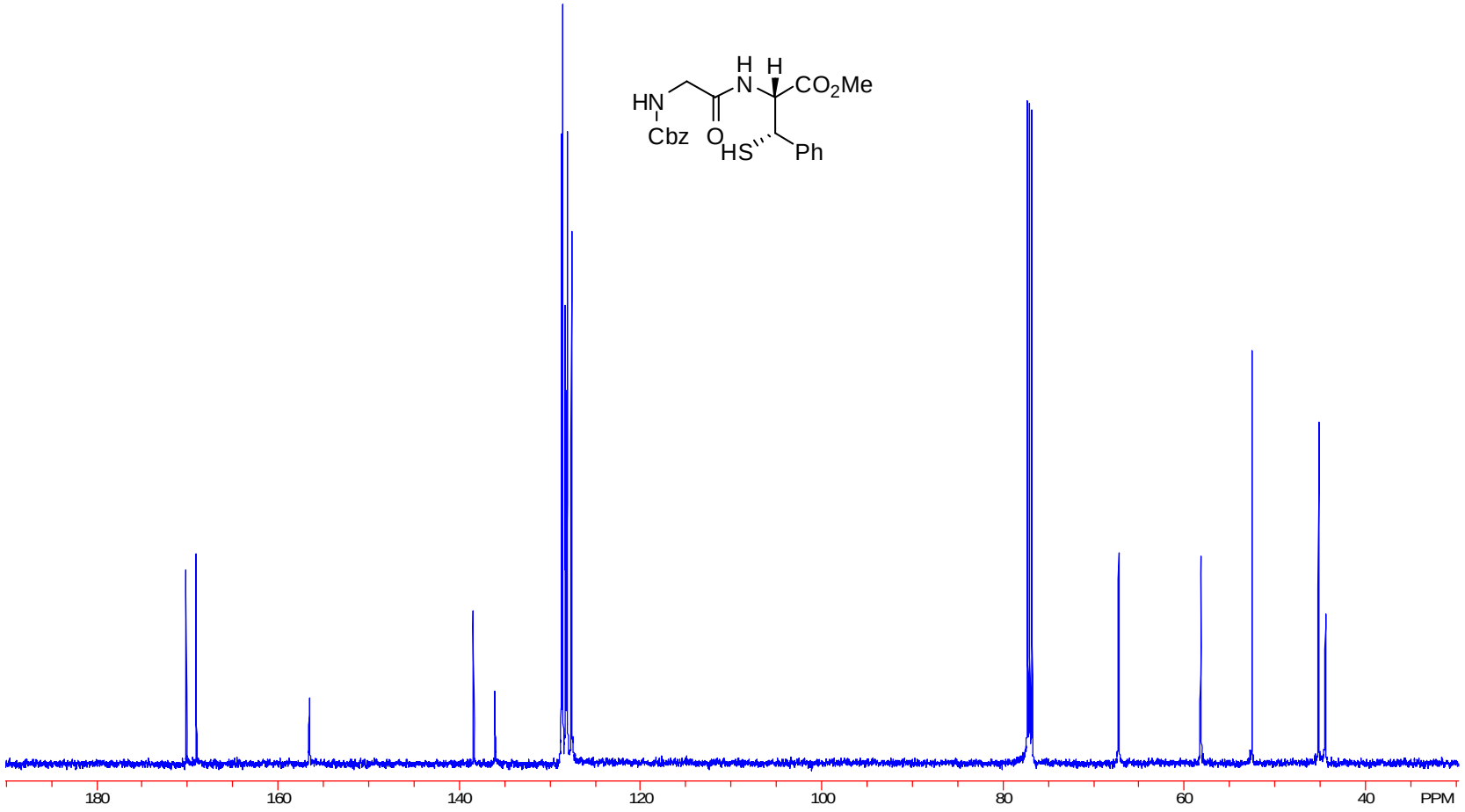
Boc-L-Met-SEt (10)



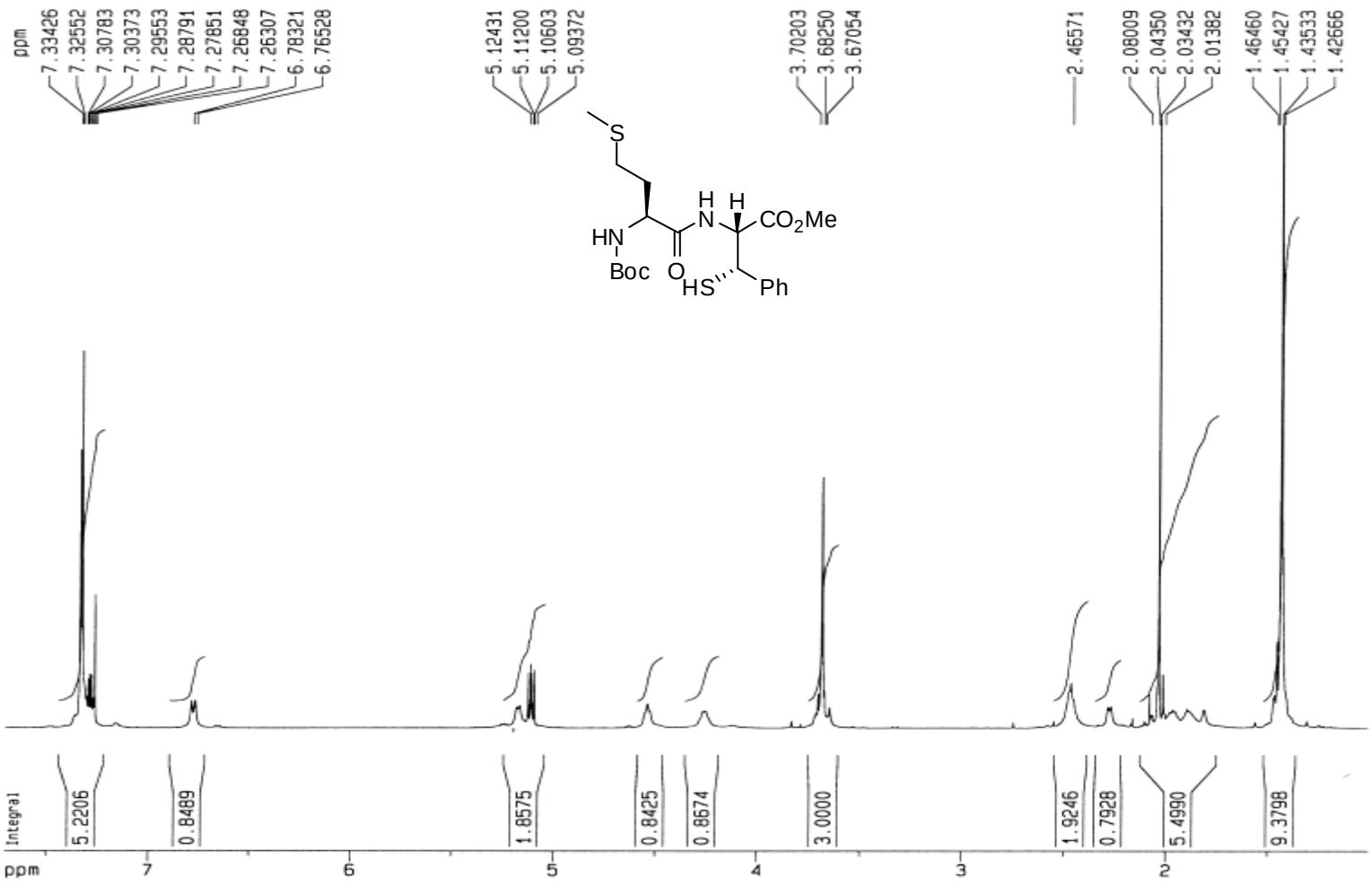
Cbz- L-Gly-(β-SH)-L-Phe-OMe (11)



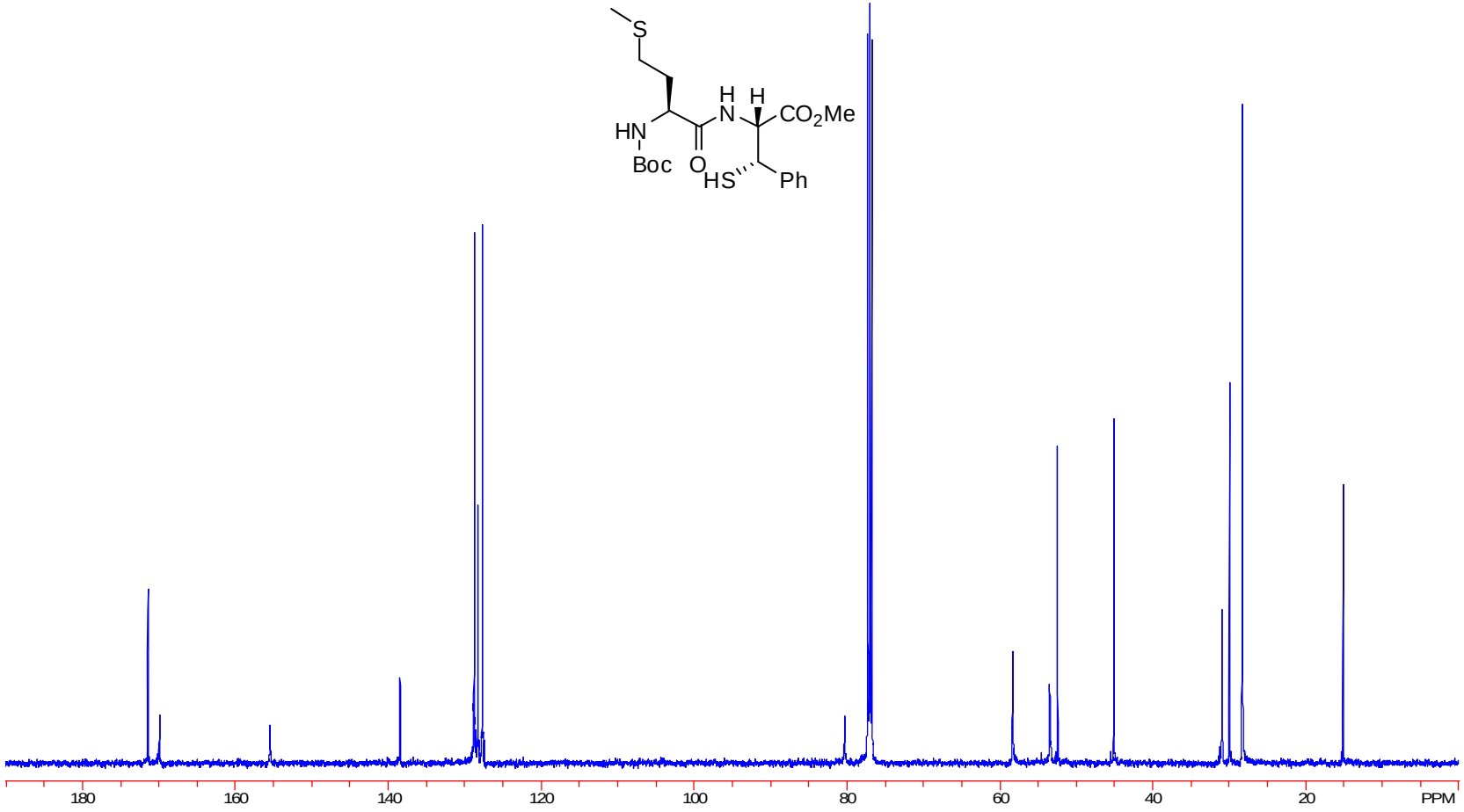
Cbz- L-Gly-(β-SH)-L-Phe-OMe (11)



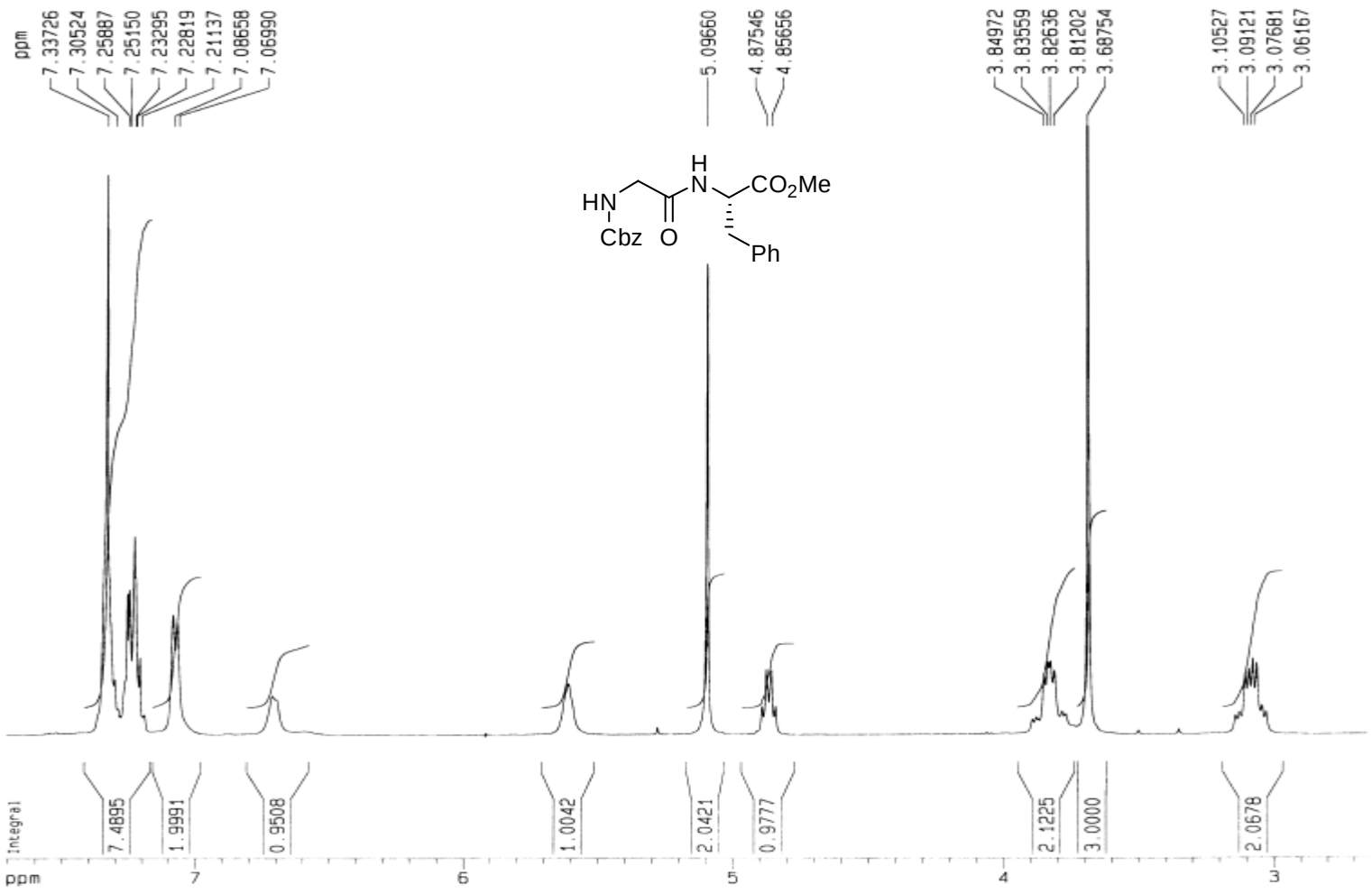
Boc-L-Met-(β-SH)-L-Phe-OMe (12)



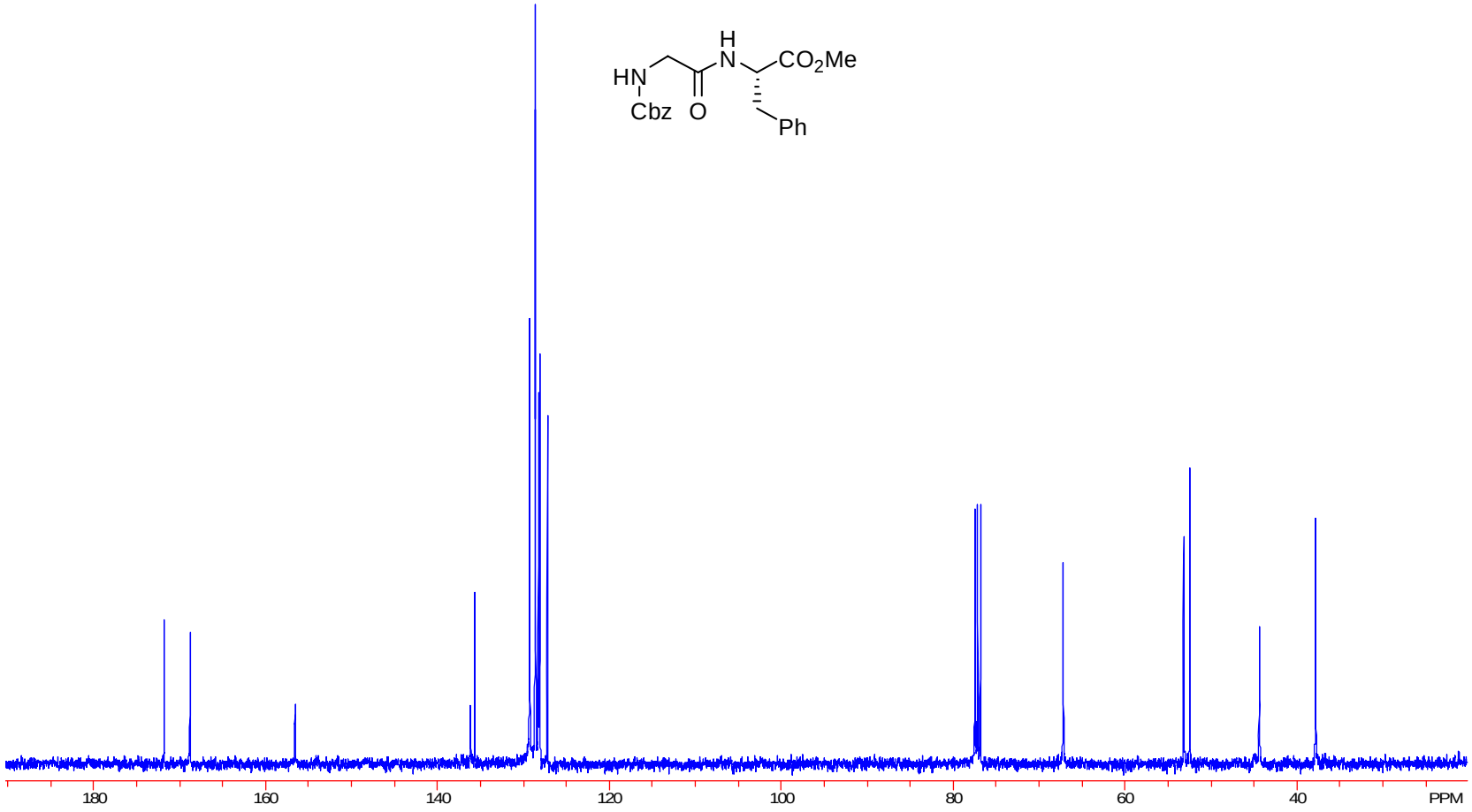
Boc-L-Met-(β-SH)-L-Phe-OMe (12)



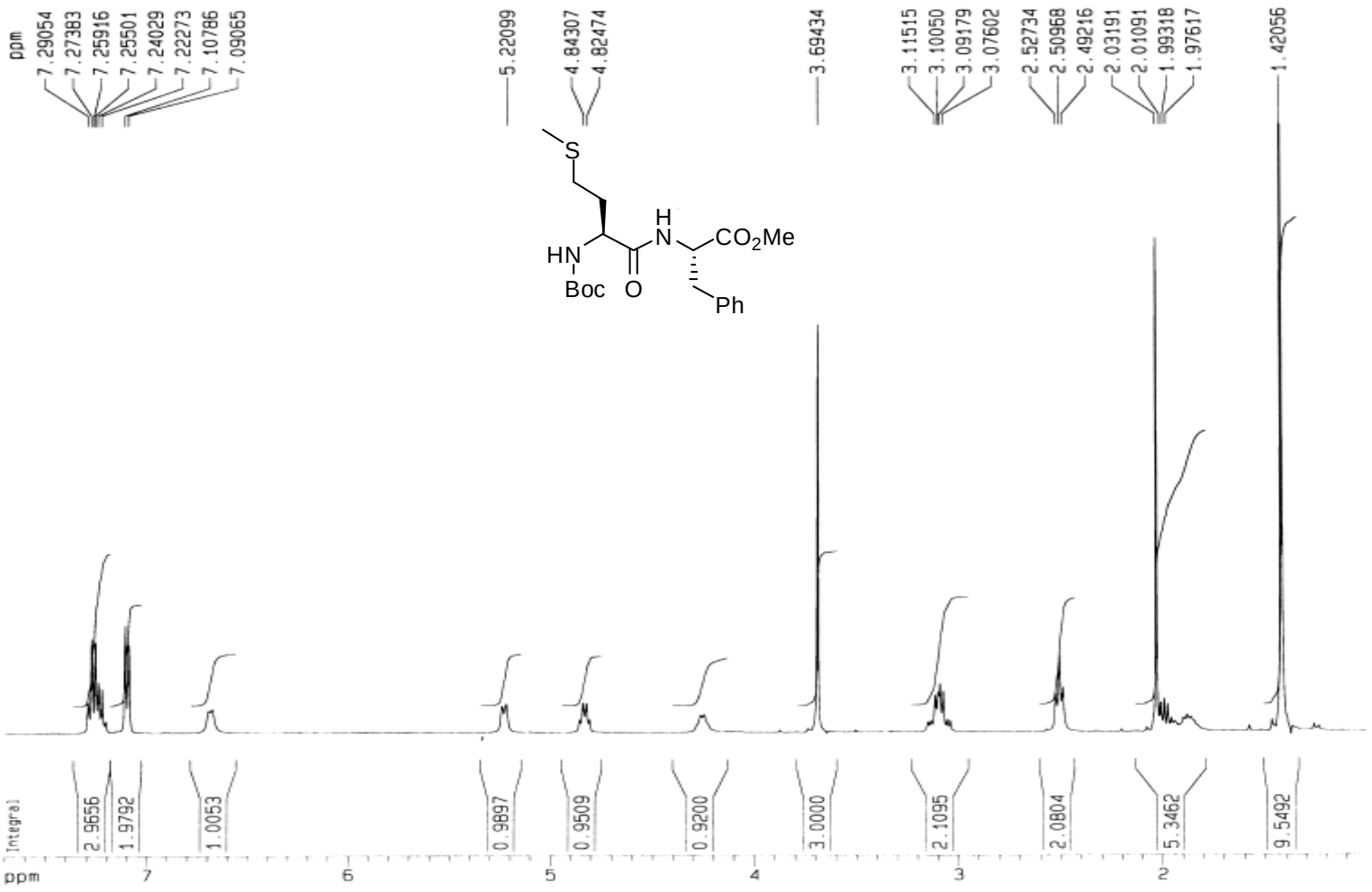
Cbz-L-Gly-L-Phe-OMe (13)



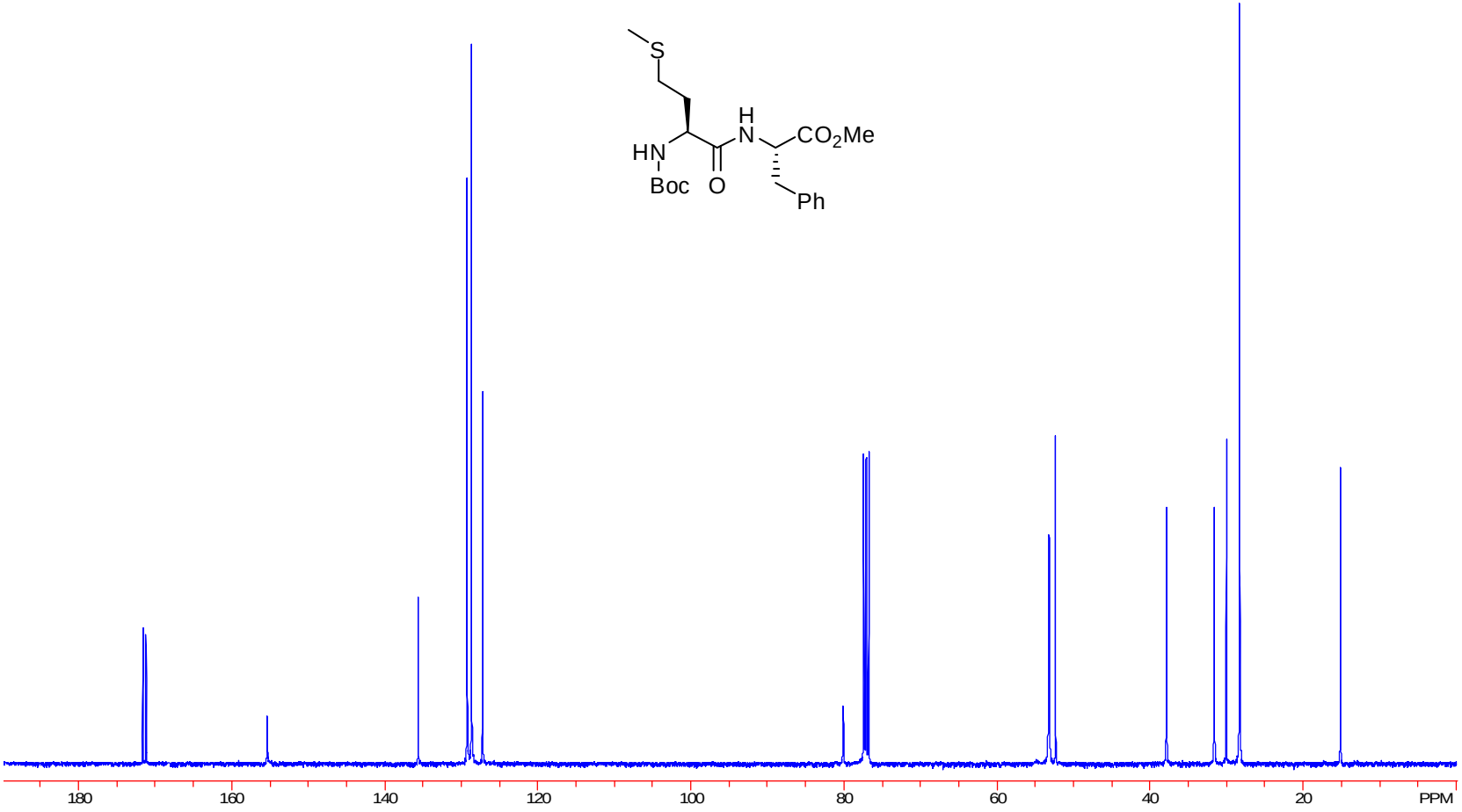
Cbz-L-Gly-L-Phe-OMe (13)



Boc-L-Met-L-Phe-OMe (14)

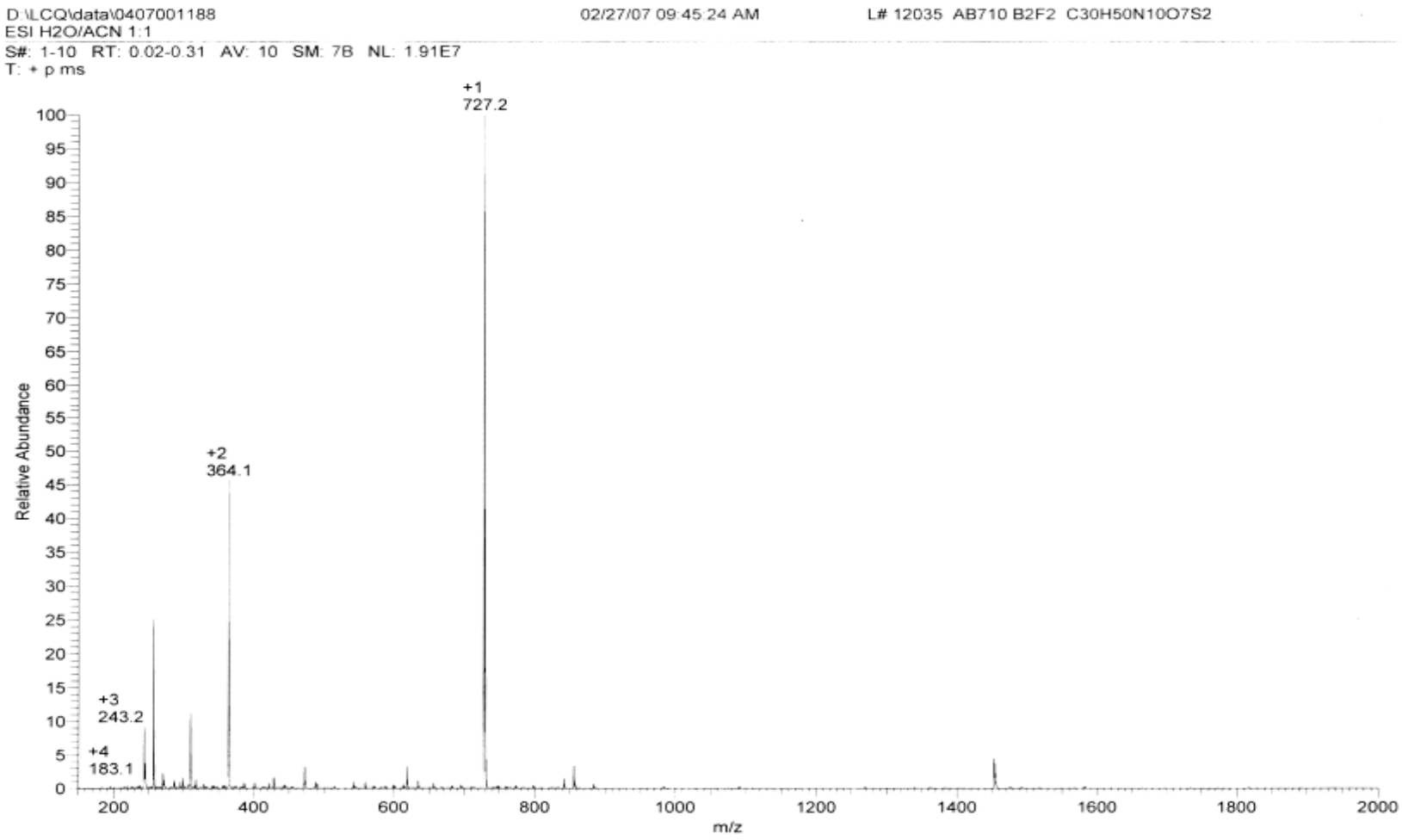


Boc-L-Met-L-Phe-OMe (14)



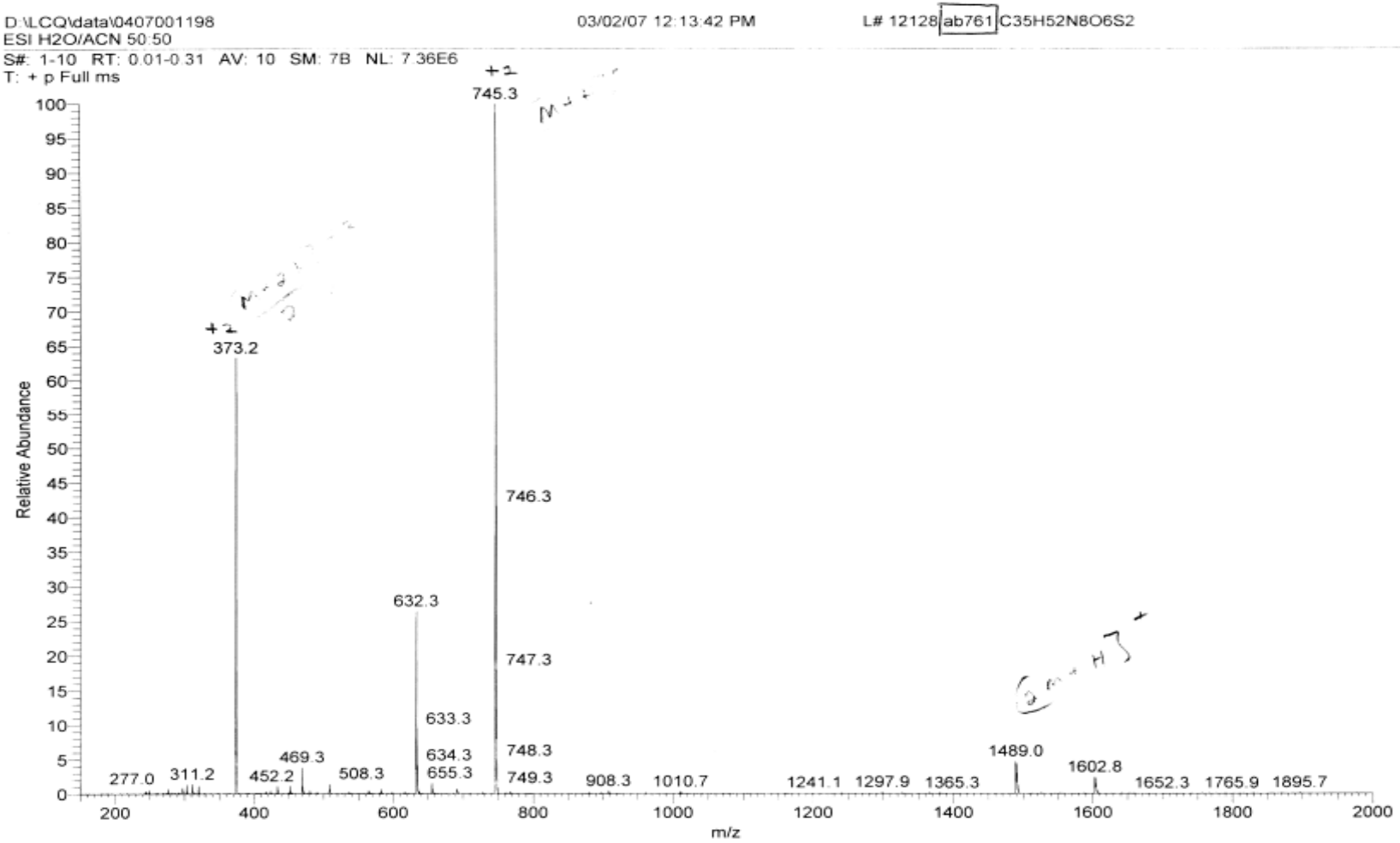
HPLC Trace of β-(EtSS)FRANK (15)

ESI-MS of β-(EtSS)FRANK (15)



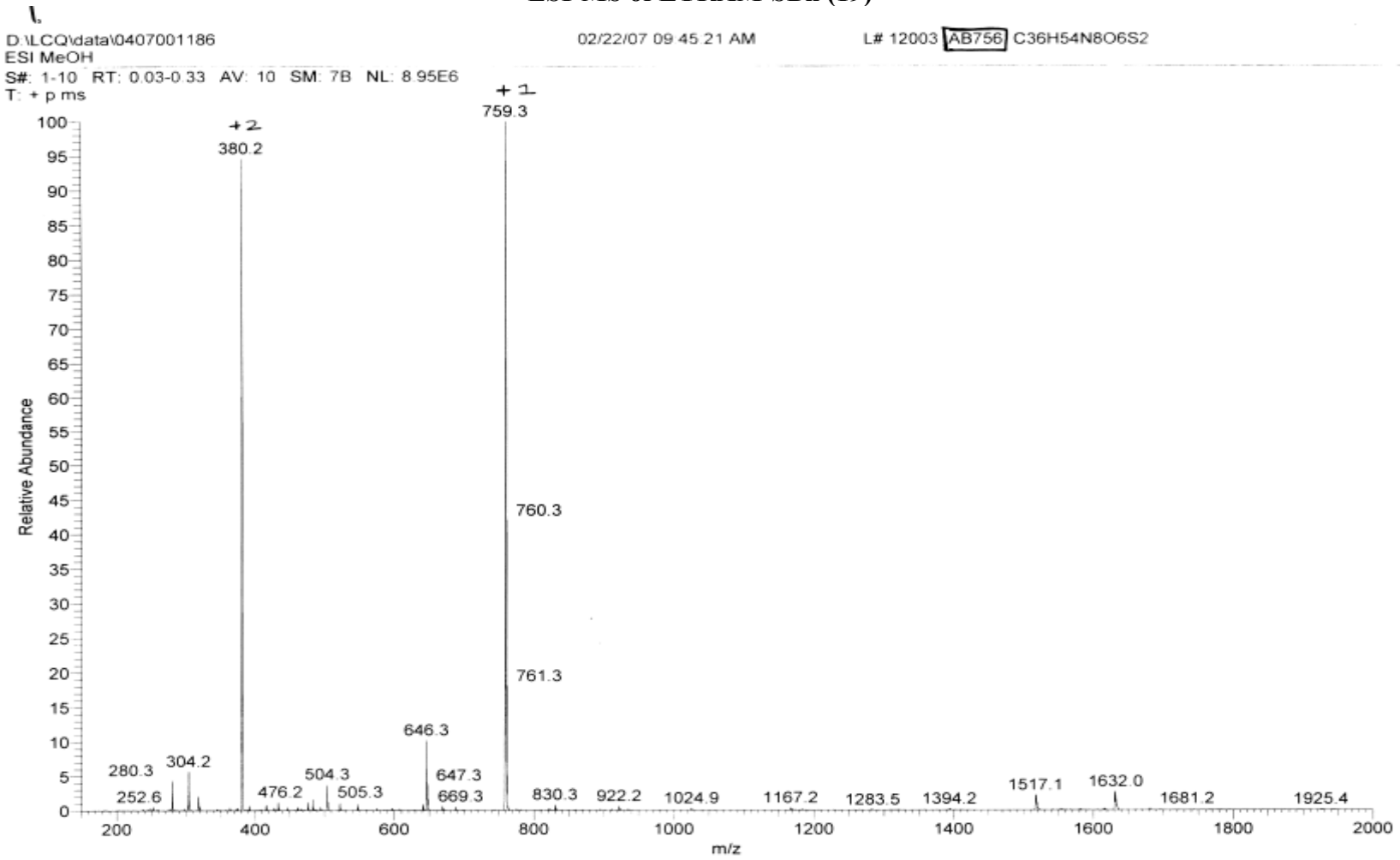
HPLC Trace of LYRMG-SBn (18)

ESI-MS of LYRMG-SBn (18)



HPLC Trace of LYRAM-SBn (19)

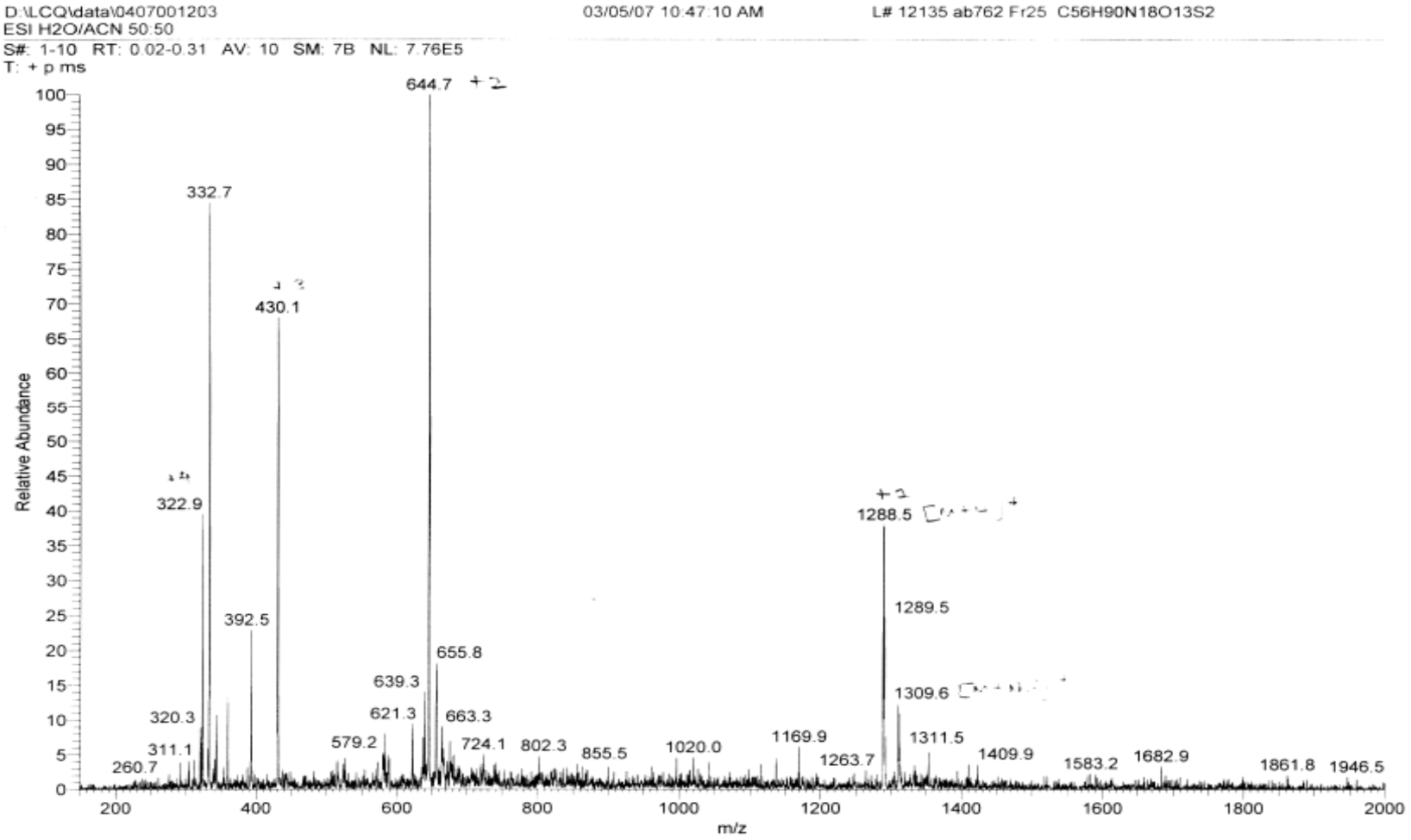
ESI-MS of LYRAM-SBn (19)



HPLC Trace of LYRMG-(β-SH)FRANK (20)

```
Title          :
Run File       : C:\star\data\ab762.run
Method File    : C:\Documents and Settings\Administrator\My Documents\restored\star\Abhisek\ab762.mth
Sample ID      : Manual Sample

Injection Date: 3/5/2007 6:20 PM    Calculation Date: 3/5/2007 7:13 PM

Operator       :                    Detector Type: 330 UV-Vis. PDA
Workstation:                        Bus Address  : 71
Instrument : Varian Star #1         Sample Rate  : 0.63 Hz
Channel    : 1 = 253.60 nm          Run Time     : 53.253 min

** Star Chromatography Workstation Version 6.00 ** 01896-34c0-ea4-0de0 **

Chart Speed =   0.37 cm/min  Attenuation = 40          Zero Offset = 3%
Start Time  =   0.000  min   End Time    = 53.253  min  Min / Tick  = 1.00
```

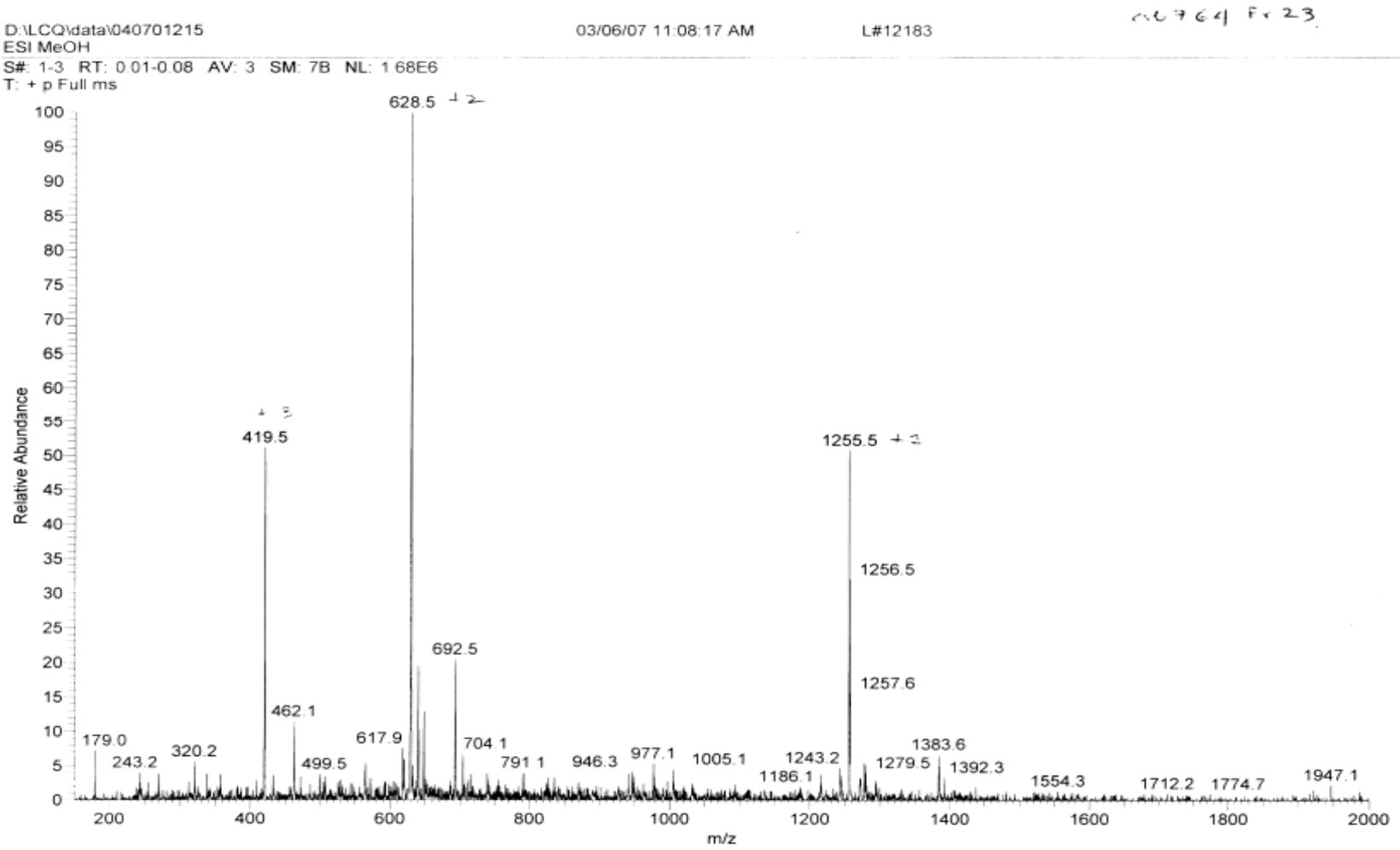
ESI-MS of LYRMG-(β-SH)FRANK (20)



HPLC Trace of LYRAM-(β-SH)FRANK (21)

```
Title          :
Run File       : C:\star\data\ab764.run
Method File    : C:\Documents and Settings\Administrator\My Documents\restored\star\Abhisek\ab764.mth
Sample ID      : Manual Sample

Injection Date: 3/6/2007 11:37 AM    Calculation Date: 3/6/2007 12:33 PM

Operator       :                     Detector Type: 330 UV-Vis. PDA
Workstation:                         Bus Address  : 71
Instrument : Varian Star #1          Sample Rate  : 0.63 Hz
Channel    : 1 = 253.60 nm           Run Time     : 56.053 min

** Star Chromatography Workstation Version 6.00 ** 01896-34c0-ea4-04e0 **

Chart Speed =   0.35 cm/min  Attenuation = 55          Zero Offset = 40
Start Time  =   0.000  min   End Time    = 56.053 min  Min / Tick  = 1.00
```

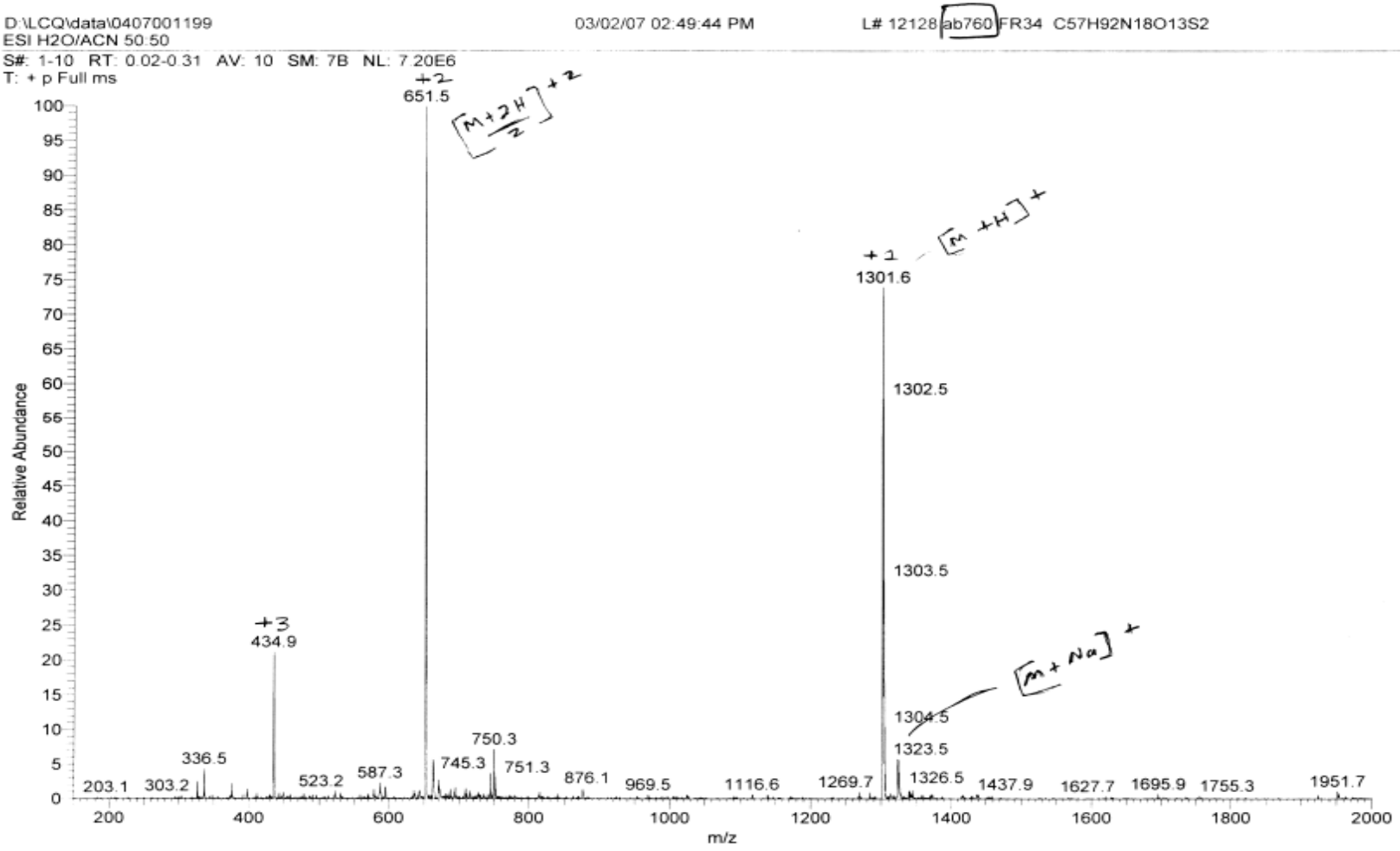
ESI-MS of LYRAM-(β-SH)FRANK (21)



HPLC Trace of LYRMGFRANK (22)

```
Title        :
Run File     : C:\star\data\ab760.run
Method File  : C:\Documents and Settings\Administrator\My Documents\restored\star\Abhisek\ab760.mth
Sample ID    : Manual Sample

Injection Date: 3/2/2007 3:38 PM    Calculation Date: 3/2/2007 4:30 PM

Operator     :                      Detector Type: 330 UV-Vis. PDA
Workstation:                        Bus Address  : 71
Instrument : Varian Star #1         Sample Rate  : 0.63 Hz
Channel    : 1 = 253.60 nm          Run Time     : 52.000 min

** Star Chromatography Workstation Version 6.00 ** 01896-34c0-0a4-04e0 **

Chart Speed =   0.38 cm/min  Attenuation = 33          Zero Offset = 4%
Start Time  =   0.000  min   End Time    = 52.000 min  Min / Tick  = 1.00
```

ESI-MS of LYRMGFRANK (22)



HPLC Trace of LYRAMFRANK (23)

Title :
Run File : C:\star\data\ab763.run
Method File : C:\Documents and Settings\Administrator\My Documents\restored\star\Abhisek\ab763.mth
Sample ID : Manual Sample

Injection Date: 3/12/2007 3:44 PM Calculation Date: 3/12/2007 4:41 PM

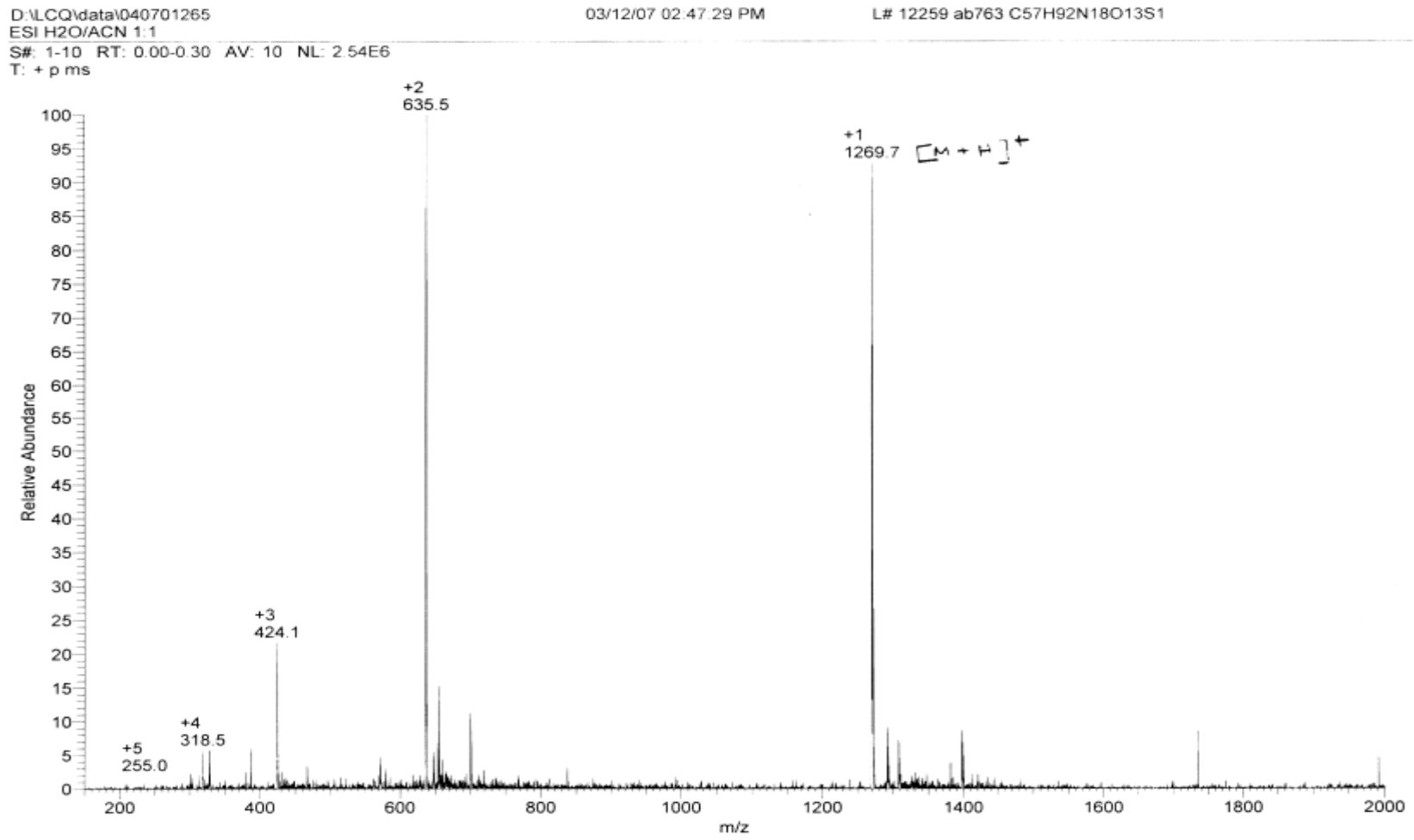
Operator :
Workstation:
Instrument : Varian Star #1
Channel : 1 = 253.60 nm

Detector Type: 330 UV-Vis. PDA
Bus Address : 71
Sample Rate : 0.63 Hz
Run Time : 57.440 min

** Star Chromatography Workstation Version 6.00 ** 01896-34c0-ea4-04e0 **

Chart Speed = 0.34 cm/min Attenuation = 55 Zero Offset = 4%
Start Time = 0.000 min End Time = 57.440 min Min / Tick = 1.00

ESI-MS of LYRAMFRANK (23)



LYRAM-(β-SH)FRANK (21) produced by the HATU method showing epimerization