Supporting Information

Solid Phase Synthesis of Peptidyl Thioacids Employing a 9-Fluorenylmethyl Thioester-Based Linker in Conjunction with Boc Chemistry.

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[2-(*tert*-Butoxycarbonylamino)-9*H*-fluoren-9-yl]methyl 4-methylbenzenesulfonate (2)	**-**	**S-18, S-19**
(2-Amino-9*H*-fluoren-9-yl)methyl 4-methylbenzenesulfonate (3)	**-**	**S-20, S-21**
***N*-[9-(Tosyloxymethyl)-9*H*-fluoren-2-yl]succinamic acid (4)**	**-**	**S-22,**

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Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH (23)	**S-14**	**S-40, S-41, S-42**
Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH (24)	**S-14**	**S-43, S-44, S-45**
Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH (25)	**S-15**	**S-46, S-47, S-48**
Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH **(26)** 	**S-15**	**S-46, S-49, S-50**
Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH (27)	**S-15**	**S-51, S-52, S-53**
Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SBn (28)	**S-16**	**S-54, S-55**
Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Allo)-SBn (29)	**S-17**	**S-56, S-57**
Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-$NHSO_2$-C_6H_4-NHAc (30)	**S-17**	**S-58, S-59**

Figure 1. ^{1}H-NMR spectrum at 500 MHz (CD_3OD) of the tetrapeptidyl thioacid **22** on release from the resin and without purification.

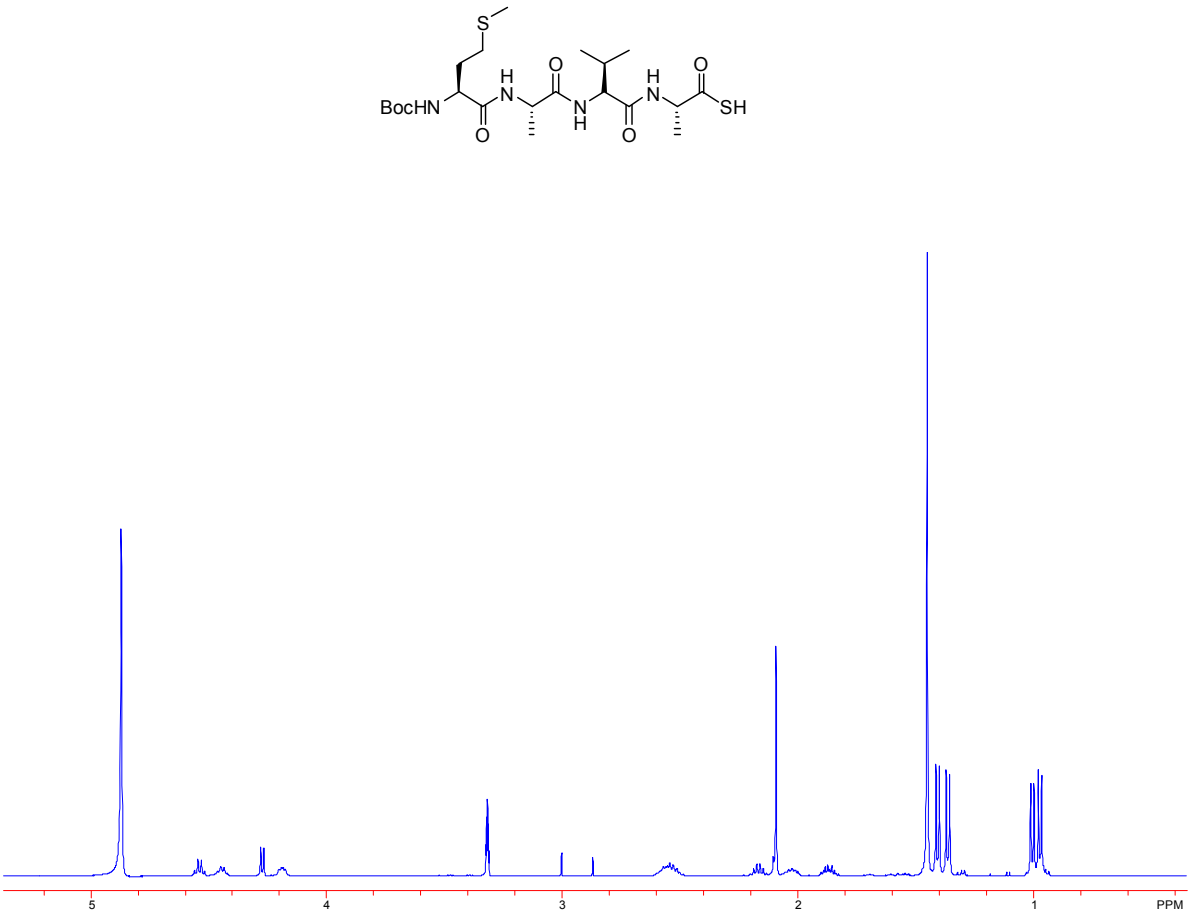


Figure 2. ^{13}C-NMR spectrum at 125 MHz ($CDCl_3$) of the tetrapeptidyl thioacid **22** on release from the resin and without purification.

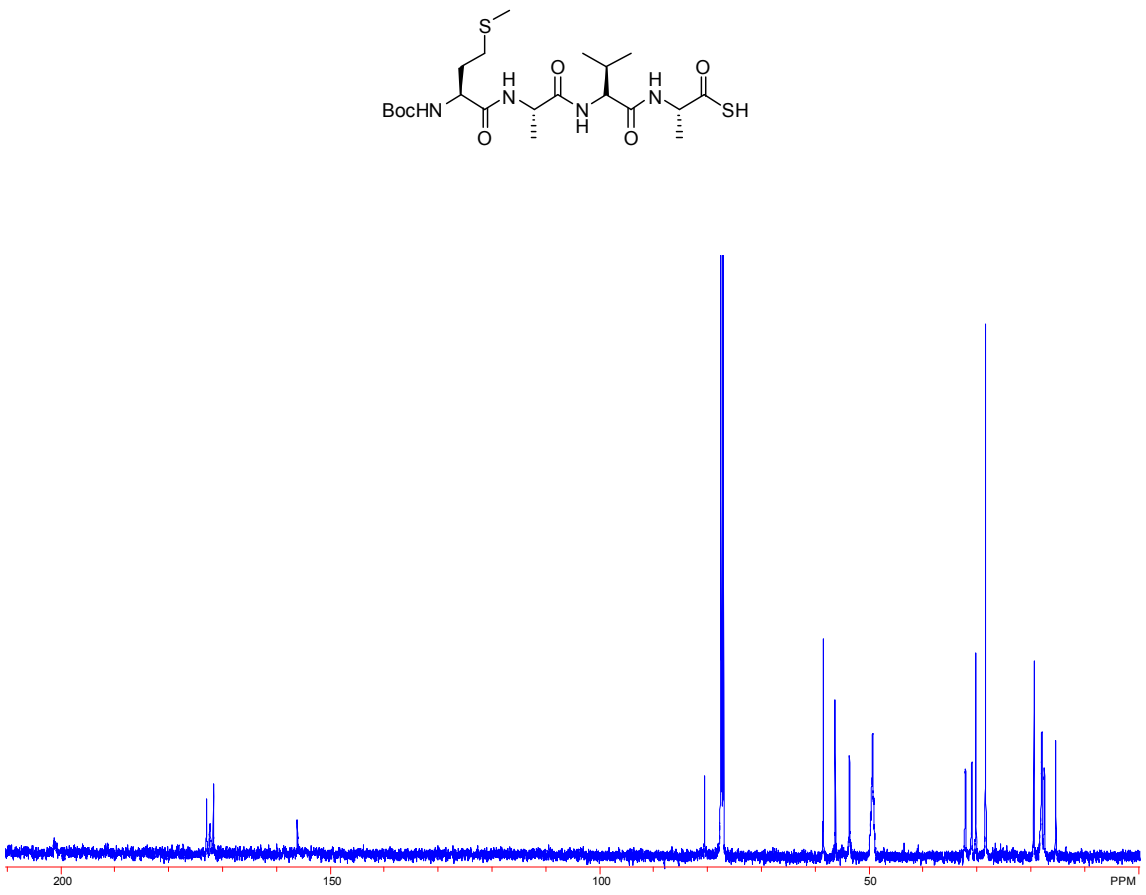
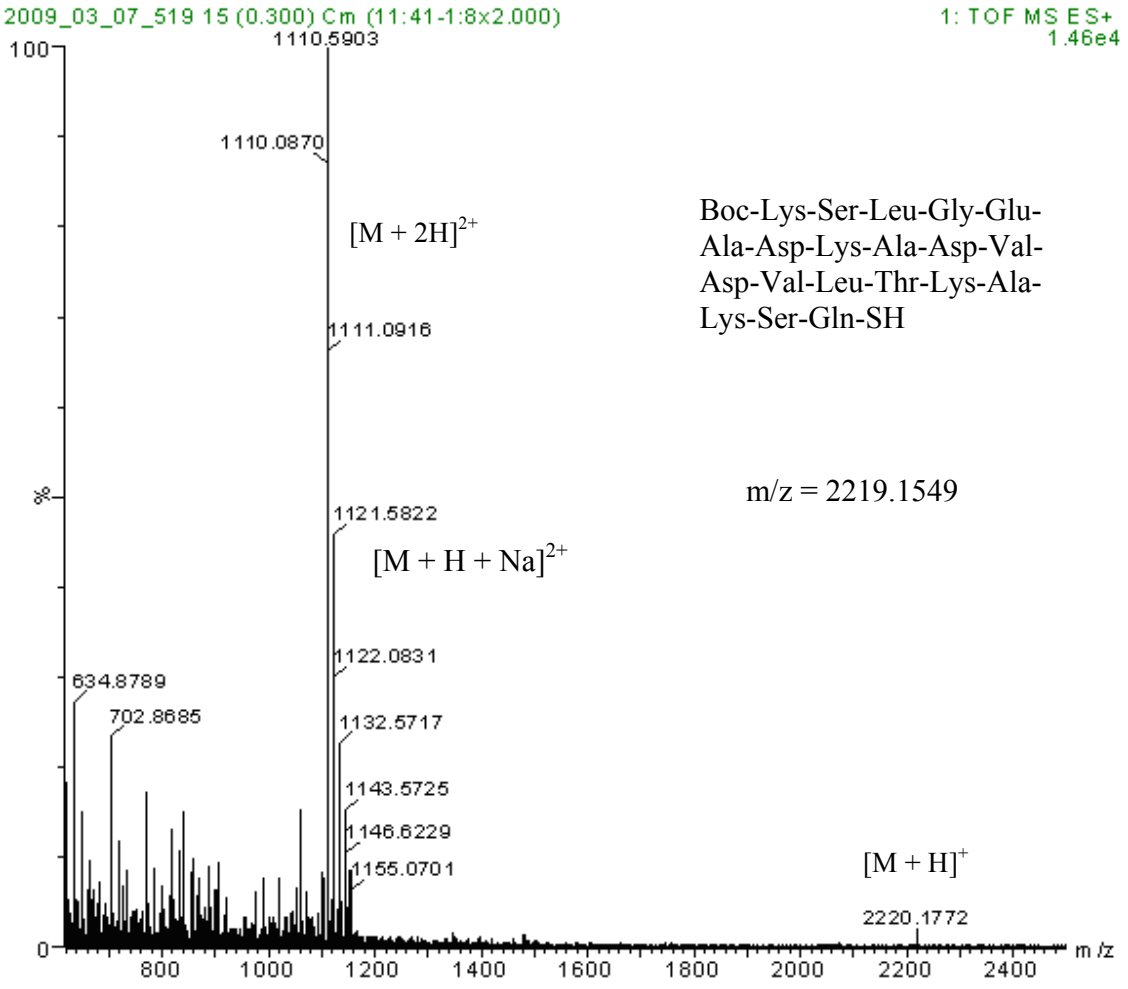


Figure 3. ESI-TOF mass spectrum of thioacid **27** on release from the resin and without chromatographic purification.



General Information. Unless otherwise stated ^{1}H and ^{13}C NMR were recorded in $CDCl_3$ solution. Optical rotations were recorded in $CHCl_3$ solution, unless otherwise stated. All organic extracts were dried over sodium sulfate, and concentrated under aspirator vacuum. Chromatographic purifications were carried out over silica gel. All peptide thioacids syntheses were carried out on a 0.1 mmol scale with 1% DVB cross linked aminomethyl polystyrene resin (244 mg, resin loding 0.41 mmol/g) in a 10 mL manual synthesizer glass reaction vessel with a Teflon-lined screw cap. The peptide resin was shaken during the both N^{α}-*tert*-butoxycarbonyl deprotection and coupling steps. After each coupling step, formation of desired peptide thioacid was confirmed by cleavage of small amount (~ 5 mg) of solid resin using a 20% solution of piperidine in DMF for 20 min., followed by examination by ESI-TOF mass spectrometry. Isolated yields following SPPS were determined based the theoretical yield calculated for the use of 0.1 mmol of resin with a loading of 0.41 mmol/g, taking into account the aliquots removed for monitoring.

Reverse phase HPLC (RP-HPLC) purification was performed with 215 and 254 nm UV detection, using a C-18 analytical and preparative columns (250 × 4.6) and (250 × 21.4), respectively. All runs used linear gradients of A in B (A: CH_3CN and B: Water).

***N*-*tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (7):** To a stirred solution of *N*-*tert*-butoxycarbonyl-L-serine (2.0 g, 9.8 mmol) and K_2CO_3 (1.35 g, 9.8 mmol) in DMF (10 mL) was added allyl bromide (1.2 mL, 14.7 mmol) at 0 ^{o}C. The reaction mixture was stirred at room temperature for 2 h, after which the DMF was removed under high vacuum and the crude mixture was dissolved in EtOAc and washed

with water, brine and dried. Evaporation of the solvent, afforded crude *N*-*tert*-butoxycarbonyl-L-serine allyl ester, which was taken forward without purification.

To a stirred solution of the crude *N*-*tert*-butoxycarbonyl-L-serine allyl ester (2.3 g, 9.8 mmol) and Fmoc-Cl (3.8 g, 14.7 mmol) in dichloromethane (10 mL) at 0 °C was added dropwise pyridine (1.2 mL, 14.7 mmol). After the addition was complete, the reaction mixture was allowed to warm room temperature and stirred at room temperature for 3 h. Then the reaction mixture was diluted with dichloromethane (20 mL) and the organic layer was washed with water, brine, dried and concentrated. Chromatographic purification using 10% ethyl acetate in hexane afforded **7** (3.9 g, 86%). Light yellow syrup; $[\alpha]^{23}_{D}$ +9.9 (*c* 0.75); ^{1}H NMR (500 MHz) δ: 7.79-7.78 (d, *J* = 7.5 Hz, 2H), 7.62-7.60 (d, *J* = 7.5 Hz, 2H), 7.44-7.41 (t, *J* = 7.5 Hz, 2H), 7.36-7.32 (dt, *J* = 2.5, 7.5 Hz, 2H), 5.97-5.90 (m, 1H), 5.41-5.33 (m, 2H), 5.27-5.25 (d, *J* = 10.5 Hz, 1H), 4.71-4.69 (d, *J* = 7.0 Hz, 1H), 4.66-4.58 (m, 2H), 4.49-4.46 (dd, *J* = 3.5, 11.0 Hz, 1H), 4.42-4.41 (d, *J* = 8.0 Hz, 2H), 4.28-4.25 (t, *J* = 7.5 Hz, 1H), 1.48 (s, 9H); ^{13}C NMR (125 MHz) δ: 169.4, 155.4, 155.0, 143.4, 141.5, 131.5, 128.2, 127.4, 125.4, 120.3, 119.3, 80.7, 70.5, 67.9, 66.7, 53.3, 46.9, 28.5; ESI-HRMS Calcd for $C_{26}H_{29}NO_7$ $[M + Na]^+$: 490.1842. Found: 490.1824.

***N*-*tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine (8):** A mixture of $Pd(OAc)_2$ (170 mg, 0.8 mmol) and PPh_3 (1.67 g, 6.4 mmol) was stirred in dichloromethane (10 mL) at room temperature for 5 min until a clear yellow solution of tetrakis(triphenylphosphine)palladium(0) was obtained. This solution was transferred *via* cannula to a stirred solution of *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (**7**) (3.0 g, 6.4 mmol) and phenylsilane (1.6 mL, 12.8 mmol) in

dichloromethane (20 mL) at room temperature and the stirring was continued at same temperature for 1.5 h. Then the reaction mixture was diluted with dichloromethane (20 mL) and was washed with water, brine, dried and concentrated. Chromatographic purification using 50% ethyl acetate in hexane afforded **8** (2.5 g, 91%). White solid, crystallized from ethyl acetate/hexane, mp: 65-66 ^{o}C. $[\alpha]^{22}_{D}$ +19.4 (*c* 1.7); ^{1}H NMR (500 MHz) δ: 7.77-7.75 (d, *J* = 7.5 Hz, 2H), 7.60-7.59 (d, *J* = 7.0 Hz, 2H), 7.42-7.39 (t, *J* = 7.5 Hz, 2H), 7.33-7.04 (t, *J* = 7.0 Hz, 2H), 5.44-5.43 (d, *J* = 7.5 Hz, 1H), 4.68 (br s, 1H), 4.61-4.59 (d, *J* = 8.5 Hz, 1H), 4.52-4.51 (d, *J* = 8.5 Hz, 1H), 4.42-4.40 (d, *J* = 7.5 Hz, 2H), 4.26-4.23 (t, *J* = 7.5 Hz, 1H), 1.47 (s, 9H); ^{13}C NMR (125 MHz) δ: 173.7, 155.7, 155.0, 143.4, 141.5, 128.2, 127.4, 125.4, 120.3, 81.1, 70.5, 67.6, 53.2, 46.9, 28.5; ESI-HRMS Calcd for $C_{23}H_{25}NO_7$ $[M + Na]^+$: 450.1529. Found: 450.1526.

***N*-*tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-threonine allyl ester (9):** Following the same procedure as for the synthesis of *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (**7**), compound **9** was synthesized from *N*-*tert*-butoxycarbonyl-L-threonine in 92% yield. Light yellow syrup; $[\alpha]^{23}_{D}$ +14.0 (*c* 1.0); ^{1}H NMR (500 MHz) δ: 7.79-7.77 (d, *J* = 7.0 Hz, 2H), 7.61-7.59 (d, *J* = 7.5 Hz, 2H), 7.44-7.41 (t, *J* = 7.5 Hz, 2H), 7.36-7.33 (tt, *J* = 1.5, 7.5 Hz, 2H), 5.93-5.85 (m, 1H), 5.39-5.30 (m, 3H), 5.21-5.19 (d, *J* = 10.5 Hz, 1H), 4.68-4.63 (m, 2H), 4.56-4.54 (dd, *J* = 2.5, 9.7 Hz, 1H), 4.43-4.35 (m, 2H), 4.26-4.23 (t, *J* = 7.5 Hz, 1H), 1.50 (s, 9H), 1.42-1.41 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR (125 MHz) δ: 169.8, 156.1, 154.4, 143.5, 141.5, 131.6, 128.2, 127.4, 125.4, 120.3, 119.3, 80.6, 75.0, 70.3, 66.6, 57.4, 46.9, 28.5, 17.1; ESI-HRMS Calcd for $C_{27}H_{31}NO_7$ $[M + Na]^+$: 504.1998. Found: 504.1974.

***N*-*tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-threonine (10):** Following the same procedure as for the synthesis of *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine (**8**), compound **10** was synthesized from *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-threonine allyl ester (**9**), in 90% yield. White solid, crystallized from ethyl acetate/hexane, mp: 69-70 ^{o}C. $[\alpha]^{23}_{D}$ +19.6 (*c* 1.34); ^{1}H NMR (500 MHz) δ: 7.75-7.73 (dd, *J* = 3.0, 7.5 Hz, 2H), 7.58-7.55 (t, *J* = 8.5 Hz, 2H, 7.41-7.38 (dt, *J* = 3.0, 7.5 Hz, 2H), 7.32-7.29 (t, *J* = 7.5 Hz, 2H), 5.36-5.33 (m, 2H), 4.50-4.48 (d, *J* = 8.0 Hz, 1H), 4.39-4.34 (m, 2H), 4.22-4.19 (t, *J* = 7.5 Hz, 1H), 1.47 (s, 9H), 1.38-1.37 (d, *J* = 6.5 Hz); ^{13}C NMR (125 MHz) δ: 174.4, 156.3, 154.4, 143.6, 143.4, 141.5, 128.1, 127.4, 125.4, 120.3, 80.8, 74.9, 70.2, 57.4, 46.9, 28.5, 17.2; ESI-HRMS Calcd for $C_{24}H_{27}NO_7$ $[M + Na]^+$: 464.1685. Found: 464.1688.

***N*-*tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-tyrosine allyl ester (11):** Following the same procedure as for the synthesis of *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (**7**), compound **11** was synthesized from *N*-*tert*-butoxycarbonyl-L-tyrosine in 95% yield. Light yellow syrup; $[\alpha]^{23}_{D}$ +14.7 (*c* 1.5); ^{1}H NMR (500 MHz) δ: 7.82-7.80 (d, *J* = 7.5 Hz, 2H), 7.67-7.66 (d, *J* = 7.5 Hz, 2H), 7.47-7.33 (t, *J* = 7.5 Hz, 2H), 7.38-7.35 (t, *J* = 7.5 Hz, 2H), 7.20-7.18 (d, *J* = 8.5 Hz, 2H), 7.14-7.13 (d, *J* = 8.5 Hz, 2H), 5.60-5.82 (m, 1H), 5.34-5.26 (dd, *J* = 14.0, 25.0 Hz, 2H), 5.04-5.03 (d, *J* = 7.0 Hz, 1H), 4.63-4.62 (t, *J* = 3.0 Hz, 3H), 4.55-4.4.54 (d, *J* = 7.0 Hz, 2H), 4.36-4.33 (t, *J* = 7.0 Hz, 1H), 3.18-3.14 (dd, *J* = 5.5, 13.5 Hz, 1H), 3.12-3.07 (dd, *J* = 5.5, 13.5 Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (125 MHz) δ: 171.6, 155.3, 153.8, 150.4, 143.4, 141.6, 134.2, 131.7, 130.7, 128.3, 127.5, 127.3, 125.4, 121.3, 120.4, 120.3, 119.3, 80.3,

70.7, 66.3, 54.6, 47.0, 37.9, 28.6; ESI-HRMS Calcd for $C_{32}H_{33}NO_7$ $[M + Na]^+$: 566.2155. Found: 566.2128.

***N*-*tert*-Butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-tyrosine (12):** Following the same procedure as for the synthesis of *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-serine (**8**), compound **12** was synthesized from *N*-*tert*-butoxycarbonyl-*O*-(9-fluorenylmethoxycarbonyl)-L-tyrosine allyl ester (**11**), in 92% yield. White solid, crystallized from ethyl acetate/hexane, mp: 84-85 ^oC. $[\alpha]^{22}_D$ -9.3 (*c* 0.6); ^1H NMR (500 MHz) δ: 7.81-7.79 (d, *J* = 7.5 Hz, 2H), 7.66-7.65 (d, *J* = 7.5 Hz, 2H), 7.46-7.43 (t, *J* = 7.5 Hz, 2H), 7.37-7.34 (t, *J* = 7.5 Hz, 2H), 7.26-7.24 (d, *J* = 8.5 Hz, 2H), 7.14-7.12 (d, *J* = 8.5 Hz, 2H), 4.77-4.76 (d, *J* = 7.5 Hz, 1H), 4.55-4.53 (d, *J* = 7.5 Hz, 2H), 4.36-4.33 (t, *J* = 7.5 Hz, 1H), 3.87 (br s, 1H), 3.69-3.67 (dd, *J* = 3.5, 11 Hz, 1H), 3.59-3.56 (dd, *J* = 5.0, 10.8 Hz, 1H), 1.43 (s, 9H); ^{13}C NMR (125 MHz) δ: 173.7, 156.3, 153.9, 150.0, 143.4, 141.6, 136.0, 130.6, 128.2, 127.5, 125.4, 121.3, 120.4, 80.1, 70.7, 64.4, 53.9, 47.0, 28.6; ESI-HRMS Calcd for $C_{29}H_{29}NO_7$ $[M + Na]^+$: 526.1842. Found: 526.1844.

Synthesis of Boc-L-Met-L-Ala-L-Val-L-Ala-SH (22) employing the DIC/HOBt activation method:

(a) Derivatization of aminomethyl polystyrene resin with *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (**5**). In a 10 mL glass reaction vessel, aminomethyl polystyrene resin (244 mg, 0.1 mmol) was swelled in DMF (2 mL) for 30 min, after which the solvent was removed by filtration. To a stirred solution of **5** (114 mg, 0.2 mmol) and HOBt (27 mg, 0.2 mmol) in DMF (1 mL) was added DIC (31 μL, 0.2 mmol) at room temperature. This mixture was stirred for 30 min before the activated HOBt ester

of **5** was added to the reaction vessel with an additional DMF (1 mL) which was then shaken for 2 h before the resin was washed thoroughly using DMF (3 × 2 mL) and CH_2Cl_2 (3 × 2 mL).

(b) Deprotection of trityl group from derivatized aminomethyl polystyrene resin. To the reaction vessel containing the derivatized resin was added dichloromethane /TFA [v/v 1:1, 1.5 mL, with Et_3SiH (65 μL, 0.4 mmol)]. After shaking for 1 h the thiol derivatized resin was washed thoroughly using dichloromethane (3 × 2 mL) and a 5% solution of DIPEA in dichloromethane (2 × 2 mL).

(c) Coupling reaction. A stirred solution of Boc-Ala-OH (75 mg, 0.4 mmol) and HOBt (54 mg, 0.4 mmol) in DMF (1 mL) was treated with DIC (62 μL, 0.4 mmol) and then stirred for 30 min before it was added to the thiol derivatized resin with additional DMF (1 mL) and the resulting mixture shaken for 3 h, after which the derivatized resin was washed with DMF (3 × 2 mL) and dichloromethane (3 × 2 mL).

(d) TFA deprotection of N^{α}-Boc group. The derivatized resin was treated with TFA in dichloromethane (25%, 1.5 mL), and shaken for 30 min. The deprotection step was repeated with fresh TFA in dichloromethane (25%, 1.5 mL) for additional 30 min, after which the derivatized resin was washed thoroughly using dichloromethane (3 × 2 mL) and 5% solution of DIPEA in dichloromethane (2 × 2 mL).

(e) The subsequent coupling and N^{α}-Boc deprotection steps were carried out as described in steps (c) and (d) respectively using an appropriate Boc-protected amino acid.

(f) Isolation of Boc-peptide thioacid form the derivatized resin. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin was transferred to a round bottom flask (25 mL) equipped with a magnetic stir bar. A solution of piperidine in

DMF (20%, 10 mL) was added into the flask containing the swollen resin and the mixture was stirred for 20 min, after which the solid was filtered off and the solution was diluted with EtOAc (20 mL). Then the organic layer was washed successively with 0.5 N aq HCl (2 × 10 mL), brine, and then dried. Evaporation of the solvent in a rotary evaporator afforded the peptide thioacid **22** (48 mg, 95%) as white amorphous solid that decomposed prior to melting. ^{1}H NMR (500 MHz, CD_3OD) δ: 4.56-4.52 (q, *J* = 7.0 Hz, 1H), 4.46-4.42 (q, *J* = 7.0 Hz, 1H), 4.28-4.27 (d, *J* = 6.0 Hz, 1H), 2.57-2.51(m, 2H), 2.19-2.15 (q, *J* = 6.5 Hz, 1H), 2.09 (s, 3H), 2.06-2.00 (m, 1H), 1.90-1.84 (m, 1H), 1.45 (s, 9H) 1.41-1.40 (d, *J* = 7.5 Hz, 3H), 1.37-1.36 (d, *J* = 7.0 Hz, 3H), 1.01-1.00 (d, *J* = 6.5 Hz, 3H), 0.98-0.96 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 201.3, 172.9, 172.3, 171.7, 156.1, 80.5, 58.5, 56.3, 53.7, 49.4, 32.1, 31.0, 30.2, 28.4, 19.4, 18.0, 17.5, 15.4; ESI-HRMS Calcd for $C_{21}H_{37}N_4O_6S_2$ $[M - H]^-$: 505.2155. Found: 505.2136.

Synthesis of Boc-L-Met-L-Ala-L-Val-L-Ala-SH (22) employing the HBTU and *i*Pr$_2$NEt activation method:

(a) Derivatization of aminomethyl polystyrene resin with *N*-[9-(tritylthiomethyl)-9*H*-fluoren-2-yl]succinamic acid (**5**). In a 10 mL glass reaction vessel, aminomethyl polystyrene resin (244 mg, 0.1 mmol) was swelled in DMF (2 mL) for 30 min, after which the solvent was removed by filtration. To a stirred solution of **5** (114 mg, 0.2 mmol) and HBTU (75 mg, 0.2 mmol) in DMF (1 mL) was added DIPEA (50 µL, 0.3 mmol) at room temperature. This mixture was stirred for 20 min before it was added to the reaction vessel containing the swollen resin with an additional DMF (1 mL) which was then shaken for 2 h before the resin was washed thoroughly using DMF (3 × 2 mL) and dichloromethane (3 × 2 mL).

(b) Deprotection of trityl group from derivatized aminomethyl polystyrene resin. To the reaction vessel containing the derivatized resin was added dichloromethane/TFA [v/v 1:1, 1.5 mL, containing Et_3SiH (65 μL, 0.4 mmol)]. After shaking for 1 h the thiol derivatized resin was washed thoroughly with dichloromethane (3 × 2 mL) and a 5% solution of DIPEA in dichloromethane (2 × 2 mL).

(c) Coupling reaction. A stirred solution of Boc-Ala-OH (75 mg, 0.4 mmol) and HBTU (150 mg, 0.4 mmol) in DMF (1 mL) was treated with DIPEA (100 μL, 0.6 mmol) and then stirred for 20 min before it was added to the thiol derivatized resin with additional DMF (1 mL) and the resulting mixture shaken for 3 h, after which the derivatized resin was washed with DMF (3 × 2 mL) and dichloromethane (3 × 2 mL).

(d) TFA deprotection of N^{α}-Boc group. The derivatized resin was treated with TFA in dichloromethane (25%, 1.5 mL), and shaken for 30 min. The deprotection step was repeated with fresh TFA in dichloromethane (25%, 1.5 mL) for an additional 30 min, after which the derivatized resin was washed thoroughly using dichloromethane (3 × 2 mL) and 5% solution of DIPEA in dichloromethane (2 × 2 mL).

(e) The subsequent coupling and N^{α}-Boc deprotection steps were carried out as described in steps (c) and (d) respectively using an appropriate Boc-protected amino acid.

(f) Isolation of Boc-peptide thioacid form the derivatized resin. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin was transferred to a round bottom flask (25 mL) equipped with a magnetic stir bar. A solution of piperidine in DMF (20%, 10 mL) was added into the flask and the mixture was stirred for 20 min, after which the solid was filtered off and the solution was diluted with EtOAc (20 mL). Then the organic layer was washed successively with 0.5 N aq HCl (2 × 10 mL), brine, and

then dried. Evaporation of the solvent in a rotary evaporator afforded the peptide thioacid **22** (45 mg, 88%).

Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH (23): Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**22**), Alloc-peptide derivatized resin (substitution level of Alloc-peptide derivatized resin = 0.2161 mmol/g) was synthesized. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin (150 mg, 0.0324 mmol) was transferred to a round bottom flask (10 mL) equipped with a magnetic stir bar. A solution of piperidine in DMF (20%, 3 mL) was added into the flask and the reaction mixture was stirred for 20 min, after which the solid was filtered off and the solvent was removed at room temperature under vacuum. The residue was washed with dichloromethane (2 × 2 mL) to removed the *N*-[(9*H*-fluoren-9-yl)methyl]piperidine by-product from the crude peptide thioacid, which was subsequently dissolved in acetonitrile/water (v/v 1:1, 3 mL) and subjected to RP-HPLC purification (20 - 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 17 min) to afford the peptide thioacid **23** (37 mg, 80%) as white amorphous solid that decomposed prior to melting.

Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH (24): Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**22**), Boc-peptide derivatized resin (substitution level of Boc-peptide derivatized resin = 0.2137 mmol/g) was synthesized. Following the same procedure as for the isolation of **23,** and RP-HPLC purification of crude mixture (20- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 18 min) of Boc-peptide derivatized resin (150

mg, 0.0321 mmol) provided the peptide thioacid **24** (32 mg, 78%) as white amorphous solid that decomposed prior to melting.

Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH (25) and

Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**22**), a Boc-peptide derivatized resin (substitution level of Boc-peptide derivatized resin = 0.2208 mmol/g) was synthesized with the following modification of N^{α}-Boc deprotection step. After the cysteine was attached in the sequence, Et_3SiH (0.5%) was added to the solution of 25% TFA in DICHLOROMETHANE during all N^{α}-Boc deprotection steps. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin (50 mg, 0.011 mmol) was transferred to a round bottom flask (10 mL) equipped with a magnetic stir bar. A solution of piperidine in acetonitrile (50%, 0.5 mL) was added into the flask and the reaction mixture was stirred for 30 min, after which the solid was filtered off. RP-HPLC purification of the filtrate (0- 50% A in B with a flow rate of 8 mL/min over 72 min and 215 nm UV detection, retention time = 38 min and 41 min) provided the Boc-peptide thioacid **25** (6 mg, 57%) as a white amorphous solid that decomposed prior to melting and the disulfide Boc-peptide thioacid **26** (1 mg, 15%) also as a white amorphous solid that decomposed prior to melting, respectively.

Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val^{8}-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH (27): Following the same procedure as for the synthesis of Boc-Met-Ala-Val-Ala-SH (**22**), a Boc-peptide derivatized resin (substitution level of Boc-peptide derivatized resin = 0.141 mmol/g) was synthesized with the following modification on

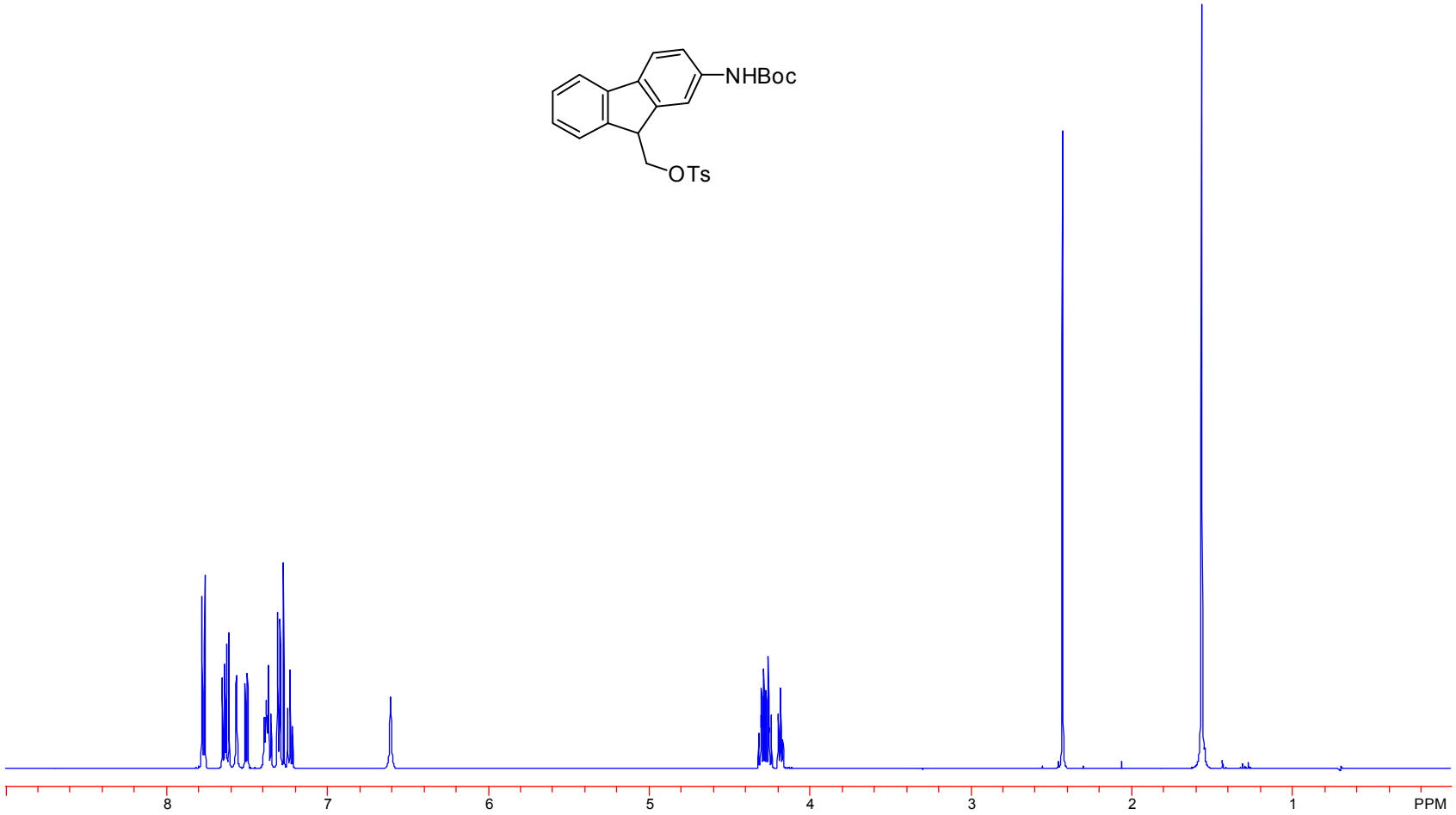
N^{α}-Boc deprotection and in coupling steps. After N^{α}-Boc deprotection from 8th aminoacid in the sequence, neutralization of TFA from solid was done with a 5% solution of DIPEA in NMP solvent and also NMP was used as coupling solvent. These modifications were followed till up to end of synthesis. After the complete synthesis of peptide sequence, the Boc-peptide derivatized resin (50 mg, 0.0071 mmol) was transferred to a round bottom flask (10 mL) equipped with a magnetic stir bar. A solution of piperidine in acetonitrile (50%, 0.5 mL) was added into the flask and the reaction mixture was stirred for 30 min. Then water (1 mL) was added in to the reaction mixture, which was subsequently stirred for 1 h before the solids which included the insoluble by-product *N*-[(9*H*-fluoren-9-yl)methyl]piperidine and the resin were filtered off. RP-HPLC purification of the filtrate (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 20 min) provided the Boc-peptide thioacid **27** (9 mg, 55%) as white amorphous solid that decomposed prior to melting.

Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SBn (28): To a stirred solution of **23** (12 mg, 0.0097 mmol) in DMF (50 µL) were added successively a solution of 2,4,6-collidine/DMF (v/v 15:85, 10 µL, 0.012 mmol) and benzyl bromide (6 µL, 0.048 mmol) at room temperature. The reaction mixture was stirred for 30 min, after which the solvent was removed at room temperature under vacuum. The crude solid was washed with dichloromethane (2 × 2 mL). The crude peptide thioester was dissolved in acetonitrile/water (v/v 1:1, 1 mL) and RP-HPLC purification (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 29 min) provided the peptide thioester **28** (12 mg, 92%) as a white amorphous solid that decomposed prior to melting.

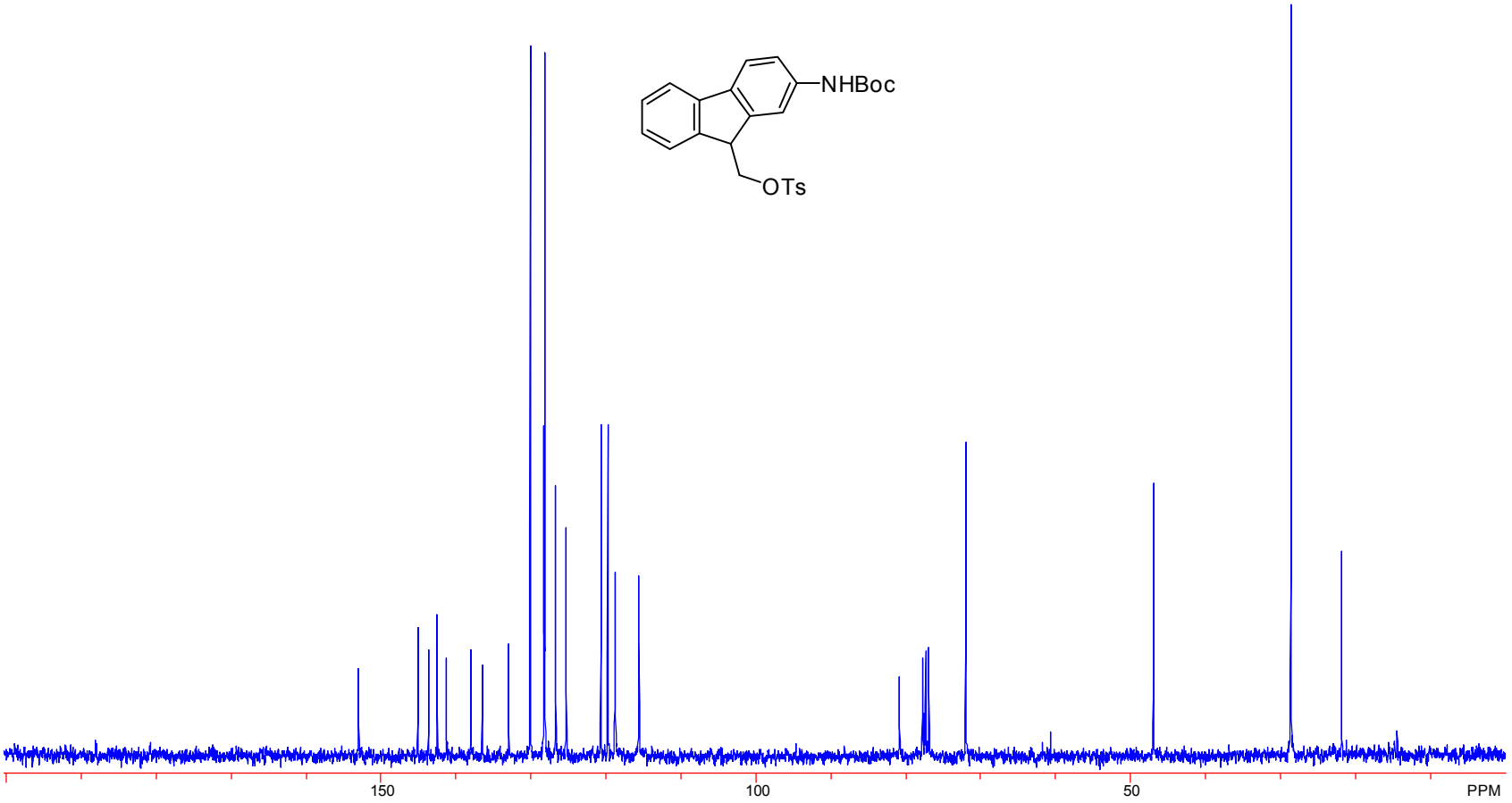
Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Allo)-SBn (29): Following the same procedure as for the synthesis of **28,** using Boc-peptide thioacid **24** and RP-HPLC purification of crude mixture (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 34 min) provided the peptide thioester **29** in 86% yield.

Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-NHSO$_2$-C$_6$H$_4$-NHAc (30): To a stirred solution of **23** (12 mg, 0.0097 mmol) in a mixture of DMF/MeOH (v/v 4:1, 125 μL) was added a solution of 2,4,6-collidine/DMF (v/v 15:85, 10 μL, 0.0116 mmol) followed by stirring for 5 min. 4-Acetamidobenzenesulfonyl azide (4 mg, 0.015 mmol) then was added at room temperature and the reaction mixture stirred for 1 h, after which the solvent was removed at room temperature under vacuum. The crude residue was washed with dichloromethane (2 × 2 mL), then crude peptide thioester was dissolved in acetonitrile/water (v/v 1:1, 1 mL) and RP-HPLC purification of crude mixture (0- 50% A in B with a flow rate of 8 mL/min over 60 min and 215 nm UV detection, retention time = 26 min) provided **30** (11 mg, 80%) as white amorphous solid that decomposed prior to melting.

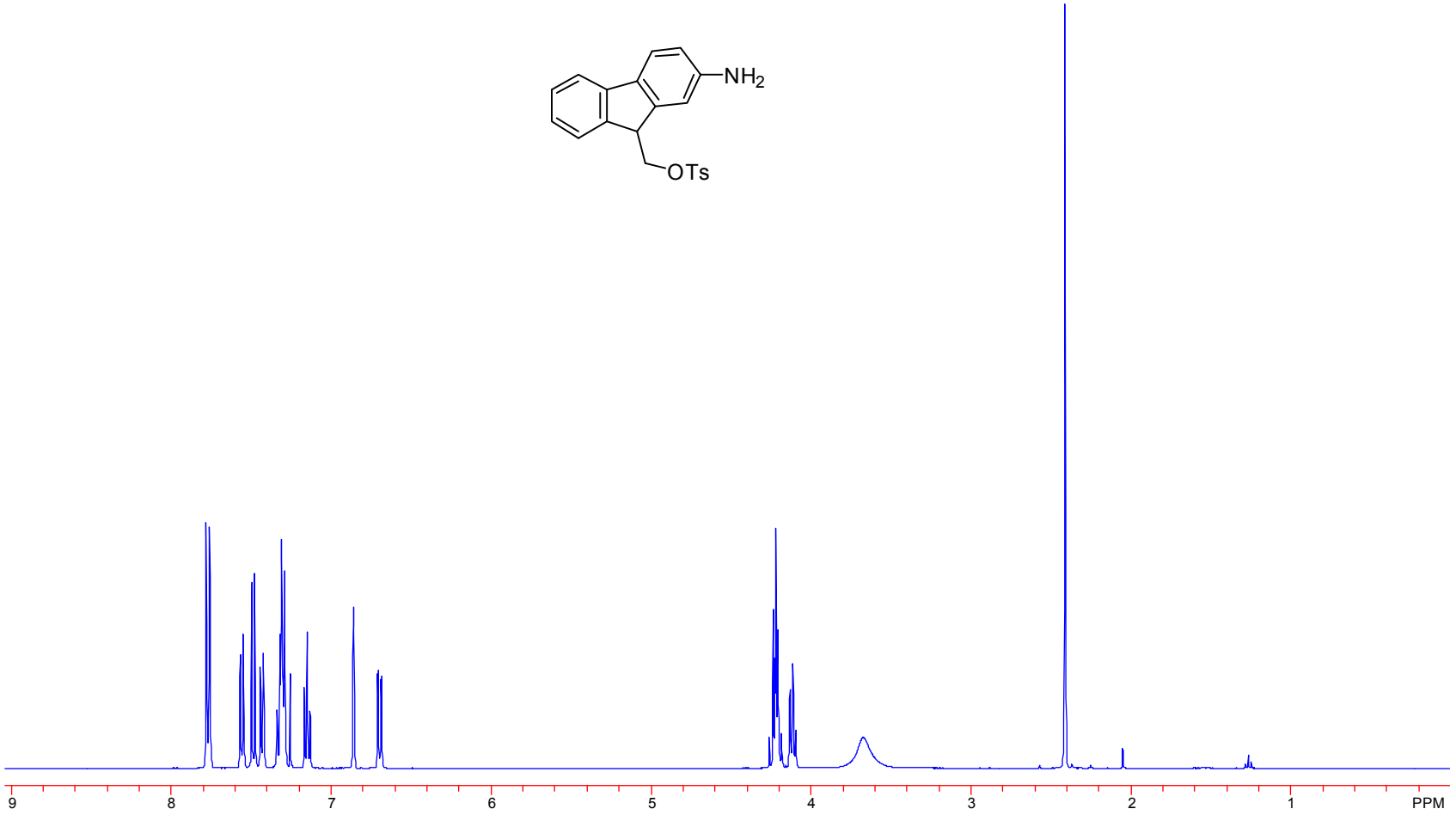
[2-(*tert*-Butoxycarbonylamino)-9*H*-fluoren-9-yl]methyl 4-methylbenzenesulfonate (2)
(500 MHz, $CDCl_3$)



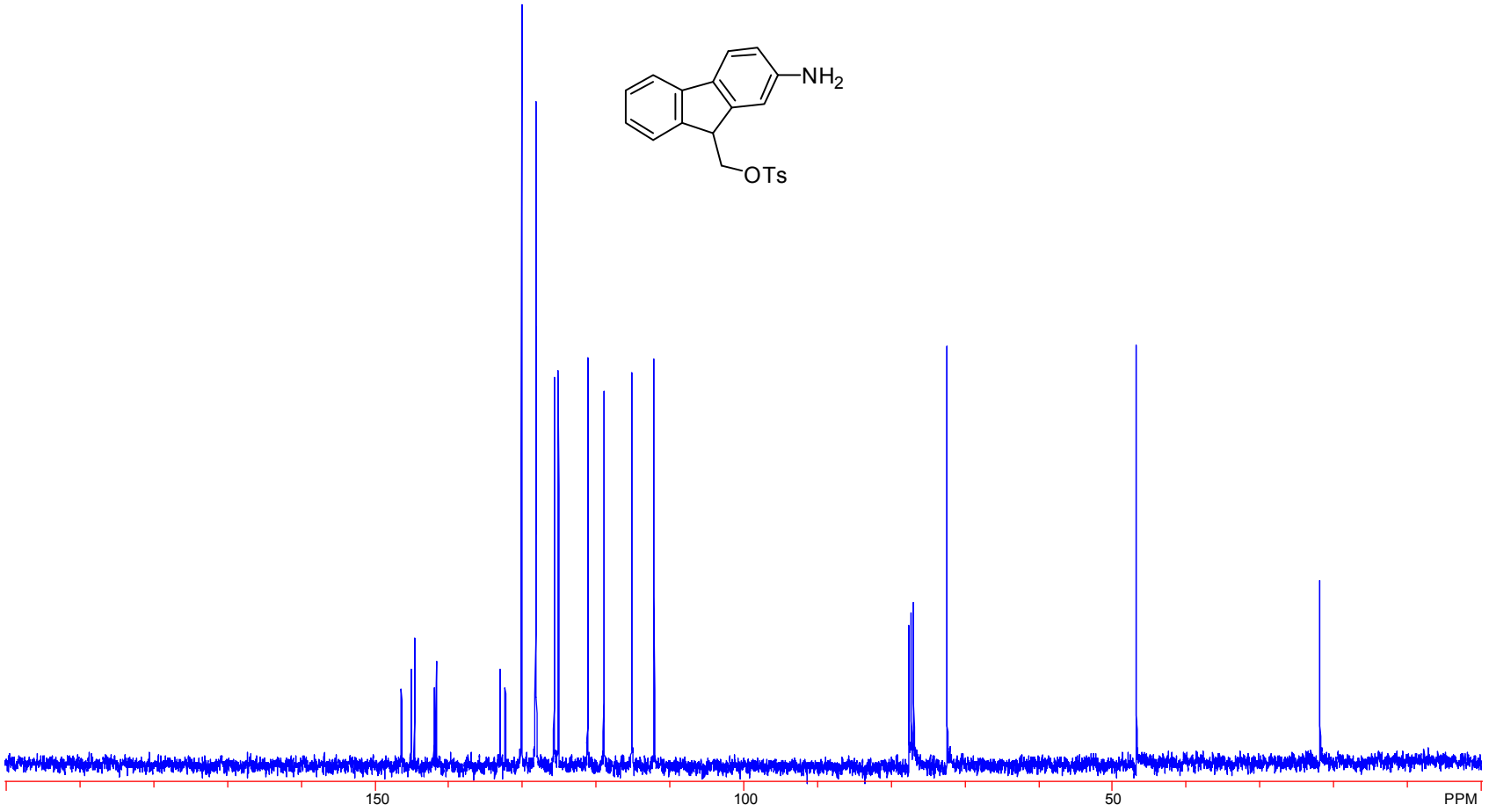
[2-(*tert*-Butoxycarbonylamino)-9*H*-fluoren-9-yl]methyl 4-methylbenzenesulfonate (2)
(125 MHz, $CDCl_3$)



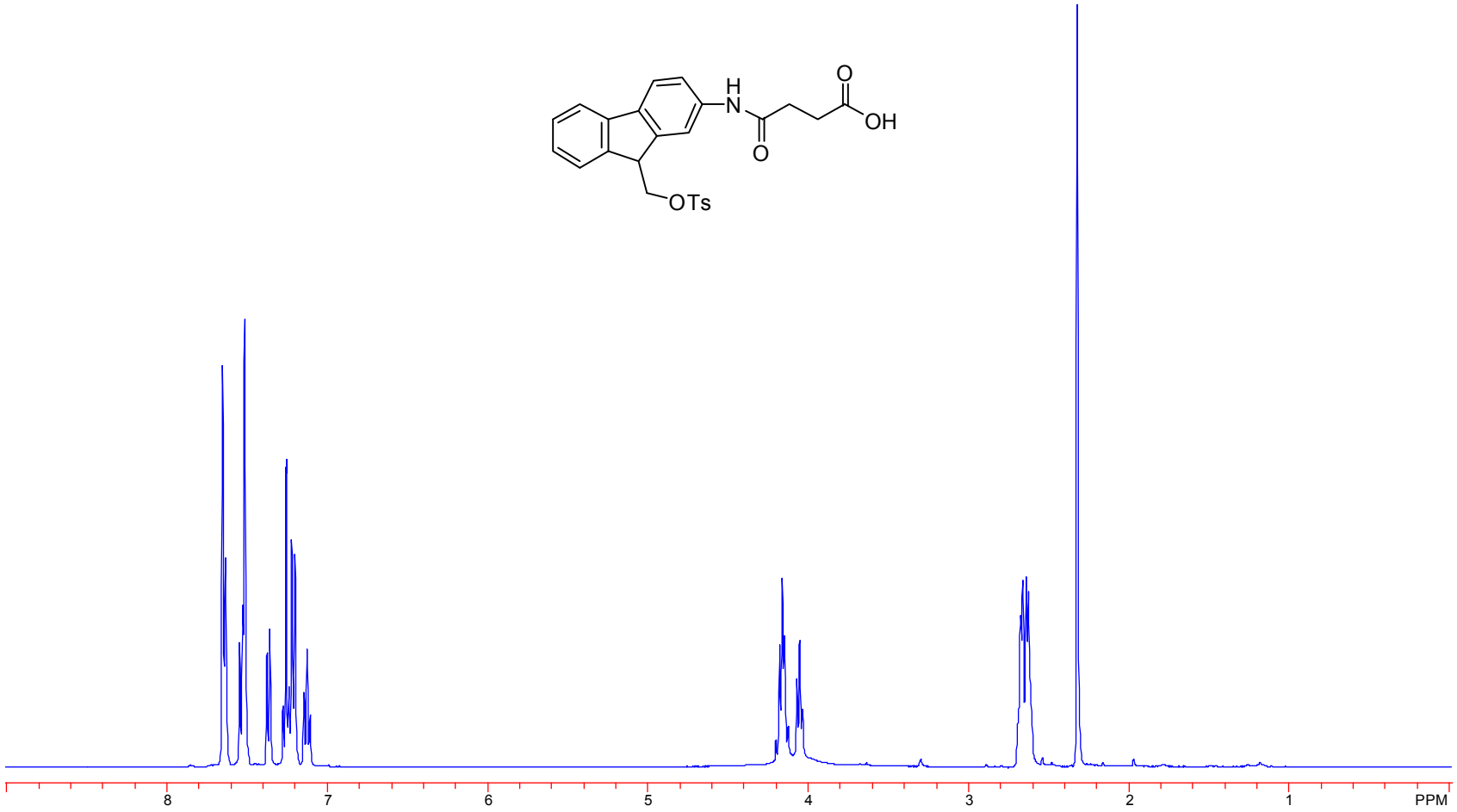
(2-Amino-9*H*-fluoren-9-yl)methyl 4-methylbenzenesulfonate (3)
(400 MHz, $CDCl_3$)



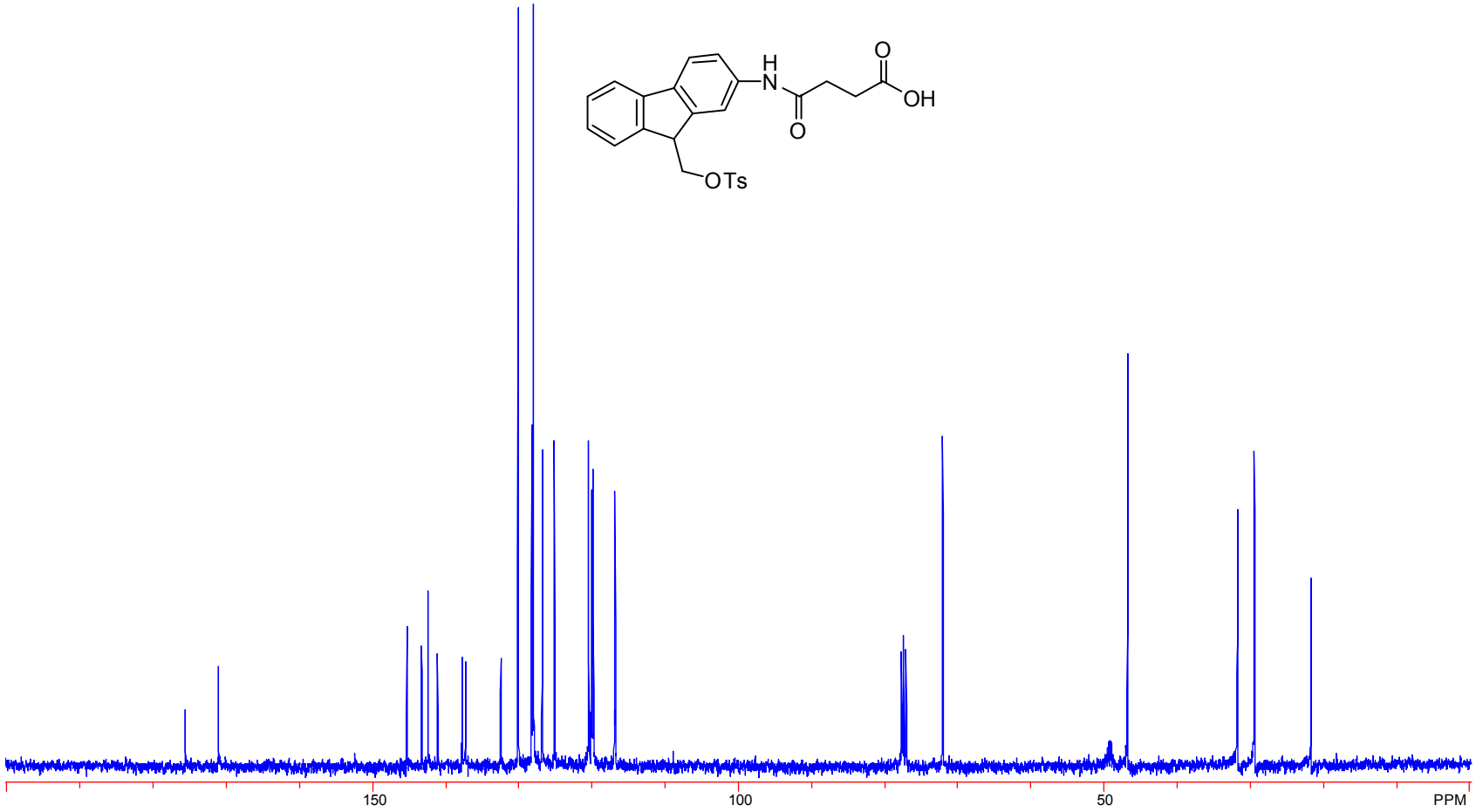
(2-Amino-9*H*-fluoren-9-yl)methyl 4-methylbenzenesulfonate (3)
(100 MHz, $CDCl_3$)



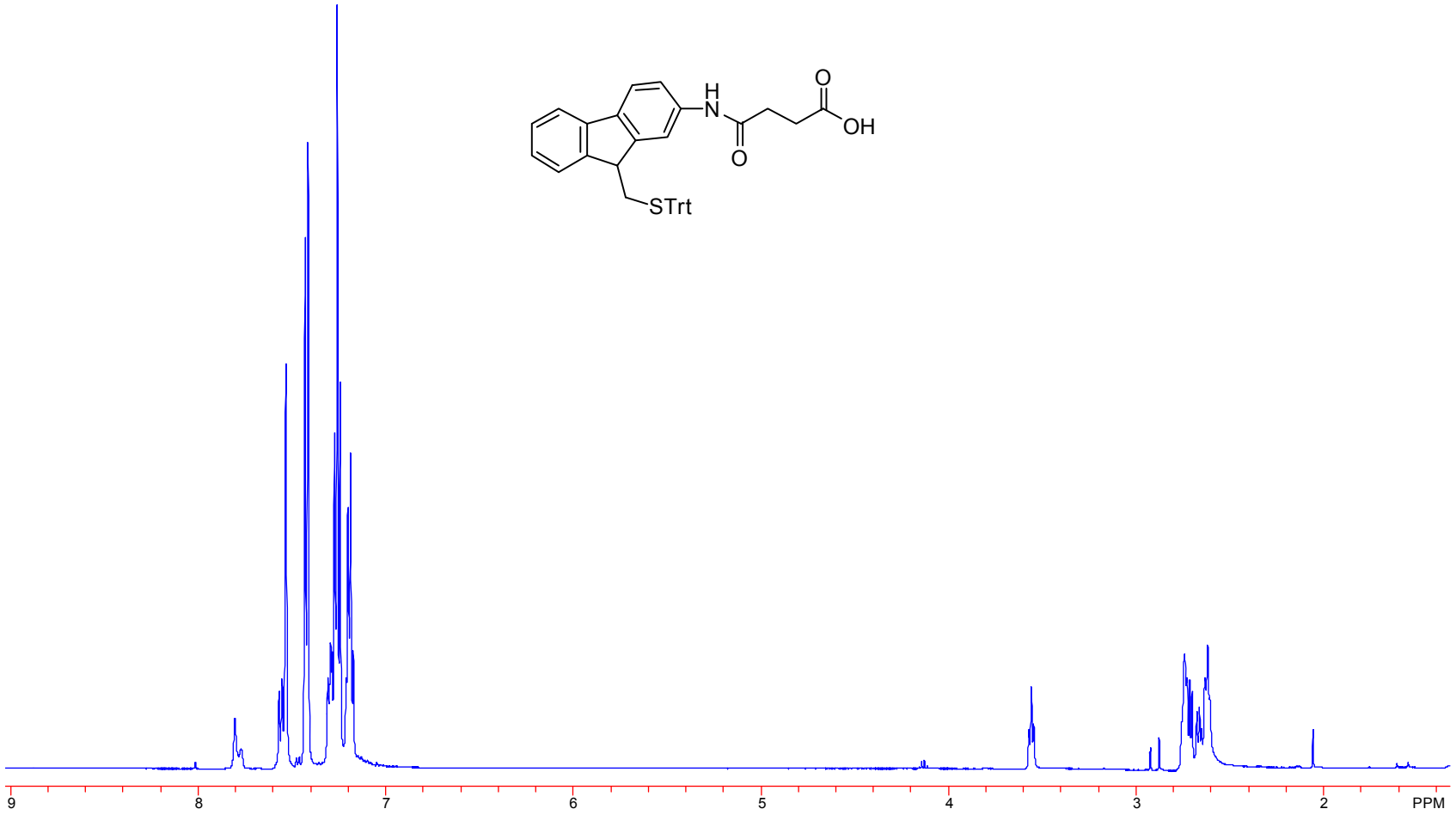
_N_-[9-(Tosyloxymethyl)-9_H_-fluoren-2-yl]succinamic acid (4)
(400 MHz, $CDCl_3$ + 2-3 drops of CD_3OD)



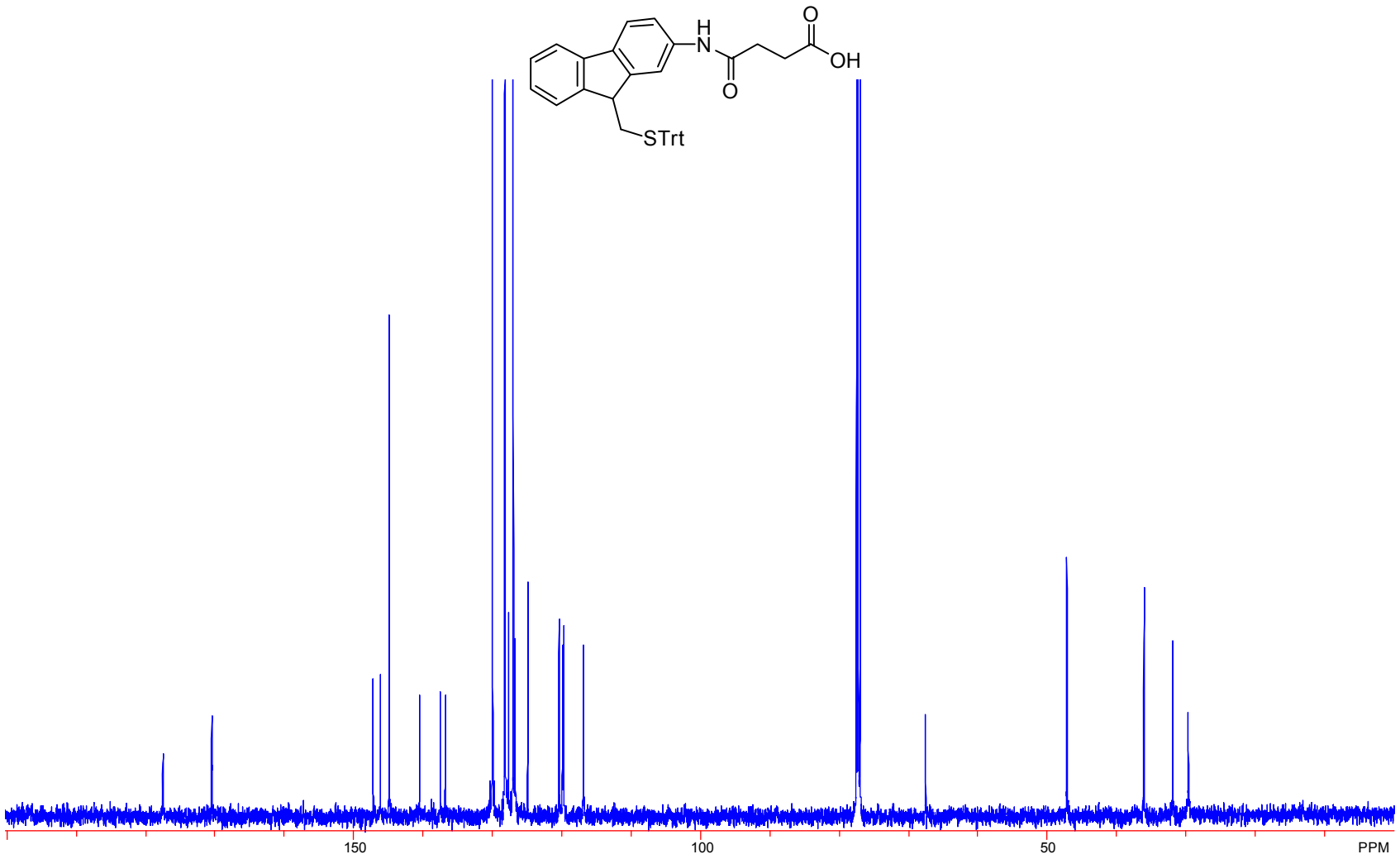
_N_-[9-(Tosyloxymethyl)-9_H_-fluoren-2-yl]succinamic acid (4)
(100 MHz, $CDCl_3$ + 2-3 drops of CD_3OD)



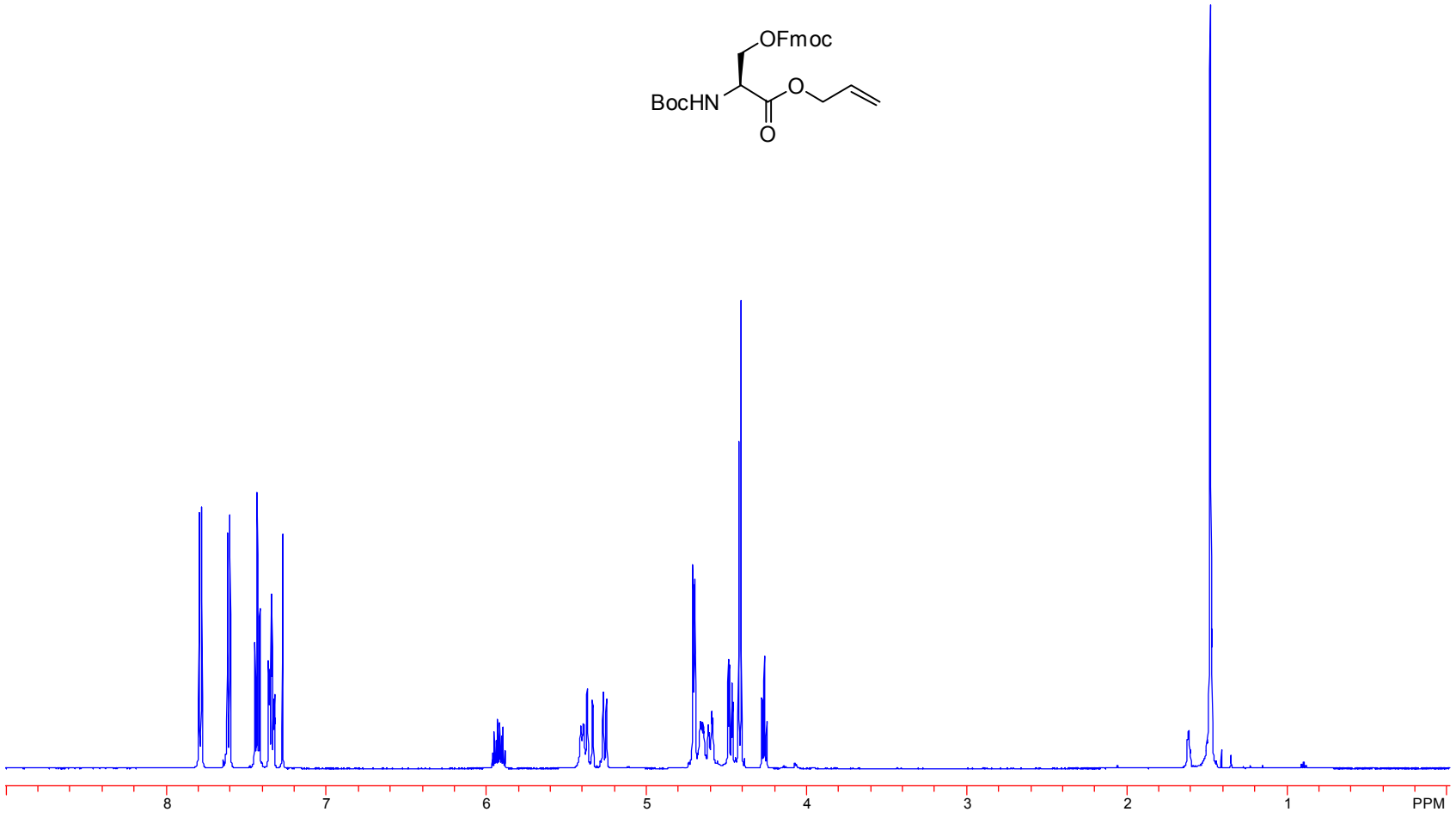
_N_-[9-(Tritylthiomethyl)-9_H_-fluoren-2-yl]succinamic acid (5)
(500 MHz, $CDCl_3$)



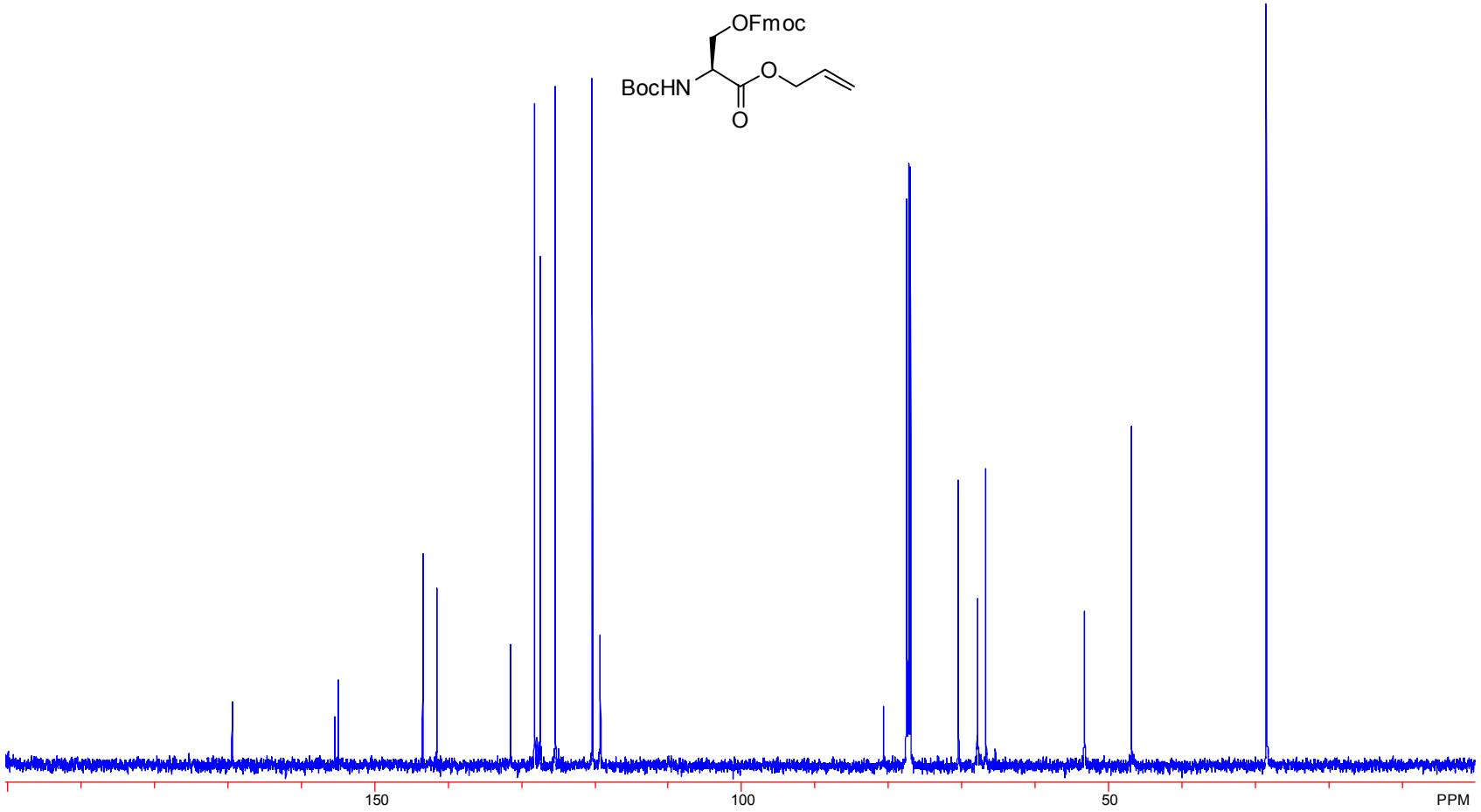
_N_-[9-(Tritylthiomethyl)-9_H_-fluoren-2-yl]succinamic acid (5)
(125 MHz, $CDCl_3$)



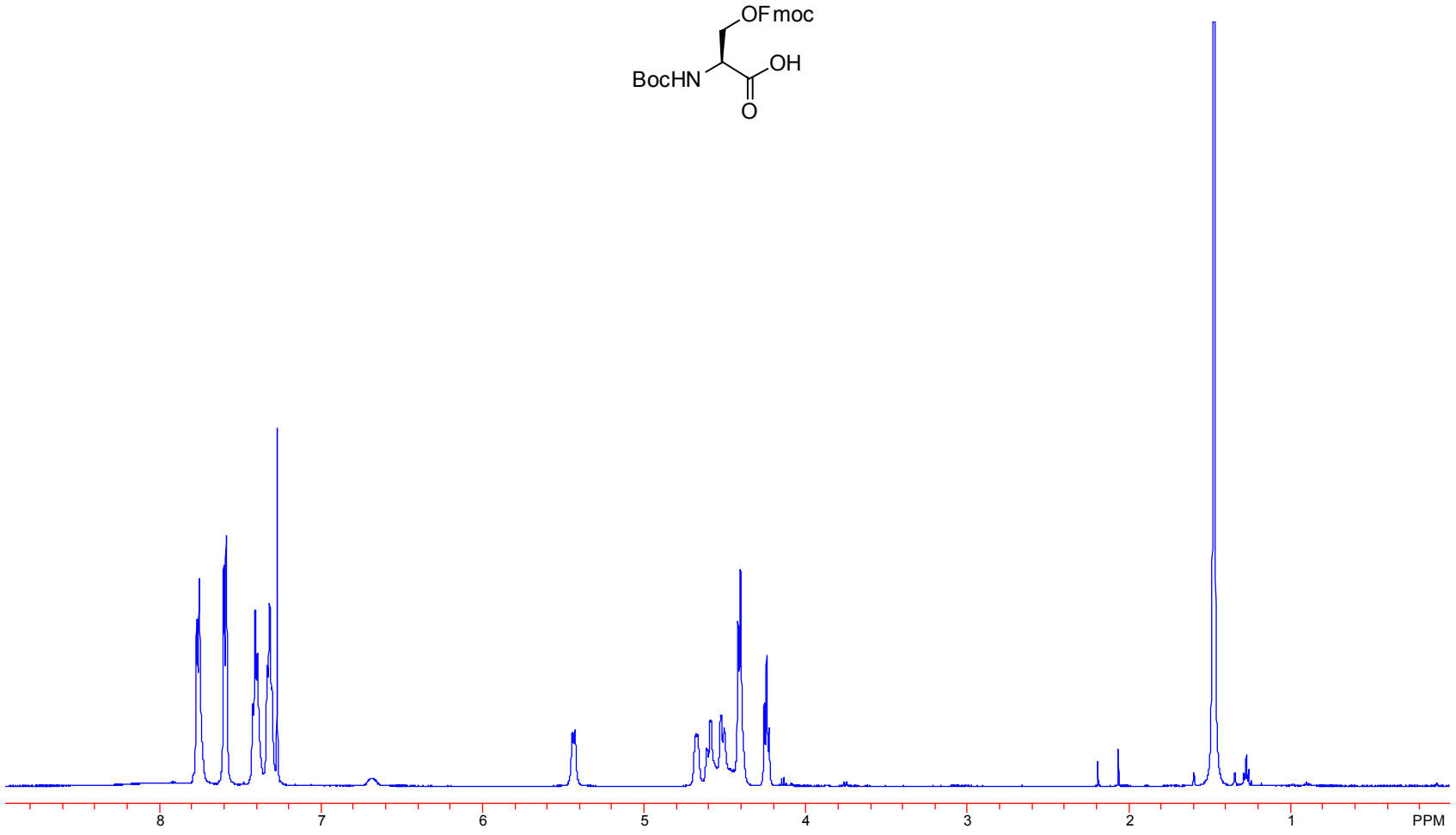
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (7)
(500 MHz, $CDCl_3$)



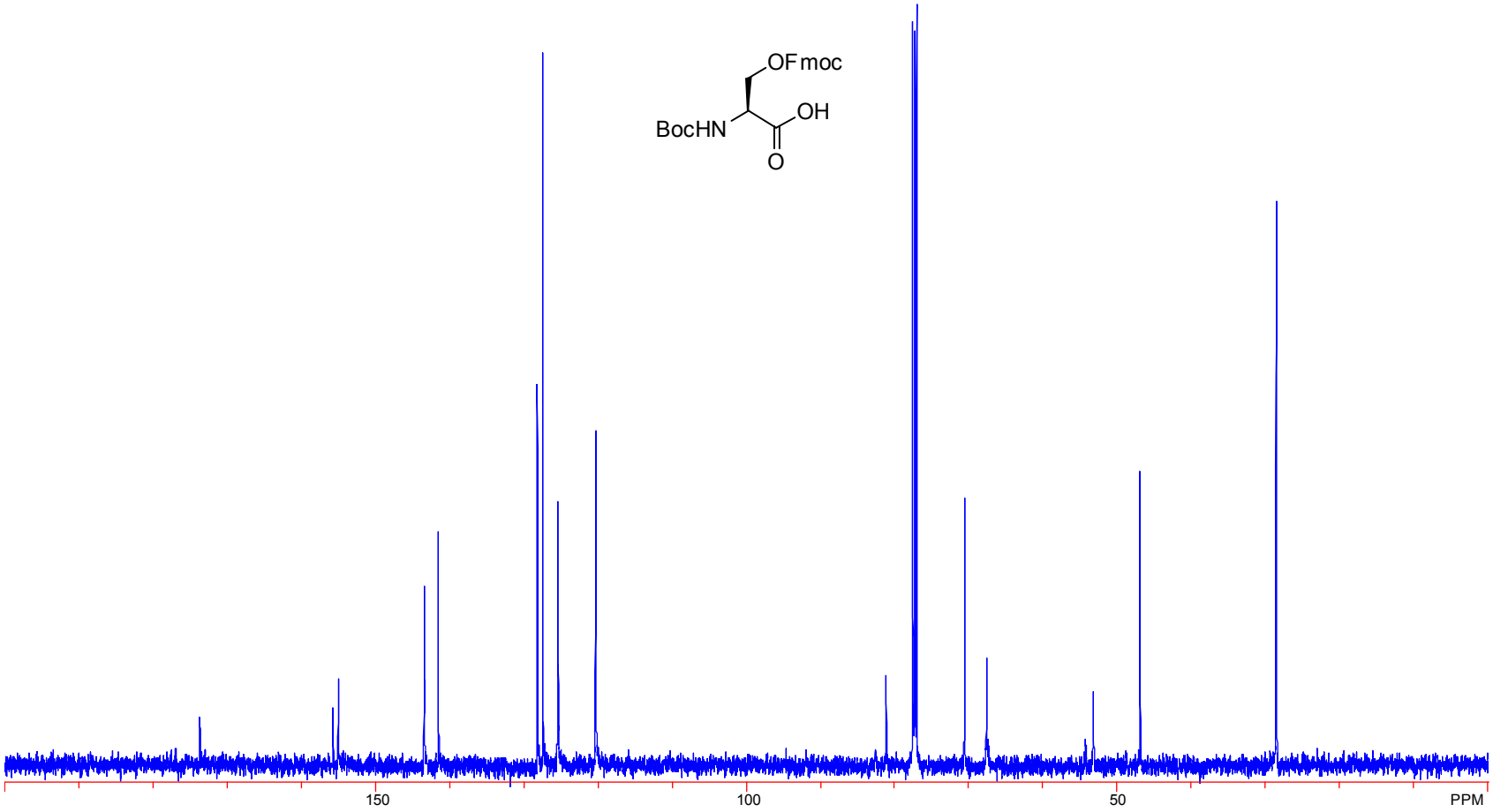
N-tert-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-serine allyl ester (7)
(125 MHz, $CDCl_3$)



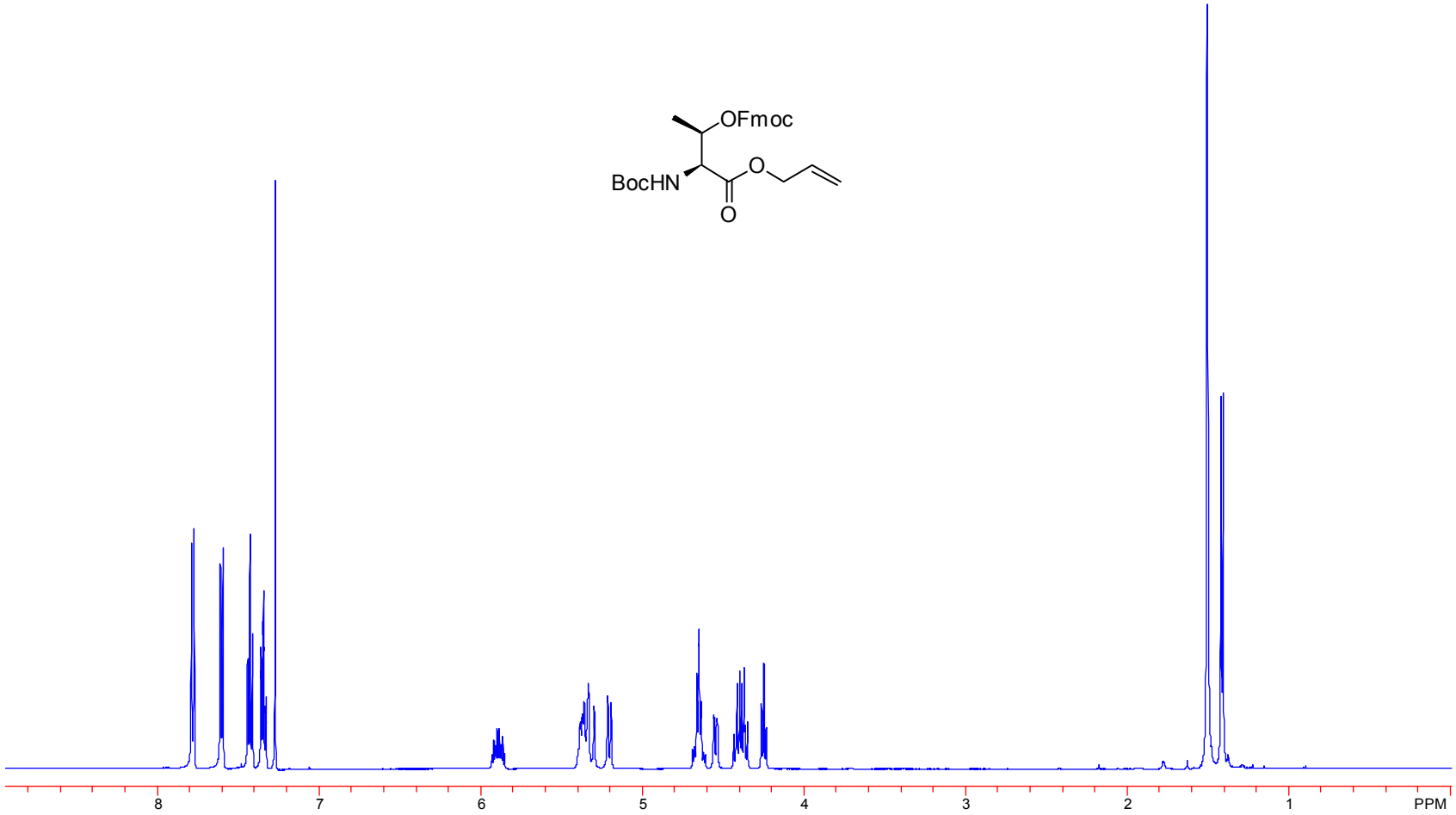
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-serine (8)
(500 MHz, $CDCl_3$)



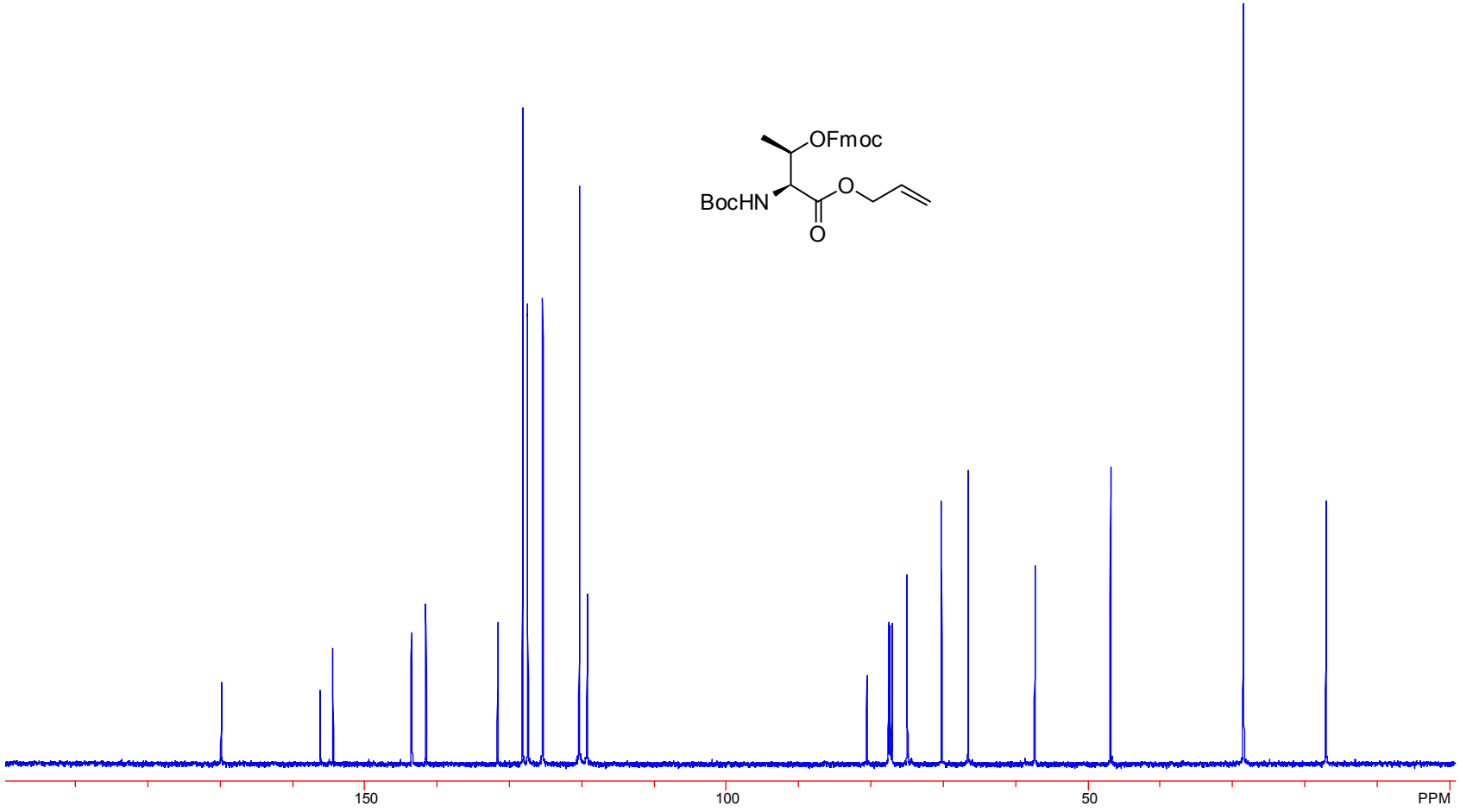
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-serine (8)
(125 MHz, $CDCl_3$)



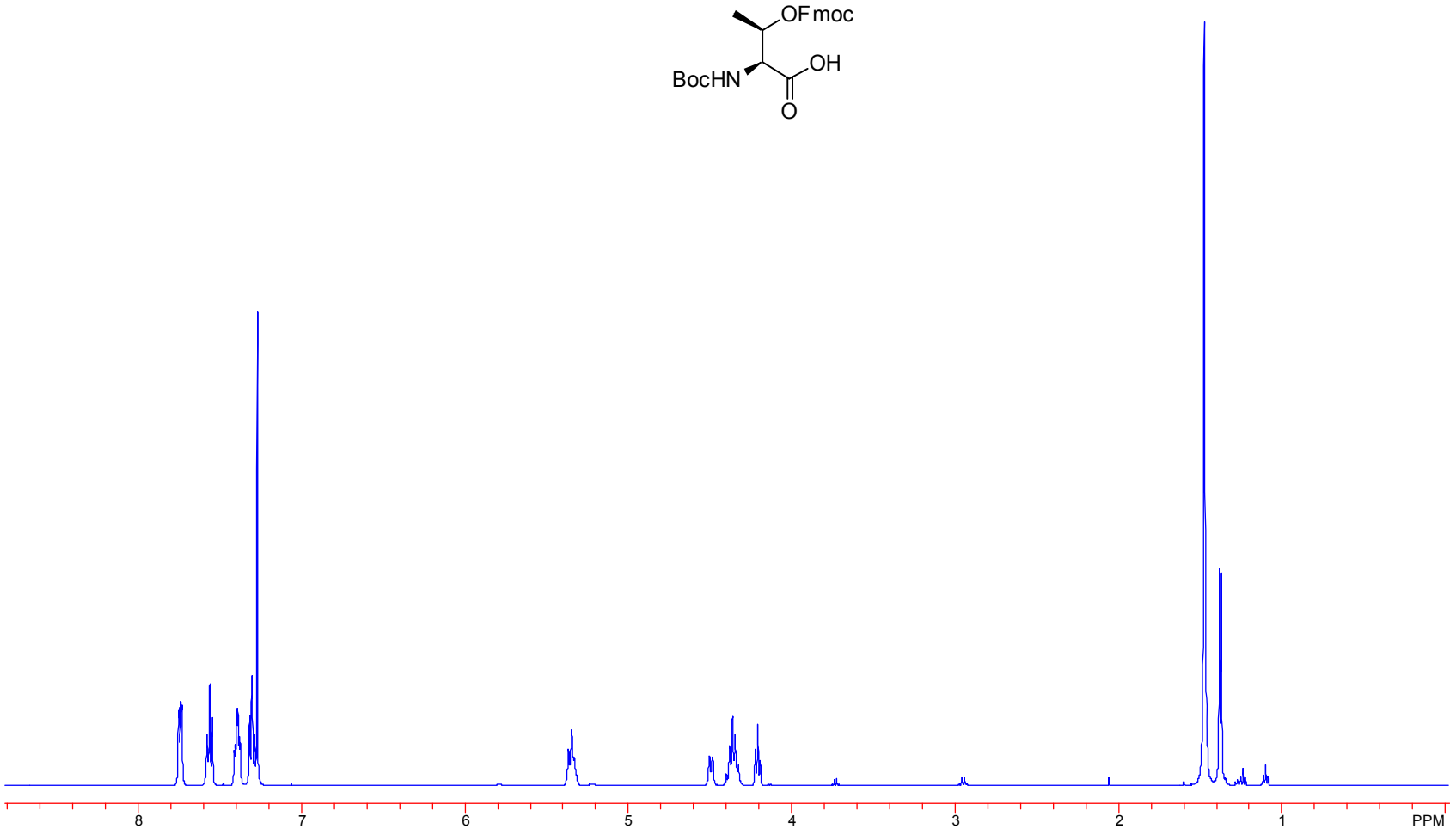
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-threonine allyl ester (9)
(500 MHz, $CDCl_3$)



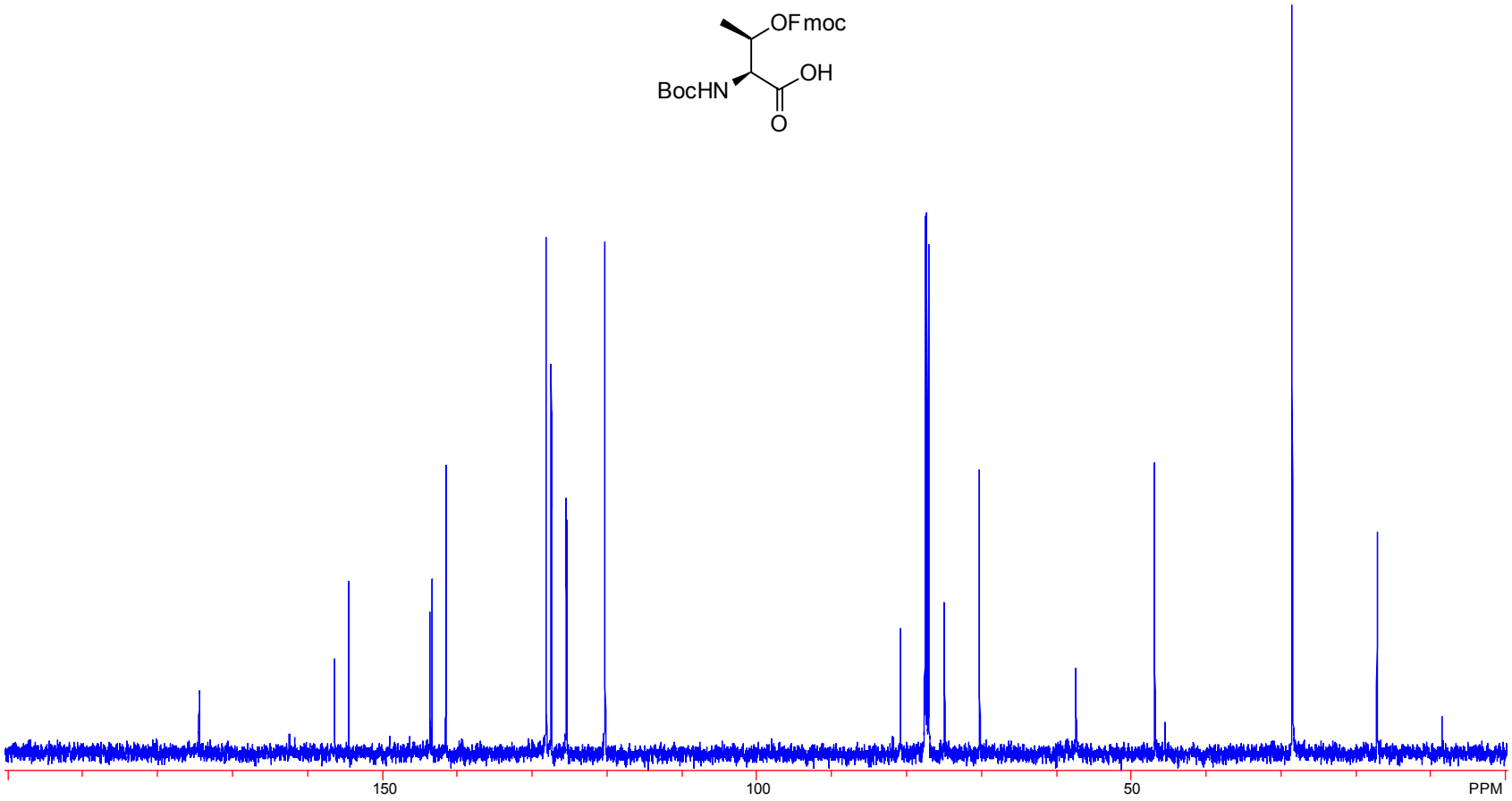
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-threonine allyl ester (9)
(125 MHz, $CDCl_3$)



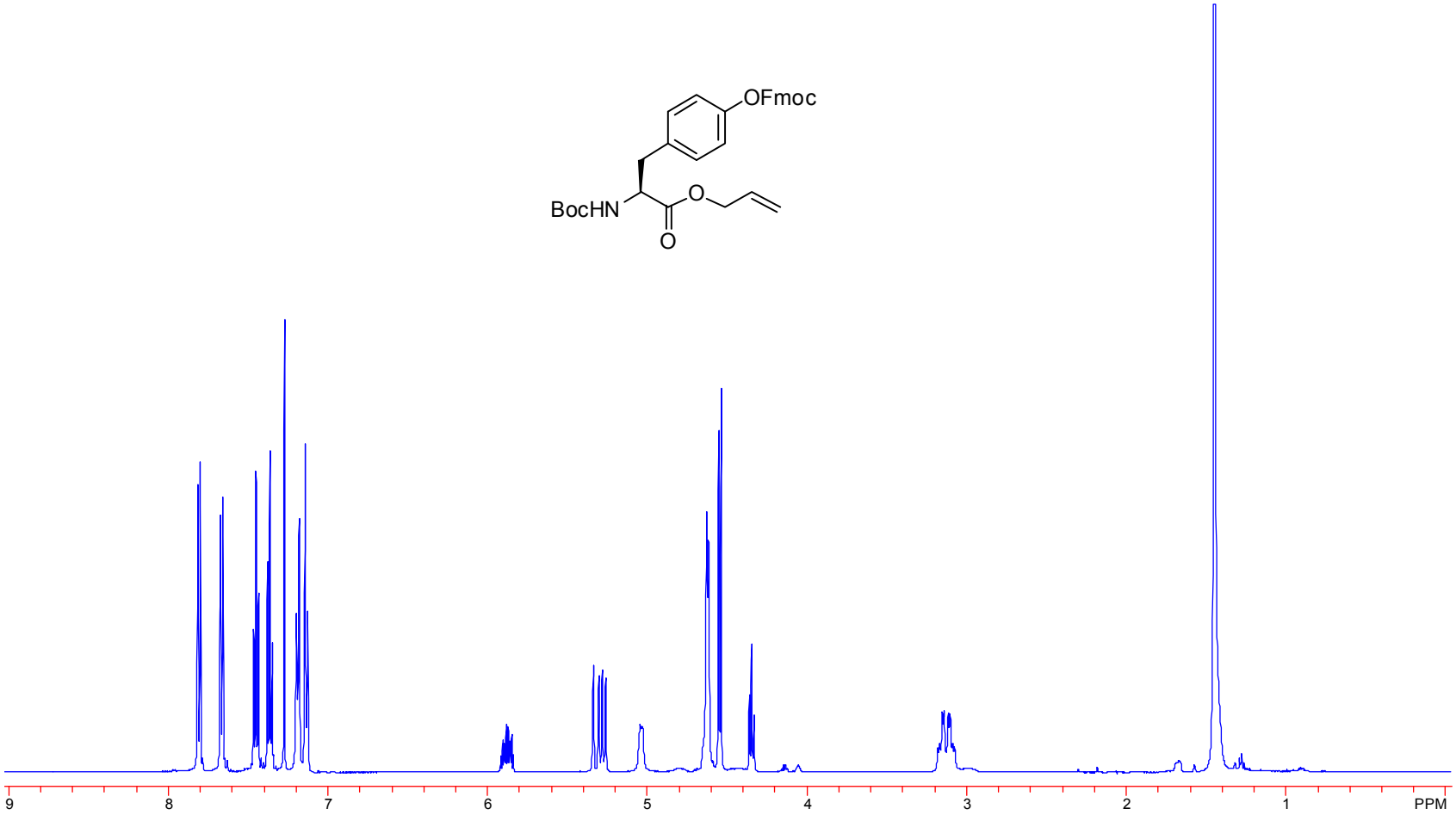
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-threonine (10)
(500 MHz, $CDCl_3$)



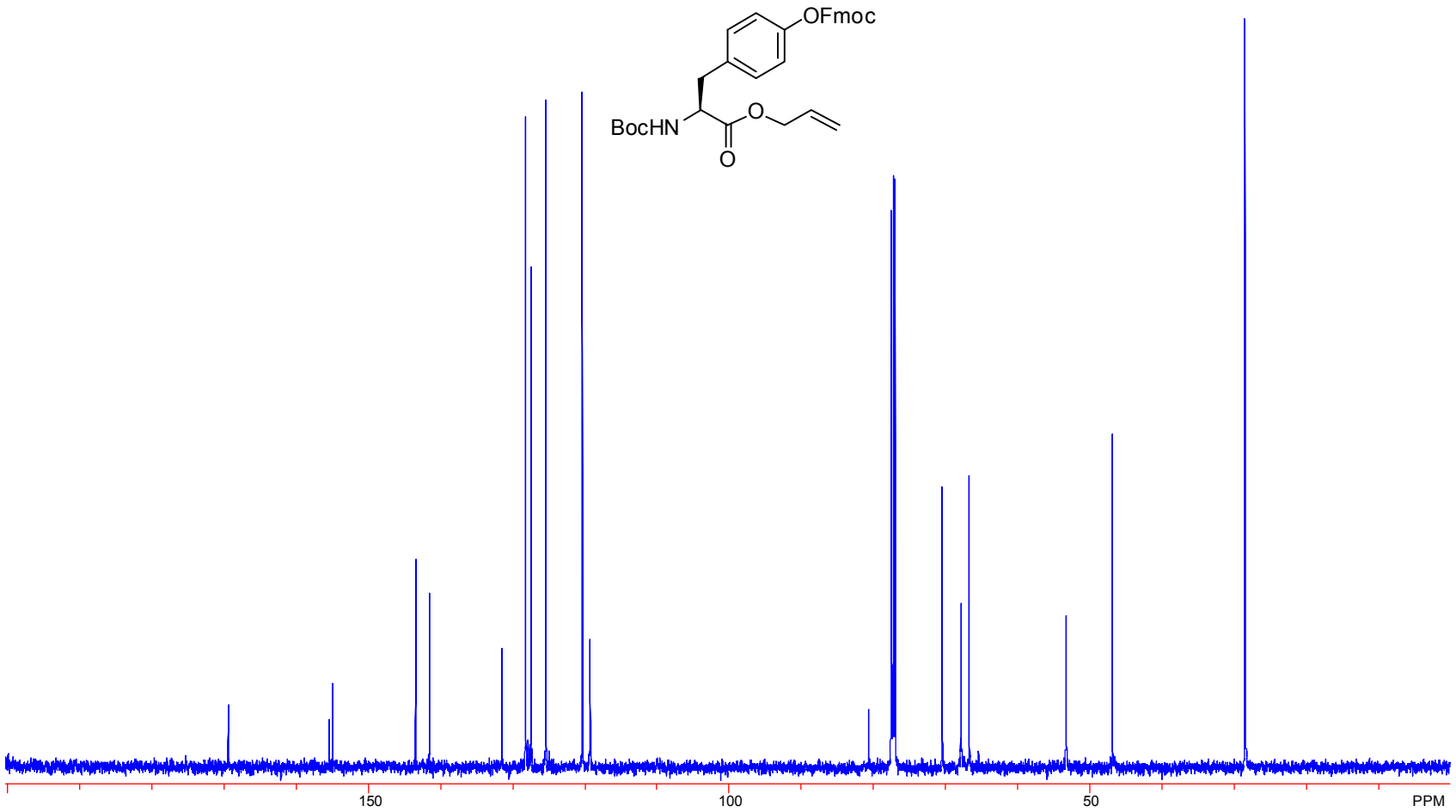
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-threonine (10)
(125 MHz, $CDCl_3$)



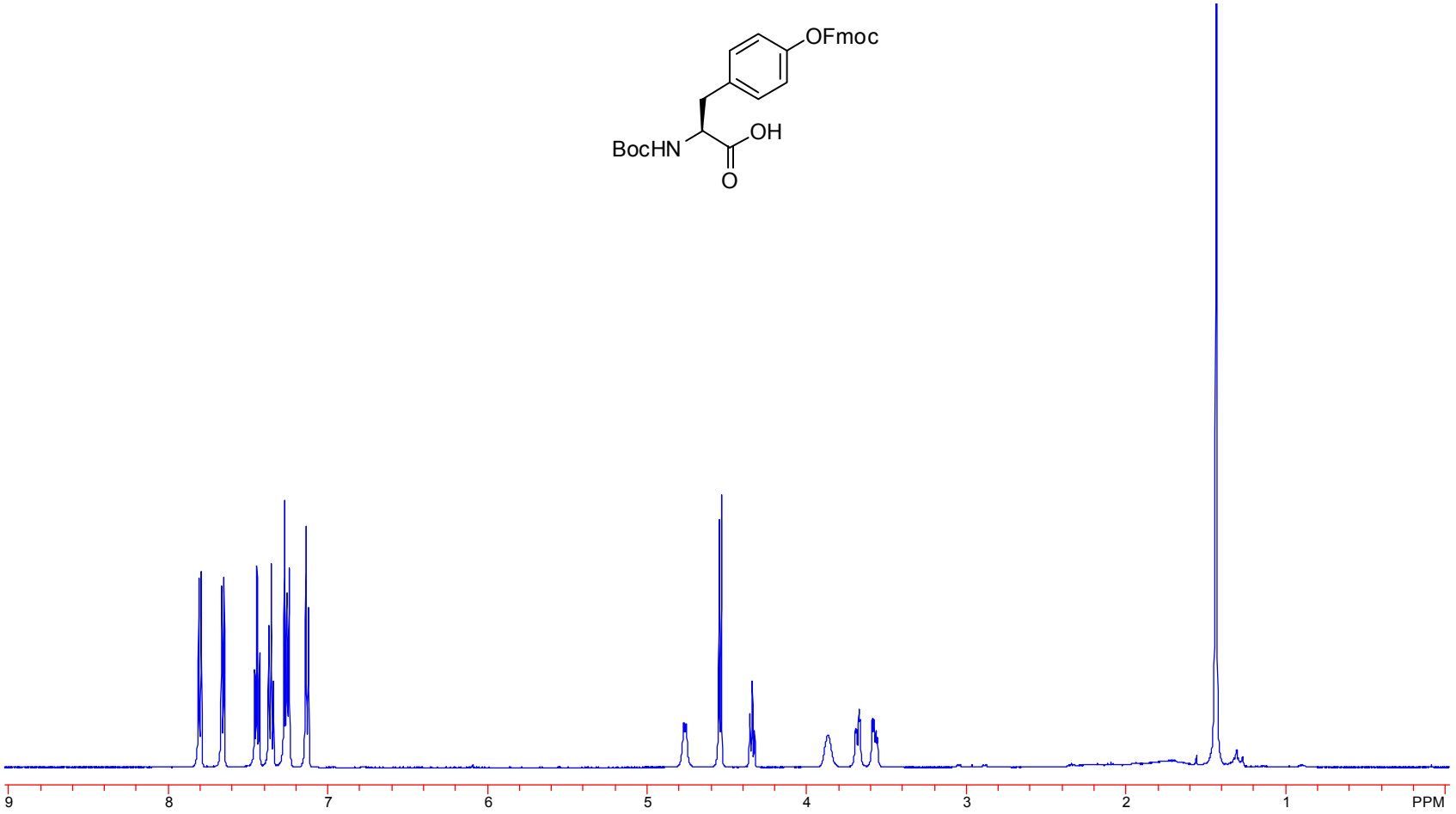
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-tyrosine allyl ester (11)
(500 MHz, $CDCl_3$)



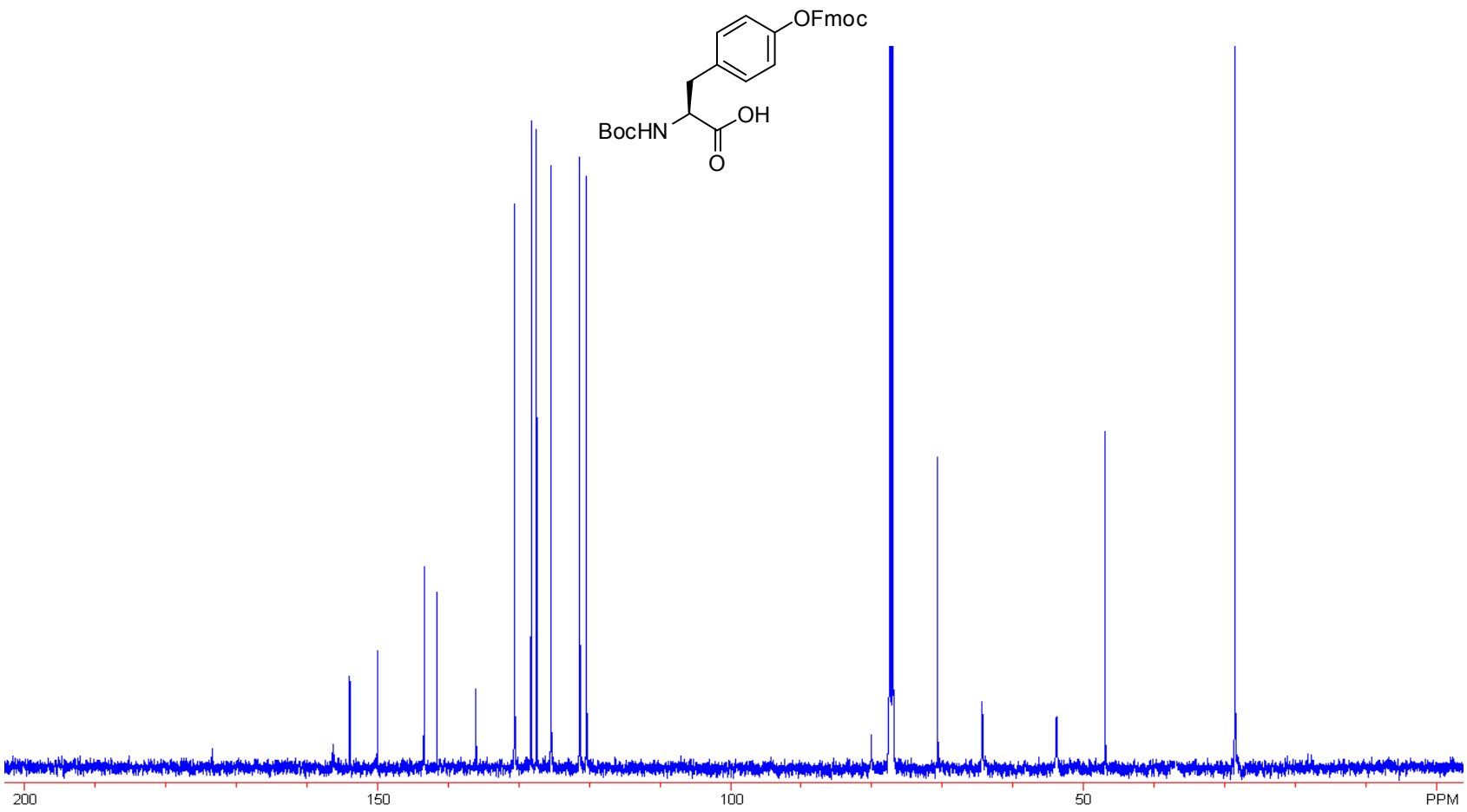
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-tyrosine allyl ester (11)
(125 MHz, $CDCl_3$)



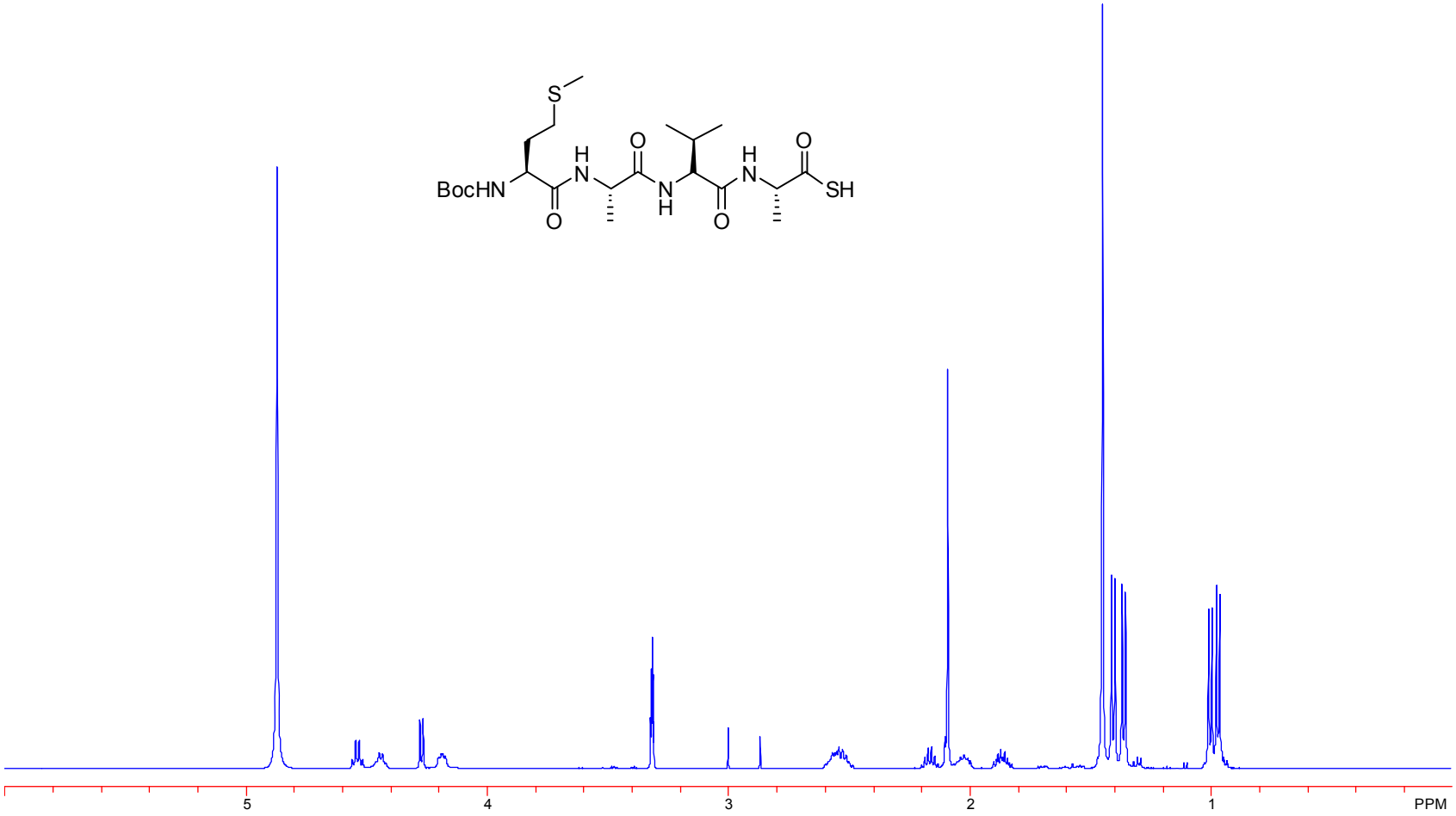
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-tyrosine (12)
(500 MHz, $CDCl_3$)



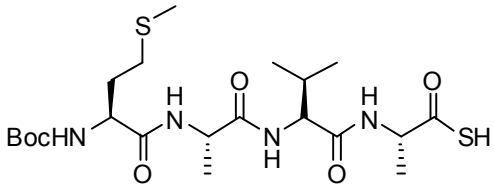
_N_-_tert_-Butoxycarbonyl-_O_-(9-fluorenylmethoxycarbonyl)-L-tyrosine (12)
(125 MHz, $CDCl_3$)



Boc-L-Met-L-Ala-L-Val-L-Ala-SH (22)
(500 MHz, CD_3OD)

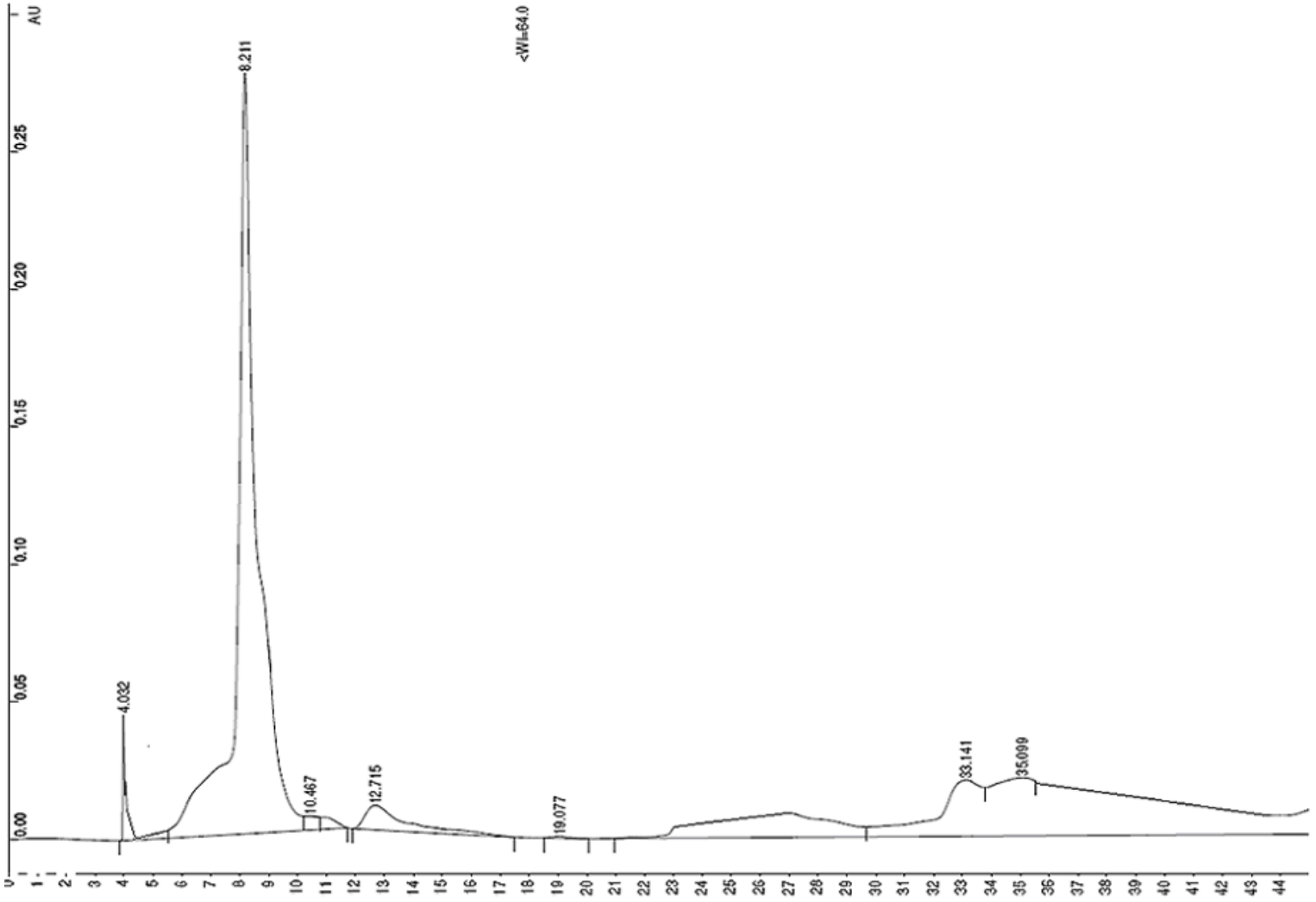


Boc-L-Met-L-Ala-L-Val-L-Ala-SH (22)
(125 MHz, $CDCl_3$)



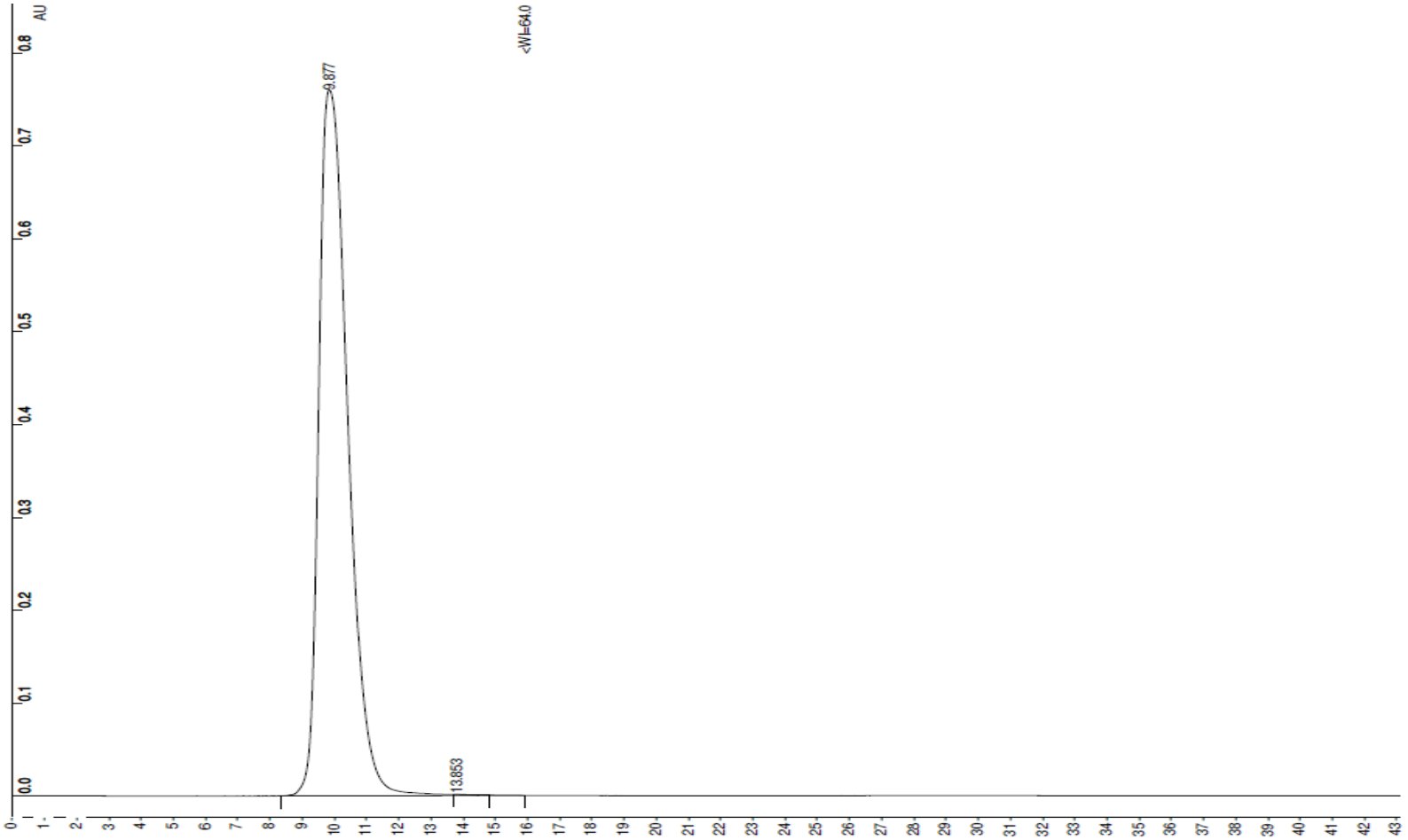
Analytical RP-HPLC trace of crude Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH (23)

Analytical RP-HPLC: 25 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 8.21 min.

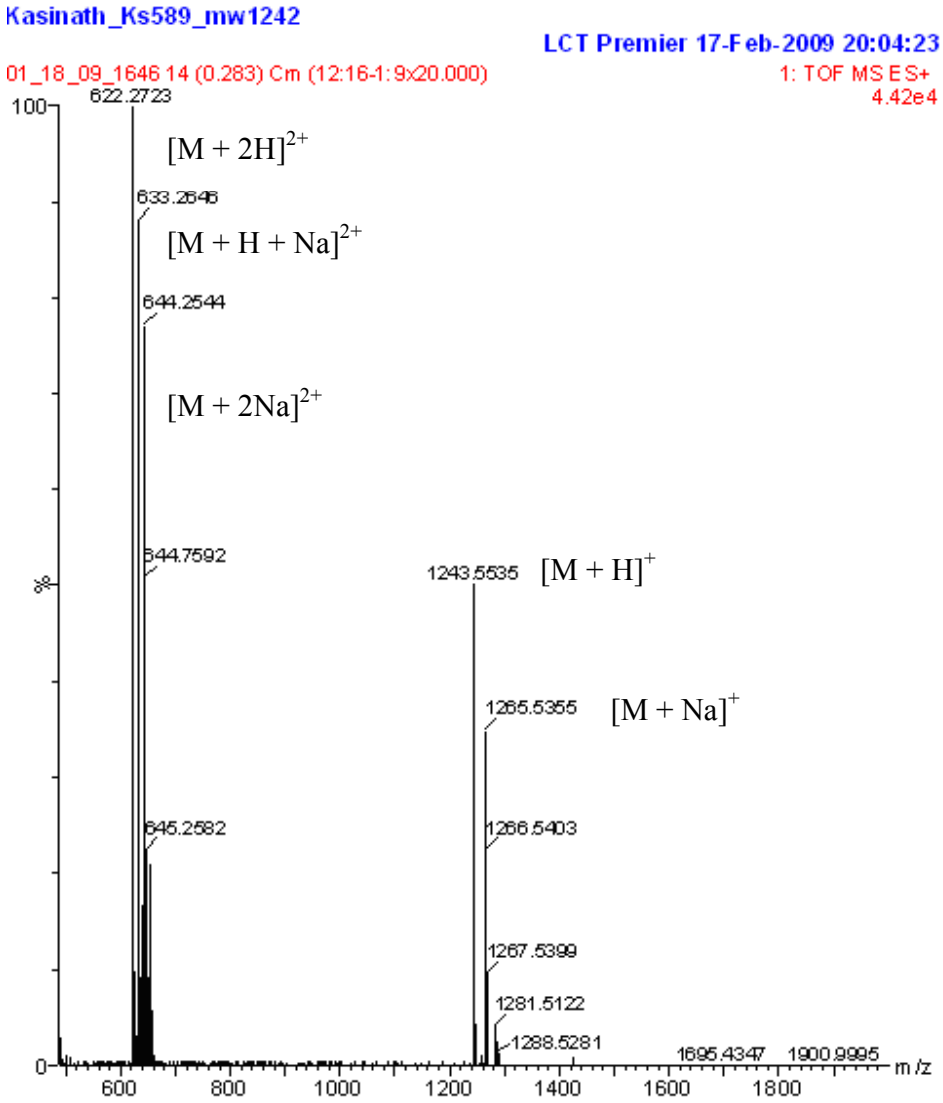


Analytical RP-HPLC trace of purified Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH (23)

Analytical RP-HPLC: 20 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 9.88 min.



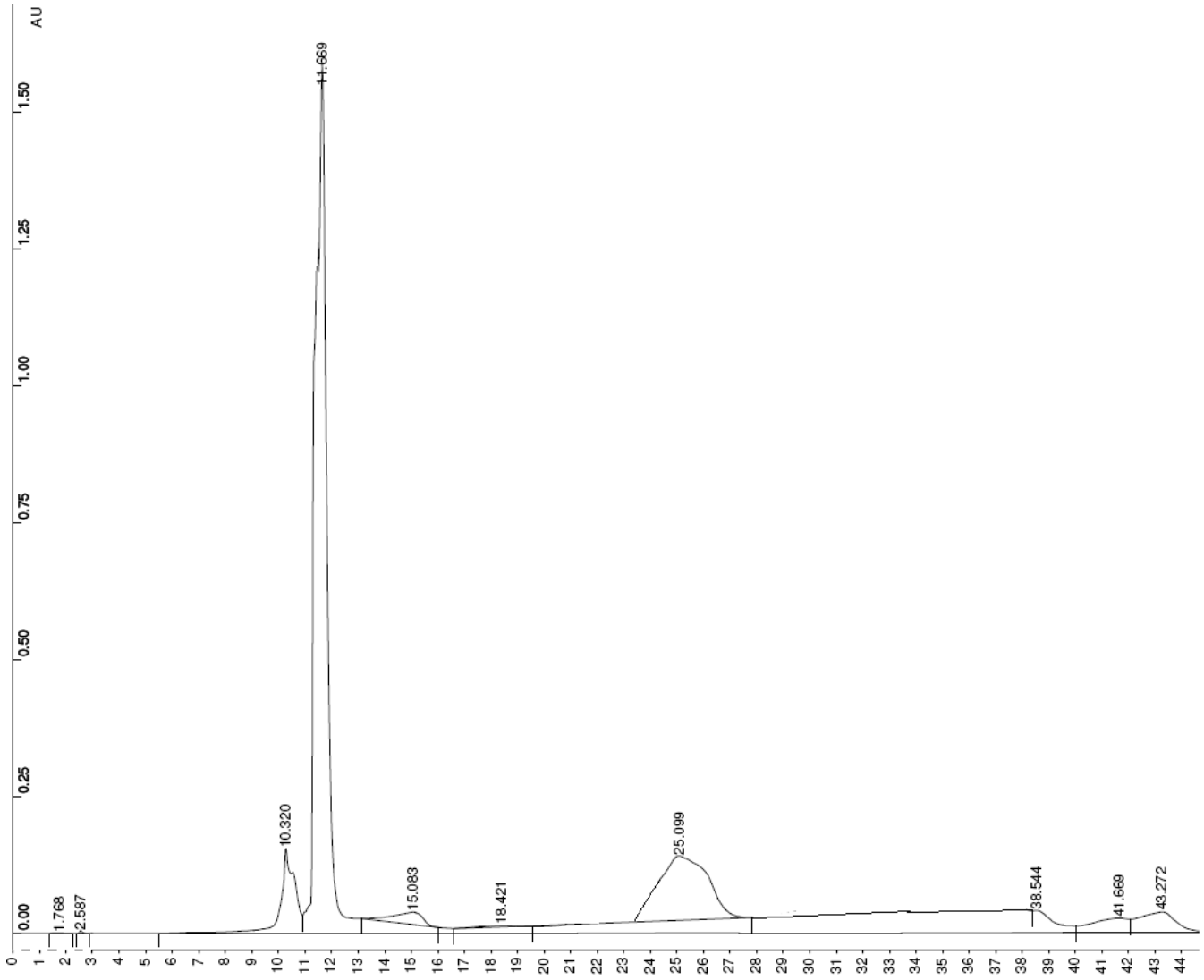
ESI-TOF mass spectrum of Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SH (23)



(m/z = 1242.5227)

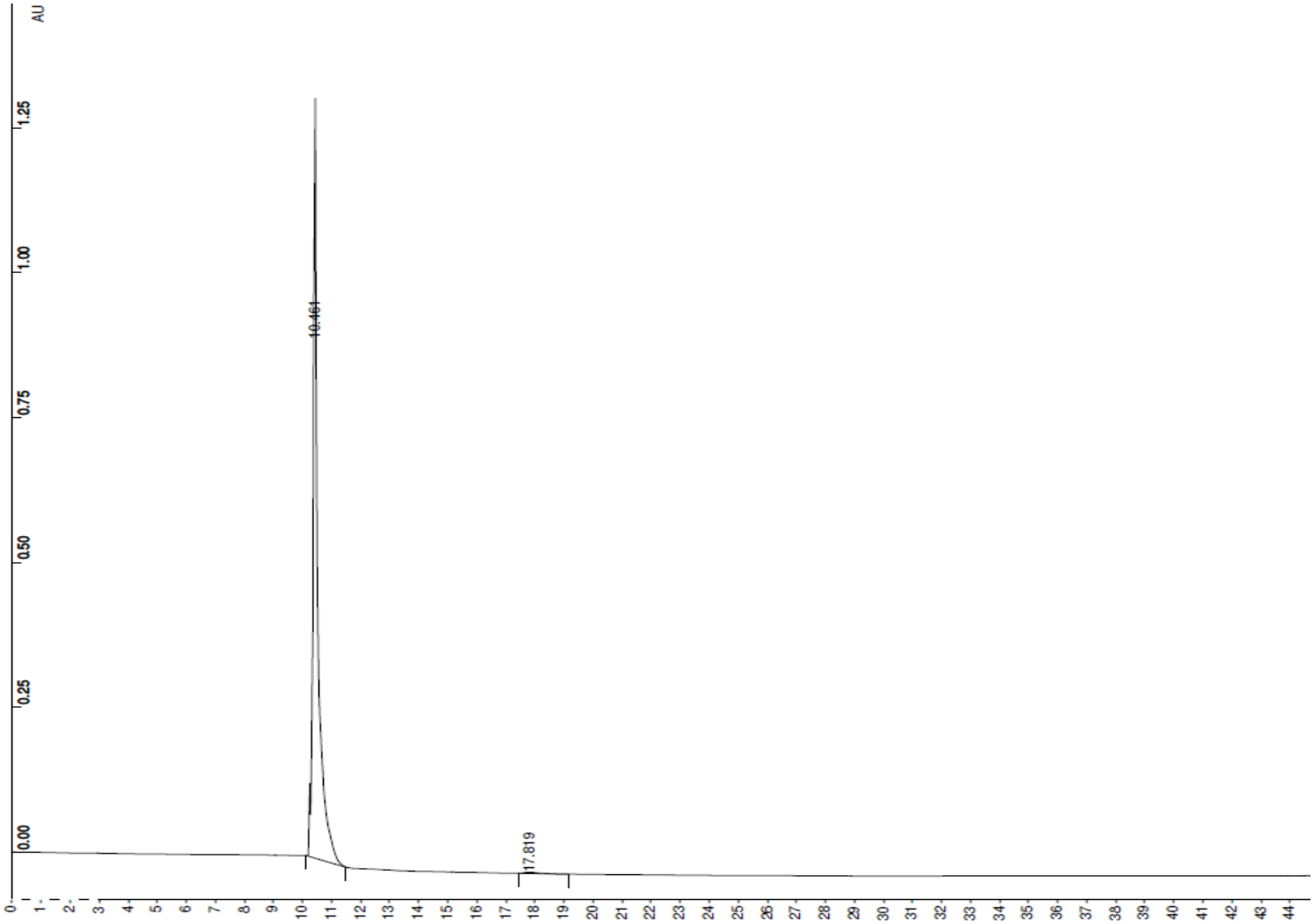
Analytical RP-HPLC trace of crude Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH (24)

Analytical RP-HPLC: 20 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 11.07 min.

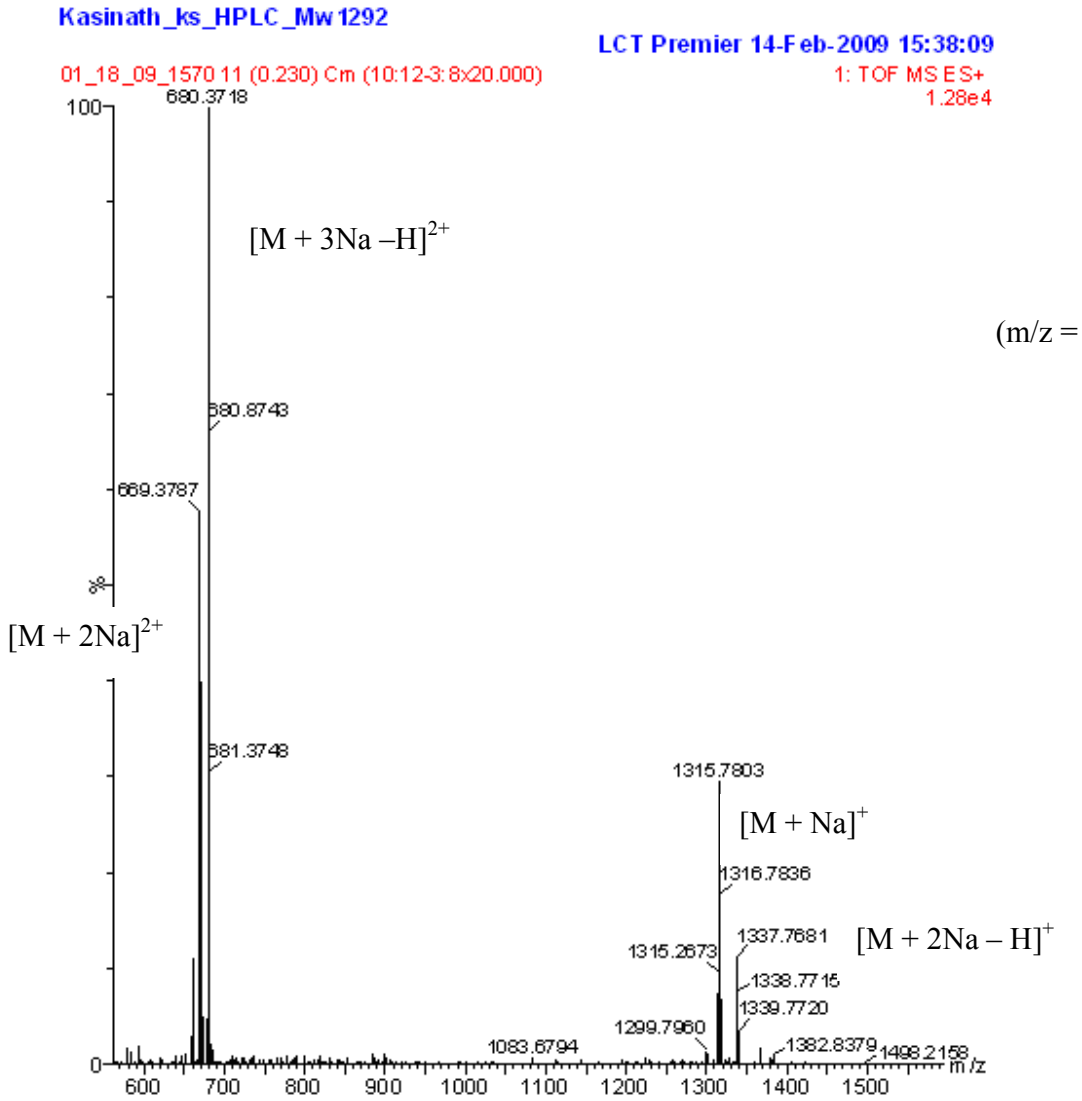


Analytical RP-HPLC trace of purified Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH (24)

Analytical RP-HPLC: 20 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 10.46 min.



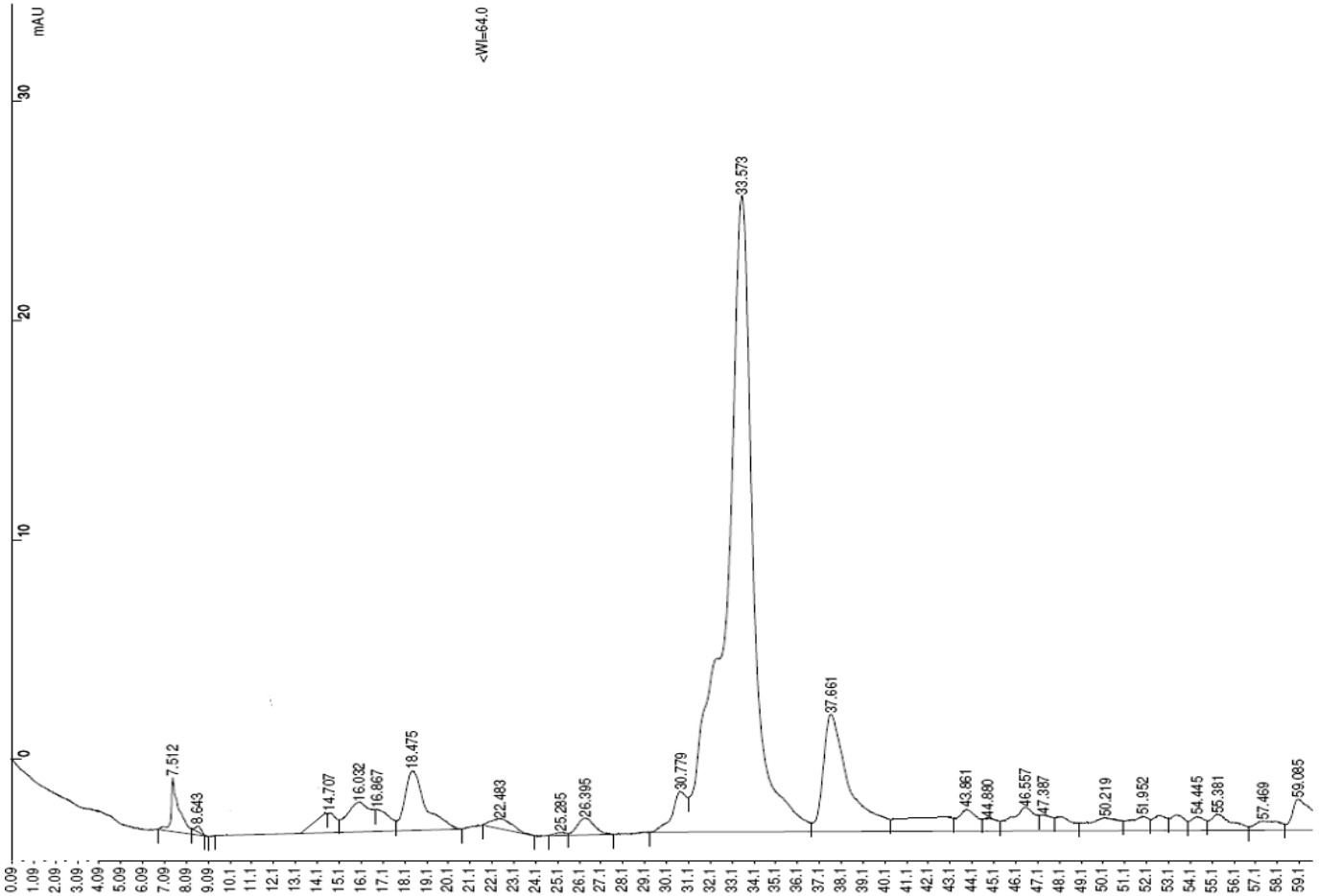
ESI-TOF mass spectrum of Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Alloc)-SH (24)



Analytical RP-HPLC trace of crude mixture of Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH (25) and

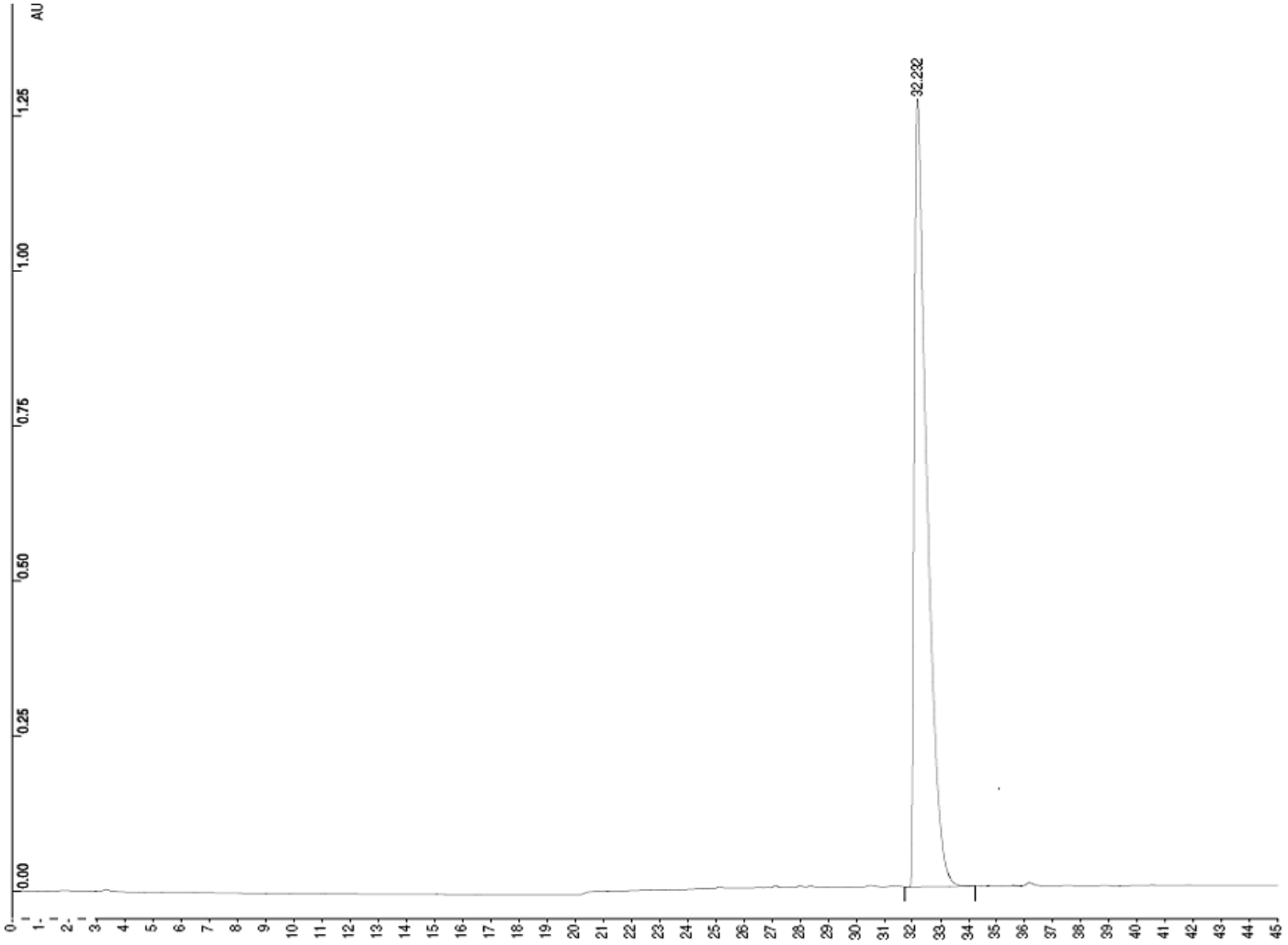
Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH **(26)**

Analytical RP-HPLC: 0 - 50% A in B over 60 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 33.57 min of **25** and 37.66 min of **26**.

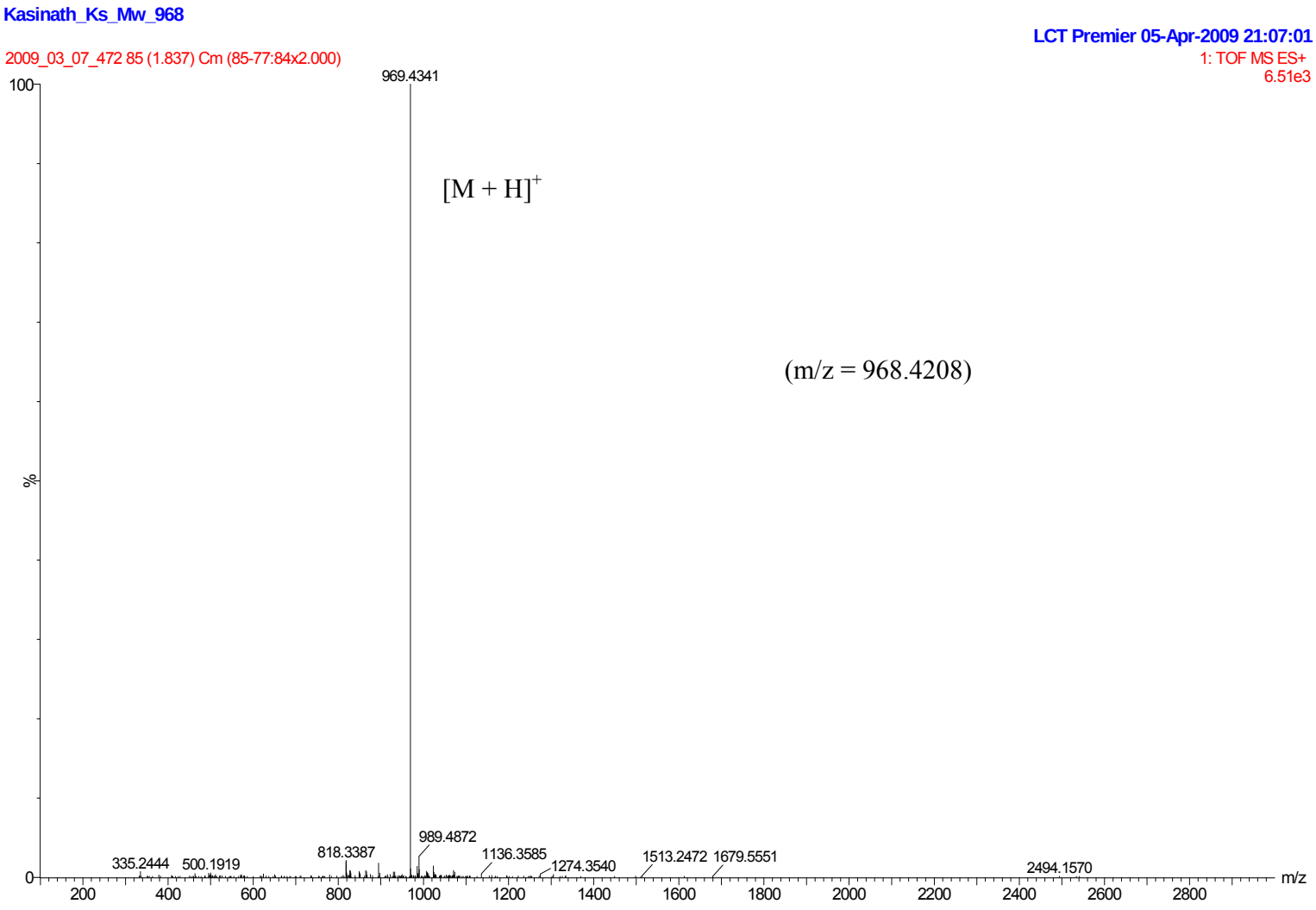


Analytical RP-HPLC trace of purified Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH (25)

Analytical RP-HPLC: 0 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 32.23 min.

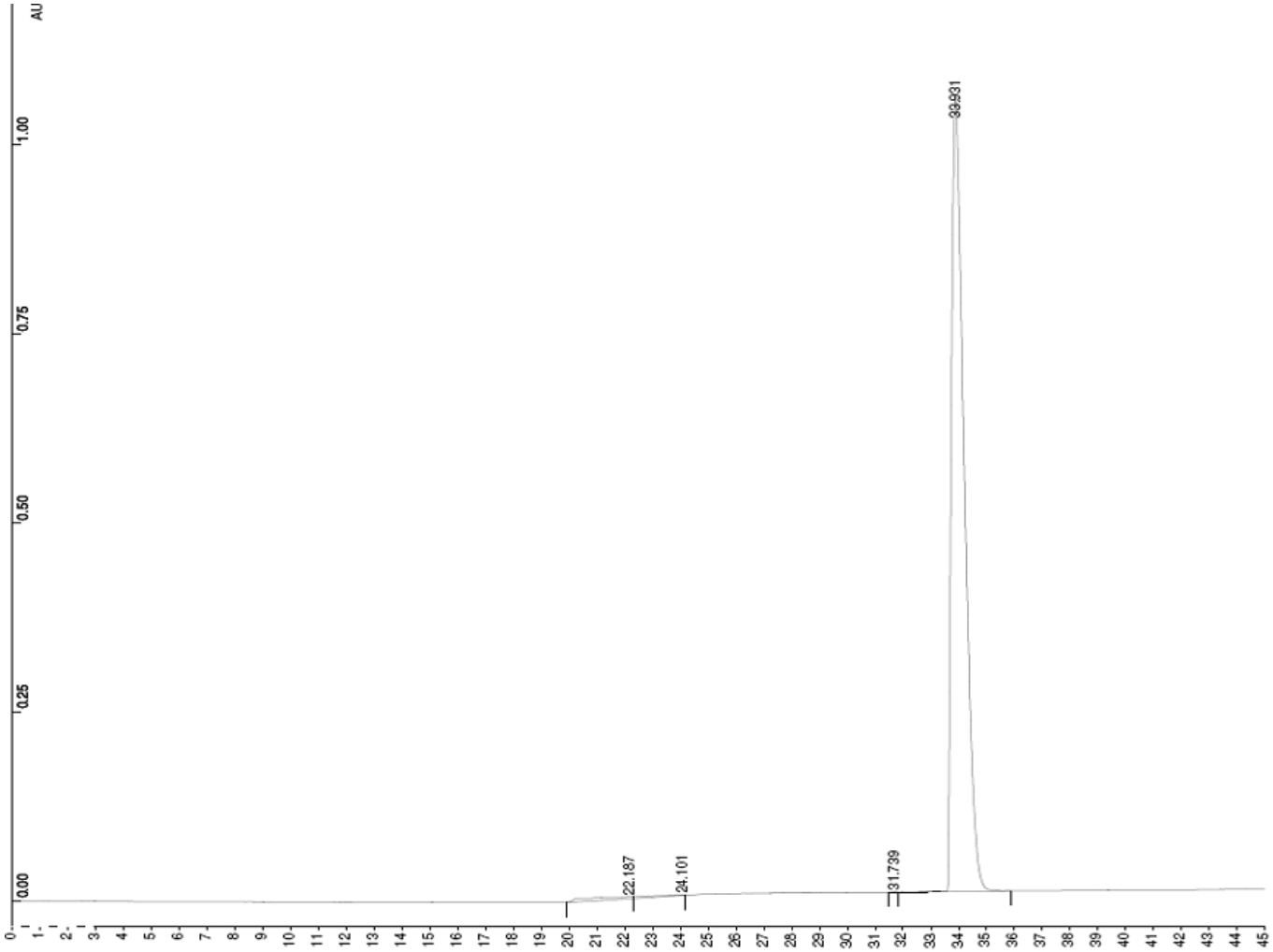


ESI-TOF mass spectrum of Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH (25)

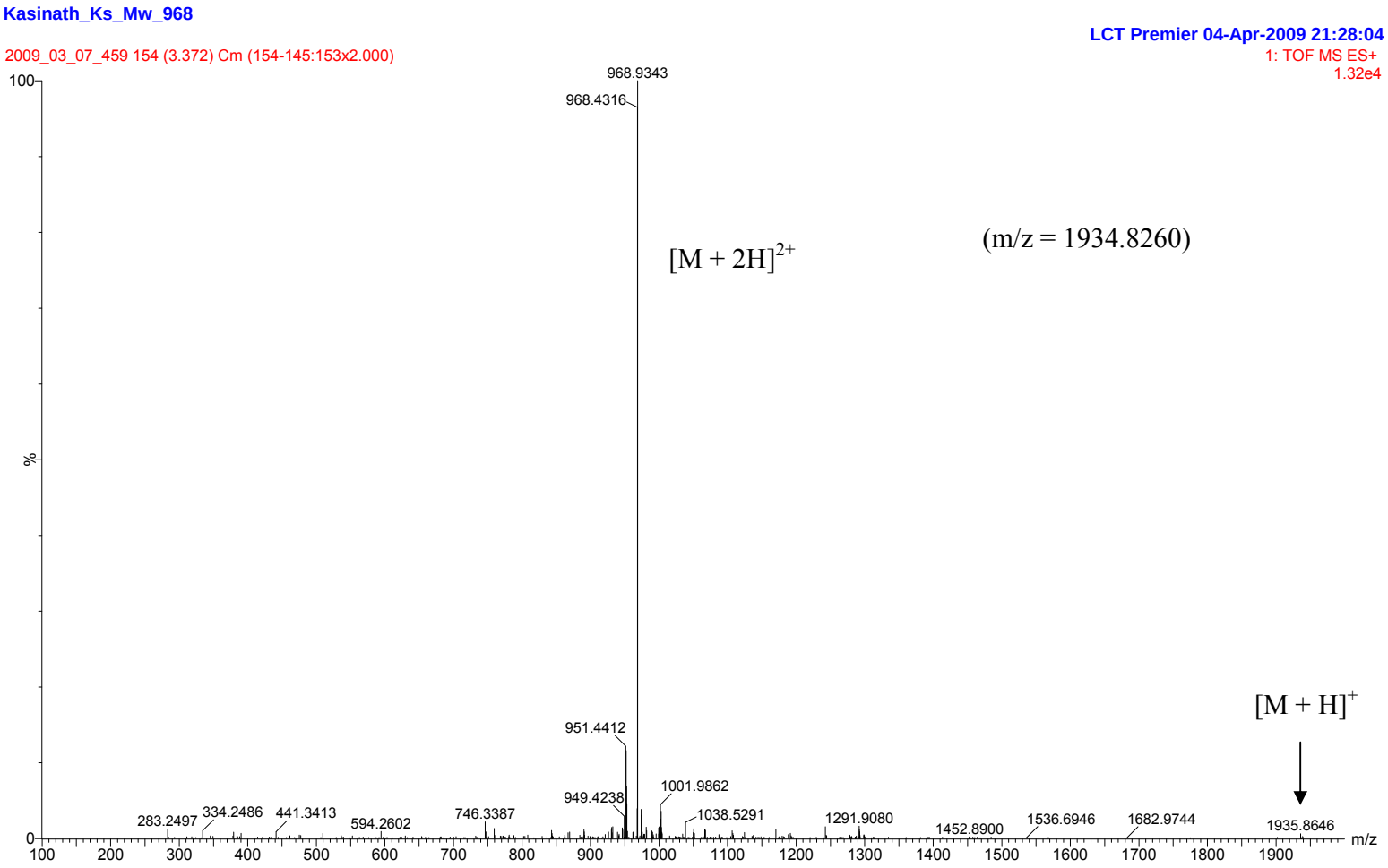


Analytical RP-HPLC trace of purified Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH **(26)**

Analytical RP-HPLC: 0 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 33.93 min.

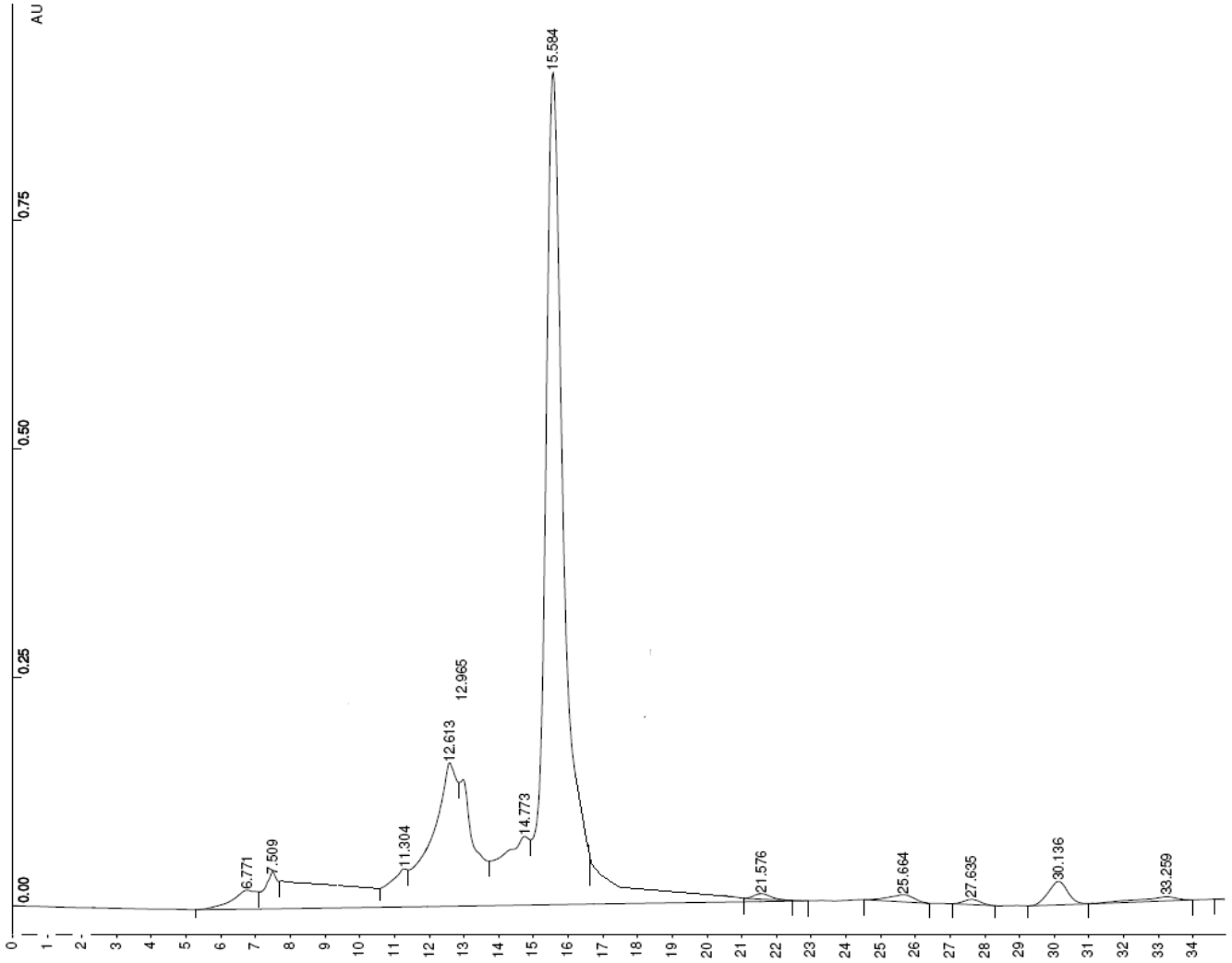


ESI-TOF mass spectrum of Boc-Ala-Ala-Thr-Cys-Phe-Ala-Arg-Asn-SH **(26)**



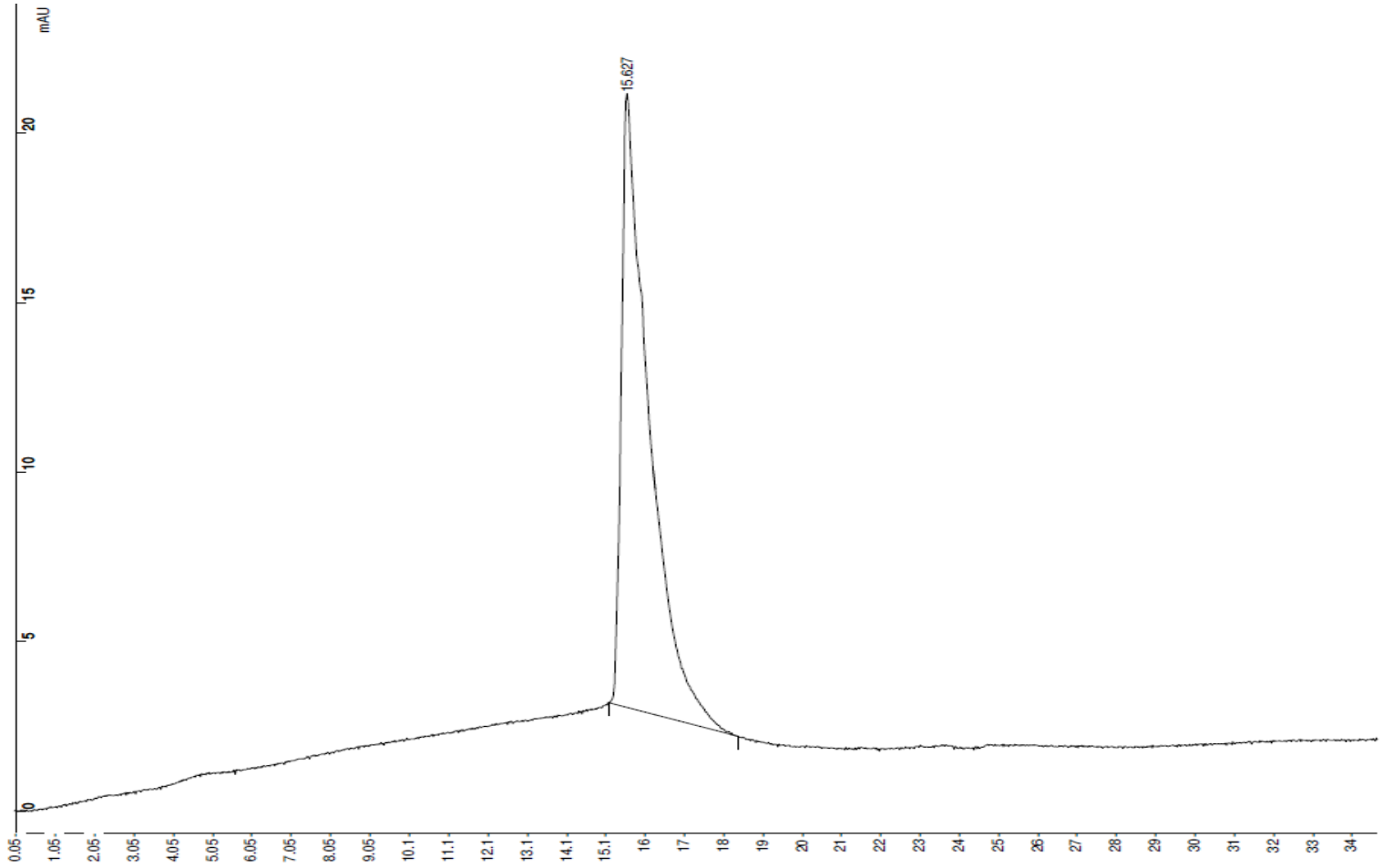
Analytical RP-HPLC trace of crude Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH (27)

Analytical RP-HPLC: 0 - 50% A in B over 35 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 15.58 min.

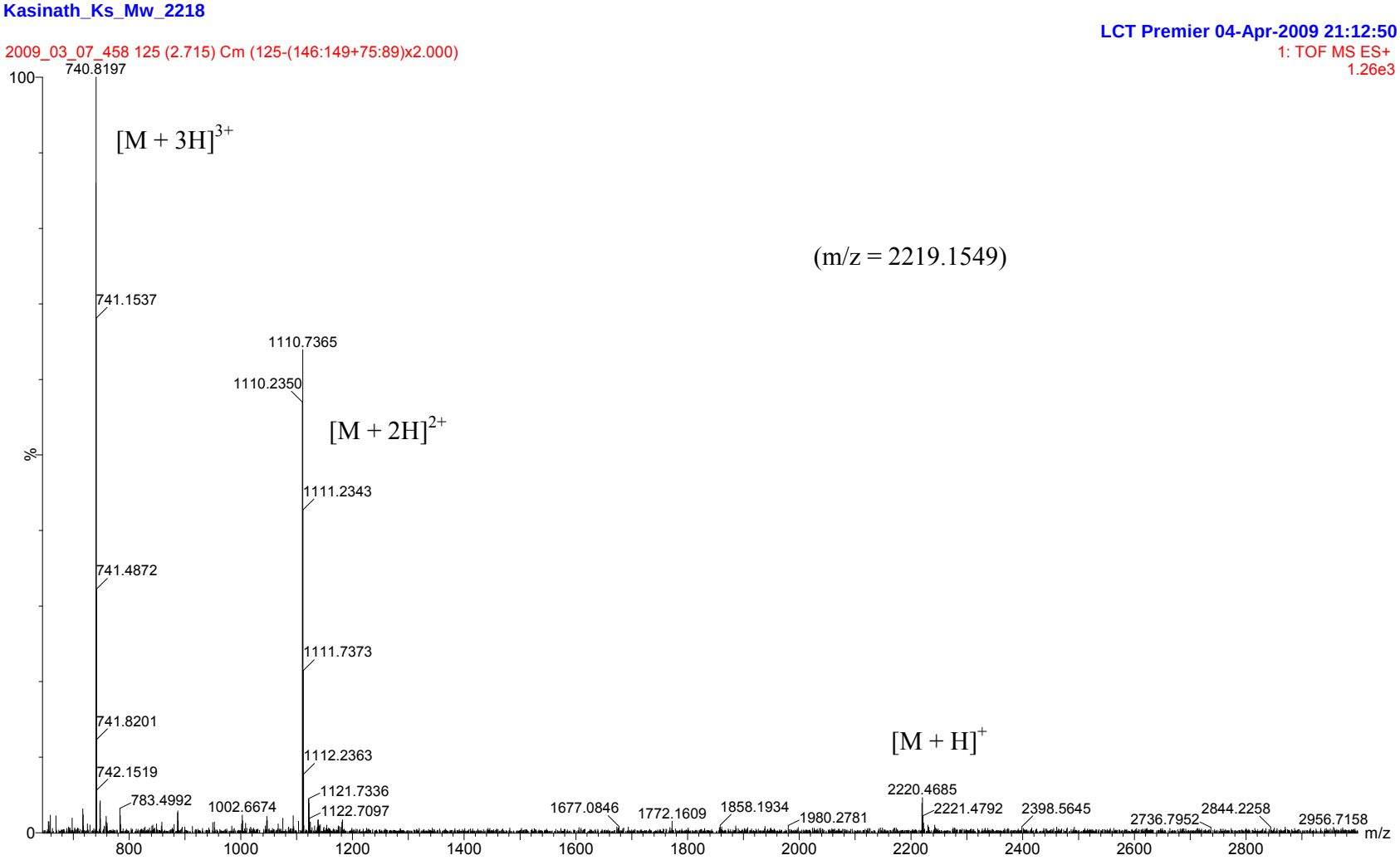


Analytical RP-HPLC trace of purified Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH (27)

Analytical RP-HPLC: 0 - 50% A in B over 35 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 15.62 min.

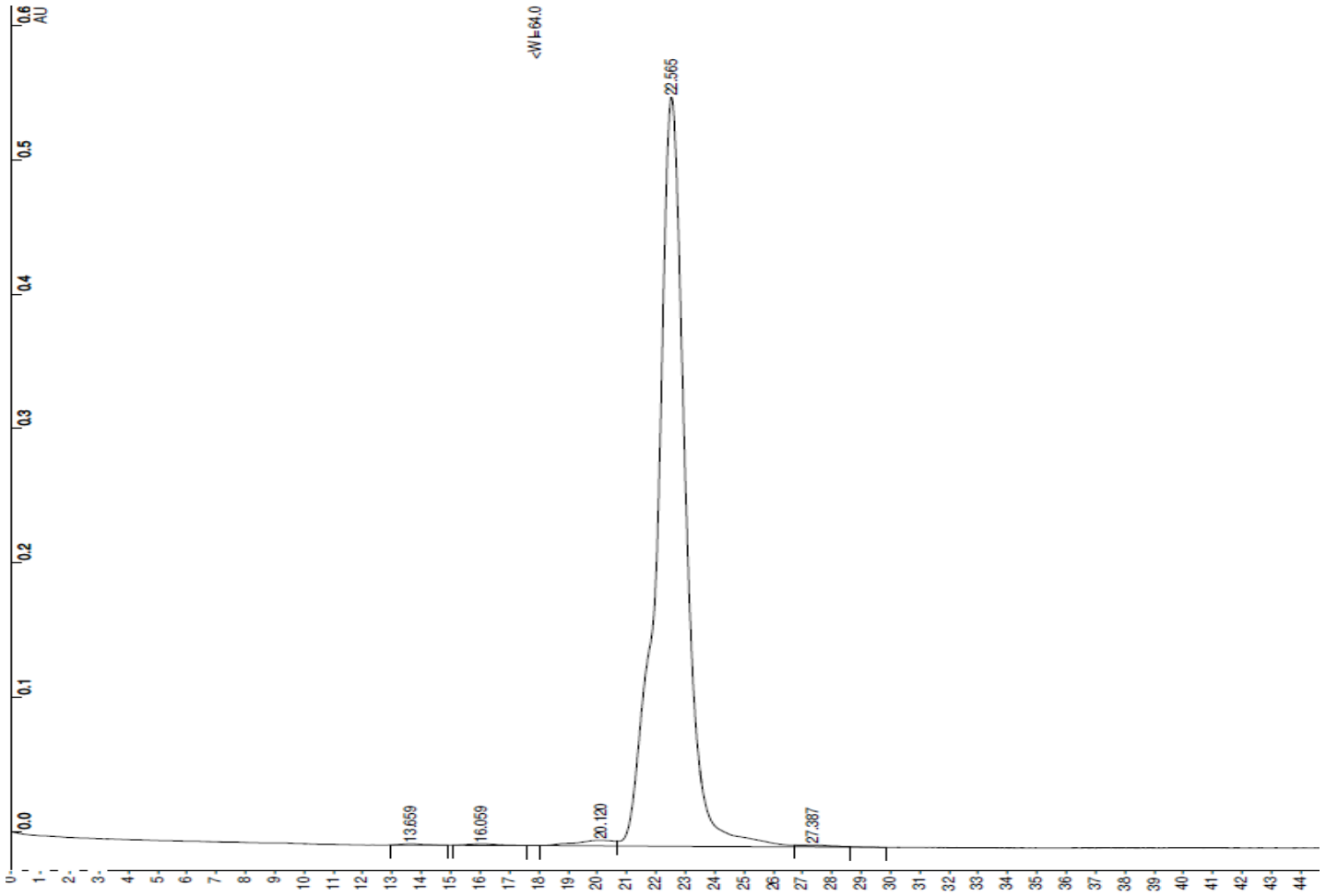


ESI-TOF mass spectrum of Boc-Lys-Ser-Leu-Gly-Glu-Ala-Asp-Lys-Ala-Asp-Val-Asp-Val-Leu-Thr-Lys-Ala-Lys-Ser-Gln-SH (27)

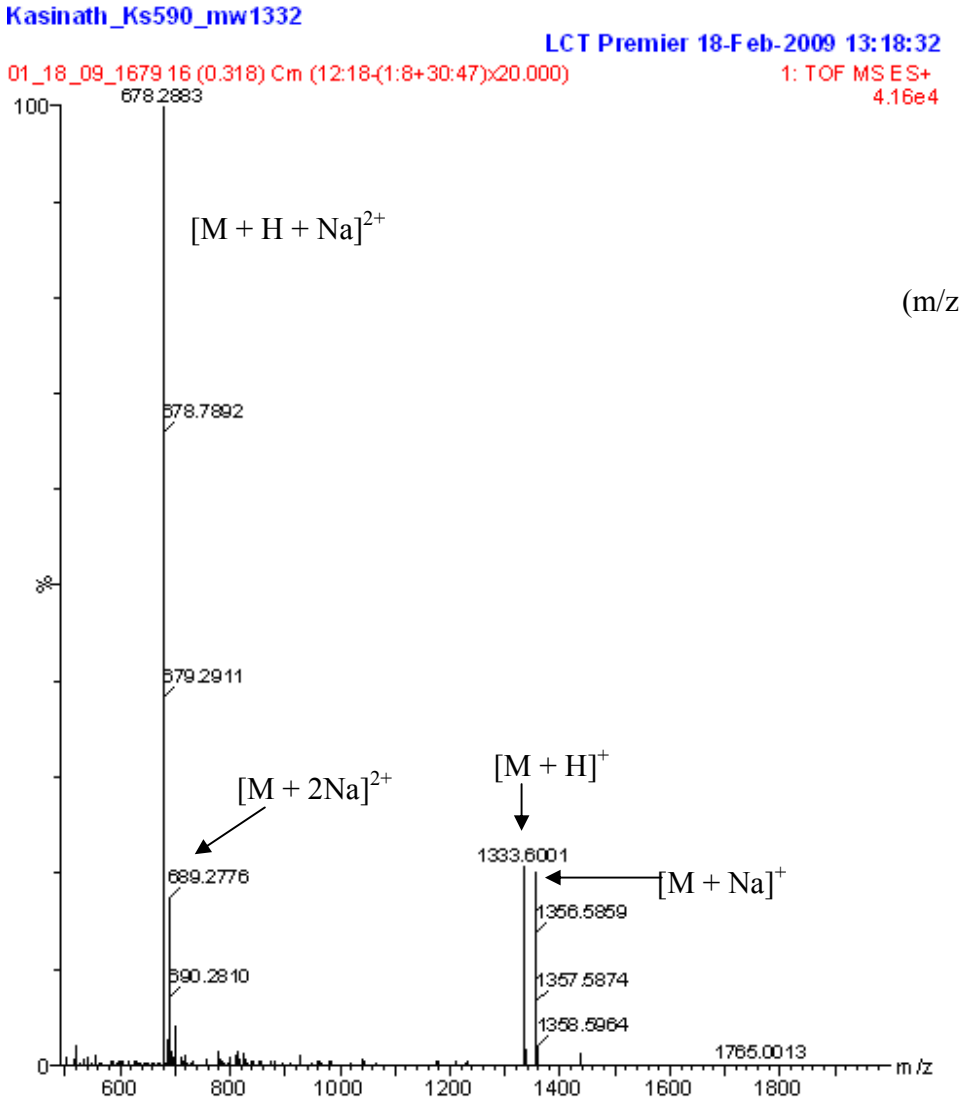


Analytical RP-HPLC trace of purified Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SBn (28)

Analytical RP-HPLC: 0 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 22.56 min.

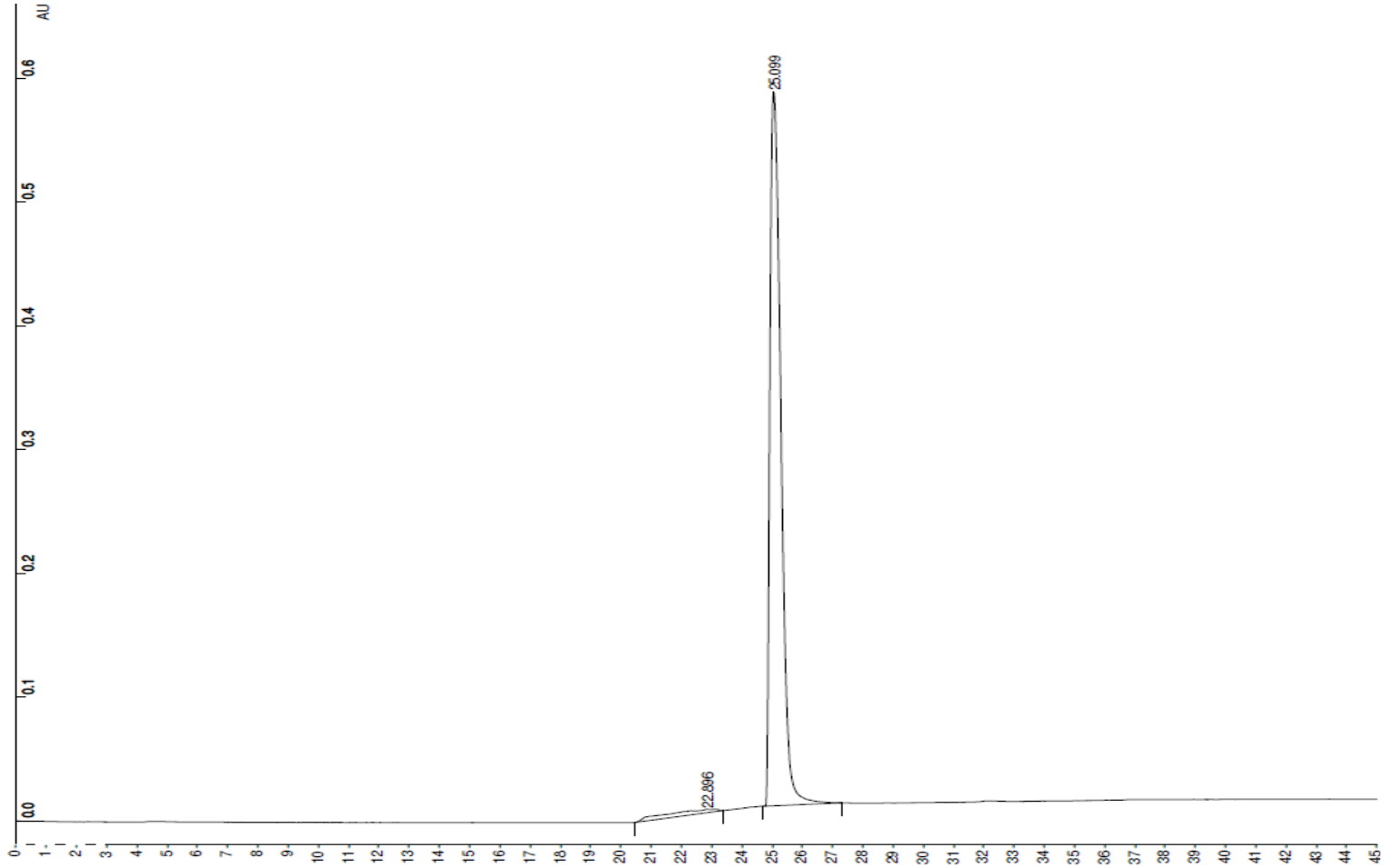


ESI-TOF mass spectrum of Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-SBn (28)

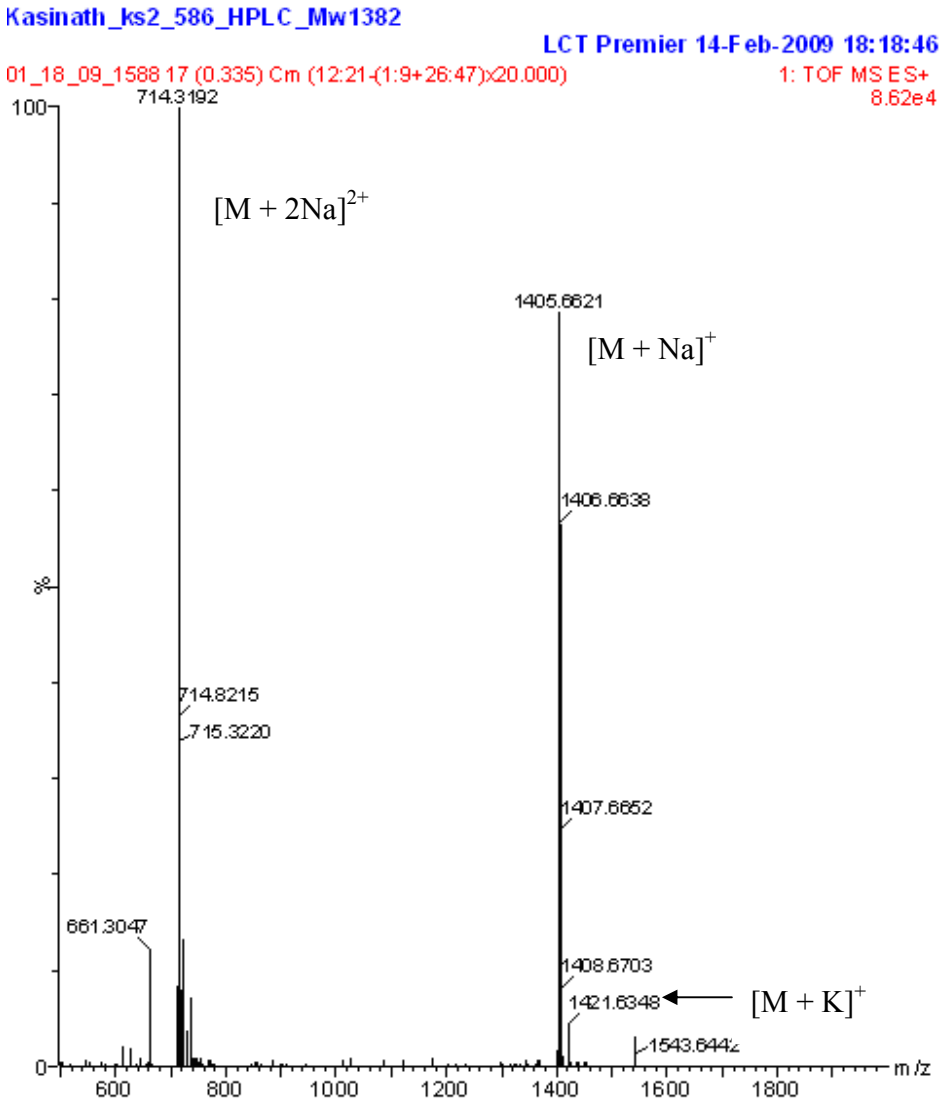


Analytical RP-HPLC trace of purified Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Allo)-SBn (29)

Analytical RP-HPLC: 0 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 25.10 min.



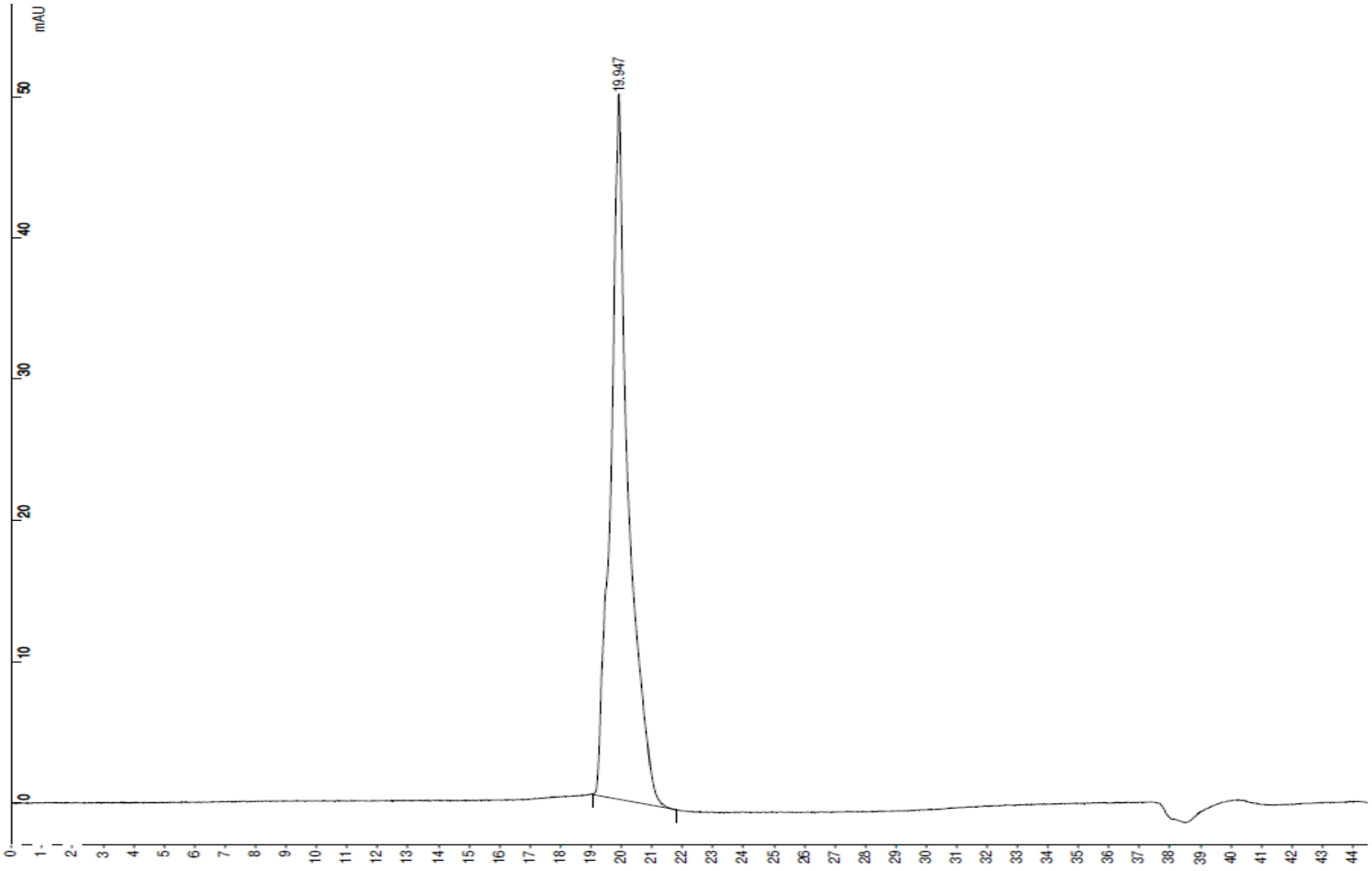
ESI-TOF mass spectrum of Boc-Ser-Ser-Tyr-Leu-Glu(OAll)-Gly-Gln-Ala-Ala-Lys(Allo)-SBn (29)



(m/z = 1382.6428)

Analytical RP-HPLC trace of purified Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-$NHSO_2$-C_6H_4-NHAc (30)

Analytical RP-HPLC: 0 - 50% A in B over 45 min with a flow rate of 1.5 mL/min and 215 nm UV detection, retention time = 19.95 min.



ESI-TOF mass spectrum of Alloc-His-Ala-Glu(OAll)-Gly-Thr-Phe-Thr-Ser-Asp(OAll)-Val-NHSO$_2$-C$_6$H$_4$-NHAc (30)