Synthesis of Mono-ADP-Ribosylated Oligopeptides using Ribosylated Amino Acids Building Blocks

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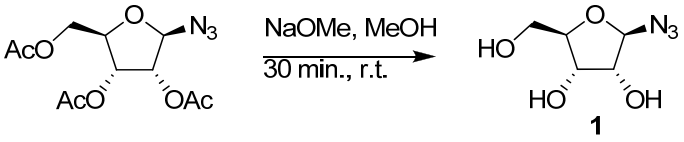
Experimental Section

General procedures

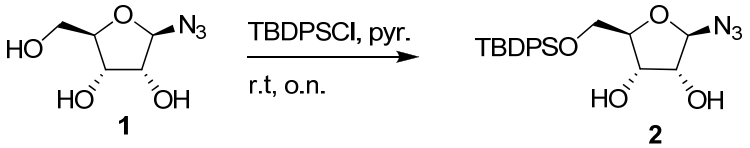
Petroleum Ether (PE) with a boiling range of 40 - 60°C was used. THF and Et_2O were distilled over $LiAlH_4$ prior to use. DCM was distilled over CaH_2 prior to use. All other solvents used under anhydrous conditions were stored over 4Å molecular sieves except for methanol which was stored over 3Å molecular sieves. Solvents used for workup and column chromatography were of technical grade and distilled before use. Unless stated otherwise, solvents were removed by rotary evaporation under reduced pressure at 40°C. Reactions were monitored by TLC-analysis using Merck 25 DC plastikfolien 60 F254 with detection by spraying with 20% H_2SO_4 in EtOH, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% sulfuric acid or by spraying with a solution of ninhydrin (3 g/L) in EtOH / AcOH (20/1 v/v), followed by charring at approx. 150°C. Column chromatography was performed on Fluka silicagel (0.04 – 0.063 mm). For LC-MS analysis was used, a JASCO HPLC-system (detection simultaneously at 214 and 254 nm) equipped with an analytical C18 column (4.6 mmD × 50 mmL, 3µ particle size) in combination with buffers A: H_2O, B: MeCN and C: 0.5% aq. TFA and coupled to a PE/SCIEX API 165 single quadruple mass spectrometer (Perkin-Elmer), unless stated otherwise. Alternatively a Thermo Finnigan LCQ Advantage MAX ion-trap mass spectrometer with an electrospray ion source coupled to Surveyor HPLC system (Thermo Finnegan) was used with the same analytical column. For reversed-phase HPLC purification of the final compounds, an automated HPLC system supplied with a C18 column (10.0 mmD × 250 mmL, 5µ particle size) was used. High resolution mass spectra spectra (except for the compounds **18**, **21** and **25**) were recorded by direct injection (2 µL of a 2 µM solution in water/acetonitrile; 50/50; v/v and 0.1% formic acid) on a mass spectrometer (Thermo Finnigan LTQ Orbitrap) equipped with an electrospray ion source in positive mode (source voltage 3.5 kV, sheath gas flow 10, capillary temperature 250 °C) with resolution R = 60000 at m/z 400 (mass range m/z = 150-2000) and dioctylpthalate (m/z = 391.2842) as a “lock mass”. For the peptide derivatives **18**, **21** and **25** high resolution mass spectra were obtained from LC-MS analysis on Thermo Finnigan LTQ Orbitrap mass spectrometer equipped with an analytical C18 column (4.6 mmD × 50 mmL, 3µ particle size). The high resolution mass spectrometer was calibrated prior to measurements with a calibration mixture (Thermo Finnigan). ^{1}H- and ^{13}C-NMR spectra were measured on a Brüker AV-400 (400 MHz), a Brüker AV-500 (500 MHz) or a Brüker DMX-600 (600 MHz). Chemical shifts are given in ppm (δ) relative to TMS (0 ppm) and coupling constants are given in Hz. Optical rotations are measured in $CHCl_3$ at a concentration of 10 mg/mL at 25 ^{0}C.



1

β-D**-ribofuranosyl azide (1)**

2,3,5-tri-*O*-acetyl- β- D- ribofuranosyl azide (2.60 g, 8.62 mmol), was dissolved in MeOH (40 mL) and NaOMe (3.3 eq., 1.54 g, 28.5 mmol) was added. After 30 min. TLC analysis (2 % MeOH/ DCM) indicated complete conversion. Amberlite-H^+ was added until pH = 6. Filtration and concentration *in vacuo* yielded the title compound as a colourless oil (1.27 g, 7.27 mmol, 85%).[13]C-NMR (100 MHz, MeOD-*d4*) δ: 96.2 (C1'), 85.2 (C4'), 76.2 (C2'), 71.8 (C3'), 63.8 (C5'). [1]H-NMR (400 MHz, MeOD-*d4*) δ: 5.17 (s, 1H, H1', $J_{1,2}$ = 1.3 Hz), 4.85 (bs, 3H, OH), 4.03 (dd, 1H, H3', $J_{2,3}$ = 4.5 Hz, $J_{3,4}$ = 6.6 Hz), 3.93 (m, 1H, H4', $J_{4,5A}$ = 3.2 Hz, $J_{4,\ 5B}$ = 5.7 Hz), 3.79 (dd, 1H, H2'), 3.71, 3.57 (AB, 2H, H5', $J_{5A,\ 5B}$ = 12.1 Hz). [α_D $CHCl_3$] = - 195.9 °. IR: 3330.3, 2113.8, 1706.1, 1234.7, 1019.0, 925.9.

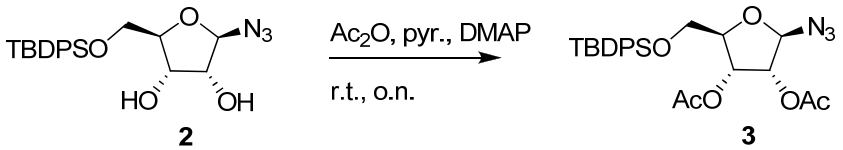


1 **2**

5-*tert*-Butyldiphenylsilyl-β-D**-ribofuranosyl azide (2)**

β-D-ribofuranosyl azide **1** (0.95 g, 3.15 mmol) was used without further purification and after coevaporation with pyridine dissolved in pyridine (40 mL). TBDPSCl (1.1 eq., 3.46 mmol, 0.9 mL) was added. The reaction mixture was stirred overnight at room temperature. After concentration *in vacuo* the residue was taken up in DCM and washed with 5 % citric acid, dried ($MgSO_4$) and concentrated. Column chromatography yielded the title compound (1.46 g, 3.22 mmol, quantitative) as a colourless oil.

[13]C-NMR (100 MHz, $CDCl_3$) δ: 135.6 (Arom.), 132.8 (Cq Arom.), 129.91, 129.87, 127.84, 127.80 (Arom.), 94.7 (C1'), 83.5 (C2'), 75.5 (C4'), 71.6 (C3'), 63.8 (C5'), 26.8 (CH_3 t-Bu), 19.2 (Cq t-Bu). [1]H-NMR (400 MHz, $CDCl_3$) δ: 7.73 – 7.67 (m, 4H, Arom.), 7.75 – 7.36 (m, 6H, Arom.), 5.31 (d, 1H, H1', $J_{1,2}$ = 1.9 Hz), 4.37 (dd, 1H, H3', $J_{2,3}$ = 4.8 Hz, $J_{3,4}$ = 6.3 Hz), 4.05 (m, 1H, H4', $J_{4,5A}$ = 3.2, $J_{4,\ 5B}$ = 4.5), 3.98 (dd, 1H, H2'), 3.88 - 3.81 (AB, 2H, H5', $J_{5A,\ 5B}$ =11.1), 1.08 (s, 9H, t-Bu).[α_D $CHCl_3$] = -61.4 °. IR: 3314.1, 2931.6, 2858.7, 2110.3, 1427.8, 1234.2, 1111.6, 988.8, 699.1, 501.6. HRMS [$C_{21}H_{27}N_3O_4Si$ + Na]$^+$: 436.1661 found, 436.1663 calc.



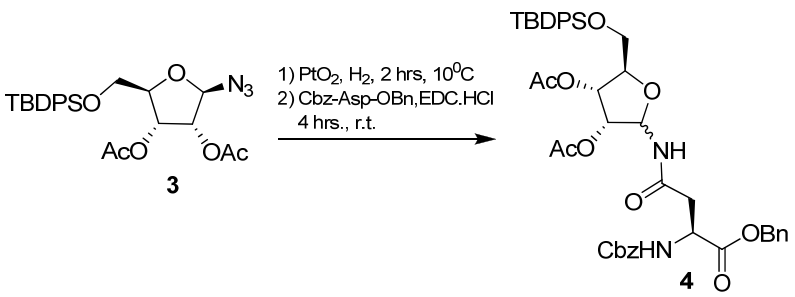
2 **3**

5- *tert*-Butyldiphenylsilyl-2, 3-di-*O*-acetyl-β-D**-ribofuranosyl azide (3)**

Ribofuranosyl azide **2** (4.13, 10 mmol) was dissolved in pyridine (20 mL) and a catalytic amount of DMAP and acetic anhydride (5 eq., 50 mmol, 4.69 mL) were added. The reaction mixture was stirred overnight at room temperature. After concentration the residue was taken up in DCM and washed with 5 % citric acid, dried ($MgSO_4$) and purified using silica gel column chromatography (PE: EA, 100/0 – 50/50). The title compound (4.88 g, 9.8 mmol, 98%) was obtained as a colourless oil.

[13]C-NMR (100 MHz, $CDCl_3$) δ: 169.4, 169.3 (CO OAc), 135.54, 134.50 (Arom.), 132.7 (Cq Arom.), 129.74, 129.67, 127.68, 127.66 (Arom.), 92.6 (C1'), 82.4 (C4'), 74.6 (C2'), 70.7 (C3'), 63.2 (C5'), 26.6 (t-Bu), 20.39, 20.37 (CH_3 OAc), 19.0 (Cq t-Bu). [1]H-NMR (400 MHz, $CDCl_3$) δ: 7.72 – 7.68 (m, 4H, arom.), 7.41 - 7.36 (m, 6H, arom.), 5.51 (t, 1H, H3', $J_{2,3}$ = 4.9 Hz, $J_{3,4}$ = 5.3), 5.41 (d, 1H, H1', $J_{1,2}$ = 3.0 Hz), 5.21 (dd, 1H, H2'), 4.21 (m, 1H, H4', $J_{4,5A}$ = 3.5 Hz, $J_{4,\ 5B}$ = 3.6 Hz), 3.88 -3.71 (AB, 2H, H5', $J_{5A,\ 5B}$ = 11.4 Hz), 2.09, 2.03 (2x s, 6H, CH_3 OAc), 1.08 (s,

9H, t-Bu). [α_D $CHCl_3$] = -53.8 °. IR: 2933.1, 2858.7, 2112.2, 1751.8, 1214.1, 1230.2, 1109.6, 701.6, 502.0. HRMS [$C_{25}H_{31}N_3O_6Si$ + Na]$^+$: 520.1866 found, 520.1874 calc.

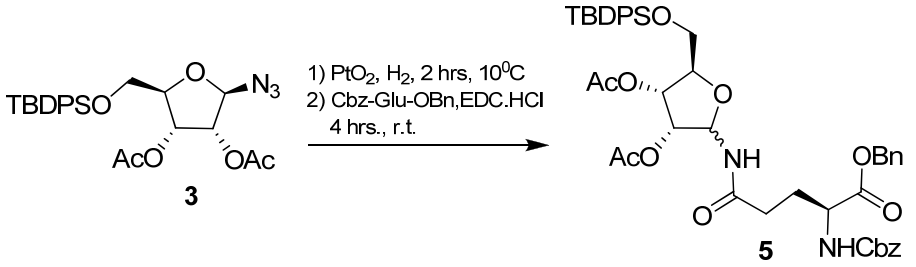


***N*$^{\gamma}$-(5-*tert*-Butyldiphenylsilyl-2,3-di-*O*-acetyl-α -D-ribosyl)-*N*$^{\alpha}$-benzyloxycarbonyl asparagine benzyl ester (4)**

Platinum(IV)oxide (110 mg, 0.48 mmol) was added to a solution of azide **3** (1.24 g, 2.5 mmol) in EtOAc (20 mL) and purged with argon before a hydrogen gas was bubbled through the reaction mixture for 2 hours at 10 °C, upon which TLC analysis (3:2, v/v, EA/PE) showed complete disappearance of the starting material. The suspension was filtered through a pad of celite and concentrated. The residue was dissolved in DCM (30 mL) and EDC·HCl (0.53 g, 2.8 mmol) and Z-Asp-OBn (0.89 g, 2.5 mmol) were added under an argon atmosphere. After stirring for 4 hours the reaction mixture was concentrated. The residue was taken up in DCM and washed with sat. aq. $NaHCO_3$ and sat. aq. NaCl. The organic layer was dried ($MgSO_4$), concentrated and the stereo-isomers were separated using silica gel column chromatography (PE: EA, 80/20 – 50/50). The title compound was obtained as a white foam (0.65 g, 0.80 mmol, 32 %) along with the β anomer (0.31 g, 0.38 mmol, 15%).

α-anomer: ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 170.6, 169.5 (CO α-Asn, CO γ-Asn), 169.2, 169.0 (CO OAc), 158.2 (CO Cbz), 135.8 (Cq Arom.), 135.4 (Cq Arom.), 135.5 (Arom.), 132.6, 132.3 (Cq Arom.), 129.7, 128.4 – 127.7 (Arom.), 82.0 (C4'), 78.5 (C1'), 72.3 (C3'), 70.0 (C2'), 67.42, 66.9 (CH_2 Bn, CH_2 Cbz), 63.5 (C5'), 50.7 (CH, α-Asn), 38.0 (CH_2, β-Asn), 26.6 (CH_3, t-Bu), 20.6, 20.2 (CH_3, OAc), 19.0 (Cq, t-Bu). ^{1}H-NMR (400 MHz, MeOD-*d4*) δ: 7.89 (1H, NH), 7.72 - 7.68, 7.44 - 7.26 (3 x m, 20H, Arom.), 6.07 (d, 1H, H1'), 5.56 (dd, 1H, H3'), 5.46 (dd, 1H, H2'), 5.16, 5.08 (2 x m, 4H, CH_2 Bn, CH_2 Cbz), 4.68 (dd, 1H, H4'), 4.16 (m, 1H, CH α-Asn), 3.76 (ddd, 2H, H5'), 2.88 (d, 2H, CH_2 β-Asn), 2.09, 2.06 (2 x s, 6H, CH_3 OAc), 1.07 (s, 9H, t-Bu). α_D ($CHCl_3$) = + 35.8°. IR (cm^{-1}): 503.0, 698.4, 1112.8, 1211.9, 1505.7, 1733.7, 1747.7. LC-MS (50 - 90 % B in 15 min.) Rt = 9.70. HRMS [$C_{44}H_{50}N_2O_{11}Si$ + H]$^+$: 811.3263 found, 811.3211 calc.

β -anomer: ^{13}C-NMR (100 MHz, CDCl3) δ: 170.6, 170.1 (CO α-Asn, CO γ-Asn), 169.8, 169.7 (CO OAc), 156.0 (CO Cbz), 135.4 (Cq Arom.), 135.3 (Arom.), 133.1, 132.0 (Cq Arom.), 130.0, 128.4 – 127.7 (Arom.), 82.0, 81.9 (C4', C1'), 74.1 (C2'), 71.2 (C3'), 67.2, 66.8 (CH_2 Bn, CH_2 Cbz), 63.3 (C5'), 50.2 (CH α-Asn), 37.1 (CH_2 β-Asn), 26.8 (CH_3, t-Bu), 20.5, 20.4 (CH_3 OAc), 19.3 (Cq, t-Bu). ^{1}H-NMR (400 MHz, MeOD-*d4*) δ: 7.88(s, 1H, NH Asn), 7.71 - 7.69, 7.43 – 7.40, 7.32 – 7.30 (3 x m, 20H, Arom.), 5.63 (d, 1H, H1'), 5.54 (m, 1H, H3'), 5.18 (t, 1H, H2'), 5.14, 5.07 (2 x s, 4H, CH_2 Bn, CH_2 Cbz), 4.64 (m, 1H, H4'), 4.08 (m, 1H, CH α-Asn), 3.80 (m, 2H, H5'), 2.77 – 2.71 (AB, 2H, CH_2 β-Asn), 2.07, 2.03 (2 x s, 6H, CH_3 OAc), 1.07 (s, 9H, t-Bu). α_D ($CHCl_3$) = +12.4°. IR: 503.3, 698.0, 1112.3, 1214.5, 1498.7, 1747.2. LC-MS (50 - 90 % B in 15 min.) Rt = 9.63. HRMS [$C_{44}H_{50}N_2O_{11}Si$ + H]$^+$: 811.3262 found, 811.3211 calc.



***N*$^{\delta}$ -(5-*tert*-Butyldiphenylsilyl-2,3-di-*O*-acetyl-α-D-ribosyl)- *N*$^{\alpha}$-benzyloxycarbonyl glutamine benzyl ester (5)**

Platinum(IV)oxide (440 mg, 88 µmol) was added to a solution of azide **3** (5 g, 9.6 mmol) in EtOAc (100 mL) and purged with argon before hydrogen gas was bubbled through the reaction mixture for 2 hours at 10 ^{0}C, upon which TLC analysis (3:2, v/v, EA/PE) showed complete disappearance of the starting material. The suspension was filtered through a pad of celite and concentrated. The residue was dissolved in DCM (100 mL) and EDC.HCl (85 mg, 9.4 mmol) and Z-Glu-OBn (3.0 g, 8.1 mmol) were added under an argon atmosphere. After stirring for 24 hours the reaction mixture was concentrated. The residue was taken up in DCM and washed with 1N HCl and sat. aq. NaCl. The organic layer was dried ($MgSO_4$), concentrated and the stereo-isomers were separated using silica gel column chromatography (PE: EA, 100/0 – 65/35). The title compound was obtained as a white foam (3,0 g, 3.7 mmol, 46 %) along with the β anomer (1,0 g, 1,2 mmol, 15%).

α-anomer: ^{13}C-NMR ($CDCl_3$) δ: 171.8, 171.5 (CO α-Gln, CO δ-Gln), 169.3, 169.0 (CO Ac), 156.0 (CO Cbz), 135.9, 135.4 (Cq Arom.), 135.5 (Arom.), 132.7, 132.4 (Cq Arom.), 129.6. 128.4 – 127.6 (Arom.), 81.6 (C4'), 78.6 (C1'), 72.2 (C3'), 70.1 (C2'), 67.1, 66.8 (CH_2 Bn, CH_2 Cbz), 63.5 (C5'), 53.4 (CH α-Gln), 32.4 (CH_2 β-Gln), 28.1 (CH_2 γ-Gln), 26.6 (t-Bu), 20.5, 20.3 (CH_3 OAc), 218.9 (Cq t-Bu). ^{1}H-NMR ($CDCl_3$) δ: 7.69 – 7.66, 7.42 – 7.37, 7.37 – 7.27 (3 x m, 20H, Arom.), 6.72 (d, 1H, NH), 6.10 (dd, 1H, H1'), 5.82 (d, 1H, NH) 5.58 (t, 1H, H3'), 5.49 (t, 1H, H2'), 5.14, 5.07 (2x s, 4H, CH_2 Bn, CH_2 Cbz), 4.42 (m, 1H, CH α-Gln), 4.10 (m, 1H, H4'), 3.71 (m, 2H, H5'), 2.33 (m, 2H, CH_2 β-Gln), 2.21 (m, 2H, CH_2 γ-Gln), 2.09, 2.07 (2x s, 6H, OAc), 1.07 (s, 9H, t-Bu). ESI-MS: *m/z* 825.20 $[M+H]^+$. LC-MS (10 - 90 % B in 15 min), Rt = 9.94. IR (cm^{-1}): 3328, 2933,2362, 1745, 1520, 1235,1212, 1110, 1049, 741, 698, 604,502. [α_D $CHCl_3$] = + 32.6^{o}. HRMS [$C_{45}H_{52}N_2O_{11}Si$ + H]$^+$: 825.3419 found, 825.3413 calc.

β -anomer: ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 172.0, 171.6 (CO Ac), 169.8, 169.7 (CO α-Gln, CO δ-Gln), 156.1 (CO Cbz), 136.0 (Cq Arom.), 135.5, 135.4 (Arom.), 135.1, 133.1, 132.2 (Cq Arom.), 130.0, 129.93, 128.5, 128.3, 128.2, 128.0, 127.9 (Arom.), 81.9 (C1', C4'), 74.0 (C2'), 71.4 (C3'), 67.2, 66.9 (CH_2 Bn, CH_2 Cbz), 63.5 (C5'), 53.3 (CH α-Gln), 31.8 (CH_2 β-Gln), 27.7 (CH_2 γ-Gln), 26.8 (t-Bu), 20.6, 20.4 (OAc), 19.3 (Cq t-Bu). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.68 – 7.65, 7.40 – 7.25 (2 x m, 20H, Arom.), 6.47, 6.46 (d, 1H, NH δ), 5.81 – 5.78 (dd, 1H, H1'), 5.65, 5.63 (d, 1H, NH α), 5.57 – 5.55 (dd, 1H, H3'), 5.32 - 5.30 (t, 1H, H2'), 5.12, 5.04 (2x s, 4H, CH_2 Bn), 4.31 – 4.27 (m, 1H, Hα), 4.12 – 4.10 (m, 1H, H4'), 3.88 – 3.80 (AB, 2H, H5'), 2.08, 2.04 (2x s, 6H, OAc), 1.94 – 1.91 (m, 2H, Hβ), 2.13 – 2.10, 1.85 – 1.80 (2x m, 2H, Hγ), 1.10 (s, 9H, t-Bu). $J_{1,2}$ = 5.7 Hz , $J_{2,3}$ = 5.3 Hz, $J_{3,4}$ = 3.4Hz, $J_{1,\ NH}$ = 9.0, $J_{4,5A}$ = 2.9 Hz, $J_{4,\ 5B}$ = 1.9 Hz, $J_{5A,\ 5B}$ = 11.4 Hz. ESI-MS: *m/z* 825.20 $[M+H]^+$. LC-MS (10 - 90 % B in 15 min), Rt = 9.88.IR (cm^{-1}): 3335.3, 2934.0, 1745.2, 1519.9, 1240.8, 1214.4, 1110.5, 1064.7, 738.9, 699.0, 502.2. [α_D $CHCl_3$] = - 0.8 ^{o}. HRMS [$C_{45}H_{52}N_2O_{11}Si$ + H]$^+$: 825.3422 found, 825.3413 calc.

Both α-anomers of **4** and **5** showed the presence of a NOE cross-peak between H1'and H3' of ribose, whereas for the corresponding β-anomers a NOE cross-peak between H1'and H4' was observed (see F**ig. 1**.)

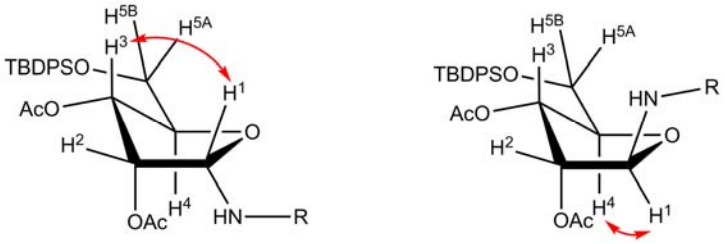
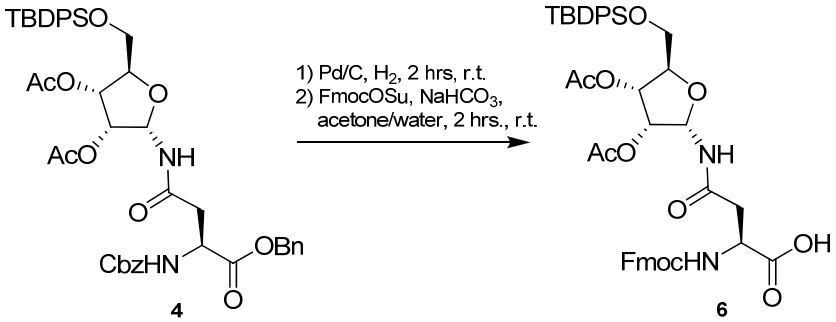
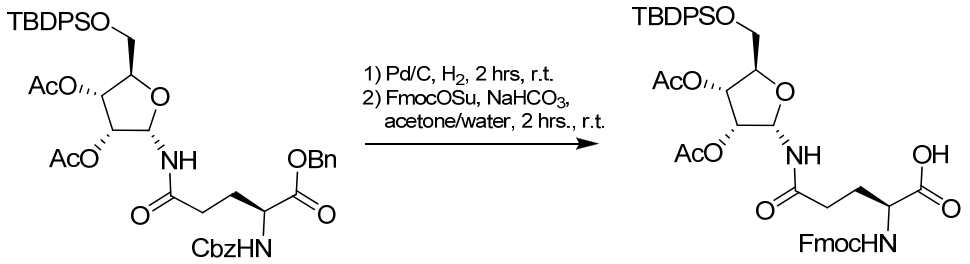


Fig. 1 NOESY observations



***N*$^{\gamma}$ -(5- *tert*-Butyldiphenylsilyl-2, 3-di-*O*-acetyl-α-D-ribosyl)- *N*$^{\alpha}$-fluorenylmethoxycarbonyl asparagine (6)**

Amino acid **4a**(1.25 g, 1.54 mmol) was dissolved in MeOH (30 mL), Pd-C (10%, 190 mg) and a drop of conc. HCl were added after purging with argon. A flow of hydrogen gas was passed through the reaction mixture for 2 hours. LC-MS analysis (CN-Column, 10-90 % B in 26 min.) showed complete consumption of the starting material and formation of the unprotected amino acid. Next, $NaHCO_3$ (190 mg, 2.3 mmol) was added and the reaction mixture was filtered over a pad of celite. The solvent was removed *in vacuo* and the residue was dissolved in H_2O/ acetone (40 mL, 1:1, v/v). Fmoc-OSu (0.51 g, 1.5 mmol) and $NaHCO_3$ (0.13 g, 1.5 mmol) were added after which the reaction mixture was stirred at room temperature for 2 hrs. The mixture was acidified to pH = 2 and acetone was removed under reduced pressure. The resulting solution was extracted with DCM, washed with citric acid 5% and H_2O, dried ($MgSO_4$) and concentrated. Purification using column chromatography (99.9 : 0.1 DCM/AcOH – 89.9/1/0.1 DCM/MeOH/AcOH) afforded the title compound as a white foam (1.1 g, 1.30 mmol, 84%). ^{13}C-NMR (100 MHz, MeOD-*d4*) δ: 173.2, 171.0 (CO γ-Asn, CO α-Asn), 170.2, 170.1 (CO OAc), 156.9 (CO Fmoc), 143.8, 143.8 (Cq Fmoc), 137.5 (Fmoc), 135.4, (Cq TBDPS), 129.7 – 119.6 (Arom. TBDPS, Fmoc), 81.2 (C4'), 79.2 (C1'), 71.5 (C3'), 70.7 (C2'), 66.8 (C5'), 63.5 (CH_2 Fmoc), 50.8 (CH α-Asn), 46.9 (CH Fmoc), 37.5 (CH_2 β-Asn), 26.1 (CH_3 t-Bu), 19.2, 19.1 (CH_3 OAc), 18.7 (Cq t-Bu). ^{1}H-NMR (400 MHz, MeOD-*d4*) δ: 7.85 (s, 1H, NH), 7.71 – 7.58, 7.40 – 7.21 (m, 18H, Arom.), 6.06 (d, 1H, H1'), 5.51 (t, 1H, H3'), 5.43 (t, 1H, H2'), 4.42 (m, 1H, H α-Asn), 4.28 (m, 2H, CH_2 Fmoc), 4.16 (t, 1H, CH Fmoc) 4.07 (d, 1H, H4'), 3.65 (m, 2H, H5'), 2.87 – 2.74 (m, 2H, CH_2 β-Asn), 2.08, 2.05 (2 x s, 6H, CH_3 OAc), 1.01 (s, 9H, CH_3 t-Bu). α_D ($CDCl_3$) = + 56.2°. LC-MS (10 – 90 % B in 15 min.), Rt = 11.34 min. IR: 503.5, 701.4, 1112.5, 1212.2, 1505.7, 1747.5. HRMS $[C_{44}H_{48}N_2O_{11}Si + H]^+$: 809.3106 found, 809.3055 calc.

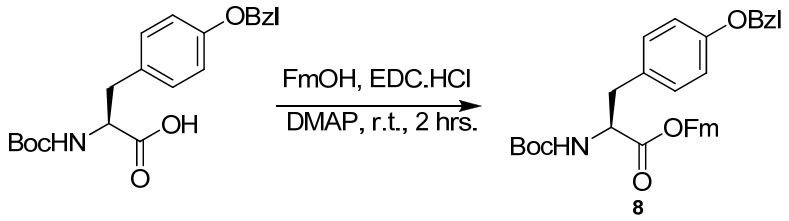


5 **7**

***N*$^{\delta}$ -(5- *tert*-Butyldiphenylsilyl-2, 3-di-*O*-acetyl-α-D-ribosyl)- *N*$^{\alpha}$-fluorenylmethoxycarbonyl glutamine (7)**

Amino acid **5** (0.2 g, 0.24 mmol) was dissolved in MeOH (5 mL), Pd-C (10%) (30 mg) and a drop of conc. HCl were added after which hydrogen gas was passed through the reaction mixture for 5 hours. LC-MS analysis (CN-Column, 10-90 % B in 26 min.) showed complete consumption of the starting material and formation of the unprotected amino acid. After filtration over a pad of celite the solvent was concentrated and dissolved in H_2O/ acetone/ dioxane (3 mL, 1:1:2, v/v/v). Fmoc-OSu (80 mg, 0.24 mmol) and $NaHCO_3$ (20 mg, 0.24 mmol) were added after which the reaction mixture was stirred overnight. The mixture was washed with 5% citric acid and after drying ($MgSO_4$) the organic layer was concentrated. Purification using column chromatography (99.9 : 0.1 DCM/AcOH – 99.4 : 0.1 : 0.5 DCM/ AcOH/ MeOH) afforded the title compound as a white foam (0.19 g, 0.23 mmol, 97%).

^{13}C-NMR (100 MHz, MeOD-*d4*) δ: 173.9, 173.7 (CO δ-Gln, CO α-Gln), 170.2, 170.0 (CO OAc), 157.2 (CO Fmoc), 143.9, 143.8, 141.2 (Cq Arom.), 135.4, 135.3 (Arom.), 132.7, 132.6 (Cq Arom.), 129.7 - 119.6(Arom. Fmoc/TBDPS), 81.1 (C4'), 79.2 (C1'), 71.5 (C3'), 70.7 (C2'), 66.7 (C5'), 63.4 (CH_2 Fmoc), 53.4 (CH α-Gln), 47.0 (CH Fmoc), 32.0 (CH_2 β-Gln), 27.3 (CH_2 γ-Gln), 26.0 (CH_3 t-Bu), 19.3, 19.1 (CH_3 OAc), 18.6 (Cq t-Bu). ^{1}H-NMR (400 MHz, MeOD-*d4*) δ: 7.89 (d, 1H, NH), 7.79 – 7.65, 7.46 – 7.28 (2x m, 18H, Arom.), 6.10 (d, 1H, H1'), 5.58 (m, 1H, H3'), 5.49 (m, 1H, H3'), 4.38 – 4.16 (m, 5H, CH Fmoc, H5', H4', H α), 3.76 - 3.71 (m, 1H, CH_2 Fmoc), 2.45 (m, 2H, CH_2 β), 2.24, 2.02 (2x m, 2H, Hγ), 2.11, 2.05 (CH_3 OAc), 1.06 (CH_3 t-Bu). ESI-MS: *m/z* 823.27 $[M+H]^+$. LC-MS (50 - 90 % B in 15 min), Rt = 8.92. IR (cm^{-1}): 2933.1, 1742.5, 1519.6, 1212.6, 1108.1, 740.0, 702.8, 502.3. α_D ($CHCl_3$) = + 33.4°. HRMS $[C_{45}H_{50}N_2O_{11}Si + H]^+$: 823.3263 found, 823.3256 calc.



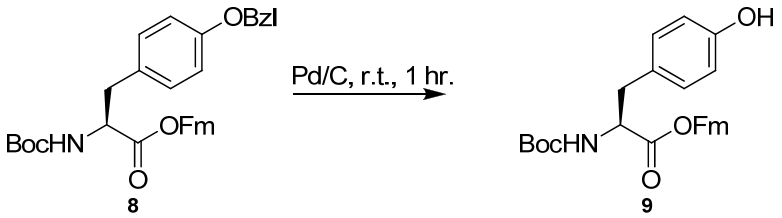
8

***N*$^{\alpha}$ -*tert*-Butoxycarbonyl-4-*O*-benzyl-L-tyrosine fluorenylmethyl ester (8)**

Boc-Tyr(Bzl)-OH (2.0 mmol, 0.74 g) was coevaporated with MeCN and dissolved in DCM (40 mL) under an argon atmosphere. To this solution were added 9-fluorenylmethanol (2.2 mmol, 0.43 g), EDC·HCl (2.2 mmol, 0,42 g) and DMAP (0.2 mmol, 32 mg). The solution was stirred at room temperature for 2 hours, washed with 5% citric acid and H_2O. The combined organic layers were dried and concentrated *in vacuo*. The crude compound was purified by silica gel column chromatography (PE/EtOAc, 95/5) to give compound **8** as a white powder (0.84 g, 76%).

^{13}C-NMR (100 MHz, $CDCl_3$): δ: 171.9 (CO α-Tyr), 157.8 (Cq Tyr.), 155.1 (CO Boc), 143.5, 143.4, 141.2 (Cq Fm), 136.9 (Cq Bn), 130.24 (Arom. Tyr.), 128.5 (Arom. Bn/Fm), 127.9 (Cq Tyr.), 127.3, 127.1 (Arom. Bn/Fm), 125.0, 120.0 (Arom. Fm), 114.9 (Arom. Tyr.), 79.8 (Cq Boc), 69.8 (CH_2 Bn), 67.0 (CH_2 Fm), 54.6 (CH α Tyr.), 46.6 (CH Fm), 37.4 (CH_2 β Tyr.), 28.3 (CH_3 Boc). ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 7.72 (d, 2H, Arom. Bn/Fm),, 7.48 (t, 2H, Arom. Bn/Fm),, 7.35 – 7.26 (m, 8H, Arom. Bn/Fm), 6.98, 6.84 (2x d, 4H, Arom. Tyr.), 5.06 (d, 1H,

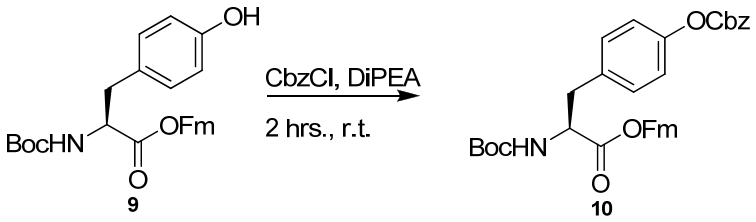
NH α-Tyr), 4.91 (s, 2H, CH_2 Bn), 4.60 (m, 1H, CH α-Tyr), 4.38 (m, 2H, CH_2 Fm), 4.10 (t, 1H, CH Fm), 2.94 (m, 2H, CH_2 β-Tyr), 1.42 (s, 9H, t-Bu Boc). IR (cm^{-1}): 3348, 2931, 1733, 1686, 1527, 1512, 1242, 1163, 1048, 736, 549. α_D ($CHCl_3$) = 0.0°. LC-MS (50 - 90 % B in 15 min), Rt = 9.62. HRMS $[C_{35}H_{35}NO_5 + H]^+$: 550.2585 found, 550.2588 calc.



***N^{α} -tert*-Butoxycarbonyl-L-tyrosine fluorenylmethyl ester (9)**

Compound **8** (1.1 mmol, 0.58 g) was coevaporated with MeCN and dissolved in n-propanol/EtOAc (9:1, 30 mL). The solution was purged with argon prior to addition of Pd-C (10%, 100 mg) and HCl (89 µL, 1.17 mmol). Hydrogen gas was bubbled trough the solution for 1 hour after which the reaction mixture was filtered over celite, concentrated and purified by silica gel column chromatography (PE/EtOAc, 90/10 → 80/20) to give compound **9** as a white powder (0.36 g, 73%).

^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.2 (CO α-Tyr), 155.4, (CO Boc), 143.5, 143.3, 141.3, 141.3 (Cq Fm), 130.4 (Arom. Tyr), 127.9, 127.9 (Arom. Fm), 127.4 (Cq Tyr), 127.2, 125.1 (Arom. Fm), 120.1, 115.6 (Arom. Tyr), 80.3 (Cq Boc), 67.2 (CH_2 Fm), 54.7 (CH α Tyr), 46.7 (CH Fm), 37.5 (CH_2 β Tyr), 28.3 (CH_3 Boc). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.78 (d, 2H, Arom. Fm), 7.48 (t, 2H, Arom. Fm), 7.34, 7.27 (2 x m, 4H, Arom. Fm), 6.89, 6.71 (2x d, 4H, Arom. Tyr), 5.12 (d, 1H, NH α-Tyr), 4.60 (m, 1H, CH α-Tyr), 4.39 (m, 2H, CH_2 Fm), 4.10 (t, 1H, CH Fm), 2.97 – 2.87 (m, 2H, CH_2 β-Tyr), 1.41 (s, 9H, Boc). IR (cm^{-1}): 3367, 2979, 1686, 1516, 1244, 1163, 530. α_D ($CHCl_3$) = -1.0°. LC-MS (50 - 90 % B in 15 min), Rt = 5.62. HRMS $[C_{28}H_{29}NO_5 + H]^+$: 460.2116 found, 460.2118 calc.

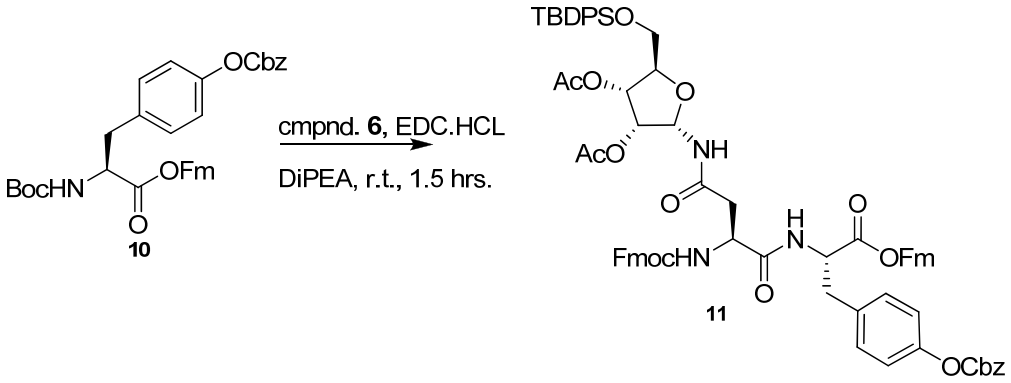


***N^{α} -tert*-Butoxycarbonyl-4-*O*-benzyloxycarbonyl- L-tyrosine fluorenylmethyl ester (10)**

Compound **9** (0.68 mmol, 0.31 g) was coevaporated with MeCN and dissolved in DCM (3.5 mL) under an argon atmosphere. To this solution were added benzylchloroformate (0.1 mL, 0.75 mmol) and DiPEA (0.12 mL, 0.75 mmol). The solution was stirred at room temperature for 2 hours, washed with 5% citric acid and H_2O. The combined organic layers were dried and concentrated *in vacuo*. The crude compound was purified by silica gel column chromatography (PE/EtOAc, 90/10 → 80/20) to give compound **10** as a white powder (0.34 g, 84%).

^{13}C-NMR (100 MHz, $CDCl_3$): δ: 171.4 (CO α-Tyr), 154.9, 153.3 (CO Cbz, CO Boc), 149.9 (Cq Tyr) 143.2, 143.1, 141.0, 141.0 (Cq Fm), 134.5 (Cq Cbz.), 133.7 (Cq Tyr), 130.2 (Arom. Tyr), 128.5, 128.4, 127.6, 126.9 (Arom. Fm/Cbz), 124.7, 120.8 (Arom. Fm), 119.8 (Arom. Tyr), 79.7 (Cq Boc), 70.0 (CH_2 Cbz), 66.8 (CH_2 Fm), 54.1 (CH α Tyr), 46.4 (CH Fm), 37.1 (CH_2 β Tyr), 28.0 (CH_3 Boc). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.83 (d, 2H, Arom. Fm), 7.62 (t, 2H, Arom. Fm), 7.58 - 7.37 (m, 9H, Arom. Fm/Cbz), 7.12 (s, 4H, Arom. Tyr), 5.32 (s, 2H, CH_2 Cbz), 5.10 (d, 1H, NH α-Tyr), 4.71 (m, 1H, CH α-Tyr), 4.52 (m, 2H, CH_2 Fm), 4.23 (t, 1H, CH Fm), 3.10 – 3.00 (m, 2H, CH_2 β-Tyr), 1.50 (s, 9H, Boc). α_D ($CHCl_3$) = + 0.4°. IR (cm^{-1}):3351,

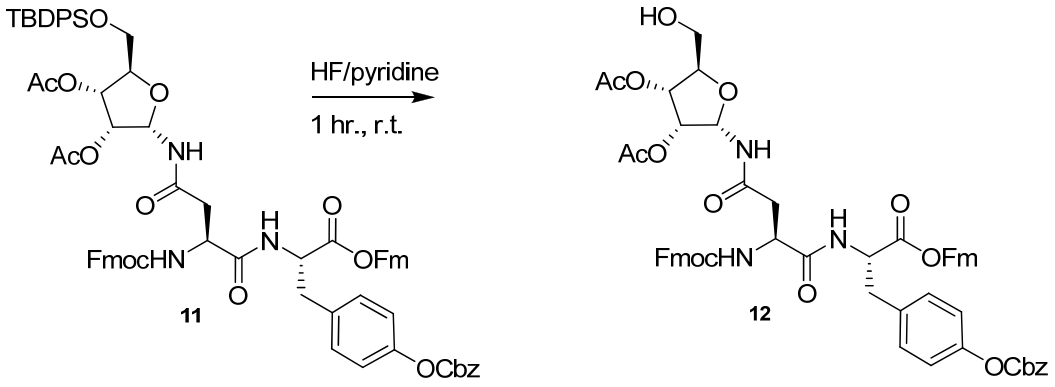
1748, 1683, 523, 1267, 1161, 738, 545. LC-MS (50 - 90 % B in 15 min), Rt = 9.34. HRMS $[C_{36}H_{35}NO_7 + H]^+$: 616.2301 found, 616.2305 calc.



***N^{γ}*-(5-*tert*-Butyldiphenylsilyl-2,3-di-*O*-acetyl-α-D-ribosyl)-*N^{α}*-fluorenylmethoxycarbonyl asparaginyl-(4-*O*-benzyloxycarbonyl)-L-tyrosine fluorenylmethyl ester (11)**

Compound **10** (0.55 mmol, 0.33 g) was dissolved in 3 M HCl/EtOAc and stirred at room temperature for 30 min. The reaction mixture was concentrated *in vacuo* and coevaporated with toluene. The residue was dissolved in DCM (10 mL) and a solution of **6** (0.5 mmol) in DCM (2.5 mL) was added, followed by EDC·HCl (0.6 mmol, 0.12 g) and DiPEA (0.6 mmol, 0.1 mL). After stirring at room temperature for 1.5 hr the reaction mixture was washed with 5% citric acid and water. The organic layer was dried ($MgSO_4$) and concentrated. The crude compound was purified by silica gel column chromatography (PE/EtOAc, 100/0 → 35/65) to give compound **11** as a white powder (0.59 g, 0.46 mmol, 84%).

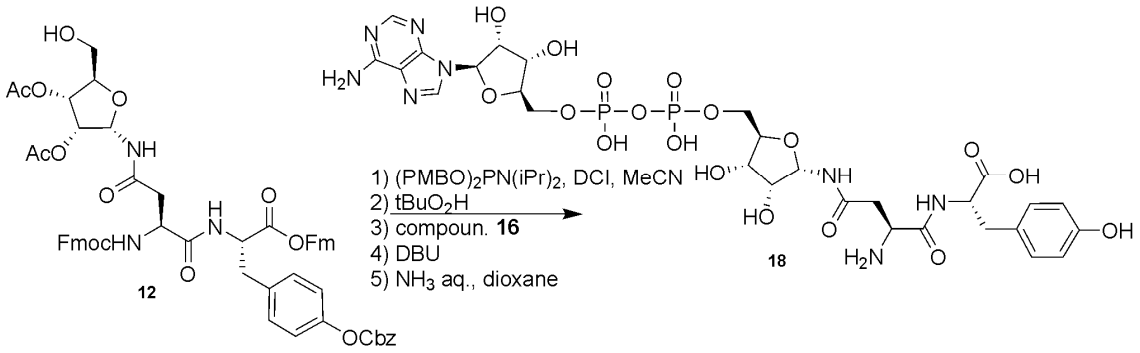
^{13}C-NMR (100 MHz, $CDCl_3$): δ 171.3 (CO α-Tyr), 170.9, 170.6 (CO OAc), 169.6, 169.2 (CO α-Asn, CO γ-Asn), 156.2 (CO Fmoc), 153.5 (CO Cbz), 150.3 (Cq Tyr), 143.8, 143.7, 143.5, 143.4, 141.4, 141.3 (Cq Fm, Cq Fmoc), 135.7 (Arom. TBDPS), 134.8 (Cq Cbz), 133.6 (Cq Tyr), 132.9, 132.7 (Cq TBDPS), 130.4, 129.9, 128.8, 128.7, 128.5, 128.0, 127.9, 127.8, 127.3, 127.2, 125.2, 125.0, 121.2, 120.0 (Arom. Fmoc, Arom. Fm, Arom. TBDPS, Arom. Cbz), 82.2 (C4'), 78.7 (C1'), 72.4 (C3'), 70.3 (CH_2 Cbz), 70.2 (C2') 67.5, 67.2 (CH_2 Fmoc, CH_2 Fm), 63.7 (C5'), 53.7 (Cα Tyr), 51.3 (Cα Asn), 47.1, 46.7 (CH Fmoc, CH Fm), 38.0 (CH_2 β-Asn), 37.0 (CH_2 β-Tyr), 26.8 (CH_3 TBDPS), 20.8, 20.6 (CH_3 OAc), 19.2 (Cq t-Bu).^{1}H-NMR (400 MHz, MeOD-d4/$CDCl_3$): δ 7.57, 7.53, 7.51 – 7.48, 7.36, 7.26, 7.03 (6x m, Arom. Fmoc, Arom. Fm, Arom. TBDPS, Arom. Tyr, 35H), 6.71 (d, 1H, NH γ-Tyr), 6.25 (d, 1H, NH α-Asn), 6.06 (dd, 1H, H1'), 5.56 (s, 1H, H3'), 5.47 (m, 1H, H2'), 5.16 (CH_2 Cbz), 4.75 (m, 1H, CH α-Asn), 4.52 (m, 1H, CH α-Tyr), 4.45, 4.34 (2 x m, 4H, CH_2 Fmoc, CH_2 Fm), 4.18 (m, 2H, CH Fmoc, CH Fm), 4.11 (s, 1H, H4'), 3.71 (s, 2H, H5'), 3.02 – 2.62 (m, 4H, CH_2 β-Asn, β-Tyr), 2.07, 2.02 (2 x s, 6H, CH_3 OAc), 1.06 (s, 9H, t-Bu). α_D ($CHCl_3$) = + 29.6°. IR (cm^{-1}): 3278, 2933, 2360, 1747, 1696, 1648, 1534, 1211, 1104, 737, 699, 503. HRMS $[C_{75}H_{73}N_3O_{15}Si + H]^+$: 1284.4903 found, 1284.4883 calc.



***N*$^{\gamma}$ -(5-Hydroxy-2,3-di-*O*-acetyl-α-D-ribosyl)- *N*$^{\alpha}$ -fluorenylmethoxycarbonyl asparaginyl-(4-*O*-benzyloxycarbonyl)-L-tyrosine fluorenylmethyl ester ; Fmoc-Asn-(*N*$^{\gamma}$ -[2,3-di-*O*-acetyl-α-D-ribosyl])-Tyr(Cbz)-OFm (12)**

Silyl protected compound **11** (0.47 mmol, 0.61 g) was dissolved in pyridine (10 mL) and treated with a HF/pyridine solution (1.0 mL) for 1 hour at room temperature. The reaction was quenched with sat. aq. $NaHCO_3$ and extracted with DCM. After washing with water the organic layer was dried ($MgSO_4$) and concentrated *in vacuo*. The crude compound was purified by silica gel column chromatography (DCM/MeOH, 99/1) to give compound **12** as a white powder (0.42 g, 0.4 mmol, 85%).

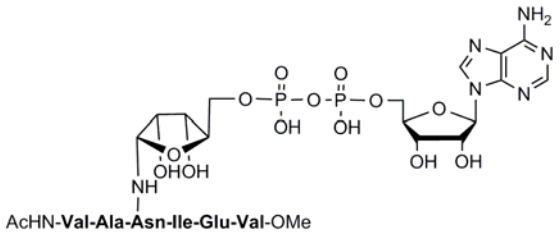
^{13}C-NMR (100 MHz, DMSO-*d6*): δ 172.0, 171.6, 170.0, 169.9, 169.8 (CO α-Tyr, CO OAc, CO α-Asn, CO γ-Asn), 156.2 (CO Fmoc), 153.4 (CO Cbz), 149.9 (Cq Tyr.), 144.3, 144.1, 143.9, 141.3, 141.2, 141.1 (Cq Fm, Cq Fmoc), 135.48 (Cq Cbz), 135.45 (Cq Tyr.), 130.7, 129.0, 128.8, 128.2, 128.1, 127.8, 127.7, 127.6, 125.8, 125.6, 121.4, 120.6 (Arom. Fmoc, Arom. Fm, Arom. Cbz), 80.5 (C4'), 79.1 (C1'), 71.5 (C3'), 70.7 (C2'), 70.2 (CH_2 Cbz), 66.7, 66.3 (CH_2 Fmoc, CH_2 Fm), 61.4 (C5'), 54.2 (Cα Tyr), 51.6 (Cα Asn), 47.0, 46.7 (CH Fmoc, CH Fm), 38.0 (CH_2 β-Asn), 36.0 (CH_2 β-Tyr), 20.9 (CH_3 OAc).^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.58, 7.45, 7.41, 7.32, 7.09 (5 x m, 25H, Arom. Fmoc, Arom. Fm, Arom. Cbz), 6.69, 6.23 (2x d, 1H, NH's), 5.95 (m, 1H, H1'), 5.40, 5.31 (2x m, 4H, H2', H3'), 5.25 (s, 2H, CH_2 Cbz), 4.79, 4,56 (2x m, 2H, H α-Asn, H α-Tyr), 4.51, 4.38, (2x m, 6H, CH_2 Fmoc, CH_2 Fm), 4.24 (m, 2H, CH Fmoc, CH Fm), 4.12 (m, 1H, H4'), 3.87 – 3.64 (m, 2H, H5'), 3.03 - 2.89, 2.56 (m, 4H, CH_2 β Asn, CH_2 β Tyr) 2.14, 2.12 (2 x s, 6H, CH_3 OAc). α_D ($CHCl_3$) = + 26.8°. IR (cm^{-1}): 3288, 2924, 2360, 1746, 1652, 1538, 1269, 1212, 1046, 736. LC-MS (10 - 90 % B in 15 min), Rt = 11.30. HRMS [$C_{59}H_{55}N_3O_{15}$ + H]$^{+}$: 1046.318 found, 1046.3705 calc.



Compound 18: H-Asn(*N*$^{\gamma}$ [5'-adenosine diphosphate-α-D-ribosyl])-Tyr-OH.

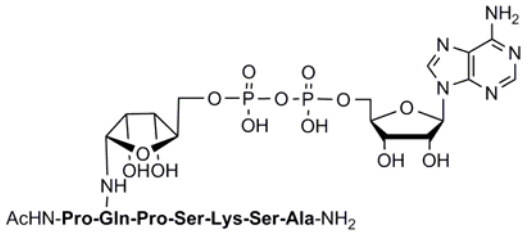
Ribosylated dipeptide **12** (0.1 mmol, 100 mg) was dissolved in dioxane/ MeCN (1:1, 4 mL) and cooled to 0 °C. Di(p-methoxybenzyl)-N,N-diisopropylphosphoramidite (0.11 mmol, 43 µL) and a solution of DCI (0.11 mmol, 13 mg) in 200 µL MeCN were added after which the reaction mixture was kept at 0 °C for 1 hour. 100 µl 5.5 M solution of $tBuO_2H$ in nonane was added and stirred for an additional hour at 0 °C. After trituration with hexane, the residue was

treated with 5% TFA in DCM to yield intermediate **14**. 6-N-Benzoyl-2', 3'-di-O-isobutyryladeninyl-5-phosporimidazolidate **16** (0.2 mmol) in 1 mL MeCN was added to the reaction mixture and stirred at room temperature for 16 hours. A silica column with isocratic elution (30% MeOH/DCM) and RP-HPLC purification 80 – 90 % MeOH in 20 mM NH_4OAc over 5 CV yielded **17** (26.2 mg, 15.4 µmol, 15.4% based on **12**).
To a solution of **17** (14.5 mg, 8.5 µmol) in dioxane/MeCN (1:1, 2 ml) 5 eq. DBU (43 µmol, 6.5 µl) was added and reacted for 30 min at room temperature. A solution of aqueous ammonia /dioxane (2:1, 6 ml) was added and reacted for 16 hours at room temperature. After concentration the title compound was obtained by means of gel filtration (4.1 mg, 4.9 µmol, 58% based on **17**).
^{31}P-NMR (162 MHz, D_2O): δ -10.45, -10.58, -10.75, -10.88. LC-MS (0 - 50 % MeCN in 10 mM NH_4OAc in 15 min), Rt = 4.0. HRMS $[C_{28}H_{38}N_8O_{18}P_2 + H]^+$: 837.1861 found, 837.1852 calc.



Compound 21; Ac-Val-Ala-Asn(N^{δ} [5'-adenosine diphosphate-α-D-ribosyl])-Ile-Glu-Val-OMe

Intermediate **19** was prepared starting from Tentagel S HMB resin (100 mg, 23 µmol) via standard Fmoc based Solid phase peptide synthesis, using HCTU as coupling reagent and a five fold excess of the appropriate amino acid, using Fmoc-Val-OH, Fmoc-Ala-OH, compound **6**, Fmoc-Ile-OH and Fmoc-Glu(Dmab)-OH. An analytical sample was deprotected and cleaved off the resin using the method described below, to determing quality of **19**.The resin was treated with 0.36 mL HF/pyridine diluted with pyridine (3 mL)(0 ^{o}C→ r.t., 75 min). The resin was filtered and washed with pyridine and MeCN to give intermediate **20**.
The resin (80 mg, 18 µmol) was coevaporated with MeCN and subsequently MeCN (3 mL), di(p-methoxybenzyl)-N,N-diisopropylphosphoramidite (0.23 mmol, 90 µL) and a solution of DCI (0.23 mmol, 27 mg) in 1 mL MeCN were added after which the reaction mixture was kept at 0 ^{o}C for 1 hour. A 0.5 M solution of I_2 in pyridine was added until the reaction mixture kept the dark brown colour and stirred for an additional hour at 0 ^{o}C. 6-N-benzoyl-2', 3'-di-O-isobutyryladeninyl-5'-phosphate monoester (0.23 mmol, 0.14 g) was added to the reaction mixture and stirred at room temperature for 24 hours. The resin was filtered and washed with MeCN and treated with 2% hydrazine hydrate in DMF (3 times 5 min.). The resin was filtered, washed with MeCN and treated with 7N NH_3/MeOH (10 mL) for 16 hours. Subsequent filtration into cold Et_2O and centrifuging (6.5 min at 4000 RPM) followed by decantation of the supernatant resulted in the crude title compound. RP-HPLC using a gradient of 0 – 60% MeCN in 1% TFA in H_2O followed by a gradient RP-HPLC using a gradient of 0 – 60% MeCN of 10 mM NH_4OAc, followed by lyophilisation of the appropriate fractions yielded the title compound **21** (0.78 mg, 0.6 µmol, 3.4% based on initial loading of the resin). ^{31}P-NMR (162 MHz, D_2O): δ -11.04, -11.17, -11.31, -11.44 . (EDTA was added to sharpen signals). LC-MS (0 - 50 % MeCN [1% TFA] in 15 min), Rt = 8.07. HRMS $[C_{46}H_{74}N_{12}O_{24}P_2 + H]^+$: 1241.4505 found, 1241.4486 calc.

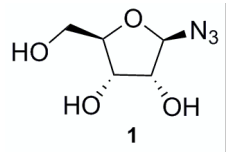


Compound 25: Ac-Pro-Gln-(N^{δ} [5'-adenosine diphosphate-α-D-ribosyl])-Pro-Ser-Lys-Ser-Ala-NH_2

Starting from Tentagel S HMB resin (150 mg, 65 µmol) using standard Fmoc based Solid Phase Peptide Synthesis intermediate **22** was prepared using Fmoc-Pro-OH, compound **7**, Fmoc-Ser(Trt)-OH, Fmoc-Lys(TFA)-OH and Fmoc-Ala-OH. The resin was repeatedly treated with 5% TFA in DCM until no yellow colour appeared upon addition of the fresh TFA solution. After washing with DCM, acetic anhydride (0.5 M, 200 µL) and DMAP (0.05 M, 16 mg) in 2 mL NMP were added and the resin was shaken for 25 min. The resin was washed with NMP and DCM prior to coupling of the last two amino acids using the standard cycle to obtain **23.** An analytical sample was deprotected and cleaved off the resin using the method described below, to determing quality of **24.** The resin was coevaporated with MeCN and treated with a HF/pyridine (0.9 mL) diluted with pyridine (7.5 mL) (0 ^{o}C→ r.t., 75 min). The resin was filtered and washed with pyridine and MeCN to obtain **24**. The resin was coevaporated with MeCN and subsequently MeCN (8 mL), di(*p*-methoxybenzyl)-*N*,*N*-diisopropylphosphoramidite (10 eq., 250 µL), dry 3Å molsieves and a solution of DCI (10 eq., 75 mg) in 1 mL MeCN were added after which the reaction mixture was kept at 0 ^{o}C for 1 hour. Next the phosphate was oxidized n using 5.5 M *t*BuO_2H (1.1 mL) in nonane for 1 hour at room temperature followed by addition of 3% TFA for 10 min. to deprotect the phosphor triester. 6-N-Benzoyl-2', 3'-di-O-isobutyryladeninyl-5-phosporimidazolidate **16** (0.4 mmol, 6 eq.) in 1 mL MeCN was added to the reaction mixture and stirred at room temperature for 16 hours. The resin was filtered, washed with MeCN and treated with 7N NH_3/MeOH (10 mL) for 16 hours. Subsequent filtration into cold Et_2O and centrifuging (6.5 min at 4000 RPM) followed by decantation of the supernatant resulted in the crude title compound. Preparative RP-HPLC using a gradient of 2.5 – 40% MeCN in 10 mM aq. NH_4OAc, followed by lyophilisation of the appropriate fractions yielded the title compound (1.3 mg, 1.0 µmol, 1.5% based on initial loading of the resin) .

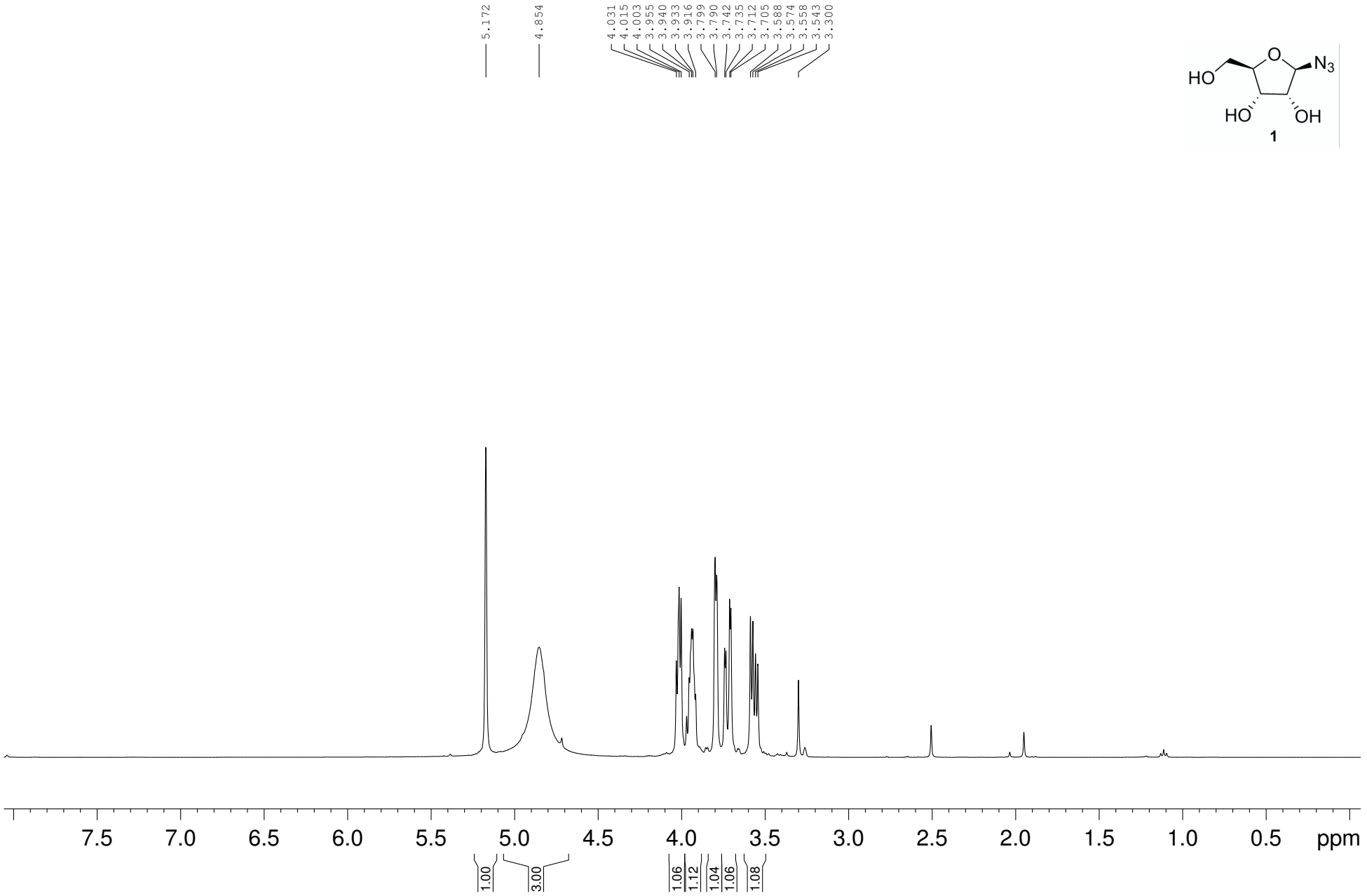
^{31}P-NMR (162 MHz, D_2O): δ -10.46, -10.59, -10.74, -10.87. LC-MS (0 - 50 % MeCN [1% TFA] in 15 min), Rt = 4.49. HRMS $[C_{47}H_{75}N_{15}O_{24}P_2 + H]^+$: 1296.4675 found, 1296.4657 calc.

13C-NMR, 100 MHz, MeOD-d4, cmpnd 1

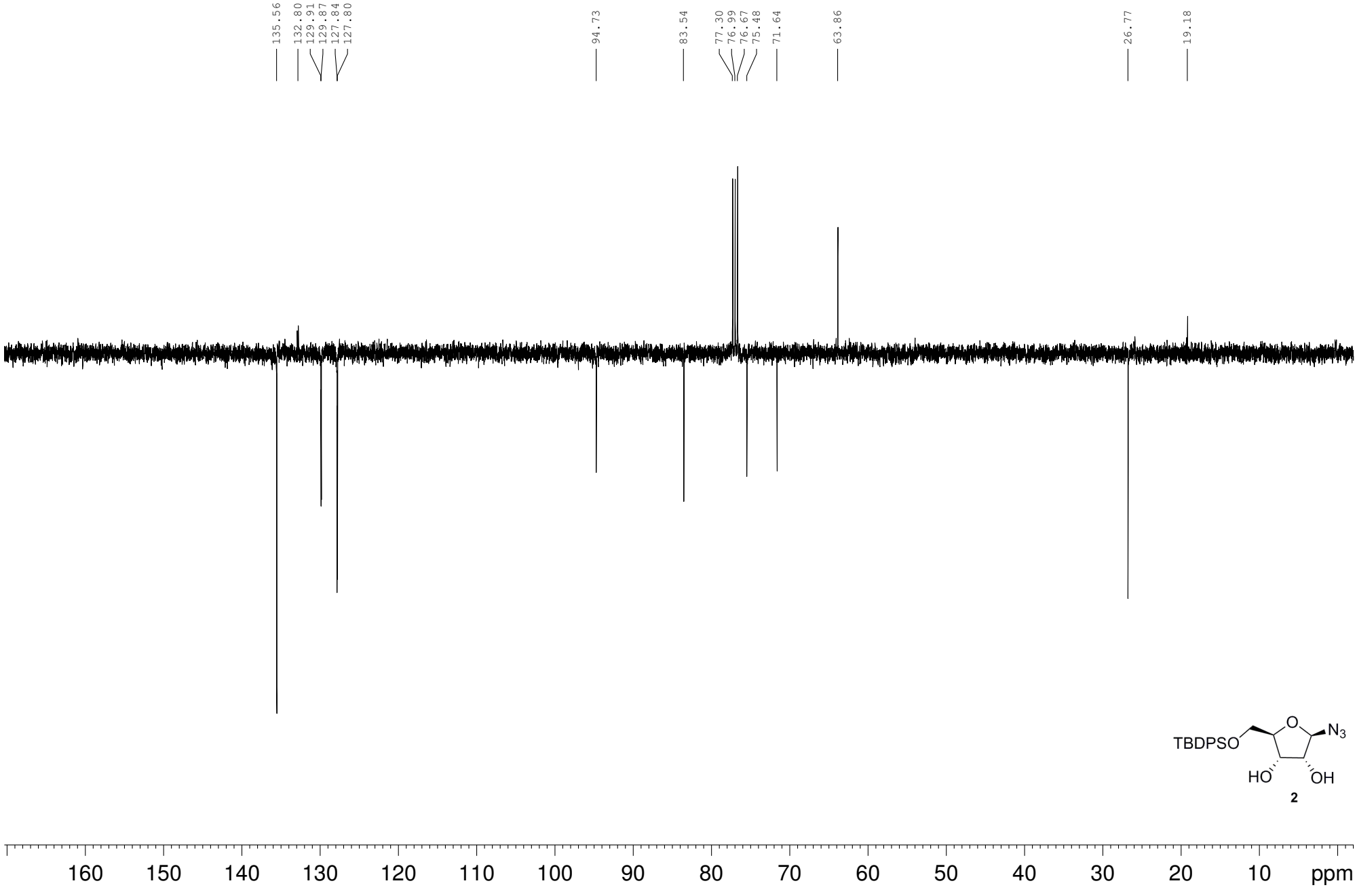


1

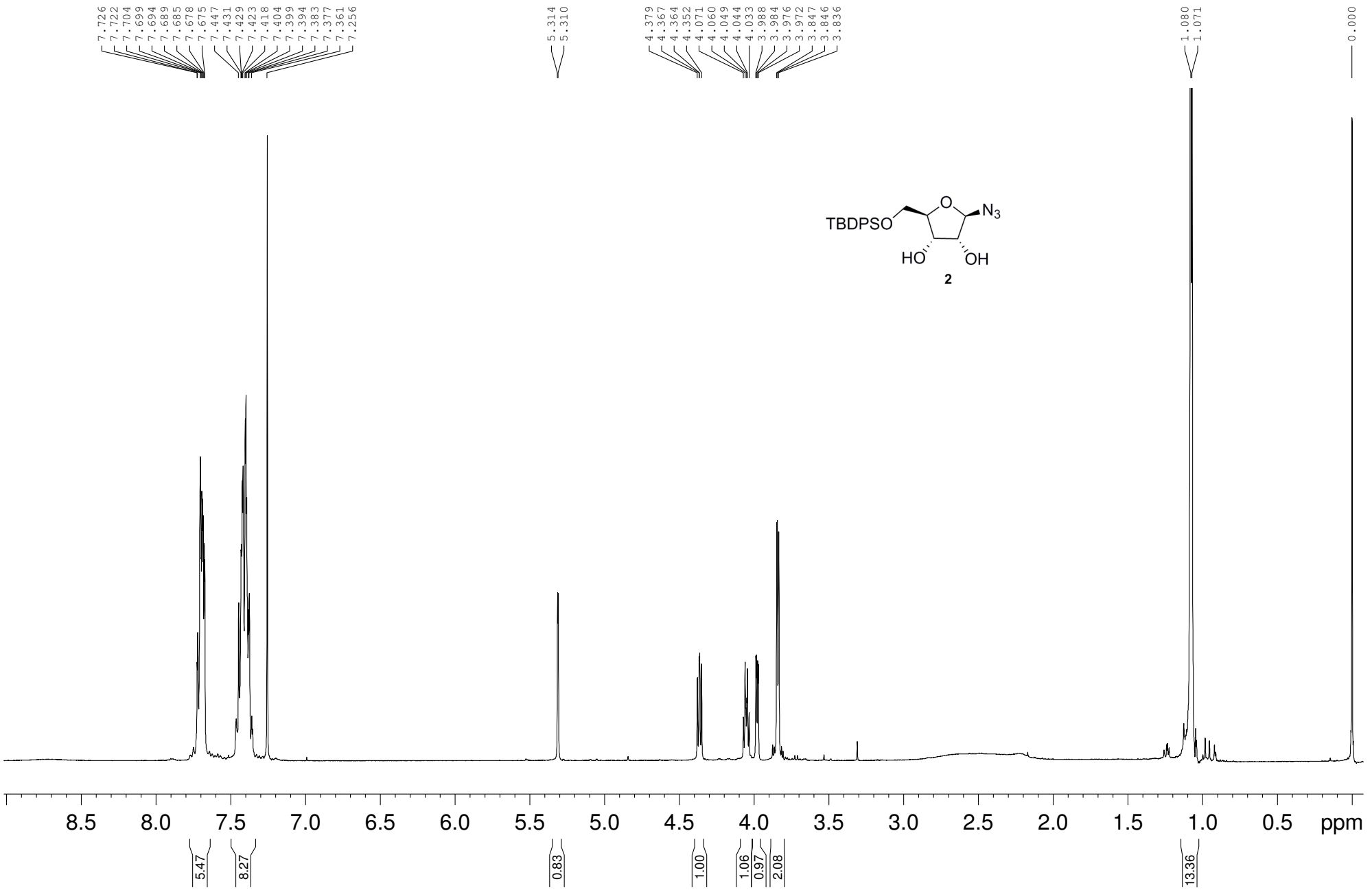
1H NMR, 400 MHz, MeOD-d4, cmpnd 1



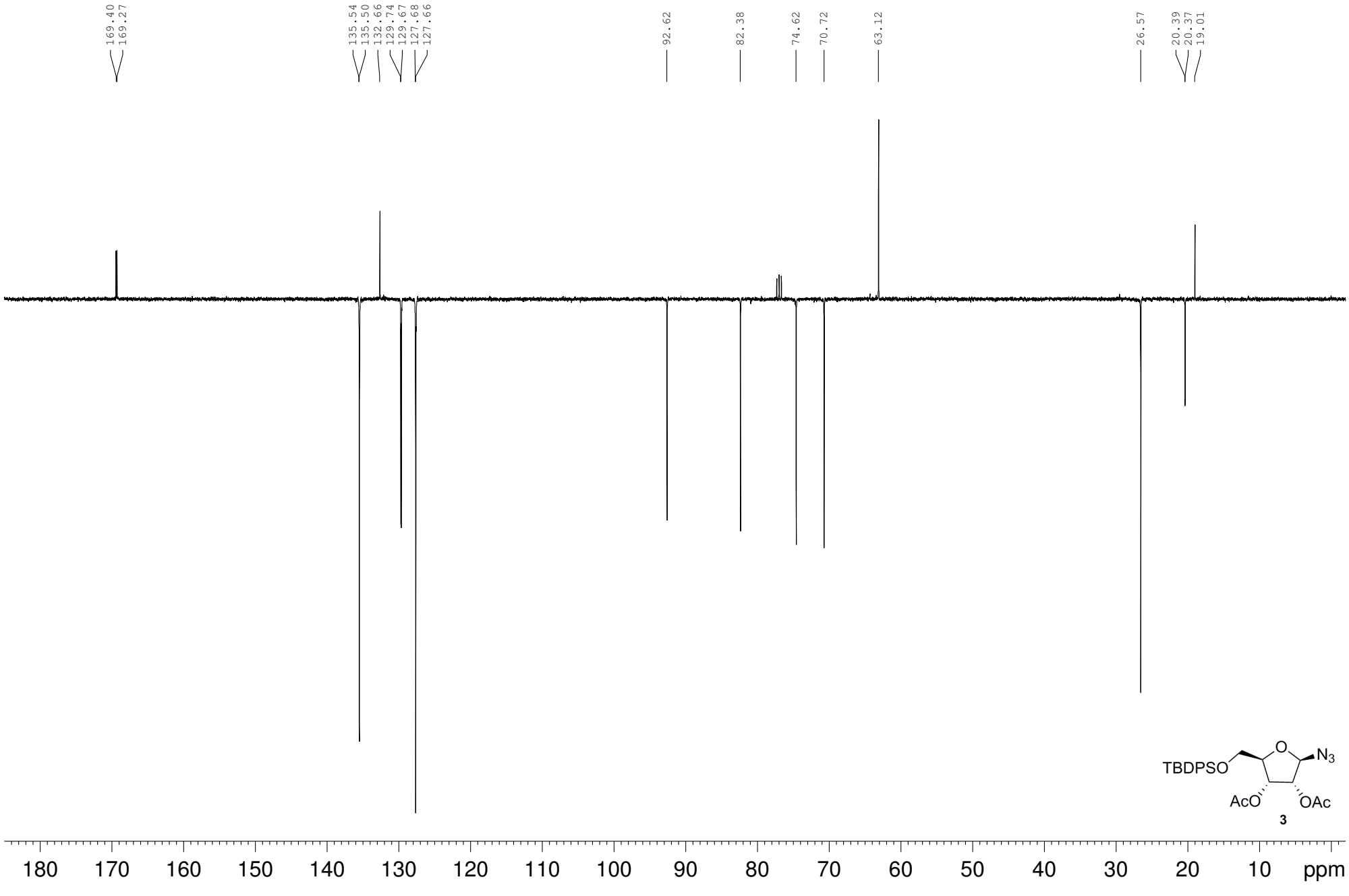
13C-NMR, 100 MHz, CDCl3, cmpnd. 2



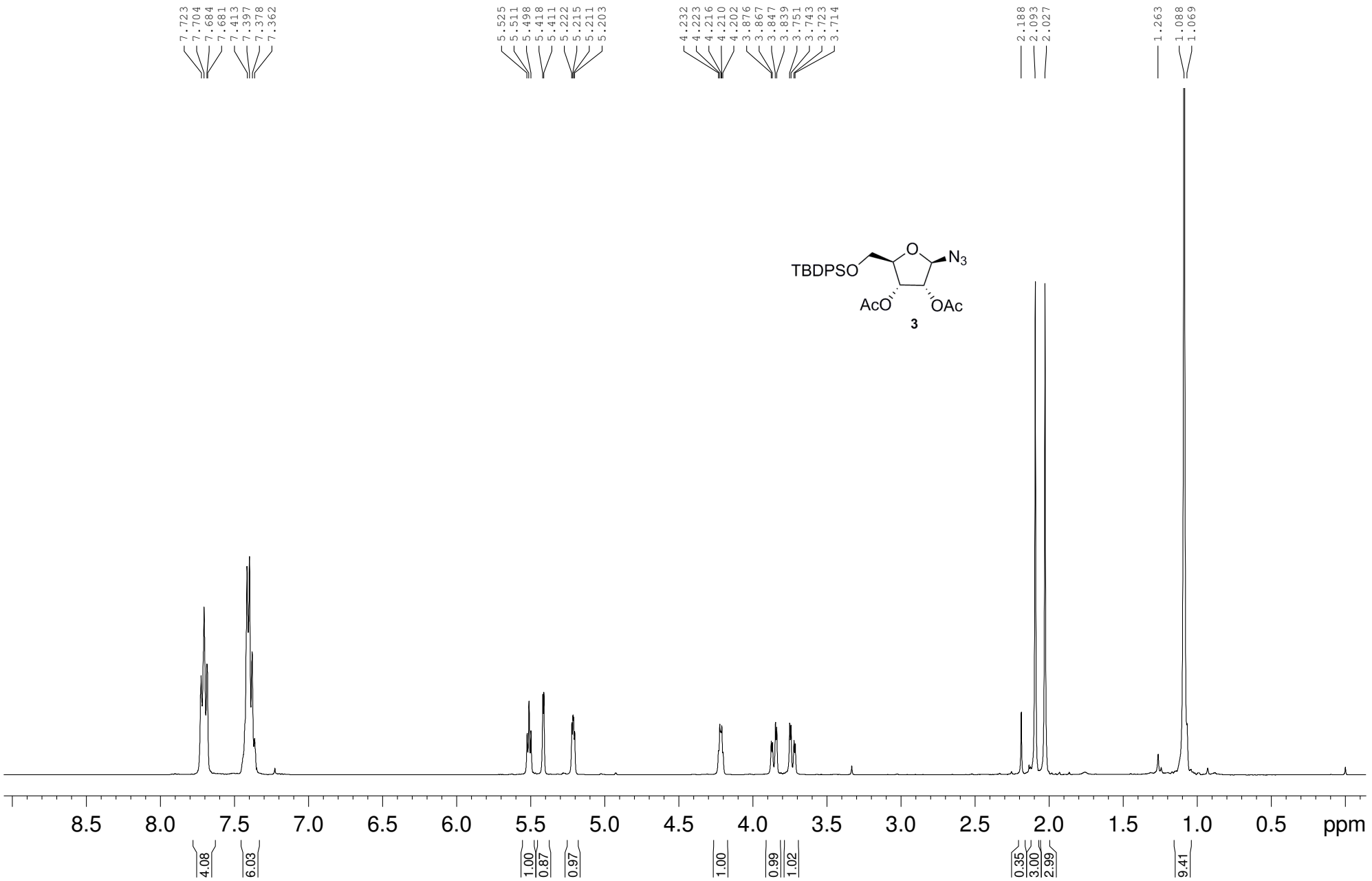
H-NMR, 400 MHz, CDCl3, cmpnd. 2



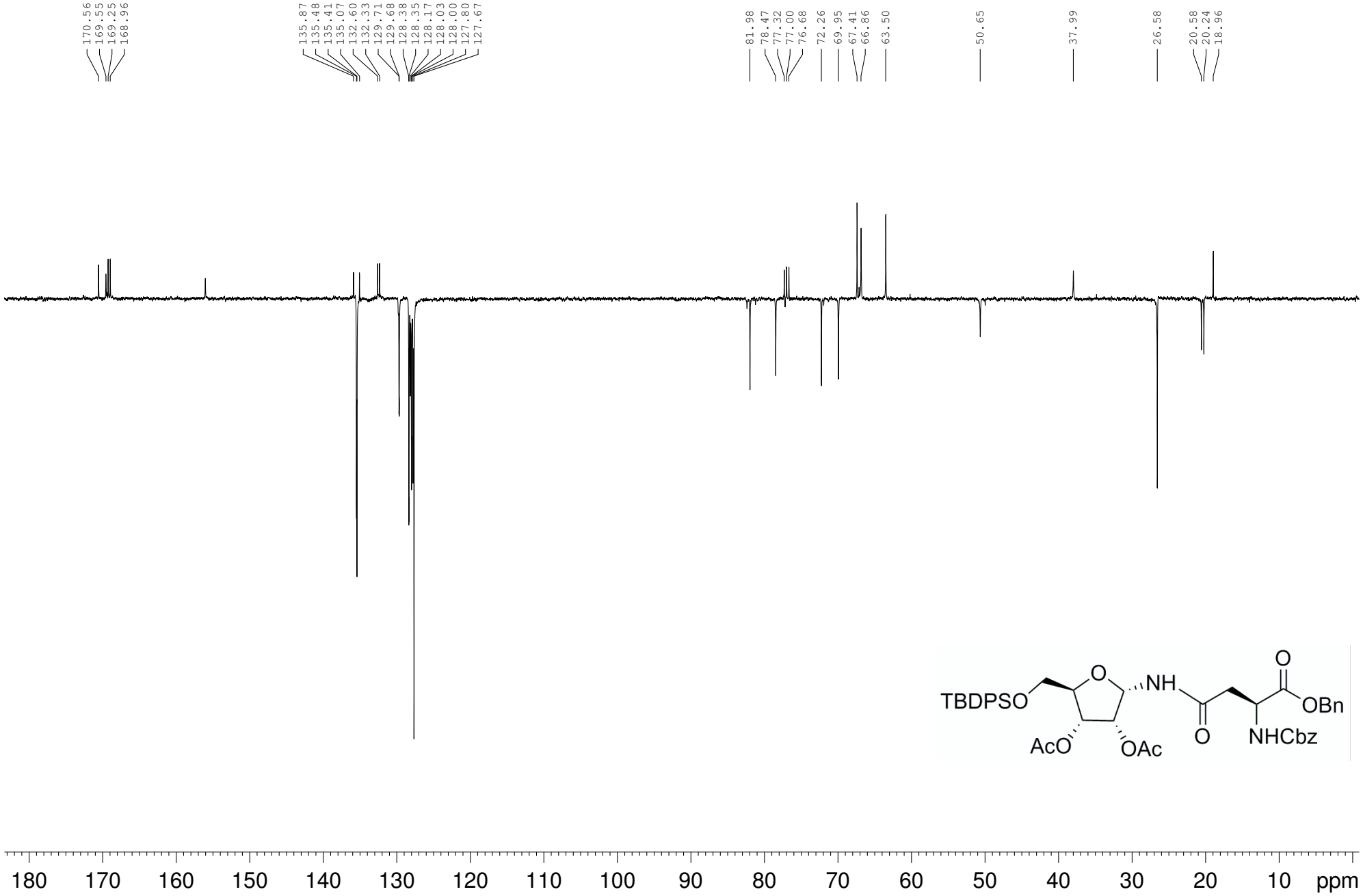
13C-NMR, 100 MHz, CDCl3, cmpnd. 3



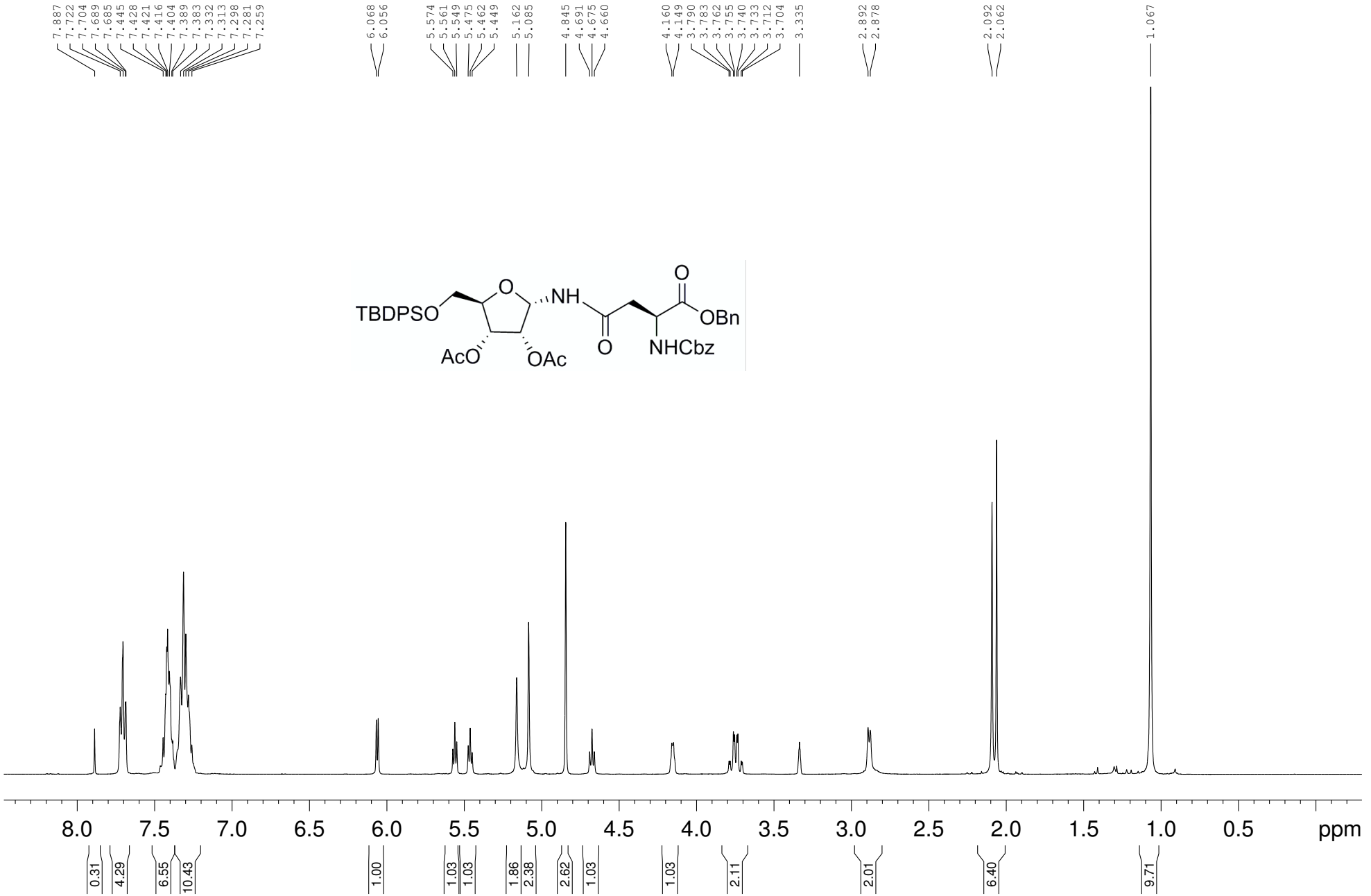
H-NMR, 400 MHz, CDCl3, cmpnd. 3

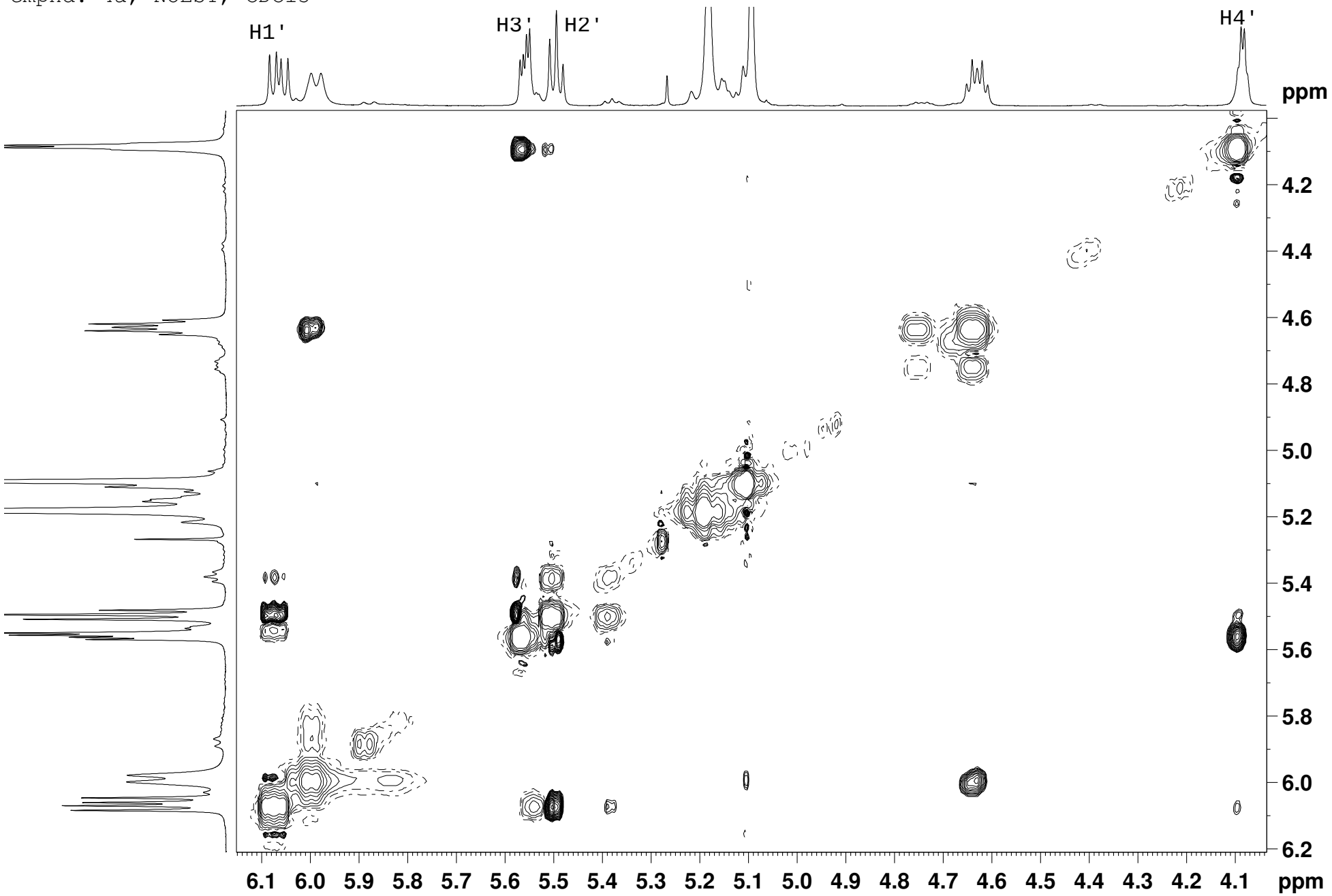


13C-NMR, 100MHz, CDCl3, cmpnd 4A

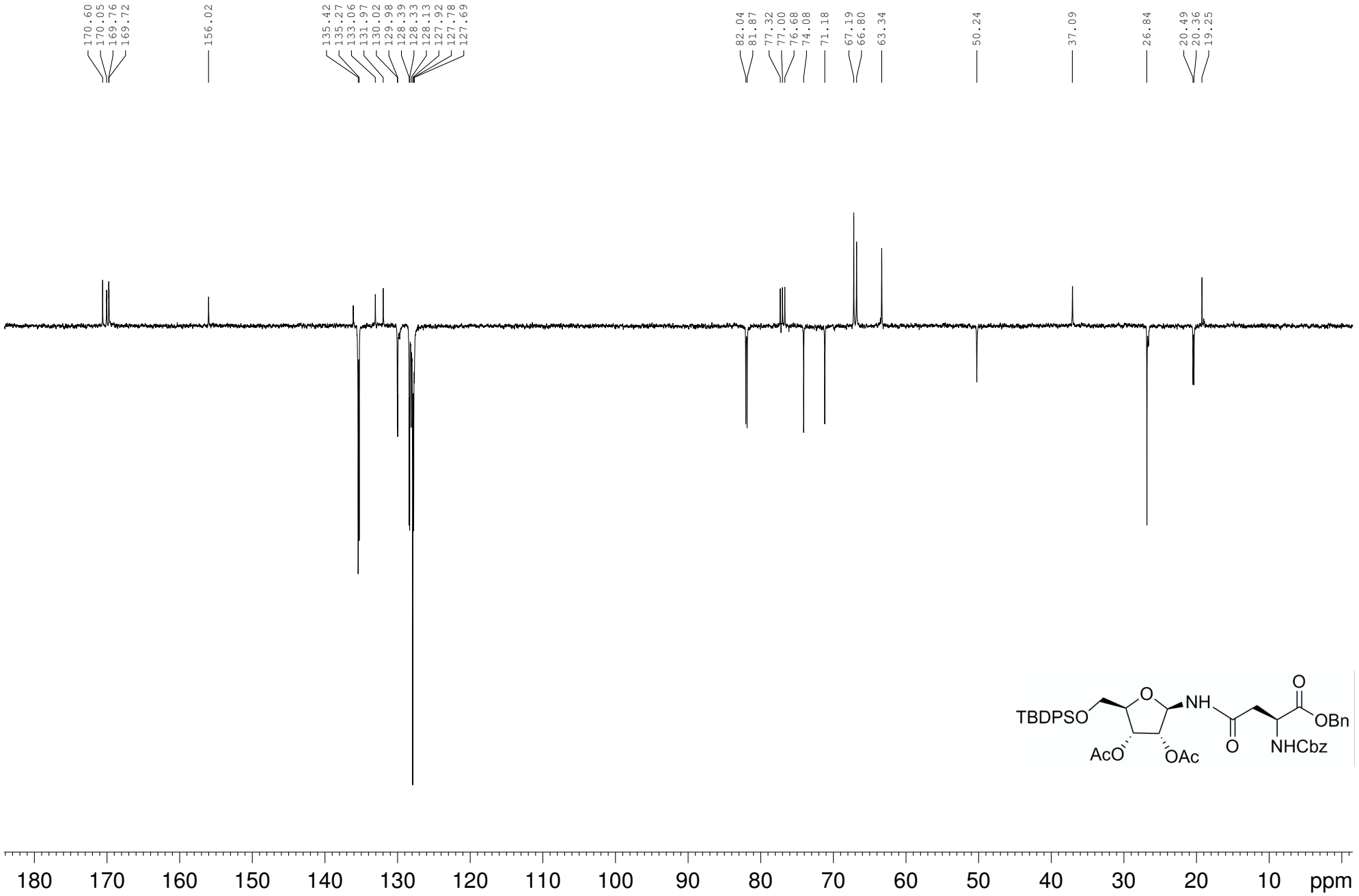


1H-NMR, 400 MHz, MeOD-d4, cmpnd 4A

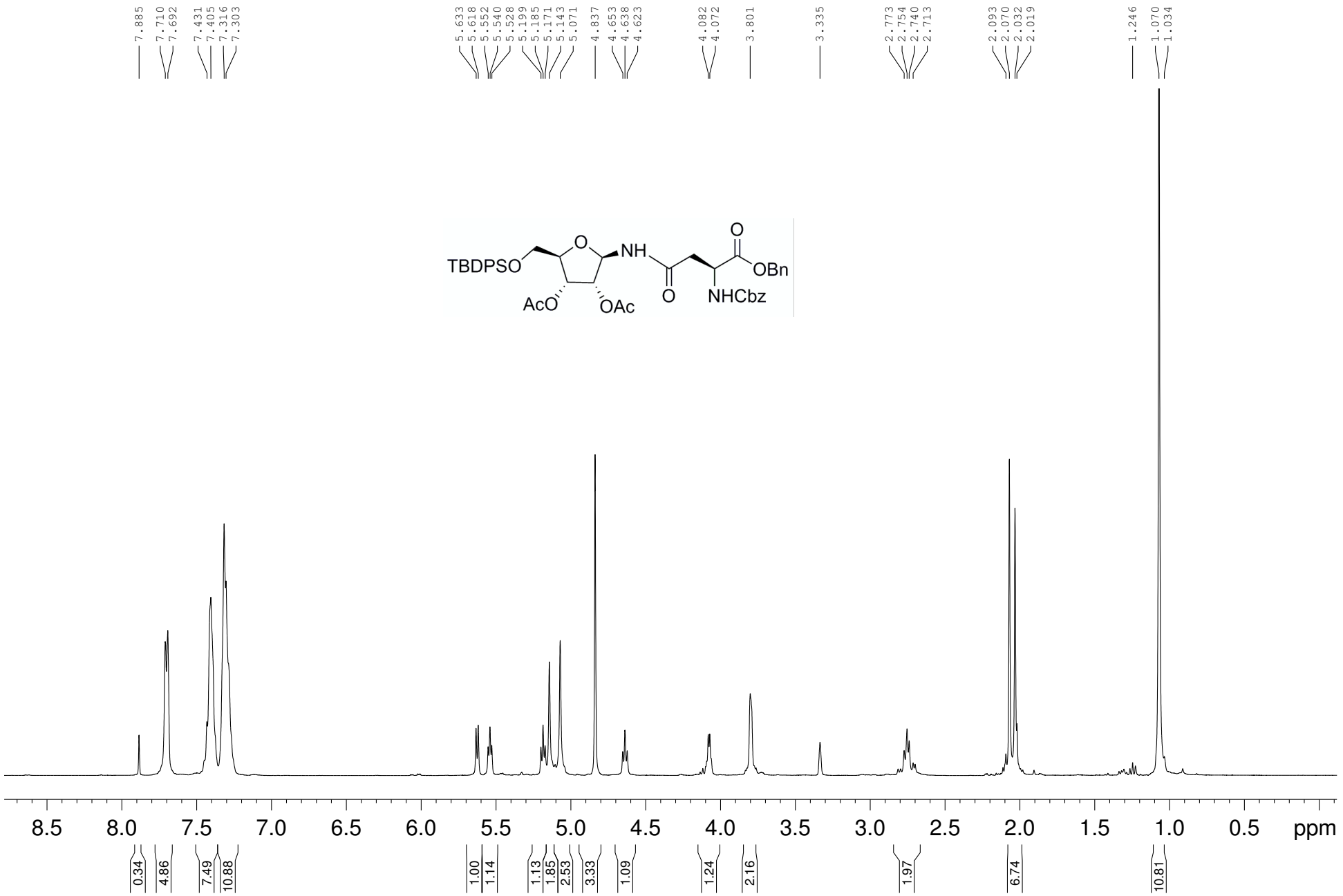


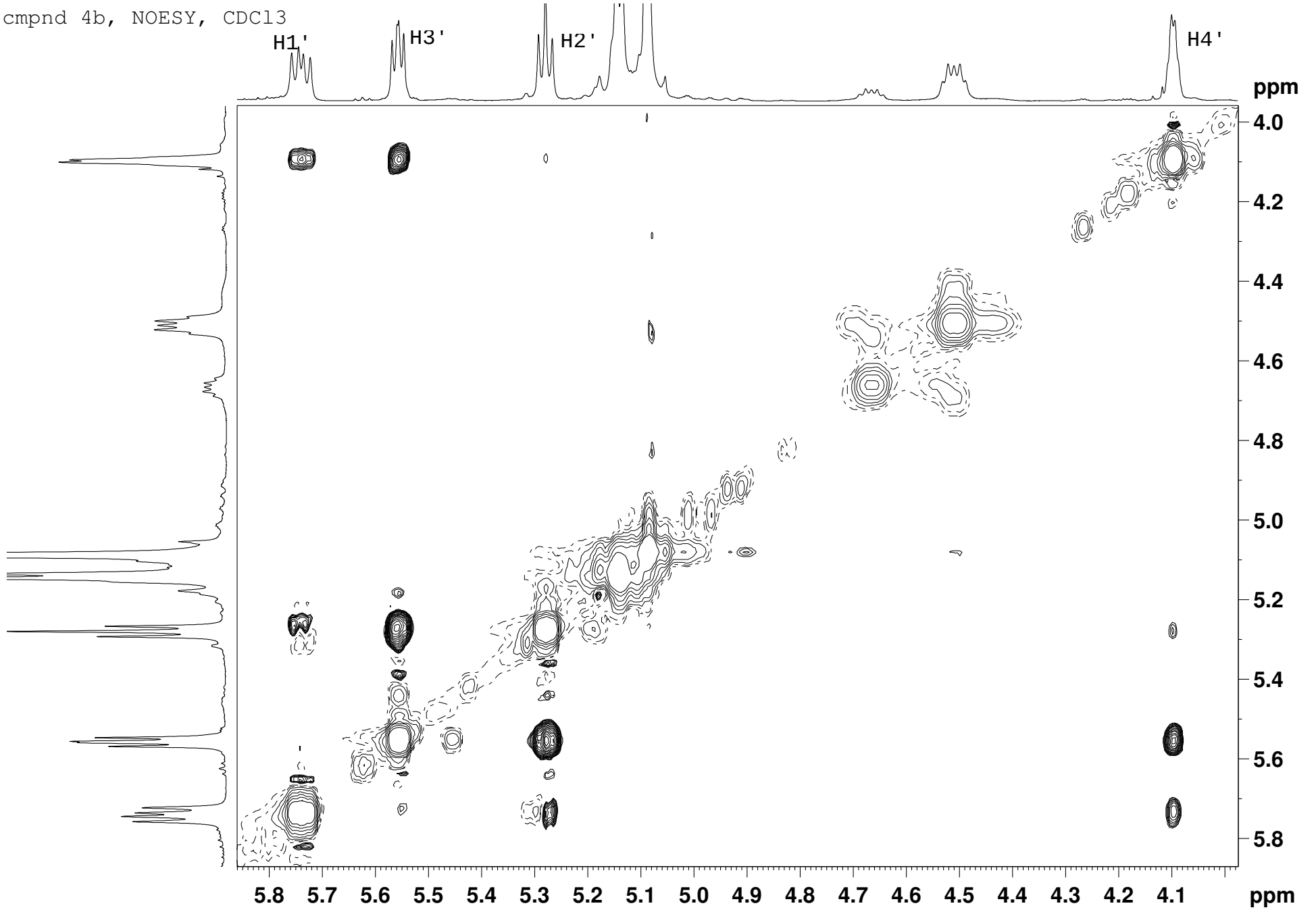

cmpnd. 4a, NOESY, CDCl3
H1'
H3'
H2'
H4'
ppm
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
6.0
6.2
6.1 6.0 5.9 5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1
ppm

13C-NMR, 100 MHz, CDCl3, cmpnd 4B

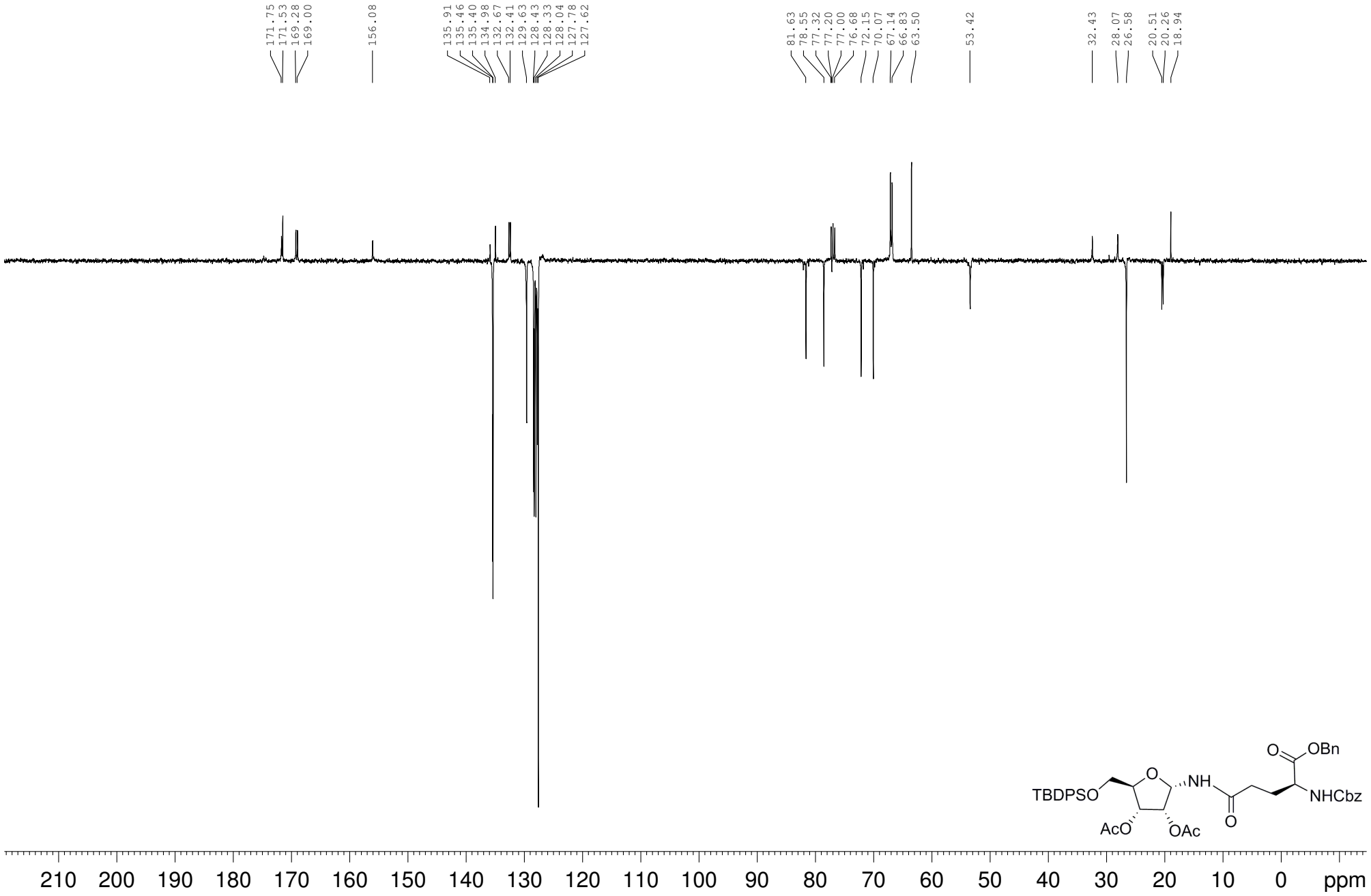


1H-NMR, 400 MHz, MeOD-d4, cmpnd 4B

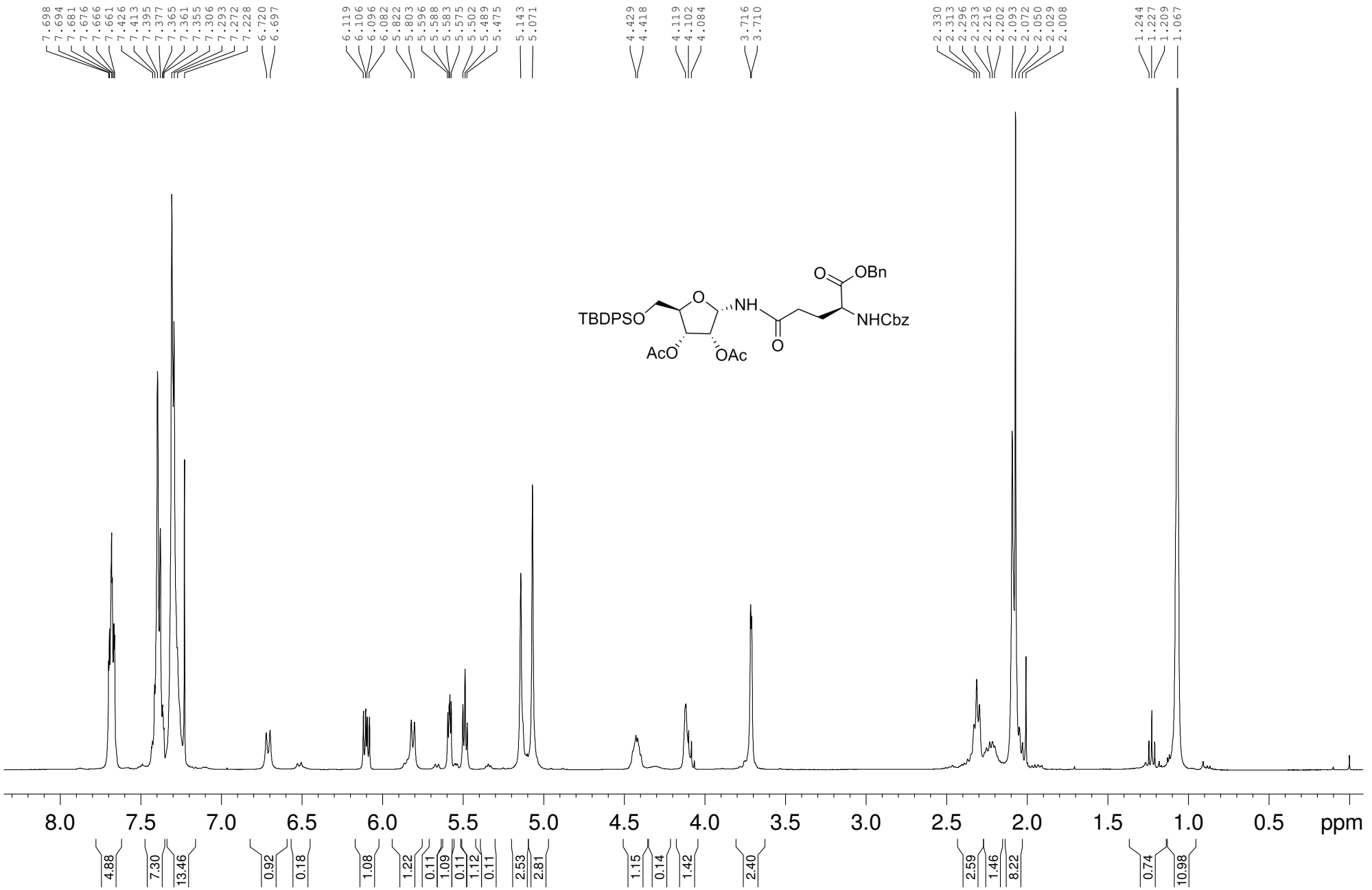



cmpnd 4b, NOESY, CDCl3
H1'
H3'
H2'
H4'
ppm
4.0
4.2
4.4
4.6
4.8
5.0
5.2
5.4
5.6
5.8
5.8 5.7 5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1
ppm

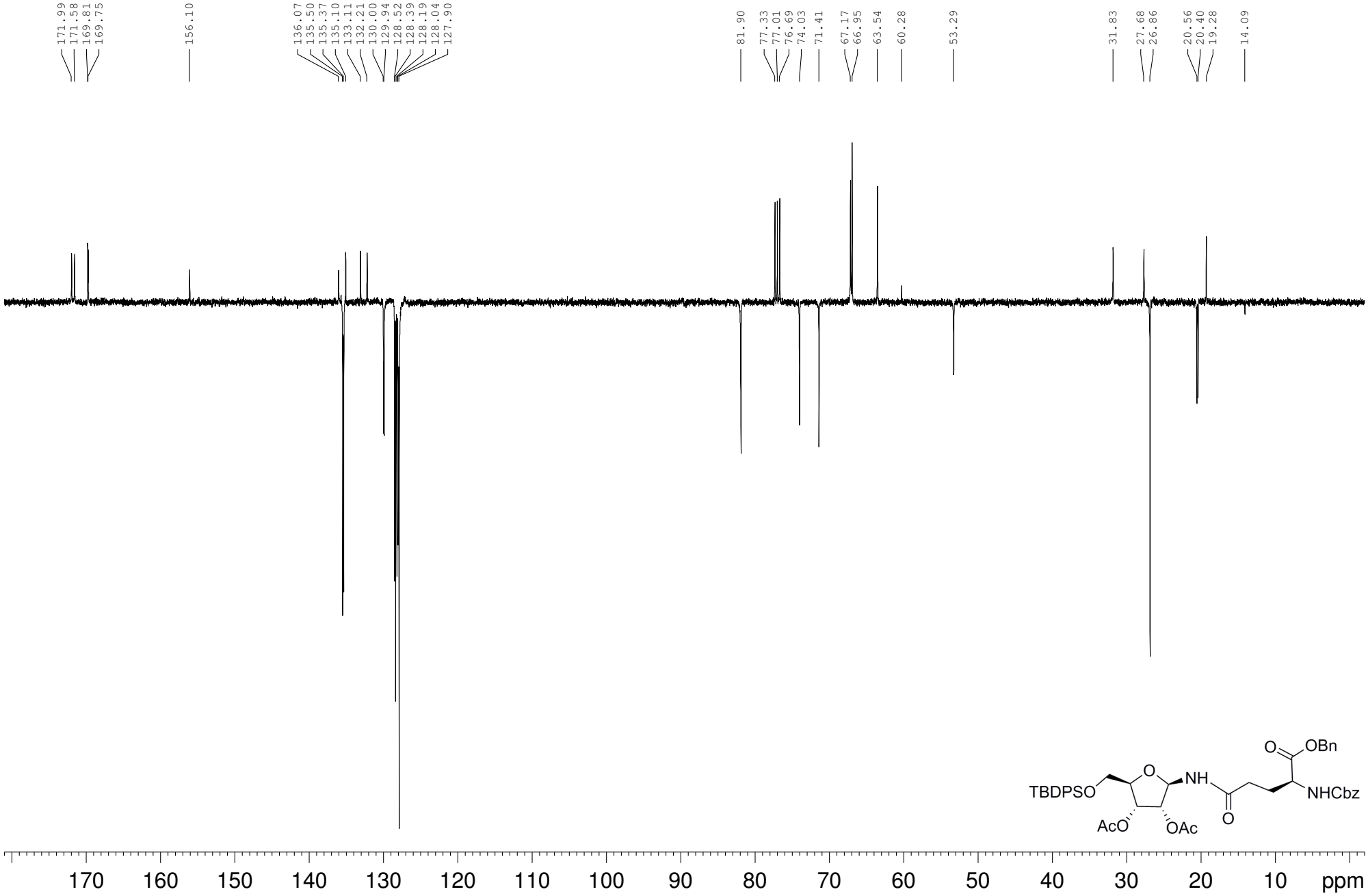
13C-NMR, 100 MHz, CDCl3, cmpnd. 5a



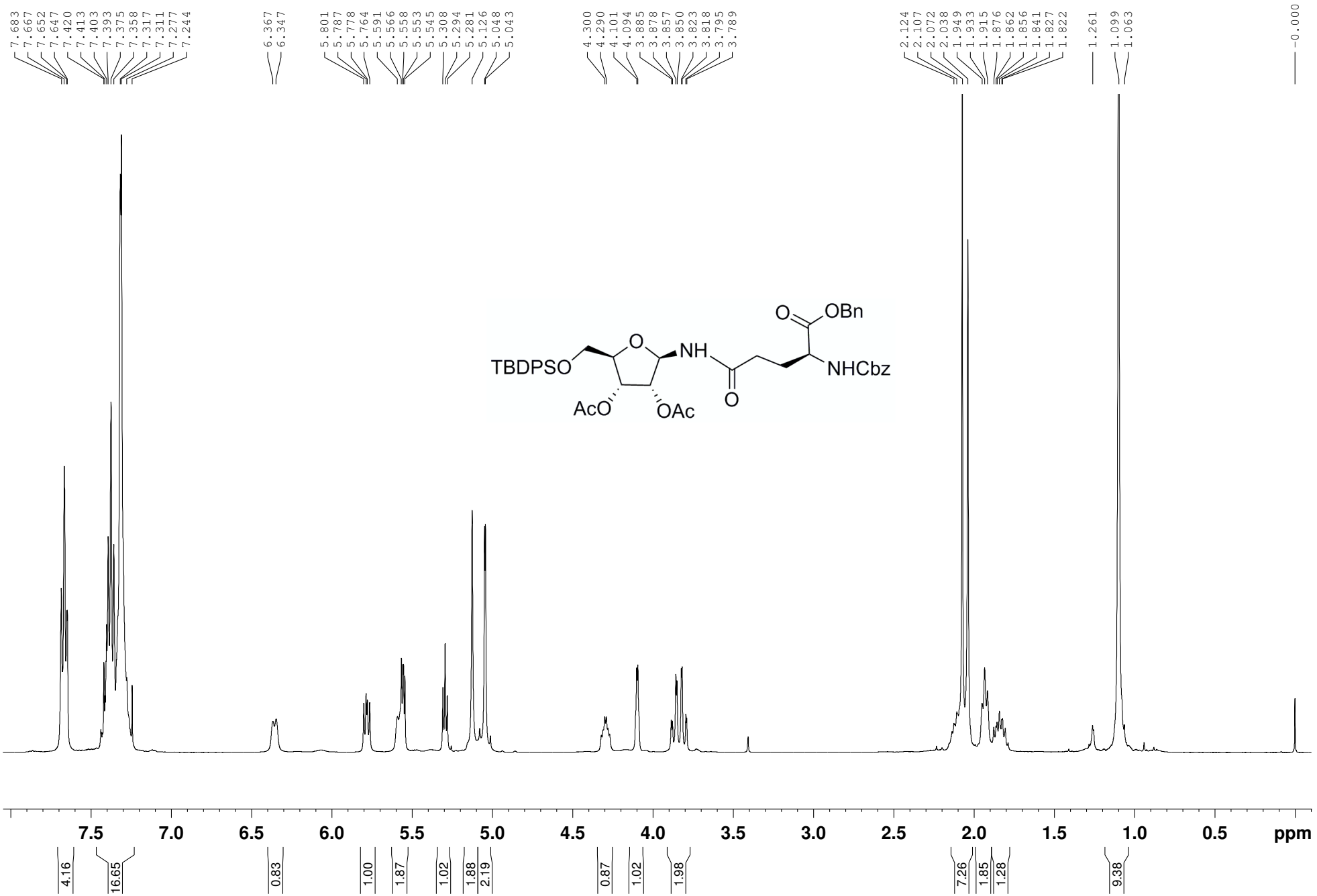
H-NMR, 400 MHz, CDCl3, cmpnd 5a



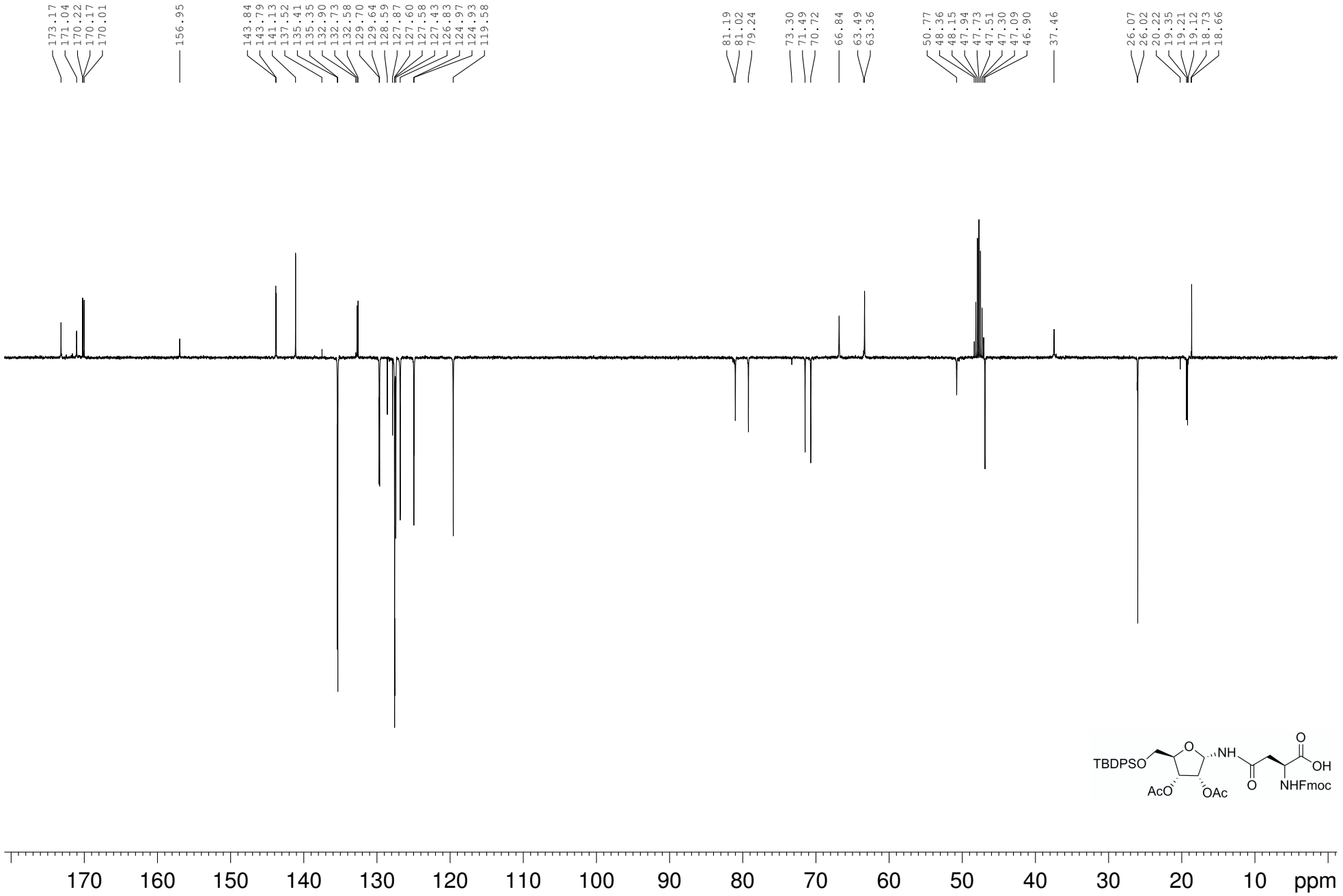
13C-NMR, 100 MHz, CDCl3, cmpnd. 5b



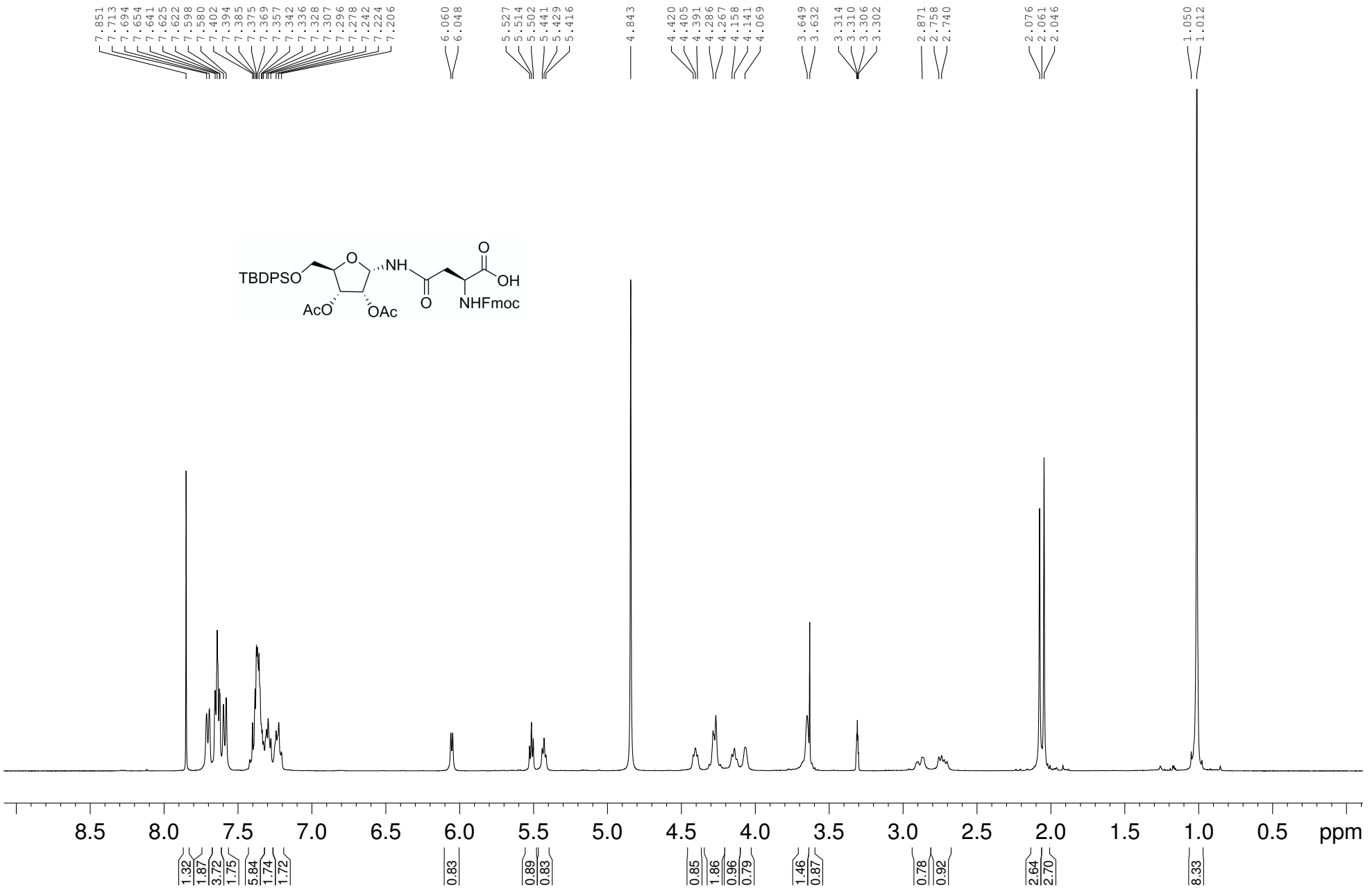
cmpnd. 5b, 400 MHz, 1H-NMR, CDCl3



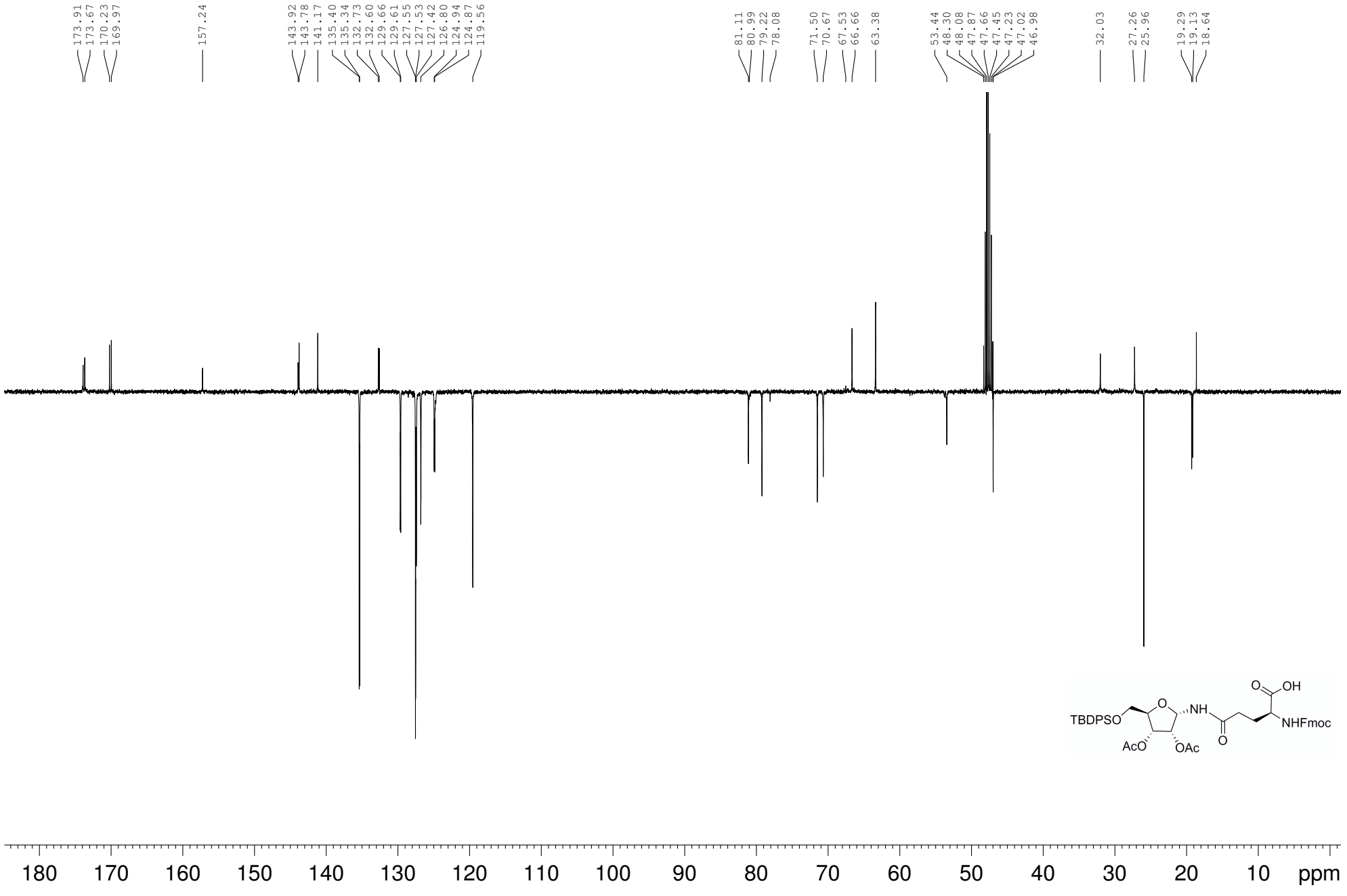
13C-NMR, 100 MHz, MeOD-d4, cmpnd 6



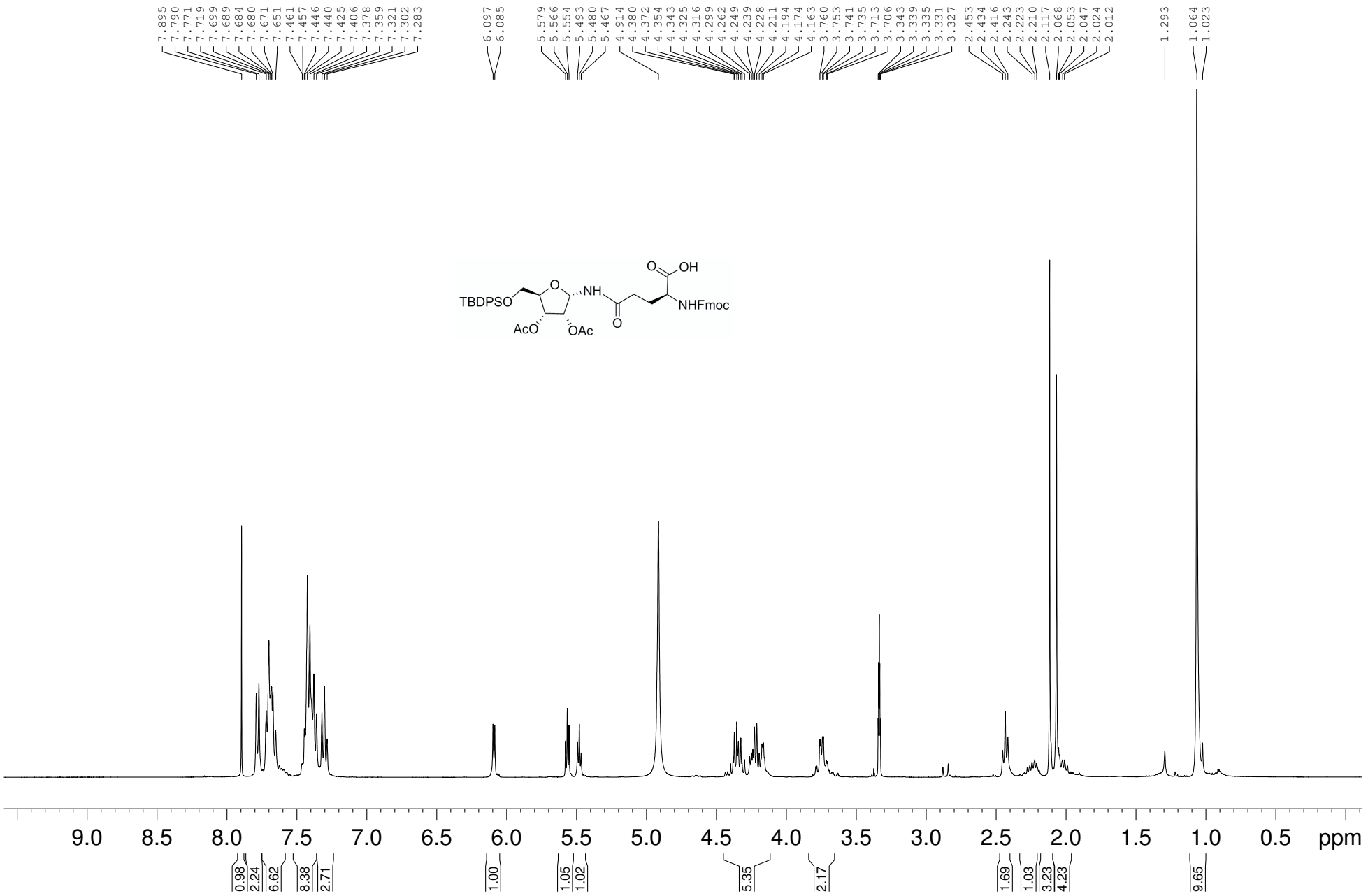
1H NMR, 400 MHz, MeOD-d4, cmpnd 6



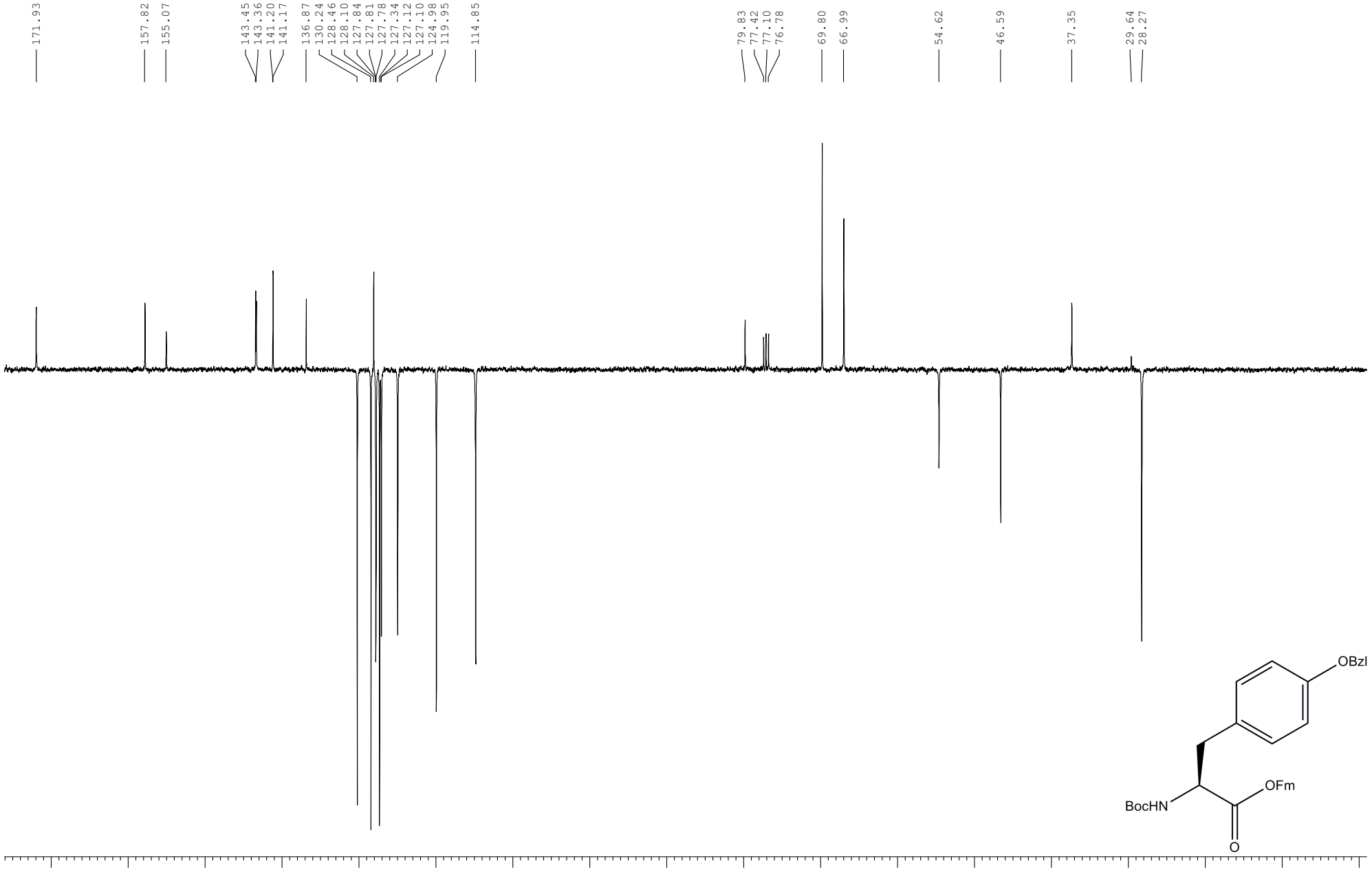
13C-NMR, 100 MHz, MeOD-d4, cmpnd 7



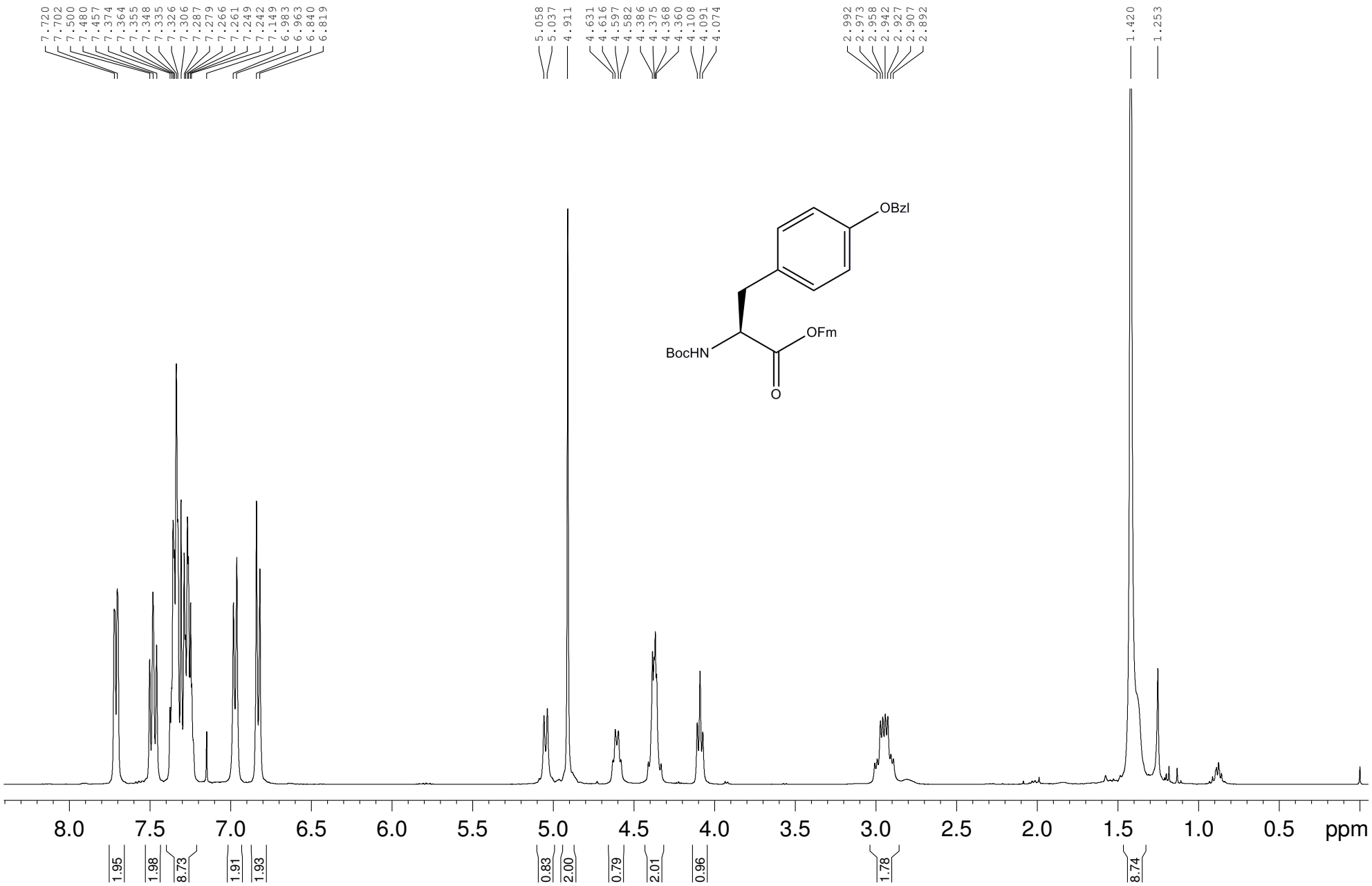
1H-NMR, 400 MHz, MeOD-d4, cmpnd 7



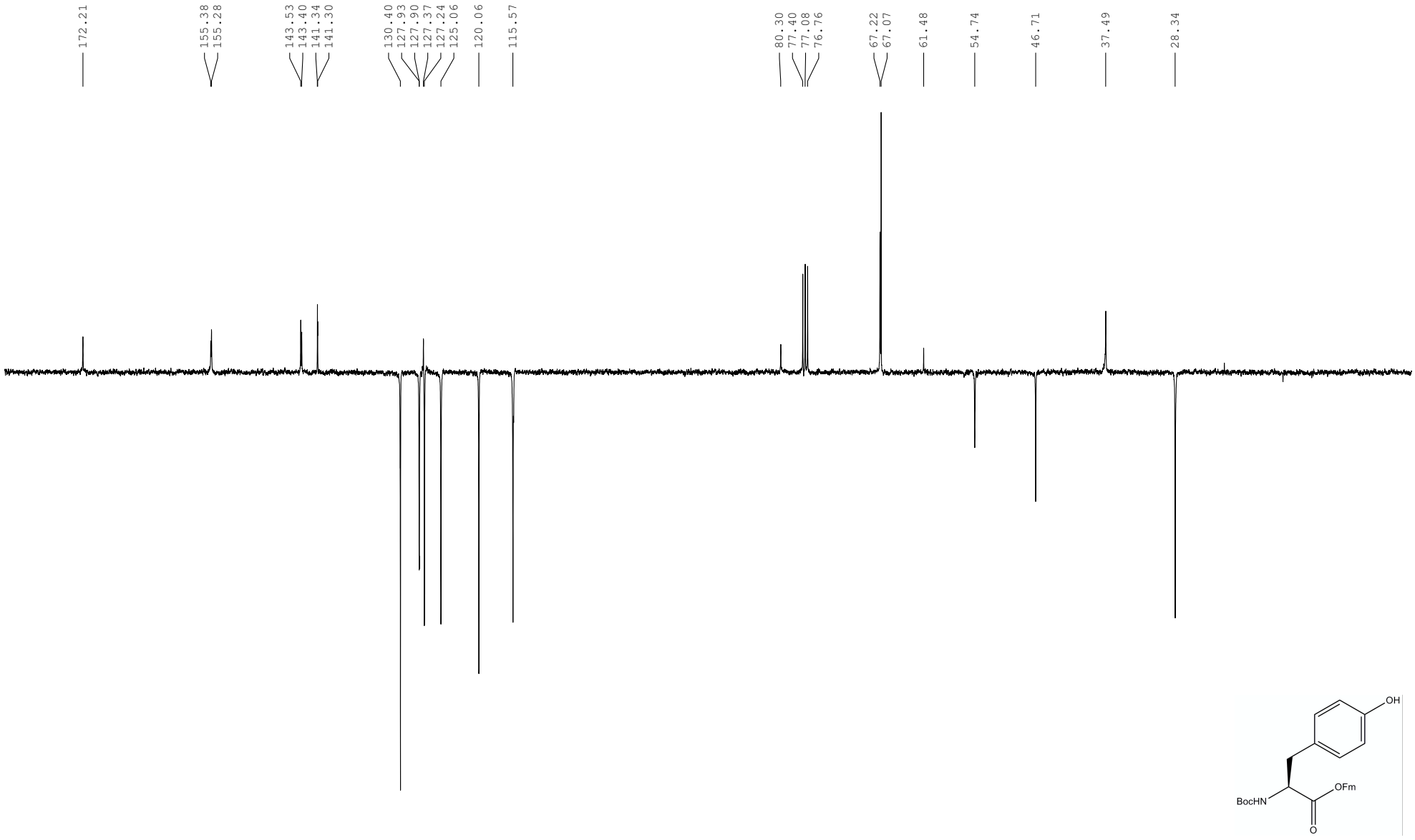
13C-NMR, 100 MHz, CDCl3, cmpnd. 8



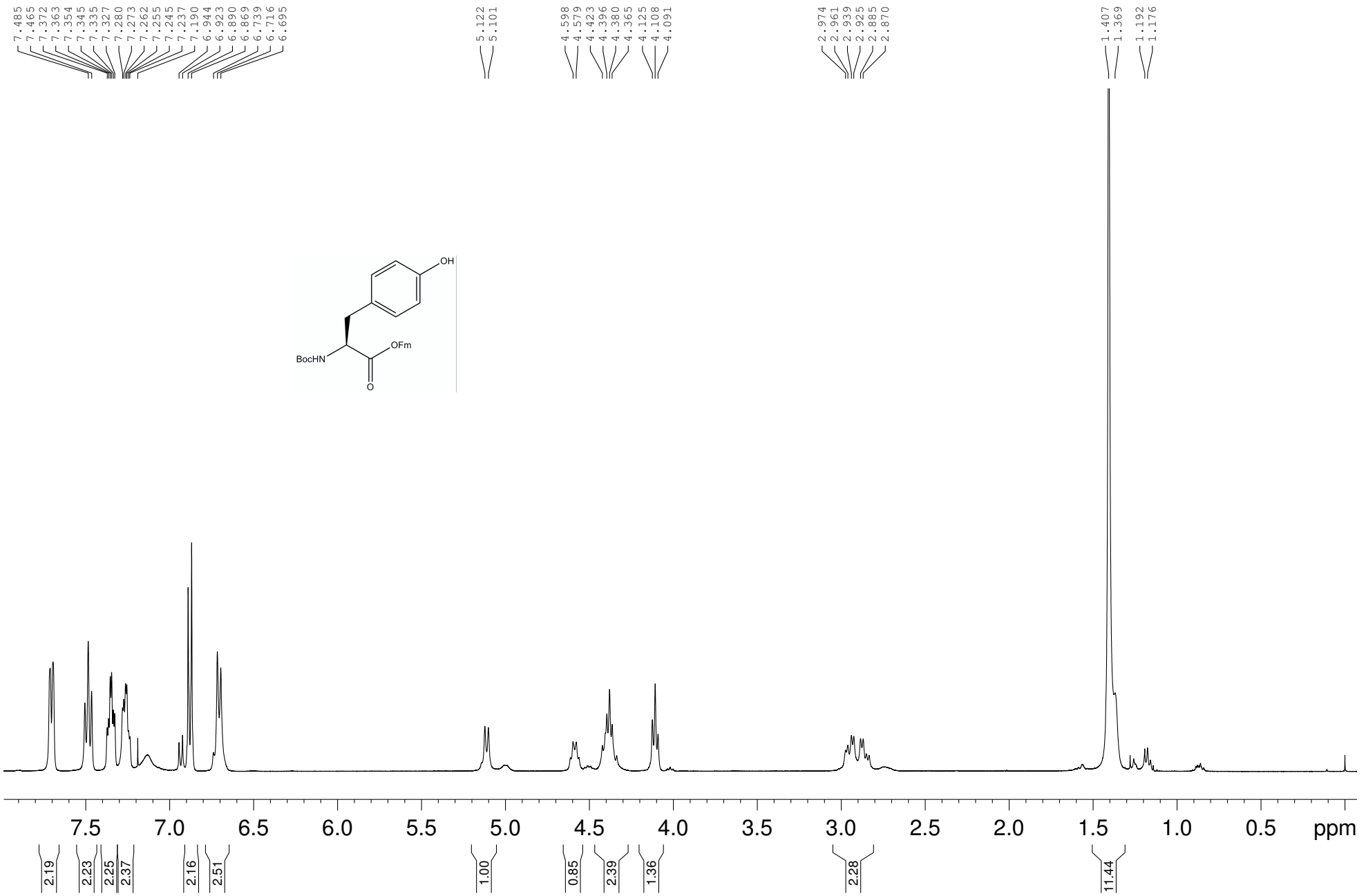
H-NMR, 400 MHz, CDCl3, cmpnd. 8



13C-NMR, 100 MHz, CDCl3, cmpnd 9

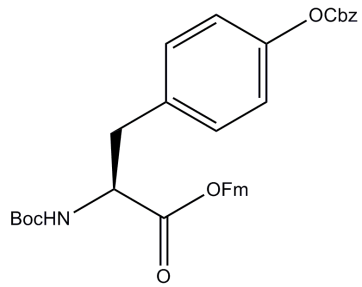


1H-nmr,400 MHz, CDCl3, cmpnd 9



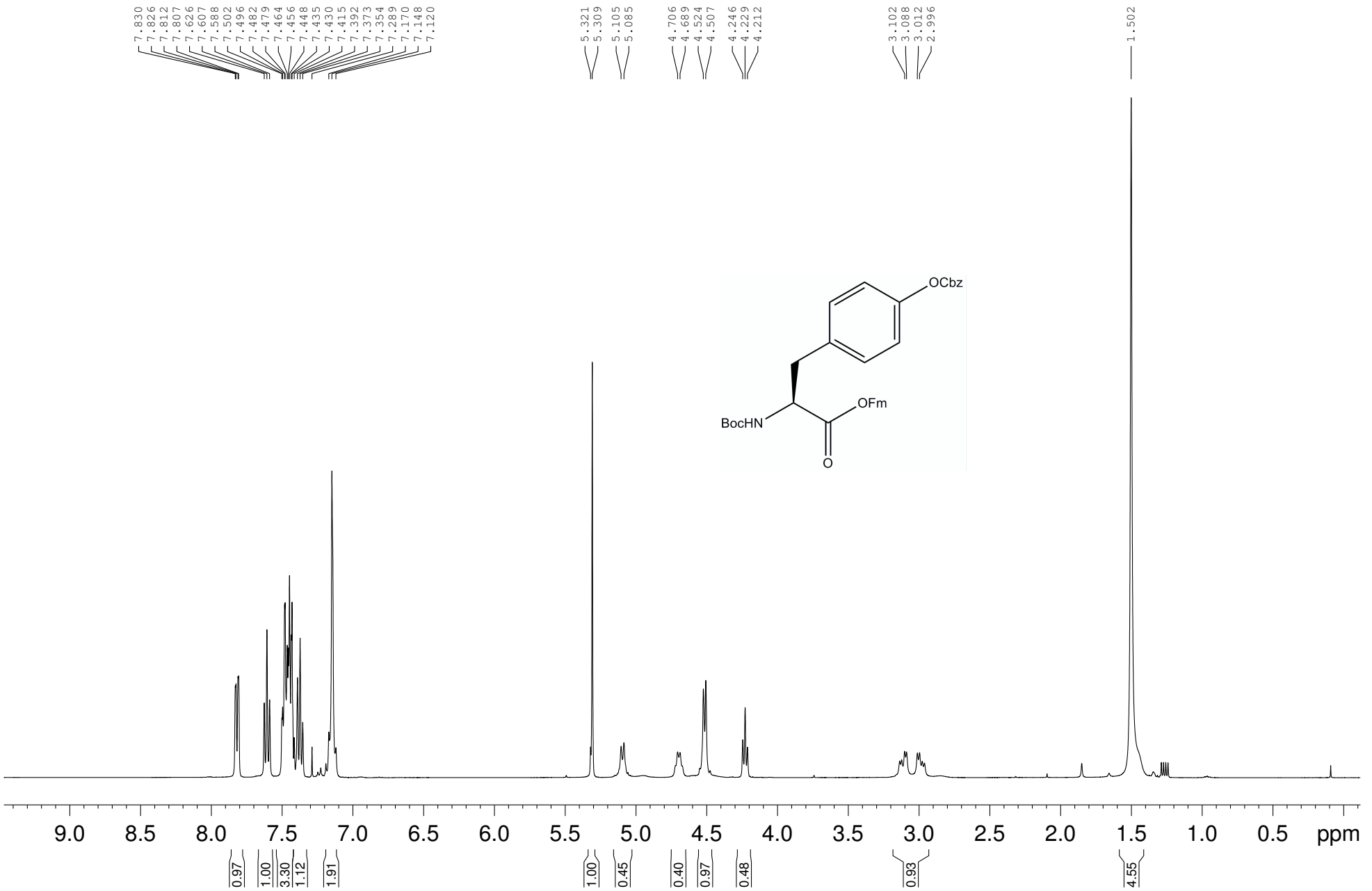
13C-NMR, 100 MHz, CDCl3, cmpnd.10

171.43
154.87
153.25
149.87
143.23
143.12
141.05
140.99
134.51
133.71
130.16
130.04
128.46
128.39
128.24
127.63
126.93
124.71
120.77
119.79
79.68
77.32
77.00
76.68
69.99
68.89
66.75
54.14
46.40
37.10
28.04

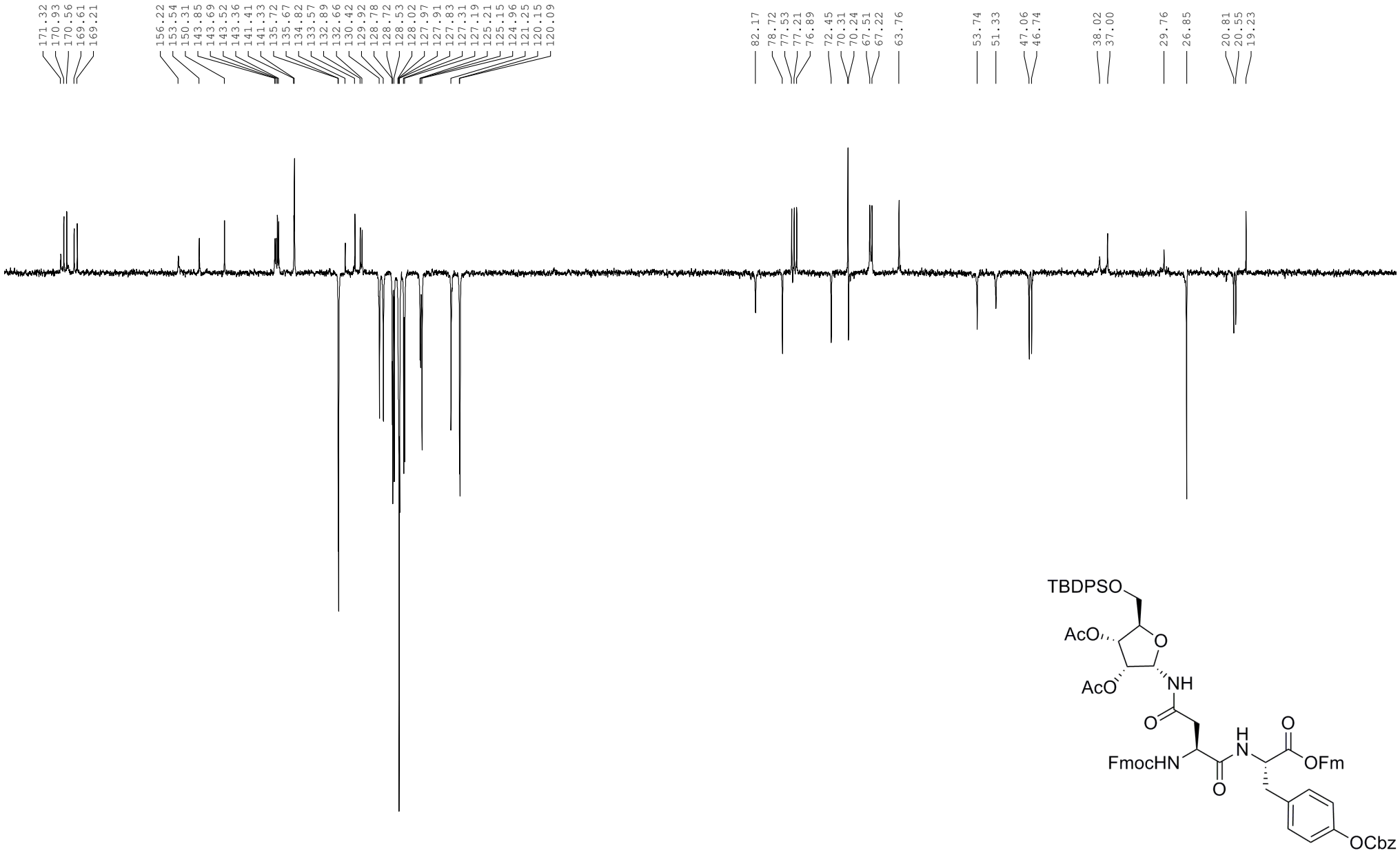


170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

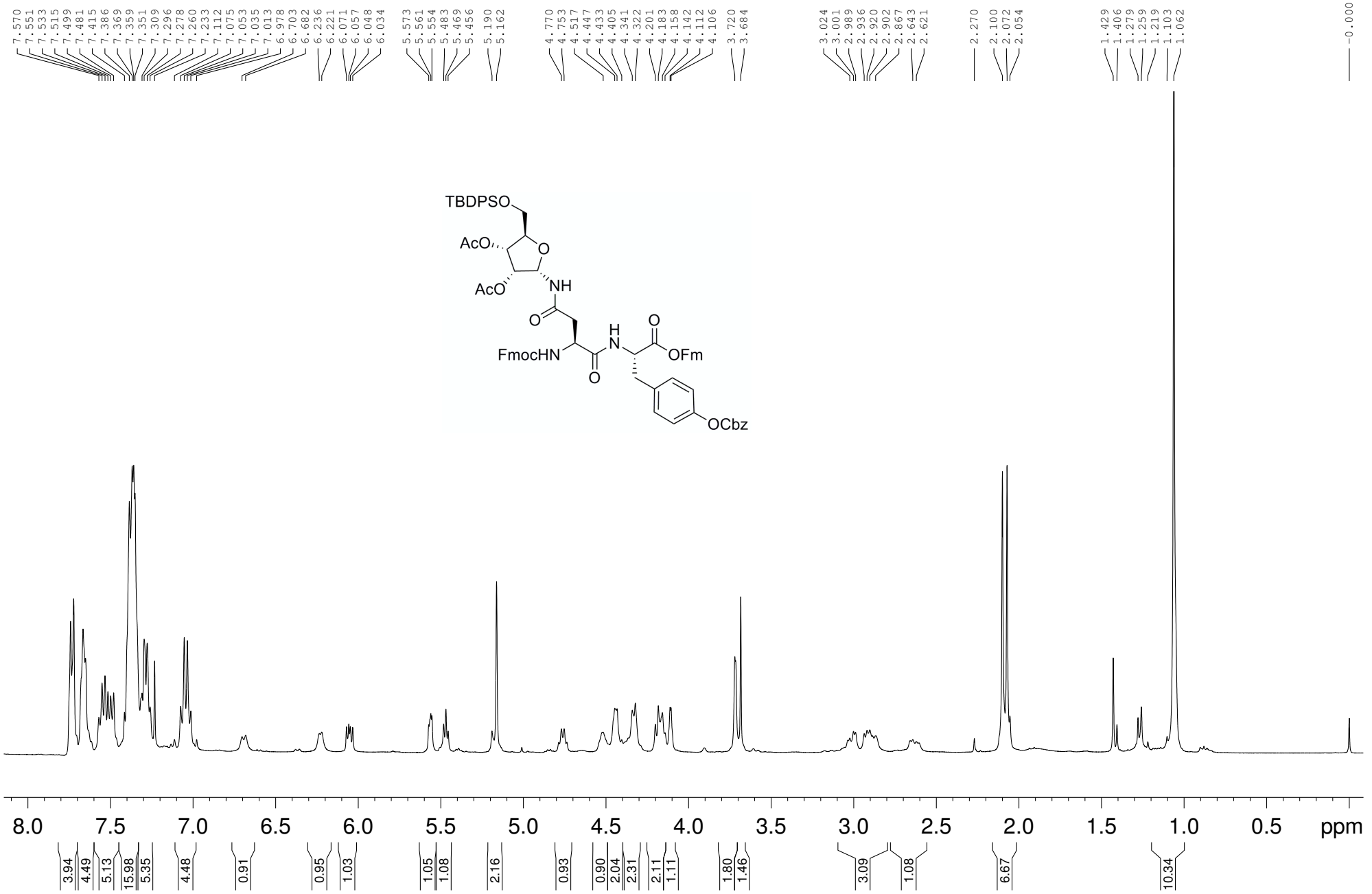
1H-NMR, 400 MHz, CDCl3, cmpnd 10



13C-NMR, 100 MHz, CDCl3, cmpnd. 11

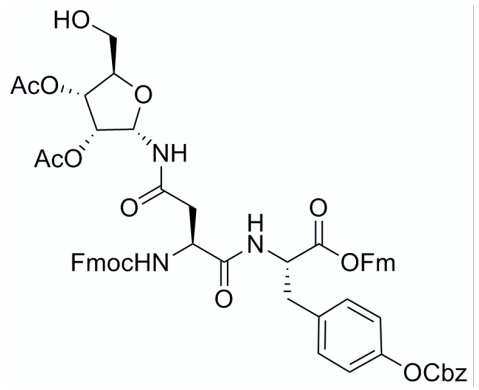


1H-NMR, 400 MHz, MeOD-d4/CDCl3, cmpnd 11



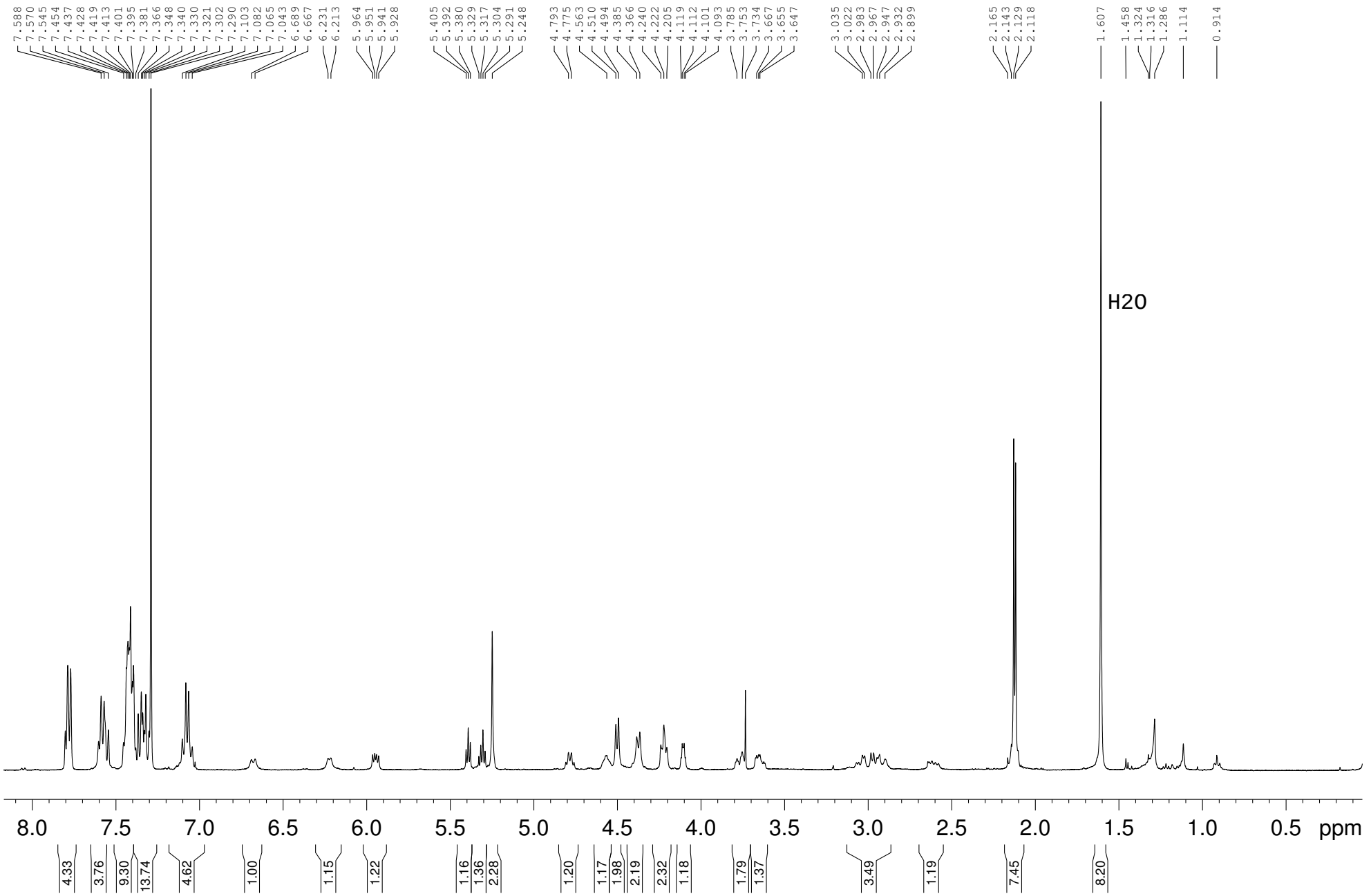
13C-NMR, 100 MHz, DMSO-d6, cpmnd 12

172.04
171.62
171.08
170.03
169.93
169.84
156.21
153.40
149.93
144.26
144.06
143.96
141.25
141.21
141.15
135.48
135.45
130.81
130.66
129.40
129.03
128.81
128.21
128.09
127.76
127.66
127.56
127.24
125.78
125.64
121.85
121.42
120.55
120.32
115.57
110.21
80.48
79.10
71.47
70.66
70.21
68.59
66.65
66.28
64.26
61.36
60.23
54.19
51.63
50.56
47.04
46.69
40.61
40.40
40.19
39.98
39.77
39.56
39.36
38.02
35.98
29.47
28.47
21.91
21.77
21.22
20.85
14.55

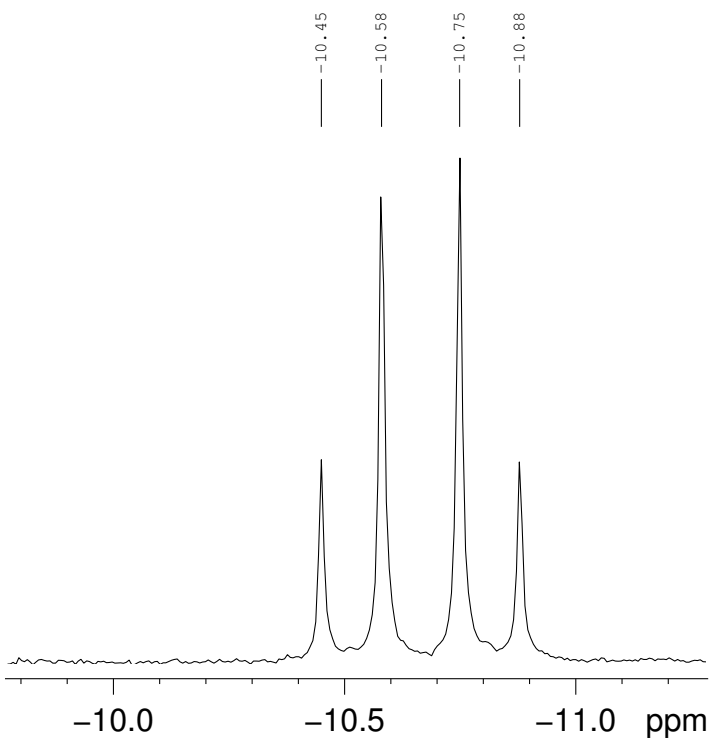


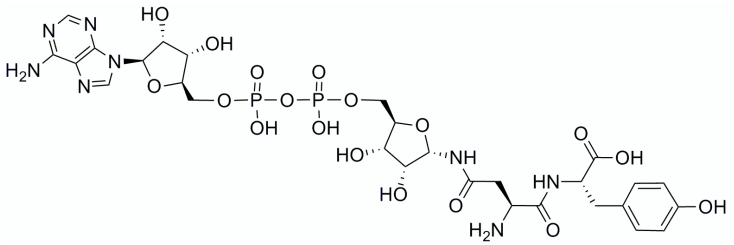
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

1H-NMR, 400 MHz, CDCl3, cmpnd 12

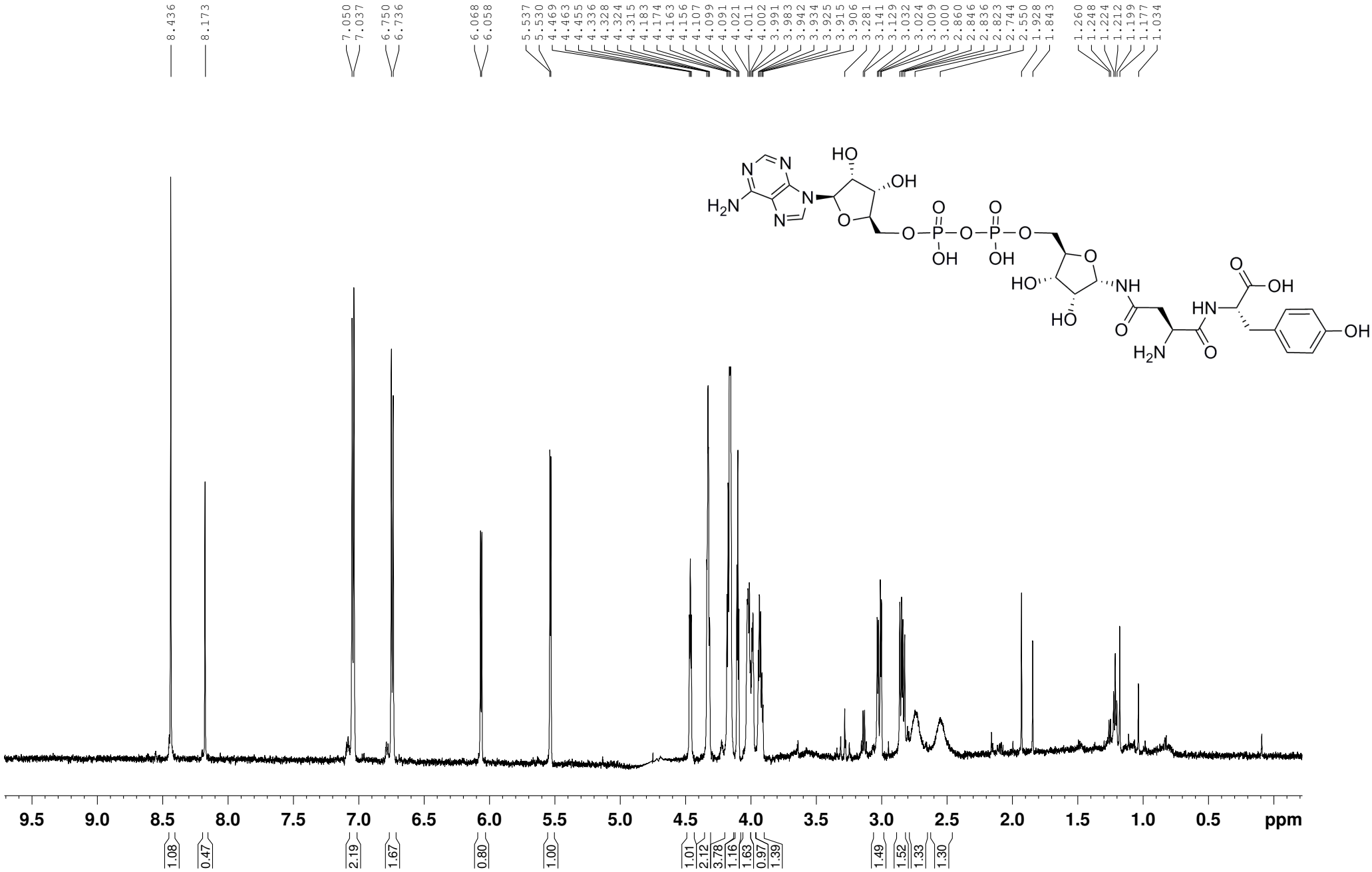


31P-NMR, D2O, 162 MHz, cpmnd 18

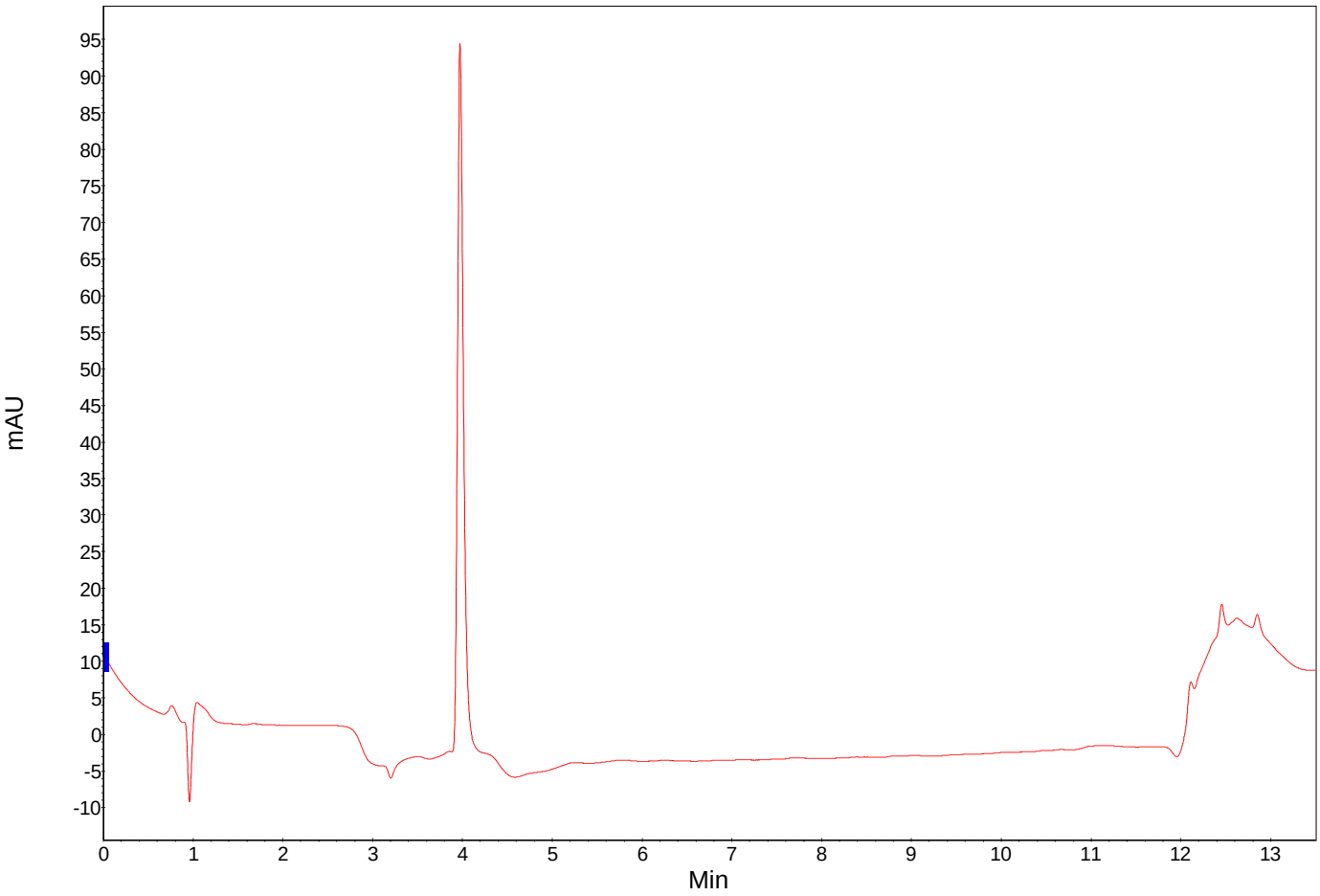




H-NMR, D2O, 600 MHz, cmpnd. 18



cmpnd 18, LC-MS (0 -50% B in 13.5 min), UV detection at 214 nm



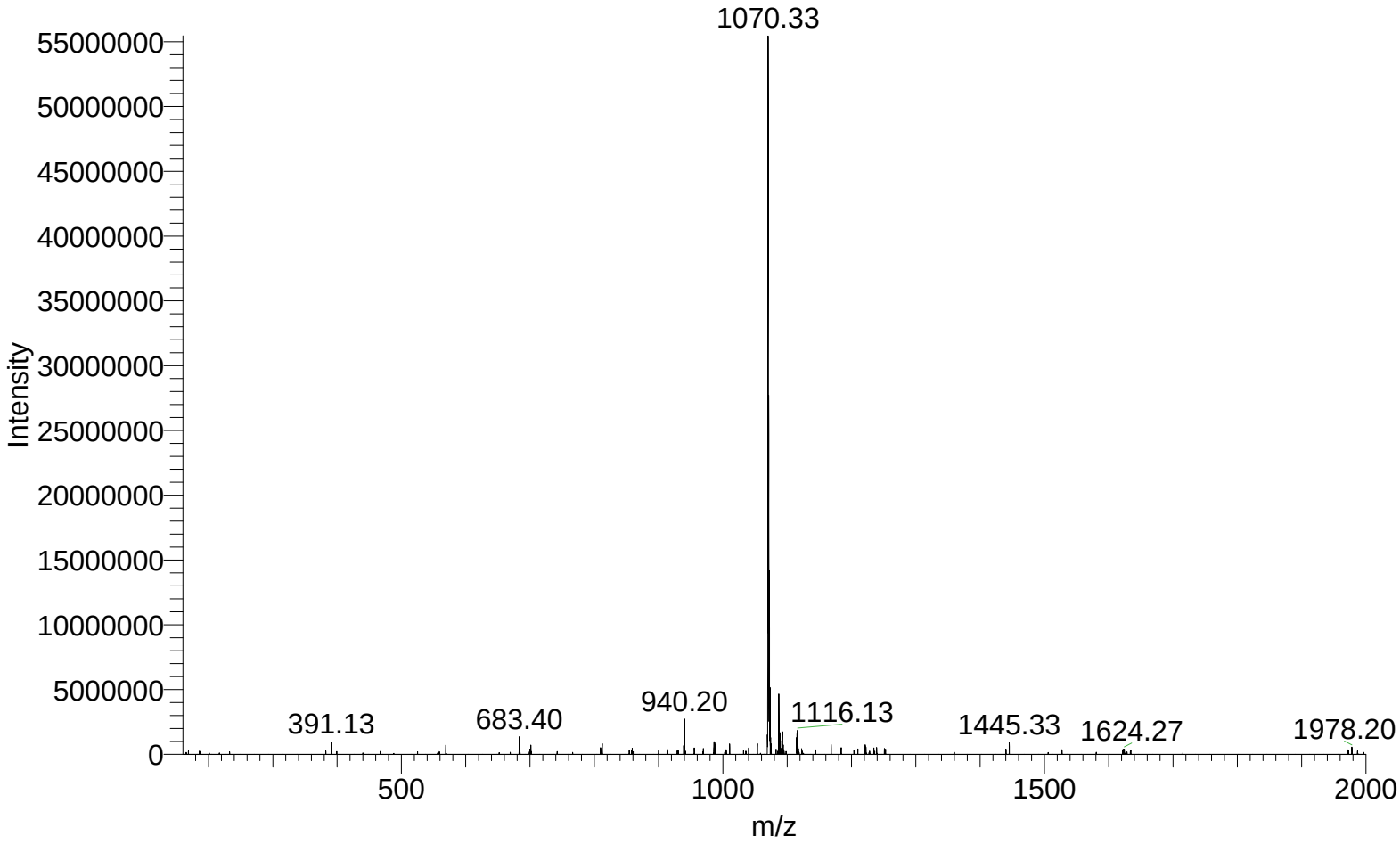
RT: 0.00 - 14.60

LC-MS trace of crude cmpnd **19**

NL:
3.02E5
Total Scan
PDA

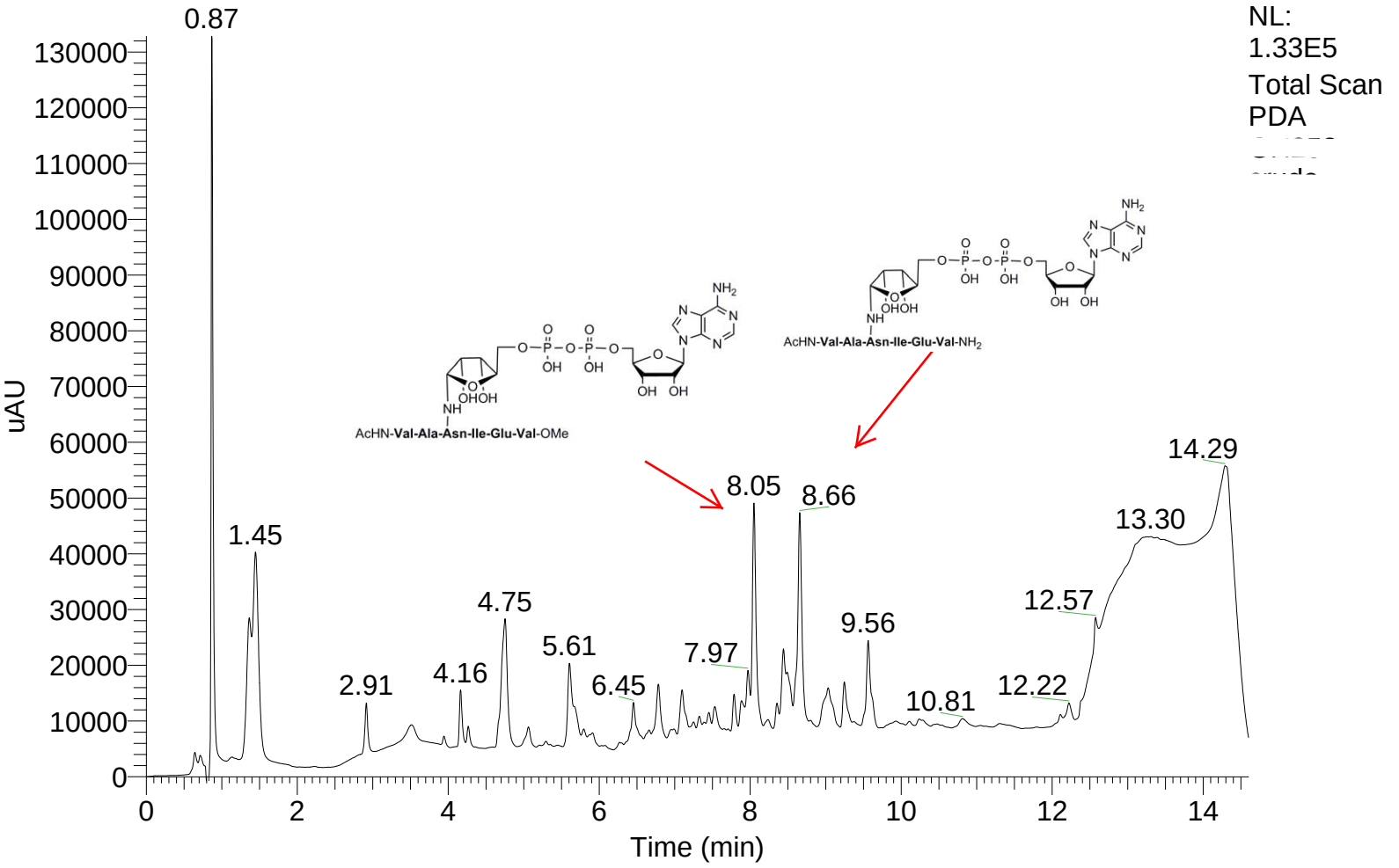
RT: 9.44 AV: 1 NL: 5.55E7

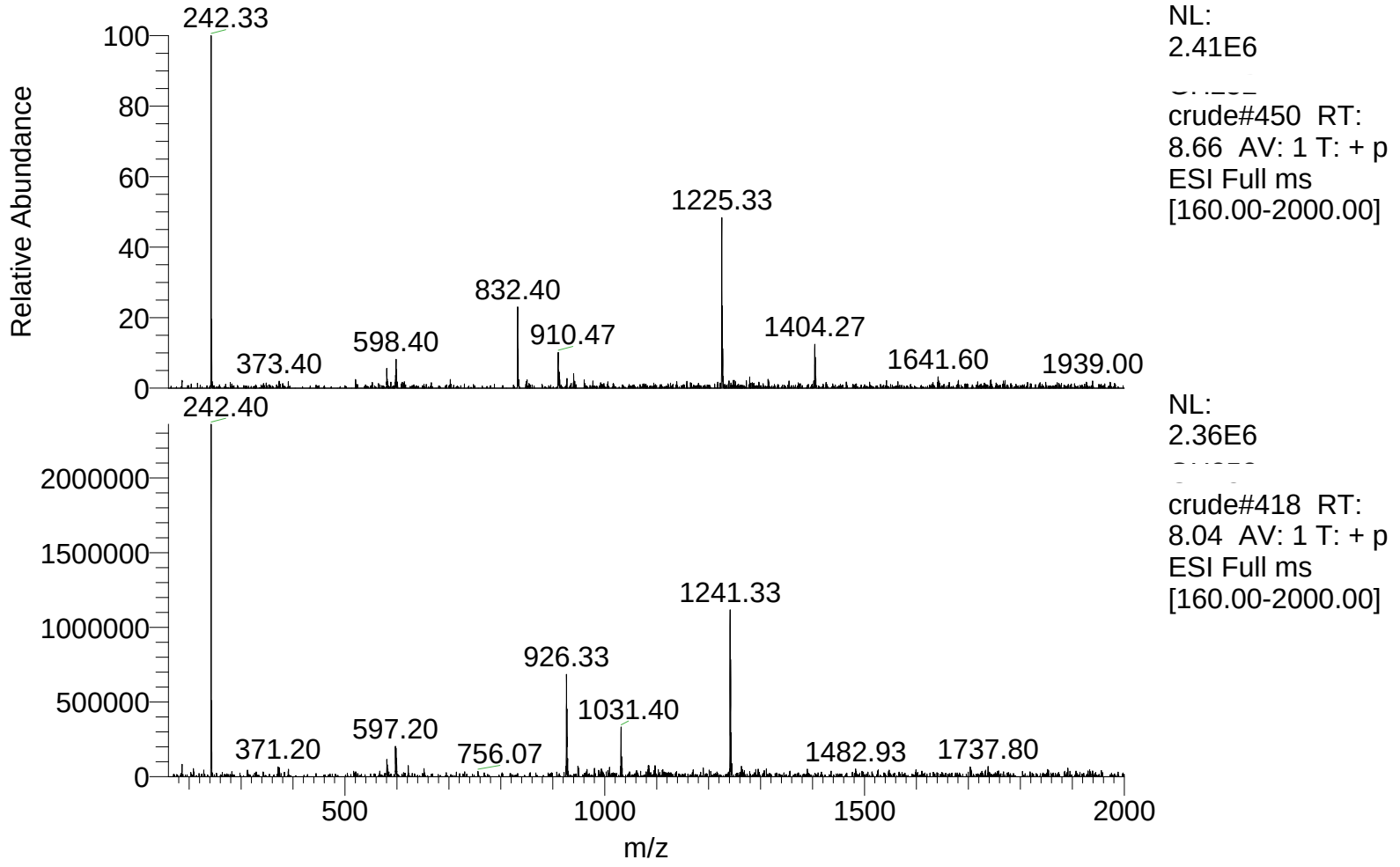
T: + p ESI Full ms [160.00-2000.00]



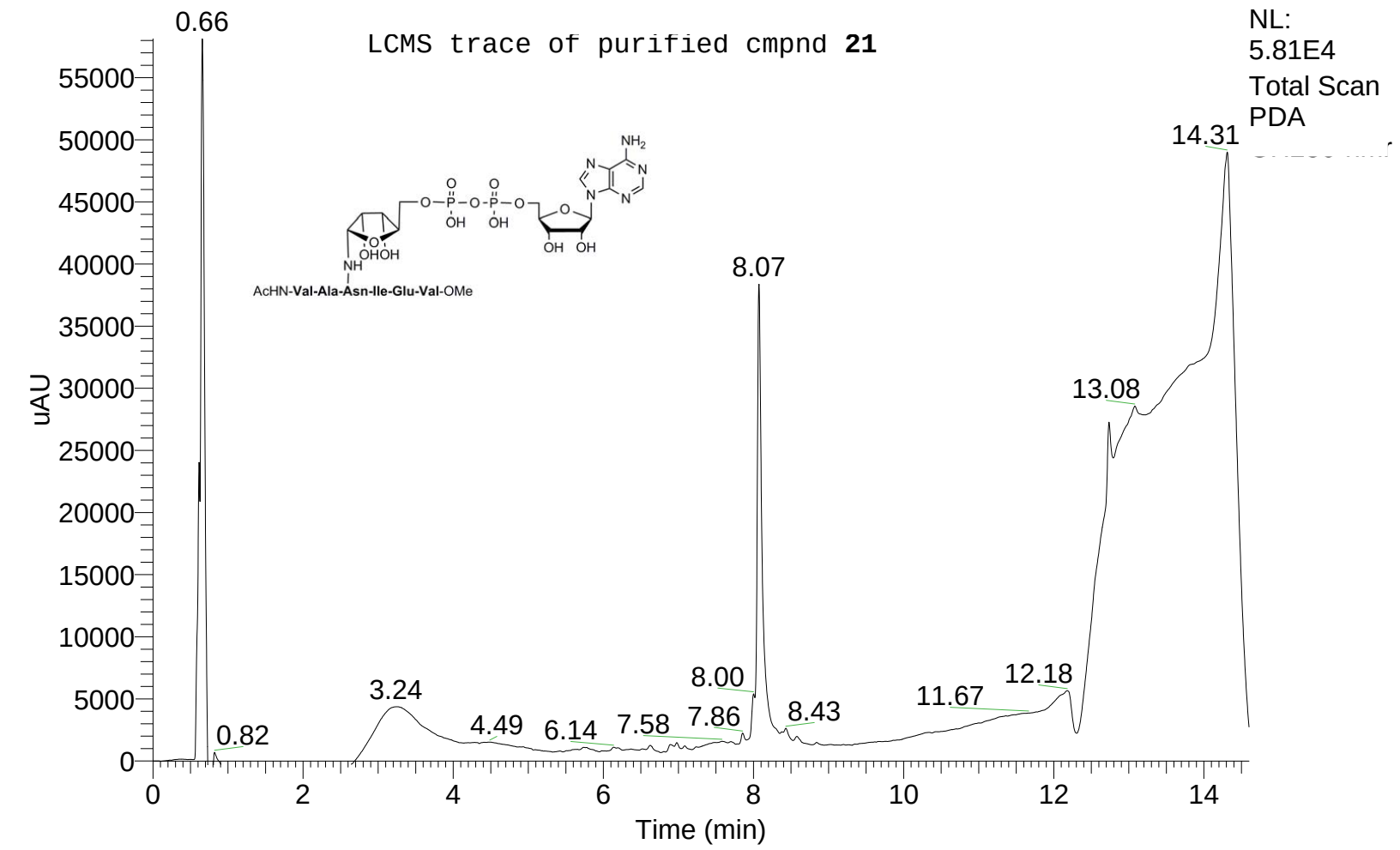
LC-MS trace of crude cmpnd. **21**

RT: 0.00 - 14.60



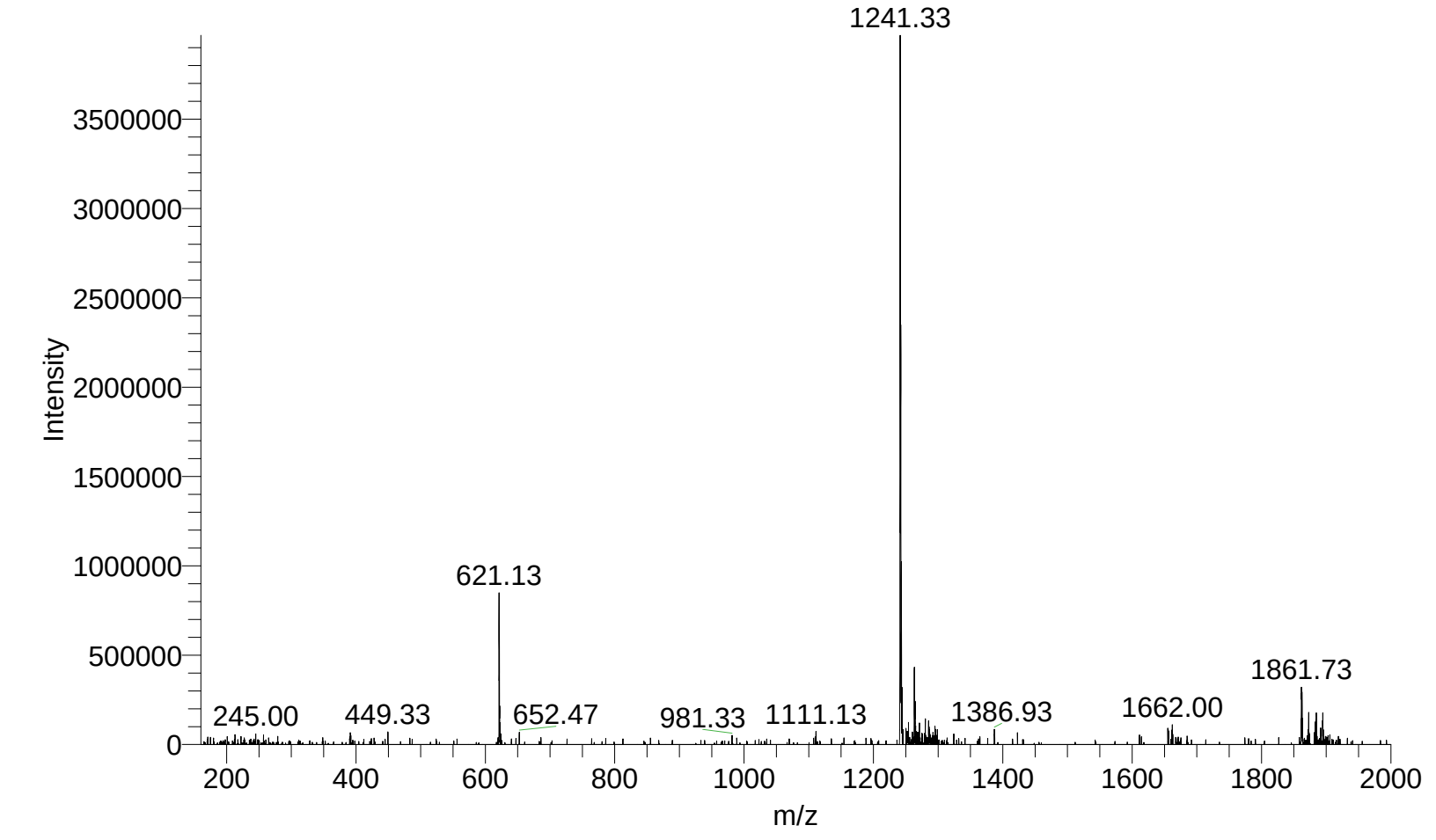


RT: 0.00 - 14.60

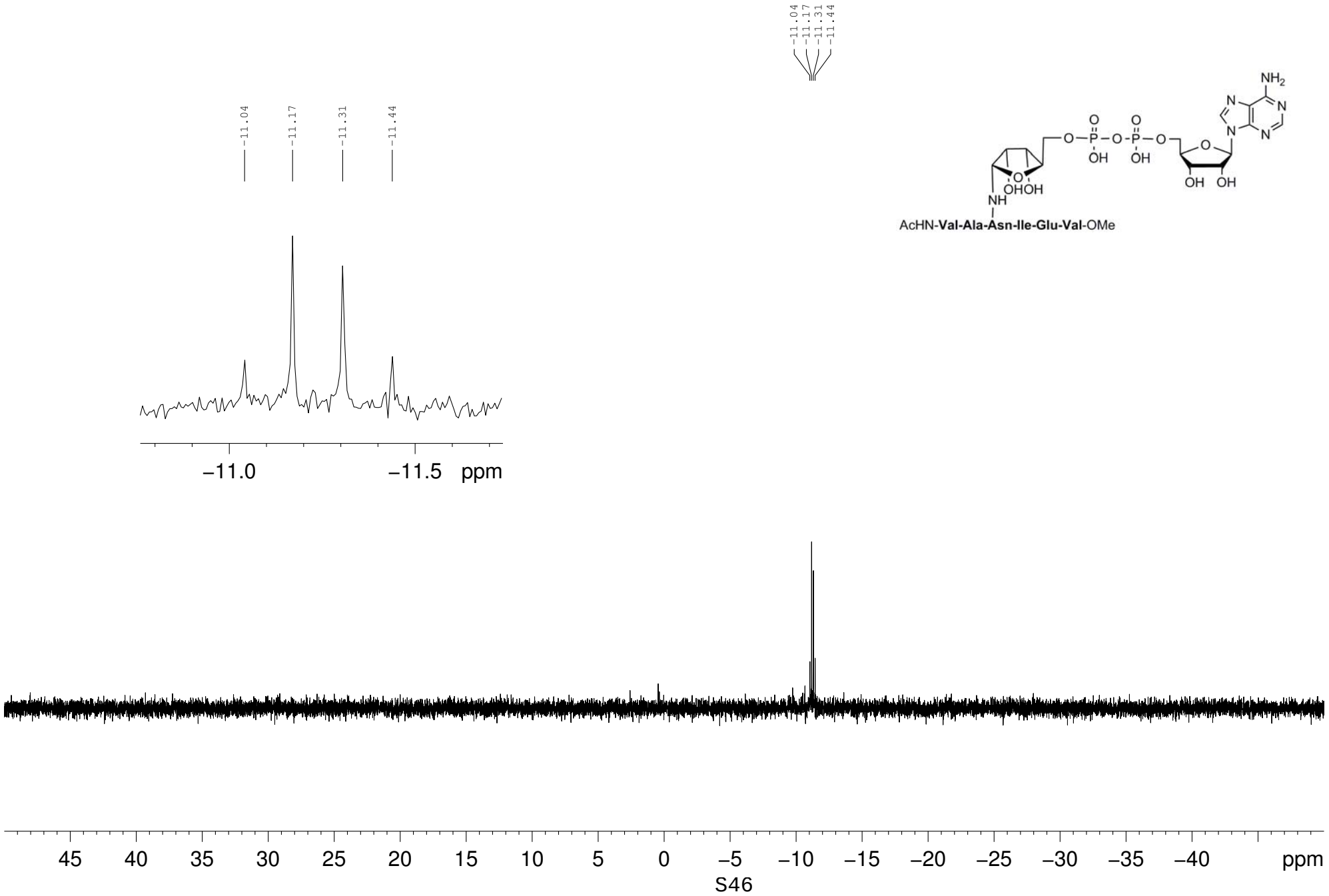


RT: 8.09 AV: 1 NL: 3.97E6

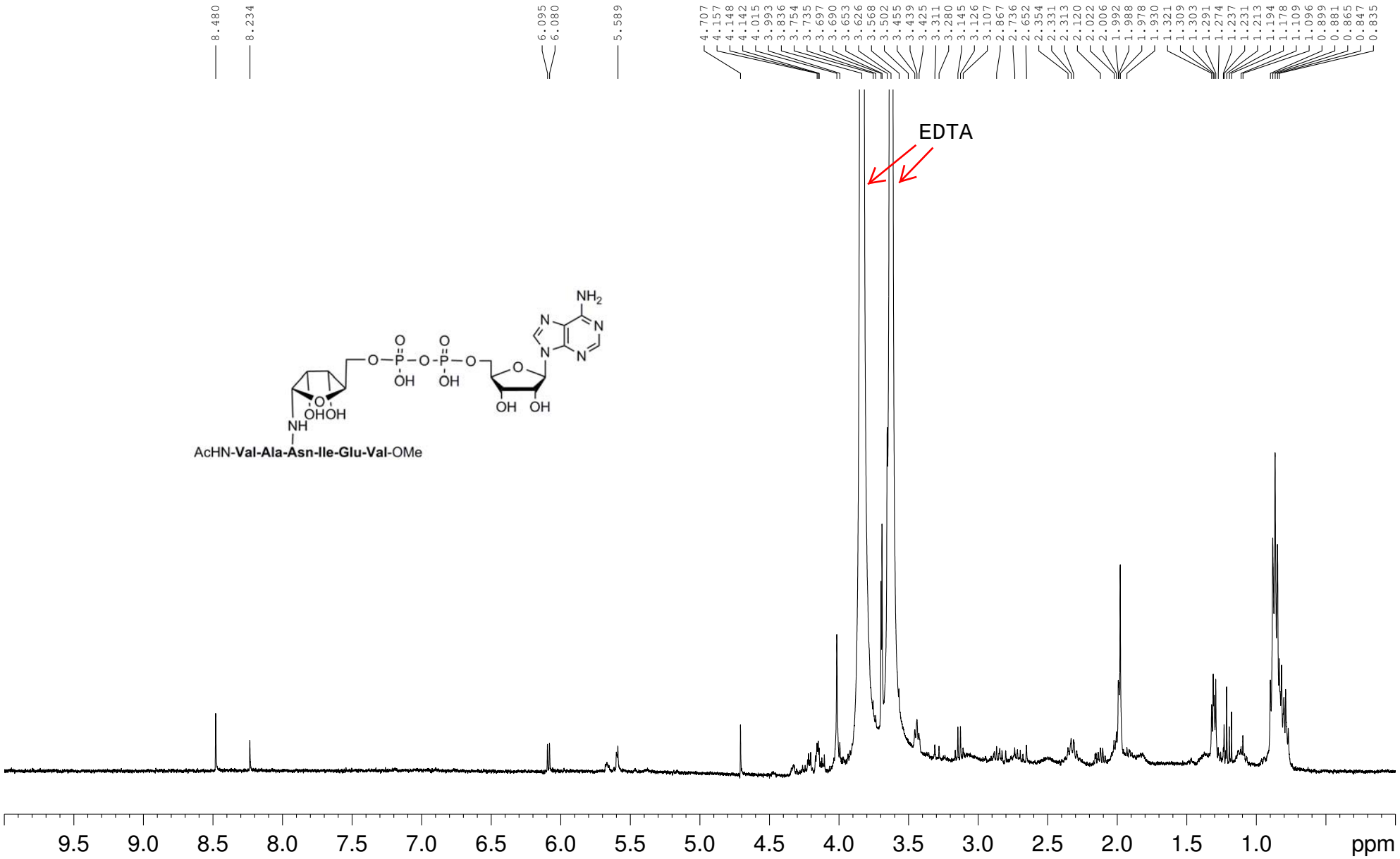
T: + p ESI Full ms [160.00-2000.00]



31p-nmr. 162 MHz, D2O, cmpnd 21

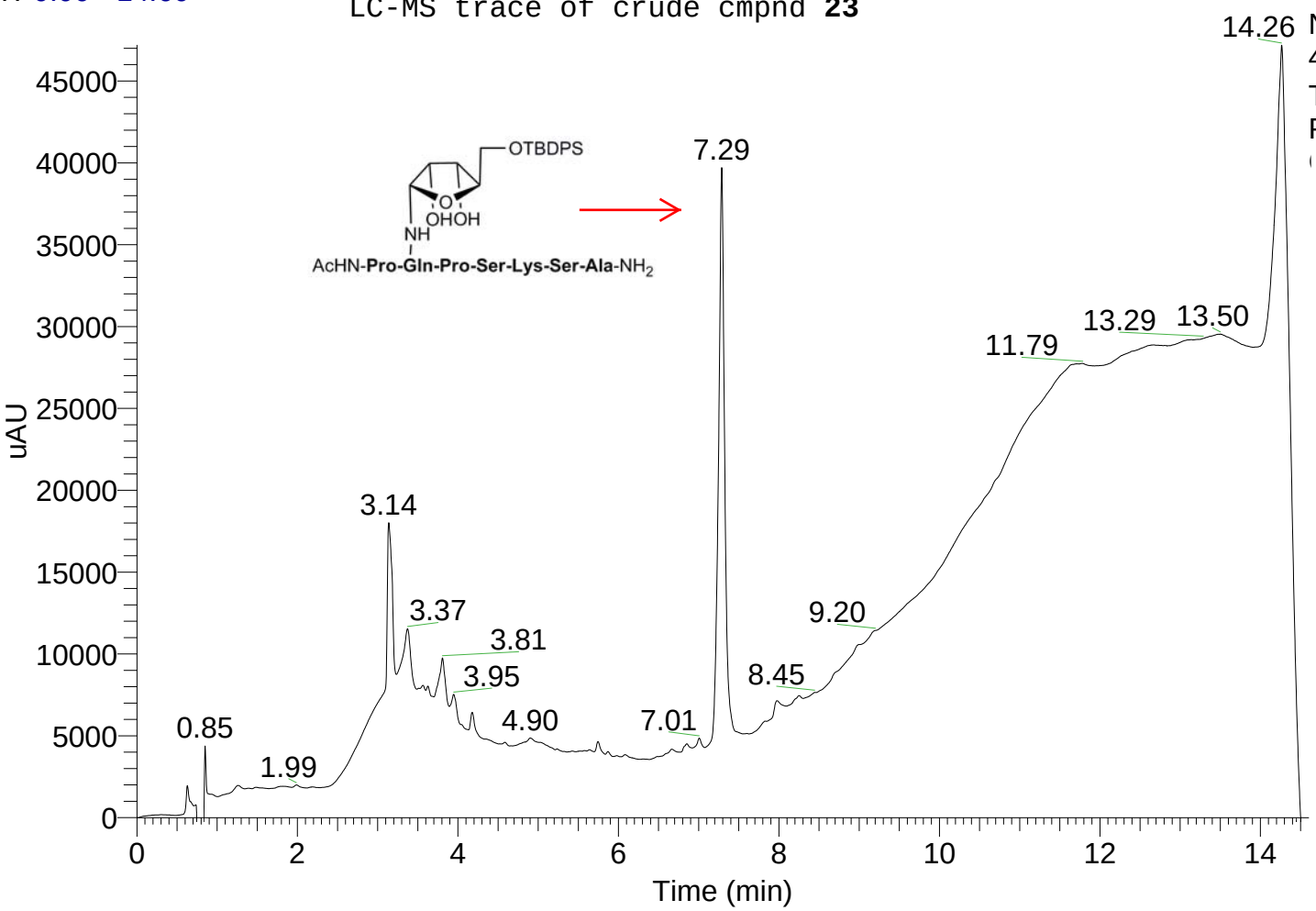


1H-NMR, 400 MHz, D2O, cmpnd 21H



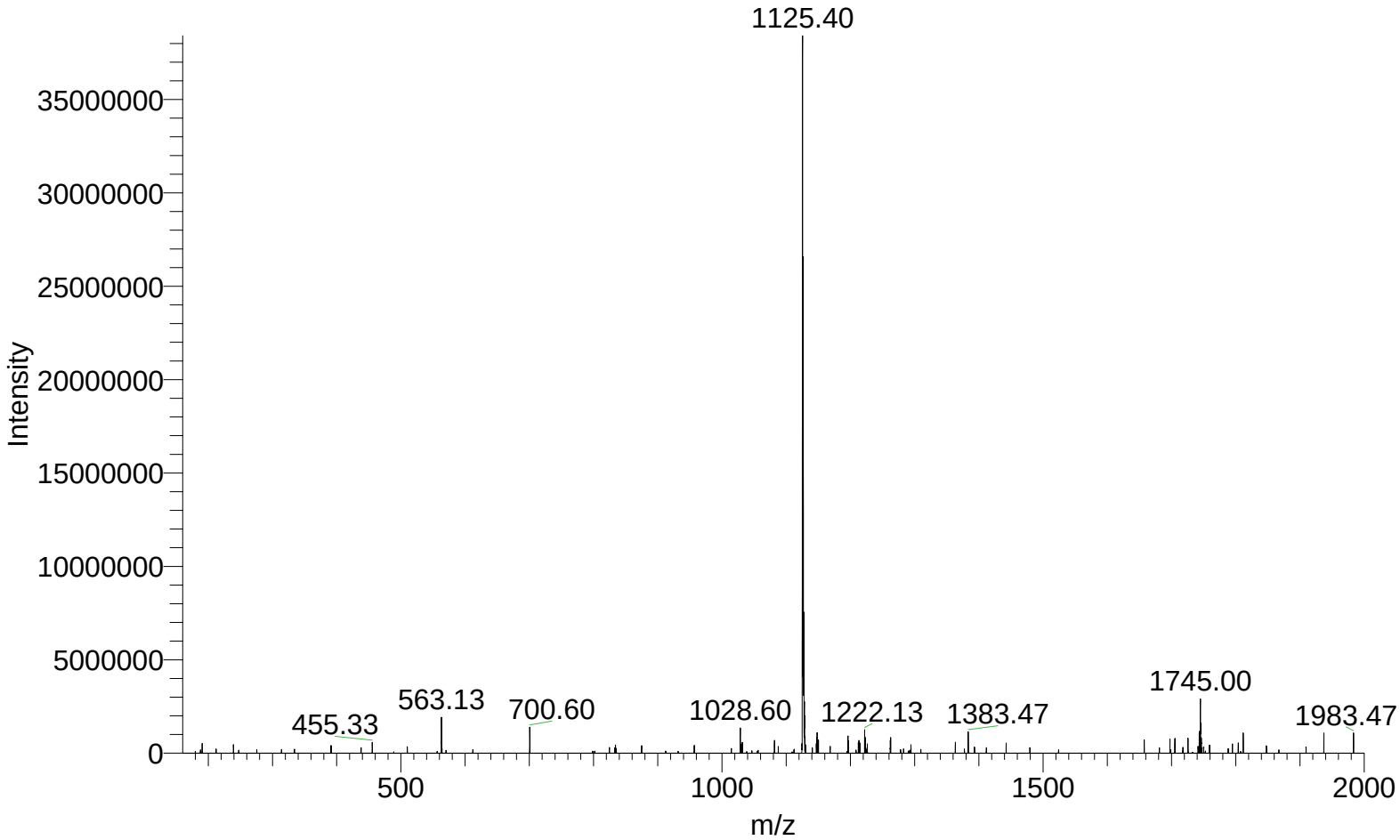
RT: 0.00 - 14.60

LC-MS trace of crude cmpnd **23**

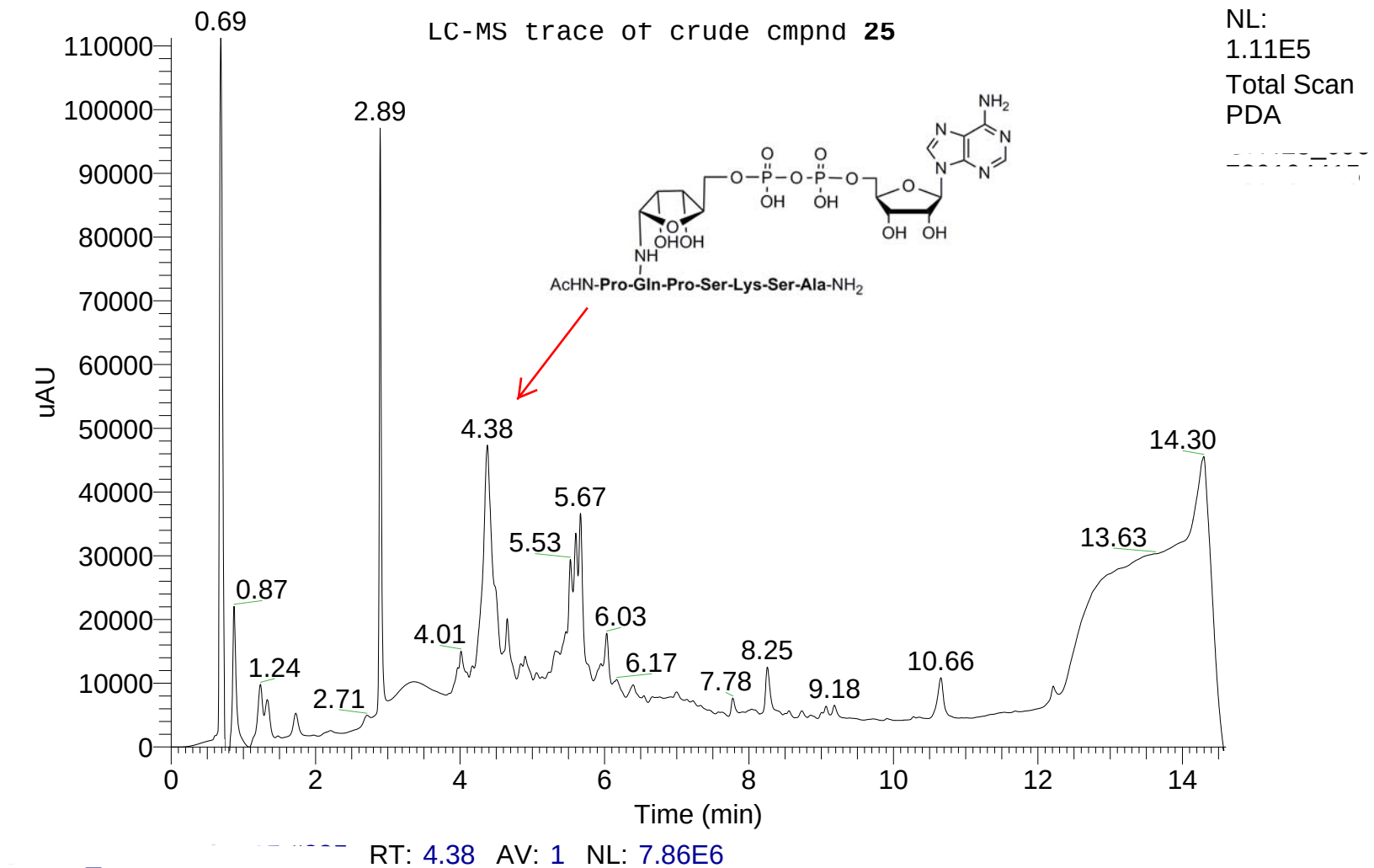


RT: 7.27 AV: 1 NL: 3.84E7

T: + p ESI Full ms [160.00-2000.00]

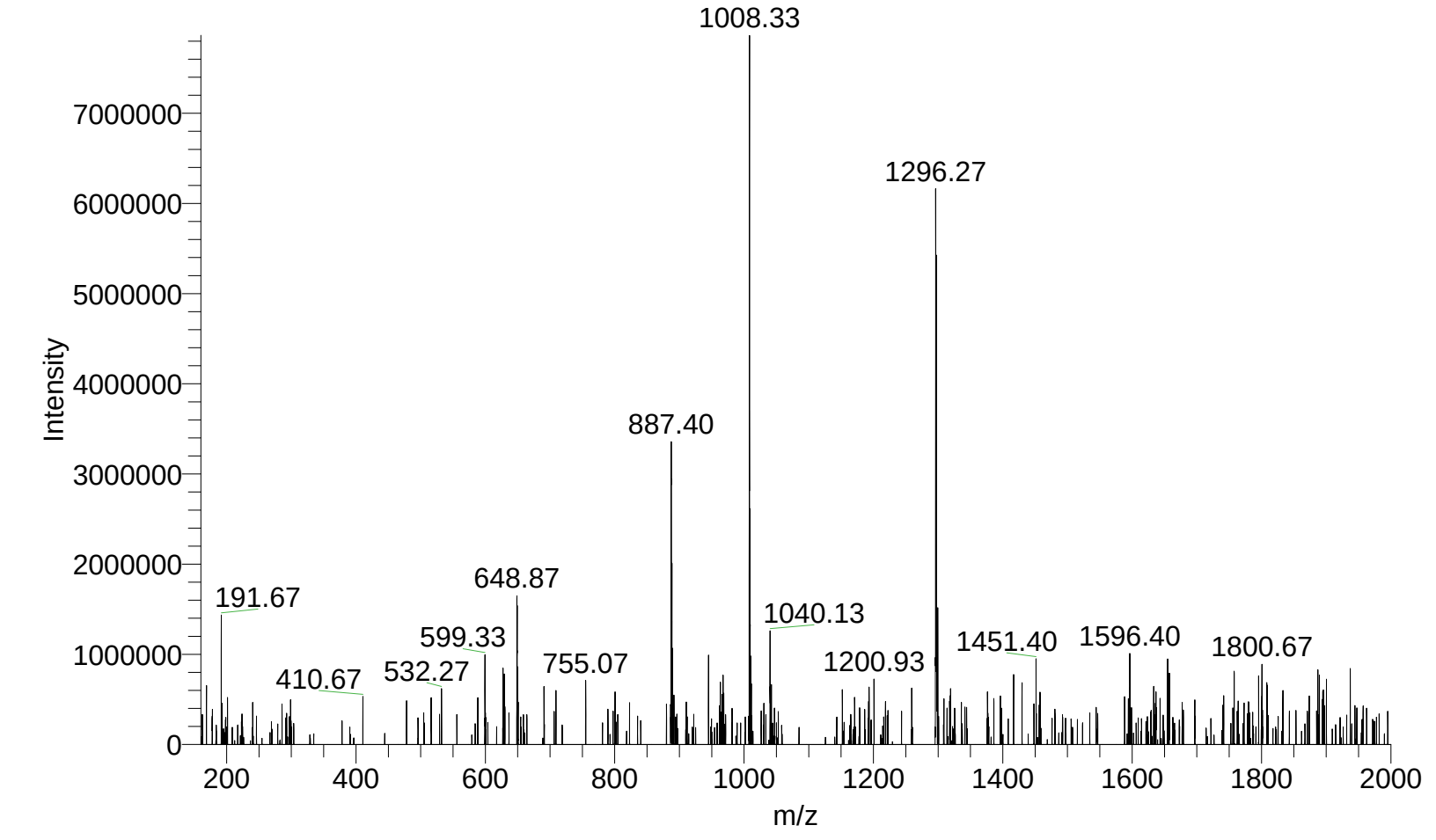


RT: 0.00 - 14.60

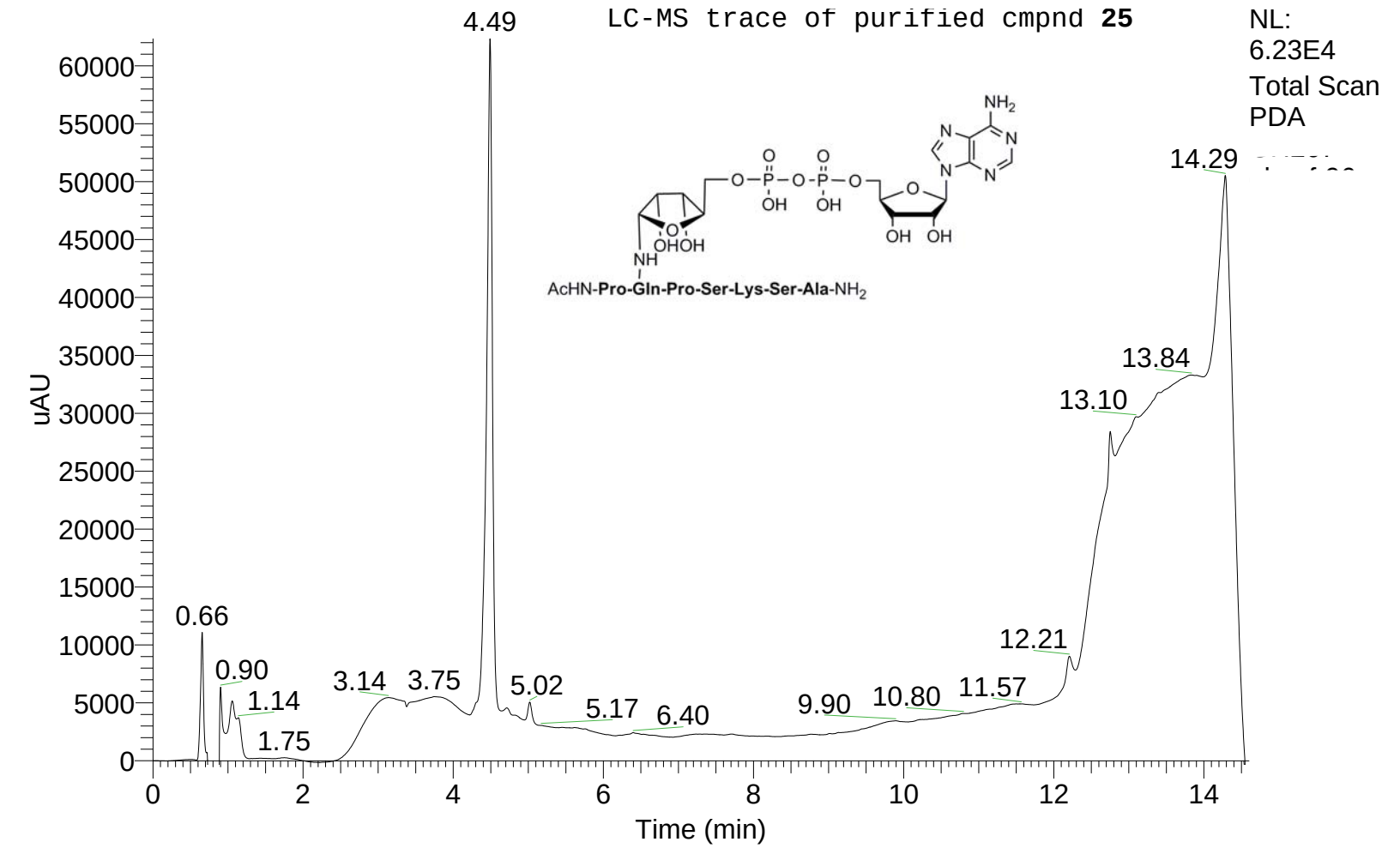


RT: 4.38 AV: 1 NL: 7.86E6

T: + p ESI Full ms [160.00-2000.00]

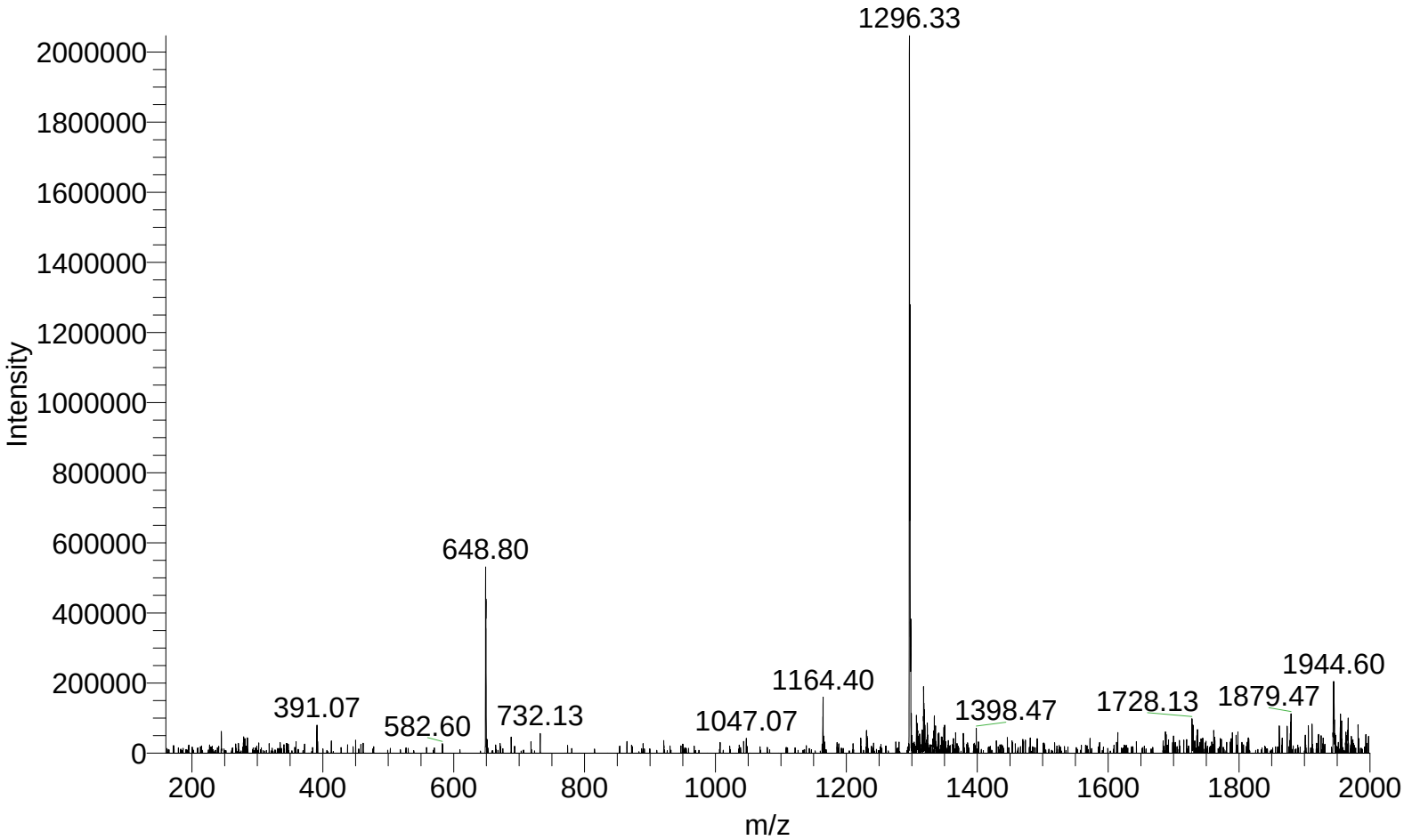


RT: 0.00 - 14.60

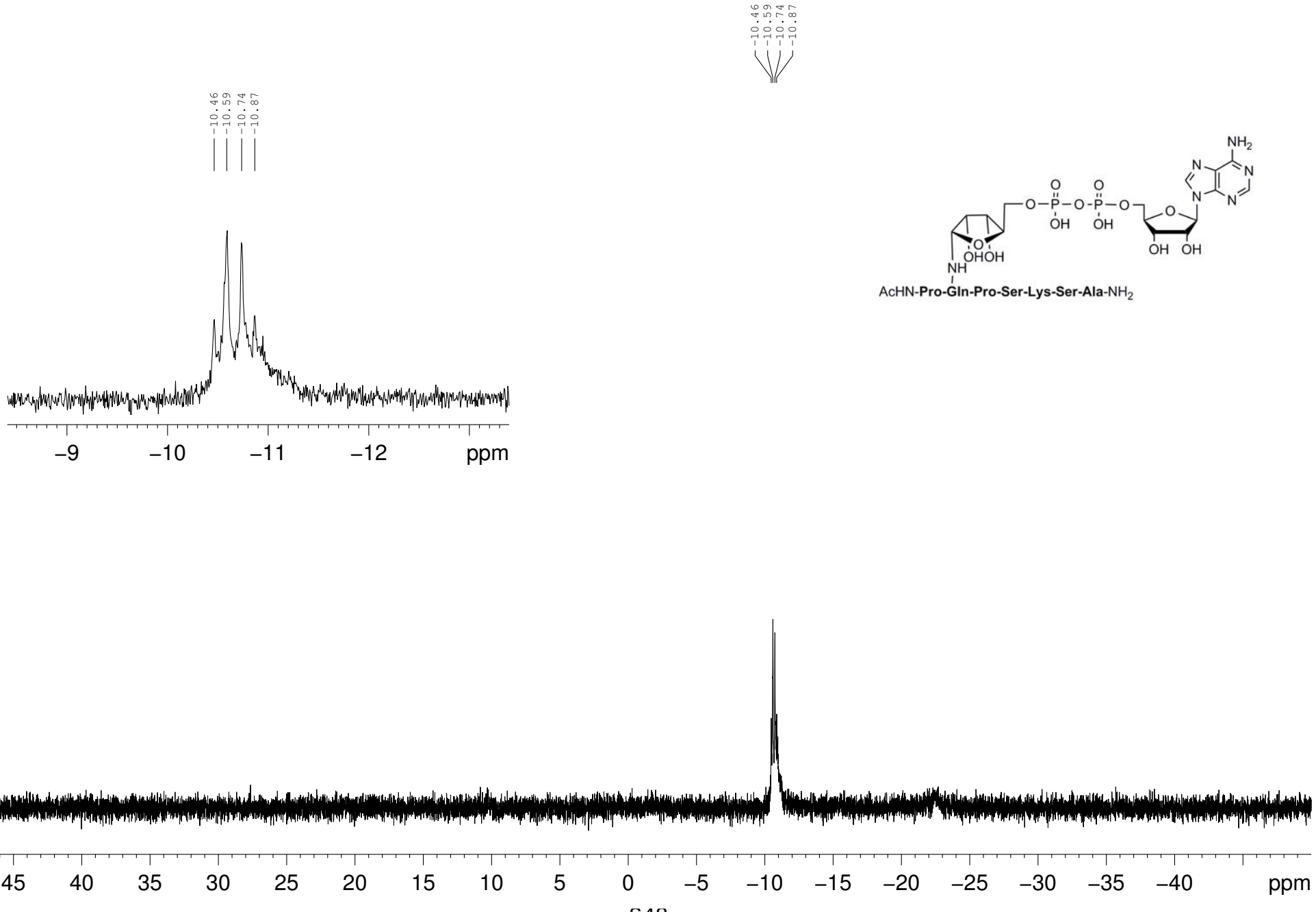


RT: 4.48 AV: 1 NL: 2.05E6

T: + p ESI Full ms [160.00-2000.00]



31P-NMR, 162 MHz, D2O, cmpnd 25



1H-NMR, 400 MHz, D2O, cmpnd 25

8.417
8.168

6.051
6.036
5.661
5.600
5.546
5.536
5.378
5.345
5.032
4.989
4.791
4.696
4.536
4.427
4.294
4.200
4.122
4.042
3.987
3.950
3.899
3.759
3.659
3.608
3.581
3.554
3.538
3.448
3.290
3.277
3.201
3.179
3.173
2.922
2.903
2.883
2.857
2.821
2.759
2.746
2.675
2.608
2.594
2.561
2.548
2.479
2.402
2.383
2.368
2.349
2.303
2.181
2.162
2.144
2.117
2.099
2.080
2.061
2.036
2.016
1.970
1.959
1.895
1.819
1.602
1.490
1.319
1.302
1.219
1.145
1.081
1.009
0.762