Simple and efficient Fmoc removal in ionic liquid.

Maria Luisa Di Gioia,[a] Paola Costanzo,[d] Antonio De Nino,[b] Loredana Maiuolo,[b] Monica Nardi,[b,c] Fabrizio Olivito[d] and Antonio Procopio[d]*

[a]Dipartimento di Farmacia e Scienze della Salute e della Nutrizione, Università della Calabria, Arcavacata di Rende (CS), 87036, Italy

[b] Dipartimento di Chimica, Università della Calabria, Cubo 12C, 87036, Arcavacata di Rende (CS), Italy

[c] Dipartimento di Agraria, Università Telematica San Raffaele, Via di Val Cannuta, 00166,247, Rome, Italy

[d]Dipartimento di Scienze della Salute, Università Magna Graecia, Viale Europa, 88100, Germaneto (CZ), Italia.

Supplementary Material

Experimental Section

Commercially available reagents were purchased from Sigma-Aldrich Chemical Co. (Milano, Italy) and used as supplied unless stated otherwise. All syntheses were carried out in atmospheric conditions. ^{1}H NMR spectra were recorded at 300 MHz, while ^{13}C NMR spectra were measured at 75 MHz. Spectral analysis was performed at 293 K on diluted solutions of each compound by using $CDCl_3$ as the solvent. Chemical shifts (δ) are reported in ppm and referenced to $CDCl_3$ (7.25 ppm for ^{1}H and 77.0 ppm for ^{13}C spectra). Coupling costants (J) are reported in Hertz (Hz). Reaction mixtures were monitored by thin layer chromatography (TLC) using Merck Silica gel 60-F_{254} precoated glass plates, and UV light (254 nm) or 0.2% ninhydrin in ethanol and charring as visualizing agent. Evaporation of solvents was performed at reduced pressure using a rotary vacuum evaporator. Chiral GC analysis were carried out using a Thermo Gas Chromatograph instrument. Chiral GC analyses of enantiomeric compounds Ac-DL-AlaOMe and Ac-L-AlaOMe were performed by using a 25 m × 0.25 mm, diethyl tertbutyldimethylisilyl-β-cyclodextrine chiral capillary column.

The GC-MS Shimadzu workstation is constituted by a GC 2010 (provided of a 30 m-QUADREX 007–5MS capillary column, operating in "split" mode, 1 ml min−1 flow of He as carrier gas) and a 2010 quadrupole mass-detector. LC-MS analysis were carried using an Agilent 6540 UHD Accurate - Mass Q-TOF LC–MS (Agilent, Santa Clara, CA) fitted with a electrospray ionisation source (Dual AJS ESI) operating in positive ion mode. Chromatographic separation was achieved using a C18 RP

analytical column (Poroshell 120, SB-C18, 50 × 2.1 mm, 2.7 μm) at 30°C with a elution gradient from 5% to 95% of B over 13 min., A being H_2O (0.1% FA) and B CH_3CN (0.1% FA). Flow rate was 0.4 ml/min.

N-Fmoc amines **1a–i** and *N*-Fmoc α-amino acid methyl esters **1j–u** were prepared according to previously published protocol.[14b] Spectral data of **1a–p** agreed with those already reported for the same compounds prepared as previously reported.[14b]

Spectroscopic data (1q-1u)

***N*-(9-Fluorenylmethoxycarbonyl) glycine *t*-butyl ester (1q):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.68- 7.52 (m, 4 H, Ar*H*), 7.32-7.22 (m, 4H, Ar*H*), 6.38 (br s, 1H, N*H*), 3.97 (t, *J* = 6.0 Hz, 1 H, CH_{Fmoc}), 3.82 (d, *J* = 5.1 Hz, 2 H, CH_{2Fmoc}), 3.23 (s, 2 H, CH_2), 1.38 (s, 9H, C(CH_3)$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 171.8, 8, 156.1, 145.9, 141.9, 128.9, 127.5, 124.8, 120.2, 81.4, 52.7, 42.4, 31.7, 28.3.

HRMS (ESI) for ($C_{21}H_{13}NO_4$)+ Na]$^+$: calcd 376.1525, found 376.1518 $(M+Na)^+$

***N*-(9-Fluorenylmethoxycarbonyl) phenylalanine benzyl ester (1r):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.78 (d, *J*= 7.2 Hz, 2 H, Ar*H*), 7.62 (d, *J*= 7.2 Hz, 2H, Ar*H*), 7.35- 7.17 (m, 12 H, Ar*H*), 7.00-6.97 (m, 2H, Ar*H* + N*H*), 5.12 (dd, *J*= 12.0 Hz, *J* = 9.6 Hz, 1 H, α-C*H*), 4.82- 4.79 (m, 2 H, OCH_2Ph), 4.14 (m, 1 H, CH_{Fmoc}), 4.10-4.04 (m, 2 H, CH_{2Fmoc}), 3.01-3.05 (m, 2 H, CH_2Ph) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.1, 8, 158.6, 144.2, 141.6, 137.0, 136.0, 135.9, 129.4, 128.6, 128.5, 128.4, 127.6, 126.9, 124.7, 120.0. HRMS (ESI) for ($C_{31}H_{27}NO_4$)+ Na]$^+$: calcd 500.1838, found 500.1824 $(M+Na)^+$

***N*-(9-Fluorenylmethoxycarbonyl) tyrosine (*O-t*-butyl) *t*-butyl ester (1s):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.75 (d, 2 H, *J*= 7.5, Ar*H*), 7.58 (d, 2H, *J* = 7.4, Ar*H*), 7.42-7.31 (m, 4H, Ar*H*), 7.11-7.03 (m, 2 H, Ar*H*), 6.93-6.88 (m, 2H, Ar*H*), 5.30 (d, 1H, *J* = 8.4 Hz, N*H*), 4.54-4.39 (m, 3 H, α-C*H* + CH_{2Fmoc}), 4.34 (t, 1H, *J* = 8.6, CH_{Fmoc}), 3.04 (m, 1 H, β-CH_2), 2.80 (m, 1 H, β-CH_2), 1.40 (s, 9H, C(CH_3)$_3$), 1.39 (s, 9H, C(CH_3)$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 170.6, 155.5, 154.2, 144.5, 143.9, 134.6, 129.1, 127.7, 127.0, 124.3, 120.3, 119.7, 82.6, 81.1, 66.9, 55.2, 47.3, 37.9, 28.8, 27.9. HRMS (ESI) for ($C_{32}H_{37}NO_5$)+ H]$^+$: calcd 516.2750, found 516.2664, 538.2570 (M+Na)$^+$.

***N*-(9-Fluorenylmethoxycarbonyl) glutamic acid (*O-tert*-butyl) methyl ester (1t):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.69 (d, *J* = 7.5 Hz, 2 H, Ar*H*), 7.52 (d, *J*= 4.8 Hz, 2H, Ar*H*), 7.35-7.21 (m, 4H, Ar*H*), 5.42 (d, *J*= 8.1 Hz, 1 H, N*H*), 4.74-4.38 (m., 3 H, α-C*H*+ CH_{2Fmoc}), 3.68 (s, 3H, OCH_3), 2.32–2.22 (m, 2 H, β-CH_2), 2.13 (m, 1H, γ-C*H*), 2.06 (m, 1H, γ-C*H*), 1.37 (s, 9 H, C(CH_3)$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 171.4, 170.9, 154.9, 142.7, 140.3, 126.6, 126.0, 124.0, 118.9, 79.8, 66.0, 52.4, 51.4, 46.1, 30.4, 27.0, 26.2 HRMS (ESI) for ($C_{25}H_{29}NO_6$)+ H]$^+$: calcd 440,2073, found 440.2135, 462.1891 (M+ Na)$^+$.

***N*-(9-Fluorenylmethoxycarbonyl) lysine (*N*-Boc) methyl ester (1u):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.53 (d, *J* = 6.9 Hz, 2 H, Ar*H*), 7.33 (d, *J* = 7.5 Hz, 2H, Ar*H*), 7.26-7.19 (m, 4H, Ar*H*), 5.39 (d, *J* = 7.8 Hz, 1 H, N*H*), 4.53 (m, 1 H, N*H*), 4.34-4.27 (m, 3 H, α-C*H* + CH_{2Fmoc}), 4.17 (t, *J* = 6.9 Hz, 1H, CH_{Fmoc}), 3.67 (s, 3H,

OCH_3), 3.04- 3.02 (m, 2 H, ε-CH_2), 1.77 (m, 1H, β-CH), 1.63 (m, 1H, β-CH), 1.42-1.16 (m, 4H, γ-CH_2 + δ-CH_2), 1.36 (s, 9 H, C(CH_3)$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.9, 156.1, 155.9, 143.7, 141.3, 127.6, 127.0, 125.1, 119.9, 80.0, 66.9, 53.7, 52.4, 47.1, 40.2, 32.1, 29.6, 28.4, 22.3.

HRMS (ESI) for [($C_{27}H_{34}N_2O_6$+ H]$^+$: calcd 483,2495 found 483.2484, 505.2306 (M+Na)$^+$.

General procedure for the *N*-Fmoc removal of amines 1a-i in [Bmim][BF_4].

To a magnetically stirred mixture of *N*-Fmoc protected amines **1a-i** (1 mmol) and [Bmim][BF_4] (1 mL), Et_3N (3 mmol) was added and the mixture was stirred at room temperature for 4-8 min. TLC monitored the reaction. Diethyl ether was added after the completion of reaction and the IL settled at the bottom. The supernatant was decanted off and the IL was washed with Et_2O (3 × 2 mL). The combined Et_2O extracts were acidified with an aqueous solution of 1N HCl and separated. The aqueous phase was then basified with sat. aq $NaHCO_3$ and finally extracted with diethyl ether. The organic phase was dried over Na_2SO_4 and filtered. The products were isolated after evaporation of the diethyl ether to yield the free amines **2a-i** in 80-93 % yields. Spectroscopic data showed full consistency of the spectra with the pure products.

General procedure for the *N*-Fmoc removal of amino acid methyl esters 1j-u in [Bmim][BF_4].

To a magnetically stirred mixture of *N*-Fmoc amino acid methyl esters **1j-u** (1 mmol) and [Bmim][BF_4] (1 mL), Et_3N (3 mmol) was added and the mixture was stirred at room temperature for 8-15 min. TLC monitored the reaction. Diethyl ether was added after the completion of reaction and the IL settled at the bottom. The supernatant was decanted off and the IL was washed with Et_2O (3 × 2 mL). The combined Et_2O extracts were acidified with an aqueous solution of 1N HCl (for compounds **1q-u** bearing acid-sensitive protecting group a 5% aqueous solution of citric acid was used) and separated. The aqueous phase was then basified with sat. aq $NaHCO_3$ and finally extracted with diethyl ether. The organic phase was dried over Na_2SO_4 and filtered. The products were isolated after evaporation of the diethyl ether to yield the free amino acid methyl ester **2j-u** in 75-88% yields. Compounds **2j-u** were acetylated in order to perform GC/MS analysis. *N*-Acetylation was achieved dissolving **2j-u** in DCM (5 mL) and adding acetic anhydride (1 mL) and a 9% aqueous solution of $NaHCO_3$ (5 mL). The mixture was maintained under magnetic stirring at room temperature for 4 h. The organic layer was separated and the aqueous phase was extracted with three additional portions of DCM (3 x 10 mL). The combined organic layers were washed with a 9% aqueous solution of $NaHCO_3$, twice with aqueous HCl 1 N (or a 5% aqueous solution of citric acid), once with brine and finally dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford

the corresponding *N*-acetyl derivatives **3j-u** as colourless oil in quantitative yield. Spectroscopic data for **3j-n** compared to those reported in the literature.[2a]

Spectroscopic data (3j-3u).

N*-Acetyl alanine methyl ester (3j):* ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.28 (s, 1 H, N*H*), 4.58 (m, 1 Hα-C*H*), 3.70 (s, 3 H, OCH_3), 2.02 (s, 3 H, CH_3CO), 1.40 (d, *J* = 7.2 Hz, 3 H, *CH*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 173.7, 169.5, 52.4, 48.0, 23.1, 18.6 ppm. GC/MS (EI): *m/z* (%) 145 (13) [$(M)^+$], 102 (86 (70), 59 (5), 44 (100). HRMS (ESI) for ($[C_6H_{11}NO_3]$ + Na)$^+$: calcd 168.0637, found 168.0630 $[M+Na]^+$.

N*-Acetyl valine methyl ester (3k):* ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.22 (d, 1 H, N*H*, *J* = 6.6 Hz), 4.55 (dd, *J* = 8.7, *J* = 5.1 Hz, 1 H, α-C*H*), 3.74 (s, 3 H, OCH_3), 2.13 (m, 1 H, C*H*(*C*H$_3$)$_2$), 2.01 (s, 3 H, CH_3CO), 0.93 (d, *J* = 6.9 Hz, 3 H, CH(*CH*$_3$)$_2$), 0.90 (d, *J* = 6.9 Hz, 3 H, CH(*CH*$_3$)$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.7, 170.2, 57.1, 52.1, 31.2, 23.1, 18.8, 17.8 ppm. GC/MS (CI): *m/z* (%) 214 (13) [$(M + C_3H_5)^+$], 202 (16) [$(M + C_2H_5)^+$], 174 (60) [$(M + H)^+$], 156 (9), 142 (65), 132 (50), 114 (100), 101 (7). HRMS (ESI) for ($[C_8H_{15}NO_3]$ + H)$^+$: calcd. 174.1130, found 174.1134 $[M+H]^+$.

N-Acetyl leucine methyl ester (3l). ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.50 (d, *J* = 7.68 Hz, 1H, N*H*), 4.52 (m, 1 H, α-C*H*), 3.69 (s, 3 H, OCH_3), 1.99 (s, 3 H, CH_3CO), 1.62-1.40 (m, 3 H, *CH*$_2$*CH*), 1.40–1.26 (m, 1 H, CH_2), 0.85-0.87 (m, 6 H,

CH(CH_3)$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 173.8, 170.1, 52.1, 50.6, 41.3, 24.7, 22.8, 22.7, 21.8 ppm. GC/MS (CI): *m/z* (%) 228 (20) [(M + C_3H_5)$^+$], 216 (35) [(M + C_2H_5)$^+$], 188 (100) [(M + H)$^+$], 170 (5), 156 (60), 146 (55), 128 (88), 86 (9).

HRMS (ESI) for ([$C_9H_{17}NO_3$] + Na)$^+$: calcd. 210.1106, found 210.1103 [M+Na]$^+$.

N*-Acetyl isoleucine methyl ester (3m):* ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.06 (s, 1 H, *N*H), 4.54 (d, *J* = 8.7 Hz, *J* = 4.8 Hz, 1 H, α-C*H*), 3.67 (s, 3 H, OCH_3), 1.96 (s, 3H, CH_3CO), 1.78 (m, 1 H, β-C*H*), 1.35 (ddd, *J* = 7.5 Hz, *J* = 4.8 Hz , *J* = 4.8 Hz, 1H, CH_2), 1.12 (m, 1 H, CH_2), 0.85 (s, 3 H, CH_3), 0.82 (s, 3 H, CH_3) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.7, 169.8, 56.4, 51.9, 37.9, 25.2, 23.2, 15.3, 11.5 ppm. GC/MS (CI): *m/z* (%) 228 (51) [(M + C_3H_5)$^+$], 216 (32) [(M + C_2H_5)$^+$], 188 (100) [(M + H)$^+$], 157 (76), 146 (83), 128 (68), 102 (2). HRMS (ESI) for ([$C_9H_{17}NO_3$] + H)$^+$ 188.1287, found 188.1279 [M+H]$^+$, 210.1099 [M+Na]$^+$.

***N*-Acetyl phenylalanine methyl ester (3n):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.32–7.24 (m, 3 H, Ar*H*), 7.09 (dd, *J* = 7.9 Hz, *J* = 1.8 Hz, 2H, Ar*H*), 6.02 (d, *J* = 6.6 Hz, 1 H, *NH*,), 4.88 (dt, *J*= 7.8 Hz, *J*= 5.8 Hz, 1 H, α-C*H*), 3.72 (s, 3 H, OCH_3), 3.14 (dd, *J*= 13.83 Hz, *J*= 5.7 Hz, 1H, β-C*H*), 3.07 (dd, *J*= 13.83 Hz, *J*= 5.7 Hz, 1H, β-C*H*), 1.99 (s, 3 H, CH_3CO) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.0, 169.5, 135.8, 129.2, 128.5, 127.1, 53.1, 52.2, 37.8, 23.0 ppm. HRMS (ESI) for ([$C_{12}H_{15}NO_3$] + Na)$^+$ 244.0950, found 244.0941 [M+Na]$^+$.

***N*-Acetyl *N*-methyl valine methyl ester (3o)** (two rotamers)**:** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C), (two rotamers): δ = 4.88 (d, 1 H, *J*= 10.5 Hz, α-C*H*), 3.69 and 3.65 (2s, 3 H, OCH_3), 2.94 and 2.82 (2s, 3 H, *N*-CH_3), 2.16 and 2.12 (2s, 3 H, CH_3CO), 2.27–2.19 (m, 1 H, β-C*H*), 0.95 and 0.94 (d, *J* = 6.6 Hz, 3 H, CH(CH_3)$_2$), 0.86 and 0.82 (d, *J* = 6.9 Hz, 3 H, CH(CH_3)$_2$] ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 170.86, 169.58, 66.21, 60.32, 51.01, 31.27, 26.73,21.06, 19.00, 18.82 ppm. GC/MS (CI): *m/z* (%) 228 (51) [(M + C_3H_5)$^+$], 216 (32) [(M + C_2H_5)$^+$], 188 (100) [(M + H)$^+$], 157 (76), 146 (83), 128 (68), 102 (2).

***N*-Acetyl *N*-methyl isoleucine methyl ester (3p).** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C), (two rotamers): δ = 4.99 (d, *J* = 10.5 Hz, 1 H, α-C*H*), 3.71 and 3.67 (2s, 3 H, OCH_3), 2.95 and 2.83 (2s, 3 H, N-CH_3), 2.14 and 2.10 (2s, 3 H, CH_3CO), 2.00–1.90 (m, 1 H, β-C*H*), 1.40–1.26 (m, 1 H, CH_2), 1.10–1.00 (m, 1 H, CH_2), 0.97 and 0.96 (2d, *J* = 6.9 Hz, 3 H, CH_3) ppm.

***N*-Acetyl glycine *t*-butyl ester (3q).** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 3.92 (d, *J*= 5.1 Hz, 2 H, α-C*H*), 2.04 (s, 3 H, CH_3), 1.47 (s, 9 H, C(CH_3)$_3$), ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 170.3, 169.3, 82.3, 42.1, 28.0, 22.9 ppm. HRMS (ESI) for [($C_8H_{15}NO_3$) + H]$^+$ 174.1130, found 174.1122 [M+H]$^+$, 196.0945 [M+Na]$^+$.

***N*-Acetyl phenylalanine benzyl ester (3r).** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.35–7.21 (m, 8 H, Ar*H*), 7.00 – 6.97 (m, 2 H, Ar*H*), 6.02 (d, *J* = 6.9 Hz, 1H, NH), 5.14-5.13 (m, 2 H, COOCH_2), 4.93 (m, 1 H, α-C*H*), 3.12-3.09 (m, 2 H, CH_2Ph), 1.96 (s, 3 H, CH_3CO) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 171.7, 169.9,

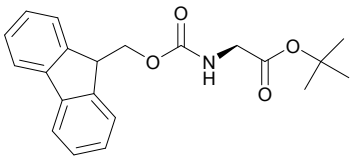
135.8, 135.2, 129.4, 128.8, 128.7, 128.6, 127.2, 67.4, 53.3, 37.9, 23.2 ppm. HRMS (ESI) for $(C_{18}H_{19}NO_3) + H]^+$: calcd 298.1443, found 298.1437 $[M + H]^+$, 320.1254 $[M + Na]^+$.

***N*-Acetyl tyrosine (*O*-*t*-butyl) *t*-butyl ester (3s).** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 7.31 (d, *J* = 8.4 Hz, 2 H, Ar*H*), δ = 7.90 (d, *J* = 8.4 Hz, 2 H, Ar*H*), 6.04 (br s, s, 1 H, N*H*), 4.74 (dd, *J* = 6.6 Hz, *J* = 5.7 Hz, 1 H, α-C*H*), 3.09–3.02 (m, 2 H, β-C*H*), 2.02 (s, 3 H, CH_3CO), 1.38 (s, 9 H, C(CH_3)$_3$), 1.40 (s, 9 H, C(CH_3)$_3$),ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ = 170.9, 169.3, 146.4, 131.1, 129.9, 124.0, 90.8, 82.4, 53.6, 37.5, 28.8, 27.9, 23.2 ppm. HRMS (ESI) for $[(C_{19}H_{29}NO_4] + H)^+$: calcd 336.2175, found 336.2133 $[M + H]^+$, 358.1987 $[M + Na]^+$.

***N*-Acetyl glutamic acid (*O*-*t*-butyl) methyl ester (3t):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.34 (d, *J* = 7.5 Hz, 1 H, NH), 4.74 (dt, *J* = 8.0 Hz, *J* = 5.1 Hz, 1 H, α-C*H*), 3.67 (s, 3H, OCH_3), 2.35–2.15 (m, 2 H, β-CH_2), 2.05 (ddd, *J* = 14.1 Hz, *J* = 7.3 Hz, *J* = 2.2 Hz, 1H, γ-C*H*), 1.95 (s, 3 H, CH_3CO), 1.87 (ddd, *J* = 14.1 Hz, *J* = 6.1 Hz, *J* = 1.3 Hz, 1H, γ-C*H*), 1.37 (s, 9 H, C(CH_3)$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.6, 172.2, 170.0, 80.8, 52.4, 51.8, 31.4, 28.0, 27.2, 23.0 ppm. HRMS (ESI) for $[(C_{12}H_{21}NO_5] + Na)^+$: calcd. 282.1317, found 282,1307 $[M + Na]^+$.

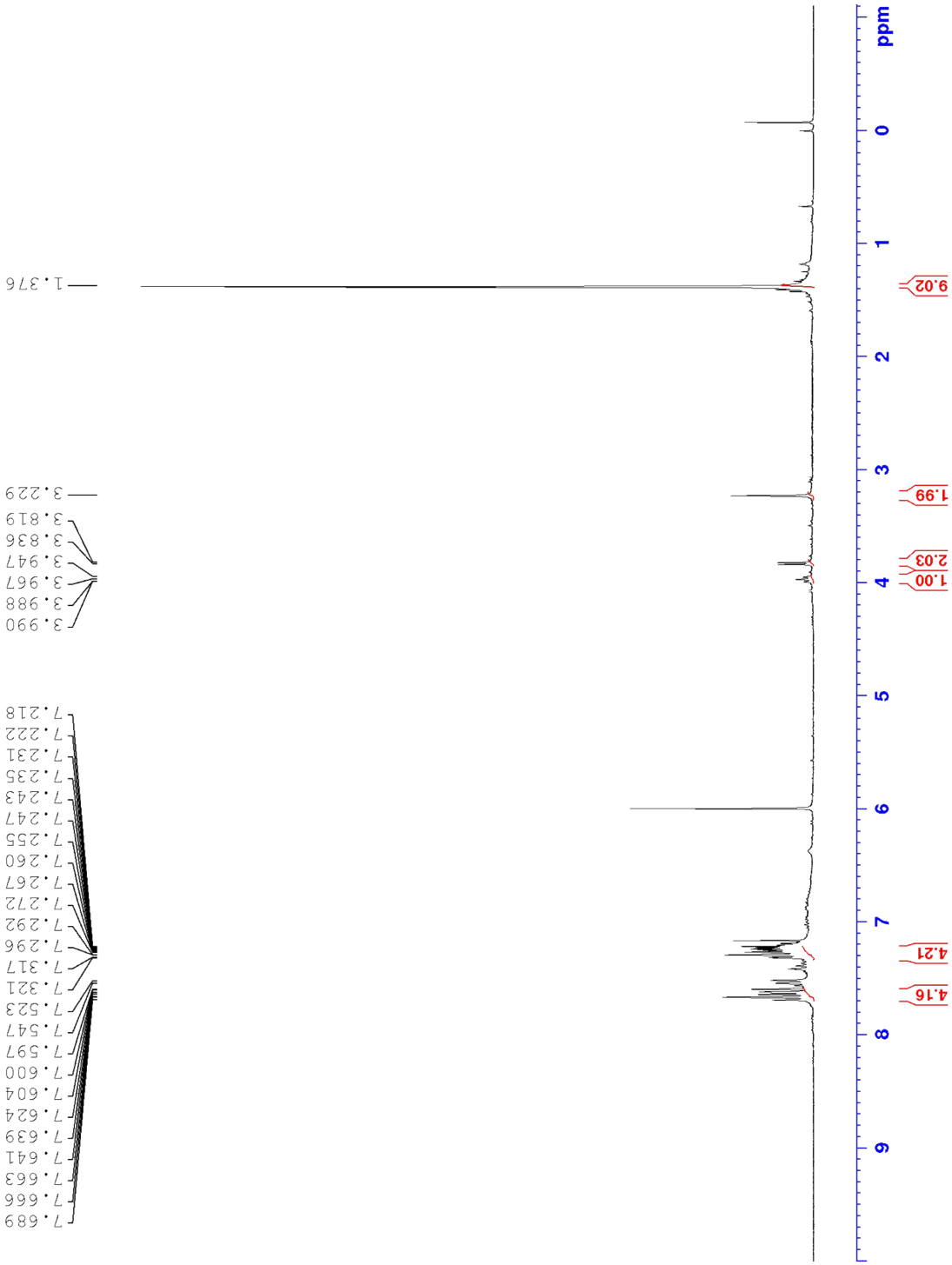
***N*-Acetyl lysine (*N*-Boc) methyl ester (3u):** ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ = 6.48 (d, *J* = 7.5 Hz, 1 H, *NH*), 4.73 (m, 1 H, N*H*), 4.50 (dd, *J* = 12.6 Hz, *J* = 7.5 Hz, 1 H, α-C*H*), 3.67 (s, 3H, OCH_3), 3.04- 2.99 (m, 2 H, ε-CH_2), 1.96 (s, 3 H, CH_3CO), 1.75 (m, 1H, β-C*H*), 1.58 (m, 1H, β-C*H*),

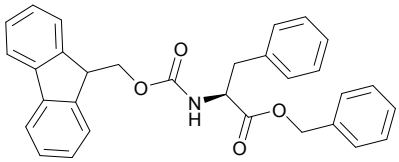
1.44-1.27 (m, 4H, γ-CH_2 + δ-CH_2), 1.36 (s, 9 H, C(CH_3)$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ= 172.1, 169.1, 155.2, 78.1, 51.3, 51.1, 38.9, 30.8, 28.6, 27.4, 21.9, 21.4 ppm. HRMS (ESI) for $[(C_{14}H_{26}N_2O_5] + H)^+$: calcd. 303.1920, found 303.1904 $[M + H]^+$, 325.1737 $[M + Na]^+$.



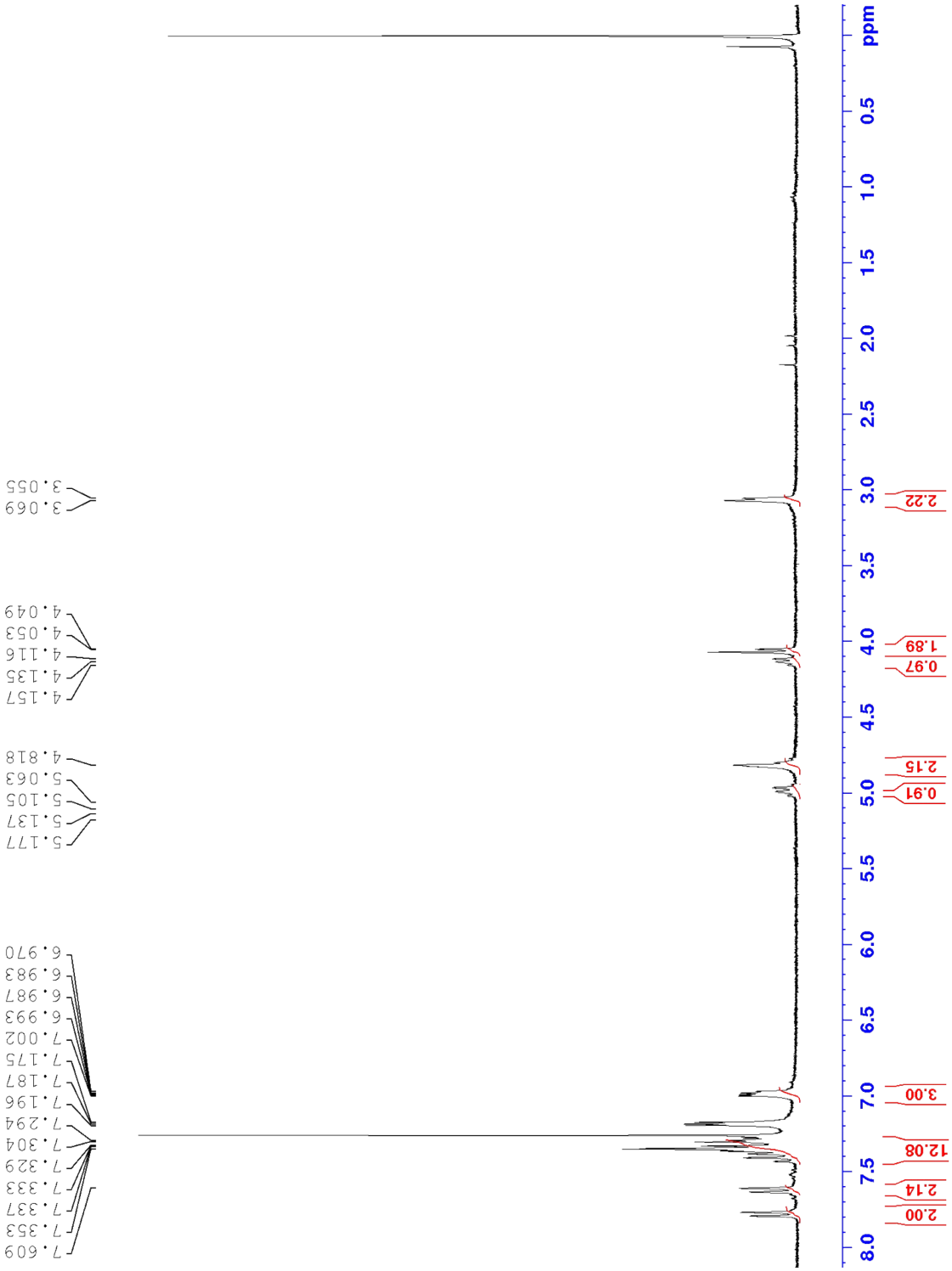
^{1}H NMR spectra (1q-1u)

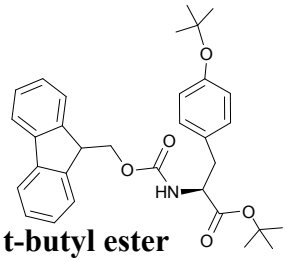
Sample 1q: *N*-(9-Fluorenylmethoxycarbonyl) glycine *t*-butyl ester



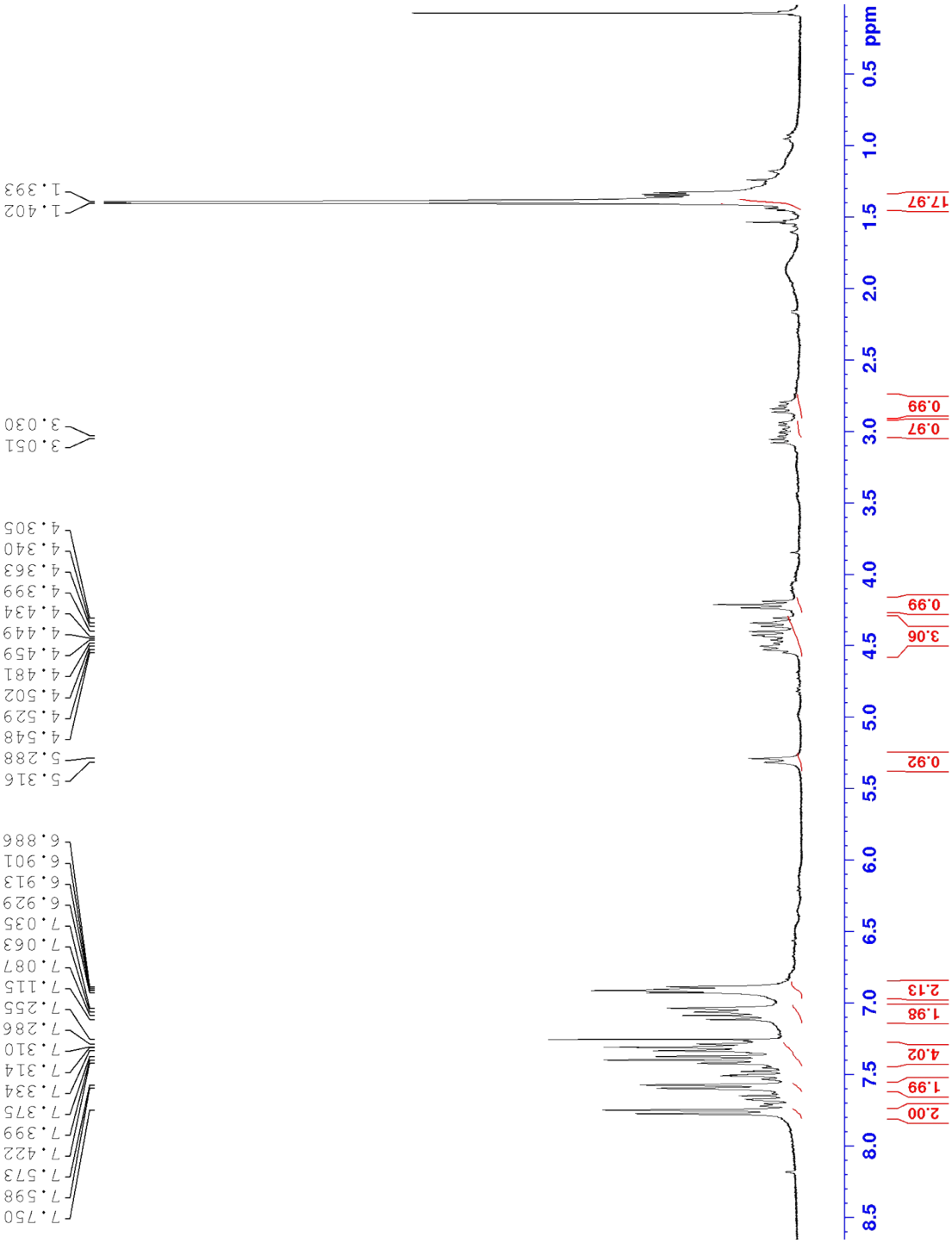


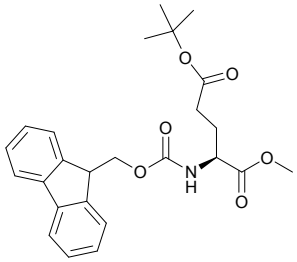
Sample 1r: *N*-(9-Fluorenylmethoxycarbonyl) phenyl alanine benzyl ester



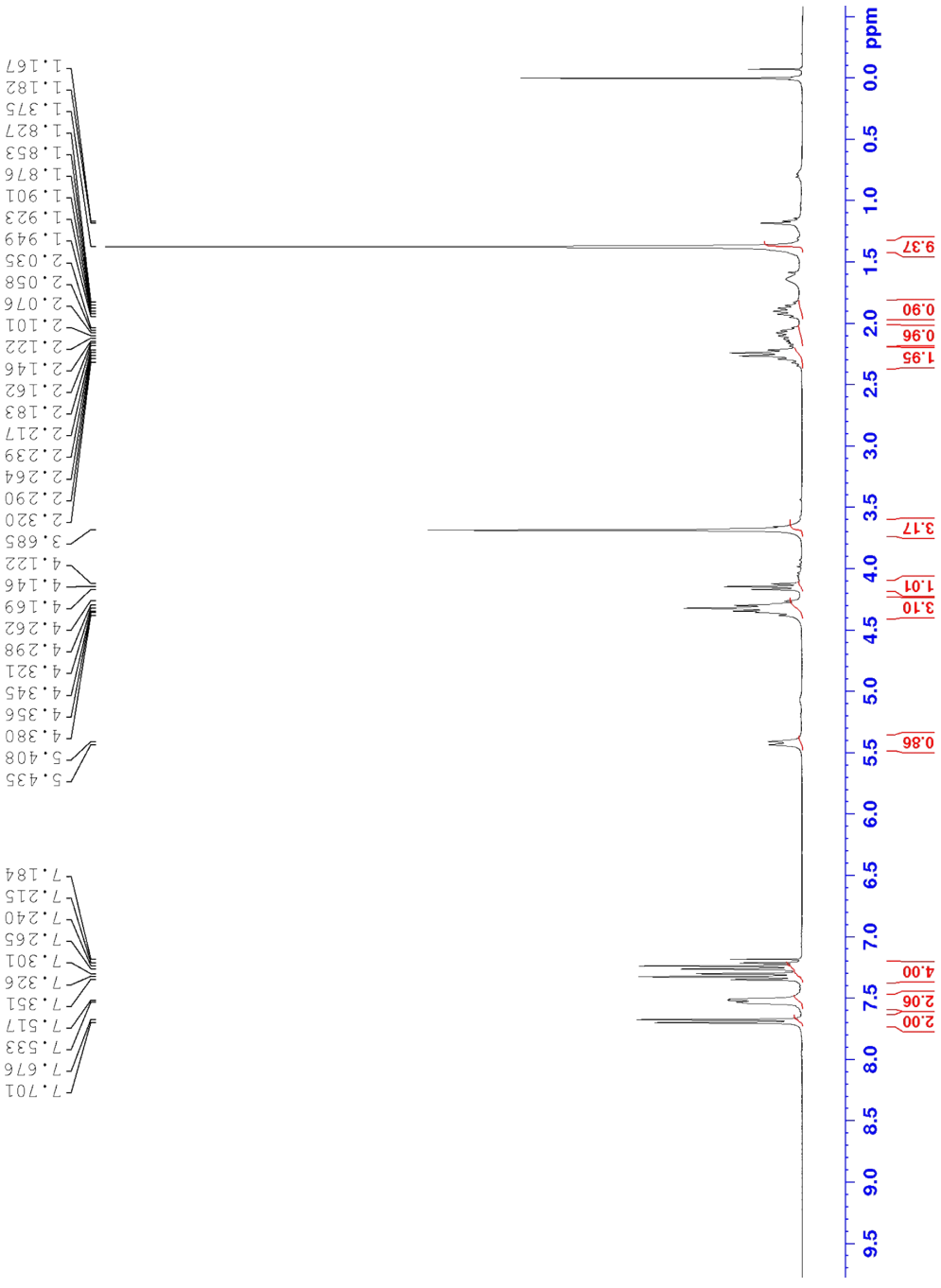


Sample 1s: *N*-(9-Fluorenylmethoxycarbonyl) tyrosine (O-*tert*-butyl) t-butyl ester

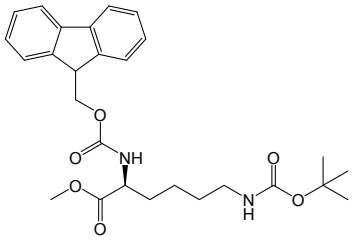


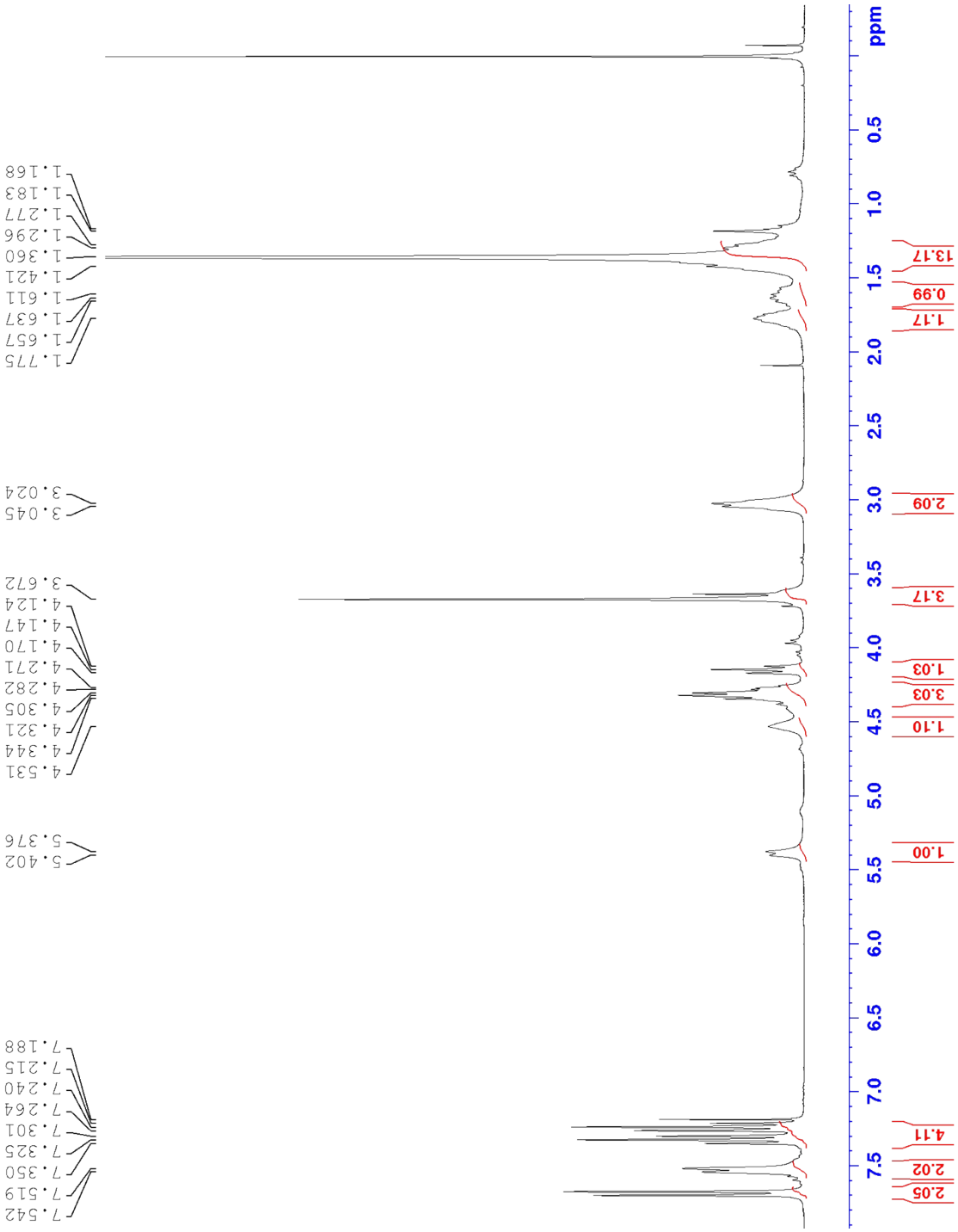


Sample 1t: *N*-(9-Fluorenylmethoxycarbonyl) glutamic acid (*O*-tert-butyl) methyl ester



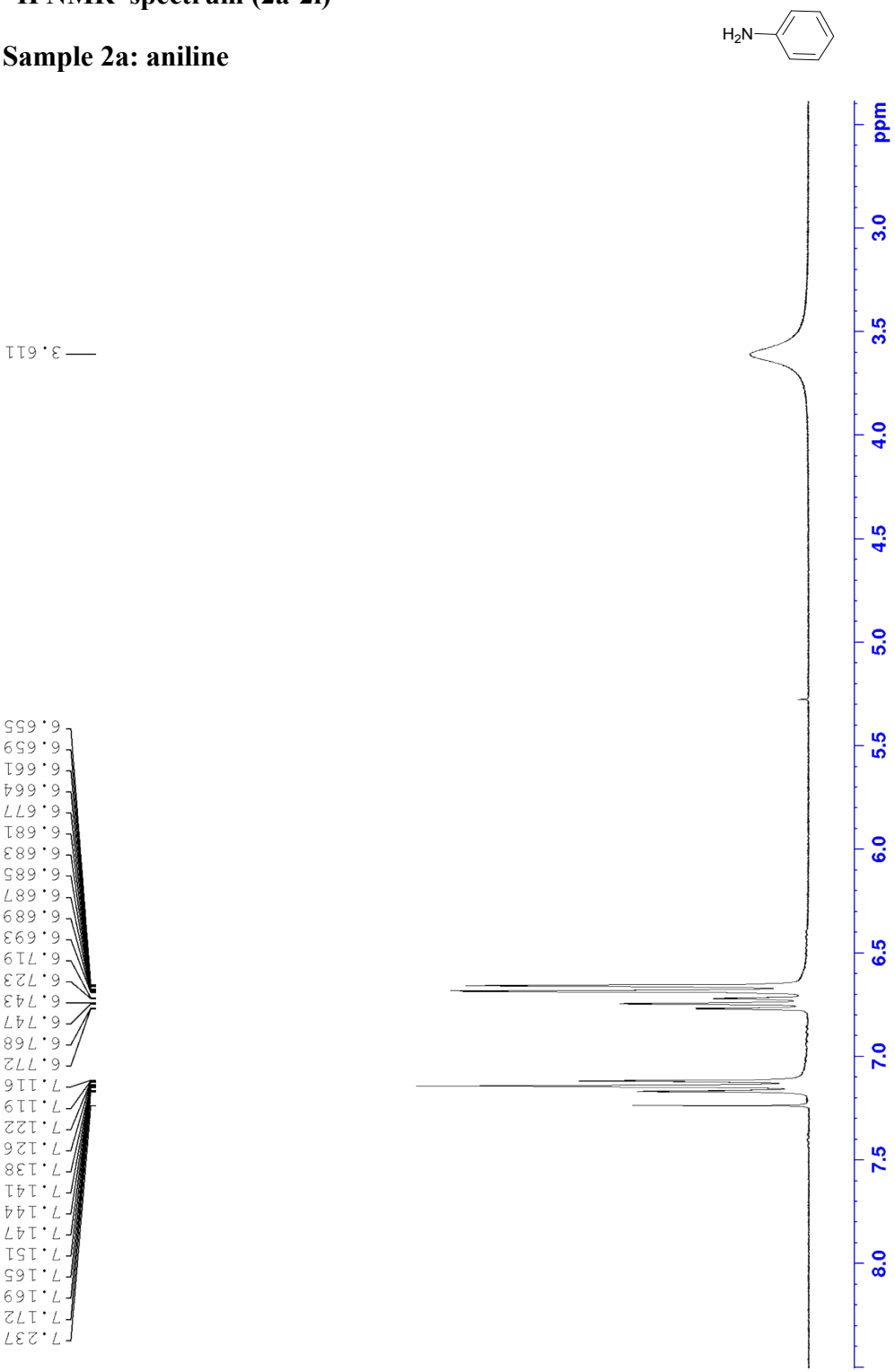
Sample 1u: *N*-Fmoc Lysine (*N*-Boc) methyl ester



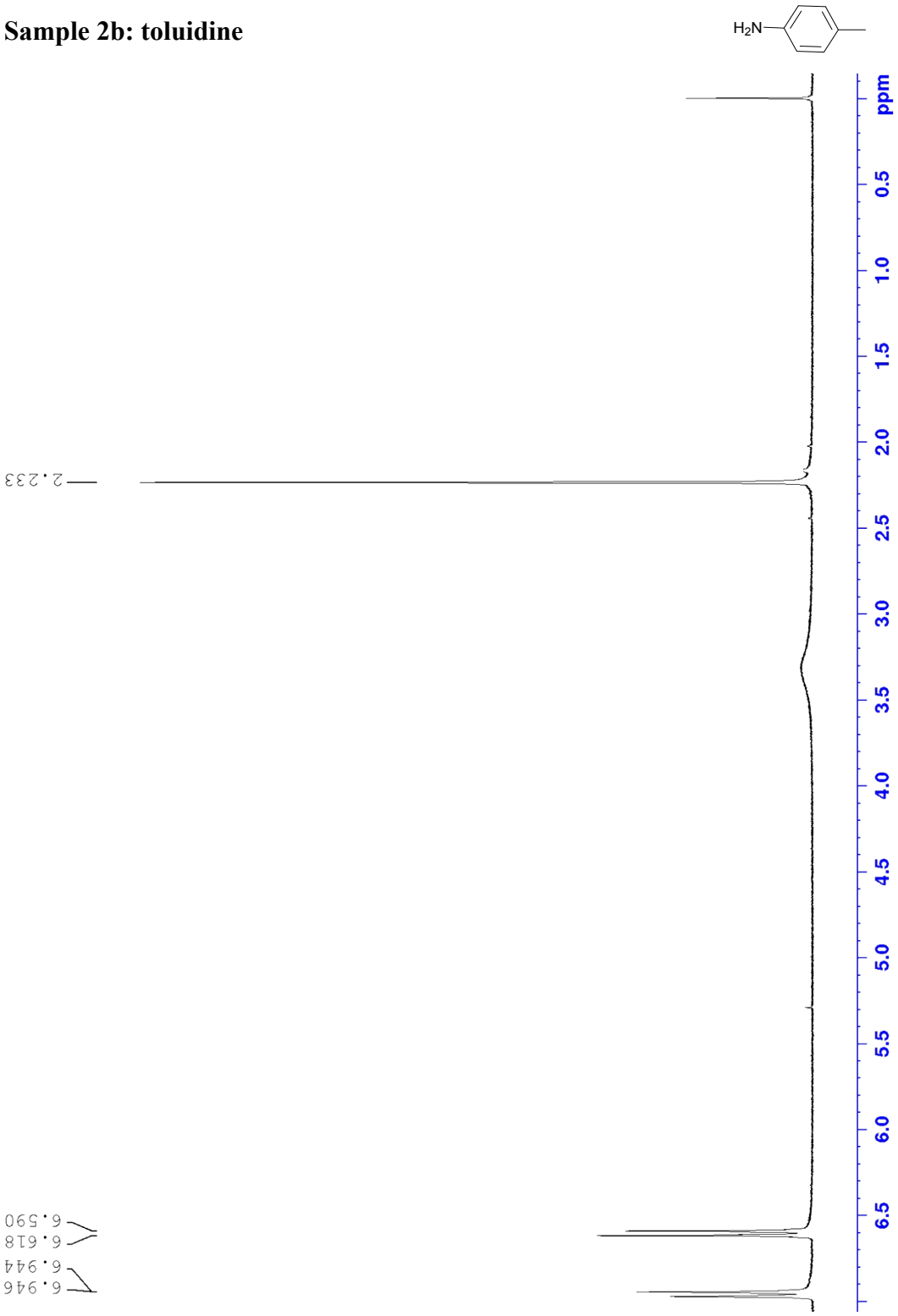


[1] H NMR spectrum (2a-2i)

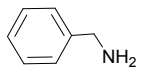
Sample 2a: aniline

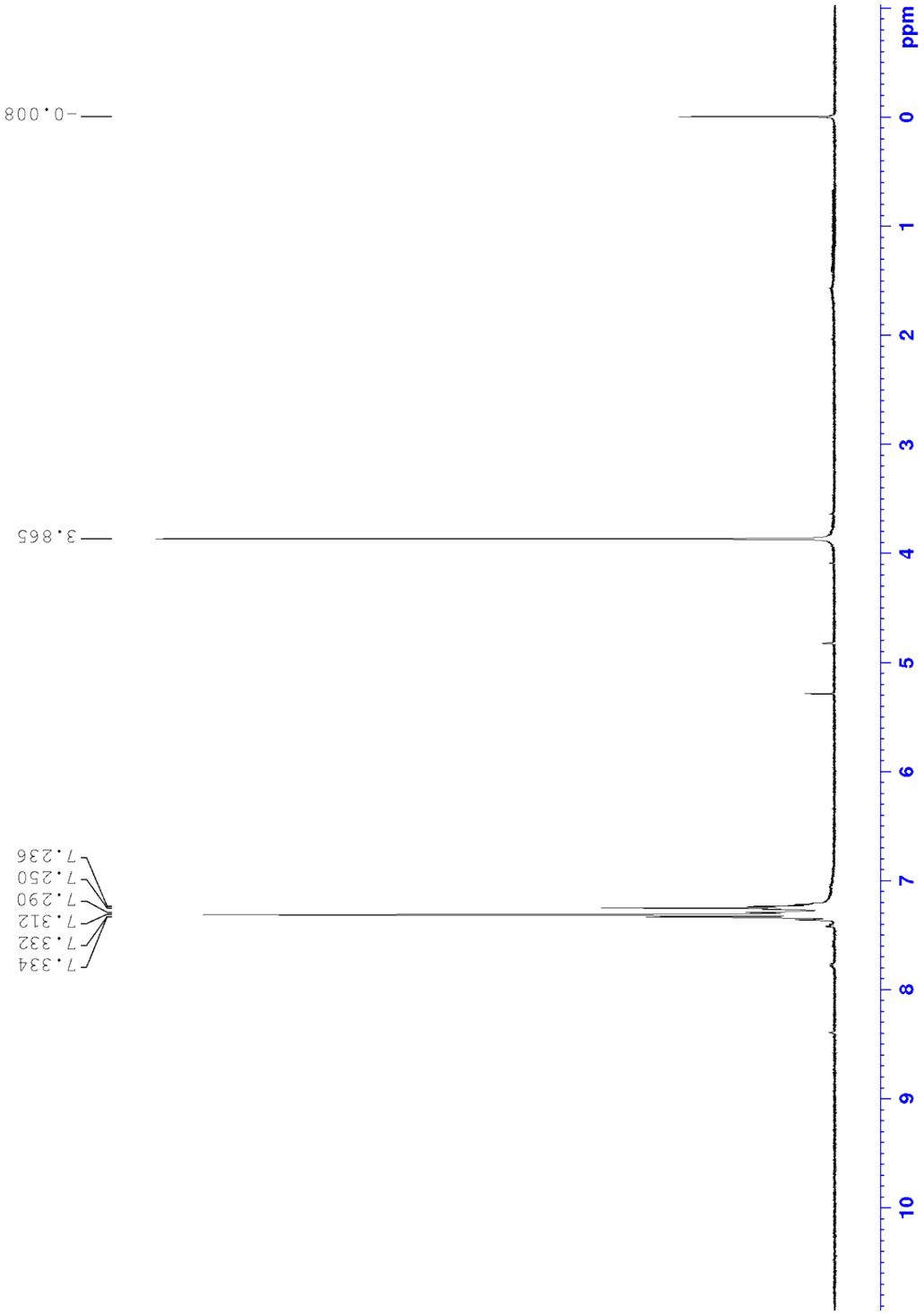


Sample 2b: toluidine

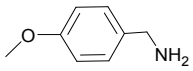


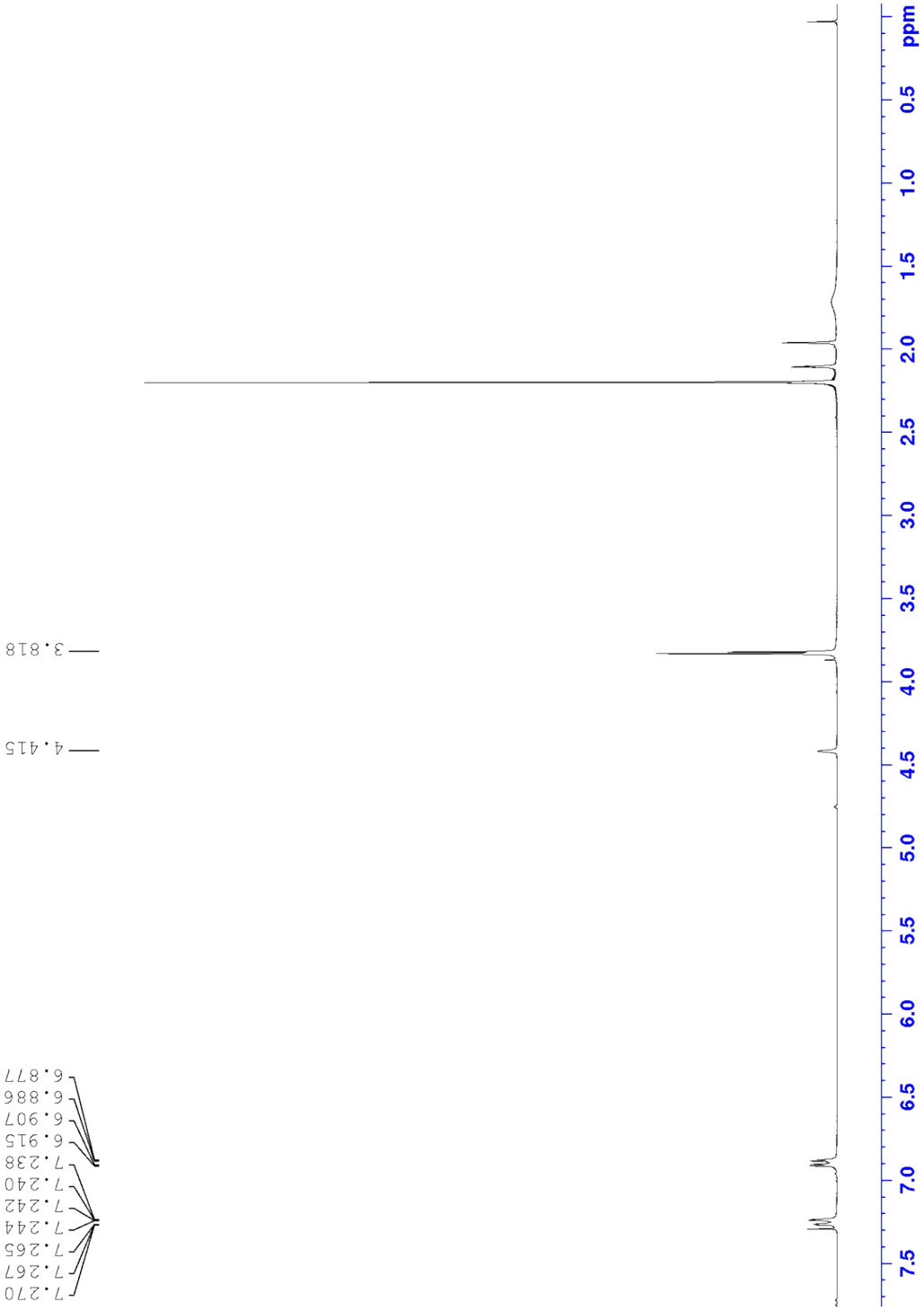
Sample 2c: benzylamine



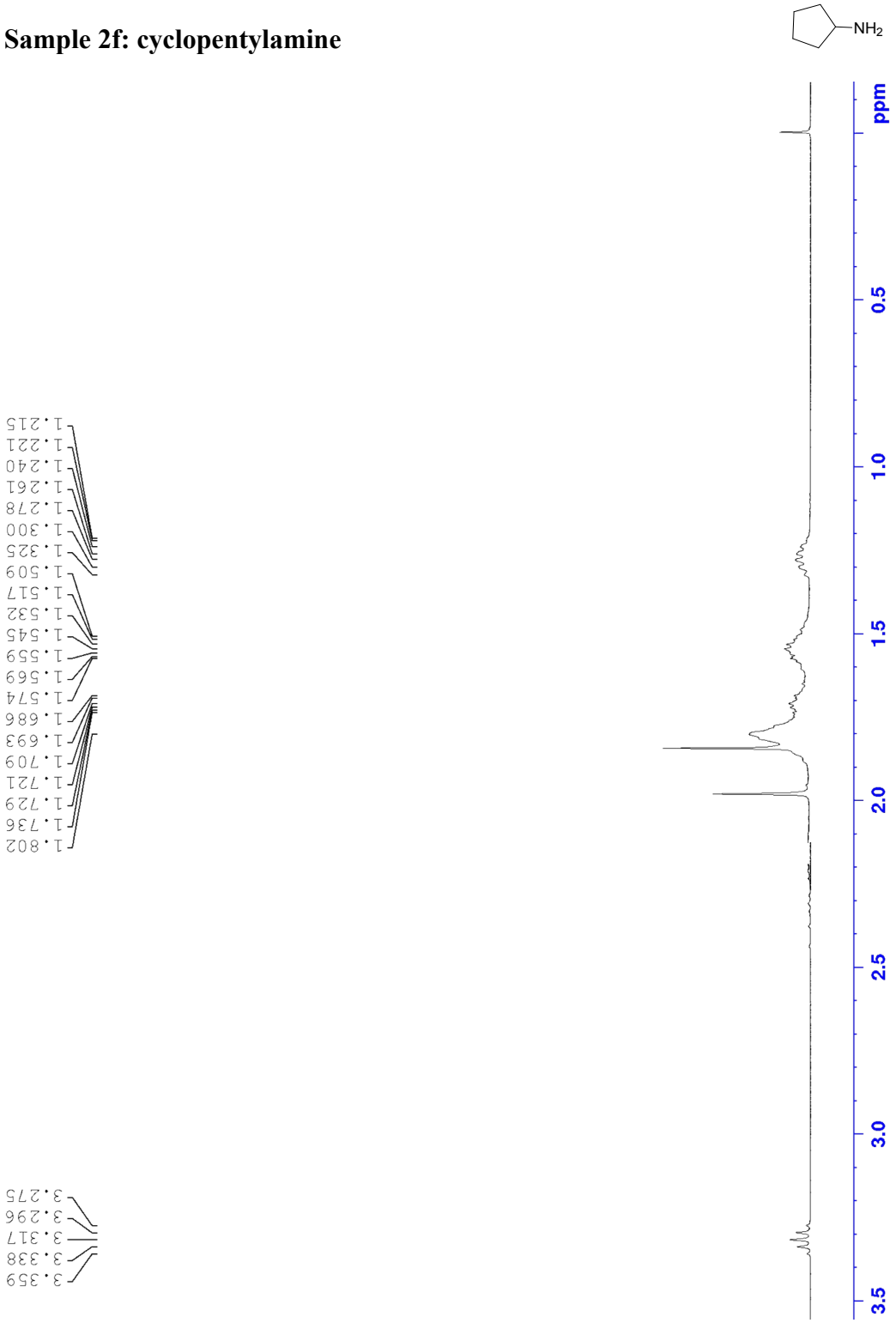


Sample 2d: p-methoxy benzylamine

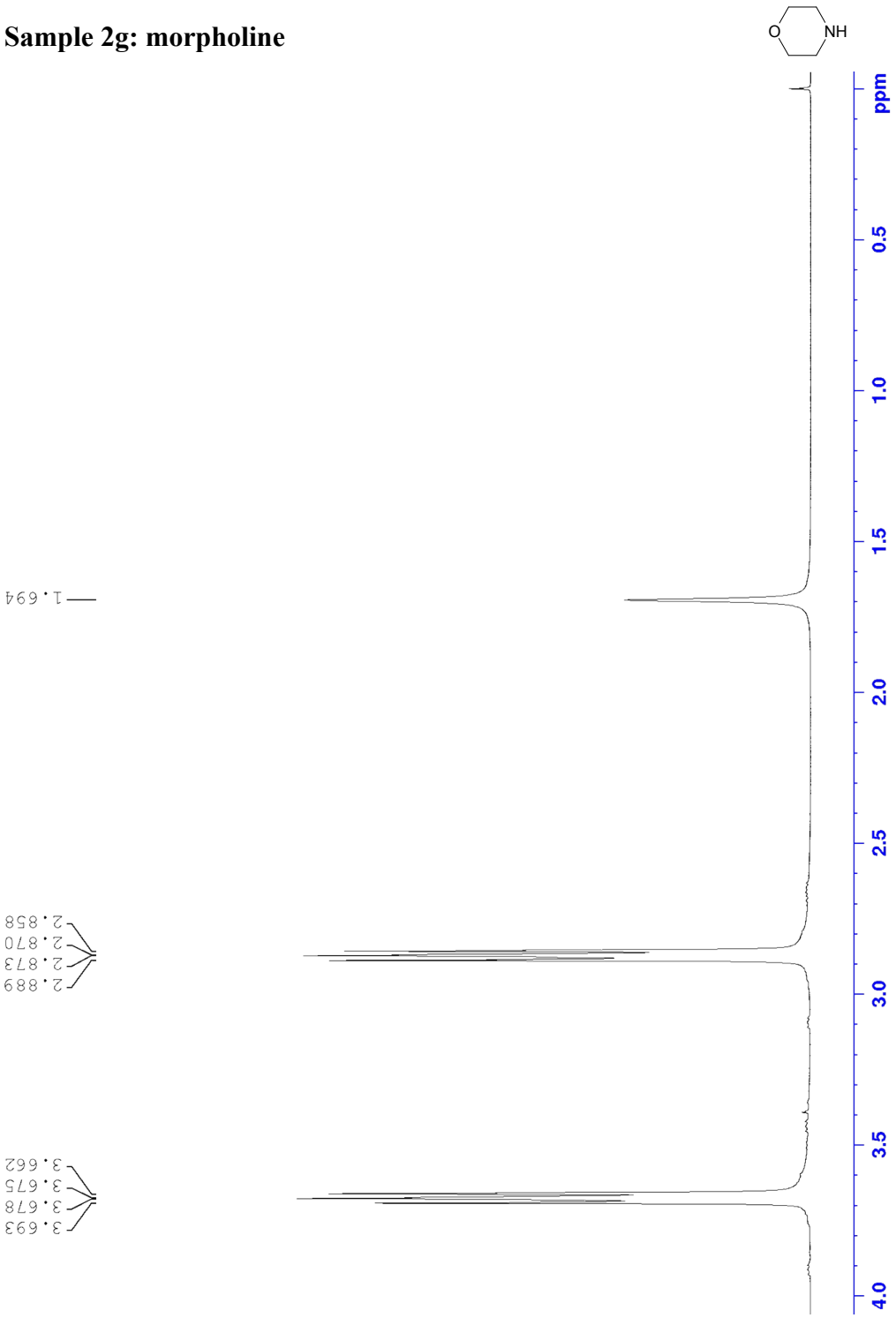




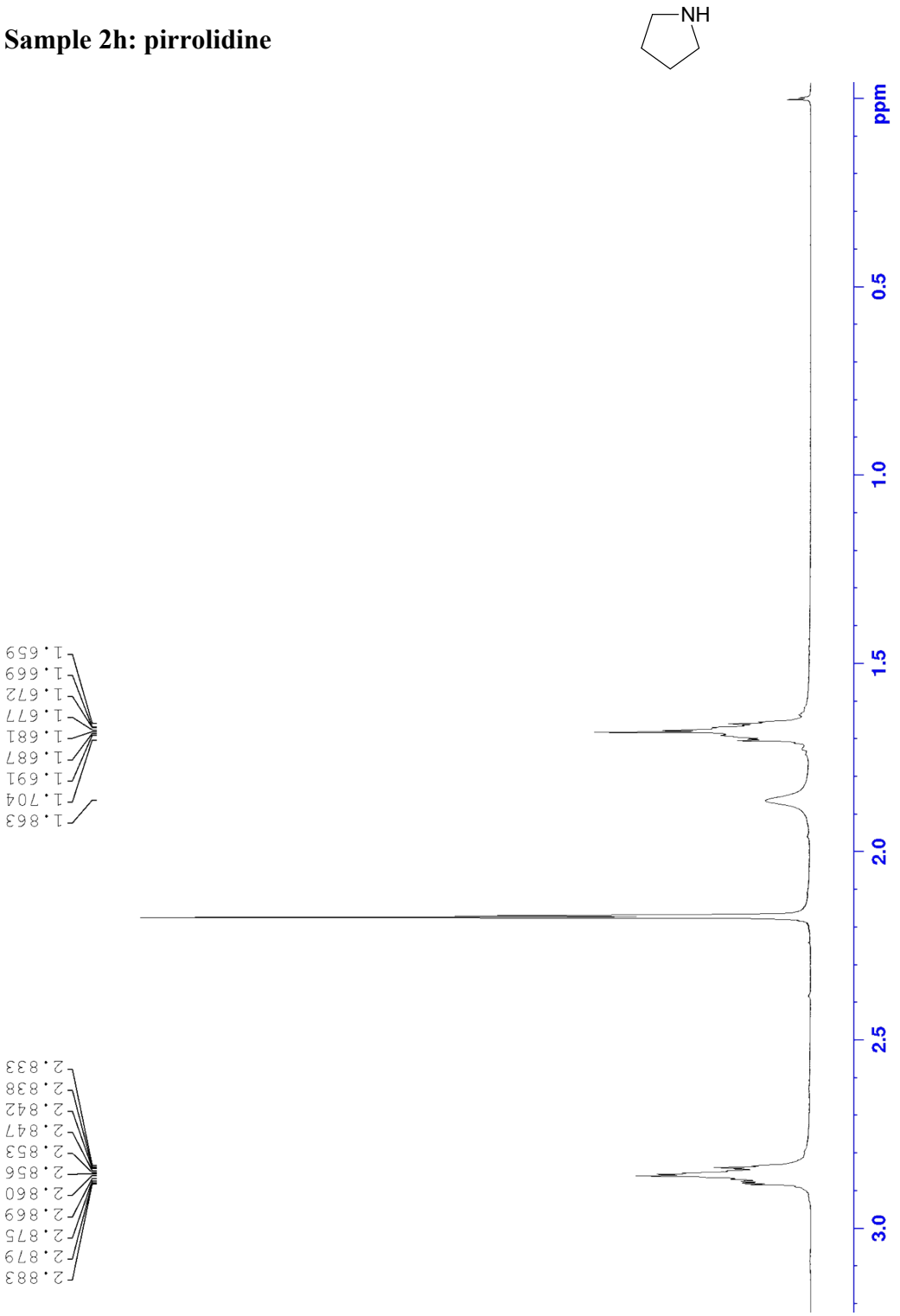
Sample 2f: cyclopentylamine



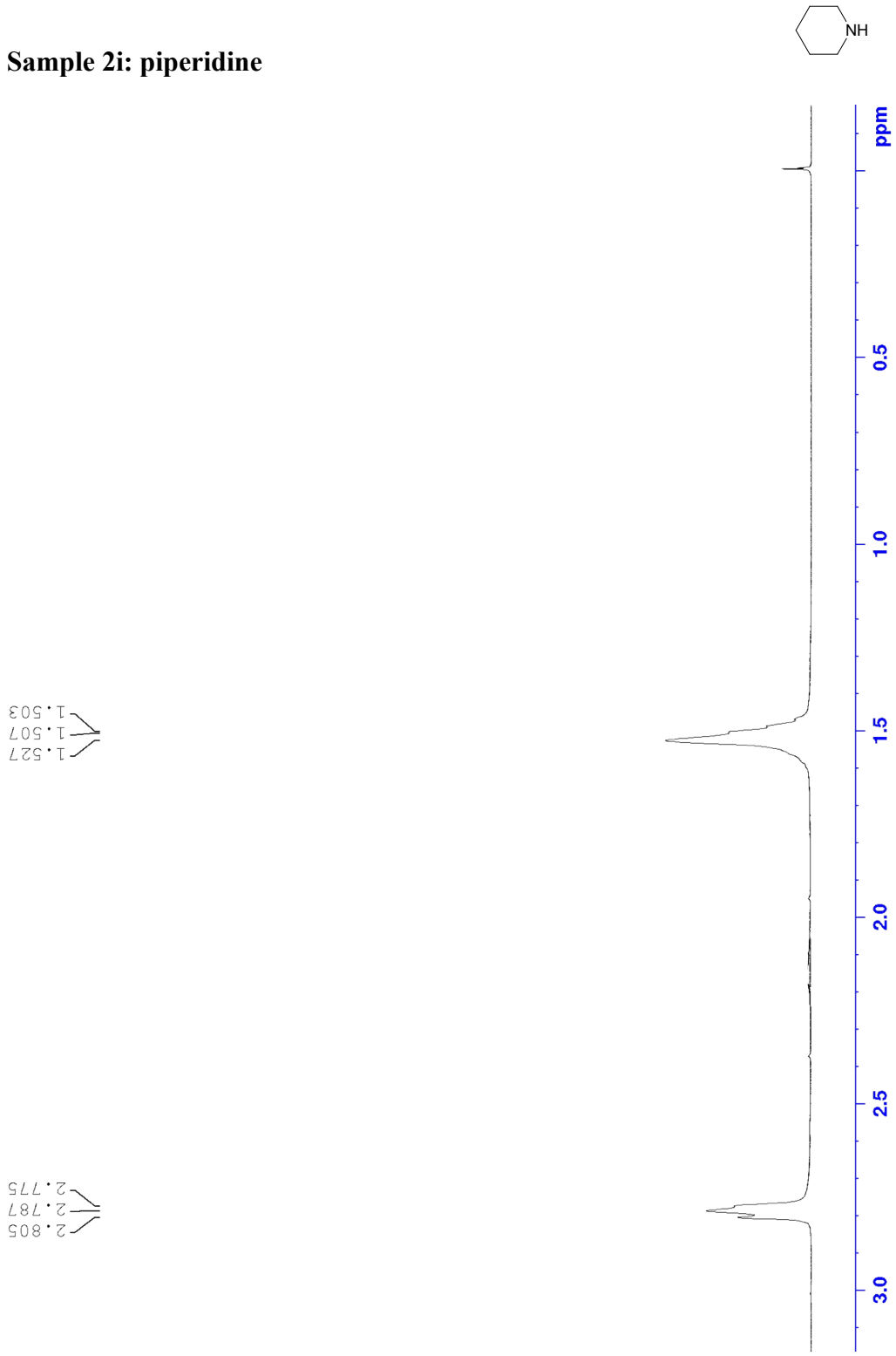
Sample 2g: morpholine



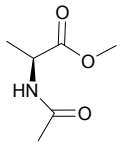
Sample 2h: pirrolidine



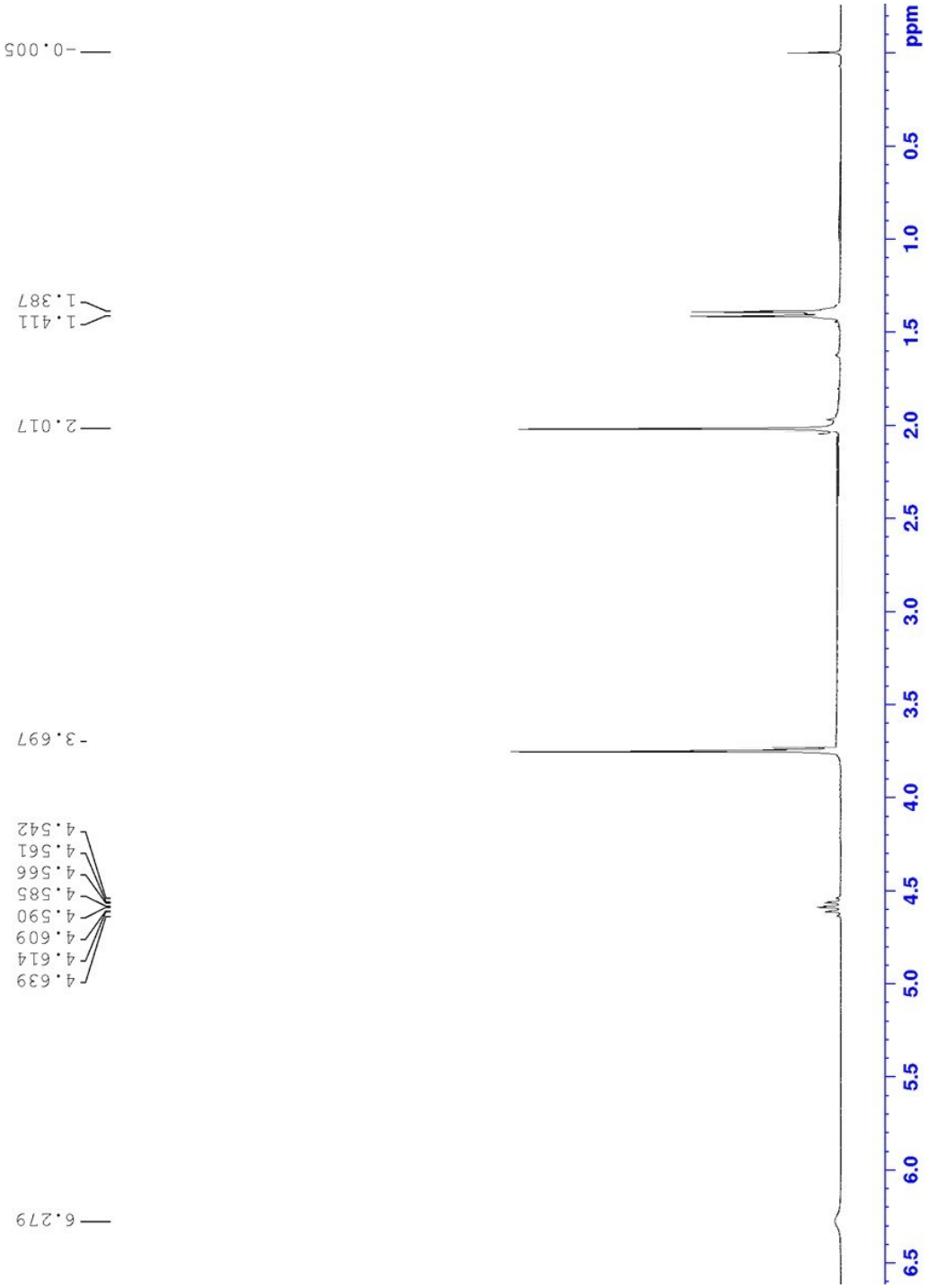
Sample 2i: piperidine



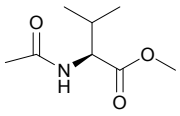
[1] H NMR spectrum (3j-3u)

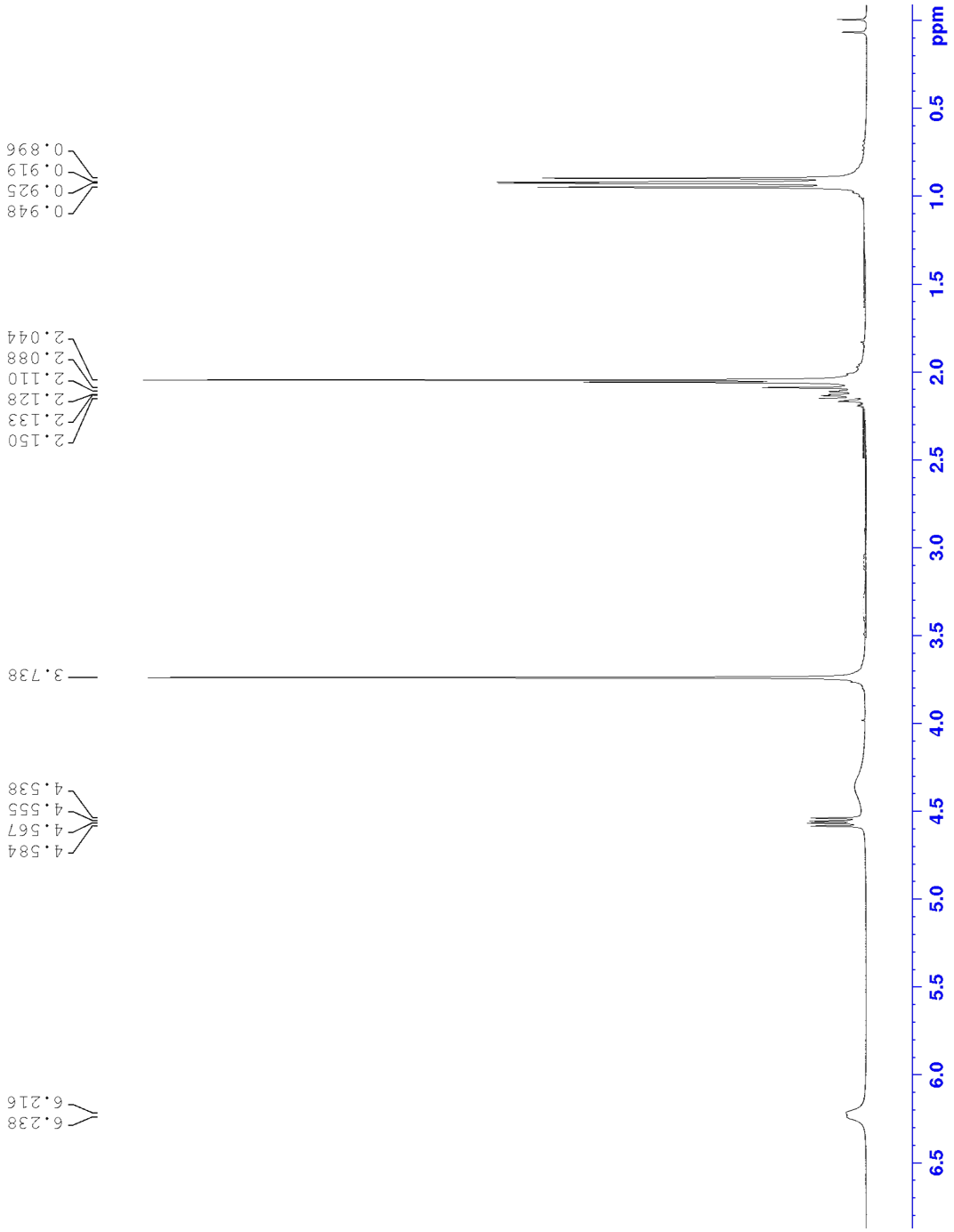


Sample 3j: *N*-Acetyl alanine methyl ester

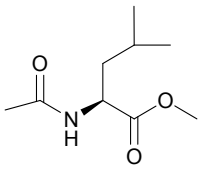


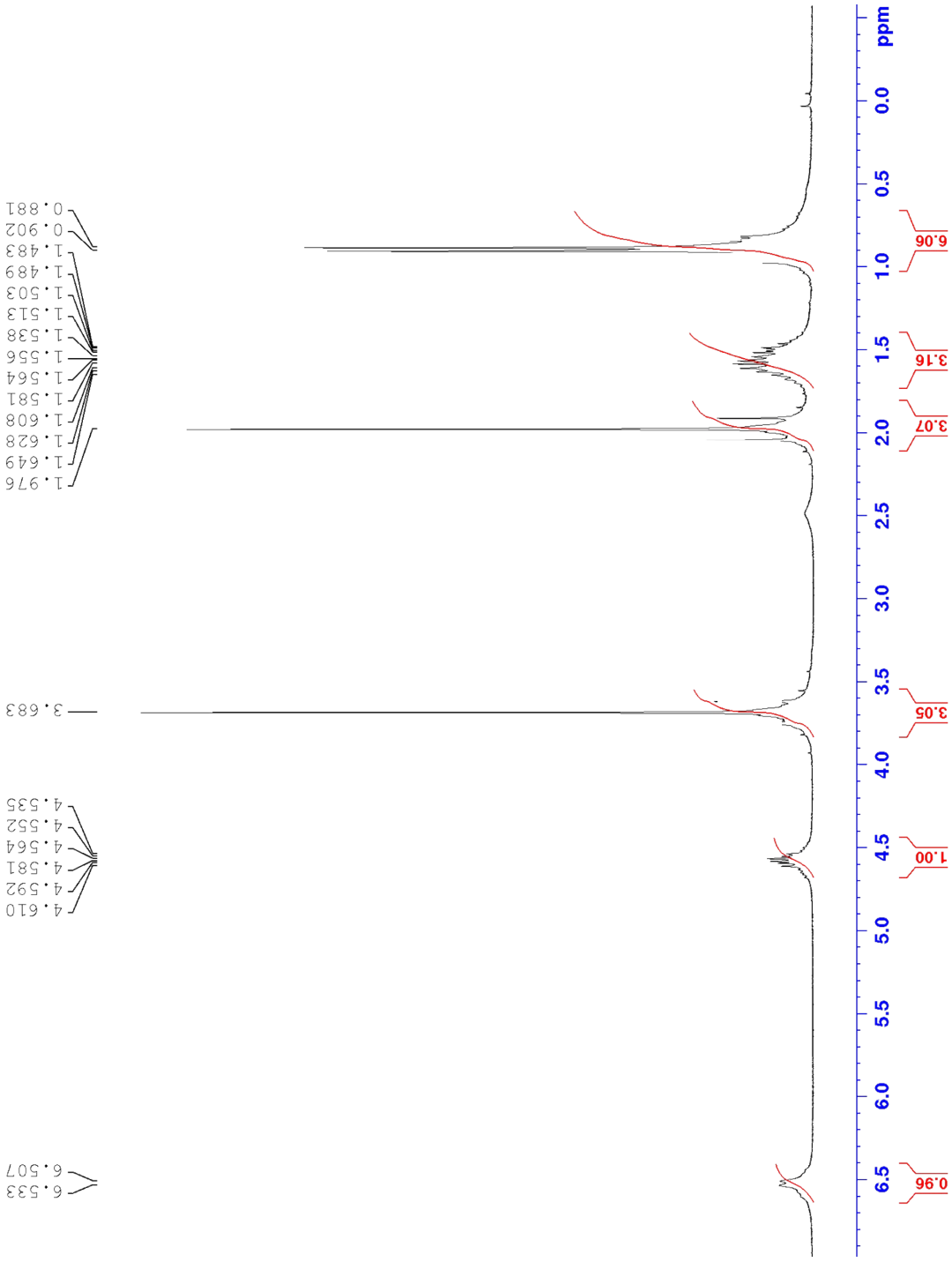
Sample 3k: *N*-Acetyl valine methyl ester



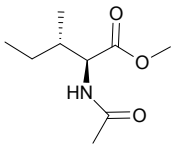


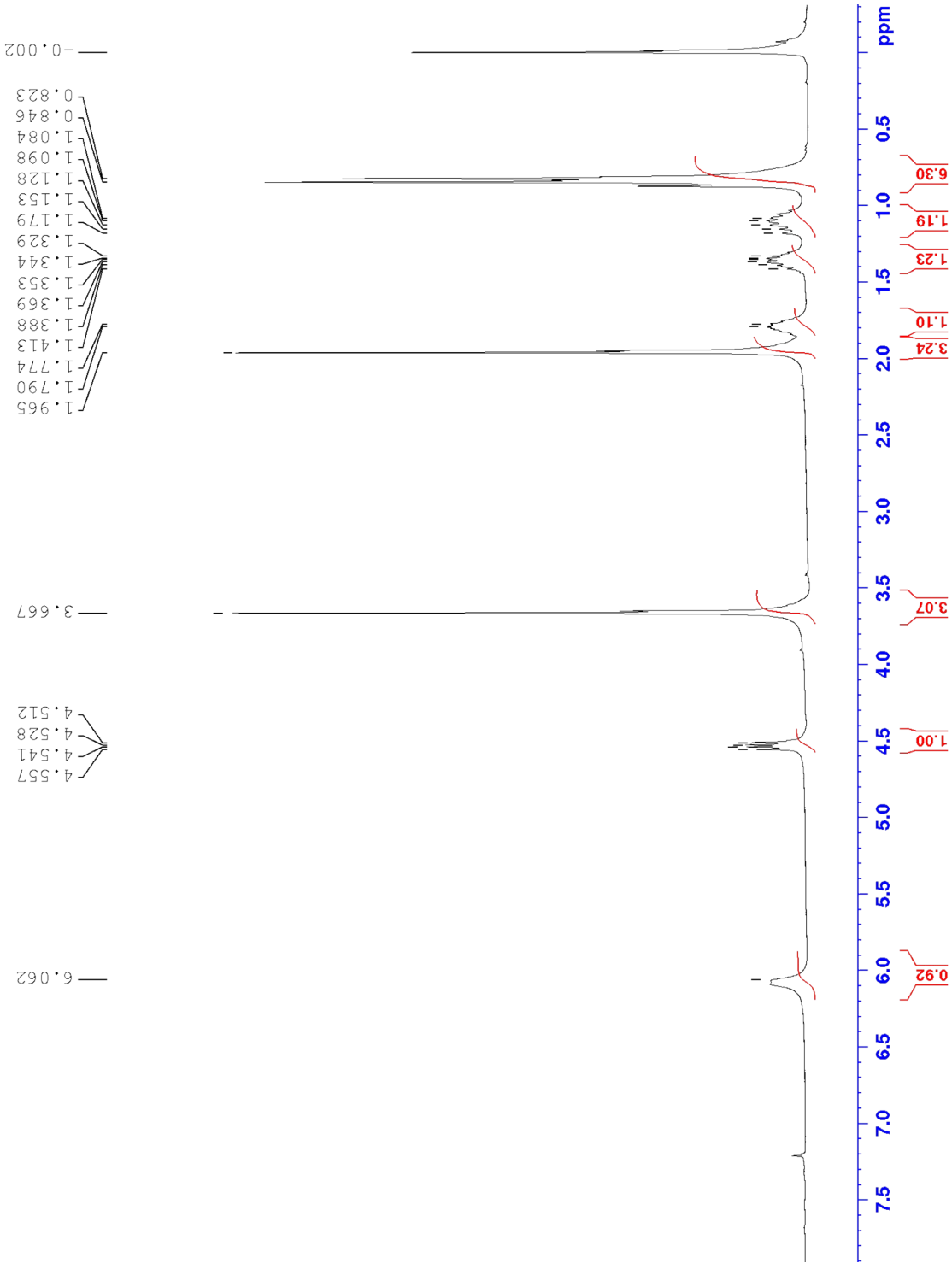
Sample 3l: *N*-Acetyl leucine methyl ester .



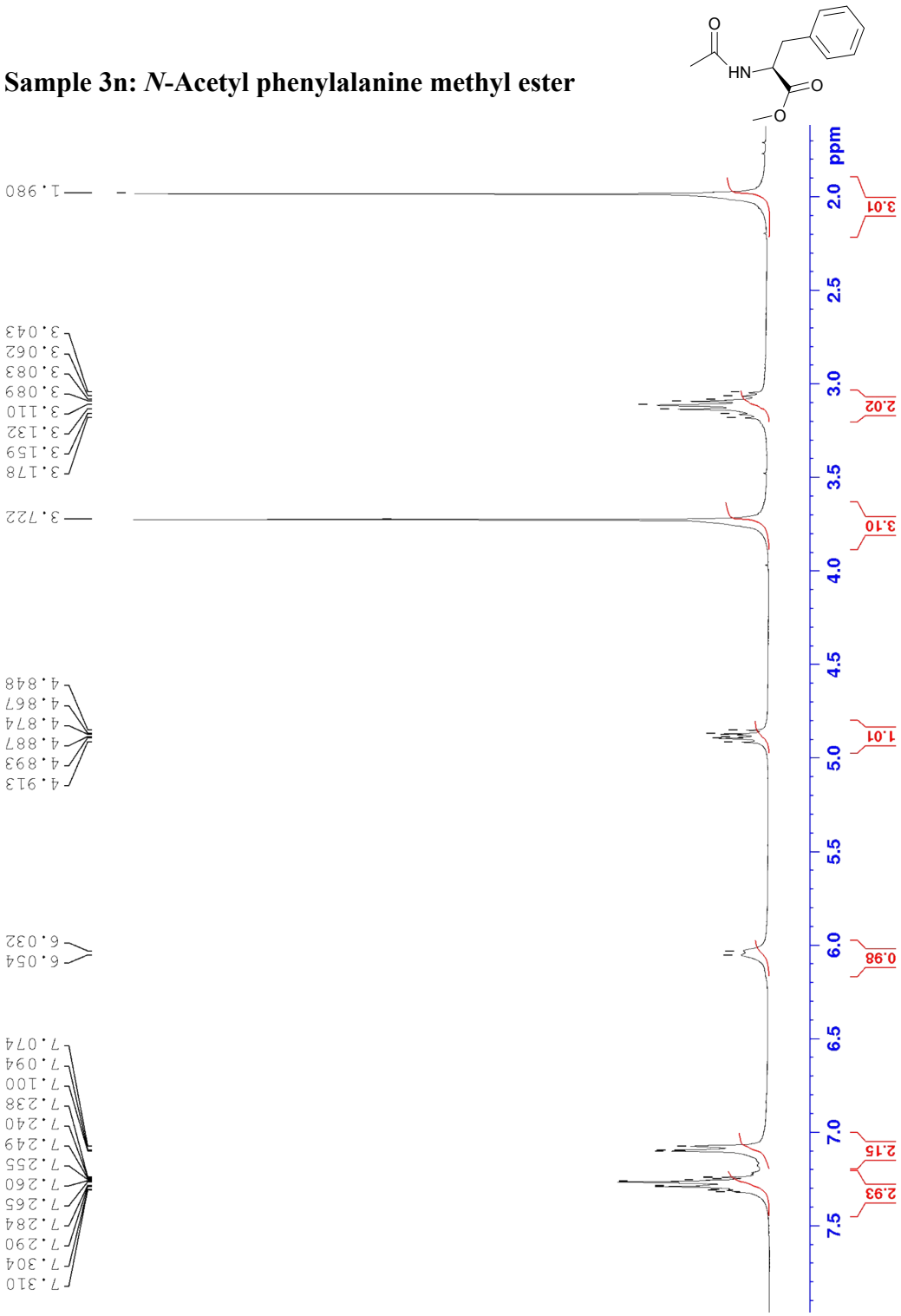


Sample 3m: *N*-Acetyl isoleucine methyl ester

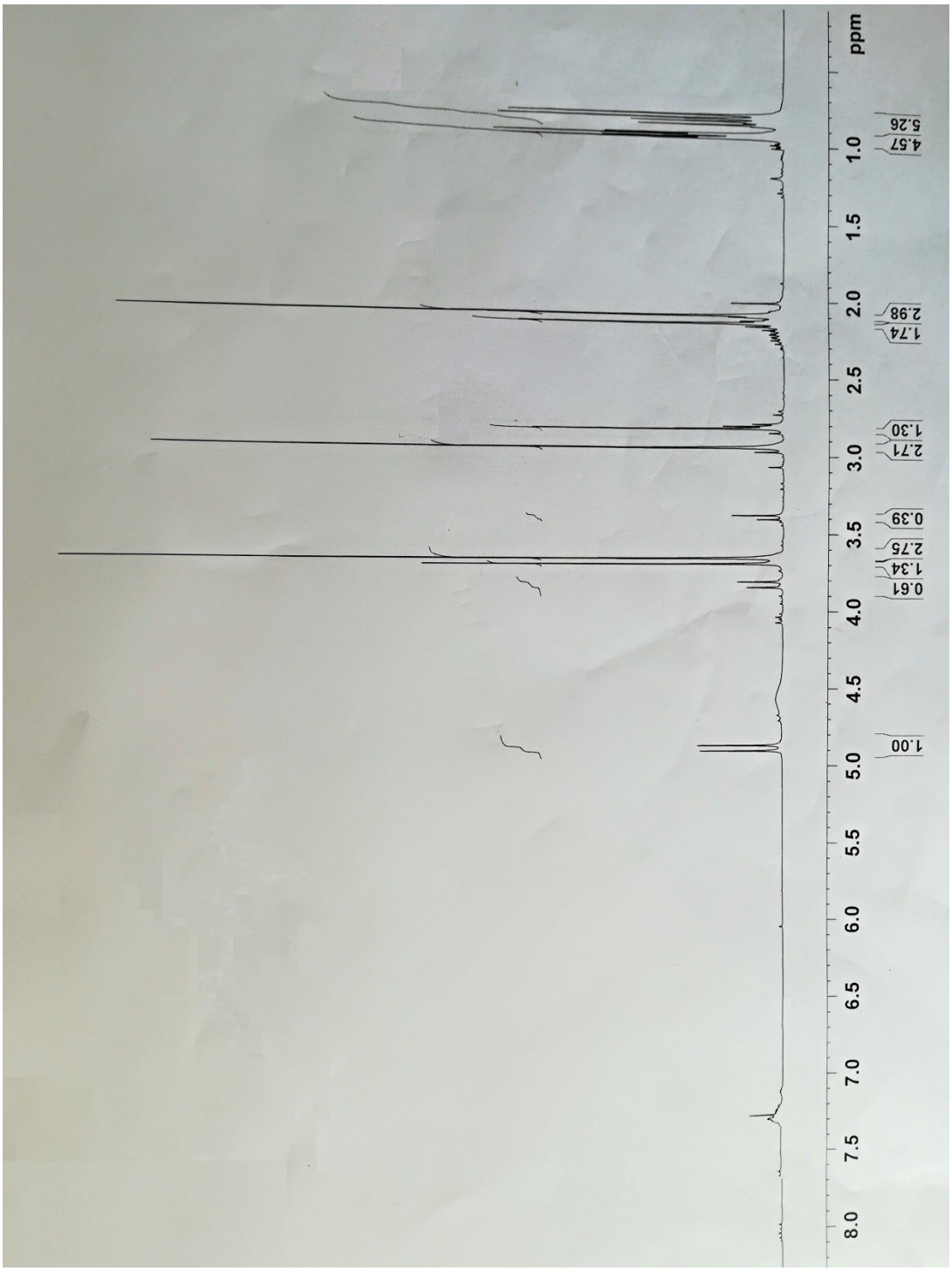




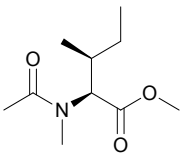
Sample 3n: *N*-Acetyl phenylalanine methyl ester

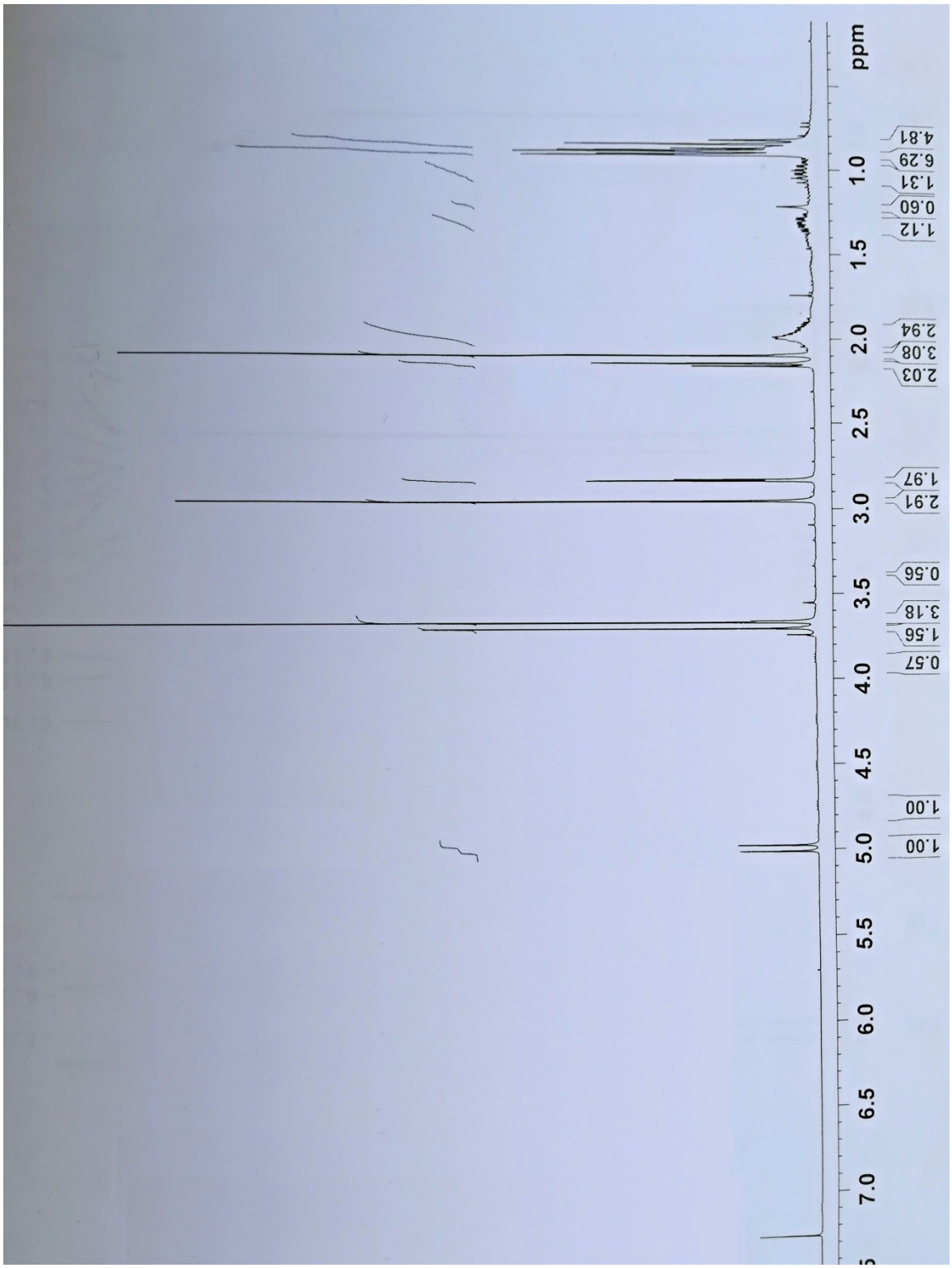


Sample 3o (two rotamers)**:** ***N*-Acetyl *N*-methyl valine methyl ester**

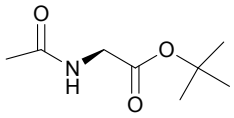


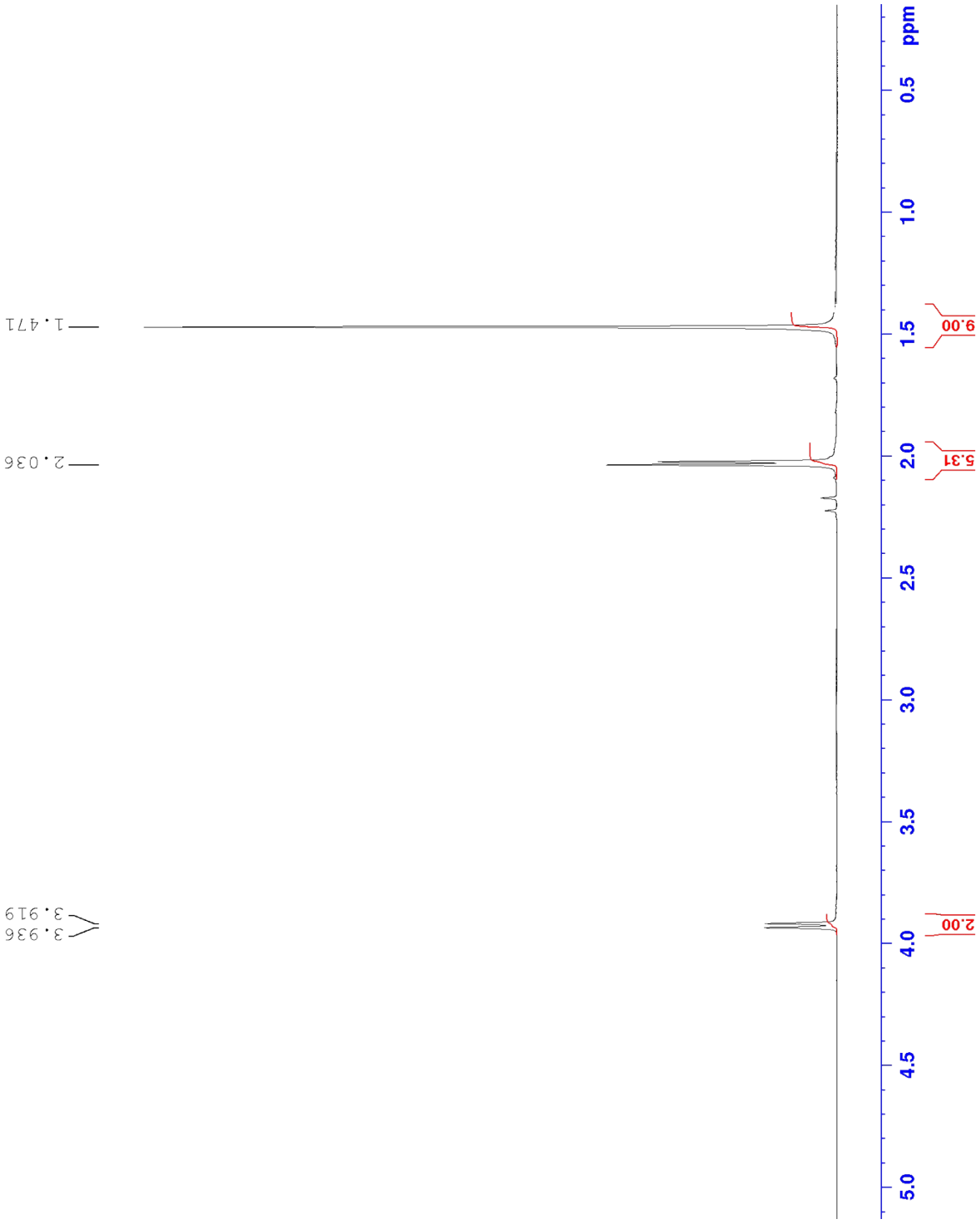
Sample 3p (two rotamers)**: *N*-Acetyl *N*-methyl isoleucine methyl ester**



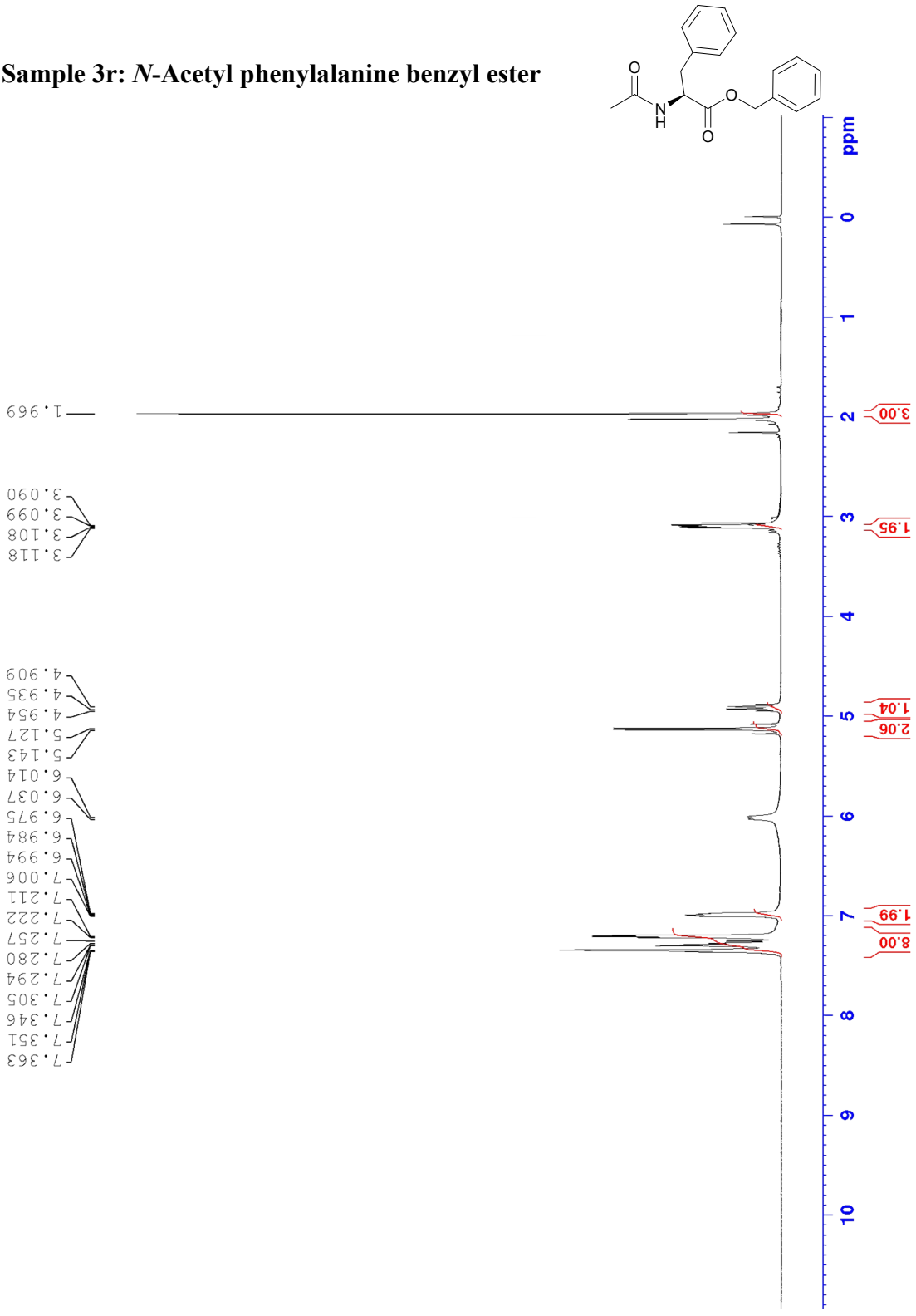


Sample 3q: *N*-Acetyl glycine *t*-butyl ester

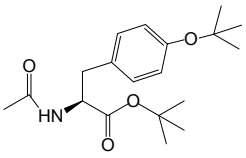


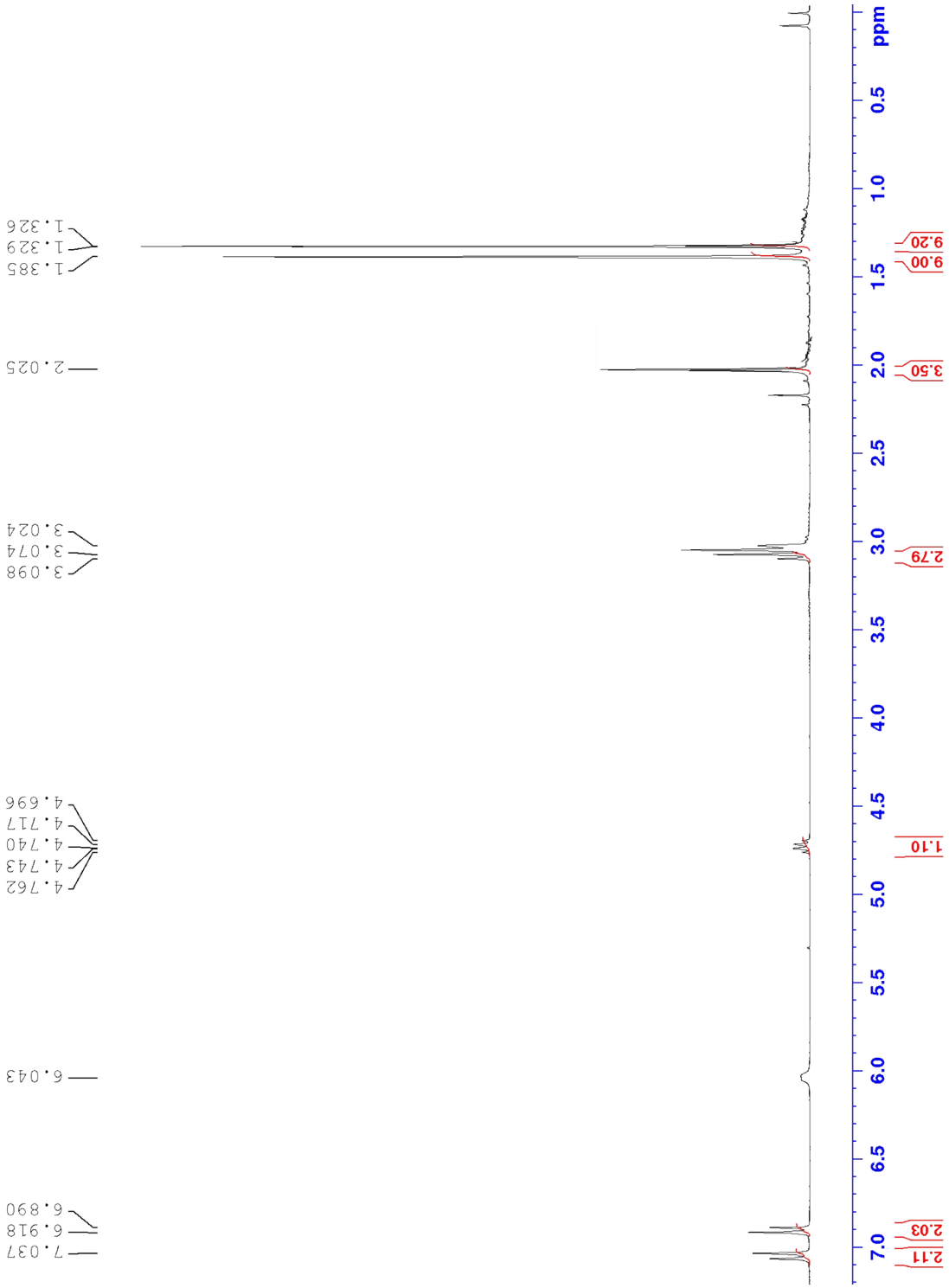


Sample 3r: *N*-Acetyl phenylalanine benzyl ester

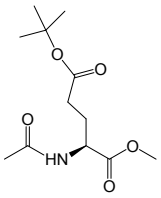


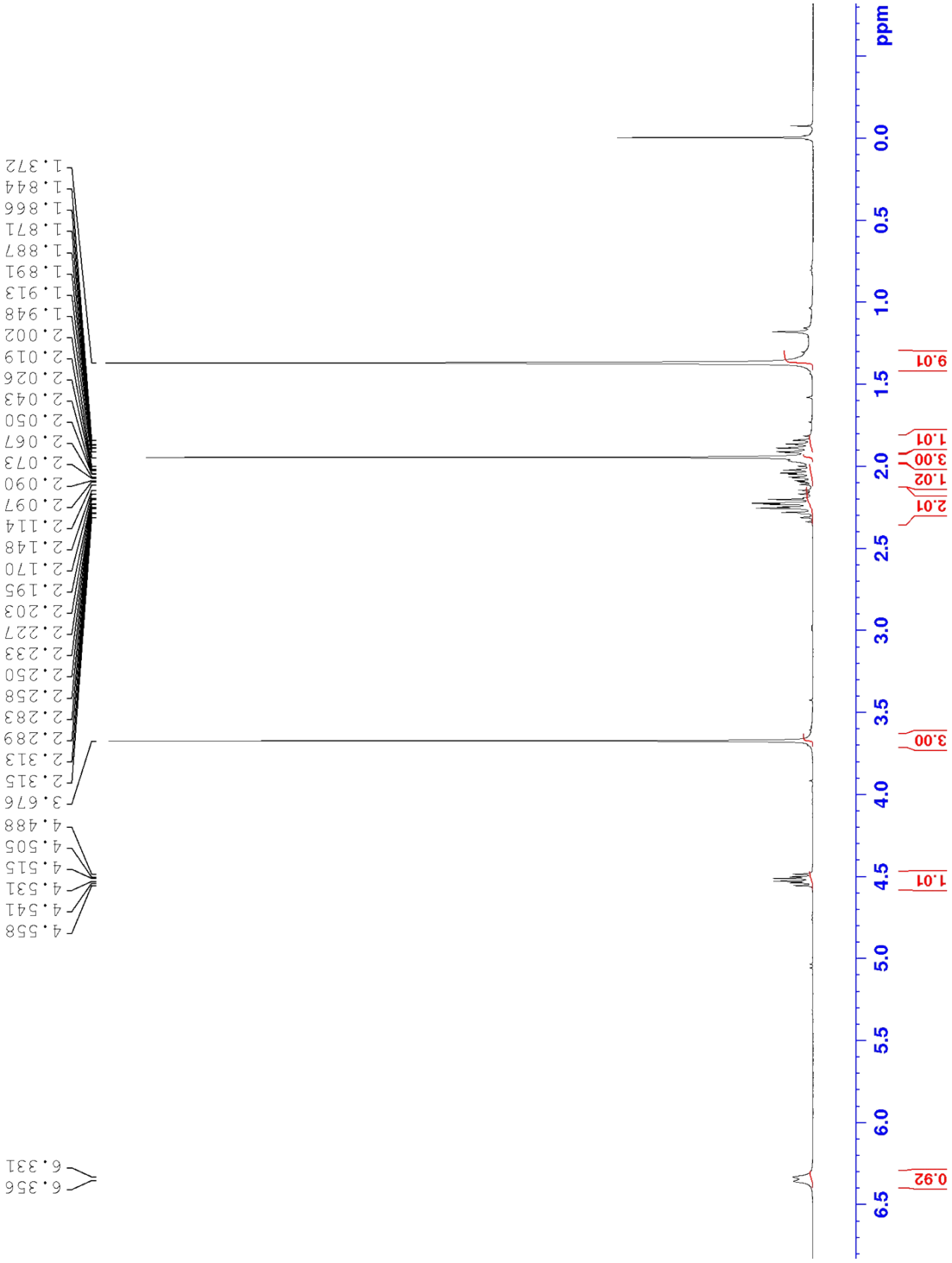
Sample 3s: *N*-Acetyl tyrosine (O-*t*-butyl) *t*-butyl ester



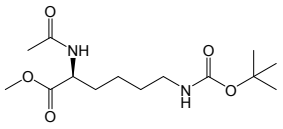


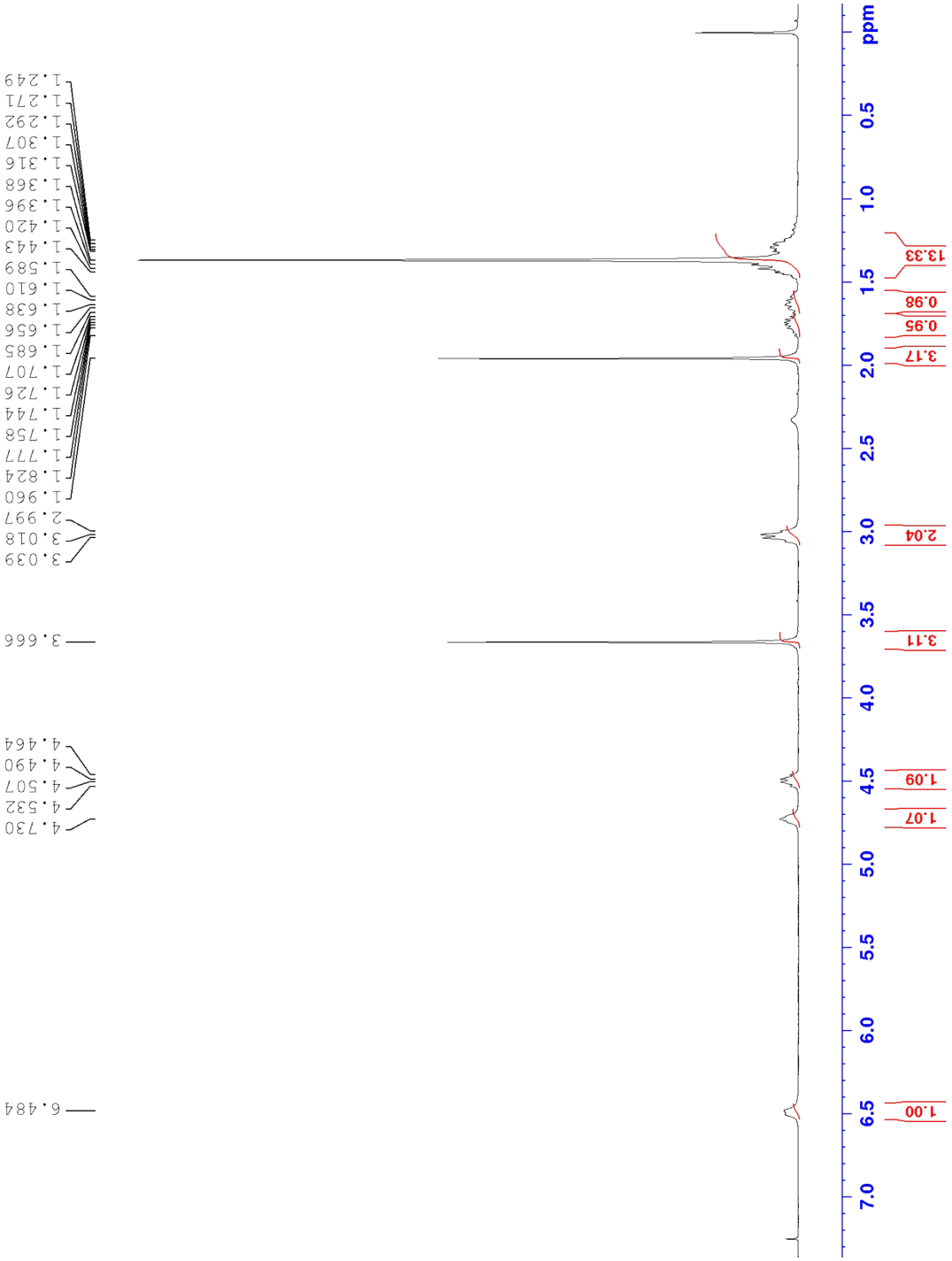
Sample 3t: *N*-Acetyl glutamic acid 5-*tert*-butyl 1-methyl ester

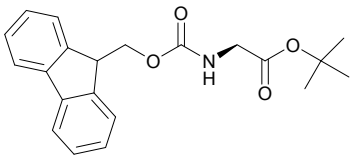




Sample 3u: *N*-Acetyl lysine (*N*-Boc) methyl ester

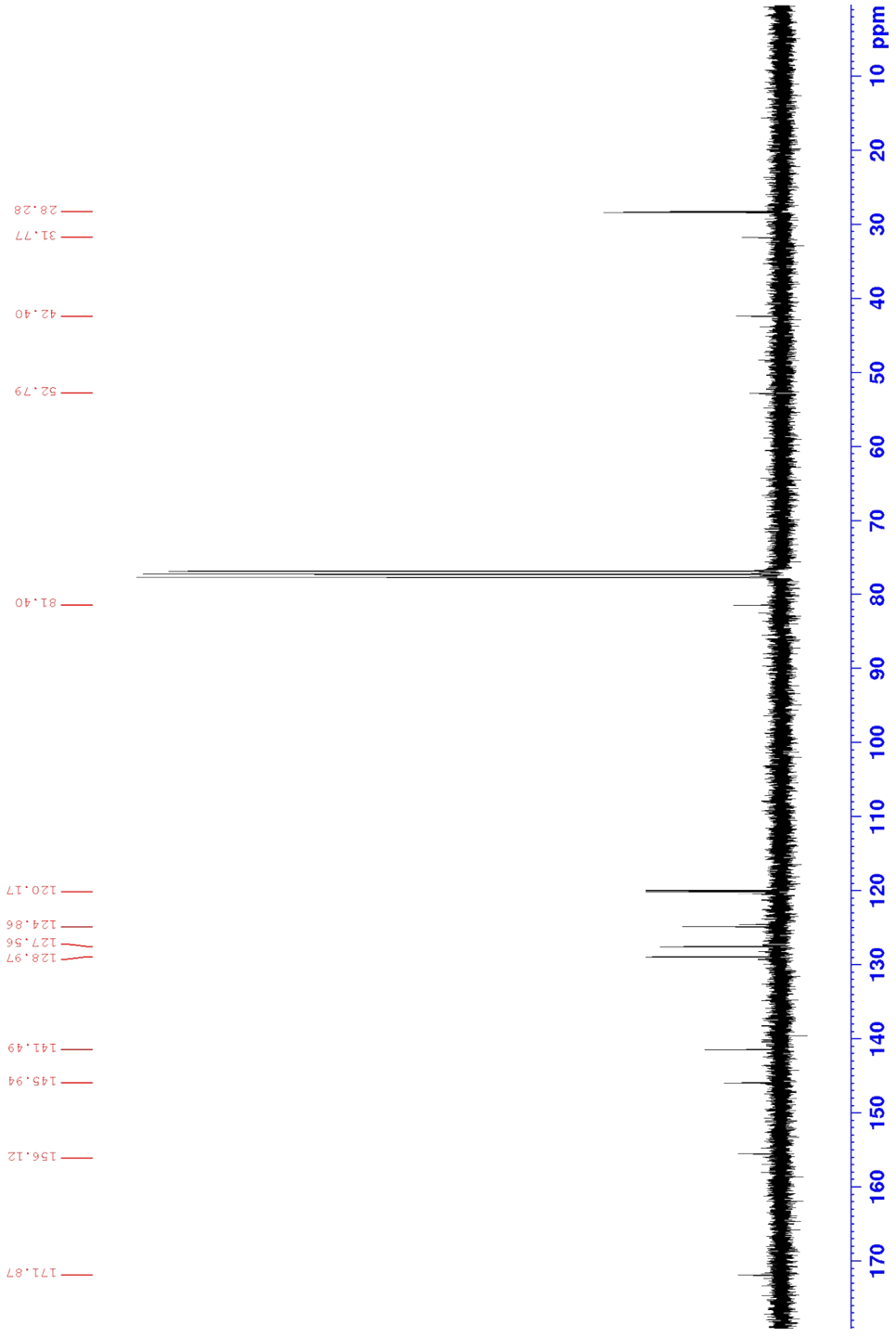


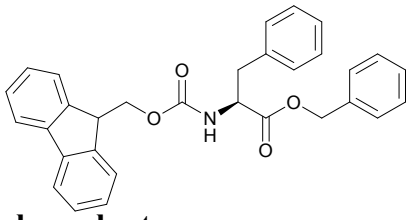




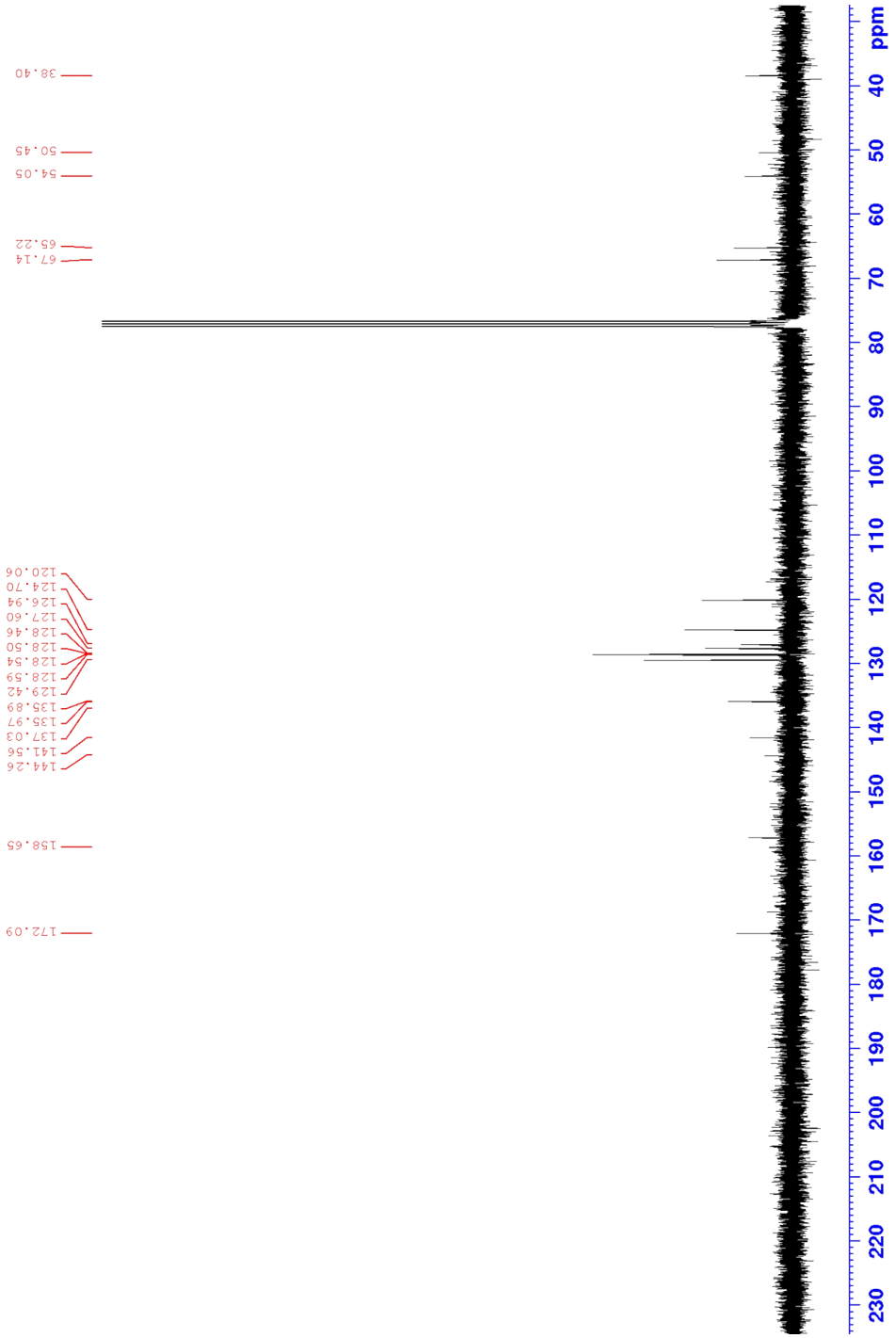
^{13}C NMR spectra (1q-1u)

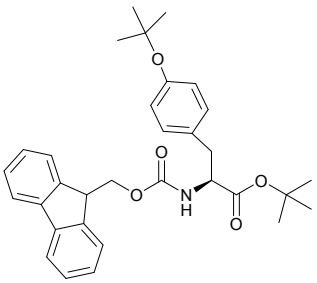
Sample 1q: *N*-(9-Fluorenylmethoxycarbonyl) glycine t-butyl ester



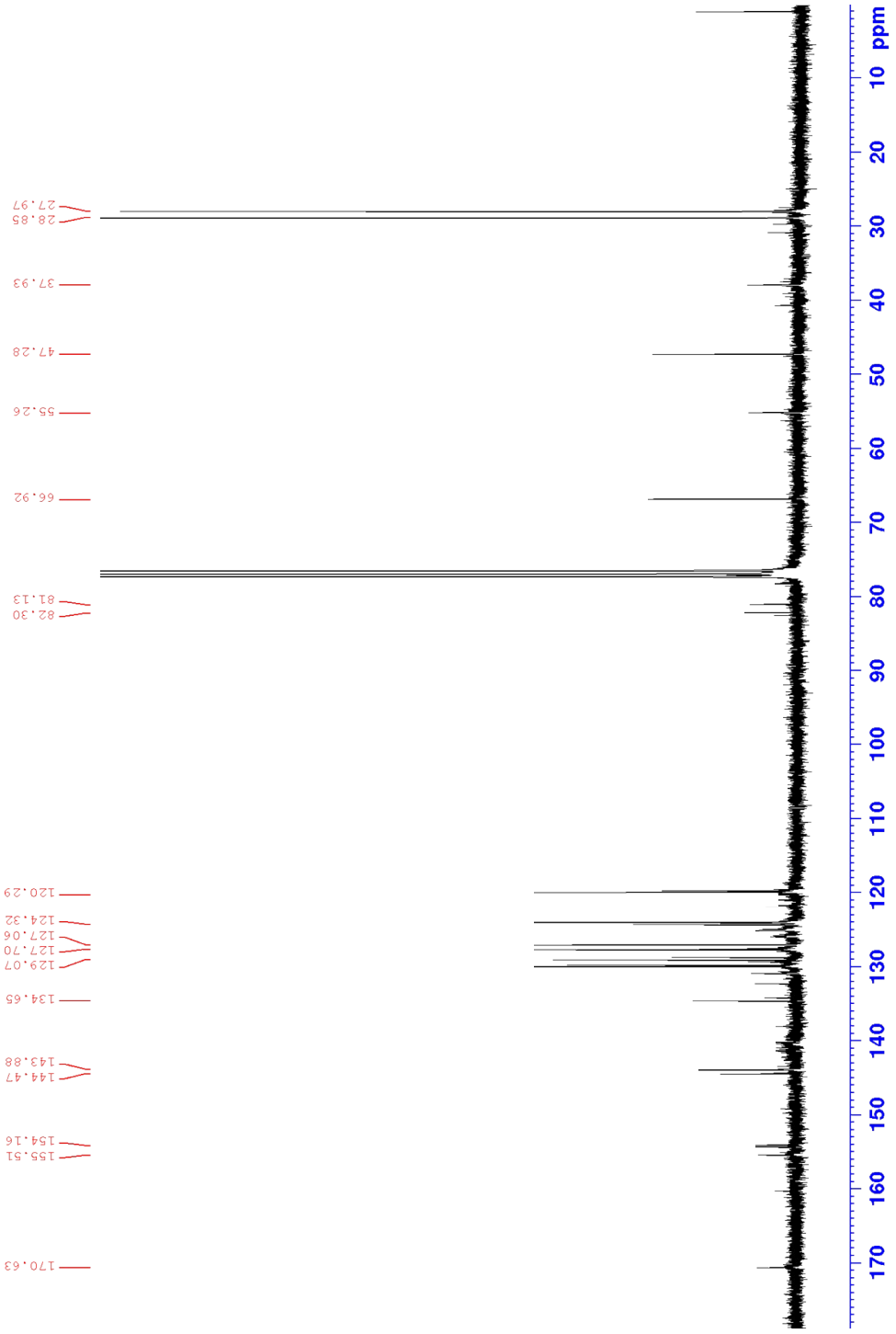


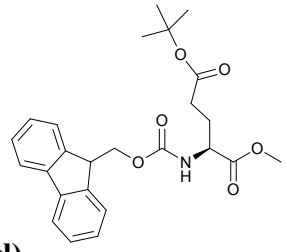
Sample 1r: *N*-(9-Fluorenylmethoxycarbonyl) phenylalanine benzyl ester



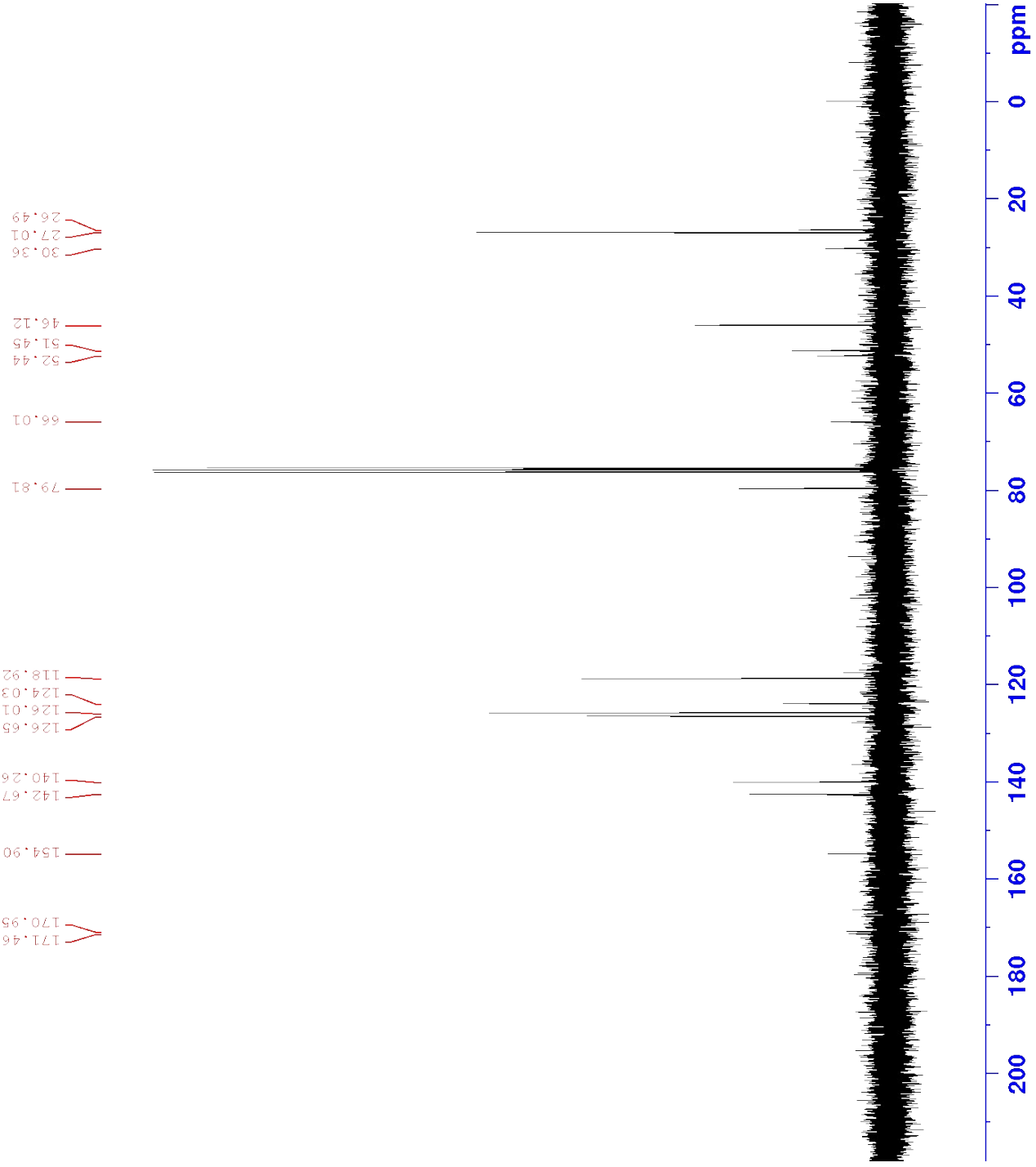


Sample 1s: *N*-(9-Fluorenylmethoxycarbonyl) tyrosine (O-*tert*-butyl) t-butyl ester

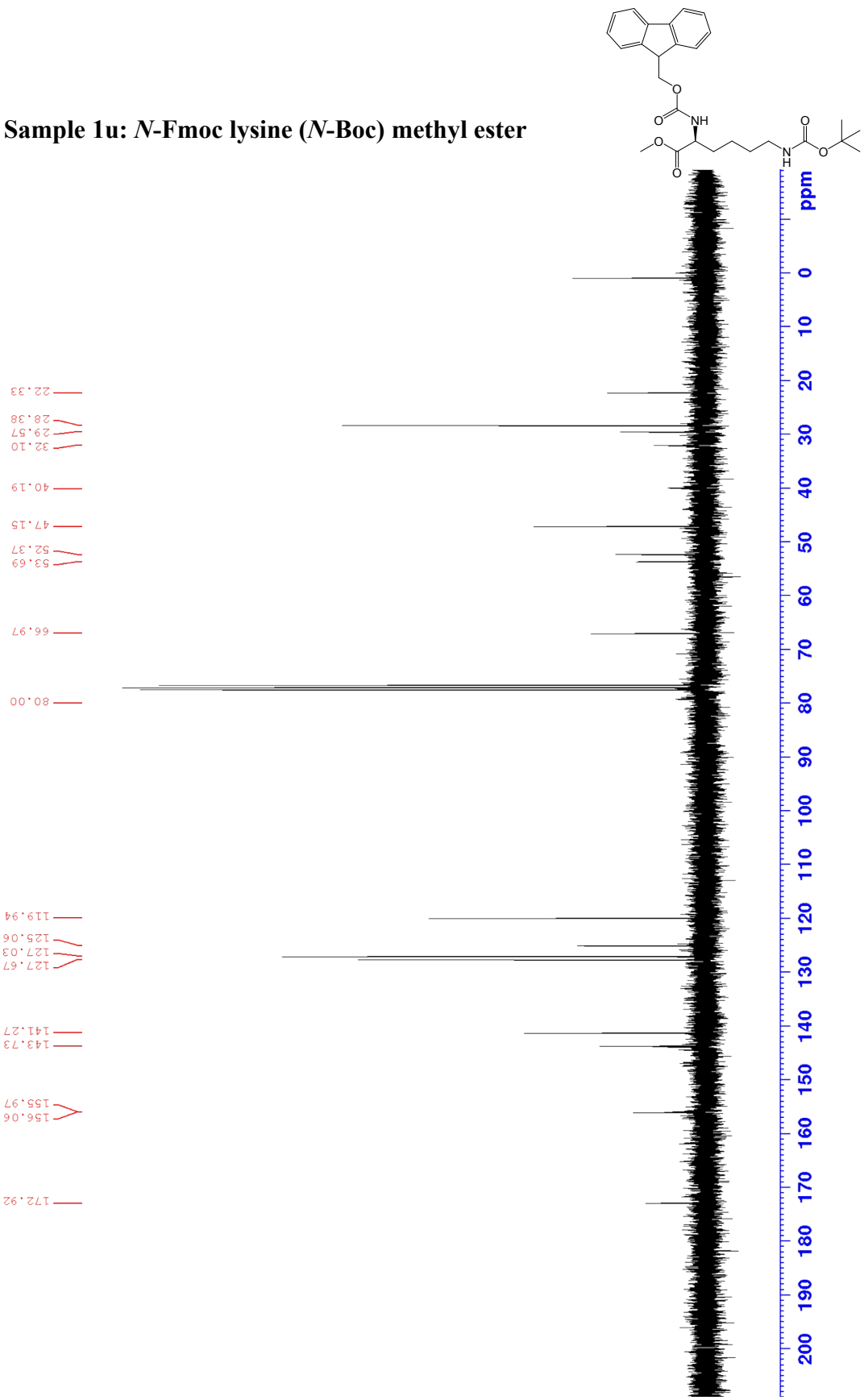




Sample 1t: *N*-(9-Fluorenylmethoxycarbonyl) glutamic acid (*O*-tert-butyl) methyl ester

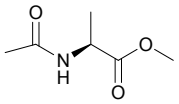


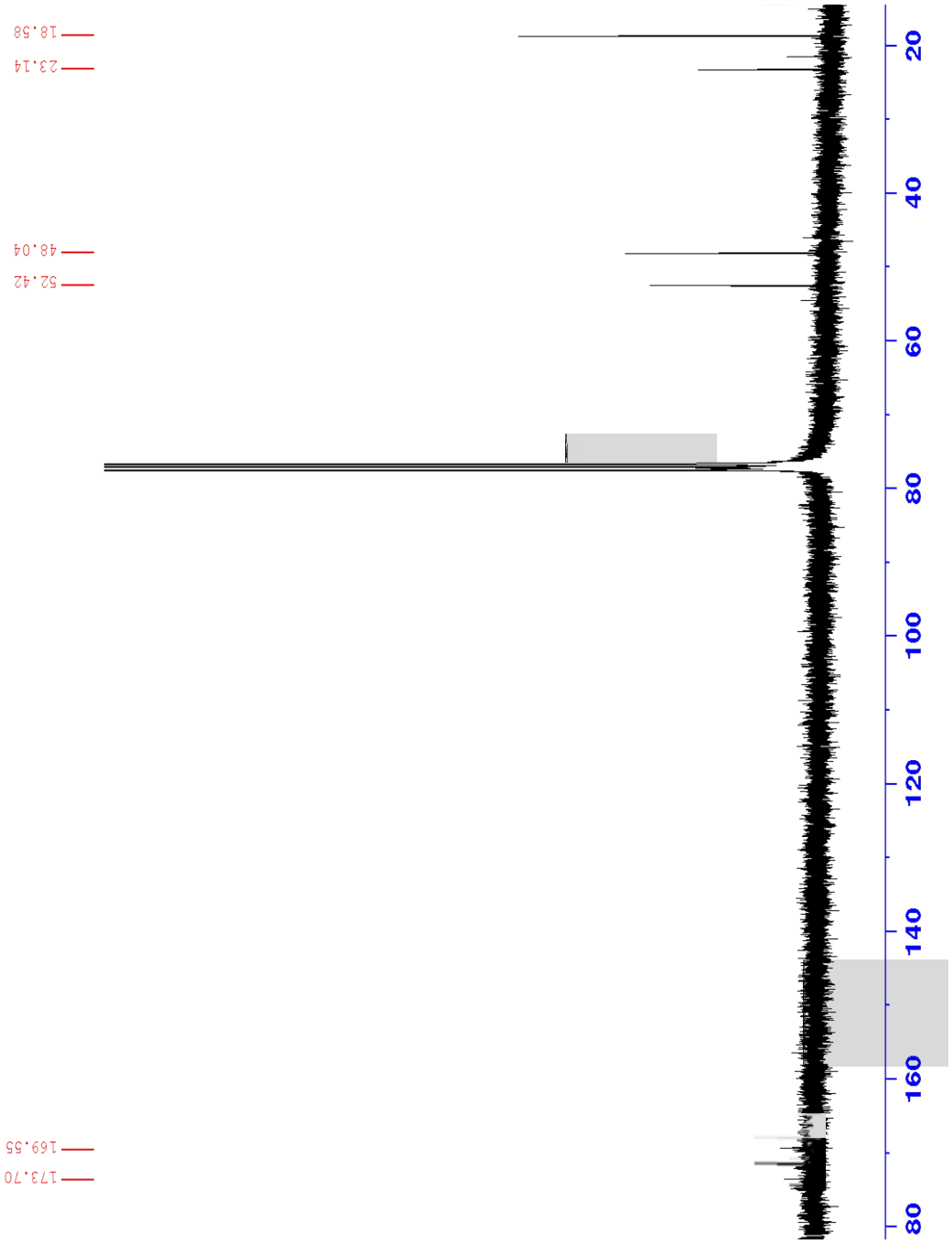
Sample 1u: *N*-Fmoc lysine (*N*-Boc) methyl ester



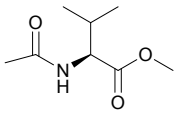
^{13}C NMR spectra (3j-3u)

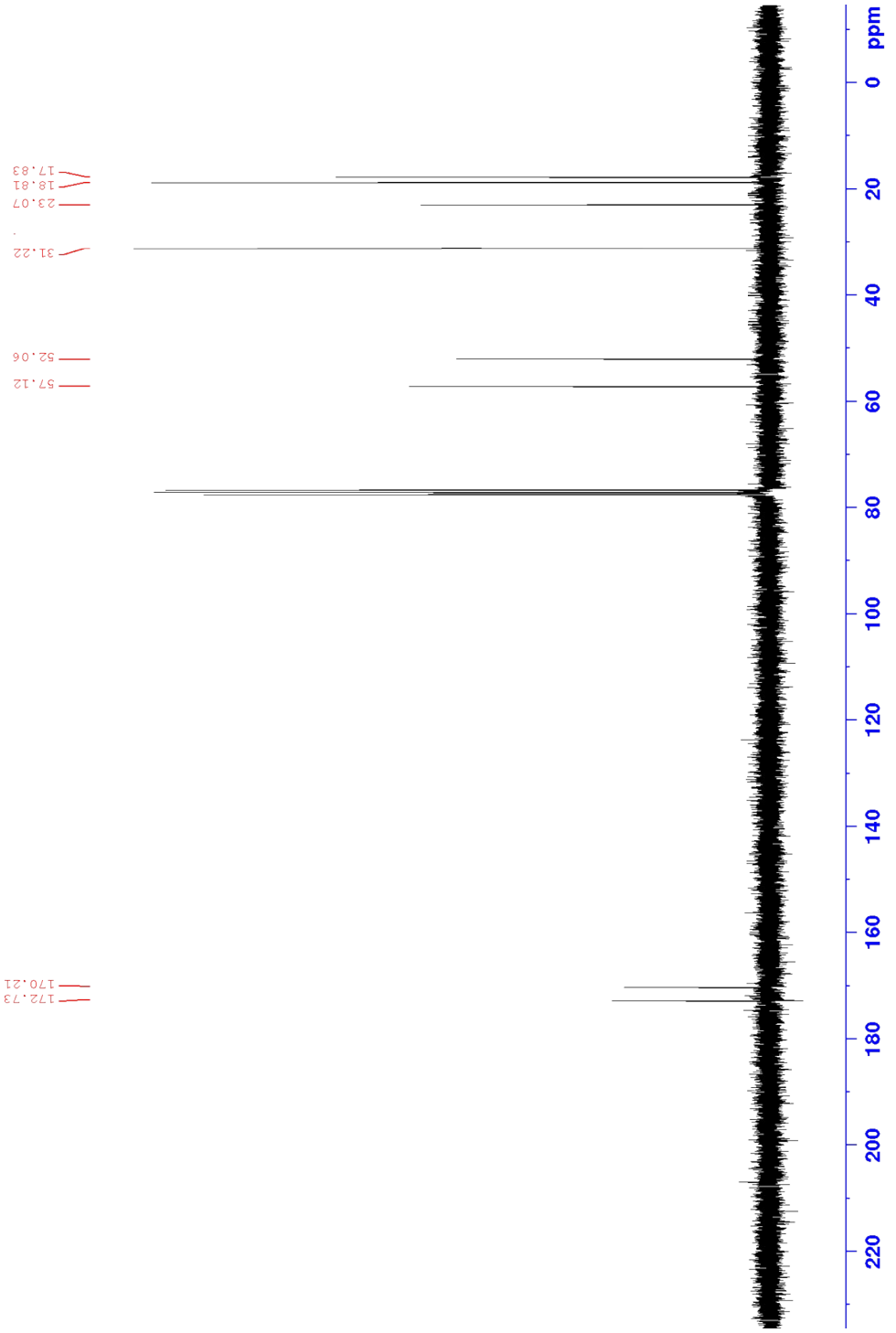
Sample 3j: *N*-Acetyl alaline methyl ester .



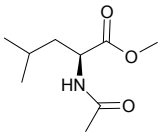


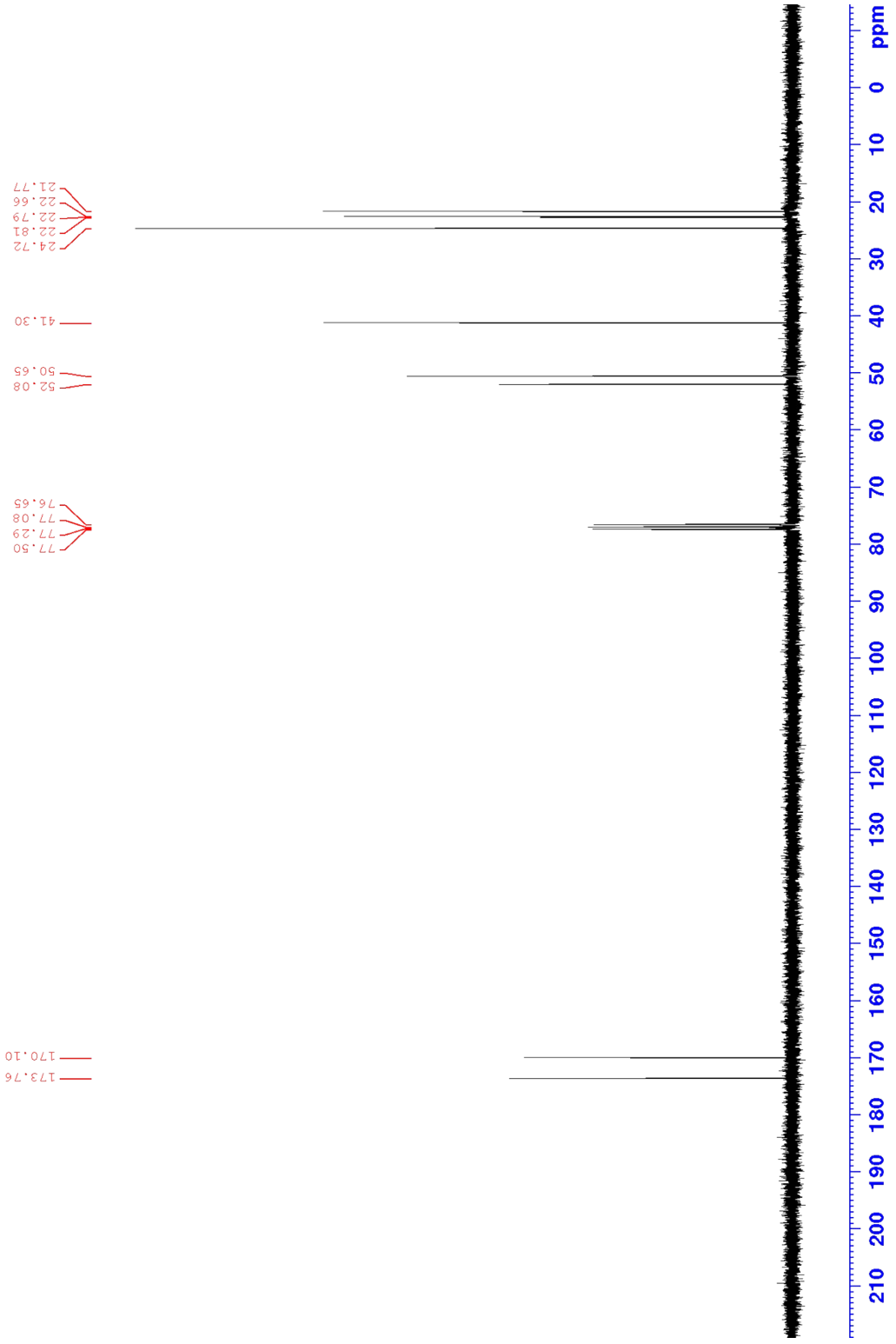
Sample 3k: *N*-Acetyl valine methyl ester .



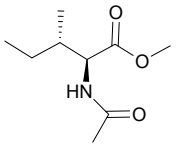


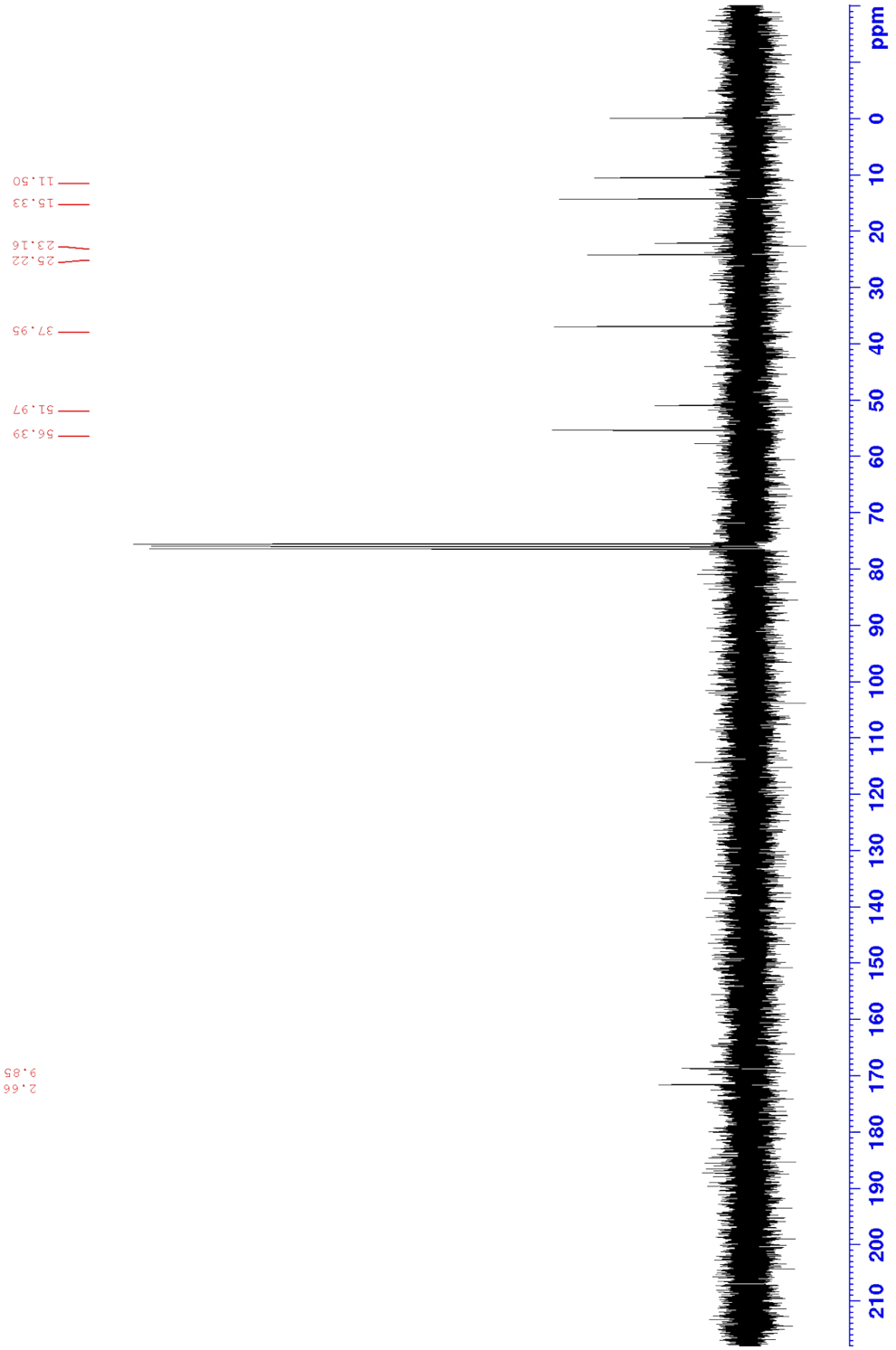
Sample 3l: *N*-Acetyl leucine methyl ester



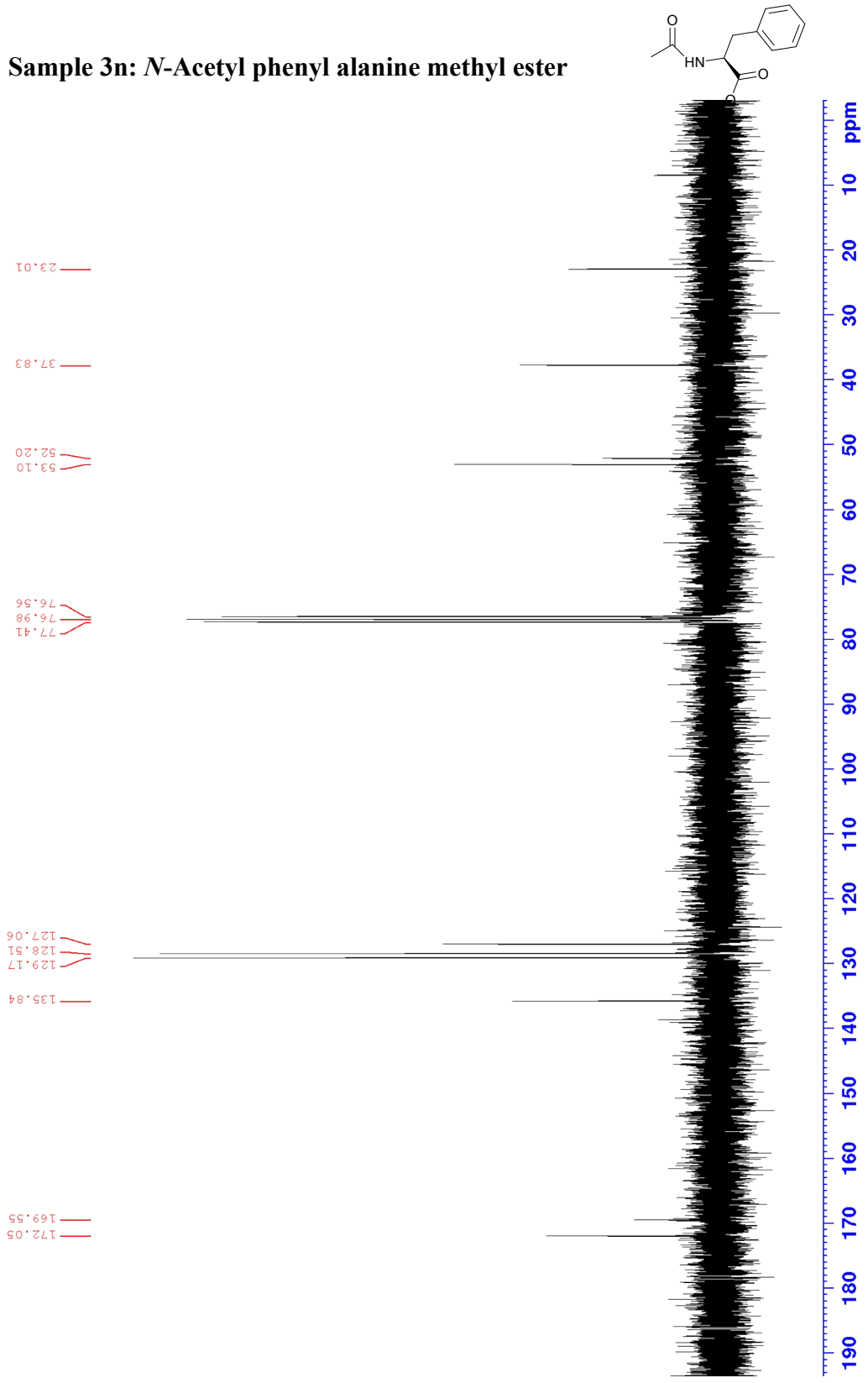


Sample 3m: *N*-Acetyl isoleucine methyl ester

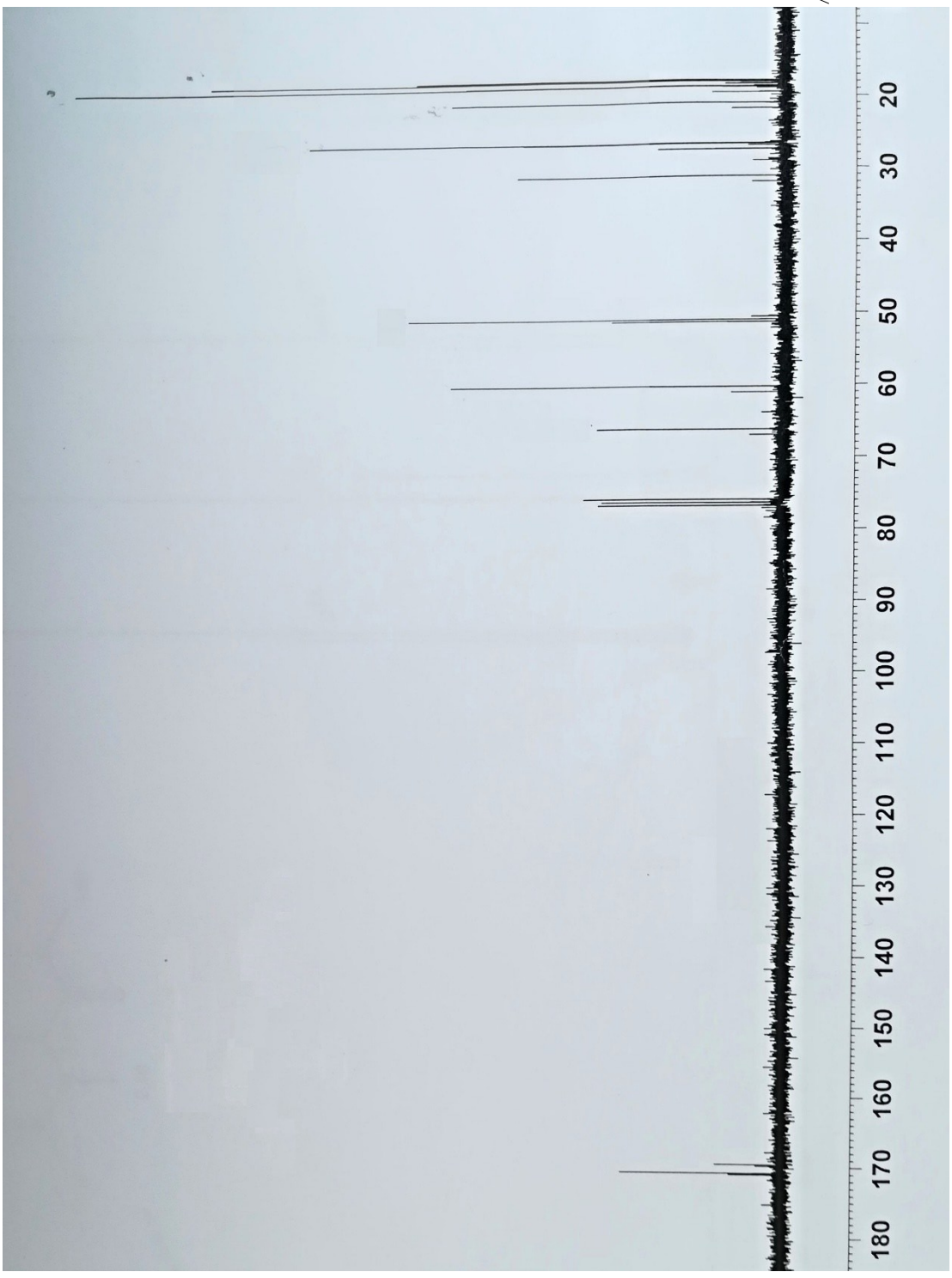




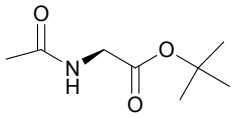
Sample 3n: *N*-Acetyl phenyl alanine methyl ester

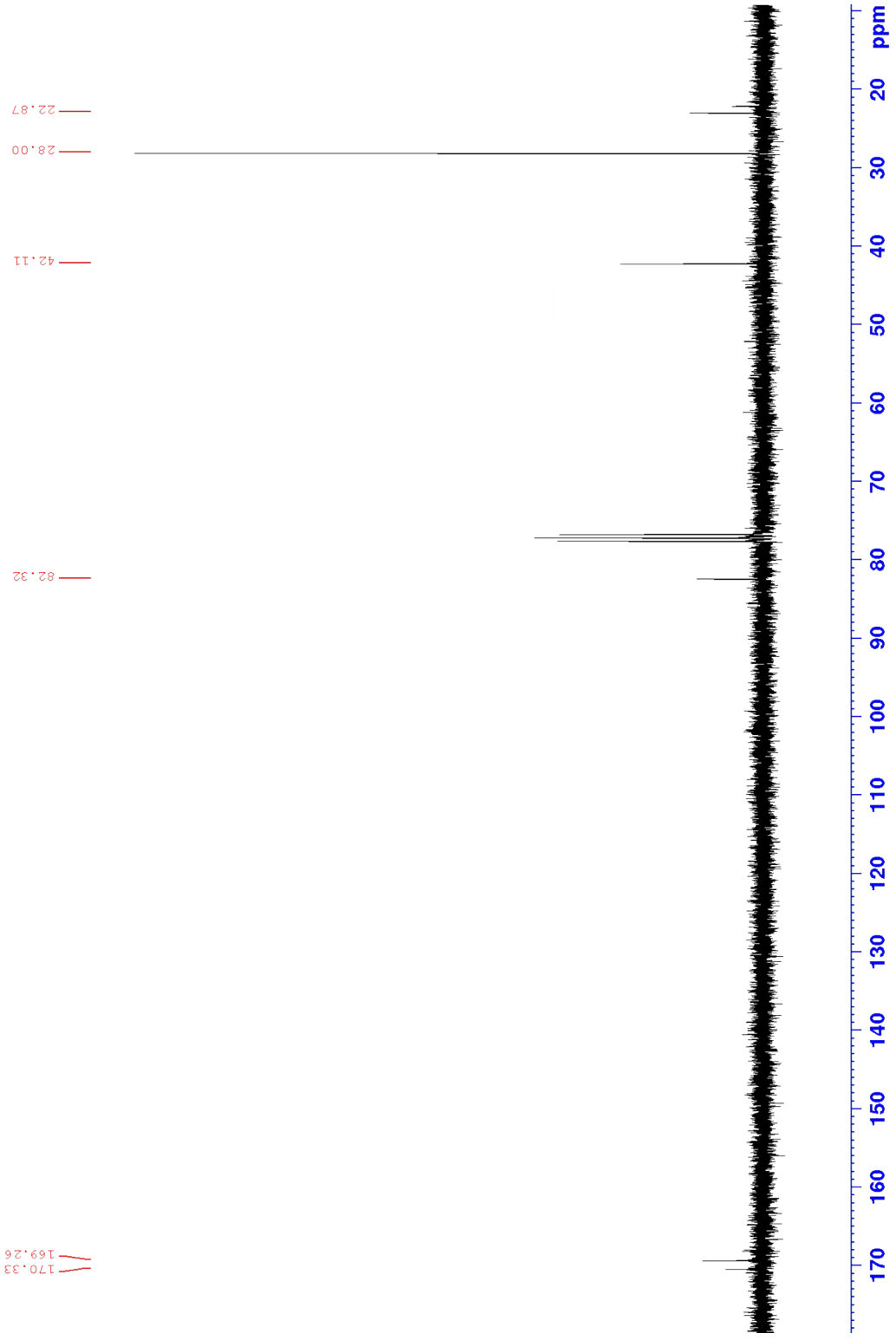


Sample 3o: N-Acetyl N-methyl valine methyl ester

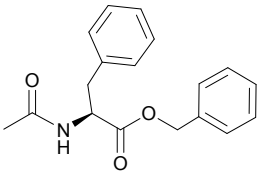


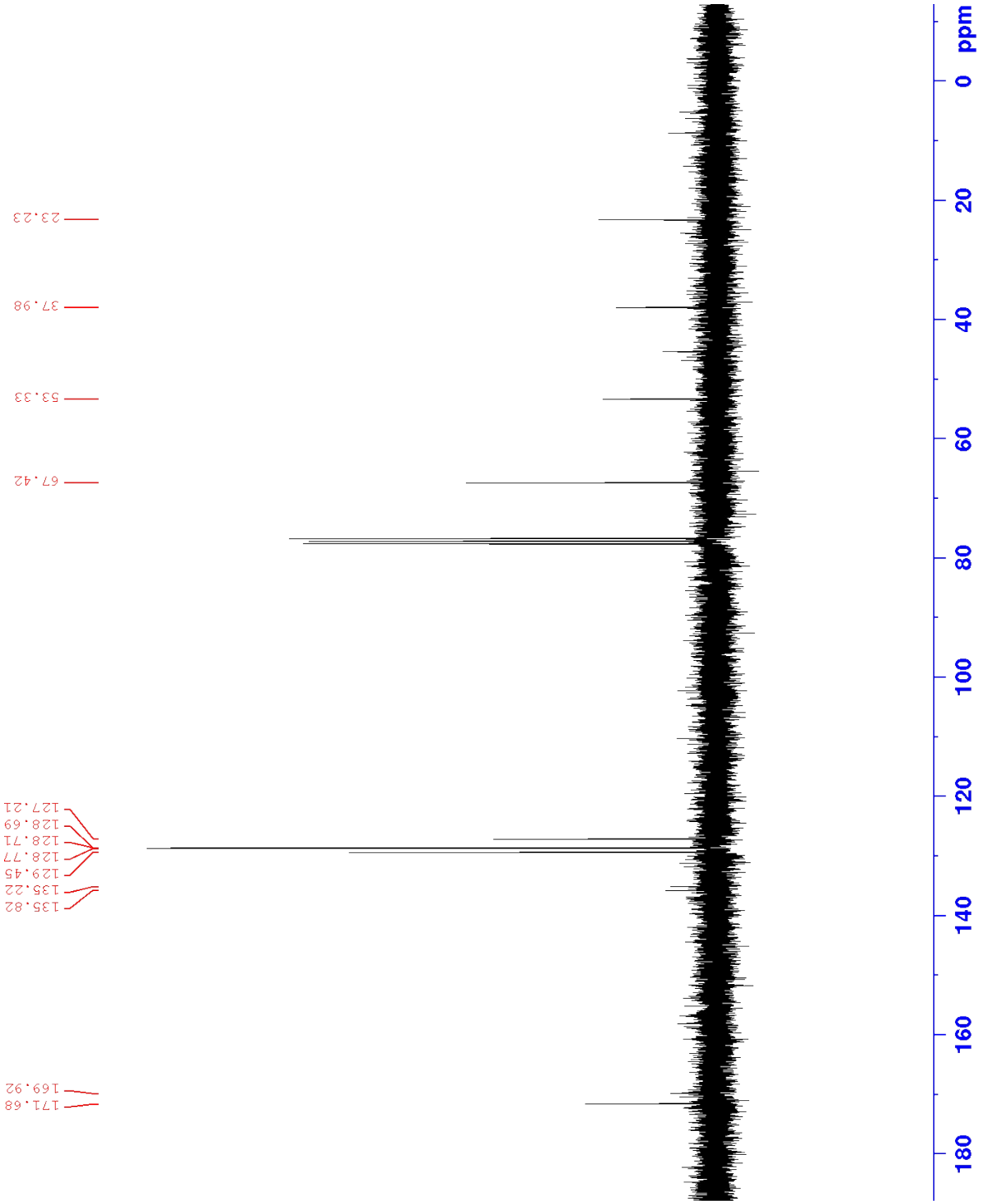
Sample 3q: *N*-Acetyl glycine *t*-butyl ester



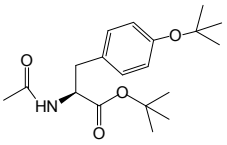


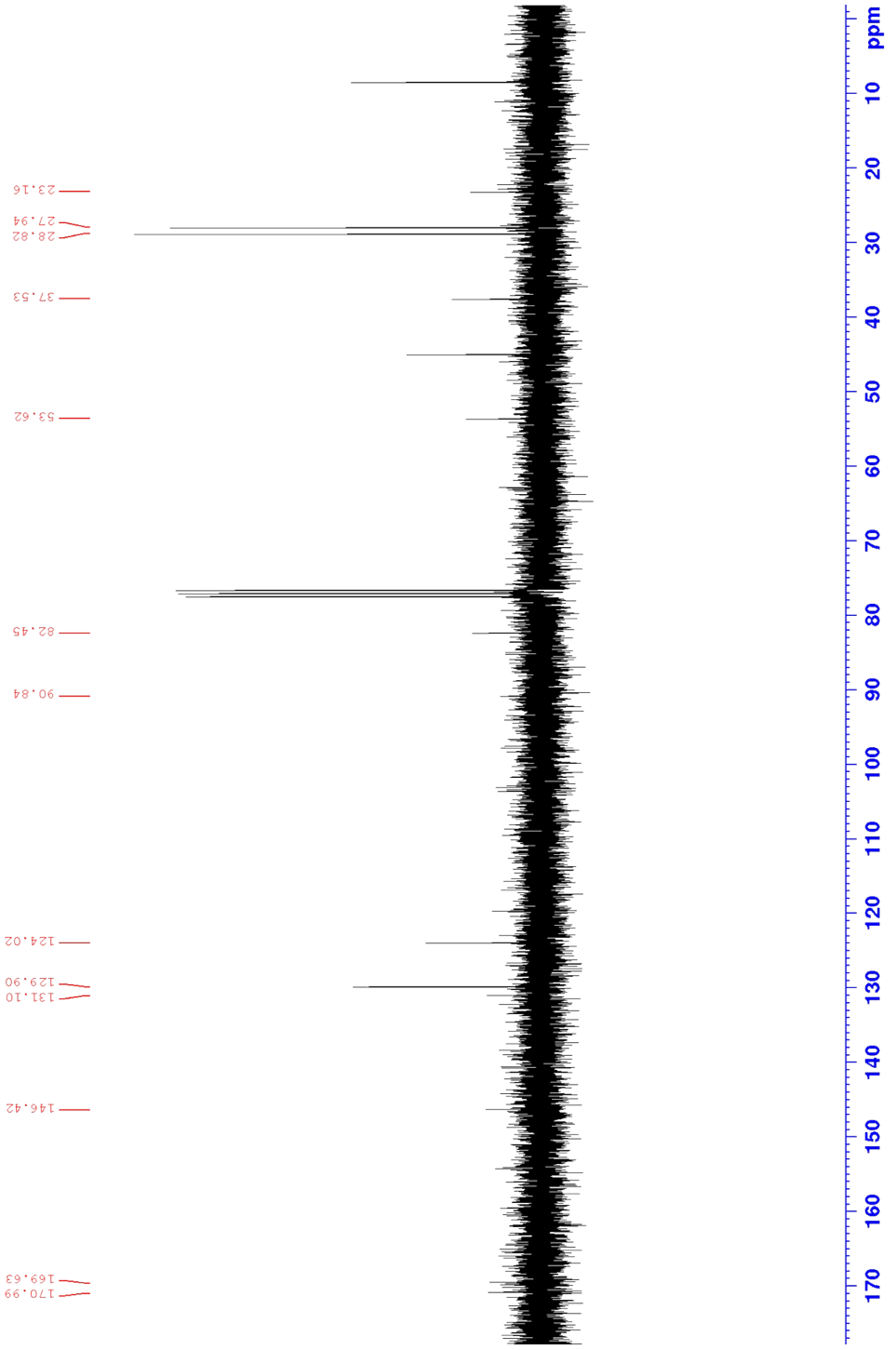
Sample 3r: *N*-Acetyl phenylalanine benzyl ester



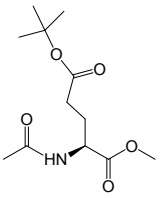


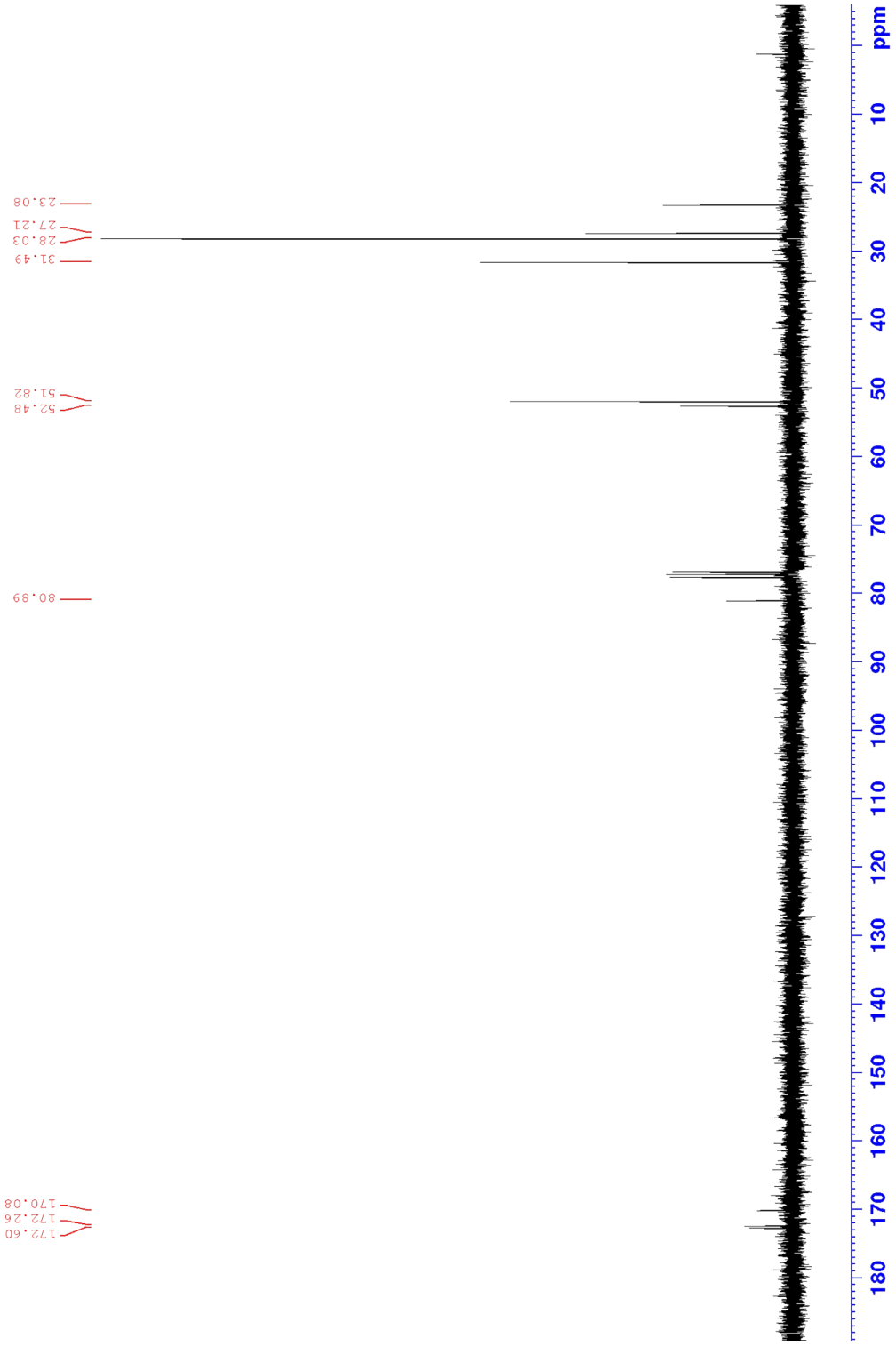
Sample 3s: *N*-Acetyl tyrosine (O-*t*-butyl) *t*-butyl ester



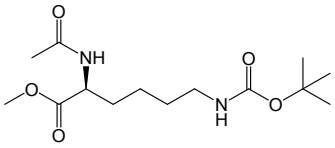


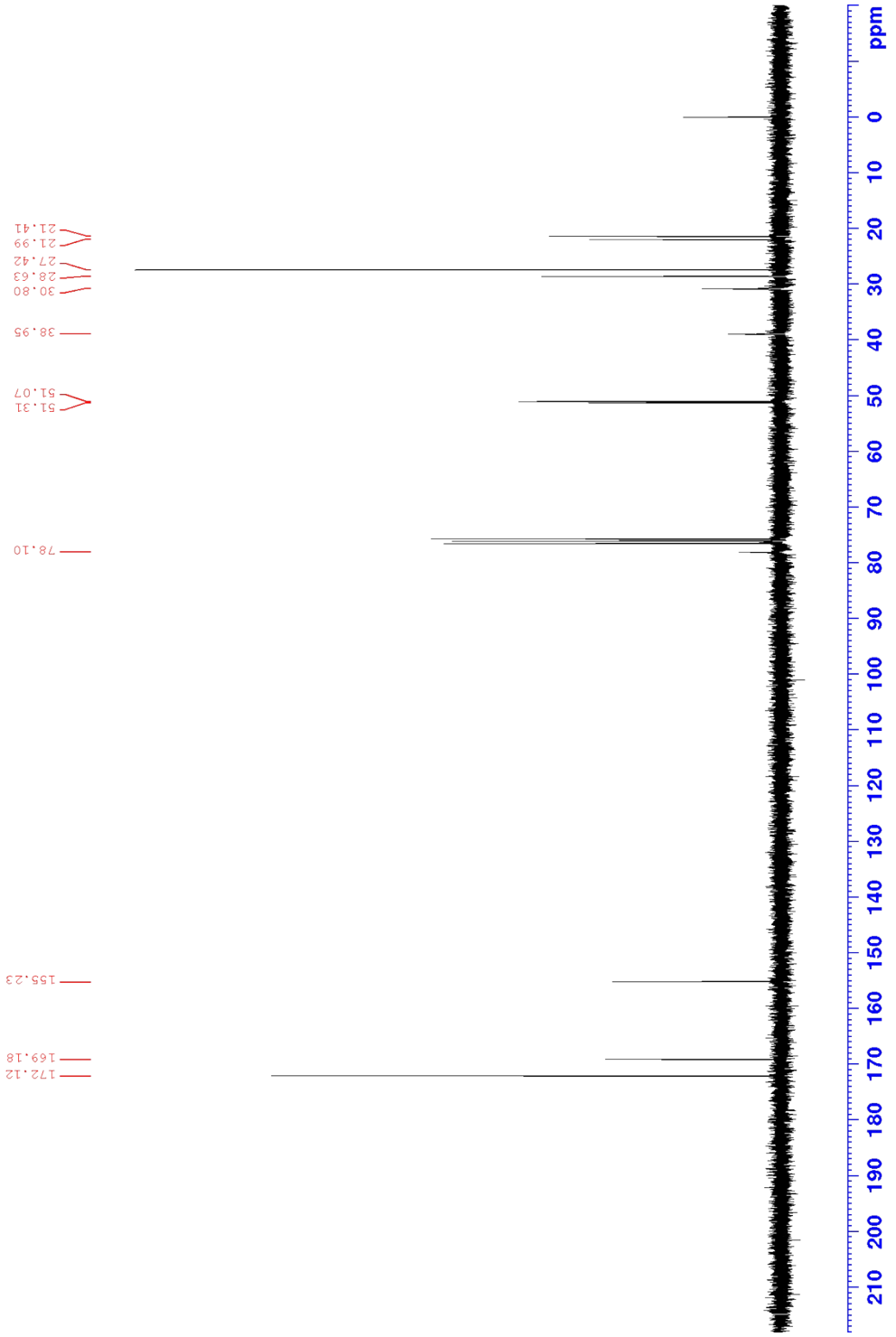
Sample 3t: *N*-Acetyl Glutamic acid 5-*tert*-butyl 1-methyl ester



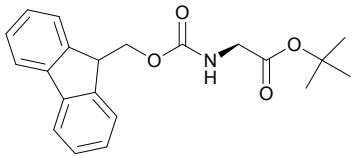


Sample 3u: *N*-Acetyl Lysine (*N*-Boc) methyl ester





HRMS (ESI) (1q-1t)

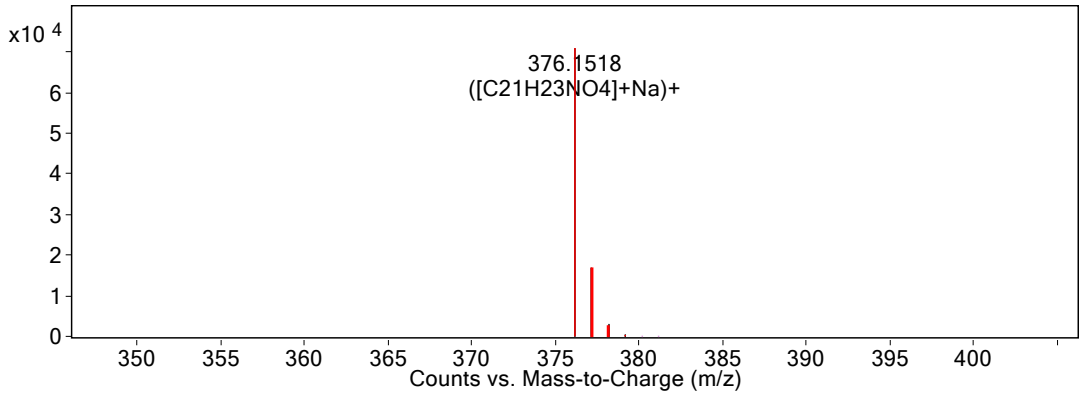


Sample 1q: *N*-(9-Fluorenylmethoxycarbonyl) glycine t-butyl ester

Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C21 H23 N O4	7,843	353,1625	70820	C21 H23 N O4	353,1627	-0,54	C21 H23 N O4	C21 H23 N O4

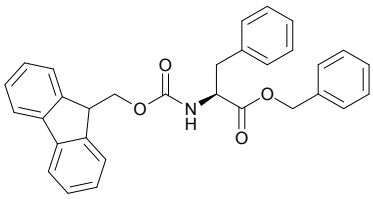
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C21 H23 N O4	376,1518	7,843	Find By Formula	353,1625



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
376,1518	1	70819,95	C21H23NO4	(M+Na)+
377,1546	1	16965,1	C21H23NO4	(M+Na)+
378,1576	1	2795,57	C21H23NO4	(M+Na)+
379,1629	1	509,69	C21H23NO4	(M+Na)+

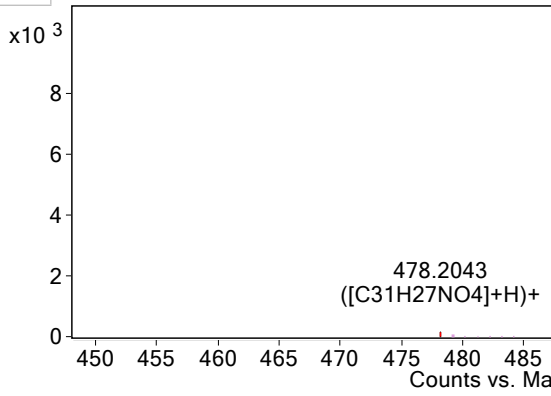
Sample 1r: *N*-Fmoc phenylalanine benzyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C31 H27 N O4	9,116	477,1936	9459	C31 H27 N O4	477,194	-0,88	C31 H27 N O4	C31 H27 N O4

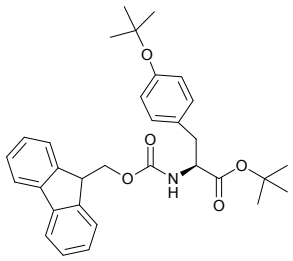
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C31 H27 N O4	500,1829	9,116	Find By Formula	477,1936



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
478,2043	1	152,58	C31H27NO4	(M+H)+
500,1829	1	9459,41	C31H27NO4	(M+Na)+
501,1856	1	3309,26	C31H27NO4	(M+Na)+
502,1894	1	678,8	C31H27NO4	(M+Na)+

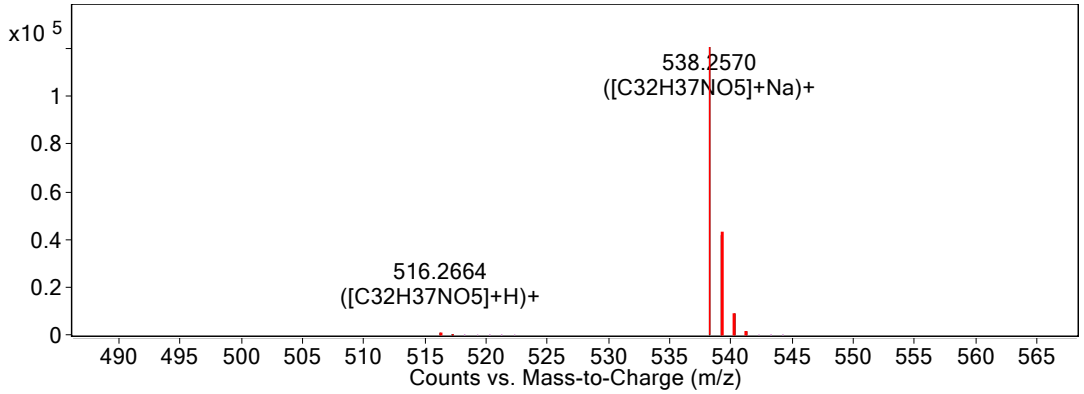
Sample 1s: *N*-Fmoc tyrosine (Otbutyl) tert butyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C32 H37 N O5	10,448	515,2675	120636	C32 H37 N O5	515,2672	0,66	C32 H37 N O5	C32 H37 N O5

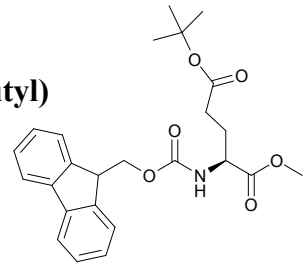
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C32 H37 N O5	538,257	10,448	Find By Formula	515,2675



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
516,2664	1	408,93	C32H37NO5	(M+H)+
517,2693	1	138,79	C32H37NO5	(M+H)+
538,257	1	120635,8	C32H37NO5	(M+Na)+
539,2596	1	41841,45	C32H37NO5	(M+Na)+
540,2624	1	8468,1	C32H37NO5	(M+Na)+
541,2688	1	1445,46	C32H37NO5	(M+Na)+

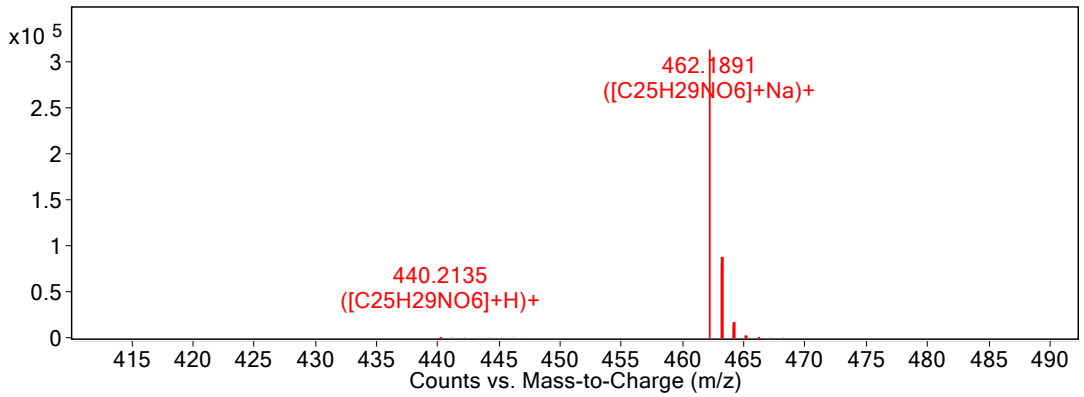
Sample 1t: *N*-(9-Fluorenylmethoxycarbonyl) Glutamic acid (O-tert-butyl) methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C25 H29 N O6	9,72	439,1998	312258	C25 H29 N O6	439,1995	0,66	C25 H29 N O6	C25 H29 N O6

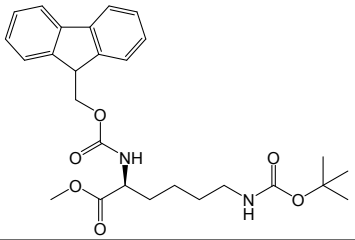
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C25 H29 N O6	462,1891	9,72	Find By Formula	439,1998



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
440,2135	1	77,61	C25H29NO6	(M+H)+
462,1891	1	312257,66	C25H29NO6	(M+Na)+
463,192	1	79635,58	C25H29NO6	(M+Na)+
464,194	1	13154,34	C25H29NO6	(M+Na)+
465,1969	1	1764,08	C25H29NO6	(M+Na)+
466,2004	1	267,16	C25H29NO6	(M+Na)+

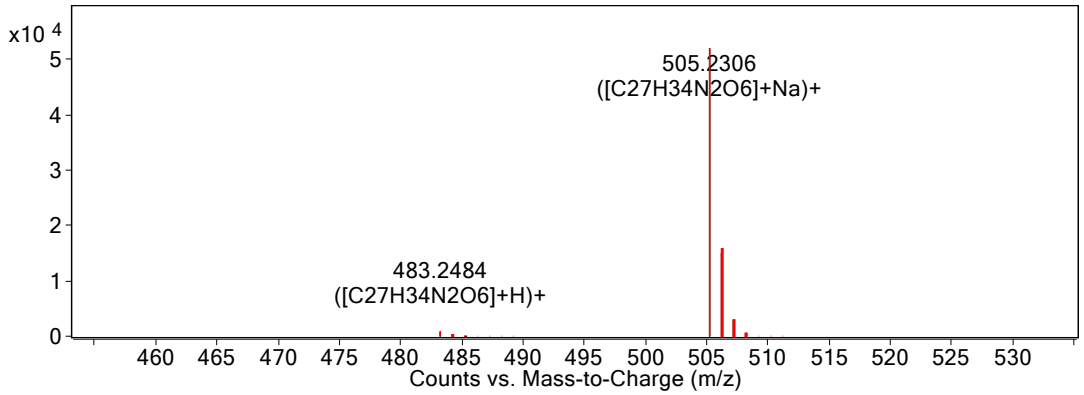
Sample 1u: *N*-Fmoc Lysine (*N*-Boc) methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C27 H34 N2 O6	8,04	482,2412	51924	C27 H34 N2 O6	482,2417	-1,08	C27 H34 N2 O6	C27 H34 N2 O6

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C27 H34 N2 O6	505,2306	8,04	Find By Formula	482,2412

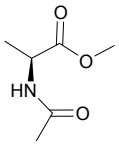


MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
483,2484	1	705,98	C27H34N2O6	(M+H)+
484,2512	1	232,07	C27H34N2O6	(M+H)+
485,2538	1	69,71	C27H34N2O6	(M+H)+
505,2306	1	51923,8	C27H34N2O6	(M+Na)+
506,2331	1	14876,35	C27H34N2O6	(M+Na)+
507,2357	1	2976,79	C27H34N2O6	(M+Na)+
508,2404	1	419,34	C27H34N2O6	(M+Na)+

HRMS (ESI) (3j-3u)

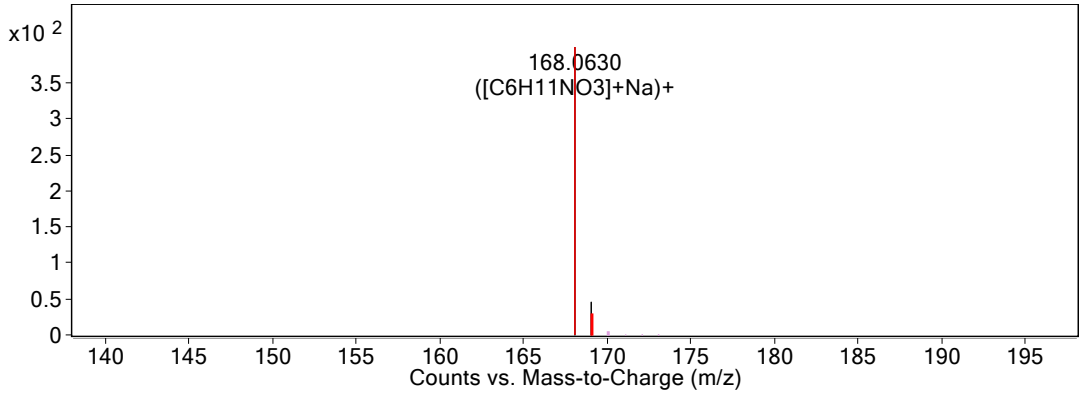
Sample 3j: *N*-Acetyl alanine methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C6 H11 N O3	1,094	145,0736	399	C6 H11 N O3	145,0739	-2,01	C6 H11 N O3	C6 H11 N O3

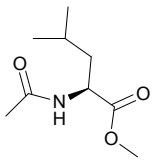
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C6 H11 N O3	168,063	1,094	Find By Formula	145,0736



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
168,063	1	399,23	C6H11NO3	(M+Na)+
169,0642	1	44,76	C6H11NO3	(M+Na)+

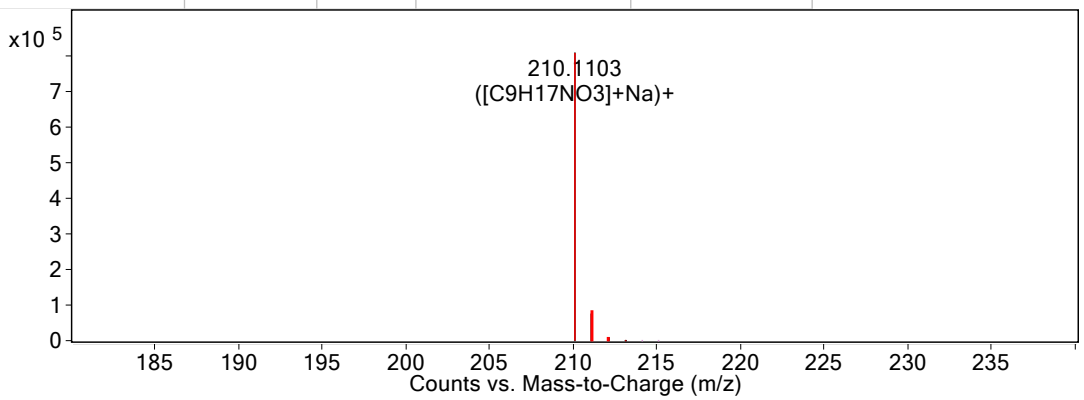
Sample 3l: *N*-Acetyl leucine methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C9 H17 N O3	0,686	187,1211	809445	C9 H17 N O3	187,1208	1,2	C9 H17 N O3	C9 H17 N O3

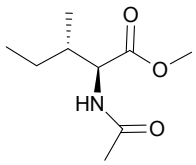
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C9 H17 N O3	210,1103	0,686	Find By Formula	187,1211



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
210,1103	1	809445,44	C9H17NO3	(M+Na)+
211,1133	1	75166,33	C9H17NO3	(M+Na)+
212,1156	1	9144,75	C9H17NO3	(M+Na)+
213,1172	1	1069,12	C9H17NO3	(M+Na)+

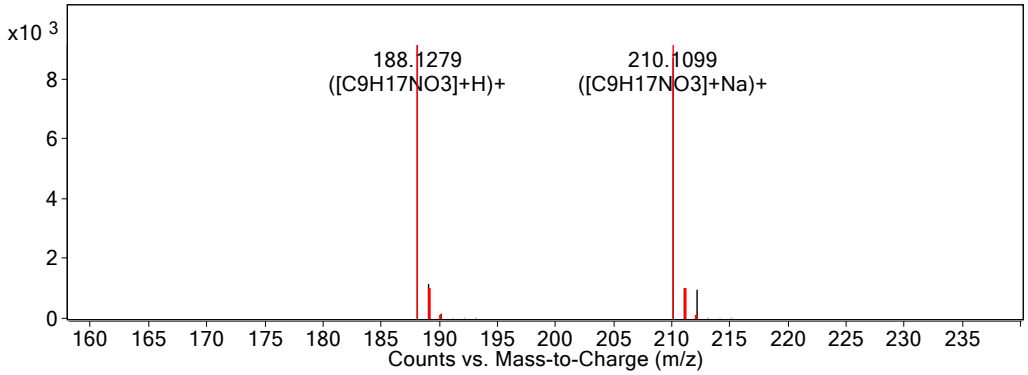
Sample 3m: *N*-Acetyl isoleucine methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C9 H17 N O3	3,261	187,1208	9107	C9 H17 N O3	187,1208	-0,46	C9 H17 N O3	C9 H17 N O3

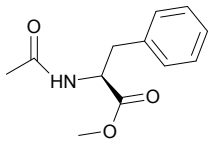
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C9 H17 N O3	188,1279	3,261	Find By Formula	187,1208



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
188,1279	1	9107,47	C9H17NO3	(M+H)+
189,1313	1	1112,44	C9H17NO3	(M+H)+
190,1322	1	147,65	C9H17NO3	(M+H)+
210,1099	1	8663,41	C9H17NO3	(M+Na)+
211,1133	1	994,68	C9H17NO3	(M+Na)+
212,1178	1	942,35	C9H17NO3	(M+Na)+

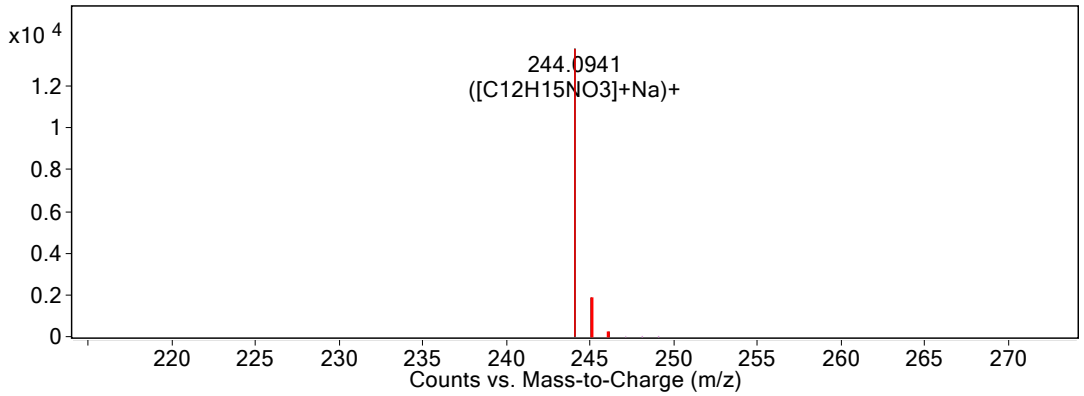
Sample 3n: *N*-Acetyl phenyl alanine methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C12 H15 N O3	3,859	221,1049	13788	C12 H15 N O3	221,1052	-1,51	C12 H15 N O3	C12 H15 N O3

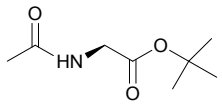
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C12 H15 N O3	244,0941	3,859	Find By Formula	221,1049



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
244,0941	1	13787,94	C12H15NO3	(M+Na)+
245,0975	1	1850,58	C12H15NO3	(M+Na)+
246,0998	1	183,8	C12H15NO3	(M+Na)+

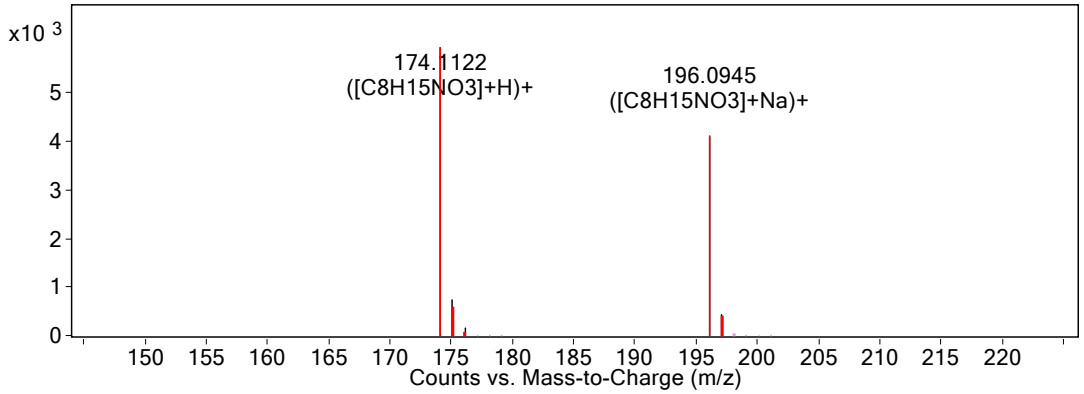
Sample 3q: *N*-Acetyl glycine *t*-butyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C8 H15 N O3	5,029	173,1051	5929	C8 H15 N O3	173,1052	-0,69	C8 H15 N O3	C8 H15 N O3

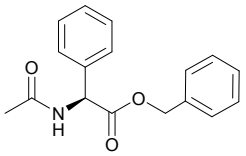
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C8 H15 N O3	174,1122	5,029	Find By Formula	173,1051



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
174,1122	1	5929,13	C8H15NO3	(M+H)+
175,1158	1	746,98	C8H15NO3	(M+H)+
176,1146	1	151,98	C8H15NO3	(M+H)+
196,0945	1	4083,82	C8H15NO3	(M+Na)+
197,0974	1	428,3	C8H15NO3	(M+Na)+

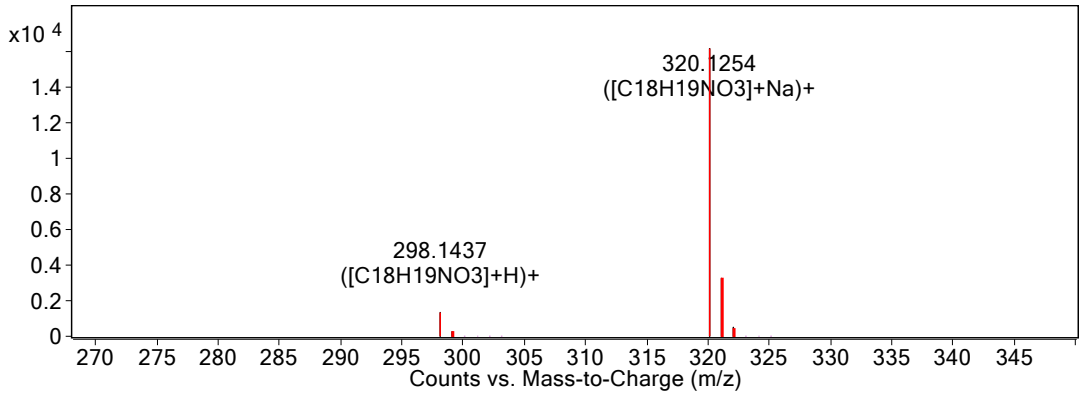
Sample 3r: *N*-Acetyl phenylalanine benzyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C18 H19 N O3	6,458	297,1363	16184	C18 H19 N O3	297,1365	-0,76	C18 H19 N O3	C18 H19 N O3

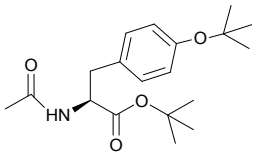
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C18 H19 N O3	320,1254	6,458	Find By Formula	297,1363



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
298,1437	1	1319,84	C18H19NO3	(M+H)+
299,15	1	291,36	C18H19NO3	(M+H)+
320,1254	1	16183,91	C18H19NO3	(M+Na)+
321,1288	1	3248,39	C18H19NO3	(M+Na)+
322,1318	1	464,67	C18H19NO3	(M+Na)+

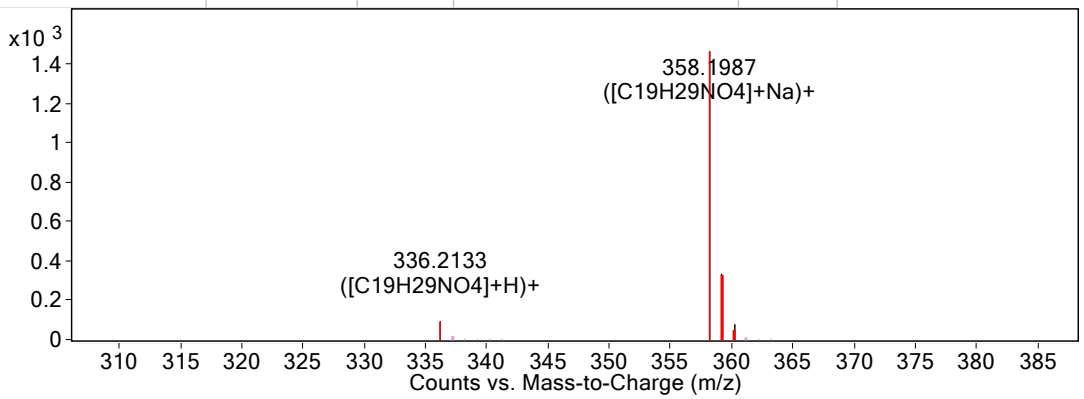
Sample 3s: *N*-Acetyl tyrosine (O-*t*-butyl) *t*-butyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C19 H29 N O4	7,134	335,2101	1463	C19 H29 N O4	335,2097	1,44	C19 H29 N O4	C19 H29 N O4

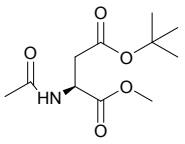
Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C19 H29 N O4	358,1987	7,134	Find By Formula	335,2101



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
336,2133	1	86,93	C19H29NO4	(M+H)+
358,1987	1	1463,28	C19H29NO4	(M+Na)+
359,2054	1	333,28	C19H29NO4	(M+Na)+
360,2119	1	72,39	C19H29NO4	(M+Na)+

Sample 3t: *N*-Acetyl glutamic acid (O-*t*-butyl) methyl ester

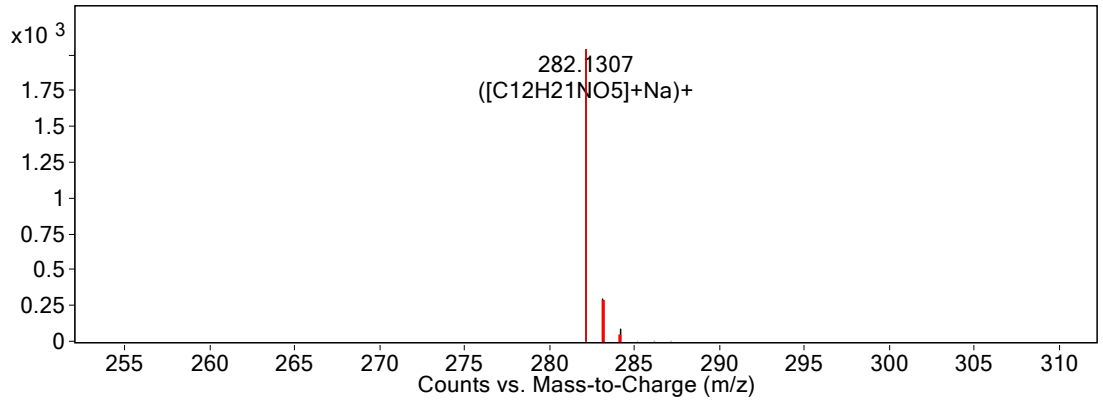


Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C12 H21 N O5	4,215	259,1413	2038	C12 H21 N O5	259,142	-2,59	C12 H21 N O5	C12 H21 N O5

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C12 H21 N O5	282,1307	4,215	Find By Formula	259,1413

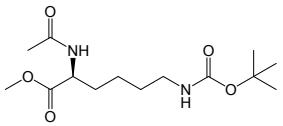
MS Zoomed Spectrum



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
282,1307	1	2038,26	C12H21NO5	(M+Na)+
283,1332	1	299,12	C12H21NO5	(M+Na)+
284,1343	1	86,64	C12H21NO5	(M+Na)+

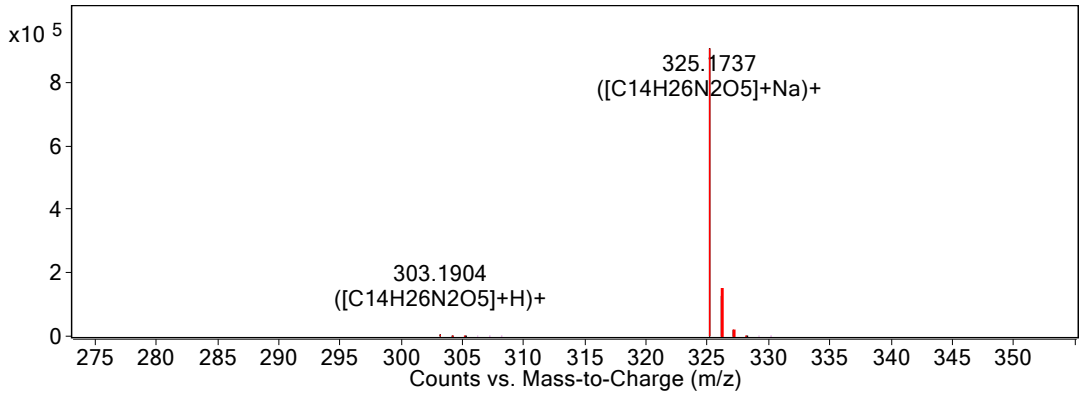
Sample 3u: N-Acetyl Lysine (N-Boc) methyl ester



Compound Table FALSO

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)	MFG Formula	DB Formula
Cpd 1: C14 H26 N2 O5	4,136	302,1845	906783	C14 H26 N2 O5	302,1842	1	C14 H26 N2 O5	C14 H26 N2 O5

Compound Label	*m/z*	RT	Algorithm	Mass
Cpd 1: C14 H26 N2 O5	325,1737	4,136	Find By Formula	302,1845



MS Spectrum Peak List

m/z	z	Abund	Formula	Ion
303,1904	1	3316,26	C14H26N2O5	(M+H)+
304,1956	1	449,96	C14H26N2O5	(M+H)+
305,1958	1	119,67	C14H26N2O5	(M+H)+
325,1737	1	906783,44	C14H26N2O5	(M+Na)+
326,1766	1	127692,35	C14H26N2O5	(M+Na)+
327,1784	1	16694,49	C14H26N2O5	(M+Na)+
328,1802	1	1730,82	C14H26N2O5	(M+Na)+