SUPPORTING INFORMATION

On the Synthesis of Conformationally Modified Peptides Through Isonitrile Chemistry: Implications for Dealing with Polypeptide Aggregation

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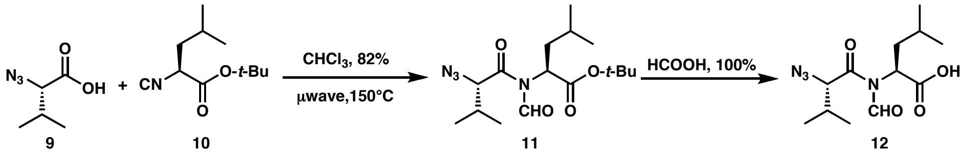
General Information. All commercial materials (Aldrich, Fluka) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher). Anhydrous THF, diethyl ether, CH_2Cl_2, *n*–PrOH, toluene, and benzene were passed through a column of alumina and used without further drying. ^1H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX-500 MHz or DRX-600 MHz at ambient temperature unless otherwise stated. Chemical shifts are reported in parts per million relative to residual solvent $CDCl_3$ (^1H, 7.26 ppm; ^{13}C, 77.0 ppm) or 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) internal reference for spectra taken in D_2O (^1H, 0 ppm; ^{13}C shifts referenced indirectly using Ξ_C = 25.144953% without temperature correction[1]). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. All ^{13}C NMR spectra were recorded with complete proton decoupling. Low-resolution mass spectral analyses were performed with a JEOL JMS-DX-303-HF mass spectrometer or Waters Micromass ZQ mass spectrometer. High-resolution mass spectral analyses were performed by the MSKCC core facility staff. All reactions were carried out in oven-dried glassware under an argon or nitrogen atmosphere unless otherwise noted. Analytical TLC was performed on E. Merck silica gel 60 F254 plates and visualized by UV fluorescence quenching and CAM staining. Flash column chromatography was performed on E. Merck silica gel 60 (40–63 mm). Yields refer to chromatographically and spectroscopically pure compounds.



(*S*)-2-((*S*)-2-azido-*N*-formyl-3-methylbutanamido)-4-methylpentanoic acid (12). To a solution of N_3-valine **9** (43 mg, 0.3 mmol) in $CHCl_3$ (1 mL) was added leucine isonitrile **10** (60 mg, 0.3 mmol). The resulting mixture was heated to 150 °C in the microwave for 30 min, then the solvent was evaporated at low pressure to give an oil. The crude residue was purified by flash chromatography using hexanes:ethyl acetate (15:1) as eluent to give the *N*-formyl dipeptide **11** (83.6 mg, 82% yield). Compound **11** (83.6 mg, 0.25 mmol) was subsequently dissolved in formic acid (2 mL). The reaction mixture was stirred at room temperature for four hours. The resulting solution was evaporated at low

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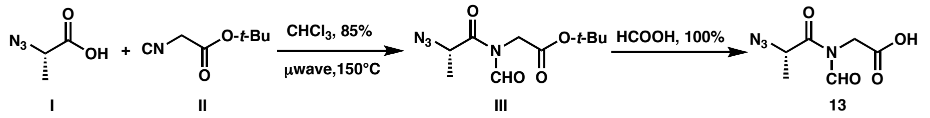
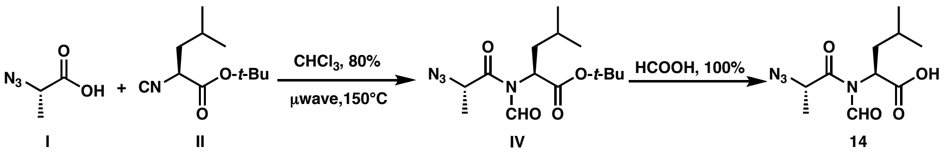
(1) Harris, R. K.; Becker, E. D.; Cabral de Menezes, S. M.; Granger, P.; Hoffman, R. E.; Zilm, K. W. *Pure Appl. Chem.* **2008**, *80*, 59–84.

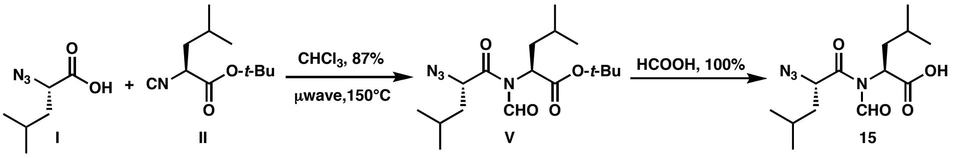
pressure to give compound **12** without further purification (70 mg, 100%). Characterization of compound **11**: $[\alpha]$= –48.3, (c =1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$), δ = 9.06 (1H, s), 5.05–5.02 (1H, dd, ^3J = 4.8 Hz, 9.8 Hz), 3.90 (1H, d, ^3J = 7.8 Hz), 2.30–2.26 (1H, m), 1.95–1.89 (1H, m), 1.77–1.71 (1H, m), 1.37 (10H, m), 1.06 (3H, d, ^3J = 6.7 Hz), 0.98 (3H, d, ^3J = 6.7 Hz), 0.88–0.85 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$), δ = 170.7, 168.4, 161.3, 82.1, 66.9, 52.8, 37.2, 30.7, 27.7, 25.2, 23.0, 19.4, 18.4; IR (thin film): 2961.2, 2116.6, 1734.7, 1679.7, 1469.5, 1369.2; Exact mass calc'd for $C_{16}H_{28}N_4O_4$ $[M+Na]^+$: 363.2, $[M+K]^+$: 379.2, found: 363.1, 379.1.



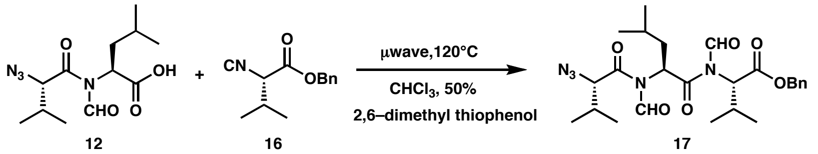
(*S*)-2-(2-azido-*N*-formylpropanamido)acetic acid (13). To a solution of N_3-alanine **I** (115 mg, 1 mmol) in $CHCl_3$ (3 mL) was added glycine isonitrile **II** (141 mg, 1 mmol). The resulting mixture was heated to 150 °C in the microwave for 30 min, then the solvent was evaporated at low pressure to give an oil. The crude residue was purified by flash chromatography using hexanes:ethyl acetate (10:1) as eluent to give the *N*-formyl dipeptide **III** (218 mg, 85% yield). Compound **III** (166 mg, 0.65 mmol) was subsequently dissolved in formic acid (4 mL). The reaction mixture was stirred at room temperature for four hours. The resulting solution was evaporated at low pressure to give compound **13** without further purification (130 mg, 100%). Characterization of compound **III**: $[\alpha]$= –58.3, (c =1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$), δ = 9.1 (1H, s), 4.30–4.27 (3H, m), 5.09 (2H, s), 1.59–1.57 (1H, d, ^3J = 6.8 Hz), 1.41 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$), δ = 171.1, 166.2, 161.4, 82.7, 55.9, 55.8, 42.1, 27.9, 15.5; IR (thin film): 2960.2, 2108.7, 1731.7, 1678.6, 1467.5, 1365.2; Exact mass calc'd for $C_{10}H_{16}N_4O_4$ $[M+Na]^+$: 279.1; $[M-H]^-$: 255.2, found: 279.2, 255.3.



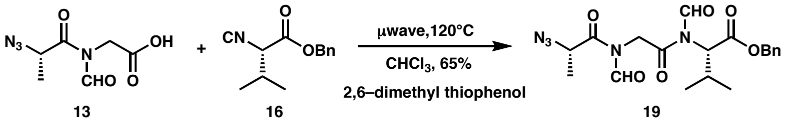
(*S*)-2-((*S*)-2-azido-*N*-formylpropanamido)-4-methylpentanoic acid (14). The procedure used was analogous to the preparation of **13** and used N_3-alanine **I** (23 mg, 0.2 mmol), leucine isonitrile **II** (59 mg, 0.3 mmol), $CHCl_3$ (1.5 mL), and formic acid (2 mL). Purification by flash chromatography using hexanes:ethyl acetate (15:1) gave the *N*–formyl dipeptide **IV** (50 mg, 80% over two steps). Characterization of compound **IV**: $[\alpha]$= –53.3, (c =1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 9.06 (1H, s), 5.05–5.02 (1H, dd, ^3J = 4.9 Hz, 9.6 Hz), 4.33–4.32 (1H, dd, ^3J = 6.7 Hz), 1.94–1.92 (1H, m), 1.82–1.80 (1H, m), 1.61 (3H, d, ^3J = 6.8 Hz, 1.45–1.39 (10H, m), 0.91–0.89 (6H, m); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 171.1, 168.5, 161.6, 82.3, 56.2, 53.1, 37.7, 28.0, 27.8, 25.3, 23.1, 21.7, 15.6; IR (thin film): 2961.2, 2108.8, 1732.7, 1678.7, 1469.5, 1368.2; Exact mass calc'd for $C_{14}H_{24}N_4O_4$ $[M+Na]^+$: 335.2, $[M-H]^-$: 311.2, found: 335.1, 311.0.



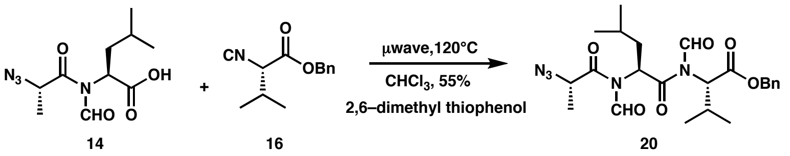
(*S*)-2-((*S*)-2-azido-*N*-formyl-4-methylpentanamido)-4-methylpentanoic acid (15). The procedure used was analogous to the preparation of **13** and used N3-leucine **I** (32 mg, 0.2 mmol), leucine isonitrile **II** (60 mg, 0.3 mmol), $CHCl_3$ (1.5 mL), and formic acid (2 mL). Purification by flash chromatography using hexanes:ethyl acetate (15:1) gave the *N*–formyl dipeptide **15** (52 mg, 87% over two steps). Characterization of compound **V**: [α]= –62.3, (c =1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 9.0 (1H, s), 5.03–5.00 (1H, dd, ^3J = 4.8 Hz, 9.7 Hz), 4.17 (1H, m), 1.92–1.70 (5H, m), 1.37 (10H, m), 0.95–0.85 (12H, m); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 171.4, 168.4, 161.3, 82.2, 59.2, 52.9, 39.0, 37.5, 27.7, 25.2, 23.0, 22.7, 21.6, 21.5; IR (thin film): 2961.2, 2116.6, 1734.7, 1679.7, 1469.5, 1369.2; Exact mass calc'd for $C_{17}H_{30}N_4O_4$ $[M+Na]^+$: 377.2, $[M+K]^+$: 393.2, found: 377.1, 393.1.



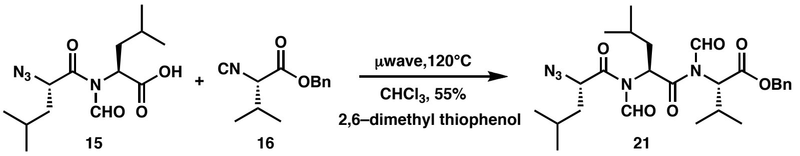
(*S*)-benzyl 2-((*S*)-2-((*S*)-2-azido-*N*-formyl-3-methylbutanamido)-*N*-formyl-4-methylpentanamido)-3-methylbutanoate (17). To a solution of dipeptide **12** (113 mg, 0.4 mmol) and valine isonitrile **16** (260 mg, 1.2 mmol) in $CHCl_3$ (4.0 mL) was added 2,6–dimethyl thiophenol (54 µL, 0.4 mmol). The resulting reaction mixture was stirred at 120 °C under microwave irradiation for two hours. The solution was cooled to room temperature and evaporated at low pressure. The crude residue was purified by flash chromatography using hexanes:ethyl acetate (20:1) as eluent to give the bis–*N*–formyl tripeptide **17** (100 mg, 50%). [α]= –27.9, (c =1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 8.95 (1H, s), 8.81 (1H, s), 7.37–7.26 (5H, m), 5.67–5.64 (1H, dd, ^3J = 6.2 Hz), 5.16–5.07 (2H, dd, ^2J = 12.2 Hz), 4.59 (1H, m), 4.23 (1H, m), 2.58–2.54 (1H, m), 2.23–2.20 (1H, m), 1.89–1.85 (2H, m), 1.54–1.52 (1H, m), 1.14–1.13 (3H, d, ^3J = 6.5 Hz), 1.02–0.90 (15H, m), 0.78 (3H, d, ^3J = 6.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 171.9, 171.8, 169.0, 161.9, 161.5, 135.2, 128.6, 128.5, 128.4, 67.8, 67.3, 60.5, 53.5, 38.6, 30.8, 28.0, 24.9, 22.8, 21.5, 21.4, 19.5, 19.2, 18.0; IR (thin film): 2965.0, 2106.9, 1728.9, 1682.6, 1467.6, 1186.0; Exact mass calc'd for $C_{25}H_{35}N_5O_6$ $[M+Na]^+$: 524.3, found: 524.4.



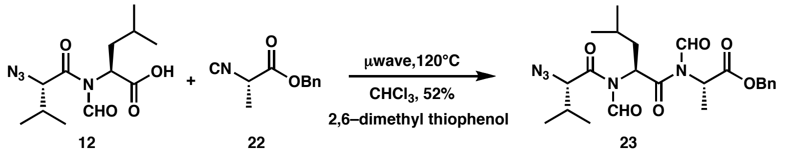
(*S*)-benzyl 2-(2-((*S*)-2-azido-*N*-formylpropanamido)-*N*-formylacetamido)-3-methylbutanoate (19) The procedure used was analogous to the preparation of **17** and used dipeptide **13** (40 mg, 0.2 mmol), valine isonitrile **16** (65 mg, 0.3 mmol), $CHCl_3$ (1.5 mL), and 2,6–dimethyl thiophenol (27 µL, 0.2 mmol). Purification by flash chromatography using hexanes:ethyl acetate (10:1) gave the bis–*N*–formyl tripeptide **19** (54 mg, 65%). [α]= –56.3, (c =1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 9.20 (1H, s), 9.02 (1H, s), 7.37–7.26 (5H, m), 5.16–5.10 (2H, m), 4.90 (2H, m), 4.80–4.77 (1H, d, ^3J = 7.8 Hz), 4.29 (1H, m), 2.53–2.48 (1H, m), 1.64–1.63 (3H, d, ^3J = 6.8 Hz), 1.12 (3H, d, ^3J = 6.5 Hz), 0.85–0.83 (3H, d, ^3J = 6.9 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 170.8, 169.1, 161.6, 135.1, 128.6, 128.4, 67.4, 59.8, 57.2, 56.0, 28.6, 21.2, 19.0, 18.9, 16.2, 16.0, 15.4; IR (thin film): 2960.2, 2113.6, 1731.2, 1679.6, 1465.4, 1365.2; Exact mass calc'd for $C_{19}H_{23}N_5O_6$ $[M+Na]^+$: 440.2, found: 440.4.



(*S*)-benzyl 2-((*S*)-2-((*S*)-2-azido-*N*-formylpropanamido)-*N*-formyl-4-methylpentanamido)-3-methylbutanoate (20) The procedure used was analogous to the preparation of **17** and used dipeptide **14** (26 mg, 0.1 mmol), valine isonitrile **16** (65 mg, 0.3 mmol), $CHCl_3$ (1.5 mL), and 2,6–dimethyl thiophenol (13 μL, 0.1 mmol). Purification by flash chromatography in hexanes:ethyl acetate (10:1) gave the bis-*N*–formyl tripeptide **20** (26 mg, 55%). [α]= –112.3, (c =1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$), δ = 8.92 (1H, s), 8.83 (1H, s), 7.36–7.26 (5H, m), 5.61–5.60 (1H, d, ^3J = 6.5 Hz), 5.15–5.09 (2H, dd, ^2J = 12.2 Hz), 4.50 (1H, m), 4.39 (1H, m), 2.58 (1H, m), 1.90–1.84 (2H, m), 1.53–1.49 (4H, m), 1.14–1.13 (3H, d, ^3J = 6.4 Hz), 0.92–0.90 (6H, m), 0.79 (3H, d, ^3J = 6.8 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 172.1, 171.9, 162.0, 161.6, 135.2, 128.6, 128.5, 128.4, 67.4, 60.6, 57.3, 38.5, 28.1, 24.9, 22.8, 21.5, 21.4, 19.3, 15.6; IR (thin film): 2963.1, 2111.7, 1740.4, 1682.6, 1445.9, 1179.2; Exact mass calc'd for $C_{23}H_{31}N_5O_6$ $[M+Na]^+$: 496.22, found: 496.39.

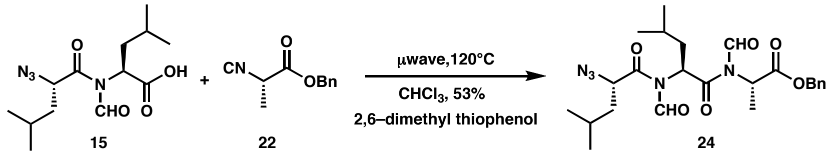


(*S*)-benzyl 2-((*S*)-2-((*R*)-2-azido-*N*-formyl-4-methylpentanamido)-*N*-formyl-4-methylpentanamido)-3-methylbutanoate (21) The procedure used was analogous to the preparation of **17** and used dipeptide **15** (60 mg, 0.2 mmol), valine isonitrile **16** (130 mg, 0.6 mmol), $CHCl_3$ (2.0 mL), and 2,6–dimethyl thiophenol (27 μL, 0.2 mmol). Purification by flash chromatography in hexanes:ethyl acetate (20:1) gave the bis–*N*–formyl tripeptide **21** (56.6 mg, 55%). [α]= –85.6, (c =1.0, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$), δ = 8.95 (1H, s), 8.80 (1H, s), 7.35–7.25 (5H, m), 5.69–5.66 (1H, t, ^3J = 7.9 Hz), 5.14–5.08 (2H, dd, ^2J = 12.3 Hz), 4.64–4.63 (1H, d, ^3J = 8.8 Hz), 4.43–4.41 (1H, m), 2.54 (1H, m), 1.87–1.53 (7H, m), 1.14–1.13 (3H, d, ^3J = 6.6 Hz), 0.92–0.76 (15H, m); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 173.1, 171.9, 169.0, 161.9, 161.4, 135.2, 128.7, 128.5, 128.3, 67.3, 60.5, 38.8, 38.7, 29.7, 28.0, 25.2, 24.9, 23.0, 22.8, 21.5, 21.4, 21.3, 19.2; IR (thin film): 2961.2, 2110.5, 1739.4, 1681.5, 1444.8, 1178.1; Exact mass calc'd for $C_{26}H_{37}N_5O_6$ $[M+Na]^+$: 538.2, found: 538.3.

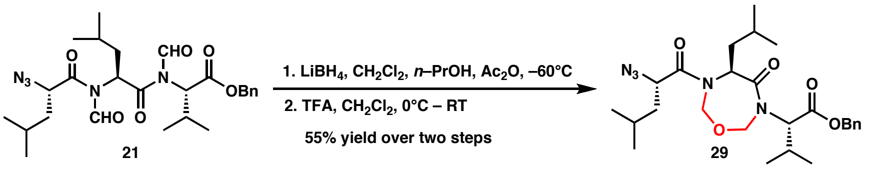


(*S*)-benzyl 2-((*S*)-2-((*S*)-2-azido-*N*-formyl-3-methylbutanamido)-*N*-formyl-4-methylpentanamido)propanoate (23) The procedure used was analogous to the preparation of **17** and used dipeptide **12** (57 mg, 0.2 mmol), alanine isonitrile **22** (113 mg, 0.6 mmol), $CHCl_3$ (2.0 mL) and 2,6–dimethyl thiophenol (27 μL, 0.2 mmol). Purification by flash chromatography in hexanes:ethyl acetate (20:1) gave the bis–*N*–formyl tripeptide **23** (49 mg, 52%). [α]= –65.6, (c =1.0, $CHCl_3$); ^{1}H NMR

(500 MHz, $CDCl_3$), δ = 8.91 (1 H, s), 8.83 (1 H, s), 7.36–7.26 (5 H, m), 5.62–5.59 (1 H, dd, ^3J = 5.45 Hz, 9.35 Hz), 5.18–5.08 (3 H, m, CH_2Ph, Leu $C^{\alpha}H$), 4.16–4.15 (1 H, d, ^3J = 7.35 Hz, Val $C^{\alpha}H$), 2.25–2.21 (1 H, m), 1.98–1.93 (1 H, m), 1.86–1.82 (1 H, m), 1.54–1.49 (3 H, d, Ala $C^{\beta}H_3$), 1.05–0.89 (12 H, m, $4CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$), δ = 171.5, 171.0, 169.5, 161.6, 160.7, 135.3, 128.6, 128.4, 128.3, 67.8, 67.5, 50.6, 38.7, 30.7, 24.8, 22.9, 21.7, 19.5, 18.2, 15.0; IR (thin film): 2964.1, 2106.9, 1740.4, 1670.7, 1444.8, 1181.2; Exact mass calc'd for $C_{23}H_{31}N_5O_6$ $[M+Na]^+$: 496.2, found: 496.2.

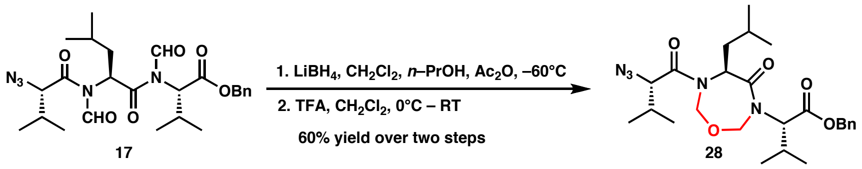


(*S*)-benzyl 2-((*S*)-2-((*S*)-2-azido-*N*-formyl-4-methylpentanamido)-*N*-formyl-4-methylpentanamido)propanoate (24) The procedure used was analogous to the preparation of **17** and used dipeptide **15** (60 mg, 0.2 mmol), alanine isonitrile **22** (113 mg, 0.6 mmol), $CHCl_3$ (2.0 mL) and 2,6–dimethyl thiophenol (27 µL, 0.2 mmol). Purification by flash chromatography in hexanes:ethyl acetate (20:1) gave the bis–*N*–formyl tripeptide **24** (52 mg, 53%). [α]= –55.6, (c =1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$), δ = 8.87 (1 H, s), 8.84 (1 H, s), 7.35–7.25 (5 H, m), 5.63–5.60 (1 H, dd, ^3J = 5.52 Hz, 9.54 Hz, Ala $C^{\alpha}H$), 5.16–5.09 (3 H, m, CH_2Ph, Leu $C^{\alpha}H$), 4.38–4.36 (1 H, d, ^3J = 4.08 Hz, 9.48 Hz, Leu $C^{\alpha}H$), 1.91–1.63 (5 H, m), 1.51 (4 H, m), 0.98–0.89 (12 H, m, $4CH_3$); ^{13}C NMR (125 MHz, $CDCl_3$), δ = 172.7, 171.1, 169.4, 161.6, 160.6, 135.2, 128.6, 128.4, 128.2, 67.5, 60.3, 50.5, 38.9, 38.6, 25.2, 24.8, 22.9, 22.8, 21.6, 21.4, 14.9; IR (thin film): 2958.3, 2112.6, 1732.7, 1680.7, 1455.0, 1180.2; Exact mass calc'd for $C_{24}H_{33}N_5O_6$ $[M+Na]^+$: 510.2, found: 510.3.

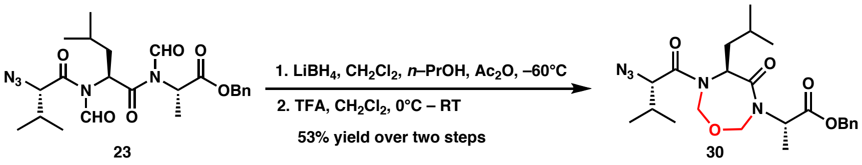


(*S*)-benzyl 2-((*S*)-6-((*S*)-2-azido-4-methylpentanoyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanoate (29) To a –60°C solution of tripeptide **21** (23 mg, 0.045 mmol) in CH_2Cl_2 (5 mL) was added 1–propanol (0.25 mL) and acetic anhydride (10 µL, 0.025 mmol). The resulting mixture was stirred at –60°C for 5 min, then a solution of $LiBH_4$ in THF (2.0 M) (0.27 mmol, 135 µL) was added. After stirring for three hours at –60°C, TLC indicated disappearance of starting material. The reaction mixture was quenched with 0.2 M Trizma hydrochloride solution (10 mL) at –60°C, then slowly warmed to room temperature and stirred until the solution became clear. The resulting mixture was extracted with CH_2Cl_2 (3 × 10 mL), then the organic phase was washed with brine and dried over $MgSO_4$. The resulting solution was evaporated at low pressure to give the crude diol, which was used in the following step without further purification. The crude diol was dissolved in dry CH_2Cl_2 and treated with TFA (50 µL, 0.36 mmol) at 0°C. The resulting mixture was warmed to room temperature slowly, then stirred at room temperature for 30 min. Finally, the solvent was evaporated at low pressure to give an oil. The crude residue was purified by flash chromatography using hexanes:ethyl acetate (15:1) as eluent to give the constrained tripeptide **29** (12 mg, 55%). [α]= +39.6, (c =1.0,

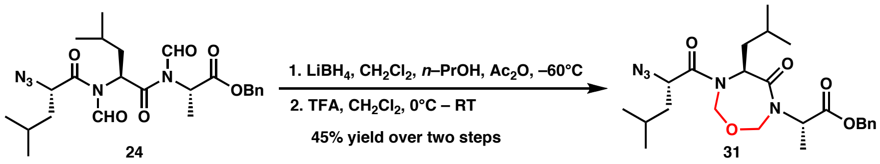
$CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, 50 °C), δ = 7.27–7.18 (5 H, m), 6.0–3.5 (9 H, m), 2.19–1.91 (7 H, m), 0.92–0.88 (18 H); IR (thin film): 2962.1, 2108.8.7, 1735.6, 1670.1, 1174.4; Exact mass calc'd for $C_{26}H_{39}N_5O_5$ $[M+Na]^+$: 524.3, found: 524.3.



(*S*)-benzyl 2-((*S*)-6-((*S*)-2-azido-3-methylbutanoyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanoate (**28**) The procedure used was analogous to the preparation of **29** and used tripeptide **17** (35 mg, 0.07 mmol), $LiBH_4$ (0.21 mL, 0,42 mmol), CH_2Cl_2 (10 mL), 1–propanol (0.5 mL), acetic anhydride (15 μL), and TFA (0.1 mL). Purification by flash chromatography in hexanes:ethyl acetate (20:1) gave the constrained tripeptide **28** (20.5 mg, 60%). [α]= +61.9, (c =1.0, $CHCl_3$); ^1H NMR (600 MHz, DMSO, 54.1 °C), δ = 7.33–7.28 (5H, m), 5.09–4.98 (7H, m), 4.49 (1H, m), 3.97 (1H, m), 2.18–2.10 (2H, m), 1.84–1.73 (2H, m), 1.48 (1H, m), 0.94–0.83 (18H, m); IR (thin film): 2963.1, 2101.1, 1735.6, 1689.3, 1469.5, 1176.4; Exact mass calc'd for $C_{25}H_{37}N_5O_5$ $[M+H]^+$: 488.3, found: 488.4.

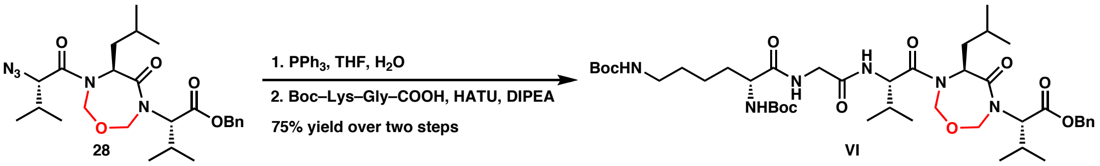


(*S*)-benzyl 2-((*S*)-6-((*S*)-2-azido-3-methylbutanoyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)propanoate (30) The procedure used was analogous to the preparation of **29** and used tripeptide **23** (33 mg, 0.07 mmol), $LiBH_4$ (0.21 mL, 0,42 mmol), CH_2Cl_2 (10 mL), 1–propanol (0.5 mL), acetic anhydride (15 μL), and TFA (0.1 mL). Purification by flash chromatography in hexanes:ethyl acetate (20:1) gave the constrained tripeptide **30** (17 mg, 53%). [α]= +45.5, (c =1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, 50 °C), δ = 7.29–7.25 (5H, m), 5.84–4.37 (8H, m), 3.48–3.36 (1H, m), 2.29–1.18 (7H, m), 0.89–0.83 (12H, m); IR (thin film): 2962.1, 2102.0, 1741.4, 1678.7, 1466.5, 1186.9; Exact mass calc'd for $C_{23}H_{33}N_5O_5$ $[M+Na]^+$: 482.2, found: 482.1.

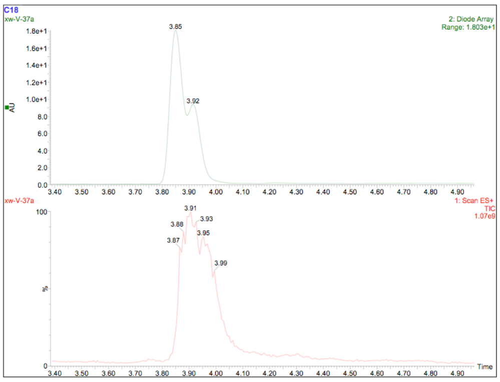


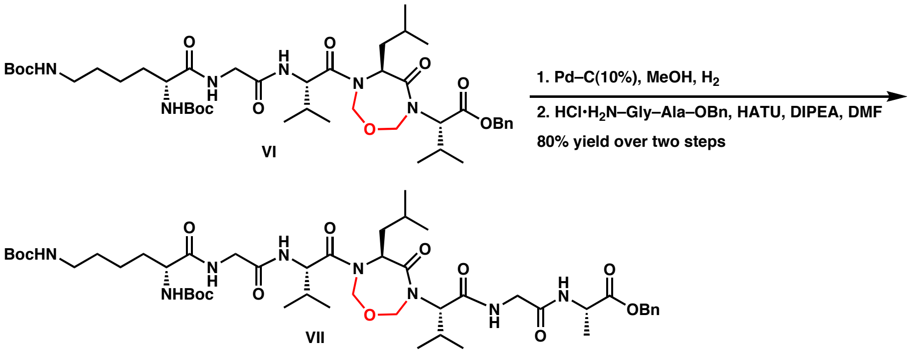
(*S*)-benzyl 2-((*S*)-6-((*S*)-2-azido-4-methylpentanoyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)propanoate (31) The procedure used was analogous to the preparation of **29** and used tripeptide **24** (34 mg, 0.07 mmol), $LiBH_4$ (0.21 mL, 0,42 mmol), CH_2Cl_2 (10 mL), 1–propanol (0.5 mL), acetic anhydride (15 μL), and TFA (0.1 mL). Purification by flash chromatography in hexanes:ethyl acetate

(20:1) gave the constrained tripeptide **31** (15 mg, 45%). [α]= +34.9, (c =1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, 50 °C), δ = 7.30–7.25 (5H, m), 5.74–3.84 (9H, m), 2.10–1.36 (9H, m), 0.96–0.87 (12H, m); IR (thin film): 2960.2, 2112.6, 1740.4, 1673.9, 1467.6, 1190.8; Exact mass calc'd for $C_{24}H_{35}N_5O_5$ $[M+Na]^+$: 496.3, found: 496.2.

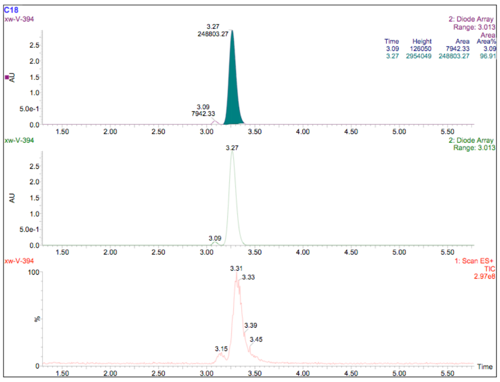


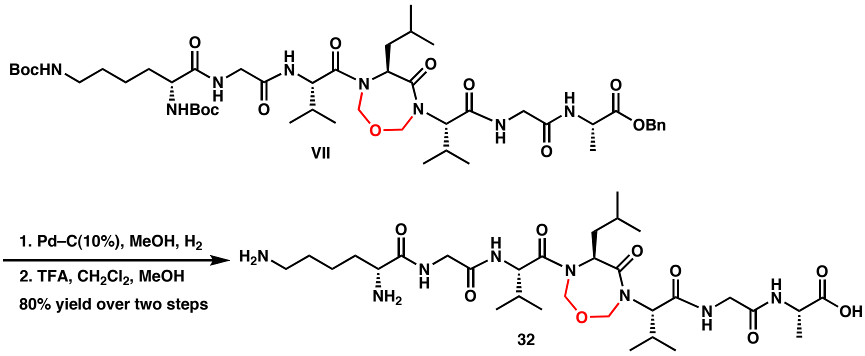
(*S*)-benzyl 2-((*S*)-6-((10*R*,16*S*)-10-(*tert*-butoxycarbonylamino)-2,2,17-trimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazaoctadecanecarbonyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanoate (VI) To a solution of **28** (25 mg, 0.05 mmol) in a mixture of THF and H_2O (6.0 mL, 5:1) was added triphenylphosphine (20 mg, 0.075 mmol). The reaction mixture was stirred at room temperature overnight, then evaporated at low pressure. The crude residue was dissolved in DMF (2.0 mL), and Boc–Lys–Gly–COOH (40 mg, 0.1 mmol) was added. The resulting mixture was treated with HATU (38 mg, 0.1 mmol) and DIPEA (26 μL, 0.15 mmol). After 30 min, LCMS analysis of the reaction mixture indicated disappearance of the starting material. The reaction mixture was purified by reverse phase HPLC to give the product **VI** (31 mg, 75%). Exact mass calc'd for $C_{43}H_{70}N_6O_{11}$ $[M+H]^+$: 847.51, found: 847.91.





(*S*)-benzyl 2-(2-((*S*)-2-((*S*)-6-((10*R*,16*S*)-10-(*tert*-butoxycarbonylamino)-2,2,17-trimethyl-4,11,14-trioxo-3-oxa-5,12,15-triazaoctadecanecarbonyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanamido)acetamido)propanoate (VII) To a solution of compound **VI** (31 mg, 0.037 mmol) in MeOH (5 mL) was added Pd–C(10%) (20 mg) and the resulting mixture was stirred under an atmosphere of hydrogen overnight. The solution was passed through a filter (0.45 μm). The resulting solution was evaporated at low pressure to give the crude deprotected pentapeptide. The residue was dissolved in DMF (2.0 mL), and HCl•H_2N–Gly–Ala–OBn (20 mg, 0.075 mmol) was added at –10°C. The resulting mixture was treated with HATU (38 mg, 0.1 mmol) and DIPEA (26 μL, 0.15 mmol) at that temperature. After 30 min, LCMS analysis of the reaction mixture indicated disappearance of the starting material. The reaction mixture was purified by reverse phase HPLC to give the product **VII** (29 mg, 80%). Exact mass calc'd for $C_{48}H_{78}N_8O_{13}$ $[M+H]^+$: 975.57, found: 976.0.

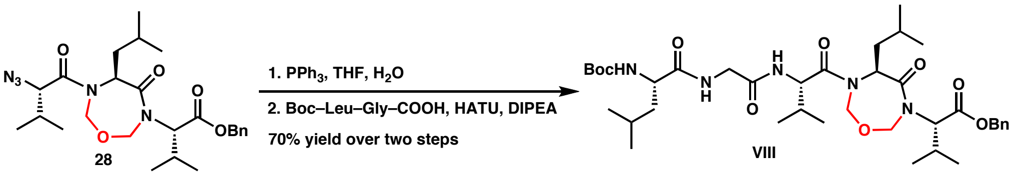




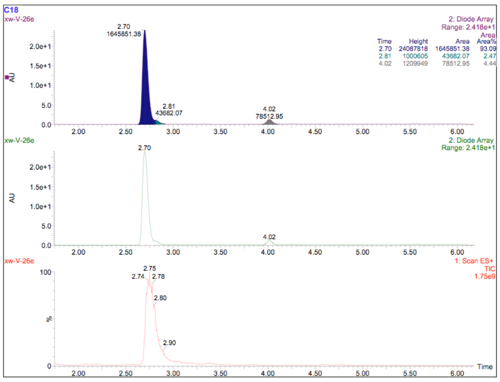
VII

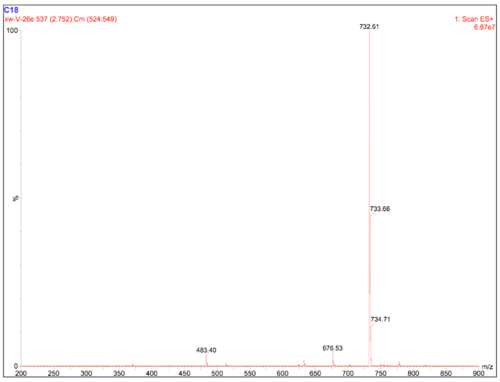
32

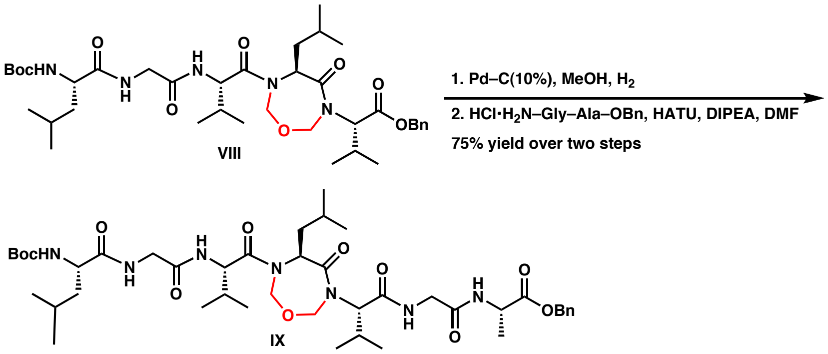
(*S*)-2-(2-((*S*)-2-((*S*)-6-((6*R*,12*S*)-6-(4-aminobutyl)-2,2,13-trimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatetradecanecarbonyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanamido)acetamido)propanoic acid (32) To a solution of compound **VII** (15 mg, 0.015 mmol) in MeOH (5 mL) was added Pd–C(10%) (20 mg) and the resulting mixture was stirred under an atmosphere of hydrogen overnight. The solution was passed through a filter (0.45 μm). The resulting solution was evaporated at low pressure to give the crude deprotected pentapeptide. The residue was dissolved in CH_2Cl_2 (1.0 mL) and treated with TFA (1.0 mL) at –10°C. The resulting mixture was stirred for two hours at that temperature. LCMS analysis of the reaction mixture indicated disappearance of the starting material. The reaction mixture was purified by reverse phase HPLC to give the product **32** (8.3 mg, 80%). [α]= +51.9, (c =1.0, MeOH); ^{1}H NMR (600 MHz, D_2O, 4 °C) δ 5.79 (d, ^3J = 12.2 Hz, 1H, one of NC**H**$_2$O), 5.37 (d, ^3J = 13.5 Hz, 1H, one of NC**H**$_2$O), 5.20 (d, ^3J = 13.5 Hz, 1H, one of NC**H**$_2$O), 4.99 (d, ^3J = 12.5 Hz, 1H, one of NC**H**$_2$O), 4.92 (d, ^3J = 4.8 Hz, 1H, Val-3 C^α**H**), 4.46 (broad dd, ^3J = 8.0, 4.4 Hz, 1H, Leu-4 C^α**H**), 4.36 (d, ^3J = 10.8 Hz, 1H, Val-5 C^α**H**), 4.24 (q, ^3J = 6.9 Hz, 1H, Ala-7 C^α**H**), 4.12 (d, ^3J = 16.9 Hz, 1H, Gly-2 C^α**H**), 4.04 (t, ^3J = 6.4 Hz, 1H, Lys-1 C^α**H**), 3.89 (s, 2H, Gly-6 C^α**H$_2$**), 3.84 (d, ^3J = 16.9 Hz, 1H, Gly-2 C^α**H**), 3.01 (t, ^3J = 7.1 Hz, 2H, Lys-1 C^ε**H$_2$**), 2.24–2.08 (m, 3H, Val-3 C$^{\beta}$**H**, Leu-4 C$^{\beta}$**H**, Val-5 C$^{\beta}$**H**), 2.00–1.86 (m, 2H, Lys-1 C$^{\beta}$**H$_2$**), 1.81–1.67 (m, 2H, Lys-1 C$^{\delta}$**H$_2$**), 1.67–1.59 (m, 1H, Leu-4 C$^{\beta}$**H**), 1.59–1.43 (m, 3H, Lys-1 C$^{\gamma}$**H$_2$**, Leu-4 C$^{\gamma}$**H**), 1.37 (d, ^3J = 7.1 Hz, 3H, Ala-7 C$^{\beta}$**H$_3$**), 1.04–0.81 (m, 15H, Val-3 C$^{\gamma}$**H$_3$** × 2, Leu-4 C$^{\delta}$**H$_3$** × 2, Val-5 C$^{\gamma}$**H$_3$**), 0.78 (d, ^3J = 6.0 Hz, 3H, Val-5 C$^{\gamma}$**H$_3$**); ^{13}C NMR (150 MHz, D$_2$O, 4 °C) δ 181.4, 180.3, 178.6, 176.3, 173.0, 172.8, 172.4, 86.6, 78.8, 66.3, 64.8, 57.6, 55.6, 52.8, 44.8, 44.6, 41.6, 38.8, 34.2, 33.1, 30.2, 29.1, 27.0, 25.2, 23.9, 23.4, 21.4, 21.3, 21.2, 19.6, 19.0; Exact mass calc'd for $C_{31}H_{56}N_8O_9$ [M+H]$^+$: 685.42, found: 685.69.



(*S*)-benzyl 2-((*S*)-5-isobutyl-6-((6*S*,12*S*)-6-isobutyl-2,2,13-trimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatetradecanecarbonyl)-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanoate (VIII) The procedure used was analogous to the preparation of **VI** and used tripeptide **28** (11 mg, 0.023 mmol), triphenylphosphine (9 mg, 0.034 mmol), Boc–Leu–Gly–COOH (13 mg, 0.045 mmol), HATU (17 mg, 0.045 mmol), and DIPEA (10 μL, 0.057 mmol). Reverse phase HPLC purification gave the constrained peptapeptide **VIII** (11.7 mg, 70%). Exact mass calc'd for $C_{38}H_{61}N_5O_9$ [M+H]$^+$: 732.45, found: 732.61.

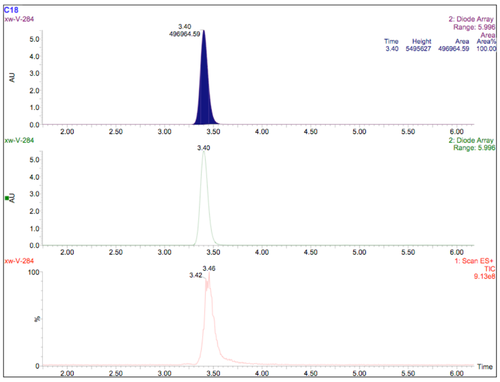


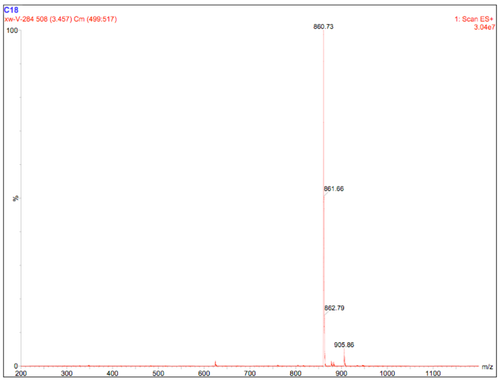


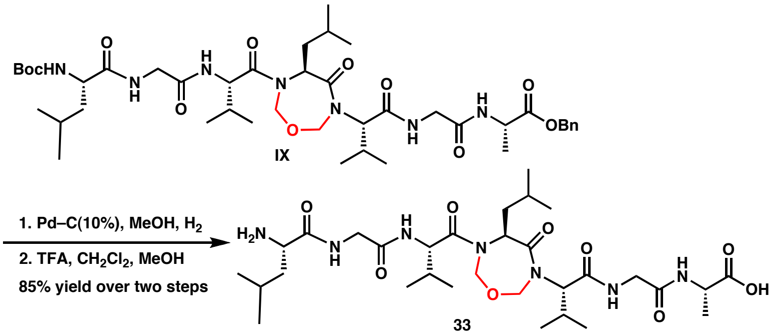


(*S*)-benzyl 2-(2-((*S*)-2-((*S*)-5-isobutyl-6-((6*S*,12*S*)-6-isobutyl-2,2,13-trimethyl-4,7,10-trioxo-3-oxa-5,8,11-triazatetradecanecarbonyl)-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanamido)acetamido)propanoate (IX) The procedure used was analogous to the preparation of **VII** and used peptapeptide **VIII** (7.3 mg, 0.01 mmol), Pd–C (10%) (20 mg), HCl•H$_2$N–Gly–Ala–OBn (3.3 mg, 0.012 mmol), HATU (5.7 mg, 0.015 mmol), and DIPEA (4.0 μL, 0.025 mmol).

Reverse phase HPLC purification gave the constrained pentapeptide **IX** (6.4 mg, 75%). Exact mass calc'd for $C_{43}H_{69}N_7O_{11}$ $[M+H]^+$: 860.51, found: 860.73.





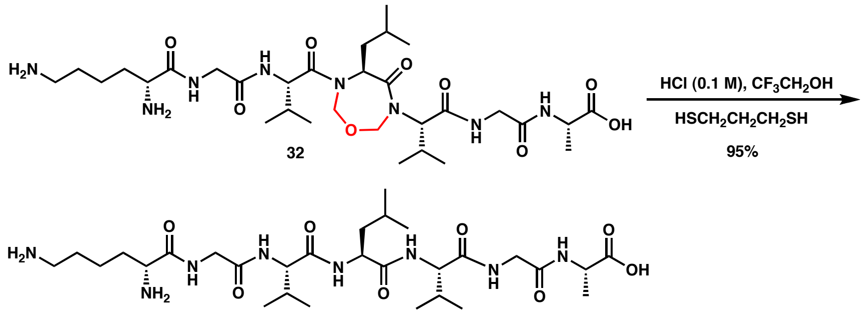


IX

33

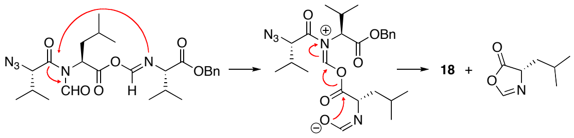
(*S*)-2-(2-((*S*)-2-((*S*)-6-((*S*)-2-(2-((*S*)-2-amino-4-methylpentanamido)acetamido)-3-methylbutanoyl)-5-isobutyl-4-oxo-1,3,6-oxadiazepan-3-yl)-3-methylbutanamido)acetamido)propanoic acid (33)

The procedure used was analogous to the preparation of **32** and used heptapeptide **IX** (6 mg, 7μmol), Pd–C (10%) (10 mg), MeOH (5 mL), CH_2Cl_2 (1.2 mL) and TFA (0.4 mL). Reverse phase HPLC purification gave the constrained heptapeptide **33** (4.0 mg, 85%). [α]= +41.8, (c =1.0, MeOH); ^{1}H NMR (600 MHz, D_2O, 4 °C) δ 5.78 (d, ^3J = 12.2 Hz, 1H, one of NC**H$_2$**O), 5.37 (d, ^3J = 13.5 Hz, 1H, one of NC**H$_2$**O), 5.20 (d, ^3J = 13.2 Hz, 1H, one of NC**H$_2$**O), 4.99 (d, ^3J = 12.2 Hz, 1H, one of NC**H$_2$**O), 4.92 (d, ^3J = 5.1 Hz, 1H, Val-3 C$^{\alpha}$**H**), 4.46 (broad dd, ^3J = 8.3, 5.1 Hz, 1H, Leu-4 C$^{\alpha}$**H**), 4.35 (d, ^3J = 10.7 Hz, 1H, Val-5 C$^{\alpha}$**H**), 4.22 (q, ^3J = 6.9 Hz, 1H, Ala-7 C$^{\alpha}$**H**), 4.09 (d, ^3J = 16.7 Hz, 1H, Gly-2 C$^{\alpha}$**H**), 4.05 (t, ^3J = 7.0 Hz, 2H, Leu-1 C$^{\alpha}$**H**), 3.89 (s, 2H, Gly-6 C$^{\alpha}$**H$_2$**), 3.85 (d, ^3J = 16.6 Hz, 1H, Gly-2 C$^{\alpha}$**H**), 2.21–2.08 (m, 3H, Val-3 C$^{\beta}$**H**, Leu-4 C$^{\beta}$**H**, Val-5 C$^{\beta}$**H**), 1.82–1.67 (m, 3H, Leu-1 C$^{\beta}$**H$_2$**, Leu-1 C$^{\gamma}$**H**), 1.66–1.58 (m, 1H, Leu-4 C$^{\beta}$**H**), 1.58–1.49 (m, 1H, Leu-4 C$^{\gamma}$**H**), 1.36 (d, ^3J = 7.2 Hz, 3H, Ala-7 C$^{\beta}$**H$_3$**), 1.05–0.81 (m, 21H, Leu-1 C$^{\delta}$**H$_3$** × 2, Val-3 C$^{\gamma}$**H$_3$** × 2, Leu-4 C$^{\delta}$**H$_3$** × 2, Val-5 C$^{\gamma}$**H$_3$**), 0.78 (d, ^3J = 6.1 Hz, 3H, Val-5 C$^{\gamma}$**H$_3$**); ^{13}C NMR (150 MHz, D_2O, 4 °C) δ 181.3, 180.4, 178.6, 176.4, 173.6, 173.0, 172.5, 86.6, 78.8, 66.4, 64.8, 57.7, 54.4, 52.8, 44.8, 44.6, 42.6, 38.8, 34.2, 30.2, 27.0, 26.5, 25.2, 24.4, 23.7, 23.4, 21.5, 21.3, 21.2, 19.6, 19.0; Exact mass calc'd for $C_{31}H_{55}N_7O_9$ [M+H]+: 670.41, found: 670.56.



To a solution of constrained heptapeptide **32** (5 mg, 7.31 μmol) in CF_3CH_2OH (2 mL) was added 1,3–propanedithiol (0.1 mL) and 0.1 M HCl (0.2 mL). The resulting mixture was stirred at room temperature for two hours. LCMS analysis of the reaction mixture indicated disappearance of the starting material. Reverse phase HPLC purification gave the linear heptapeptide **34** (4.5 mg, 95%). ^{1}H NMR (600 MHz, D_2O, 4 °C) δ 4.46–4.40 (m, 1H, Leu-4 C$^\alpha$**H**), 4.27 (q, ^3J = 7.3 Hz, 1H, Ala-7 C$^\alpha$**H**), 4.11 (d, ^3J = 7.3 Hz, 1H, Val-3 C$^\alpha$**H**), 4.10–4.02 (m, 3H, Gly-2 C$^\alpha$**H**, Val-5 C$^\alpha$**H**, Lys-1 C$^\alpha$**H**), 3.99 (d, ^3J = 16.8 Hz, 1H, Gly-2 C$^\alpha$**H**), 3.93 (s, 2H, Gly-6 C$^\alpha$**H$_2$**), 3.00 (t, ^3J = 7.6 Hz, 2H, Lys-1 C$^\varepsilon$**H$_2$**), 2.10–2.01 (m, 2H, Val-3 C$^\beta$**H**, Val-5 C$^\beta$**H**), 2.00–1.87 (m, 2H, Lys-1 C$^\beta$**H$_2$**), 1.76–1.68 (m, 2H, Lys-1 C$^\delta$**H$_2$**), 1.67–1.53 (m, 3H, Leu-4 C$^\beta$**H$_2$**, Leu-4 C$^\gamma$**H**), 1.53–1.43 (m, 2H, Lys-1 C$^\gamma$**H$_2$**), 1.39 (d, ^3J = 7.3 Hz, 3H, Ala-7 C$^\beta$**H$_3$**), 1.01–0.89 (m, 15H, Val-5 C$^\gamma$**H$_3$** × 2, Val-3 C$^\gamma$**H$_3$** × 2, Leu-4 C$^\delta$**H$_3$**), 0.87 (d, ^3J = 5.6 Hz, 3H, Leu-4 C$^\delta$**H$_3$**); ^{13}C NMR (150 MHz, D_2O, 4 °C) δ 180.8, 177.1, 176.6, 176.2, 173.5, 173.2, 172.9, 62.6, 62.2, 55.6, 54.8, 52.4, 44.9, 44.7, 42.3, 41.6, 33.0, 32.9, 32.7, 29.1, 27.0, 24.7, 23.9, 23.6, 21.1, 21.0, 20.8, 20.3, 19.4; Exact mass calc'd for $C_{29}H_{54}N_8O_8$ [M+H]+: 643.41, found: 643.57.

Rationale for Formation of 18. The structure of **18** was confirmed by ^{1}H NMR and MS evidence. A plausible mechanism for its formation might involve acyl transfer from the *N*-formyl nitrogen to the formimidate nitrogen, with expulsion of the formyl-Leu residue in the form of an oxazolone:

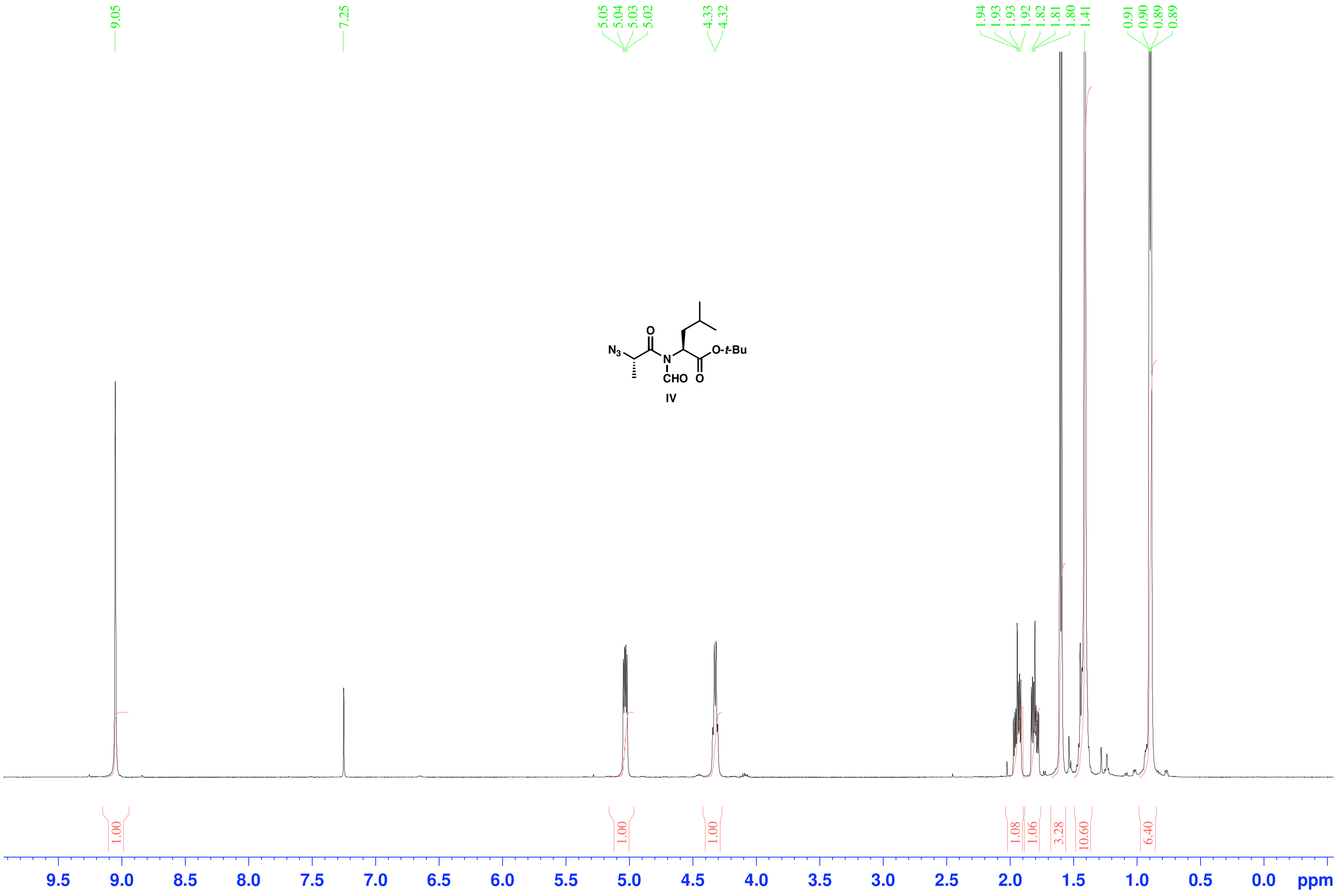


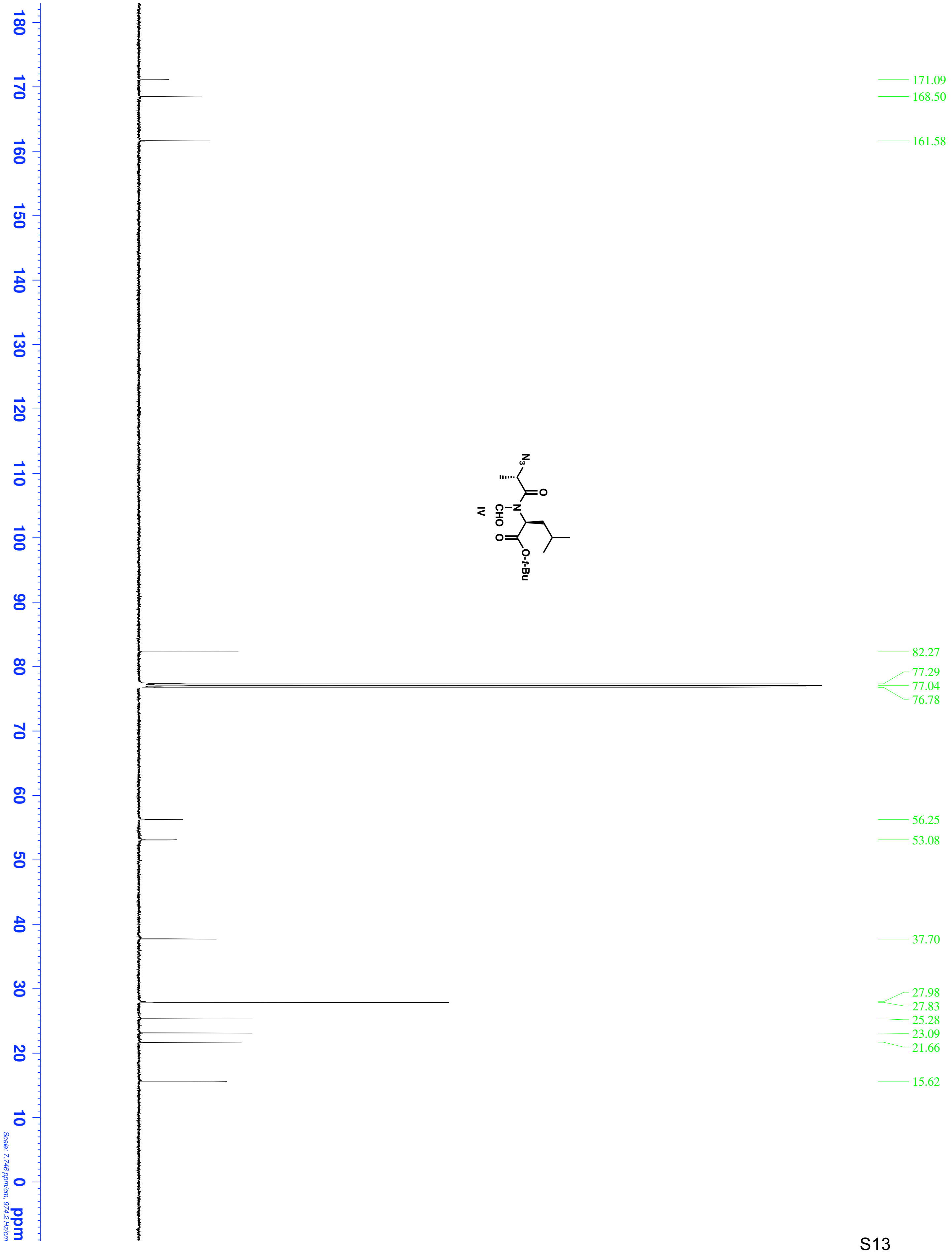
Notes on Substrate Scope.

1. Although the 2+1 coupling afforded synthetically useful yields of bis-*N*-formyl tripeptides containing bulky amino acids such as Leu and Val, we noted a precipitous decline in efficiency (yields < 5%) with couplings involving isoleucine-containing substrates.

2. When tripeptides containing glycine at any position were subjected to reduction conditions (cf. **25**→**26**, Table 3), decomposition into multiple species was observed. Analysis of the mixture by MS indicated the presence of side products derived from deformylation as well as cleavage of the peptide chain, among others. Expanding the scope of this sequence is an area of active investigation in our laboratory.

Proton24 .* wux1 xw–V–12a (1 1) CDCl3 24.0C March_06,2009_18:29 DRX 500MHz zg30 1H *.

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82.27
77.29
77.04
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56.25
53.08
37.70
27.98
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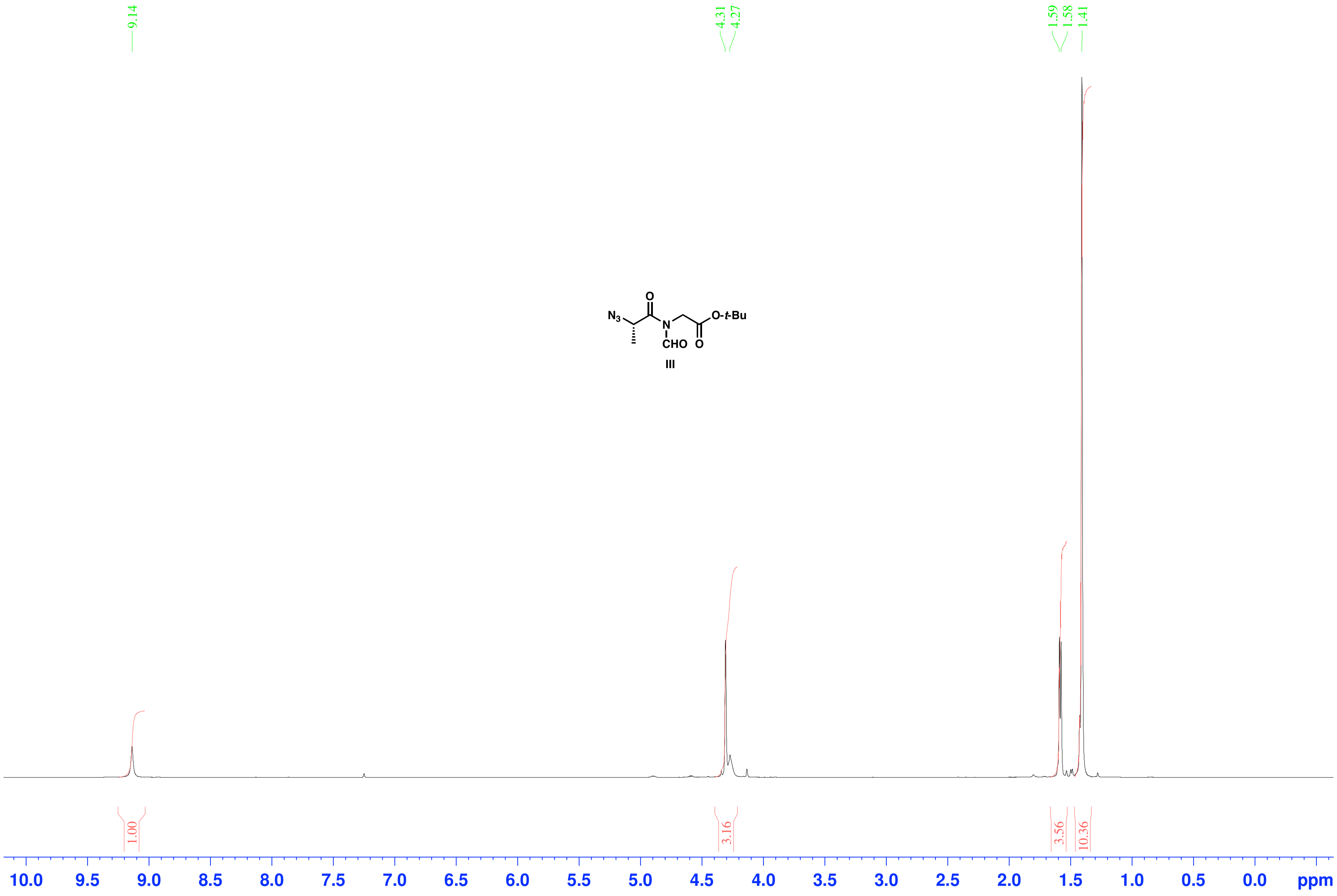
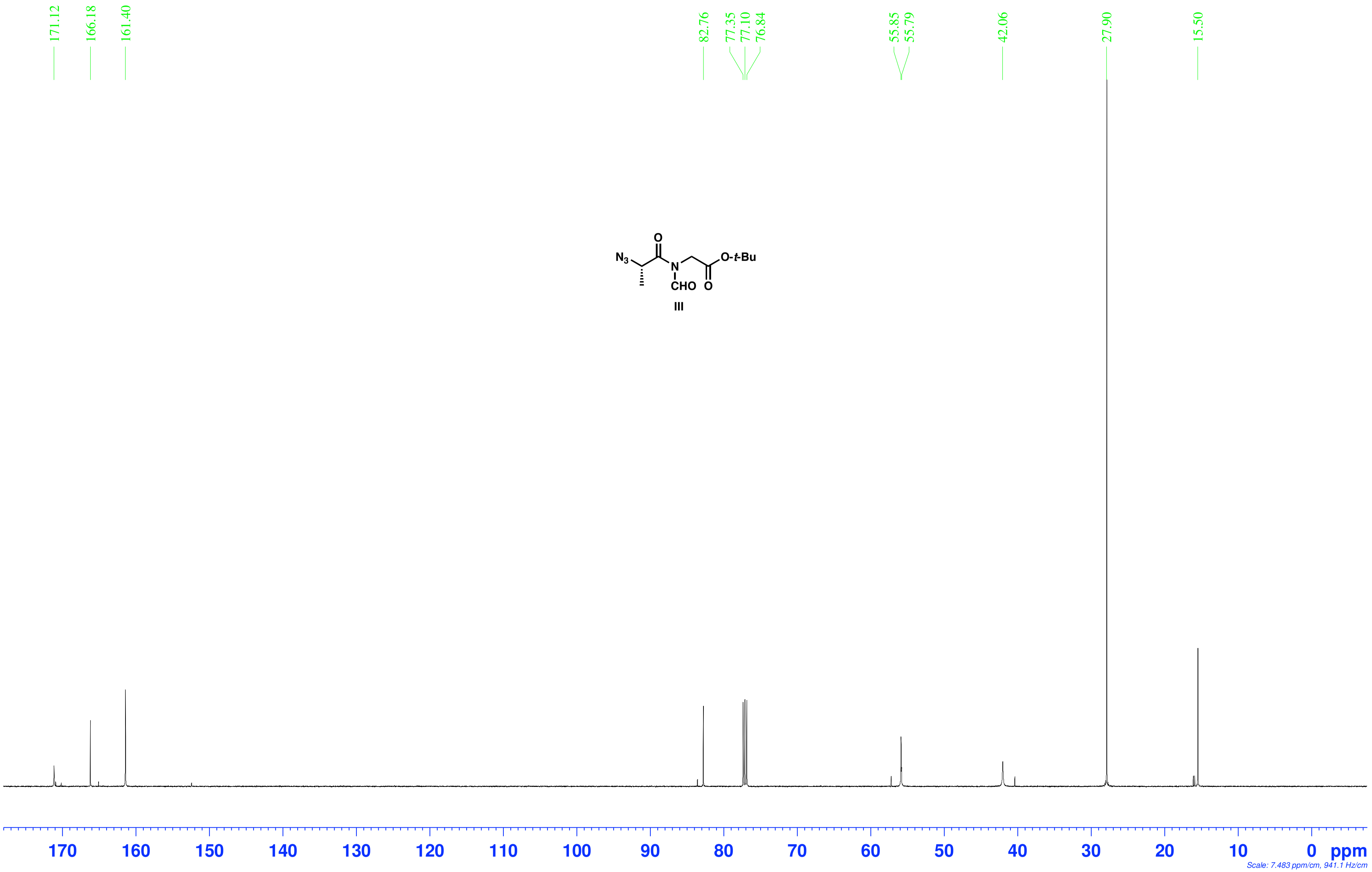
23.09
21.66
15.62
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm
Scale: 7.746 ppm/cm, 974.2 Hz/cm
N3
O
N
CHO
O
O-t-Bu
IV

Proton24 .* wux1 xw−V−45H (1 1) CDCl3 24.0C July_12,2010_11:24:20 DRX 500MHz zg30 1H *.

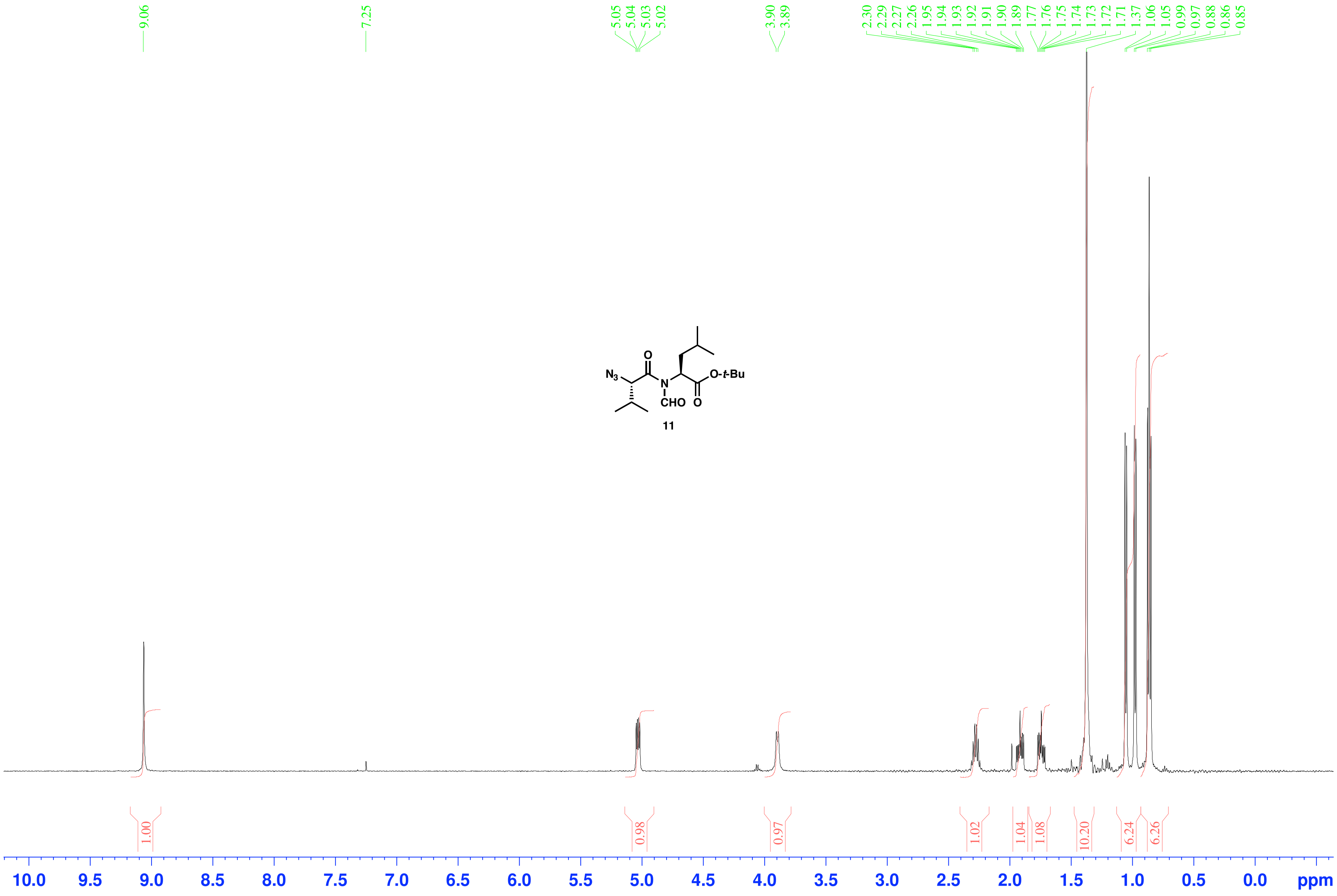


Carbon .* wux1 xw–V–45C13 (1 1) CDCl3 24.0C July_12,2010_11:28:56 DRX 500MHz zgpg30 13C; 1H O2=4.000 *.



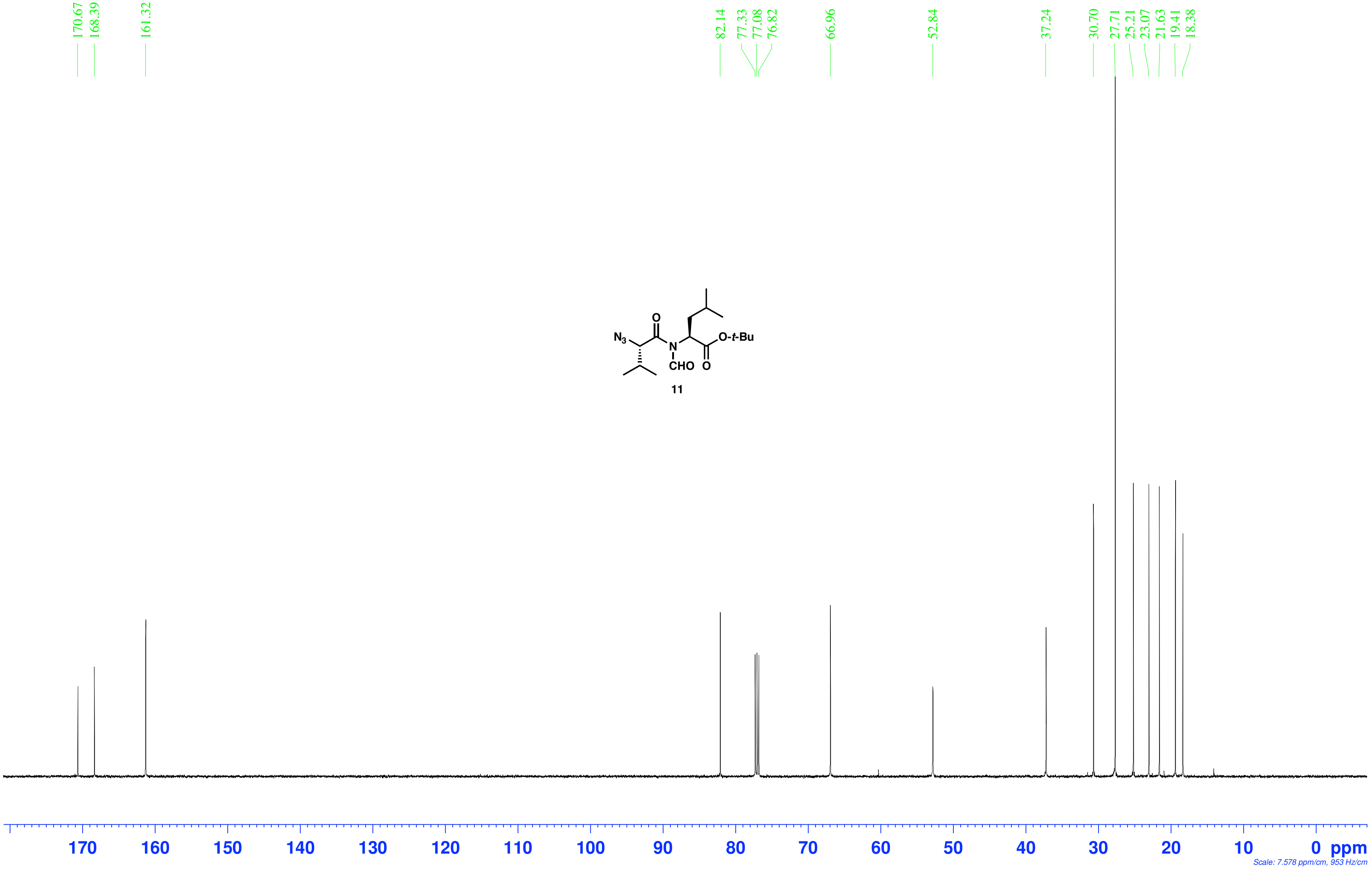
III

Proton24 .* wux1 xw–V–22H (1 1) CDCl3 24.0C February_10,2010_23:29:16 DRX 500MHz zg30 1H *.

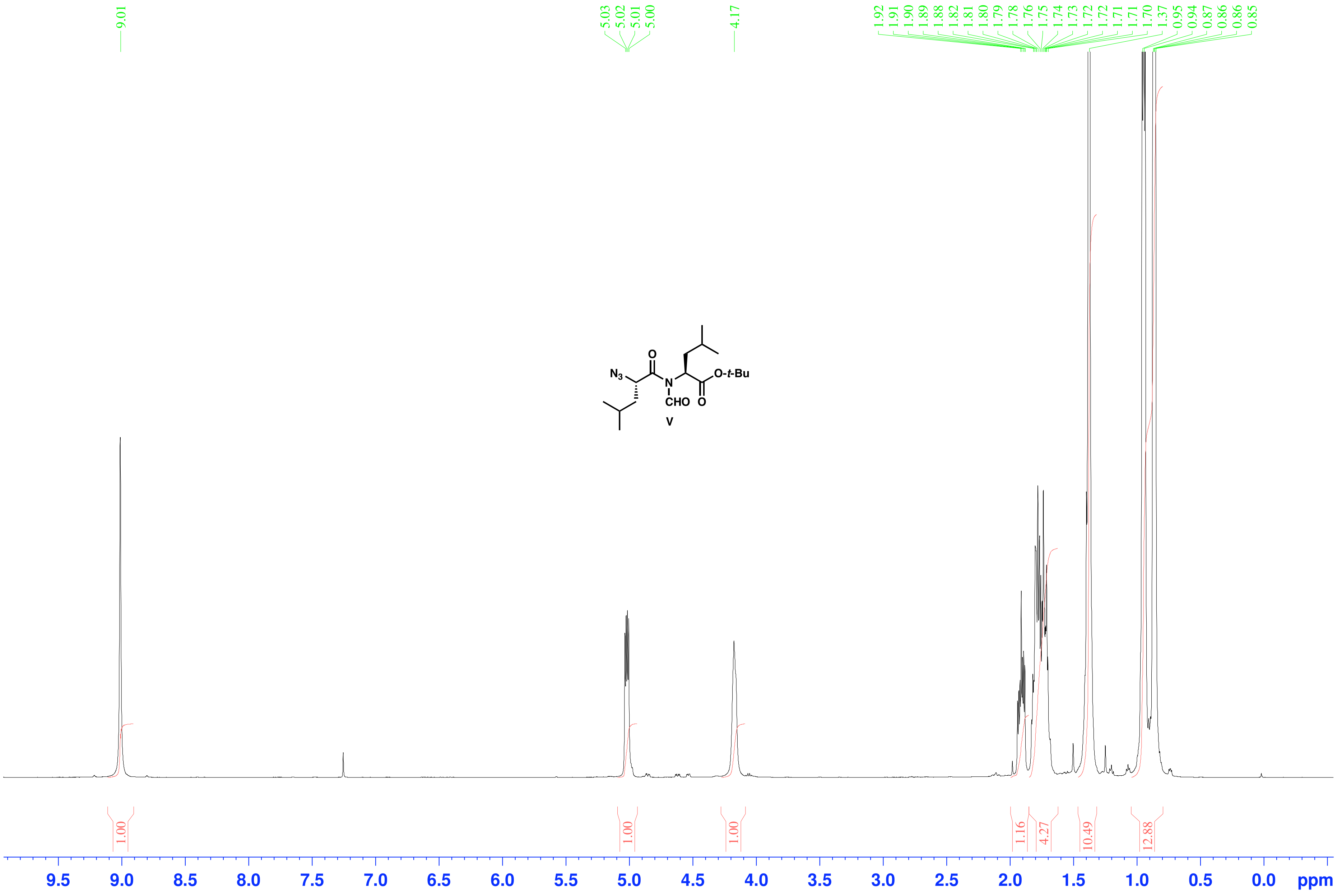


11

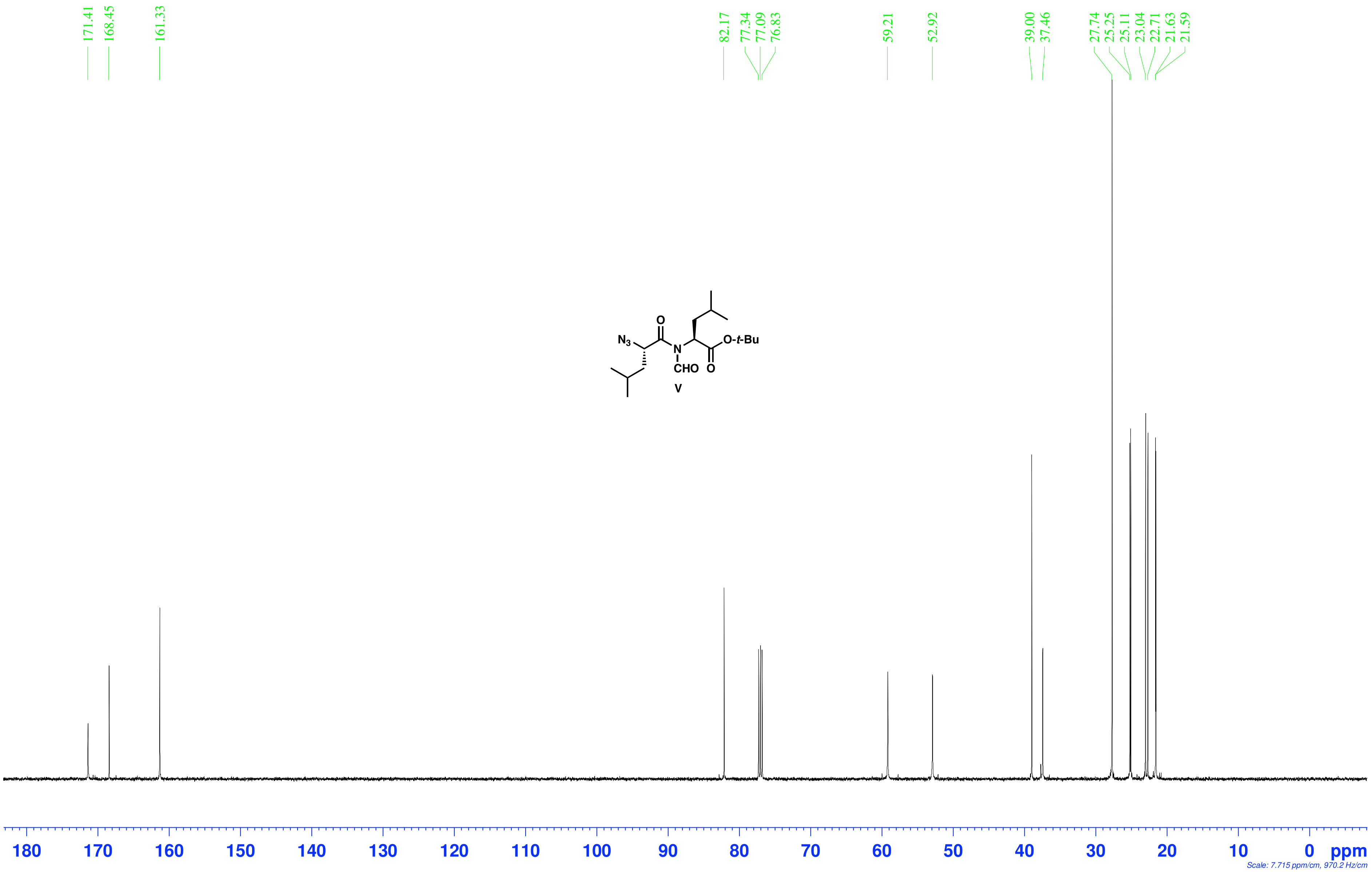
Carbon .* wux1 xw–V–22C13 (1 1) CDCl3 24.0C February_10,2010_23:32:51 DRX 500MHz zgpg30 13C; 1H O2=4.000 *.



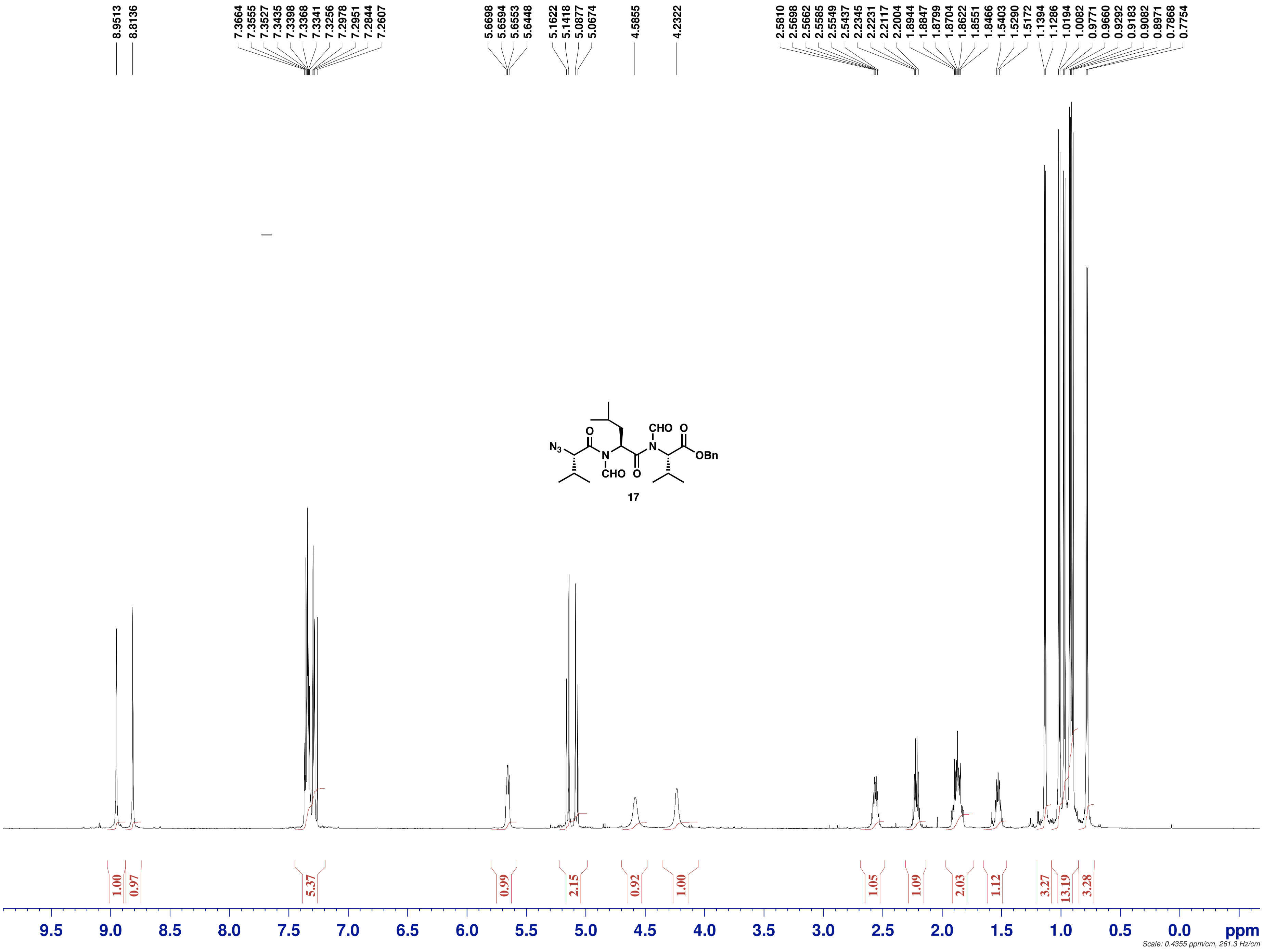
Proton24 .* wux1 xw–V–18H_ (1 1) CDCl3 24.0C January_13,2010_15:27:33 DRX 500MHz zg30 1H *.



Carbon .* wux1 xw−V−18C13 (1 1) CDCl3 24.0C January_13,2010_15:32:22 DRX 500MHz zgpg30 13C; 1H O2=4.000 *.

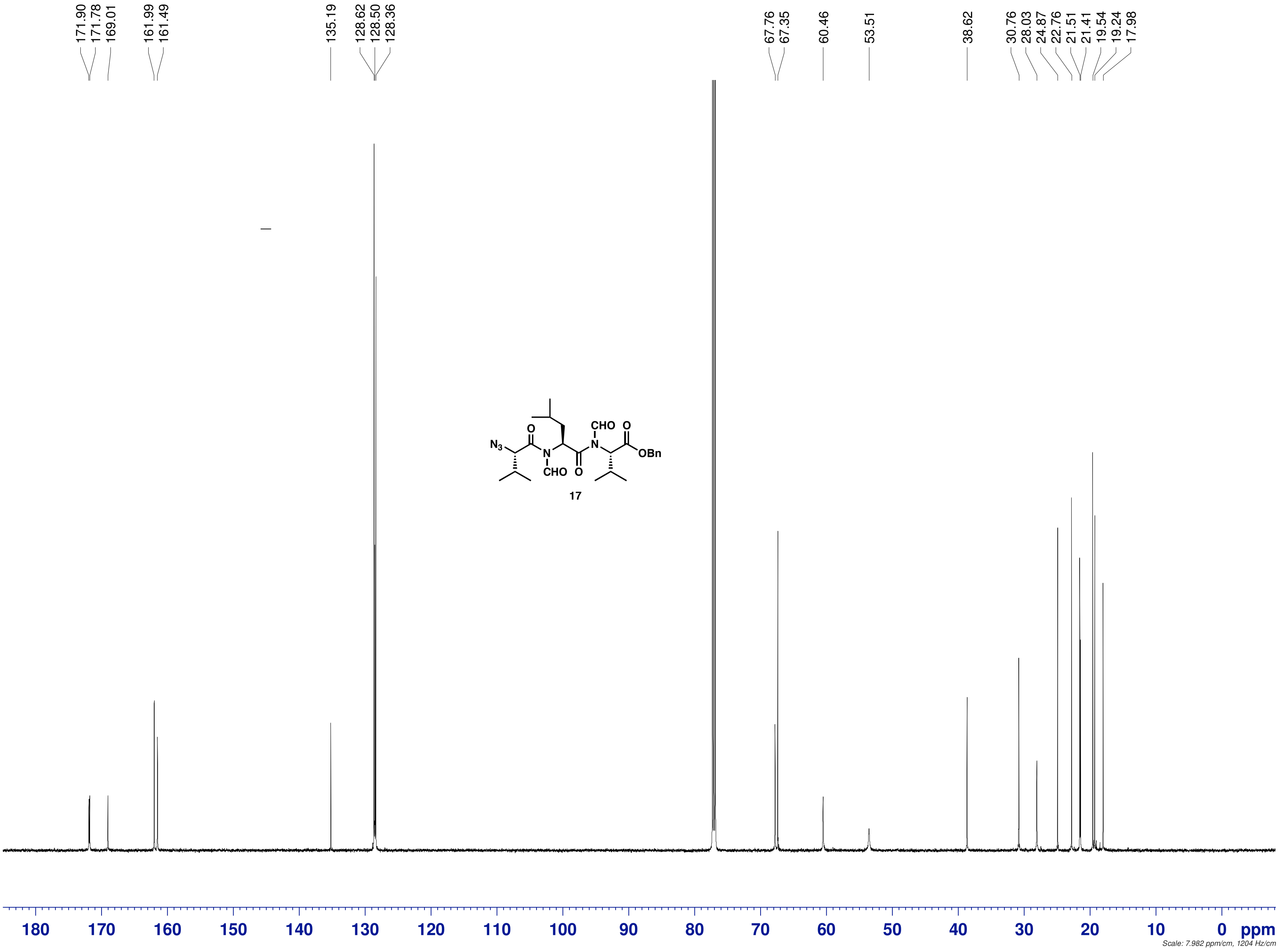


.* wux1 xw–V–23H (10 1) CDCl3   24.0C February_12,2010_16:22:38 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm   *.

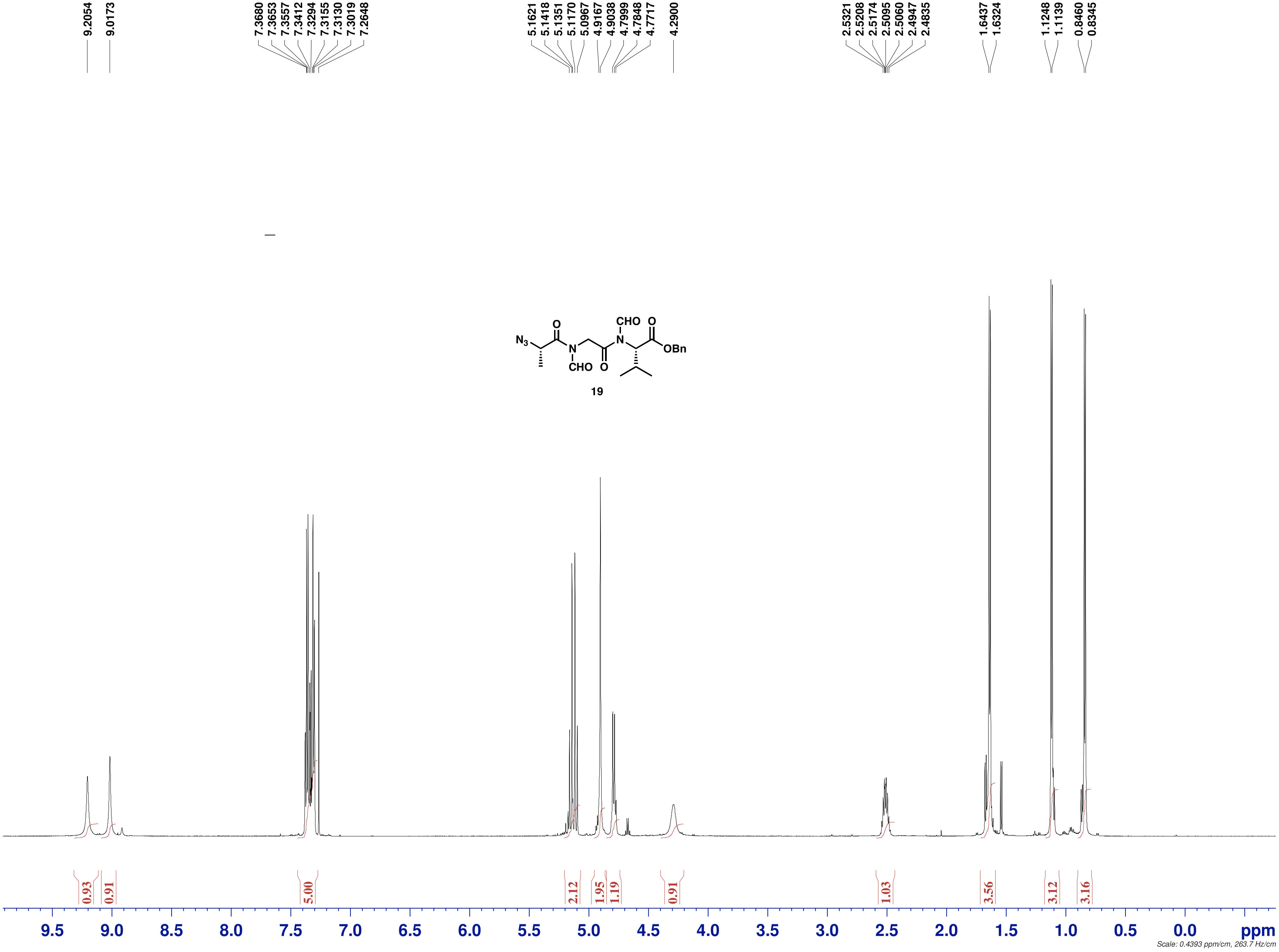


17

.* wux1 xw–V–23C13 (10 1) CDCl3   24.0C February_12,2010_17:01:27 Bruker AVIII 600MHz RRL1326: janggeum zgpg30 : 13C 110.000 ppm; 1H 4.500 ppm   *.

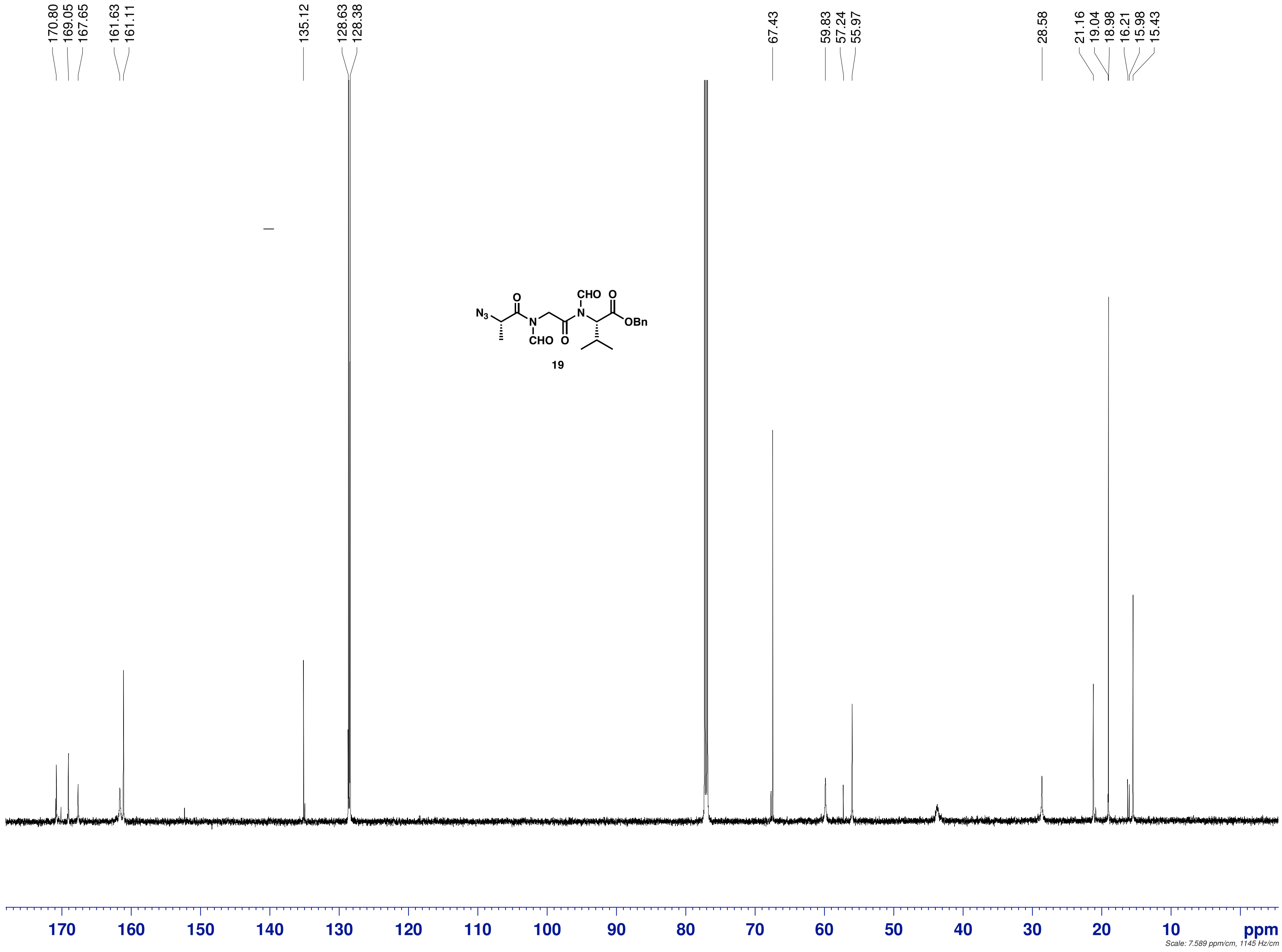


.* wux1 xw-V-46H (10 1) CDCl3   24.0C July_15,2010_18:13:01 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm   *.

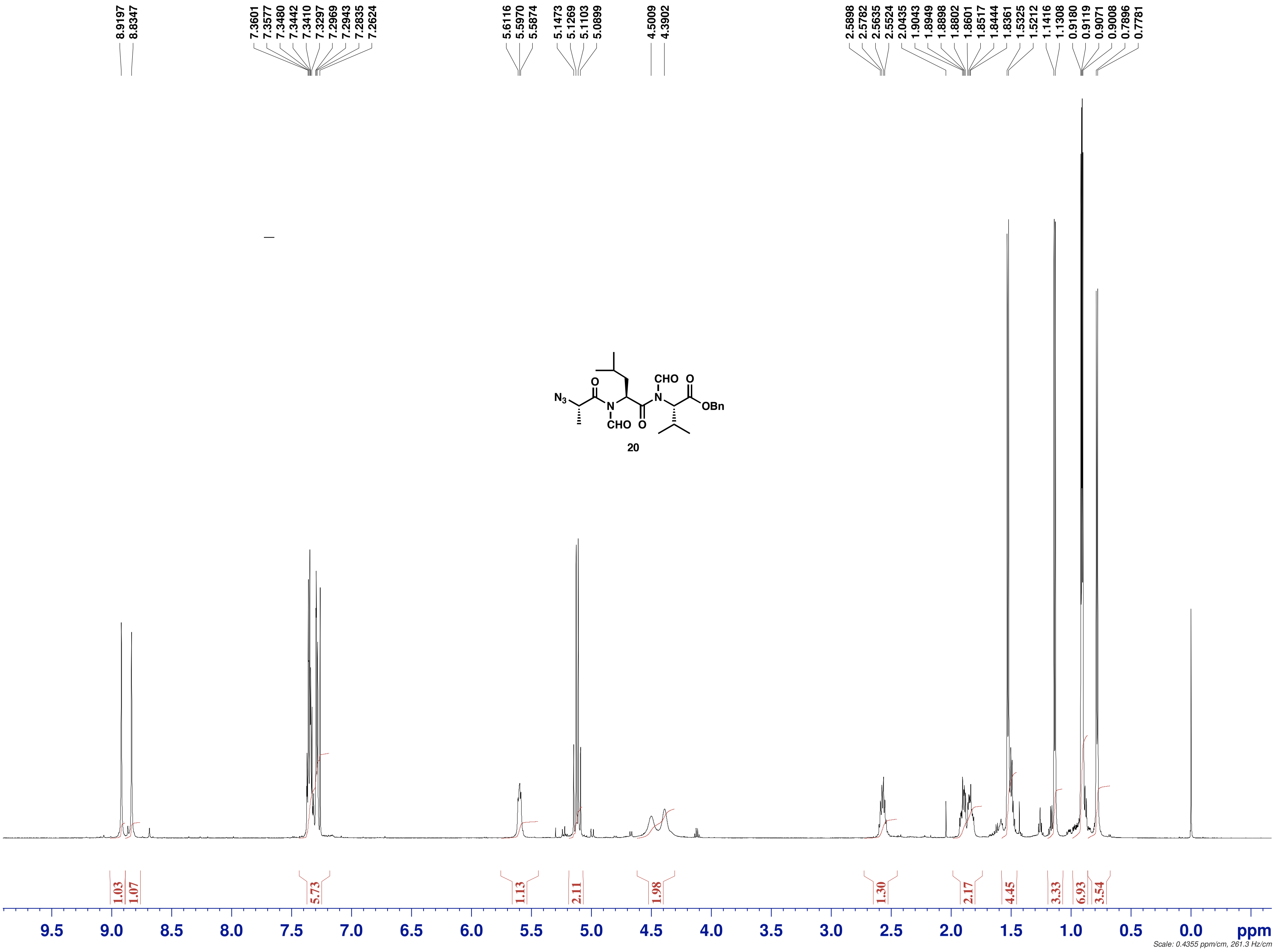


19

.* wux1 xw–V–46C13 (10 1) CDCl3   24.0C July_15,2010_18:51:29 Bruker AVIII 600MHz RRL1326: janggeum zgpg30 : 13C 110.000 ppm; 1H 4.500 ppm   *.

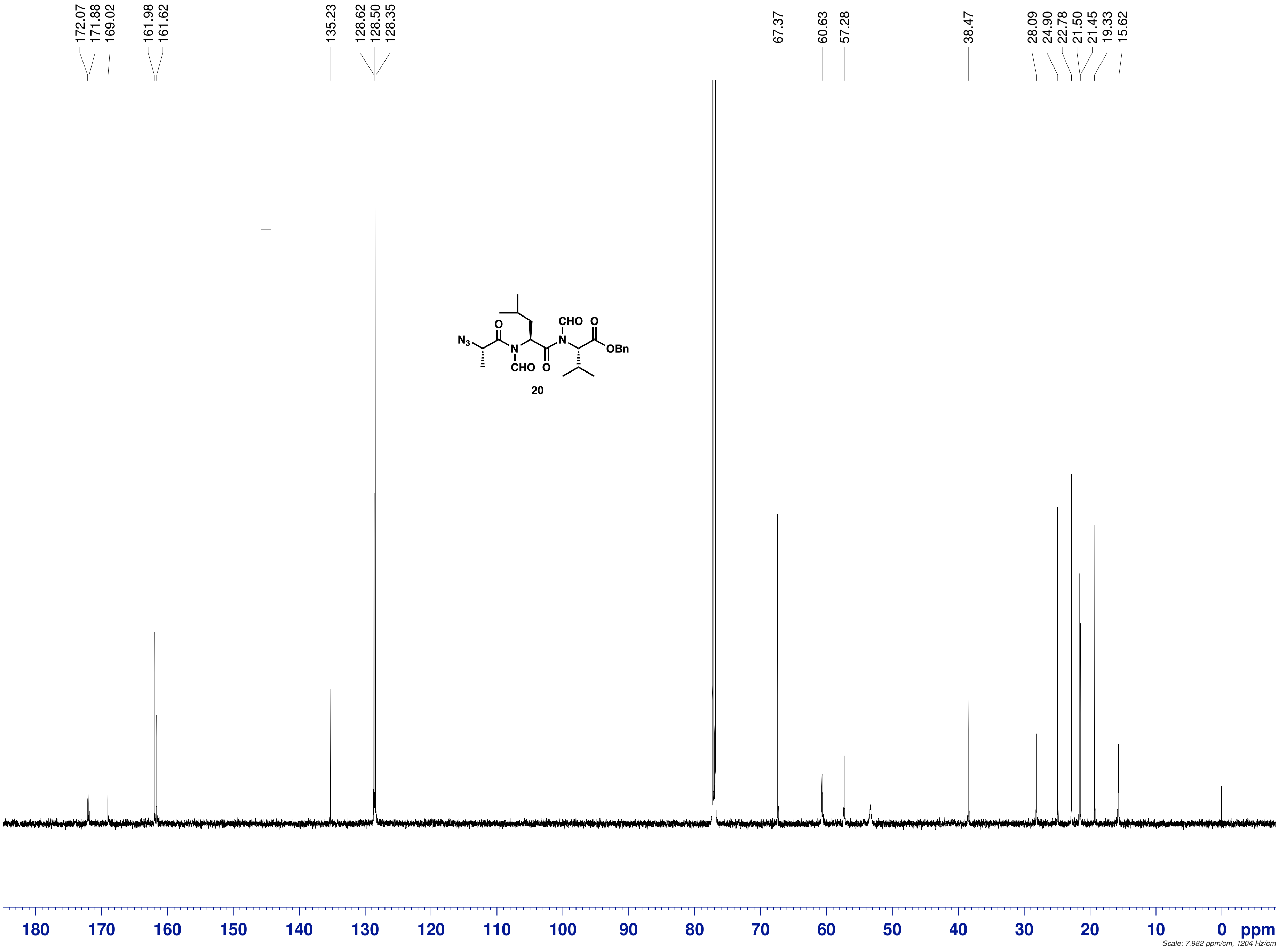


.* wux1 xw-V-36H (10 1) CDCl3   24.0C April_26,2010_14:41:30 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm   *.



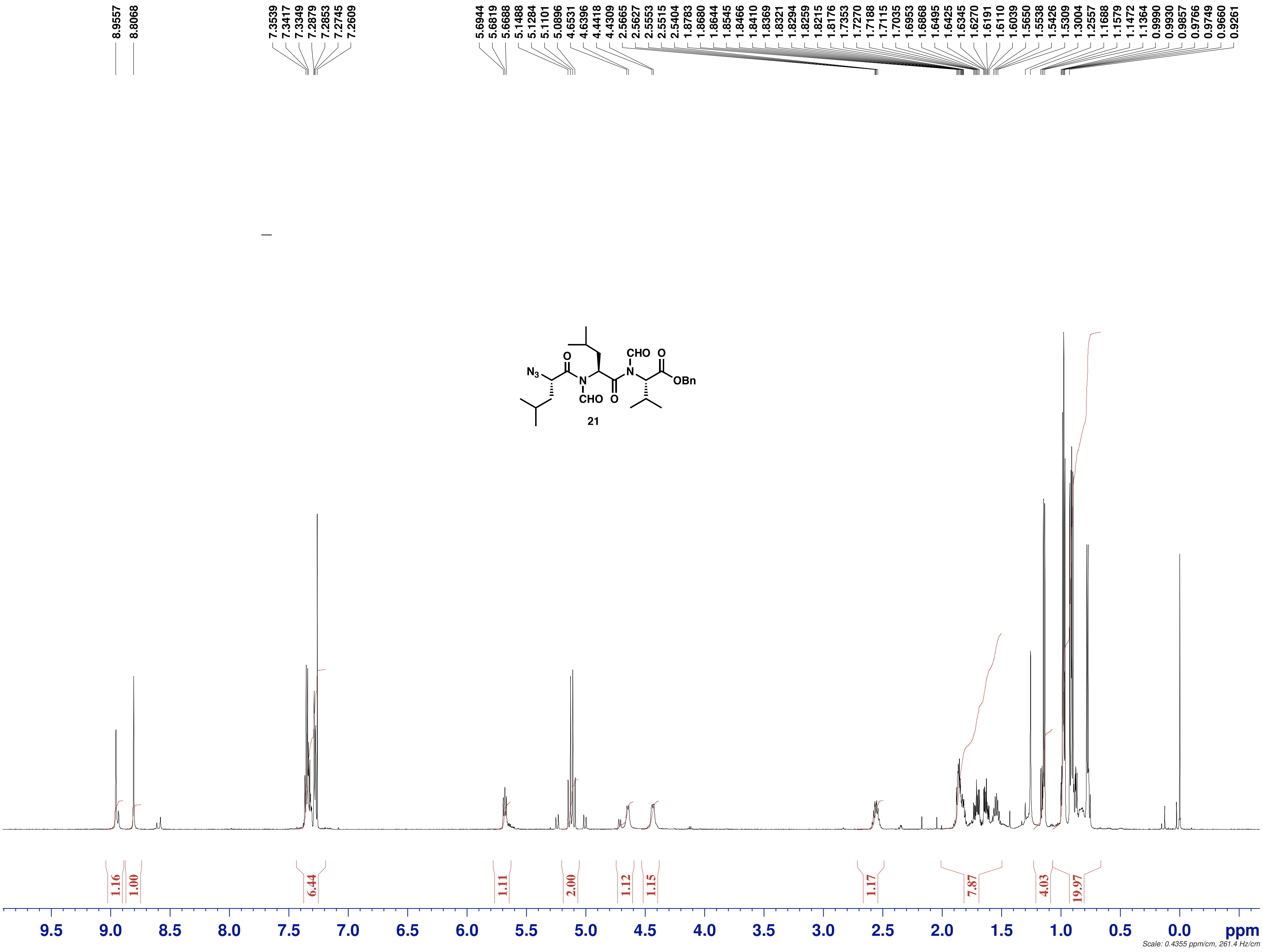
20

.* wux1 xw–V–36C13 (10 1) CDCl3   24.0C April_26,2010_14:44:27 Bruker AVIII 600MHz RRL1326: janggeum zgpg30 : 13C 110.000 ppm; 1H 4.500 ppm   *.

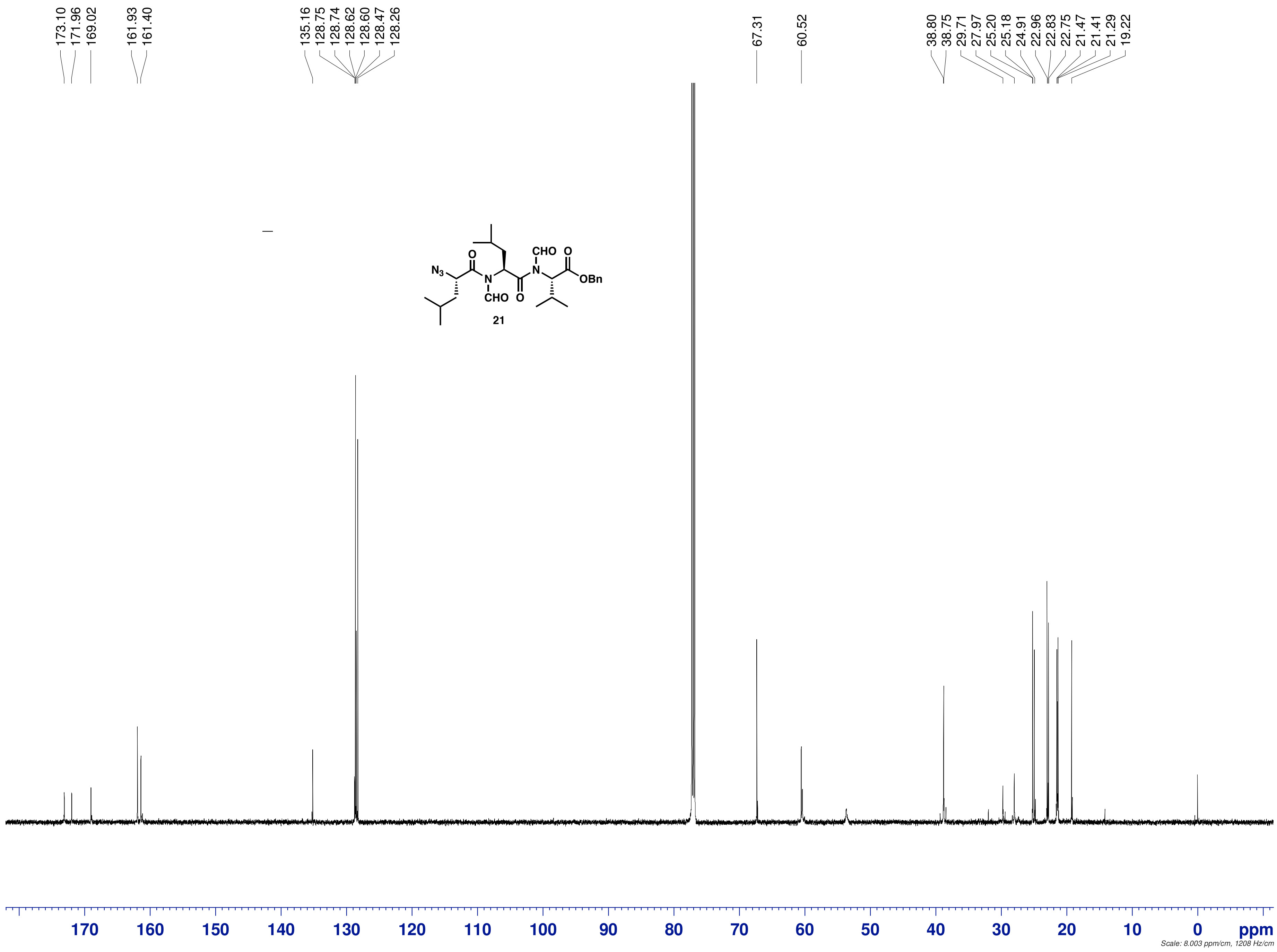


20

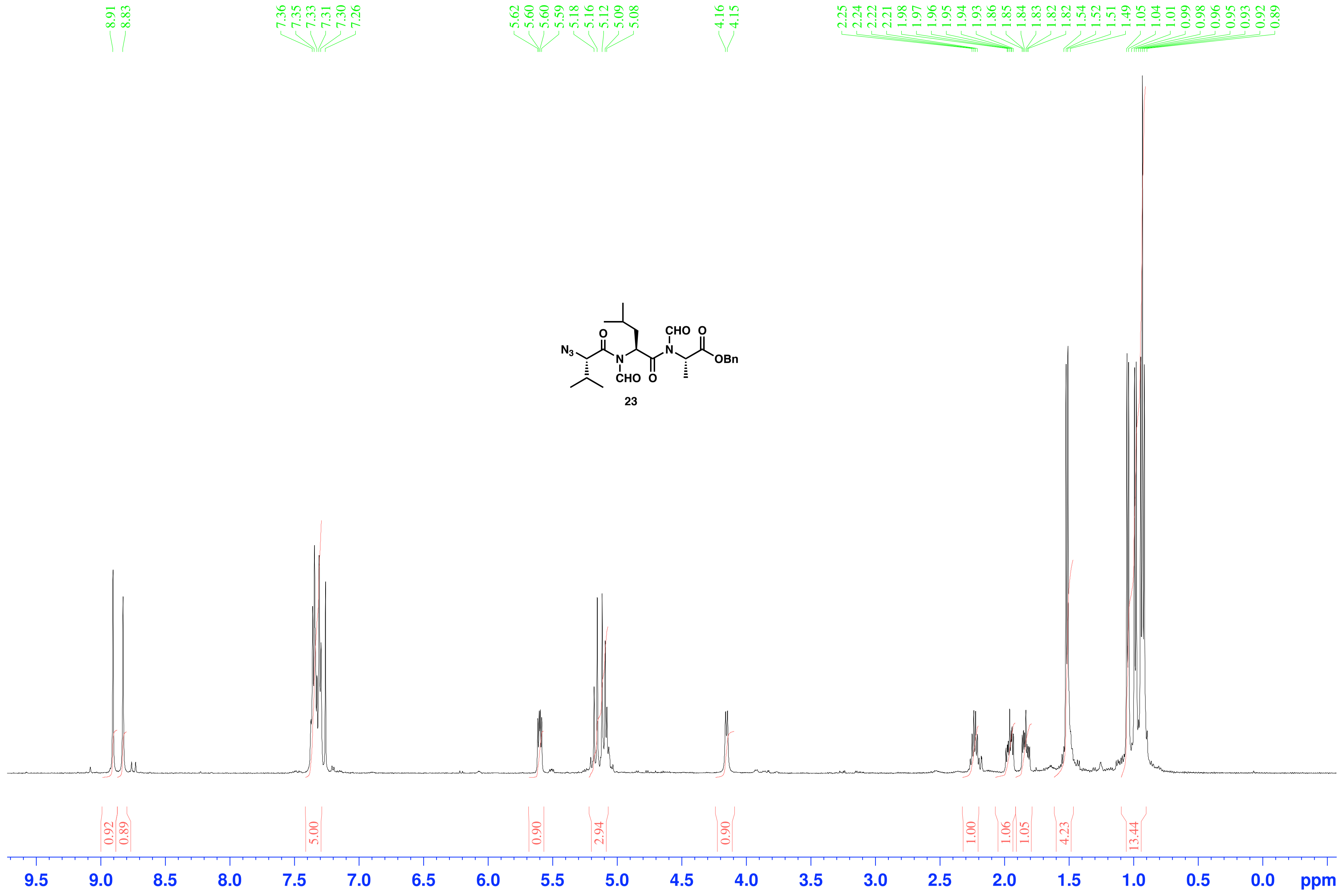
.* wux1 xw-V-20H (10 1) CDCl3   24.0C April_17,2010_22:45:21 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm   *.



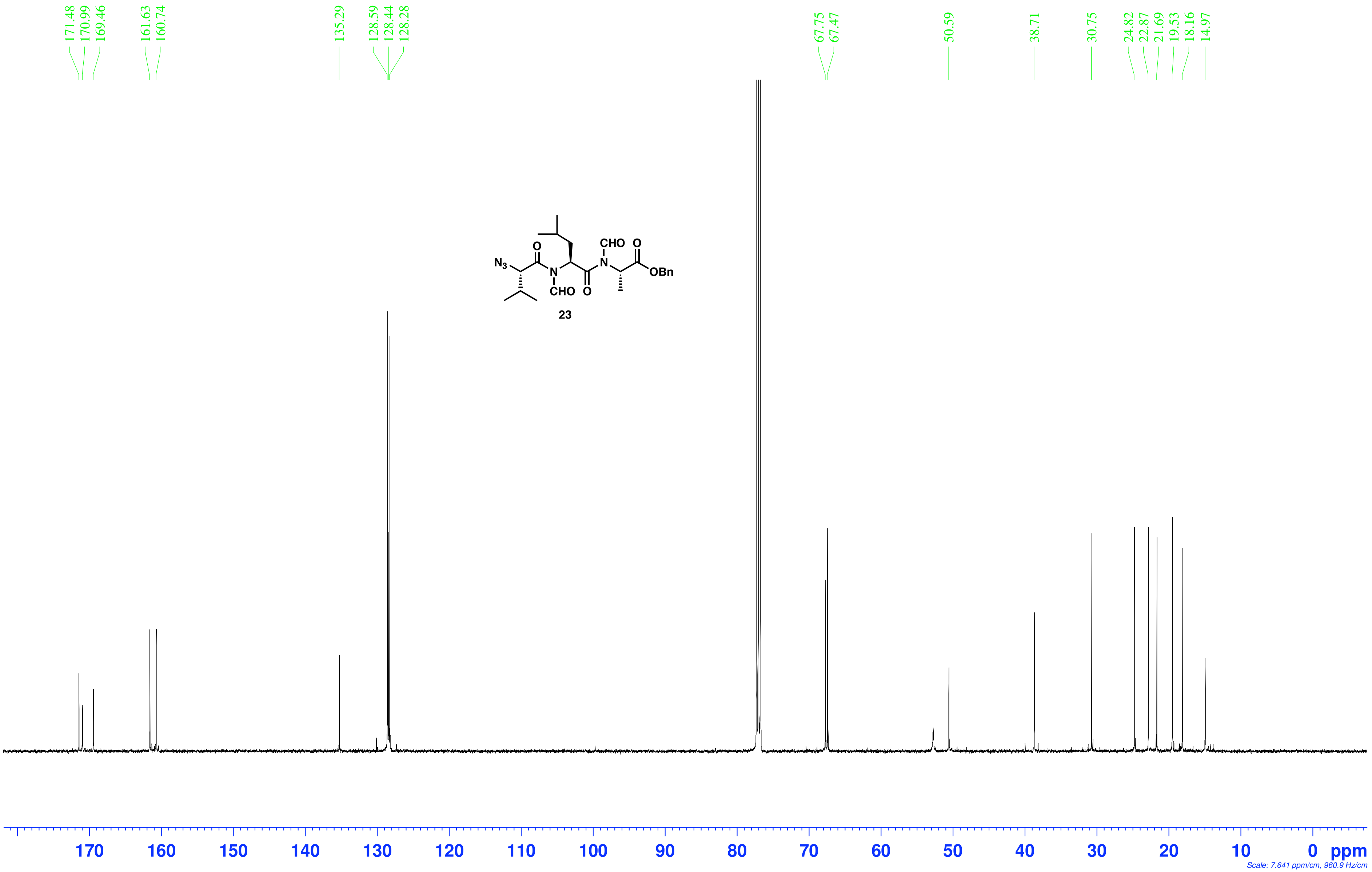
.* wux1 xw-V-20C13 (10 1) CDCl3   24.0C April_17,2010_23:58:33 Bruker AVIII 600MHz RRL1326: janggeum zgpg30 : 13C 110.000 ppm; 1H 4.500 ppm   *.


21

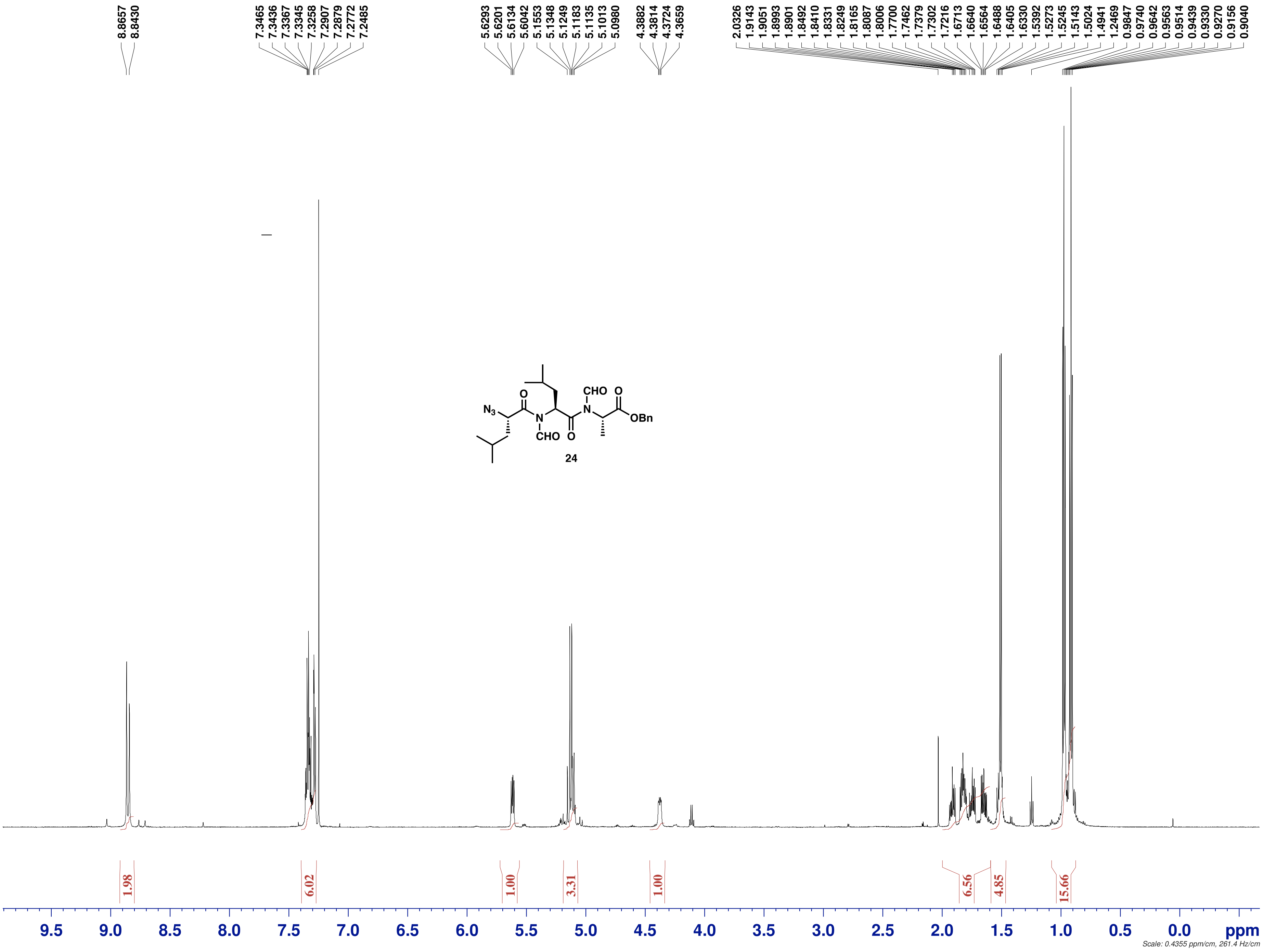
Proton24 .* wux1 xw-V-51H (1 1) CDCl3 24.0C February_04,2011_23:39:52 DRX 500MHz zg30 1H *.



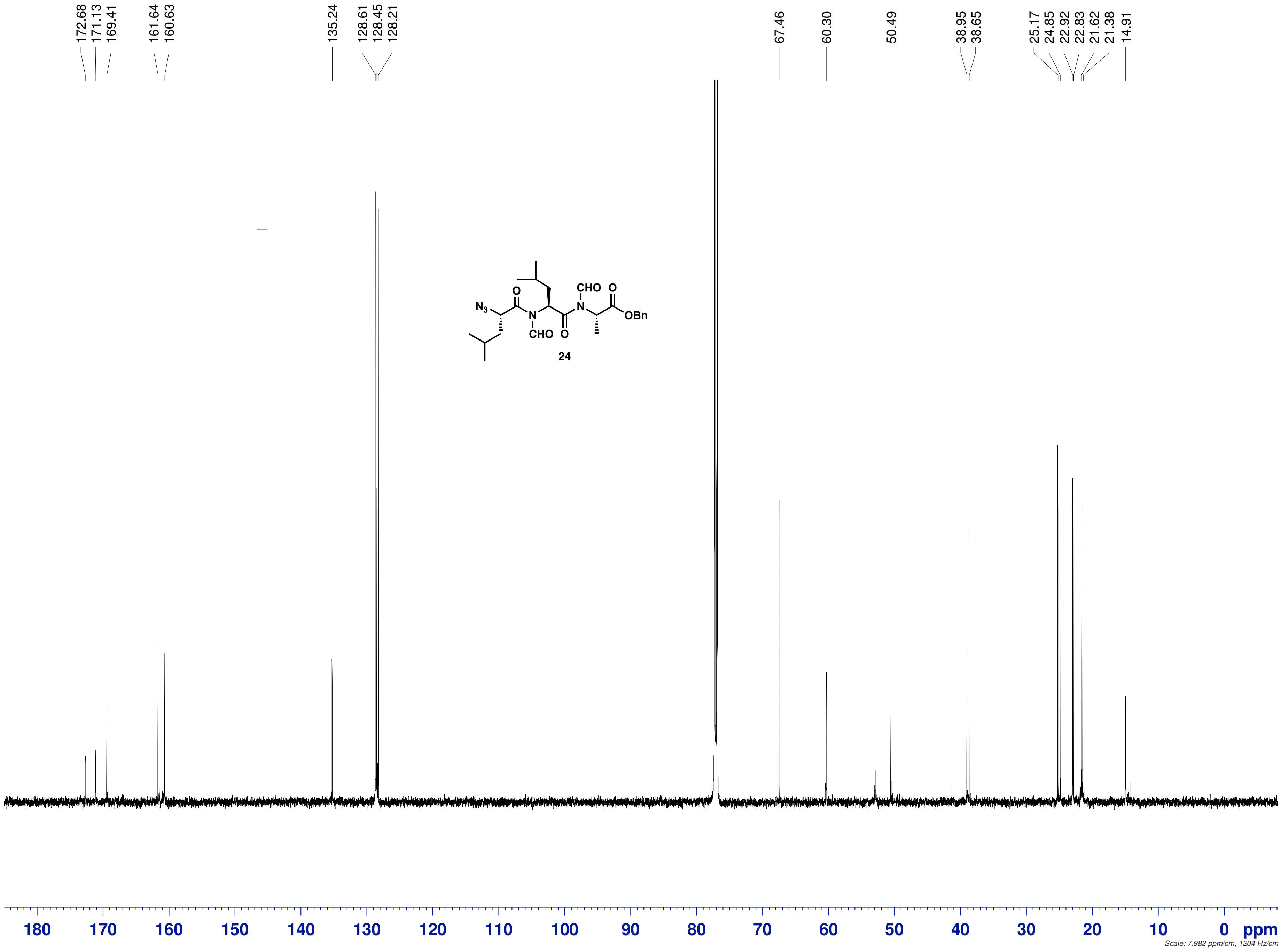
Carbon .* wux1 xw–V–51C13 (1 1) CDCl3 24.0C February_05,2011_00:08:50 DRX 500MHz zgpg30 13C; 1H O2=4.000 *.



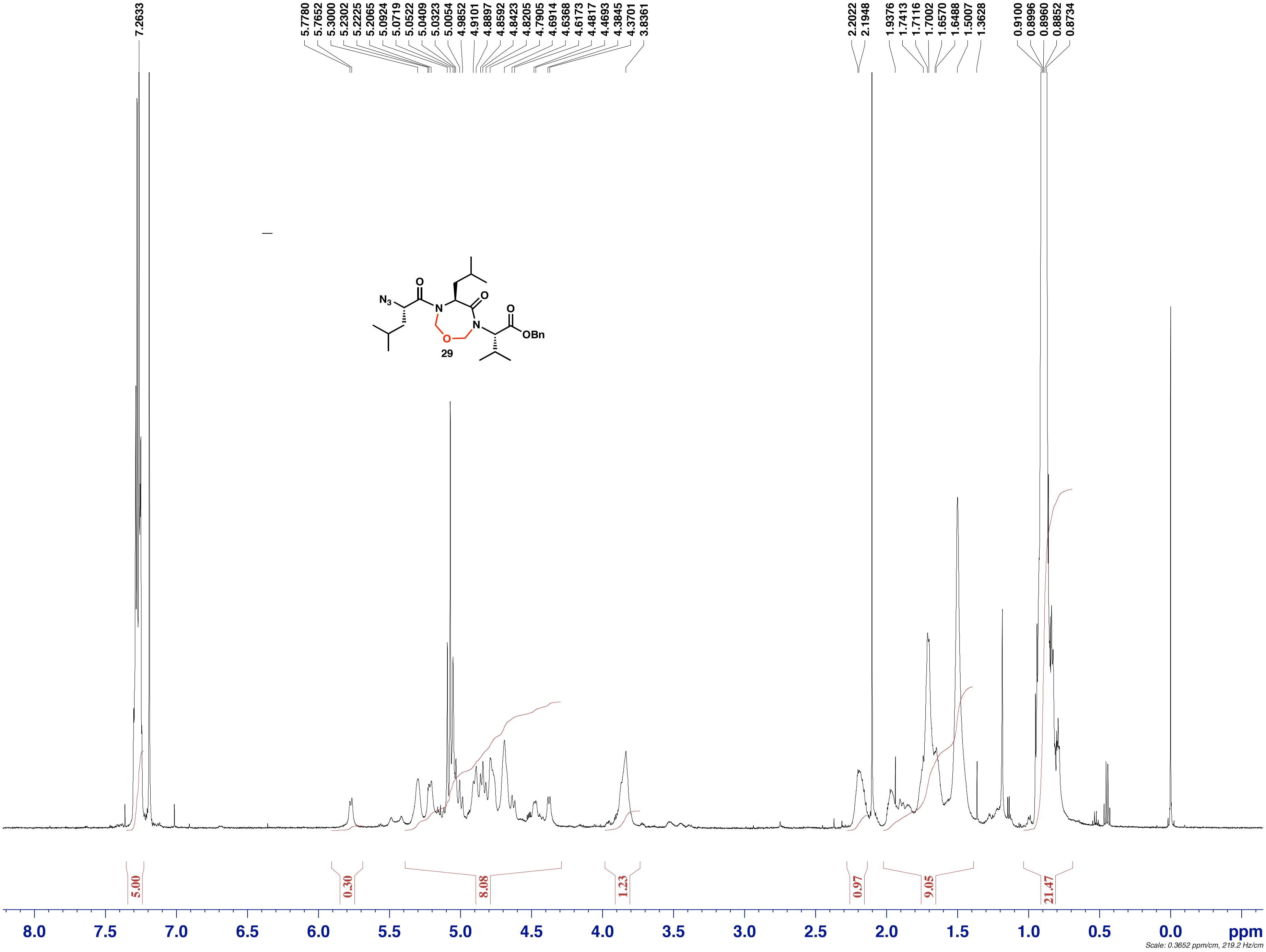
.* wux1 xw−V−50H (10 1) CDCl3   24.0C February_05,2011_13:41:33 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm   *.

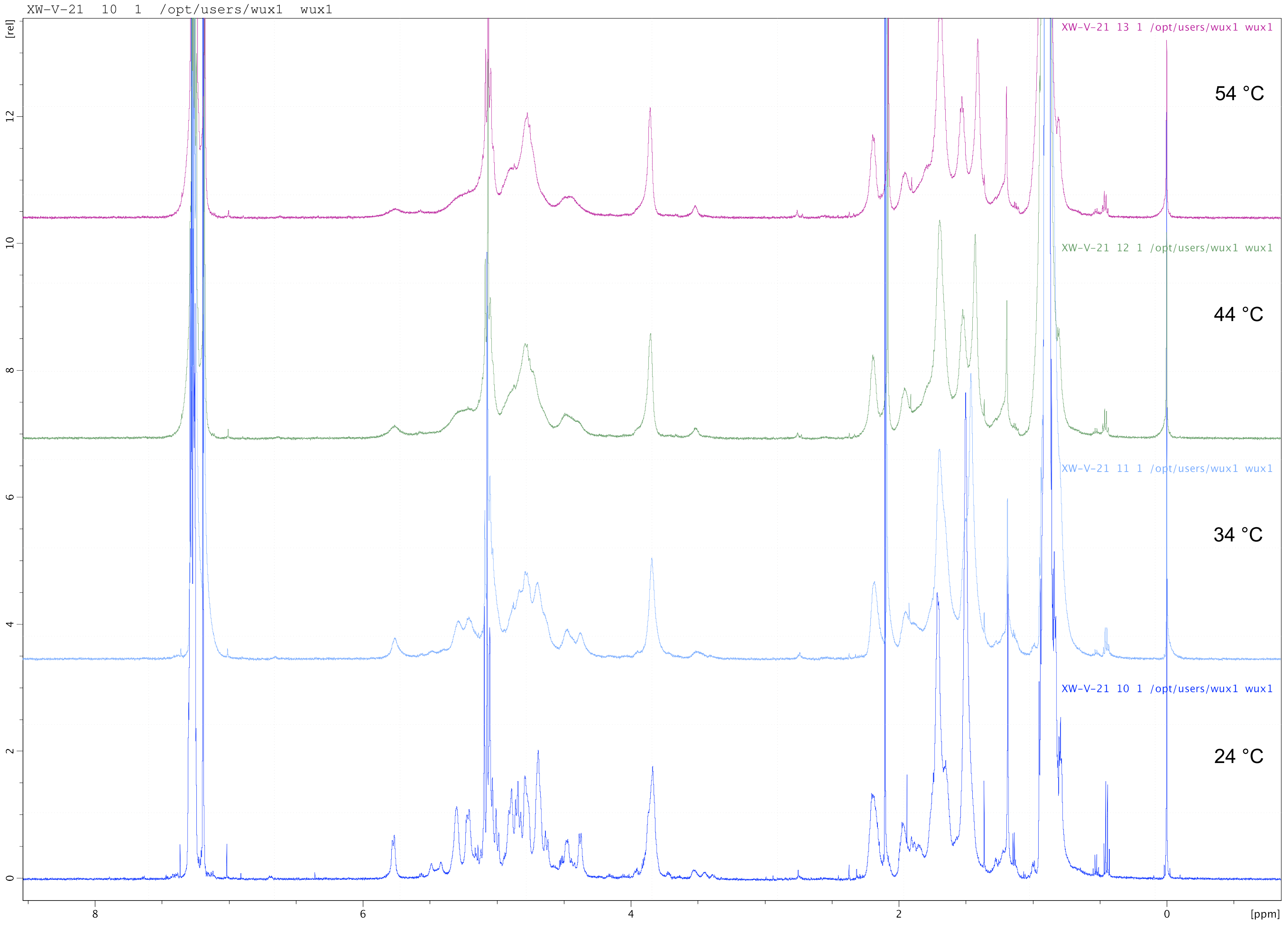


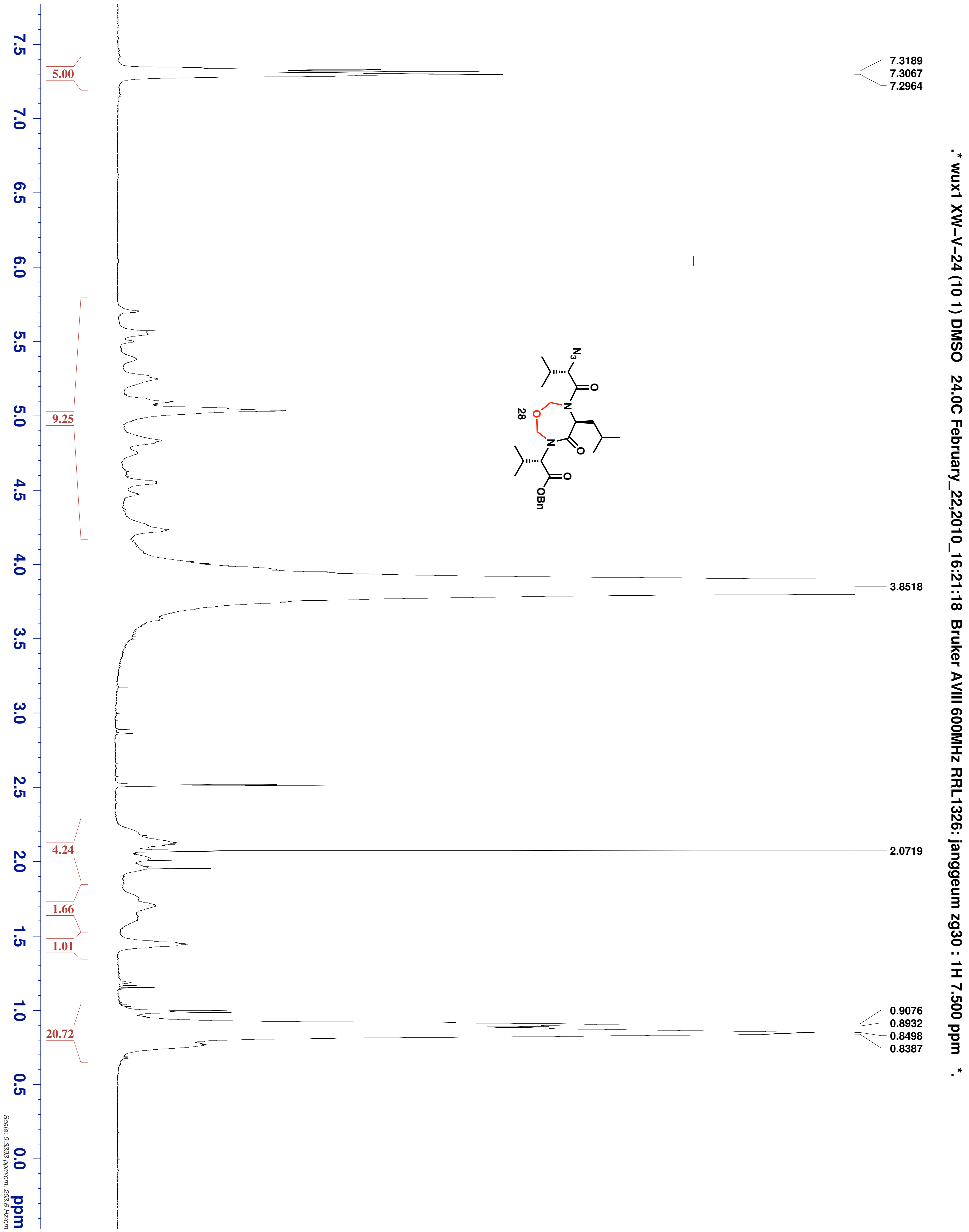
.* wux1 xw–V–50C13 (10 1) CDCl3   24.0C February_05,2011_14:20:14 Bruker AVIII 600MHz RRL1326: janggeum zgpg30 : 13C 110.000 ppm; 1H 4.500 ppm   *.

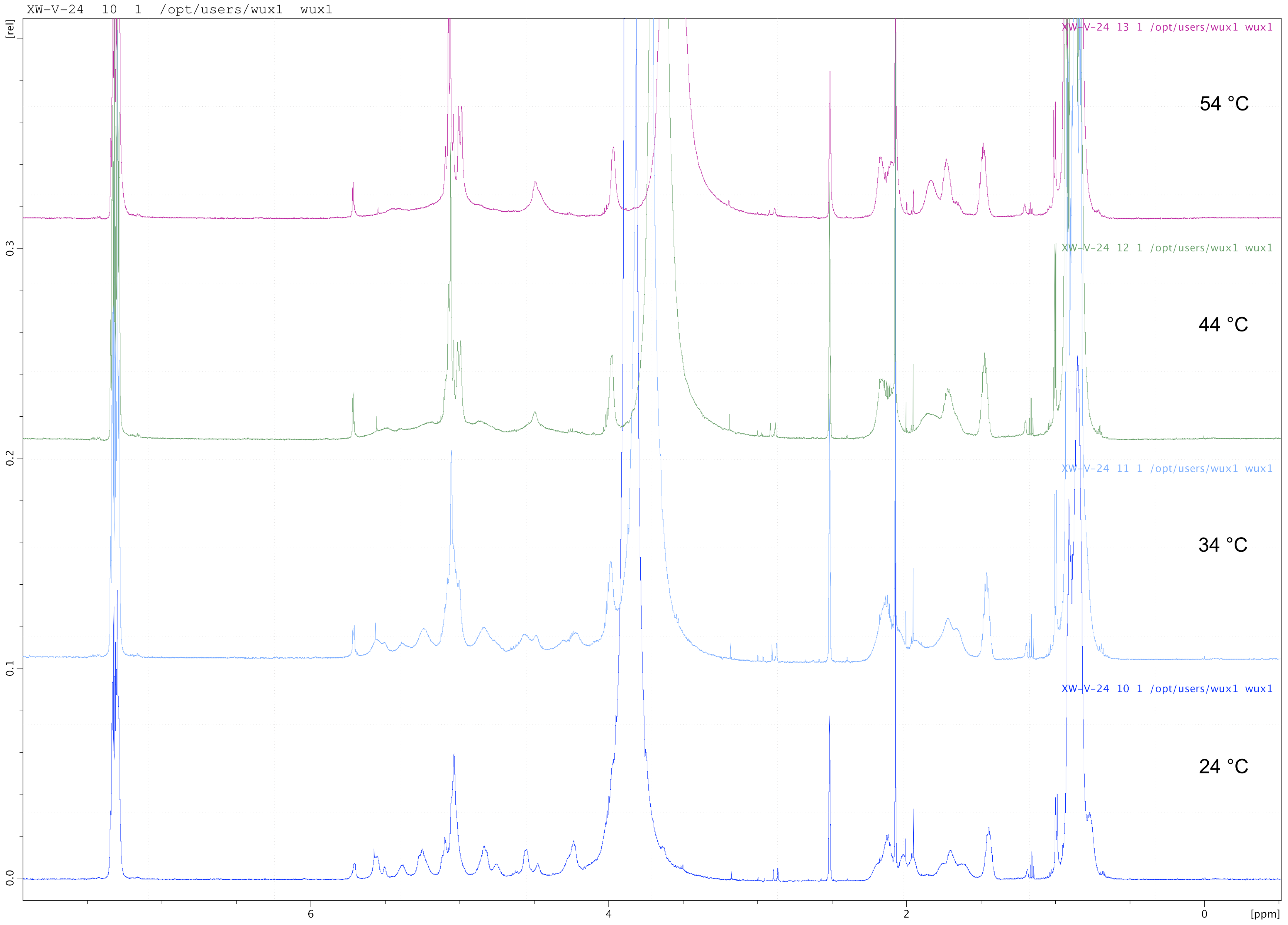


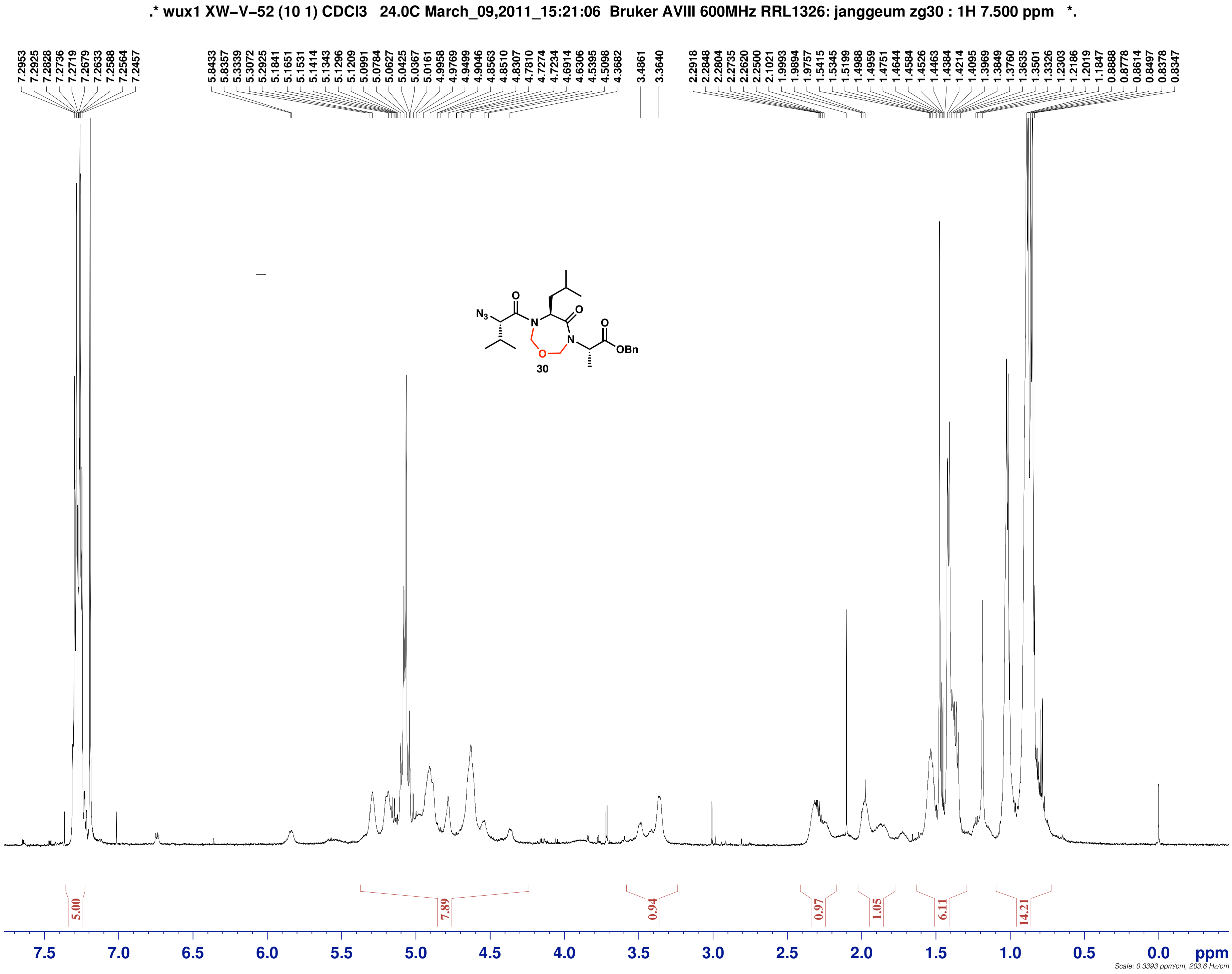
.* wux1 XW–V–21 (10 1) CDCl3 24.0C March_08,2011_15:01:25 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm *.




XW-V-21 10 1 /opt/users/wux1 wux1
[rel]
0
2
4
6
8
10
12
XW-V-21 13 1 /opt/users/wux1 wux1
54 °C
XW-V-21 12 1 /opt/users/wux1 wux1
44 °C
XW-V-21 11 1 /opt/users/wux1 wux1
34 °C
XW-V-21 10 1 /opt/users/wux1 wux1
24 °C
8
6
4
2
0
[ppm]

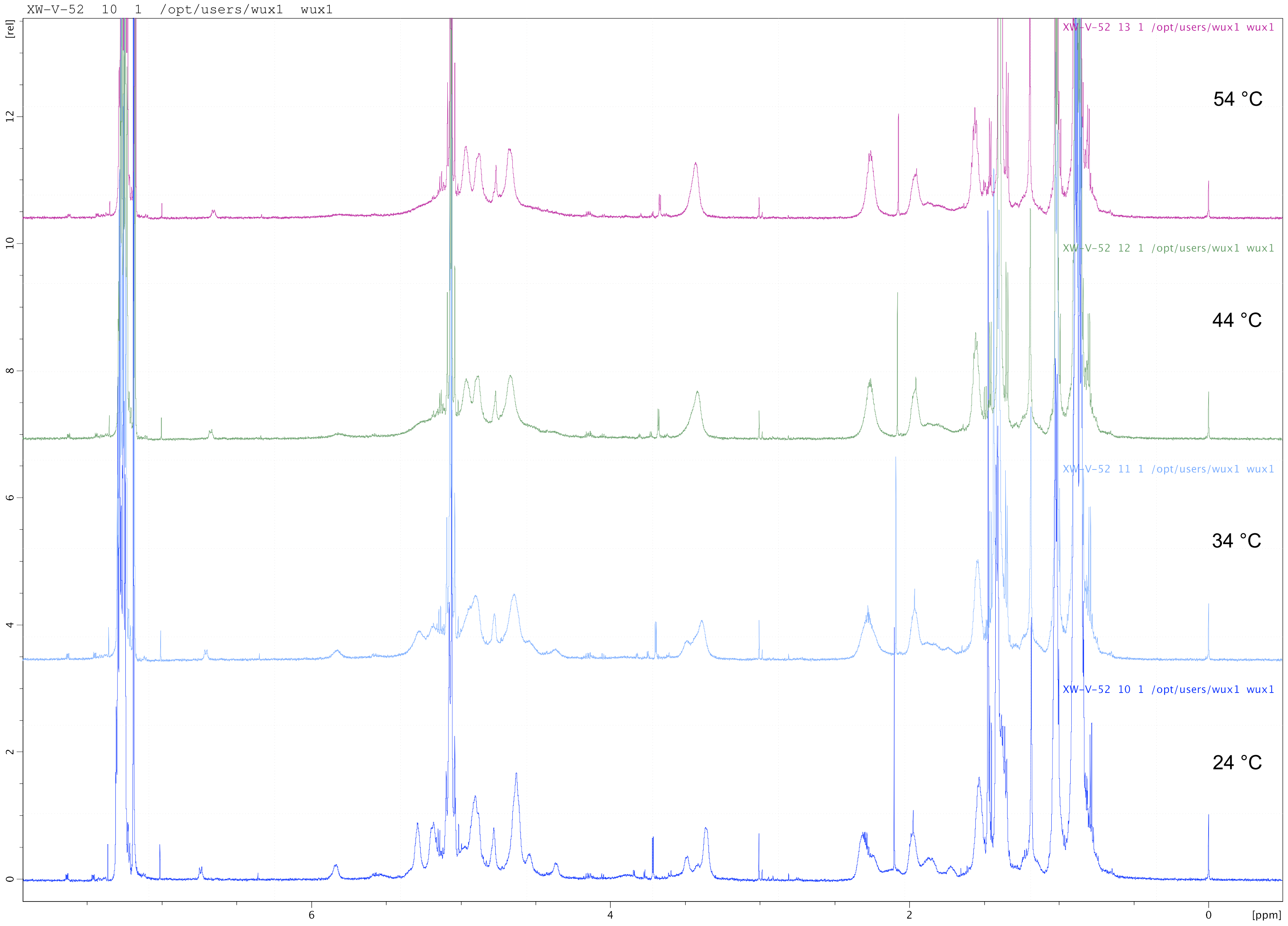

* wux1 XW–V–24 (10 1) DMSO 24.0C February_22,2010_16:21:18 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm *
7.3189
7.3067
7.2964
3.8518
2.0719
0.9076
0.8932
0.8498
0.8387
5.00
9.25
4.24
1.66
1.01
20.72
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
Scale: 0.3393 ppm/cm, 203.6 Hz/cm
N3
O
N
O
N
O
O
OBn
28

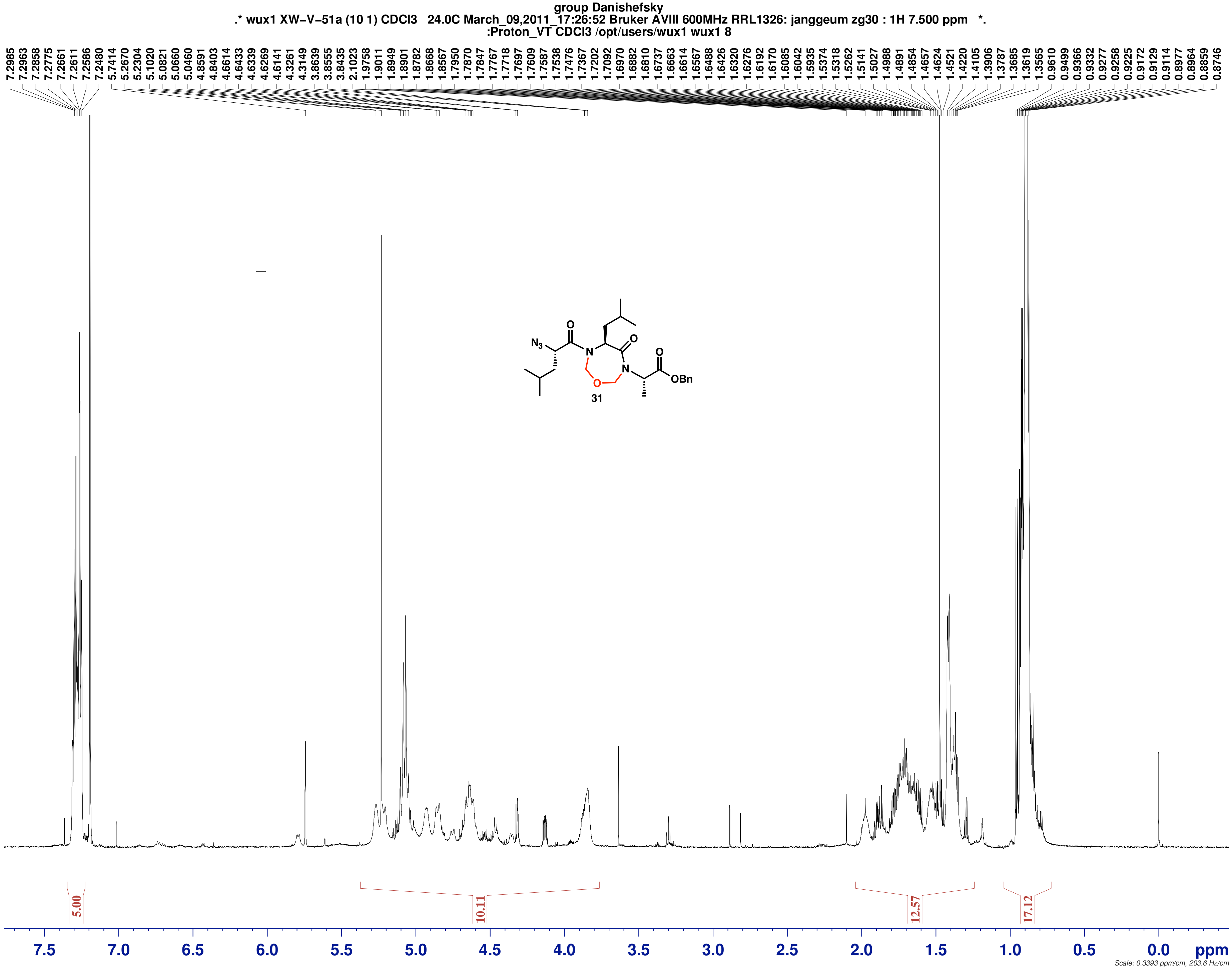

XW-V-24 10 1 /opt/users/wux1 wux1
[rel]
0.0
0.1
0.2
0.3
XW-V-24 13 1 /opt/users/wux1 wux1
54 °C
XW-V-24 12 1 /opt/users/wux1 wux1
44 °C
XW-V-24 11 1 /opt/users/wux1 wux1
34 °C
XW-V-24 10 1 /opt/users/wux1 wux1
24 °C
6
4
2
0
[ppm]

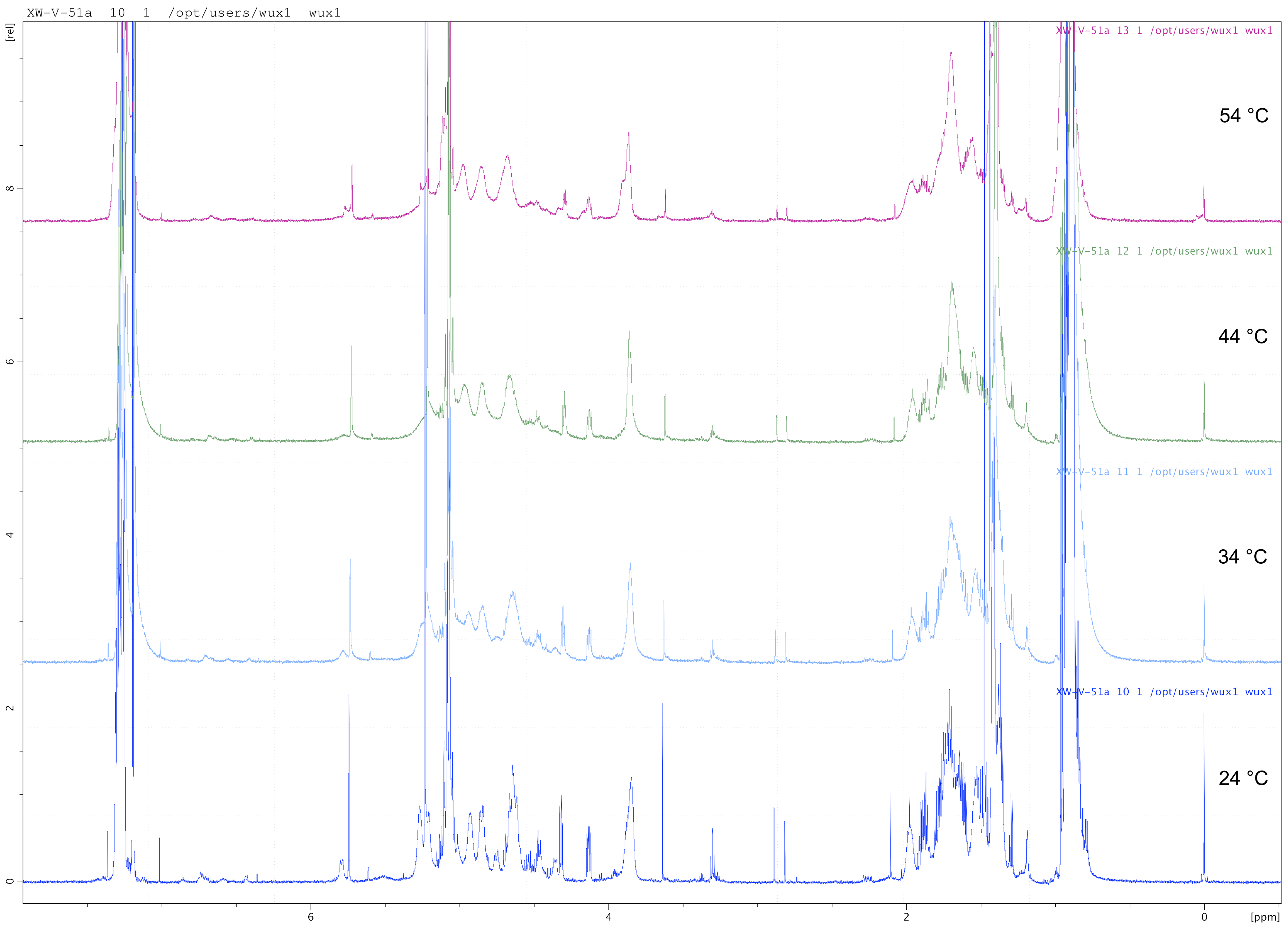


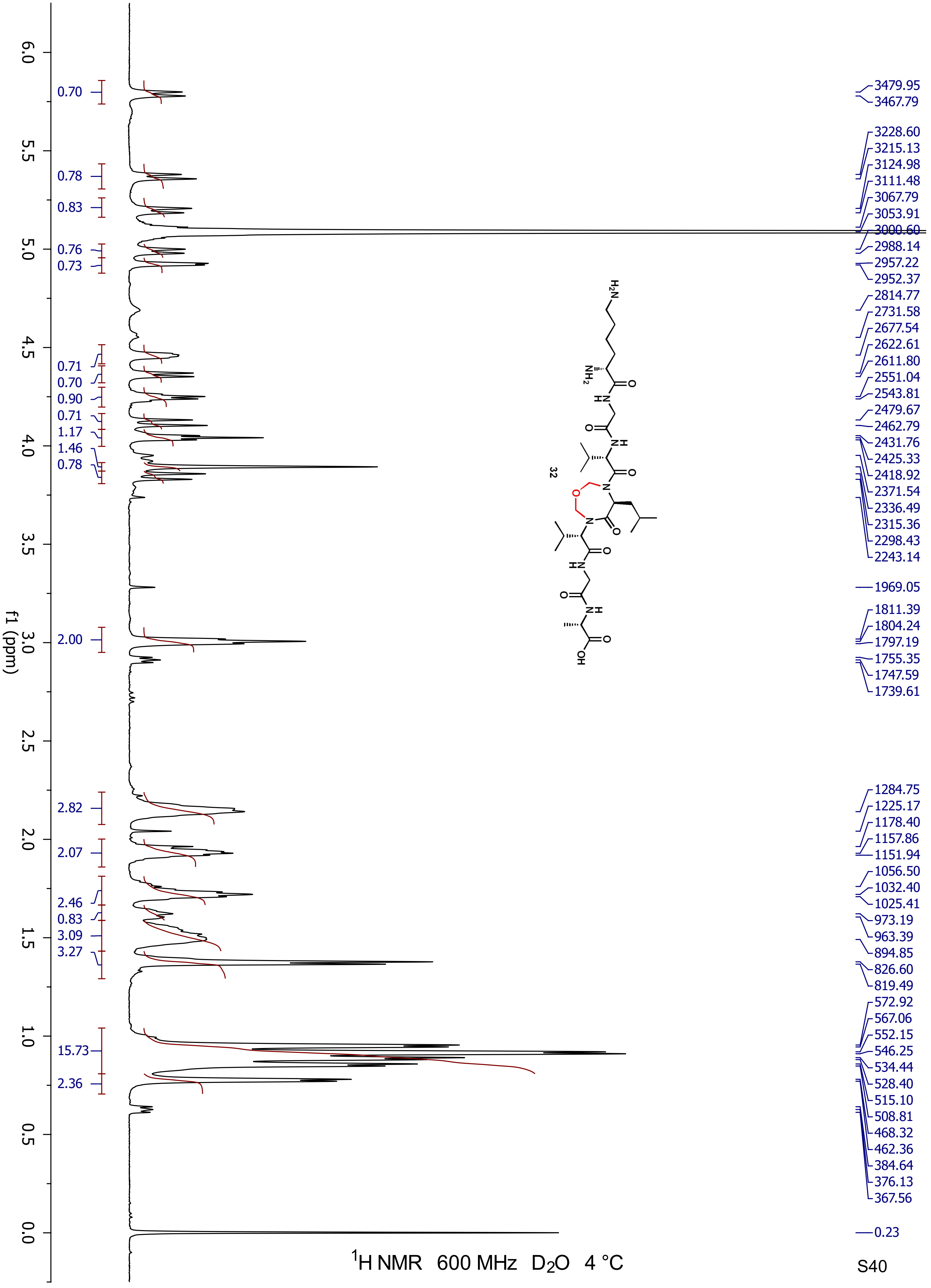
30

.* wux1 XW−V−52 (10 1) CDCl3 24.0C March_09,2011_15:21:06 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm *.
7.2953
7.2925
7.2828
7.2736
7.2719
7.2679
7.2633
7.2588
7.2564
7.2457
5.8433
5.8357
5.3339
5.3072
5.2925
5.1841
5.1651
5.1531
5.1414
5.1343
5.1296
5.1209
5.0991
5.0784
5.0627
5.0425
5.0367
5.0161
4.9958
4.9769
4.9499
4.9046
4.8563
4.8510
4.8307
4.7810
4.7274
4.7234
4.6914
4.6306
4.5395
4.5098
4.3682
3.4861
3.3640
2.2918
2.2848
2.2804
2.2735
2.2620
2.2500
2.1021
1.9993
1.9894
1.9757
1.5415
1.5345
1.5199
1.4988
1.4959
1.4751
1.4644
1.4584
1.4526
1.4463
1.4384
1.4214
1.4095
1.3969
1.3849
1.3760
1.3635
1.3501
1.3326
1.2303
1.2186
1.2019
1.1847
0.8888
0.8778
0.8614
0.8497
0.8378
0.8347
N_3
O
N
O
N
O
OBn
5.00
7.89
0.94
0.97
1.05
6.11
14.21
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
Scale: 0.3393 ppm/cm, 203.6 Hz/cm

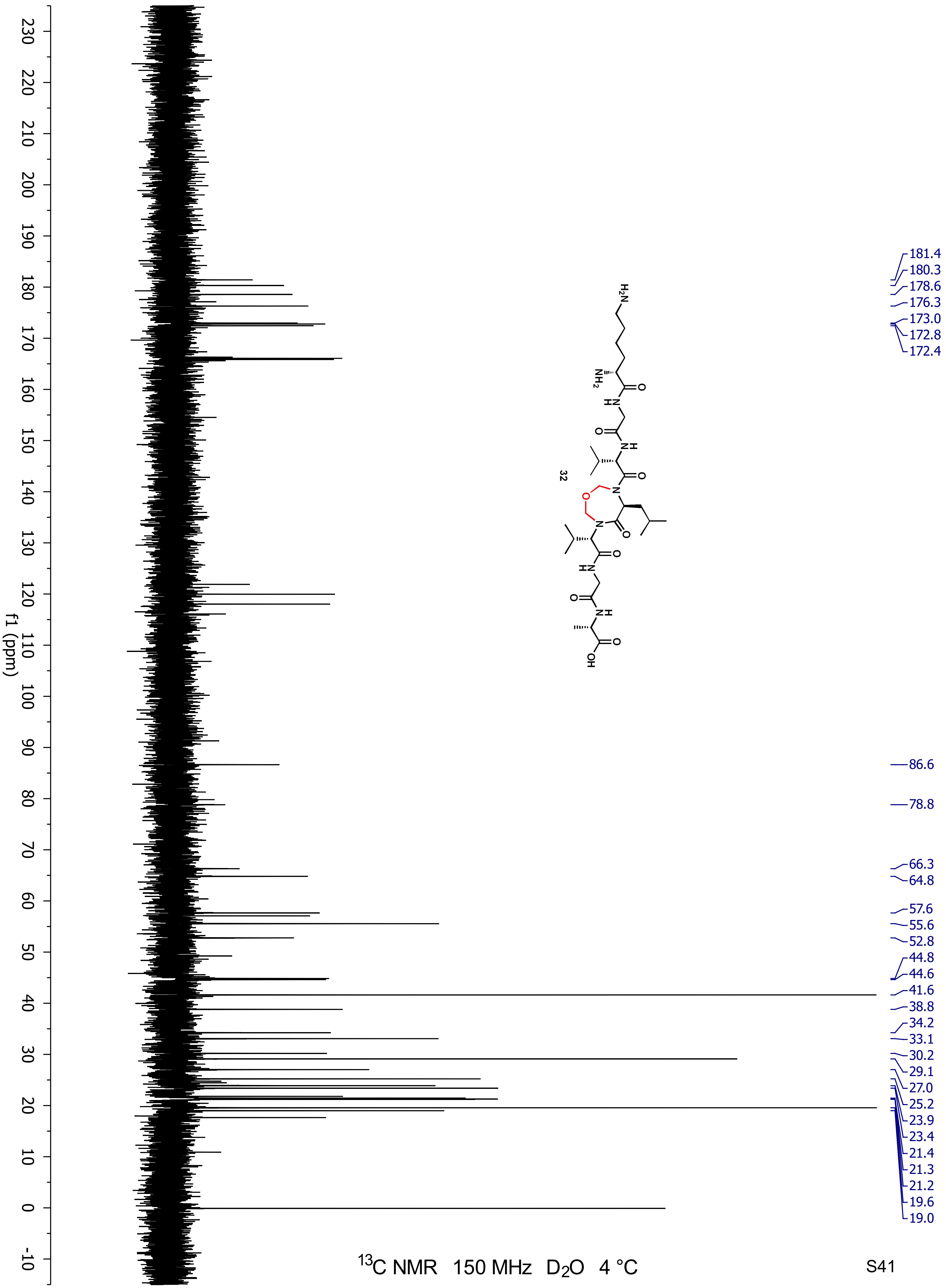

XW-V-52 10 1 /opt/users/wux1 wux1
[rel]
12
10
8
6
4
2
0
XW-V-52 13 1 /opt/users/wux1 wux1
54 °C
XW-V-52 12 1 /opt/users/wux1 wux1
44 °C
XW-V-52 11 1 /opt/users/wux1 wux1
34 °C
XW-V-52 10 1 /opt/users/wux1 wux1
24 °C
6
4
2
0
[ppm]


group Danishefsky
.* wux1 XW–V–51a (10 1) CDCl3   24.0C March_09,2011_17:26:52 Bruker AVIII 600MHz RRL1326: janggeum zg30 : 1H 7.500 ppm   *.
:Proton_VT CDCl3 /opt/users/wux1 wux1 8
7.2985
7.2963
7.2858
7.2775
7.2661
7.2611
7.2586
7.2480
5.7414
5.2670
5.2304
5.1020
5.0821
5.0660
5.0460
4.8591
4.8403
4.6614
4.6433
4.6339
4.6269
4.6141
4.3261
4.3149
3.8639
3.8555
3.8435
2.1023
1.9758
1.9011
1.8949
1.8901
1.8782
1.8668
1.8567
1.7950
1.7870
1.7847
1.7767
1.7718
1.7697
1.7609
1.7587
1.7538
1.7476
1.7367
1.7202
1.7092
1.6970
1.6882
1.6810
1.6737
1.6663
1.6614
1.6567
1.6488
1.6426
1.6320
1.6276
1.6192
1.6170
1.6085
1.6042
1.5935
1.5374
1.5318
1.5262
1.5141
1.5027
1.4988
1.4891
1.4854
1.4657
1.4624
1.4521
1.4220
1.4105
1.3906
1.3787
1.3685
1.3619
1.3565
0.9610
0.9499
0.9366
0.9332
0.9277
0.9258
0.9225
0.9172
0.9129
0.9114
0.8977
0.8964
0.8859
0.8746
N3
O
N
O
N
O
O
OBn
31
5.00
10.11
12.57
17.12
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
ppm
Scale: 0.3393 ppm/cm, 203.6 Hz/cm

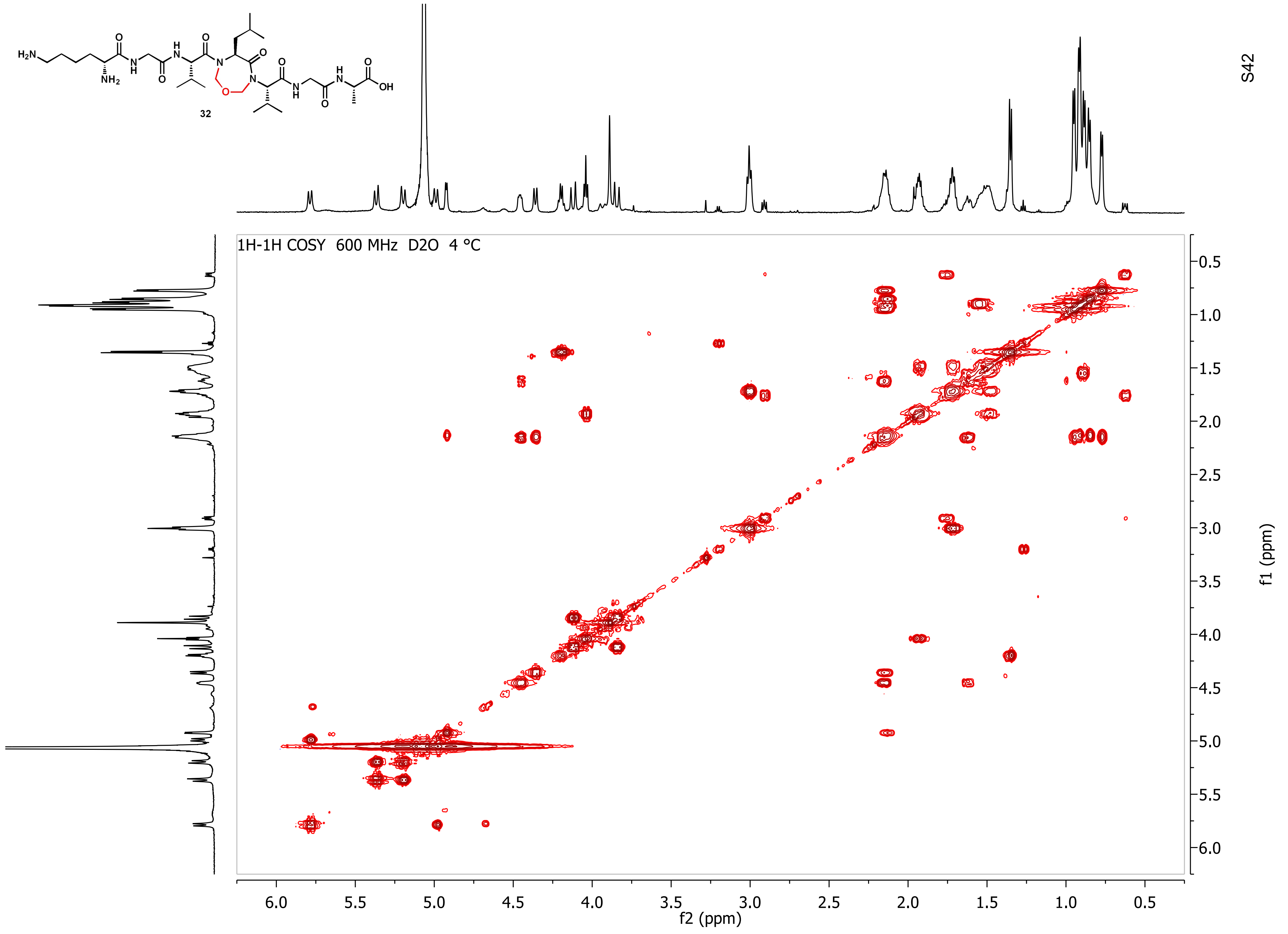

XW-V-51a 10 1 /opt/users/wux1 wux1
[rel]
8
6
4
2
0
XW-V-51a 13 1 /opt/users/wux1 wux1
54 °C
XW-V-51a 12 1 /opt/users/wux1 wux1
44 °C
XW-V-51a 11 1 /opt/users/wux1 wux1
34 °C
XW-V-51a 10 1 /opt/users/wux1 wux1
24 °C
6
4
2
0
[ppm]



^{1}H NMR   600 MHz   D$_2$O   4 °C

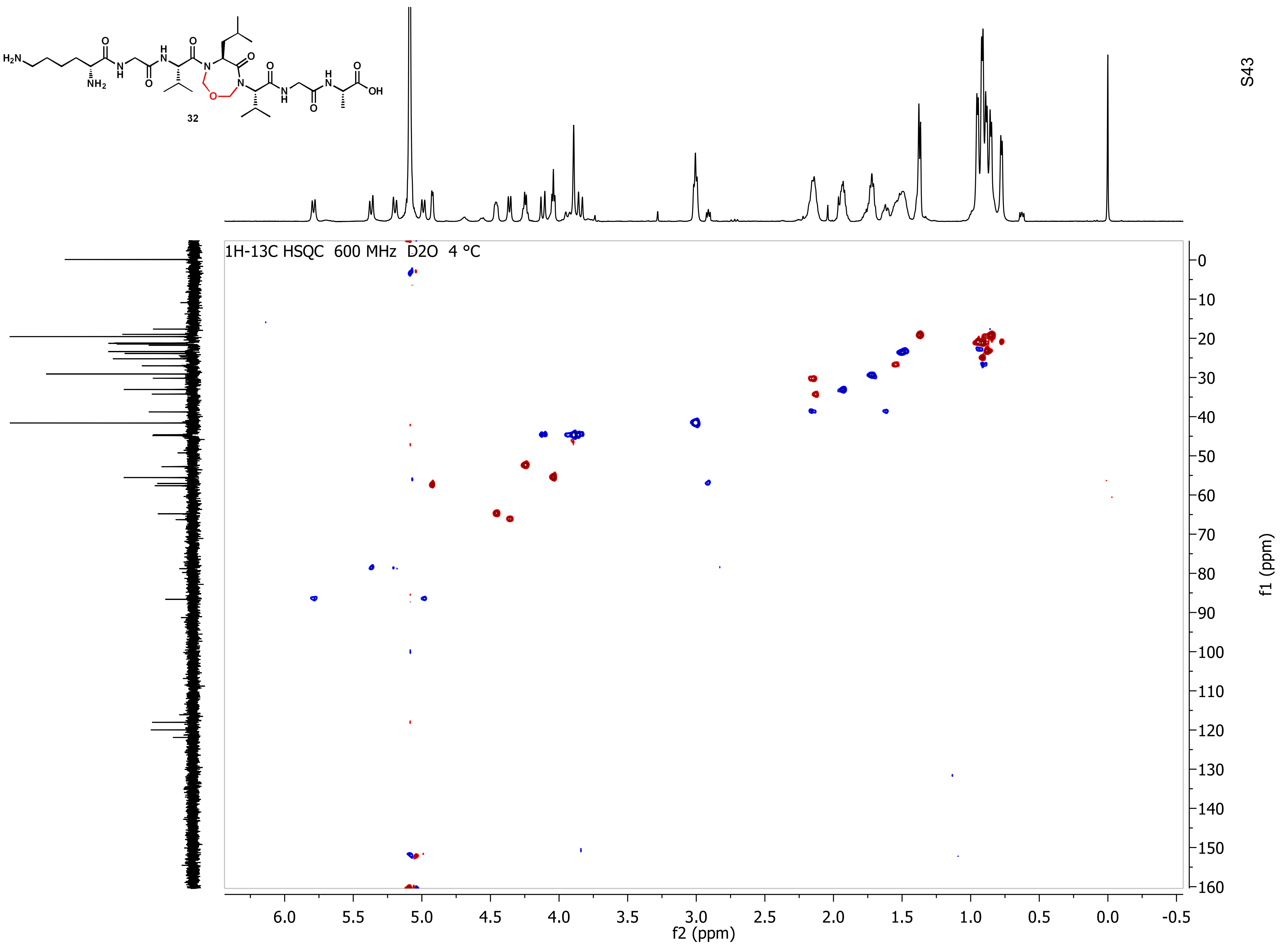


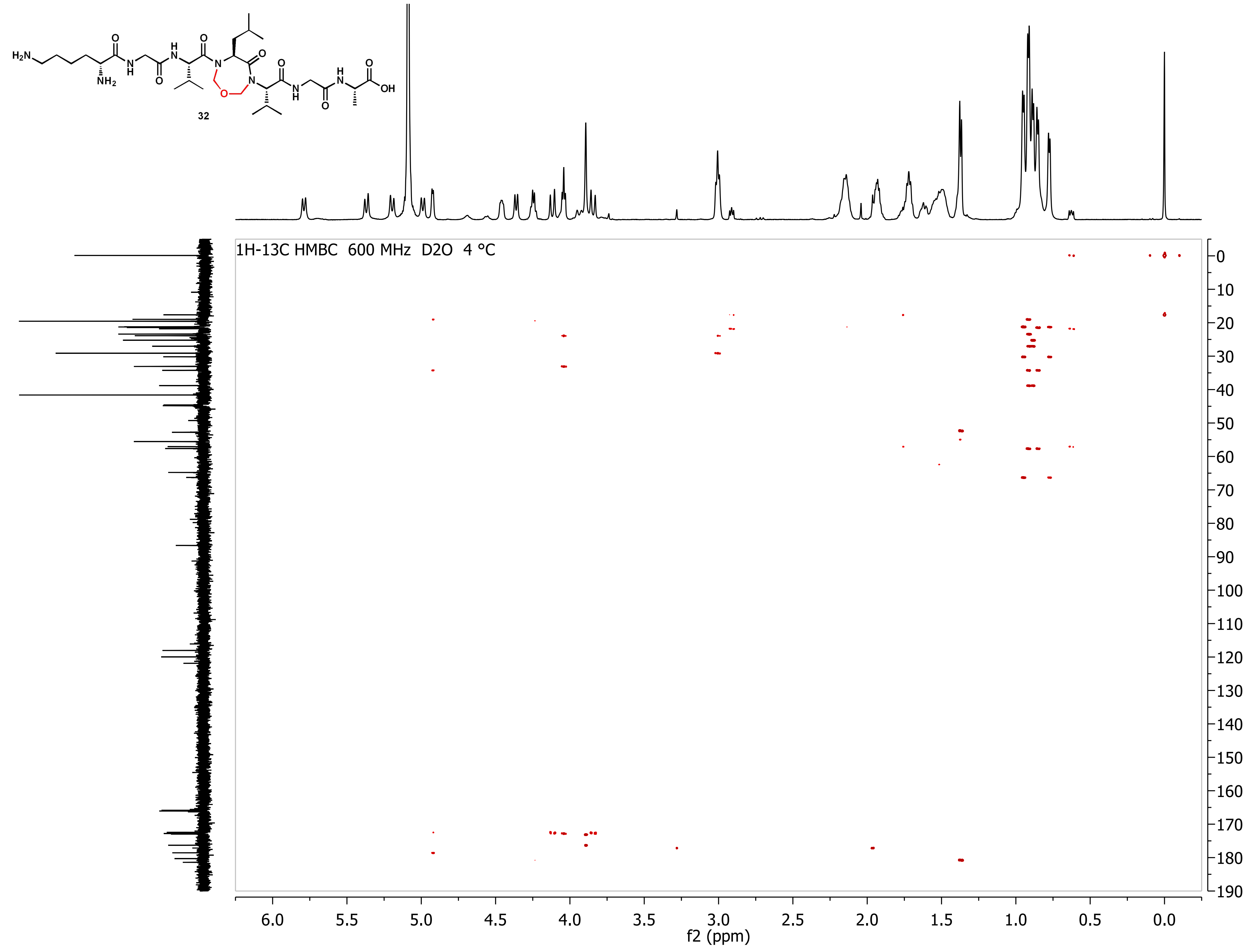
^{13}C NMR 150 MHz D_2O 4 °C



32

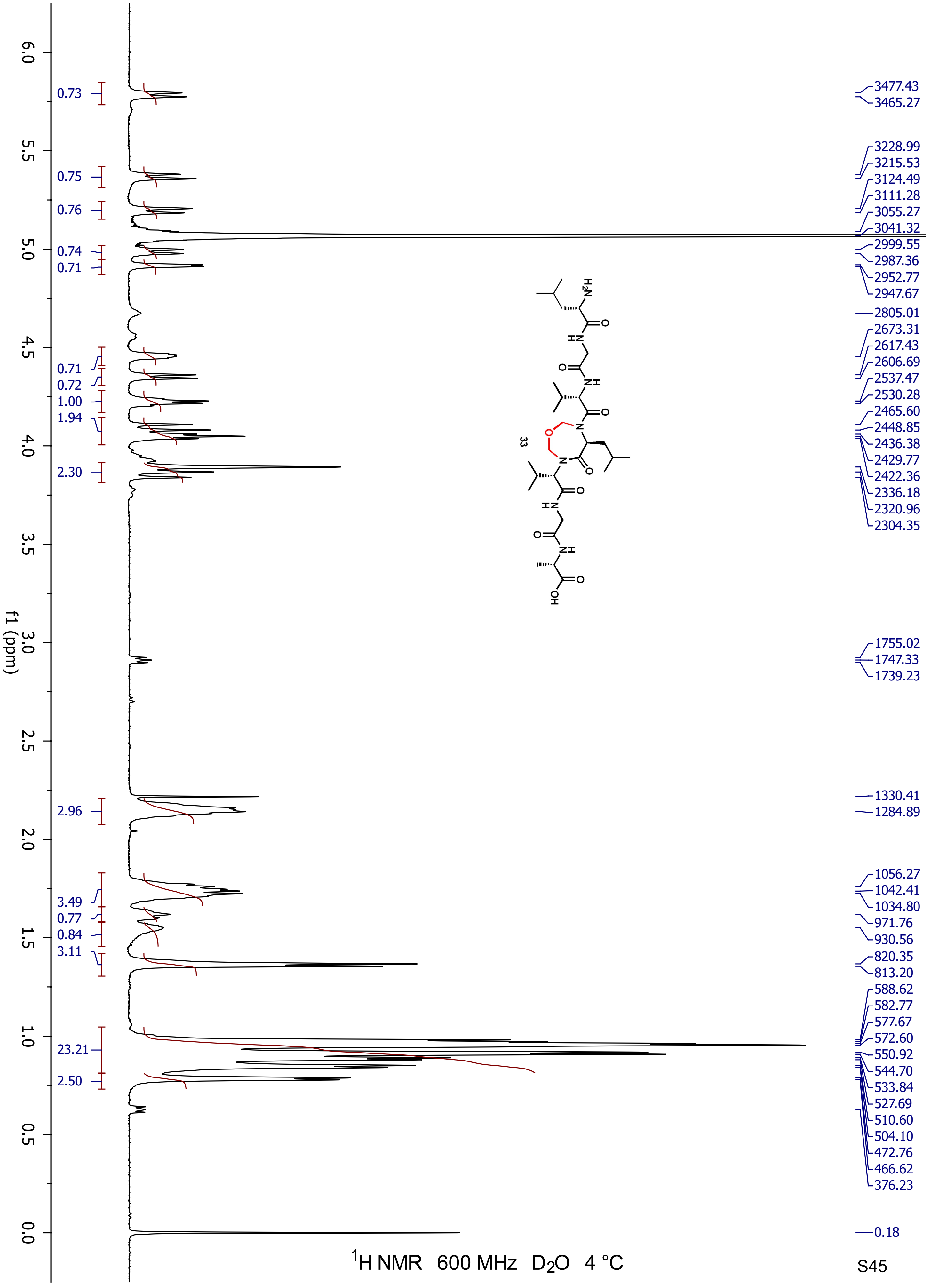
1H-1H COSY 600 MHz D2O 4 °C
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
H2N
NH2
OH


H2N
NH2
32
1H-13C HSQC 600 MHz D2O 4 °C
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
f1 (ppm)

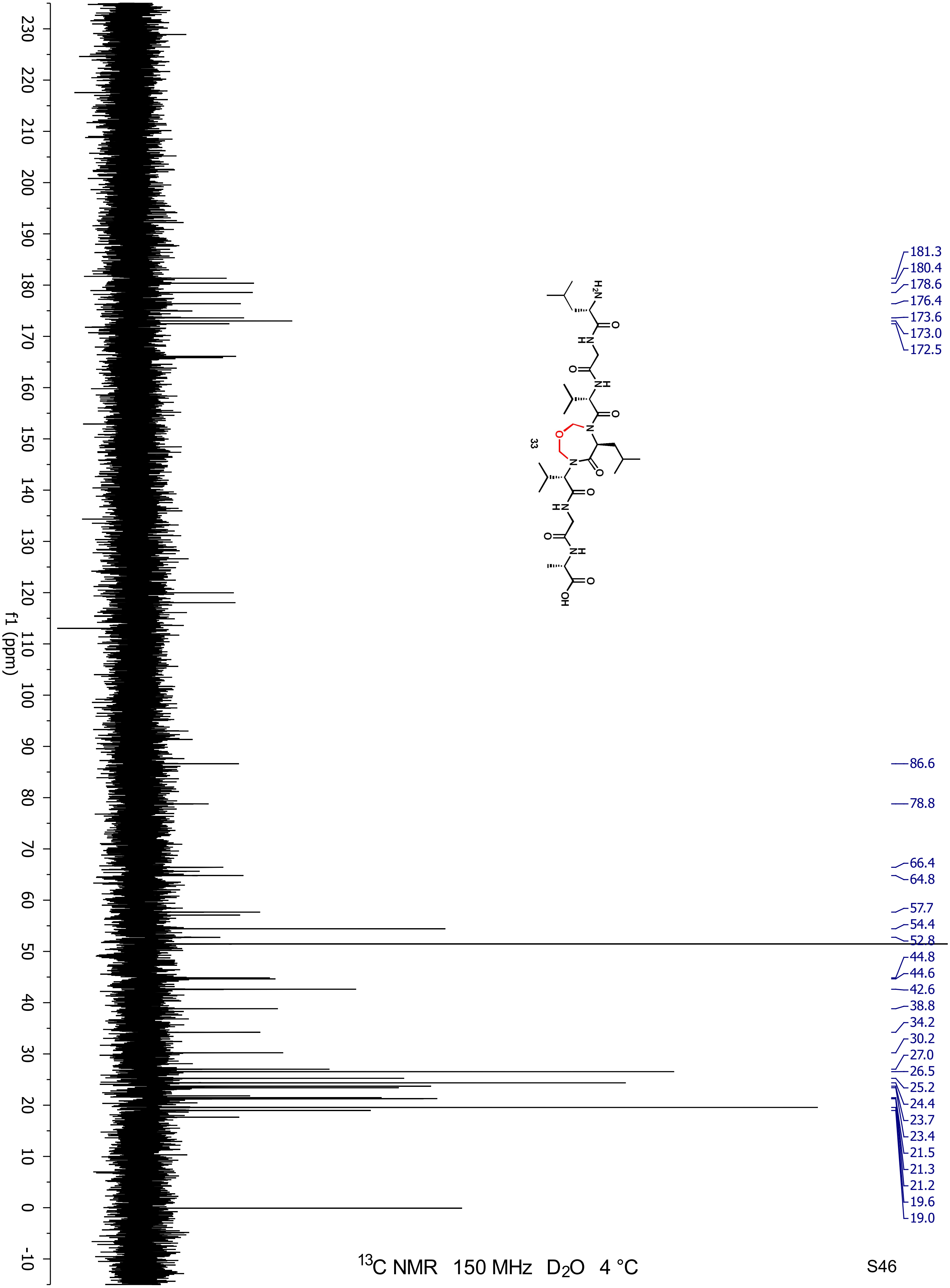


32

H2N
NH2
O
N
H
OH
1H-13C HMBC 600 MHz D2O 4 °C
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190

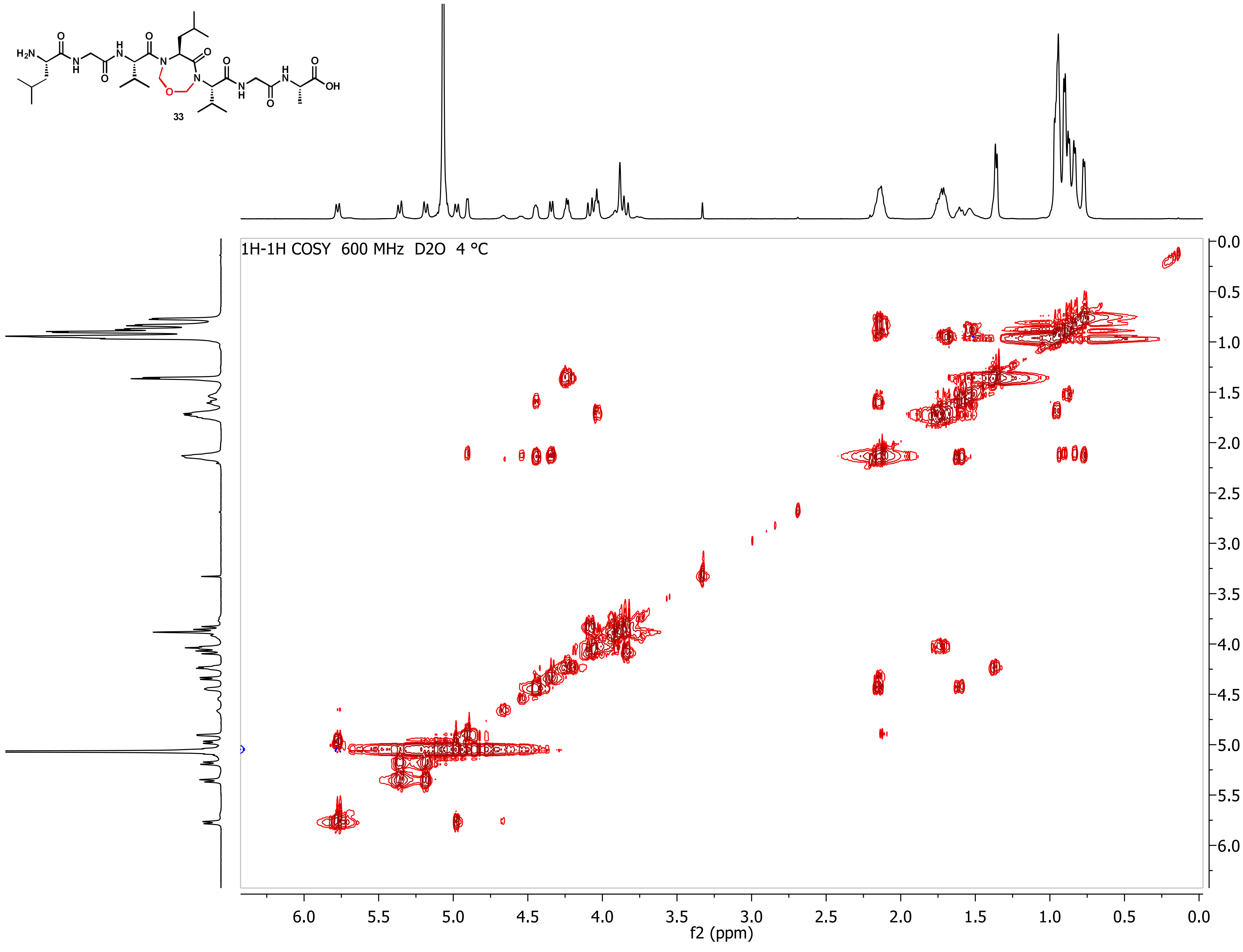


^{1}H NMR   600 MHz   D$_2$O   4 °C



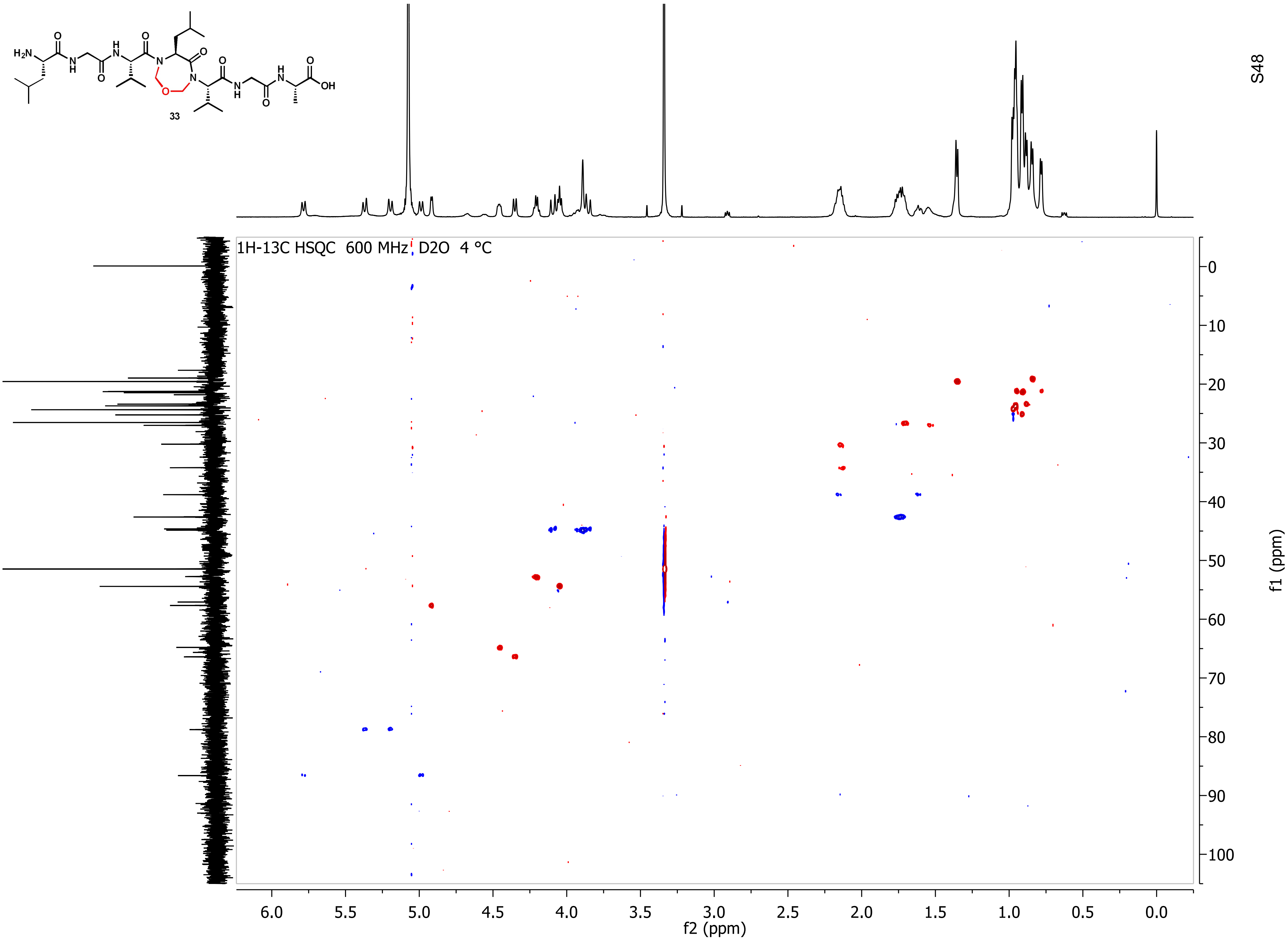
33

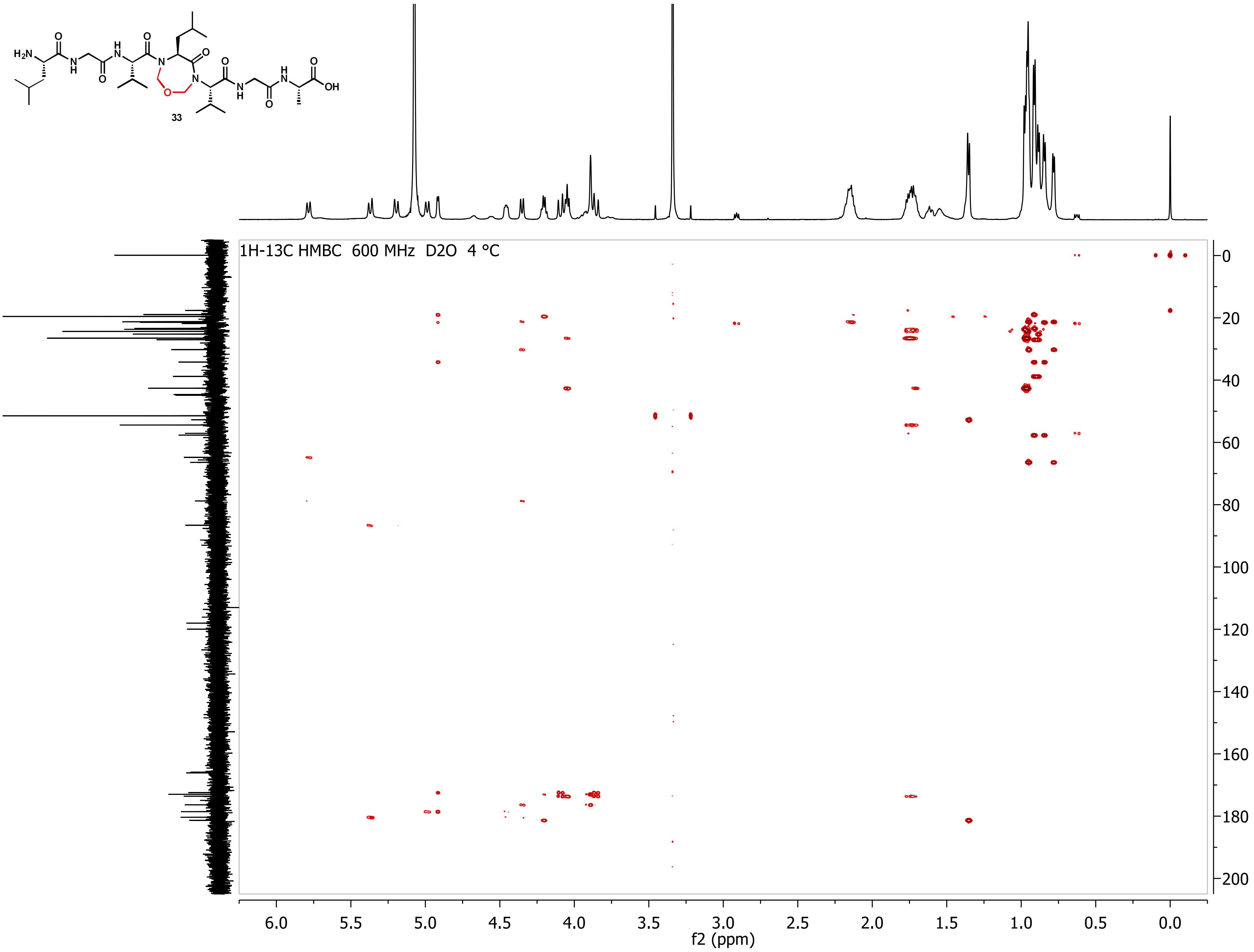
^{13}C NMR 150 MHz D_2O 4 °C

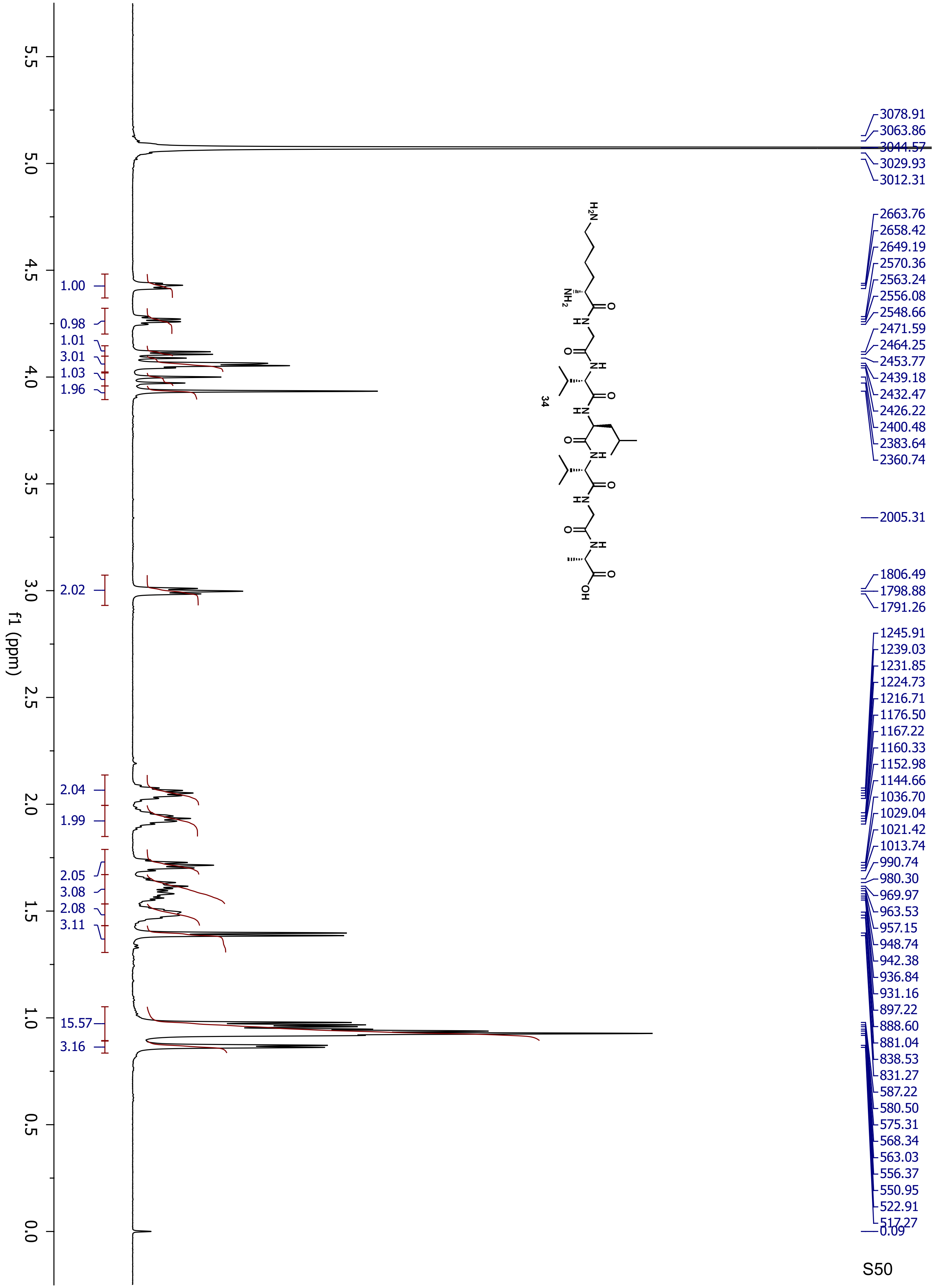


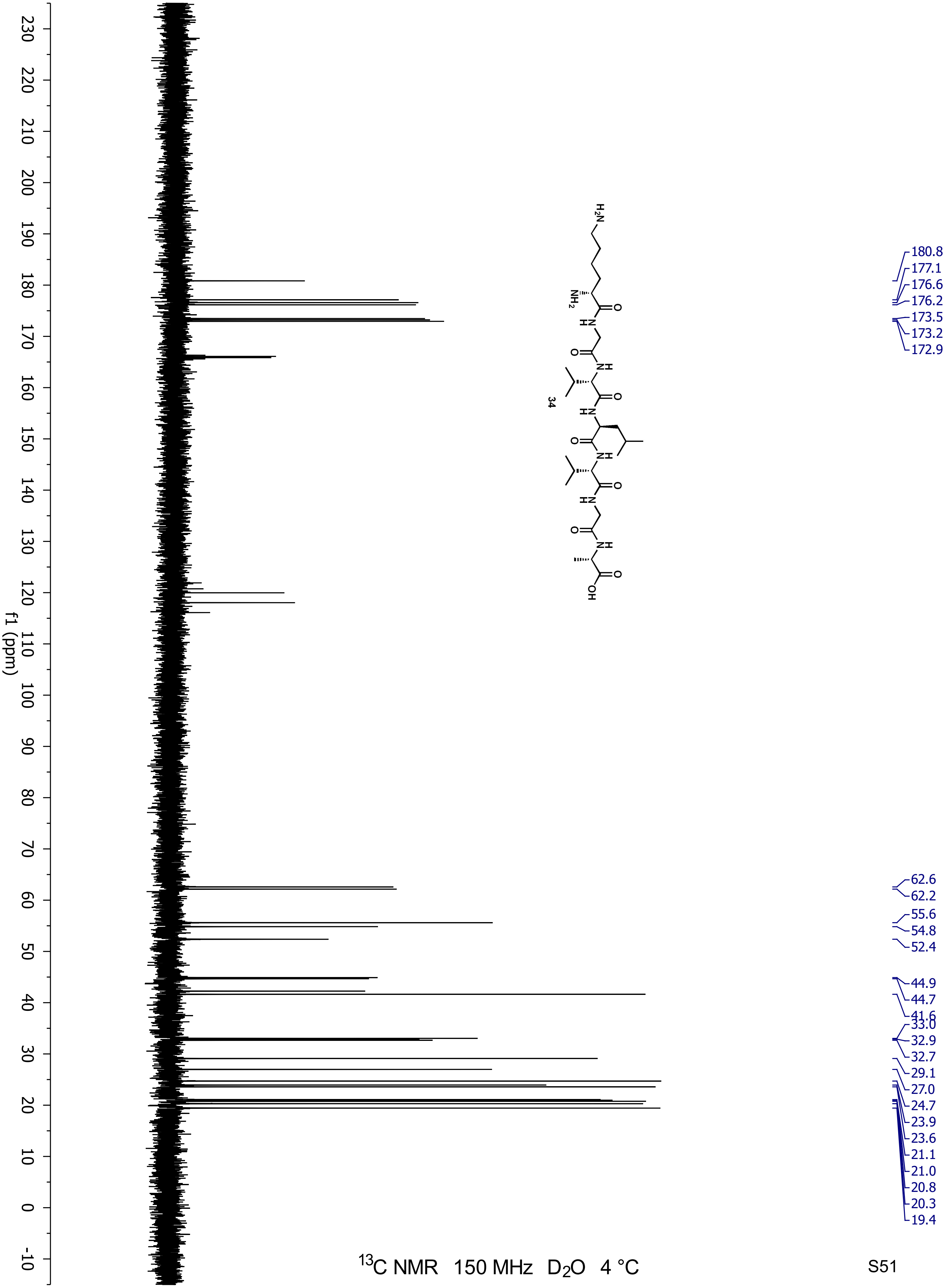
33

H2N
N
H
O
OH
1H-1H COSY 600 MHz D2O 4 °C
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
f1 (ppm)

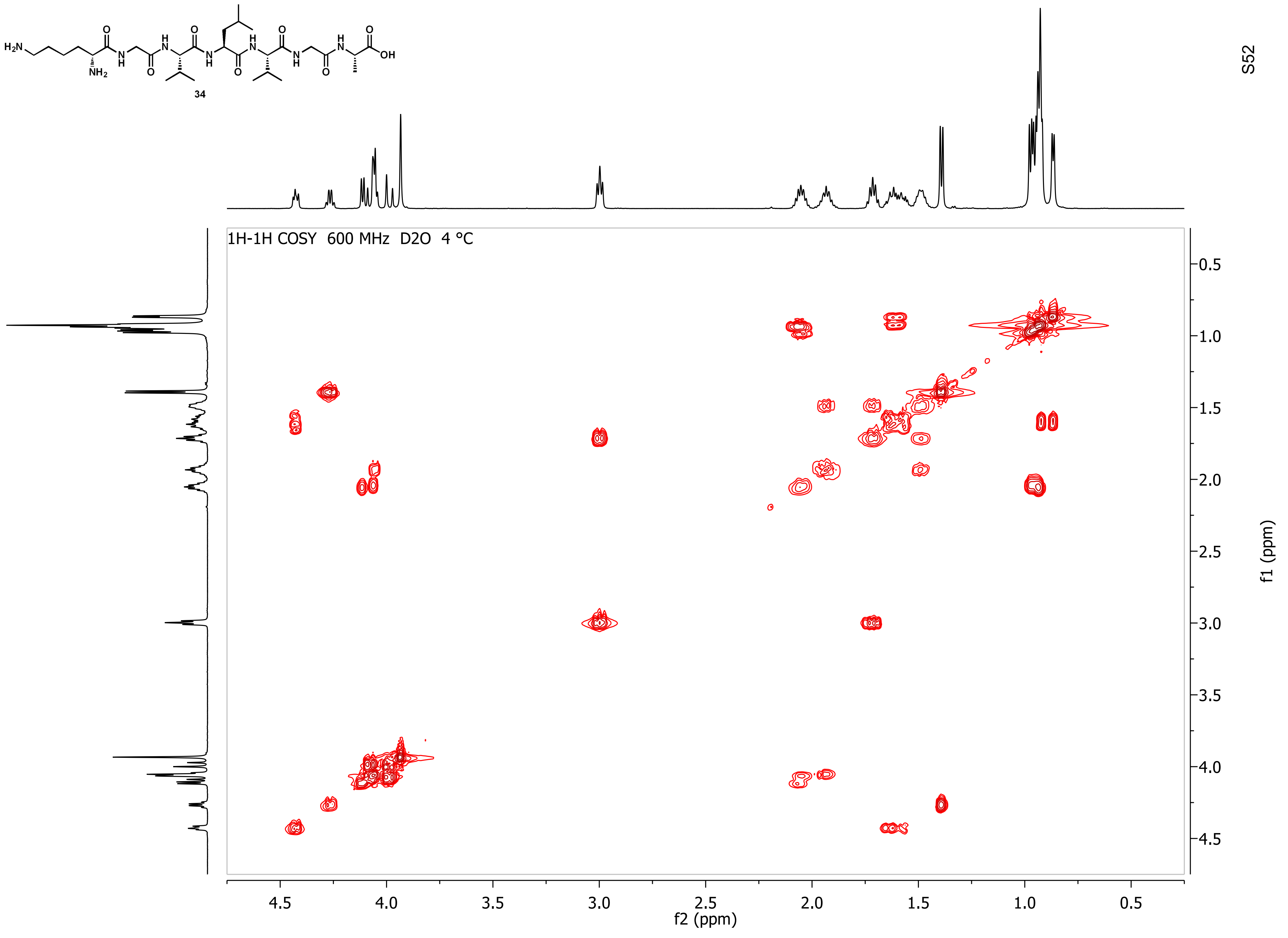
33
1H-13C HSQC 600 MHz D2O 4 °C
f2 (ppm)
f1 (ppm)
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
100


H2N
O
N
H
O
H
N
O
N
O
N
O
H
N
O
H
N
O
OH
33
1H-13C HMBC 600 MHz D2O 4 °C
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f2 (ppm)
0
20
40
60
80
100
120
140
160
180
200
f1 (ppm)

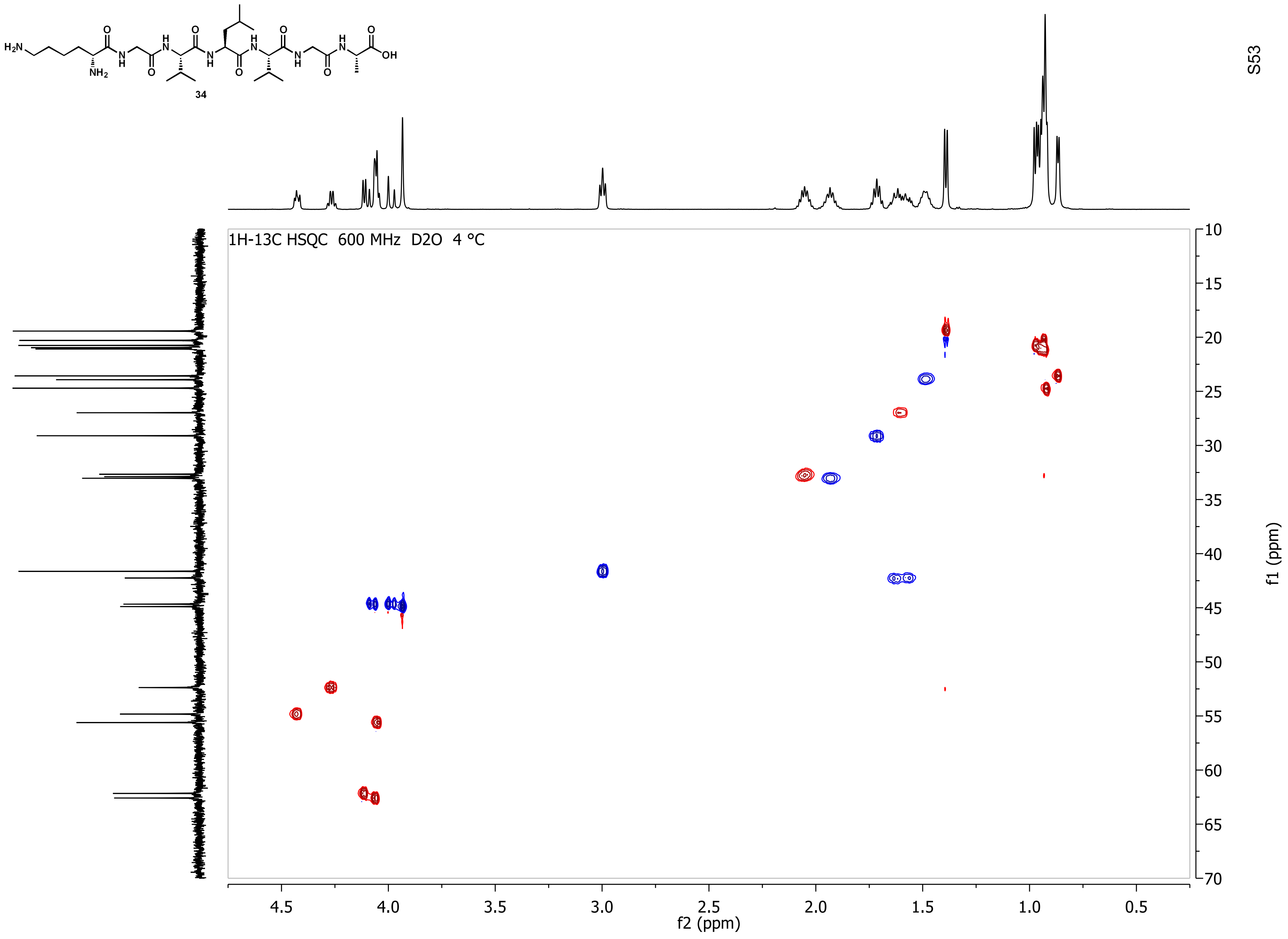

5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
1.00
0.98
1.01
3.01
1.03
1.96
2.02
2.04
1.99
2.05
3.08
2.08
3.11
15.57
3.16
3078.91
3063.86
3044.57
3029.93
3012.31
2663.76
2658.42
2649.19
2570.36
2563.24
2556.08
2548.66
2471.59
2464.25
2453.77
2439.18
2432.47
2426.22
2400.48
2383.64
2360.74
2005.31
1806.49
1798.88
1791.26
1245.91
1239.03
1231.85
1224.73
1216.71
1176.50
1167.22
1160.33
1152.98
1144.66
1036.70
1029.04
1021.42
1013.74
990.74
980.30
969.97
963.53
957.15
948.74
942.38
936.84
931.16
897.22
888.60
881.04
838.53
831.27
587.22
580.50
575.31
568.34
563.03
556.37
550.95
522.91
517.27
0.09
H_2N
NH_2
O
H
N
OH
34



^{13}C NMR 150 MHz D_2O 4 °C


H₂N
NH₂
OH
1H-1H COSY 600 MHz D2O 4 °C
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
f1 (ppm)

34



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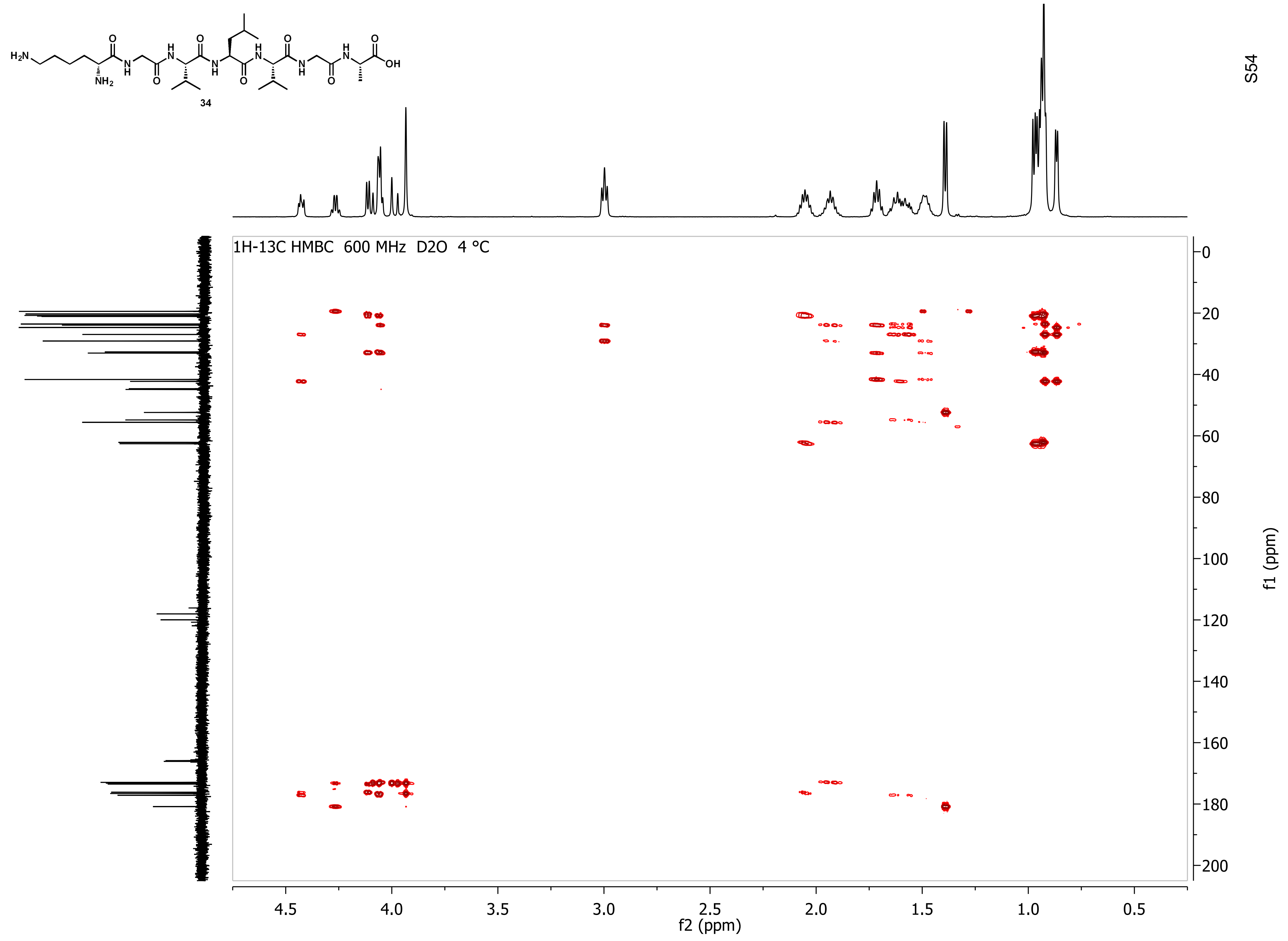
1H-13C HSQC 600 MHz D2O 4 °C
f2 (ppm)
f1 (ppm)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
10
15
20
25
30
35
40
45
50
55
60
65
70


34

1H-13C HMBC 600 MHz D2O 4 °C