SUPPORTING INFORMATION

Total Synthesis of Cyclosporine: Access to *N*-Methylated Peptides via Isonitrile Coupling Reactions

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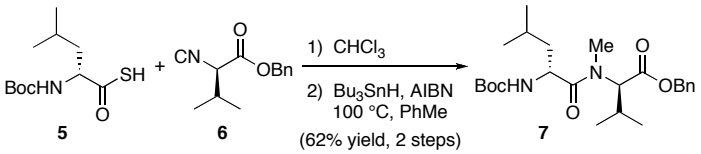
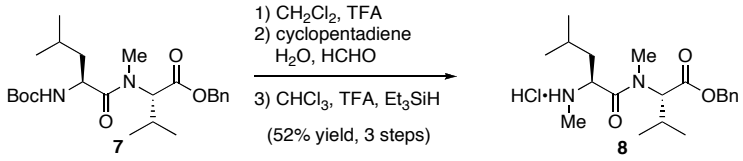
General Information. All commercial materials (Aldrich, Fluka) were used without further purification. All solvents were reagent grade or HPLC grade (Fisher). Anhydrous THF, diethyl ether, CH_2Cl_2, toluene, and benzene were passed through column of alumina and used without further drying. ^{1}H NMR spectra and ^{13}C NMR spectra were recorded on a Bruker Advance 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 $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.0 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, m = multiplet, comp. m = complex multiplet, app. = apparent, br s = broad singlet. All ^{13}C NMR spectra were recorded with complete proton decoupling. Low-resolution mass spectral analyses were performed with a JOEL 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.

Preparation of Compounds.

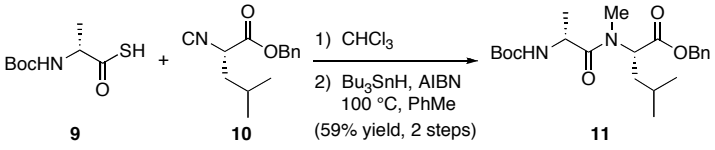


Boc-Leu-MeVal-OBn (7). To a solution of leucine thioacid **5** (0.5 mmol, 124 mg) in chloroform (2.5 mL) was added valine isonitrile **6** (0.5 mmol, 108 mg). The reaction mixture was stirred at ambient temperature overnight then evaporated at low pressure to give a yellow oil. This residue residue was immediately dissolved in toluene (10 mL), and then Bu_3SnH (2.5 mmol, 0.65 mL) and AIBN (0.5 mmol, 82 mg) were added. The reaction mixture was heated to 100 ºC for 30 min. The solvents were evaporated, and the residue was purified by chromatography using hexanes:ethyl acetate (4:1) as eluent to give dipeptide **7** (134 mg, 62% yield). $[\alpha]_D^{20} = -76.8°$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, DMSO-d_6, mixture of rotamers, major rotamer reported), δ = 7.39-7.32 (5H, m), 7.00 (1H, d, *J* = 8.1 Hz), 5.19 (1H, d, *J* = 12.3 Hz), 5.05 (1H, d, *J* = 12.3 Hz), 4.76 (1H, d, *J* = 10.5 Hz), 4.34 (1H, ddd, *J* = 4.6, 4.6, 9.8), 2.91 (3H, s), 2.16 (1H, dddd, *J* = 6.6, 6.6, 13.2, 13.2 Hz), 1.54 (1H, dddd, *J* = 6.5, 6.5, 13.1, 13.1 Hz), 1.39 (1H, ddd, *J* = 5.0, 10.0, 14.0 Hz), 1.34 (10H, app. s), 1.44 (1H, ddd, *J* = 4.6, 8.7, 13.5 Hz), 0.93 (3H, d, *J* = 6.5 Hz), 0.81 (3H, d, *J* = 6.5 Hz), 0.80 (3H, d, *J* = 6.5 Hz), 0.75 (3H, d, *J* = 6.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 174.1, 170.7, 155.8, 135.6, 128.6, 128.4, 79.5, 66.6, 61.6, 48.9, 42.1, 31.0, 28.3, 27.2, 24.5, 23.3, 21.8, 19.8, 18.7; IR (thin film) 2962.1, 1736.6, 1707.7, 1647.9, 1497.5 cm^{-1}; HRMS (ES+) $[M+H]^+$ *m/z* calculated for $[C_{24}H_{38}N_2O_5+H]^+$: 435.2859, found 435.2873.



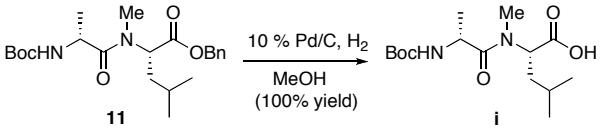
HCl•MeLeu-MeVal-OBn (8). To a solution of **7** (110 mg, 0.25 mmol) in CH_2Cl_2 (3 mL) at 0 °C was added trifluoroacetic acid (1.0 mL), and the solution was stirred for 30 min. The reaction mixture was evaporated at low pressure to provide 113 mg of the intermediate TFA salt. The salt was dissolved in H_2O (0.3 mL), and the resulting homogeneous solution was treated with freshly cracked cyclopentadiene (58 μL, 0.63 mmol) and an aqueous solution of 37% formaldehyde (30 μL, 0.32 mmol). The heterogeneous reaction mixture was vigorously stirred for 1.5 h, then

washed with hexanes to remove excess cyclopentadiene and neutralized with 5% sodium bicarbonate solution. The product was isolated by extraction with dichloromethane. The combined CH_2Cl_2 extracts were dried over magnesium sulfate and concentrated in vacuo. The residue was purified by flash chromatography to give a mixture of 2-azanorbornene adducts. To a solution of the 2-azanorbornene adducts in chloroform (2.0 mL) was added trifluoroacetic acid (0.2 mL) and triethylsilane (0.4 mL). The resulting homogeneous reaction mixture was stirred at ambient temperature under argon for 10 h. LC-MS analysis of the crude reaction mixture indicated consumption of the starting 2-azanorbornenes. The solvents were removed under reduced pressure. The crude residue was evaporated from a 1:2 mixture of CH_2Cl_2 and hexanes (10 mL) twice, then diluted with 10 mL CH_2Cl_2, 30 mL hexanes, and 10 mL 1N HCl. The layers were separated and the organic layer was washed with 1 N HCl (3 x 5 mL). The combined aqueous layers were neutralized with sat. sodium bicarbonate then extracted with CH_2Cl_2 (3 x 15 mL). The combined CH_2Cl_2 layers were then dried over Na_2SO_4, filtered, and acidified with 20 mL 1.0 N HCl in Et_2O. Removal of the solvents afforded dipeptide **8** (50.7 mg, 52% yield) as an off-white solid. $[\alpha]_D^{20} = -66.9°$ (c = 1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, DMSO-d_6, 74 ºC), δ = 7.40-7.35 (5H, m), 5.19 (1H, d, *J* = 12.3 Hz), 5.13 (1H, d, *J* = 12.4 Hz), 4.78 (1H, d, *J* = 10.0 Hz), 4.36 (1H, dd, *J* = 5.3, 7.3 Hz), 2.99 (3H, s), 2.48 (3H, s), 2.30 (1H, ddd, *J* = 6.6, 13.1, 16.4 Hz), 1.73 (1H, ddd, *J* = 6.7, 6.7, 13.4 Hz), 1.61 (1H, ddd, *J* = 6.4, 6.4, 13.9 Hz), 1.49 (1H, ddd, *J* = 6.5, 6.5, 13.4 Hz), 1.26 (1H, br s), 1.02 (3H, d, *J* = 6.5 Hz), 0.87 (3H, d, *J* = 5.9 Hz), 0.86 (3H, d, *J* = 6.2 Hz), 0.85 (3H, d, *J* = 6.7 Hz); ^{13}C NMR (150 MHz, DMSO-d_6), δ = 169.3, 168.9, 135.5, 128.5, 128.4, 128.3, 66.3, 62.2, 56.2, 38.9, 31.5, 31.4, 26.3, 23.3, 22.7, 21.8, 19.8, 18.6; IR (thin film) 2961, 2924, 2872, 2850, 2736, 2695, 1739, 1655, 1557, 1469, 1389, 1290, 1189, 1129, 1002 cm^{-1}; HRMS (ES+) $[M+H]^+$ *m/z* calculated for $[C_{20}H_{32}N_2O_3+H]^+$: 349.2491, found 349.2495.



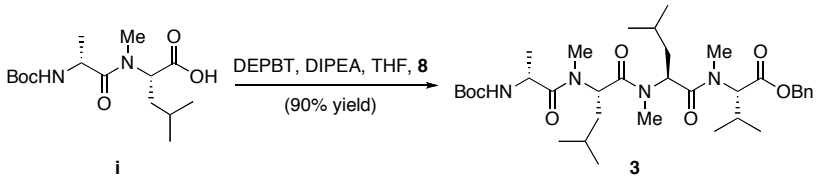
Boc-D-Ala-MeLeu-OBn (11). To a solution of alanine thioacid **9** (89 mg, 0.43 mmol) in chloroform (2.5 mL) was added leucine isonitrile **10** (100 mg, 0.43 mmol), and the reaction mixture was stirred at ambient temperature overnight. The reaction mixture was evaporated at low pressure to give a yellow oil, which was immediately dissolved in toluene (10 mL), and then Bu_3SnH (0.56 mL, 2.15 mmol) and AIBN (71 mg, 0.43 mmol) were added. The reaction mixture was heated to 100 ºC for 30 min. The solvents were evaporated, and the residue was purified by flash chromatography using hexanes:ethyl acetate (5:1) as eluent to give the product **11** (103 mg, 59%). $[\alpha]_D^{20} = -29.4°$ (c =

1.0, $CHCl_3$); ^1H NMR (600 MHz, DMSO-d_6, mixture of conformers, major conformer reported), δ = 7.39–7.33 (5H, m), 6.93 (1H, d, *J* = 7.9 Hz), 5.11 (2H, dd, *J* = 12.7, 14.3 Hz), 4.97 (1H, dd, *J* = 4.5, 11.2 Hz), 4.46 (1H, ddd, *J* = 6.8, 6.8, 14.2 Hz), 1.81 (1H, ddd, *J* = 4.1, 11.1, 14.9 Hz), 1.59 (1H, ddd, *J* = 4.5, 10.1, 14.3 Hz), 1.37 (3H, s), 1.12 (3H, d, *J* = 7.0 Hz), 0.89 (3H, d, *J* = 6.7 Hz), 0.82 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 173.8, 171.3, 155.0, 135.6, 128.6, 128.3, 128.1, 79.5, 66.9, 55.0, 46.7, 37.2, 31.4, 28.4, 26.8, 25.0, 23.2, 21.2, 19.1; IR (thin film) 2960.2, 1740.4, 1649.8, 1455.9, 1367.3 cm^{-1}; HRMS (ES+) $[M+H]^+$ *m/z* calculated for $[C_{22}H_{35}N_2O_5+H]^+$: 407.2546, found 407.2531.



11 **i**

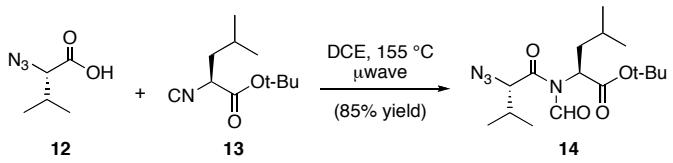
Boc-D-Ala-MeLeu-OH (i). To a solution of dipeptide **11** (80 mg, 0.197 mmol) in methanol (5 mL) was added 10% palladium on carbon (50 mg), and stirred under a balloon of hydrogen overnight. The resulting mixture was filtered and evaporated to give acid **i** (62 mg, 100% yield) as a white solid, which was used in the following step with no further purification. $[\alpha]_D^{20}$ = +13.1° (c = 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$), δ = 5.68 (1H, d, *J* = 7.9 Hz), 5.16 (1H, dd, *J* = 7.1, 8.6 Hz), 4.67 (1H, ddd, *J* = 6.9, 6.9, 14.0 Hz), 1.76 (2H, app. t, *J* = 7.0 Hz), 1.41 (10H, app. s) 1.29 (3H, d, *J* = 6.9 Hz), 0.95 (3H, d, *J* = 6.7 Hz), 0.89 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 175.3, 174.4, 155.3, 79.8, 55.3, 46.8, 37.1, 31.6, 28.4, 25.0, 23.3, 21.2, 18.8; IR (thin film) 2957.3, 1748.2, 1658.5, 1516.7, 1368.3 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{15}H_{28}N_2O_5+Na]^+$: 339.1896, found 339.1892.



i **3**

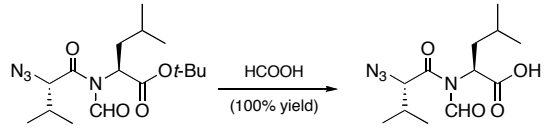
Boc-D-Ala-MeLeu-MeLeu-MeVal-OBn (3). To a solution of dipeptide acid **i** (32 mg, 0.1 mmol) in THF (5.0 mL) at –20 °C was added dipeptide **8** (35 mg, 0.1 mmol) and DEPBT (60 mg, 0.02 mmol). The resulting mixture was treated with DIPEA (18 μL, 0.1 mmol), and stirred at –20 ºC for two hours. LC–MS check indicated consumption of the starting material. The solvents were removed, and the residue was purified by chromatograph using hexanes:ethyl acetate (2:1) as eluent to give the product **3** (58 mg, 90% yield). $[\alpha]_D^{20}$ = –96.4° (c = 0.2, $CHCl_3$); ^1H NMR (600 MHz,

DMSO-d_6, ~2:1 ratio of conformers), δ = 7.38-7.32 (5H, m), 7.12 & 7.09 (1H, 2d, 1:2, *J* = 7.3 & 7.4), 5.39-5.29 (2H, comp. m), 5.27 & 5.15 (1H, 2d, 1:2, *J* = 12.5 & 12.4 Hz), 5.09 & 5.01 (1H, 2d, 2:1, *J* = 12.4 & 12.5 Hz), 4.37 (1H, app. ddd, *J* = 7.0, 7.0, 14.1 Hz), 2.85 & 2.82 (3H, 2s, 2:1), 2.84 & 2.60 (3H, 2s, 2:1), 2.73 & 2.53 (3H, 2s, 2:1), 1.61-1.30 (7H, comp. m), 1.35 (9H, br s), 1.11 (3H, d, *J* = 6.9 Hz), 0.93 (3H, 2d, 2:1, *J* = 6.5 & 6.4 Hz), 0.88-0.78 (12H, comp. m), 0.72 (3H, d, *J* = 6.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$, peaks for BOTH conformers reported), δ = 173.1, 173.0 171.9, 171.0, 170.8, 170.7, 170.6, 169.7, 169.6, 155.3, 155.2, 135.6, 135.2, 129.0, 128.6, 128.54, 128.47, 128.28, 128.25, 128.2, 79.64, 79.57, 66.8, 66.4, 64.4, 61.7, 51.5, 51.4, 51.0, 46.6, 38.5, 38.0, 37.8, 37.5, 31.1, 30.3, 30.1, 29.84, 29.82, 29.6, 28.2, 27.5, 27.2, 24.73, 24.70, 24.41, 24.36, 23.4, 22.9, 22.83, 22.79, 22.5, 22.3, 22.1, 22.0, 21.9, 19.8, 19.6, 18.63, 18.60, 18.3, 18.0; IR (thin film) 2959.2, 1737.6, 1642.1, 1456.9, 1367.3 cm^{-1}; HRMS (ES+) $[M+H]^+$ *m/z* calculated for $[C_{35}H_{58}N_4O_7+H]^+$: 647.4384, found 647.4380.



12 **13** **14**

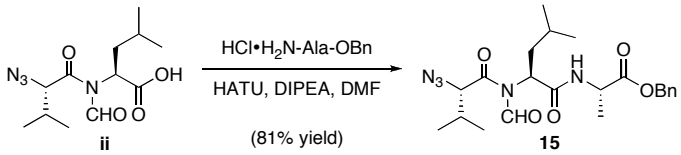
N_3-Val-(CHO)Leu-O*t*-Bu (14). To a solution of azido-valine **12** (29 mg, 0.2 mmol) in 1,2-dichloroethane (1 mL) was added leucine isonitrile **13** (39 mg, 0.2 mmol). The resulting mixture was heated to 155 °C in the microwave for 40 min, and the solution was evaporated at low pressure to give a crude oil. The residue was purified by chromatograph using hexanes:ethyl acetate (20:1) as eluent to give the *N*-formyl dipeptide **14** (58 mg, 85% yield). $[\alpha]_D^{20}$ = +36.3, (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ 9.05 (1H, s), 5.02 (1H, dd, *J* = 4.7, 9.8 Hz), 3.89 (1H, d, *J* = 7.0 Hz), 2.28 (1H, dddd, *J* = 6.7, 6.7, 13.3, 20.1 Hz), 1.92 (1H, ddd, *J* = 4.8, 9.5, 14.4 Hz), 1.88 (1H, ddd, *J* = 4.6, 9.8, 14.5 Hz), 1.38 (10H, app. s), 1.06 (3H, d, *J* = 6.7 Hz), 0.98 (3H, d, *J* = 6.7 Hz), 0.87 (3H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 6.7 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ 170.6, 168.4, 161.3, 82.2, 67.0, 52.9, 37.2, 30.7, 27.7, 25.2, 23.0, 21.6, 19.4, 18.4; IR (thin film) 2964.1, 2103.9, 1736.6, 1678.7, 1368.3 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{16}H_{28}N_4O_4+Na]^+$: 363.2008, found 363.2010.



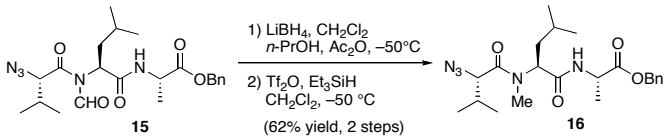
14 **ii**

N_3-Val-(CHO)Leu-OH (ii). Dipeptide **14** (34 mg, 0.1 mmol) was dissolved in formic acid (1.0 mL), and stirred at

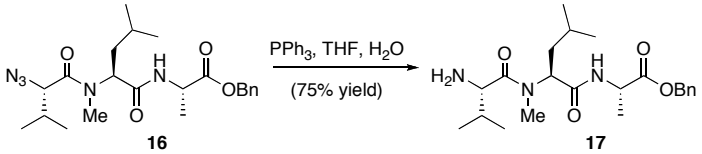
room temperature for 4.0 hours. The reaction mixture was evaporated at low pressure to give dipeptide acid **ii** (28.4 mg, 100% yield) as an oil. $[\alpha]_D^{20}$ = +18.7, (c = 1.1, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 11.51 (1H, br s), 9.14 (1H, s), 5.25 (1H, dd, *J* = 4.7, 9.7 Hz), 3.93 (1H, d, *J* = 8.0 Hz), 2.28 (1H, ddd, *J* = 6.7, 13.6, 13.6 Hz), 1.98 (1H, ddd, *J* = 4.7, 9.5, 14.4), 1.85 (1H, ddd, *J* = 4.6, 9.7, 14.4 Hz), 1.43 (1H, m), 1.09 (3H, d, *J* = 6.7 Hz), 1.01 (3H, d, *J* = 6.6 Hz), 0.91 (3H, d, *J* = 6.8 Hz), 0.90 (3H, d, *J* = 7.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 175.3, 170.6, 161.5, 67.5, 51.8, 37.0, 30.9, 25.1, 22.9, 21.5, 19.3, 18.4; IR (thin film) 2964.1, 2104.9, 1717.3.6, 1675.8, 1366.3 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{12}H_{20}N_4O_4+Na]^+$: 307.1382, found 307.1371.



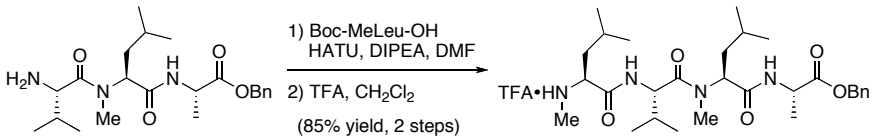
N_3-Val-(CHO)Leu-Ala-OBn (15). To a solution of **ii** (62 mg, 0.22 mmol) in DMF (1.0 mL) was added HATU (84 mg, 0.22 mmol) and DIPEA (86 μL, 0.5 mmol), and the reaction mixture was stirred for 5 min. To the resulting mixture was added HCl·NH_2-Ala-OBn (43 mg, 0.2 mmol), and stirred for 30 min. The reaction mixture was diluted with ethyl acetate (10 mL), and washed with brine. The organic phase was dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography using hexanes:ethyl acetate (6:1) as eluent to give tripeptide **15** (72 mg, 81%) as a >20:1 ratio of diastereomers. $[\alpha]_D^{20}$ = +15.4, (c = 2.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, major diastereomer), δ = 9.06 (1H, s), 7.39-7.33 (5H, m), 6.34 (1H, d, *J* = 6.8 Hz), 5.21 (1H, d, *J* = 12.2 Hz), 5.18 (1H, app. t, *J* = 5.6 Hz), 5.16 (1H, d, *J* = 12.4 Hz), 4.60 (1H, ddd, *J* = 7.1, 7.1, 14.2 Hz), 4.16 (1H, d, *J* = 7.4 Hz) 2.30 (1H, ddd, *J* = 6.7, 6.7, 13.5 Hz), 1.99 (1H, ddd, *J* = 6.7, 6.7, 13.5 Hz), 1.82 (1H, ddd, *J* = 5.6, 9.3, 14.4 Hz), 1.47 (1H, m), 1.40 (3H, d, *J* = 7.1 Hz), 1.08 (3H, d, *J* = 6.7 Hz), 1.02 (3H, d, *J* = 6.7 Hz), 0.95 (3H, d, *J* = 6.9 Hz), 0.93 (3H, d, *J* = 7.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 172.6, 171.6, 168.6, 162.2, 135.1, 128.6, 128.5, 128.1, 67.6, 67.3, 53.4, 48.5, 37.8, 30.8, 25.2, 23.0, 21.5, 19.4, 18.2, 18.1; IR (thin film) 3347.8, 2962.1, 2102.9, 1724.1, 1679.7 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{22}H_{31}N_5O_5+Na]^+$: 468.2223, found 468.2200.



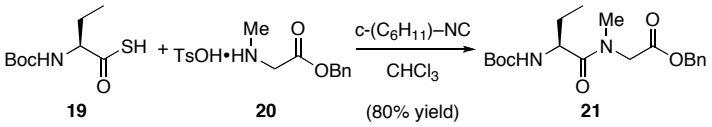
N_3-Val-MeLeu-Ala-OBn (16). To a solution of **15** (22 mg, 0.05 mmol) in dichloromethane (3 mL) at –50 °C was added 1–propanol (0.15 mL) and acetic anhydride (7.5 μL). The reaction mixture was treated with lithium borohydride (75 μL, 0.15 mmol) at –50 °C. After one hour, the reaction mixture was quenched trizma•HCl solution (0.2 M, 1.0 mL), and slowly warmed to room temperature while stirred vigorously until the solution became clear. The resulting solution was extracted with CH_2Cl_2 (2 x 5 mL), washed with brine, and dried over $MgSO_4$. The solution was evaporated at low pressure to give the crude. The residue above was dissolved in CH_2Cl_2 (2.0 mL), cooled to –50 ºC, and treated with triflic anhydride (42 μL, 0.25 mmol) and triethylsilane (0.16 mL, 1.0 mmol). The solution was stirred for two hours, neutralized with 10% sodium bicarbonate solution (1 mL), and extracted with CH_2Cl_2 (2 x 5 mL), washed with brine, and dried over $MgSO_4$. The solution was concentrated to give a crude residue, which was purified by flash chromatography using hexanes:ethyl acetate (6:1) as eluent to give tripeptide **16** (13 mg, 62%). $[\alpha]_D^{20} = -24.6$, (c = 0.2, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$), δ = 7.38–7.33 (5H, m), 6.51 (1H, d, *J* = 7.3 Hz), 5.18 (1H, d, *J* = 1.9 Hz), 5.14 (1H, d, *J* = 12.2 Hz), 4.55 (1H, ddd, *J* = 7.1, 7.1, 14.4 Hz), 3.52 (1H, d, *J* = 9.1 Hz), 2.97 (3H, s), 2.29 (1H, m), 1.73-1.64 (2H comp. m), 1.48 (1H, app. ddd, *J* = 6.5, 13.0, 19.9 Hz), 1.40 (1H m), 1.36 (3H, d, *J* = 7.1 Hz), 1.1 (3H, d, *J* = 6.7 Hz), 0.95 (3H, d, *J* = 6.4 Hz), 0.94 (3H, d, *J* = 6.4 Hz), 0.91 (3H, d, *J* = 6.5 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 172.2, 171.0, 169.9, 135.3, 128.6, 128.4, 128.2, 67.2, 64.4, 54.7, 48.1, 36.2, 30.7, 30.4, 24.9, 23.0, 22.0, 19.2, 19.0, 18.1; IR (thin film) 3326.6, 2962.1, 2097.2, 1743.3, 1680.7 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{22}H_{33}N_5O_4+Na]^+$: 454.2430, found 454.2441.



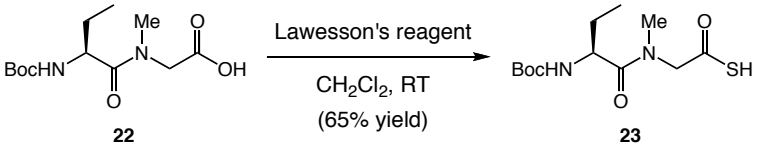
Val-MeLeu-Ala-OBn (17). To a solution of **16** (60 mg, 0.139 mmol) in a mixture of THF and H_2O (5.25 mL, 20:1) was added triphenyl phosphine (73 mg, 0.278 mmol). The reaction mixture was stirred at room temperature overnight then evaporated at low pressure. The residue was purified by flash chromatography using CH_2Cl_2:MeOH (20:1) as eluent to give the product **17** (42 mg, 75% yield). The analytical data are identical with the published values.



TFA•MeLeu-Val-MeLeu-Ala-OBn (18). To a solution of Boc-MeLeu-OH (32 mg, 0.129 mmol) in DMF (1.0 mL) was added HATU (49 mg, 0.129 mmol) and DIPEA (58 μL, 0.344 mmol). The reaction mixture was stirred for 5 min, and then compound **17** (35 mg, 0.086 mmol) was added, and the mixture was stirred for 30 min. LC–MS check showed complete consumption of the starting material. The solution was diluted with ethyl acetate, washed with water and brine, and dried over $MgSO_4$. The solvents were evaporated, and the residue was purified by chromatograph using hexanes:ethyl acetate (3:1) as eluent to give the Boc-intermediate (46 mg, 85% yield). The intermediate (46 mg, 0.073 mmol) was dissolved in CH_2Cl_2 (0.5 mL), cooled to 0 ºC, and TFA (0.5 mL) was added. The resulting mixture was stirred at 0 °C until the starting material totally disappeared. The solvent was blown down to give the crude tetrapeptide, which was used without further purification. The analytical data are identical with the published values.

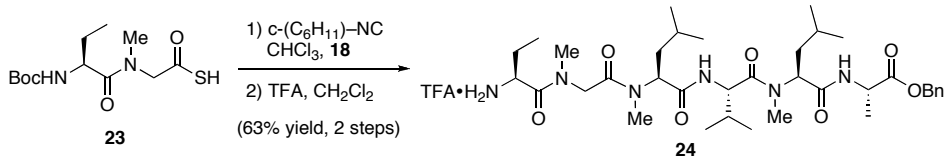


Boc-Abu-Sar-OBn (21). To a solution of **19** (44 mg, 0.2 mmol) in $CHCl_3$ (1 mL) was added compound **20** (35 mg, 0.1 mmol) and cyclohexyl isonitrile (38 μL, 0.3 mmol). The reaction mixture was stirred at room temperature for 24 hours. The solvents were evaporated, and the residue was purified by flash chromatography using hexanes:ethyl acetate (4:1) as eluent to give the product **21** (29 mg, 80% yield). The analytical data are identical with the published values.

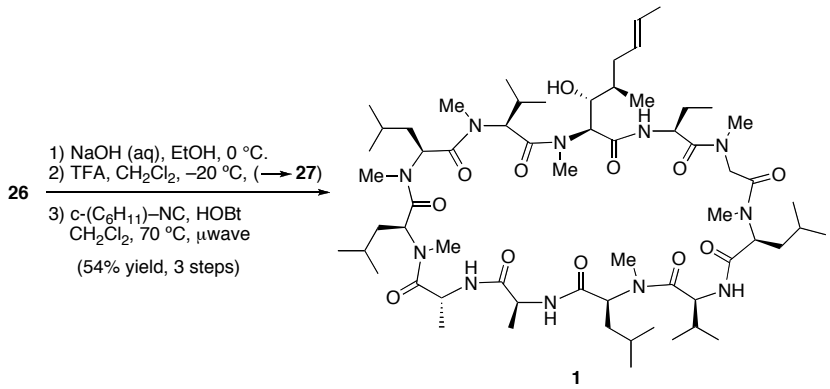


Boc-Abu-Sar-SH (23). To a solution of **22** (27 mg, 0.1 mmol) in $CHCl_3$ (1 mL) was added Lawesson' reagent (22 mg, 0.055 mmol), and the reaction mixture was stirred at room tempeature for 24 hours. The solvents were removed, and the residue was purified by flash chromatography using hexanes:ethyl acetate (2:1) as eluent to give the product **23** (19

mg, 65% yield). $[\alpha]_D^{20}$ = +73.3, (c = 2.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 5.27 (1H, d, *J* = 7.9 Hz), 4.62 (1H, dd, *J* = 7.9, 13.0 Hz), 4.49 (1H, d, *J* = 16.9 Hz), 4.05 (1H, d, *J* = 16.9 Hz), 3.17 (3H, s), 1.83 (1H, app. ddd, *J* = 6.9, 6.9, 13.4 Hz), 1.61 (1H, dddd, *J* = 7.3, 7.3, 14.6, 14.6 Hz), 1.43 (9H, s), 0.93 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 194.7, 173.3, 155.6, 79.7, 58.8, 51.3, 36.5, 28.3, 25.9, 9.6; IR (thin film) 3323.7, 2974.7, 1707.7, 1648.8, 1485.99 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{12}H_{22}N_2O_4S+Na]^+$: 313.1198, found 313.1193.

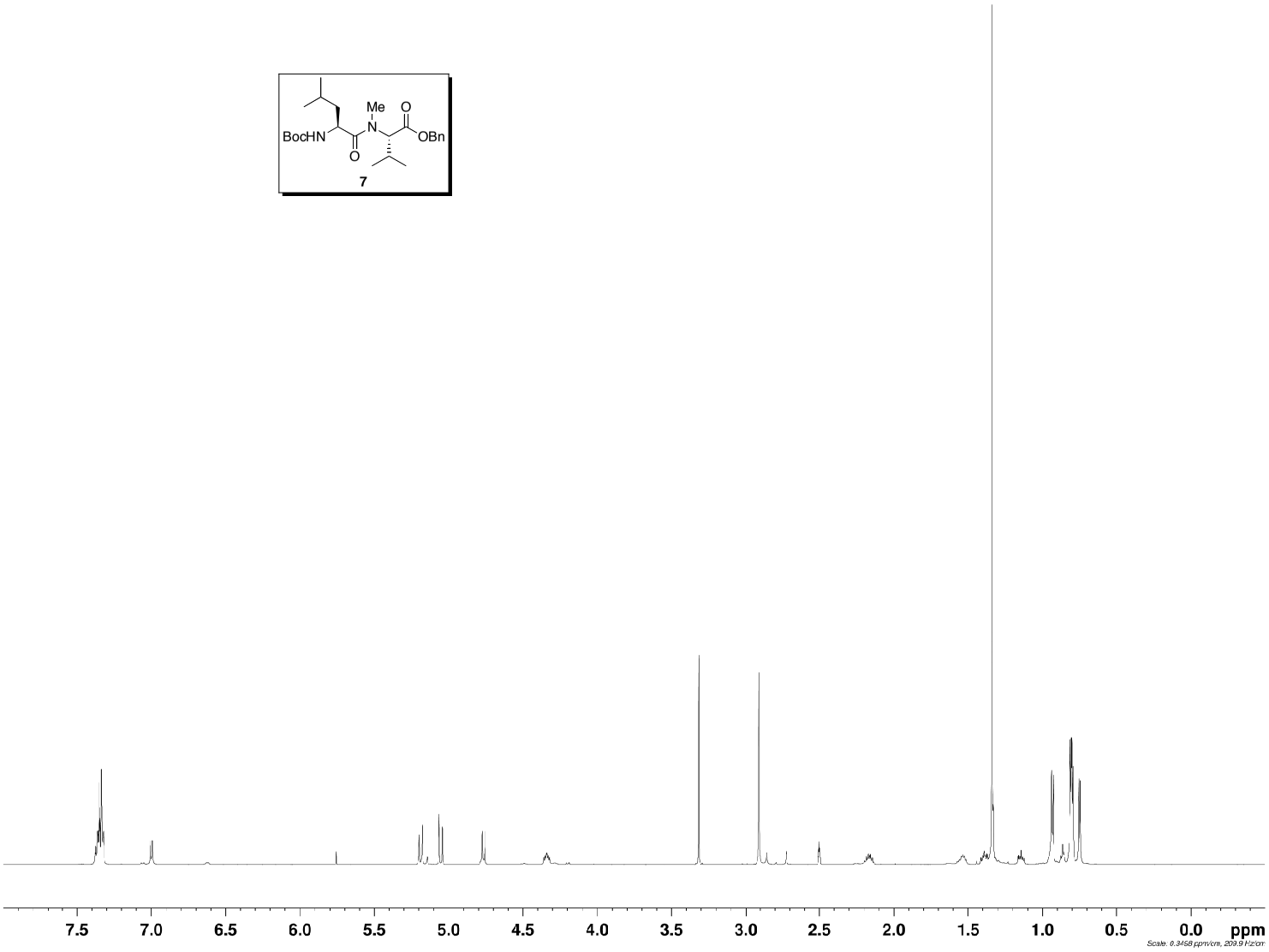


TFA•Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (24). To a solution of **23** (78 mg, 0.27 mmol) in $CHCl_3$ (4 mL) was added compound **18** (114 mg, 0.18 mmol) and cyclohexyl isonitrile (66 μL, 0.54 mmol). The reaction mixture was stirred at room temperature for 24 hours. The solvents were evaporated and the residue was purified by flash chromatography using hexanes:acetone (3:1) as eluent to give the product **24** (85 mg, 63% yield). The analytical data are identical with the published values.

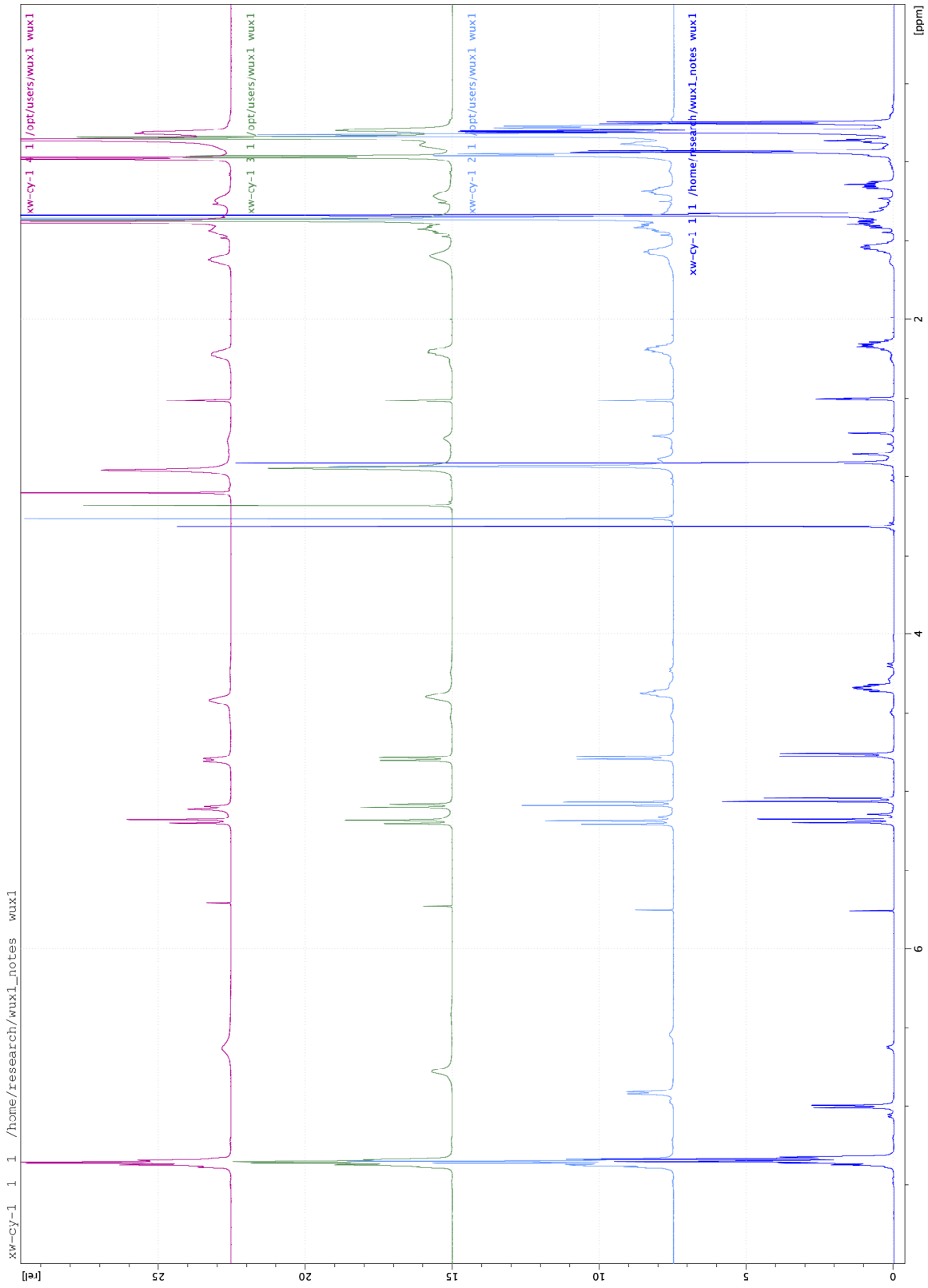


Cyclosporine A (1). To a solution of **26** (14 mg, 0.01 mmol) in ethanol (1.4 mL) at 0 °C was added aqueous sodium hydroxide (0.2 N, 0.2 mL), and the solution was stirred overnight at 0 °C until the starting material disappeared. The reaction mixture was neutralized with acetic acid and evaporated at low pressure. The residue was dissolved in dichloromethane (5 mL), washed with water then brine, and dried over $MgSO_4$. Finally, the resulting solution was evaporated to give the intermediate for next reaction. The intermediate was dissolved in CH_2Cl_2 (0.5 mL) and cooled to –20 ºC, then TFA (0.5 mL) was added, and the solution was stirred overnight until the Boc group was completely cleaved. The reaction mixture was diluted with CH_2Cl_2 (5 mL), washed with saturated $NaHCO_3$ solution then brine, and

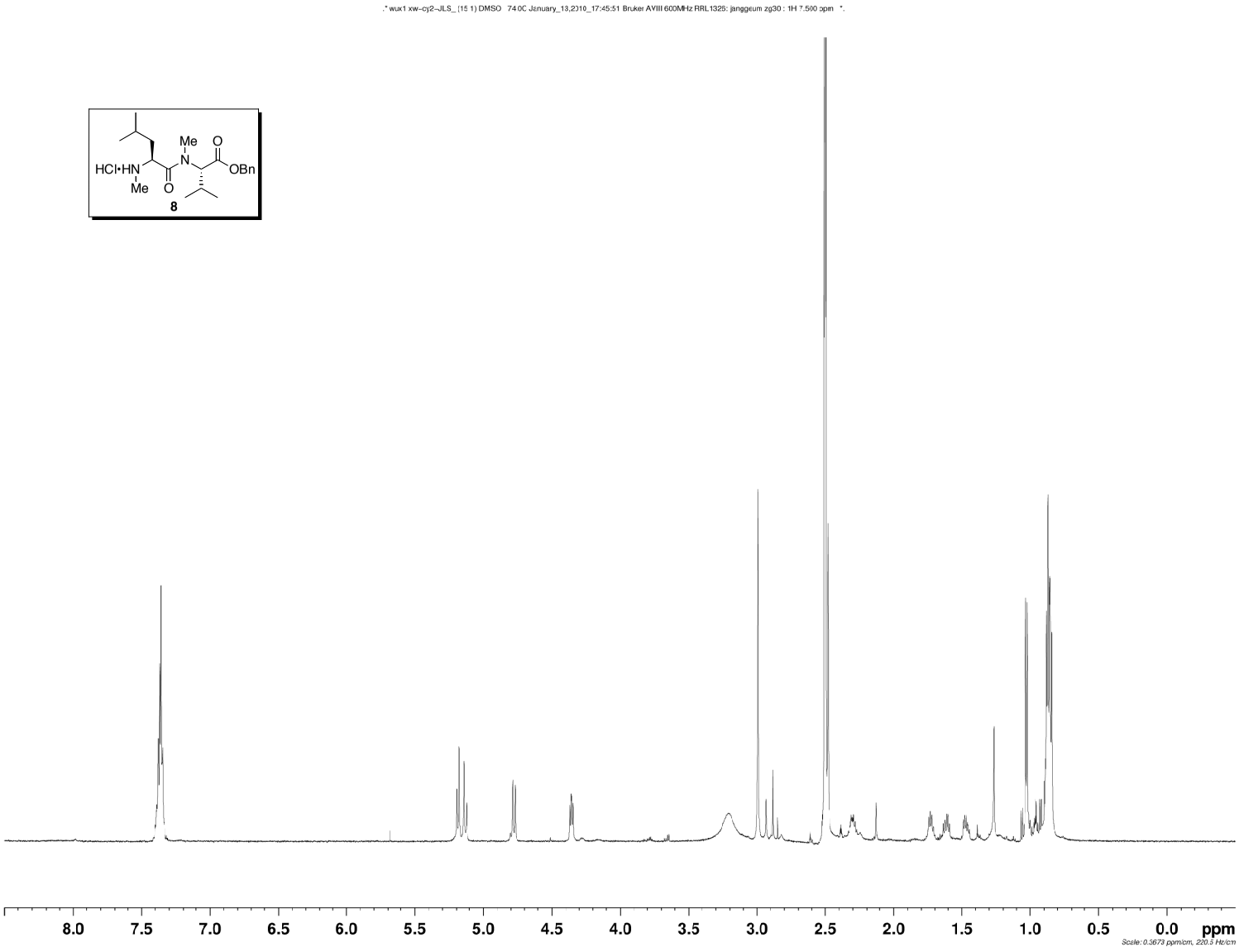
dried over $MgSO_4$. The solvents were evaporated and the crude residue was dissolved in CH_2Cl_2 (8 mL). Cyclohexyl isonitrile (6 μL, 0.048 mmol) and HOBt (2.0 mg, 0.015 mmol) in THF (0.3 mL) were added, and the resulting mixture was heated at 70 °C in the microwave for 16 hours. Finally, the residue was purified by flash chromatography using CH_2Cl_2:MeOH (20:1) as eluent to give the product **1** (6.4 mg, 54% over three steps). $[\alpha]_D^{20}$ = –267.9, (c = 0.43, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), δ = 8.00 (1H, d, *J* = 9.8 Hz), 7.62 (1H, d, *J* = 7.4 Hz), 7.47 (1H, d, *J* = 8.3 Hz), 7.16 (1H, d, *J* = 7.9 Hz), 5.70 (1H, dd, *J* = 4.3, 10.9 Hz), 5.49 (1H, d, *J* = 6.0 Hz), 5.34 (3H, comp. m), 5.12 (1H, d, *J* = 10.9 Hz), 5.06 (1H, t, *J* = 7.1 Hz), 5.04 (1H, dd, *J* = 7.4, 17.0 Hz), 4.97 (1H, dd, *J* = 5.9, 9.8 Hz), 4.83 (1H, ddd, *J* = 6.7, 6.7, 13.8 Hz), 4.72 (1H, d, *J* = 13.9 Hz), 4.65 (1H, app. t, *J* = 9.2 Hz), 4.52 (1H, ddd, *J* = 7.3, 7.3, 14.6 Hz), 3.79 (2H, m), 3.51 (3H, s), 3.40 (3H, s), 3.26 (3H, s), 3.19 (1H, d, *J* = 13.9 Hz), 3.11 (6H, br s), 2.70 (3H, s), 2.69 (3H, s), 2.45-2.39 (2H, comp. m), 2.15-2.03 (4H, comp. m), 1.99 (1H, ddd, *J* = 3.9, 10.4, 14.5 Hz), 1.78 (1H, m), 1.71 (1H, ddd, *J* = 7.2, 13.9, 13.9 Hz), 1.66-1.58 (9H, comp. m), 1.50-1.21 (4H, comp. m), 1.35 (3H, d, *J* = 7.26 Hz), 1.25 (3H, d, *J* = 6.8 Hz), 1.07 (3H, d, *J* = 6.5 Hz), 1.03 (3H, d, *J* = 6.6 Hz), 1.01 (6H, d, *J* = 6.7 Hz), 0.95 (3H, d, *J* = 6.7 Hz), 0.94 (3H, d, *J* = 6.7 Hz), 0.93 (3H, d, *J* = 6.5 Hz), 0.88 (3H, d, *J* = 6.9 Hz), 0.87 (3H, d, *J* = 6.2 Hz), 0.87 (6H, d, *J* = 6.5 Hz), 0.86 (3H, d, *J* = 7.3 Hz), 0.85 (3H, d, *J* = 6.6 Hz), 0.71 (3H, d, *J* = 6.1 Hz); ^{13}C NMR (150 MHz, $CDCl_3$), δ = 173.81, 173.75, 173.53, 173.45, 171.6, 171.3, 171.2, 170.4, 170.3, 170.1, 170.0, 129.7, 126.2, 74.7, 58.9, 57.9, 57.6, 55.5, 55.4, 55.3, 50.3, 48.7, 48.6, 48.2, 45.1 40.6, 39.5, 39.0, 37.4, 36.03, 36.95, 35.6, 34.0, 31.5, 31.3, 31.1, 29.77, 29.75, 29.5, 29.3, 29.1, 254, 24.90, 24.85, 24.6, 24.5, 23.9, 23.8, 23.7, 23.4, 21.8, 21.1, 20.3, 19.9, 18.7, 18.4, 18.2, 18.0, 17.9, 17.7, 16.8, 15.9; IR (thin film) 3322.8, 2960.2, 1639.2, 1470.5 cm^{-1}; HRMS (ES+) $[M+Na]^+$ *m/z* calculated for $[C_{62}H_{111}N_{11}O_{12}+Na]^+$: 1224.8311, found 1224.8345.



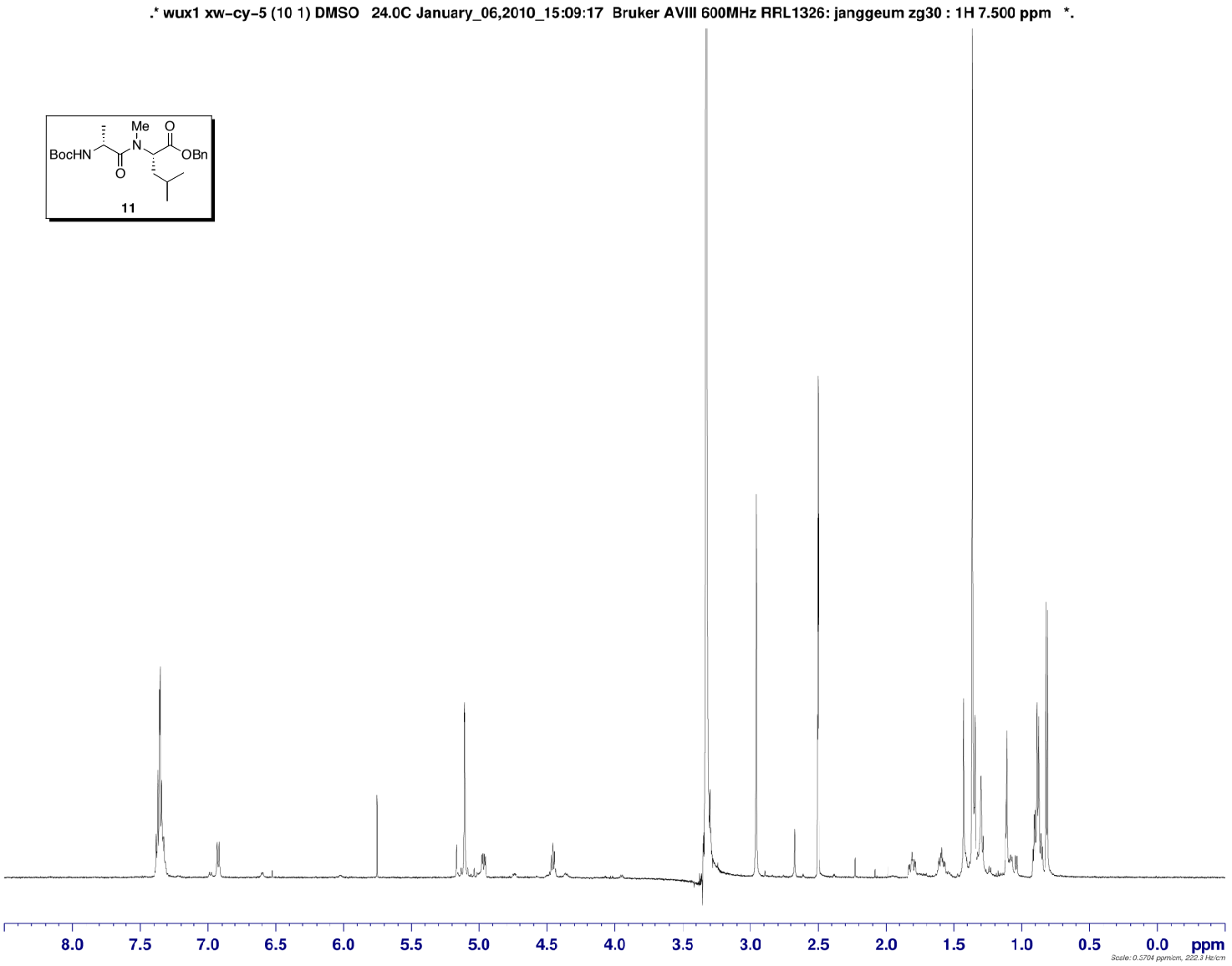
^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **7**.



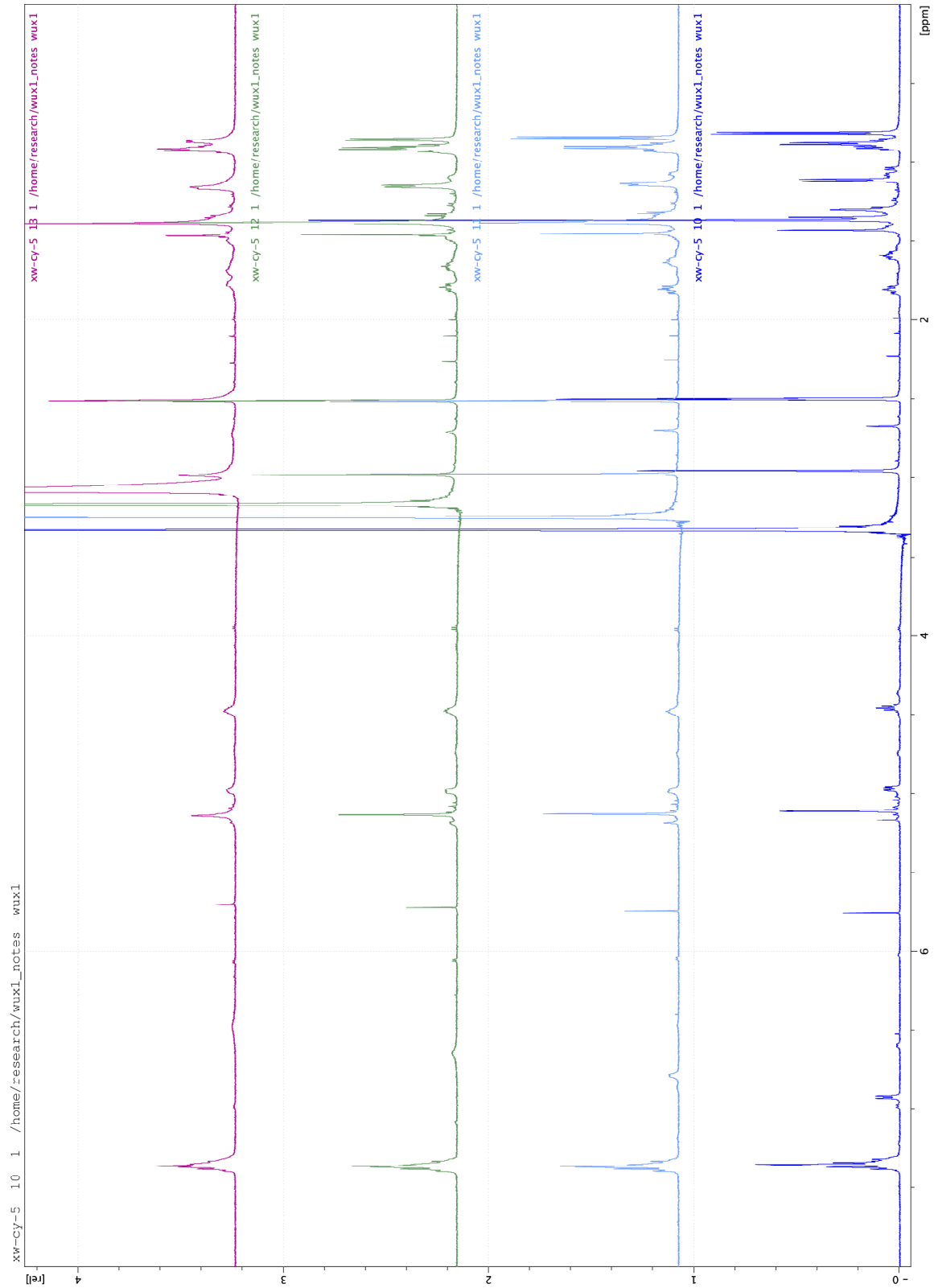
Variable Temperature ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **7**.



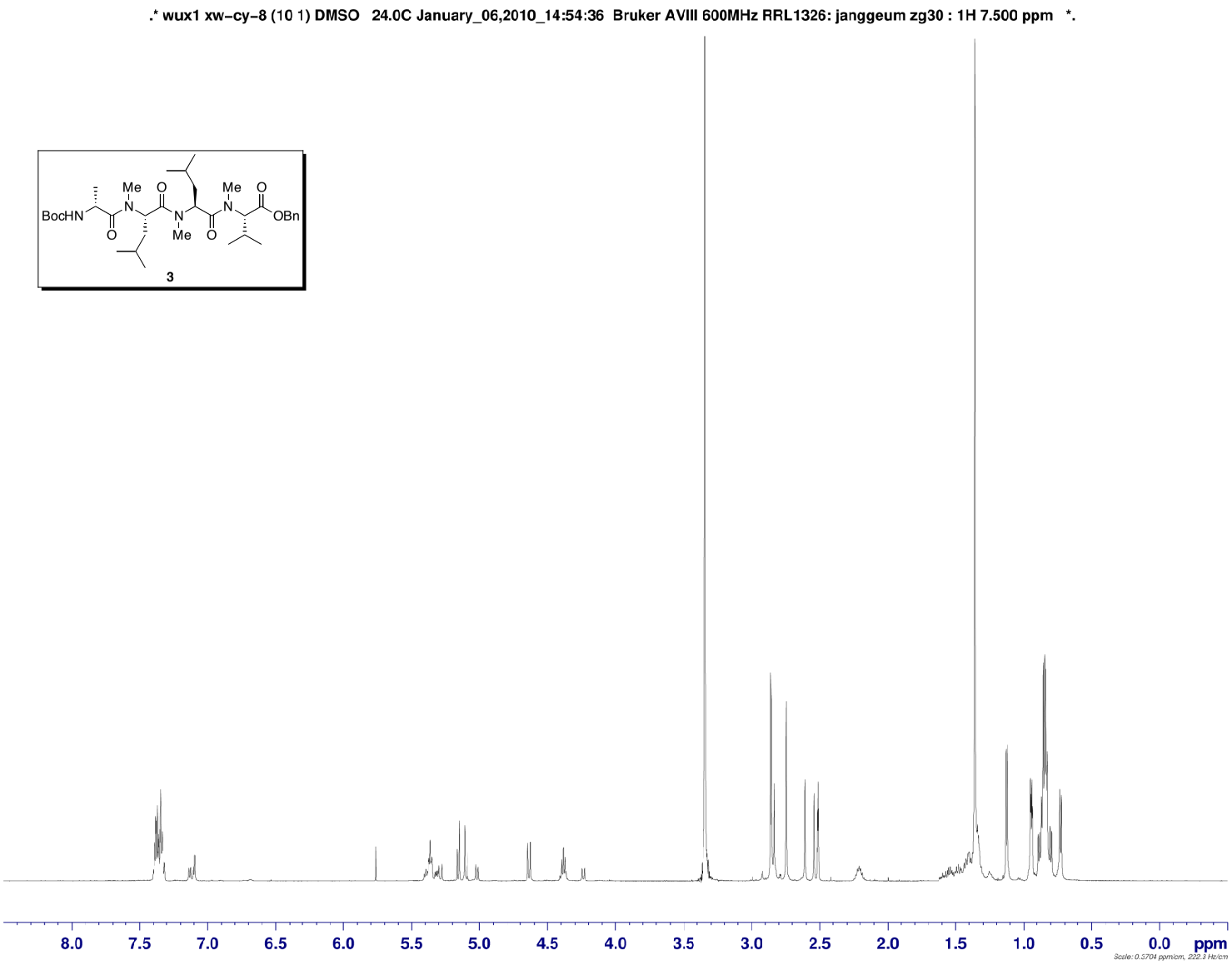
^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **8**.



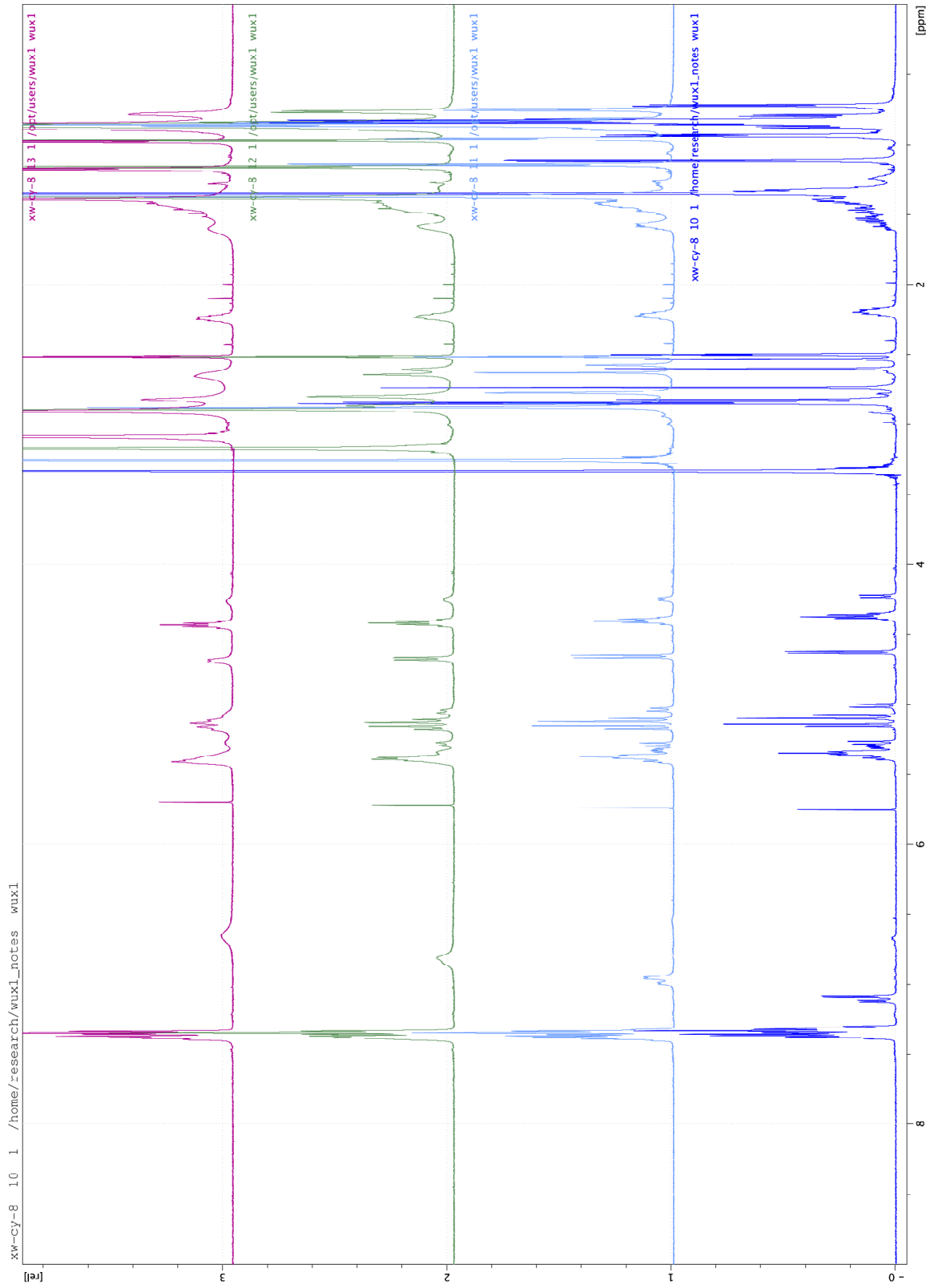
^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **11**.



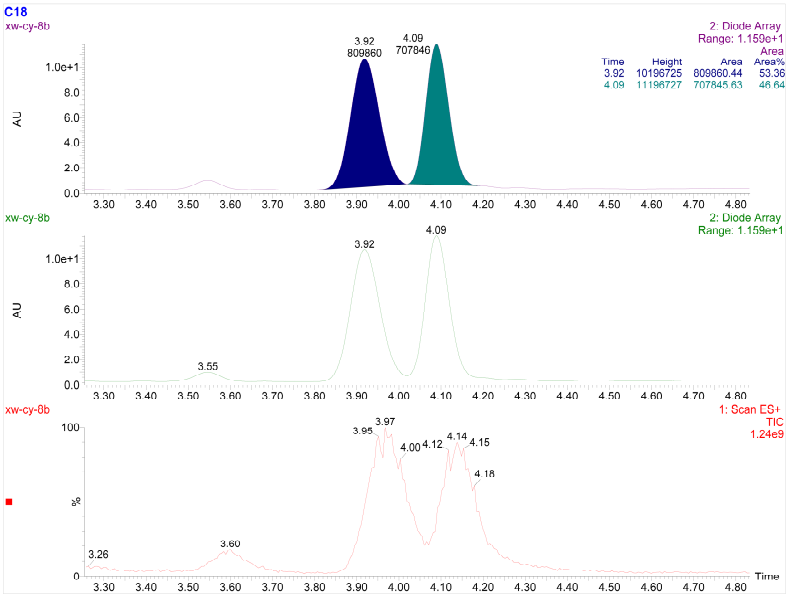
Variable Temperature ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **11**.



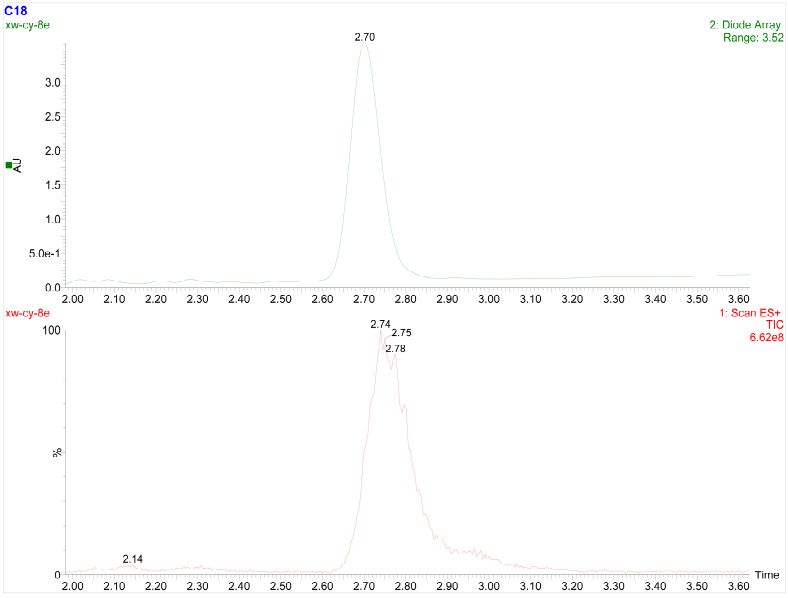
^{1}H NMR (600 MHz, DMSO-d_6) spectrum of compound **3**.



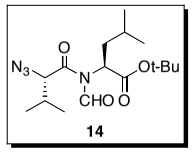
Variable Temperature ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **3**.



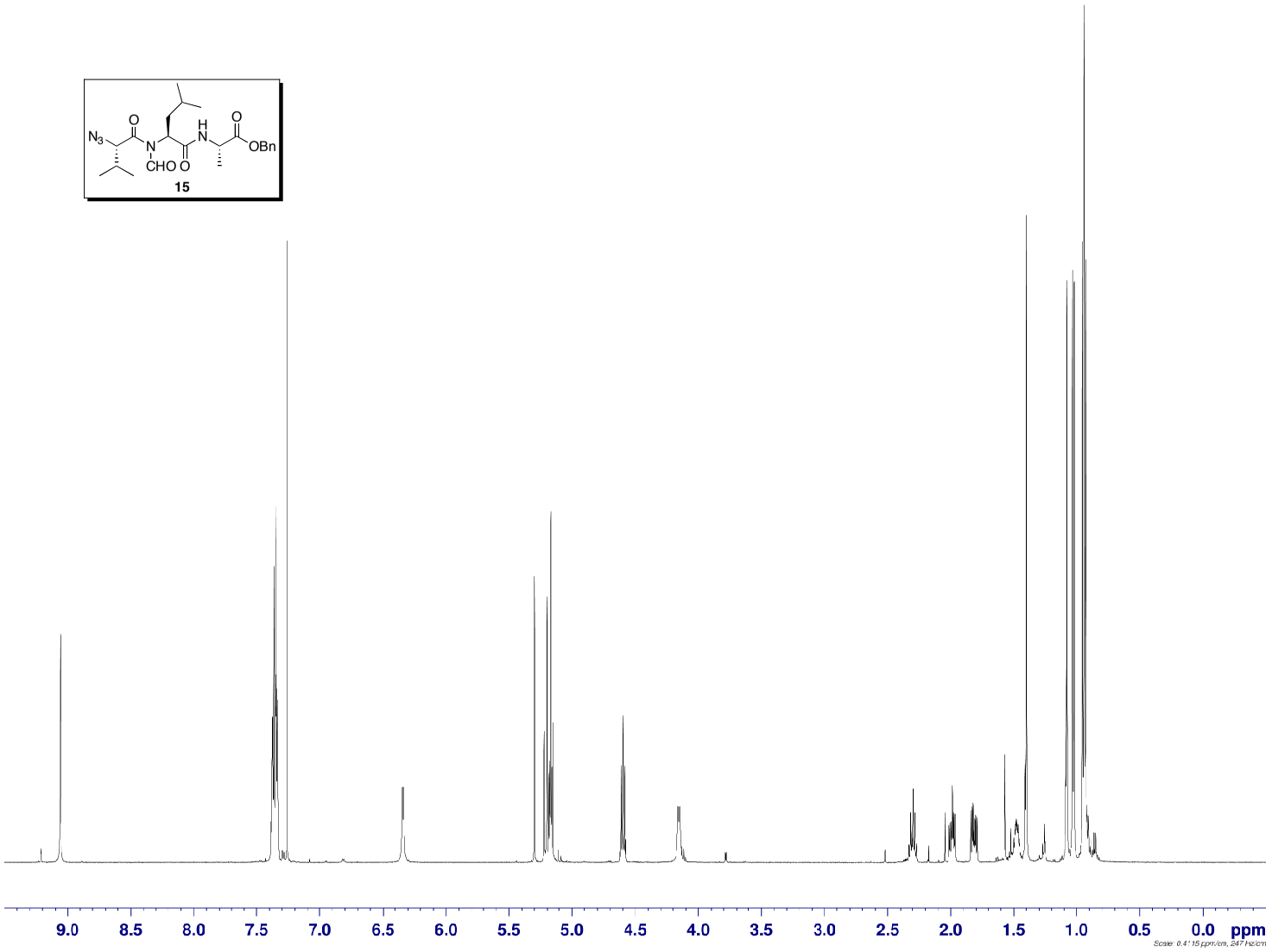
UPLC trace of racemic tetrapeptide **3**.



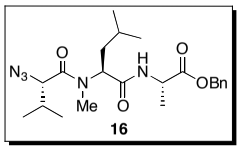
UPLC trace from DEBPT-mediated coupling to form tetrapeptide **3**.



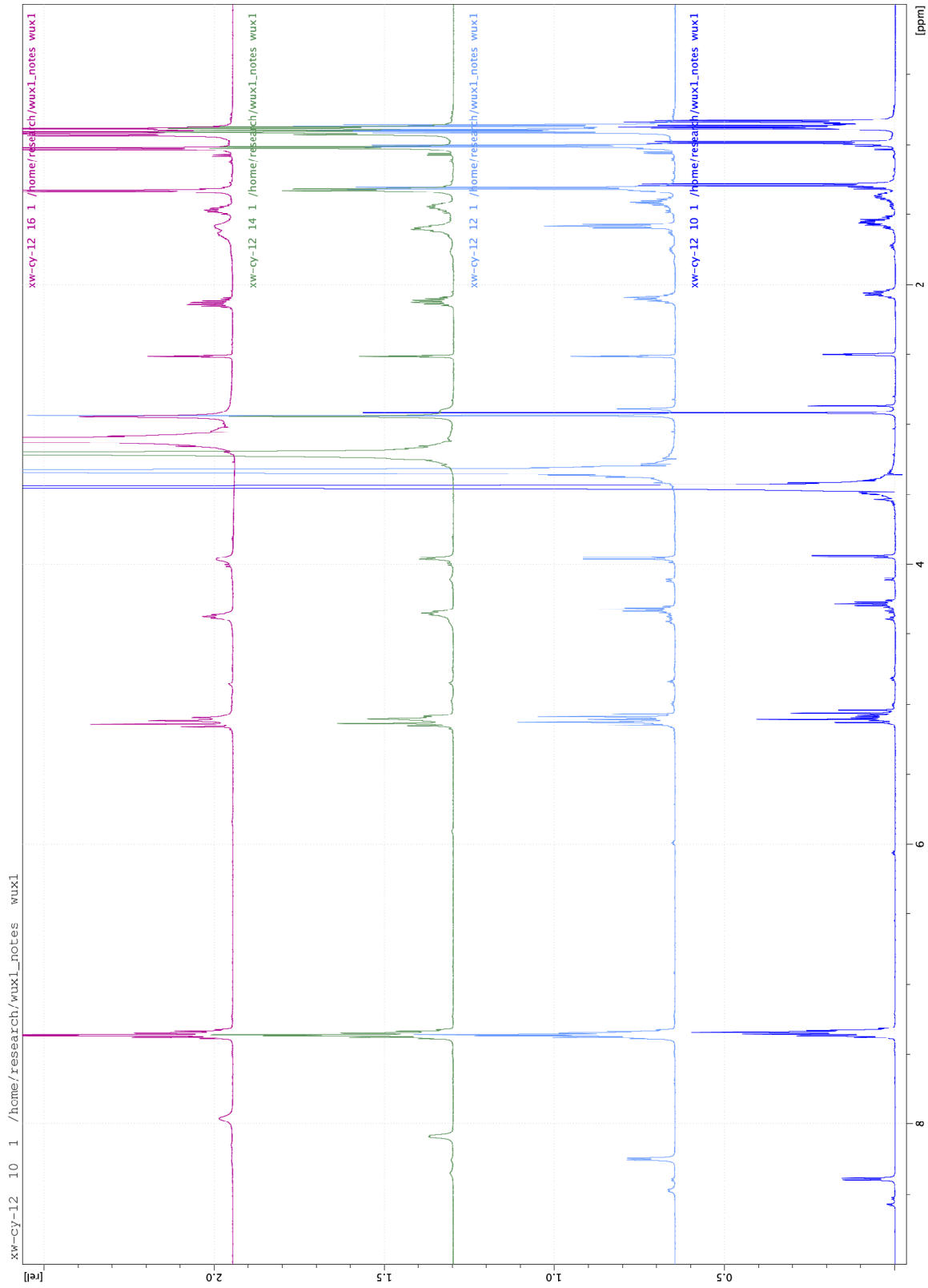
^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **14**.



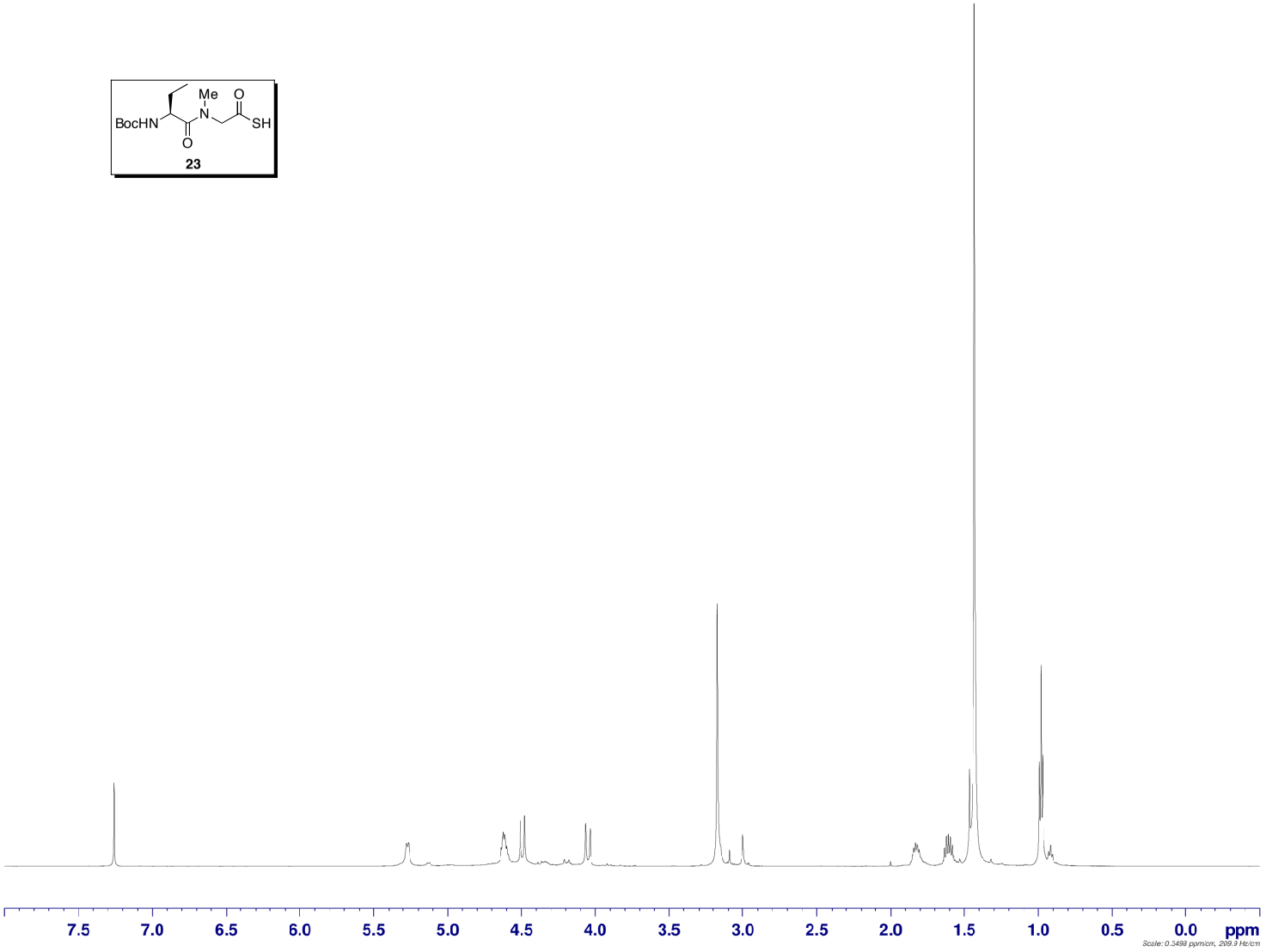
^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **15**.



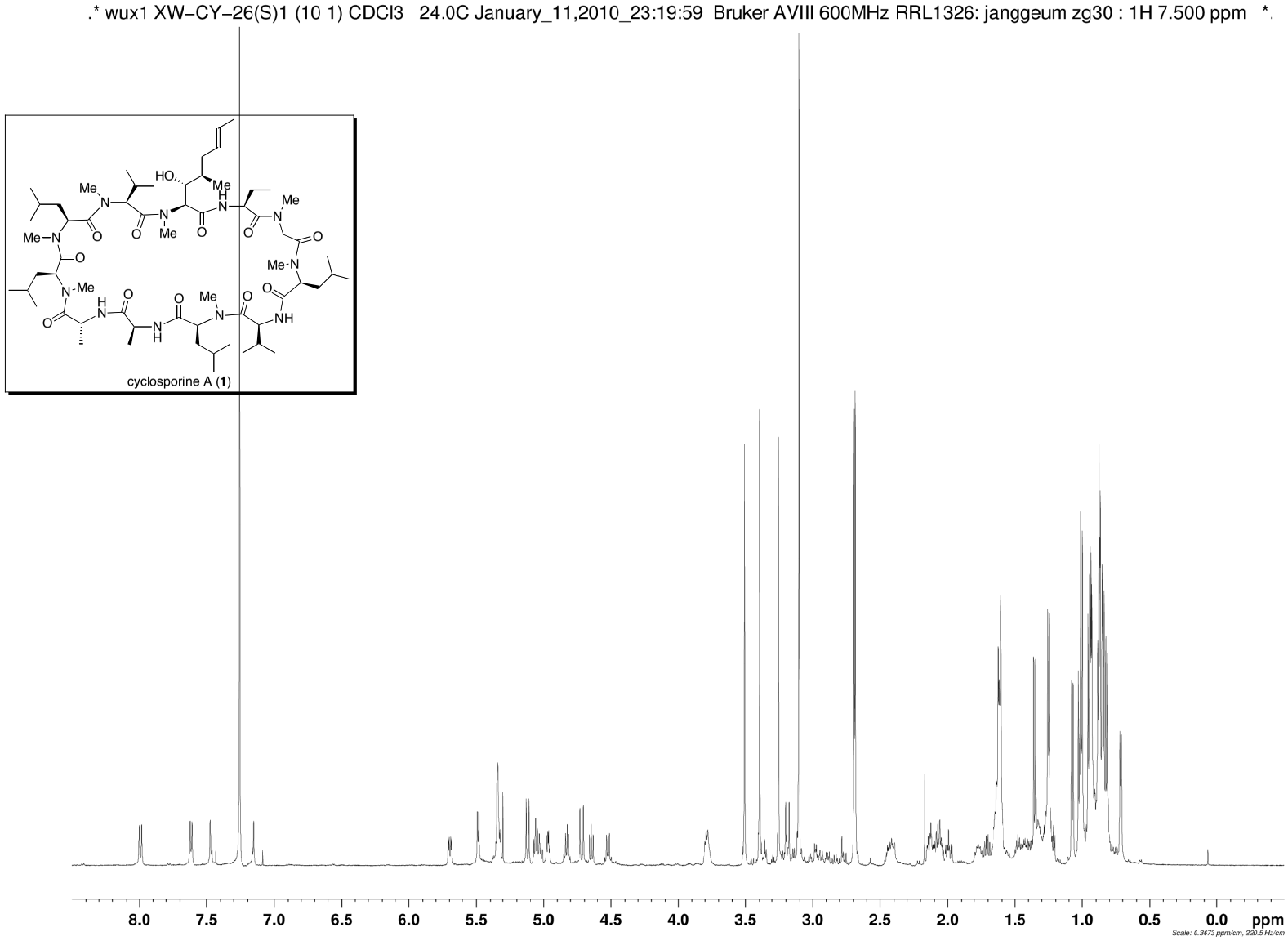
^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **16**.



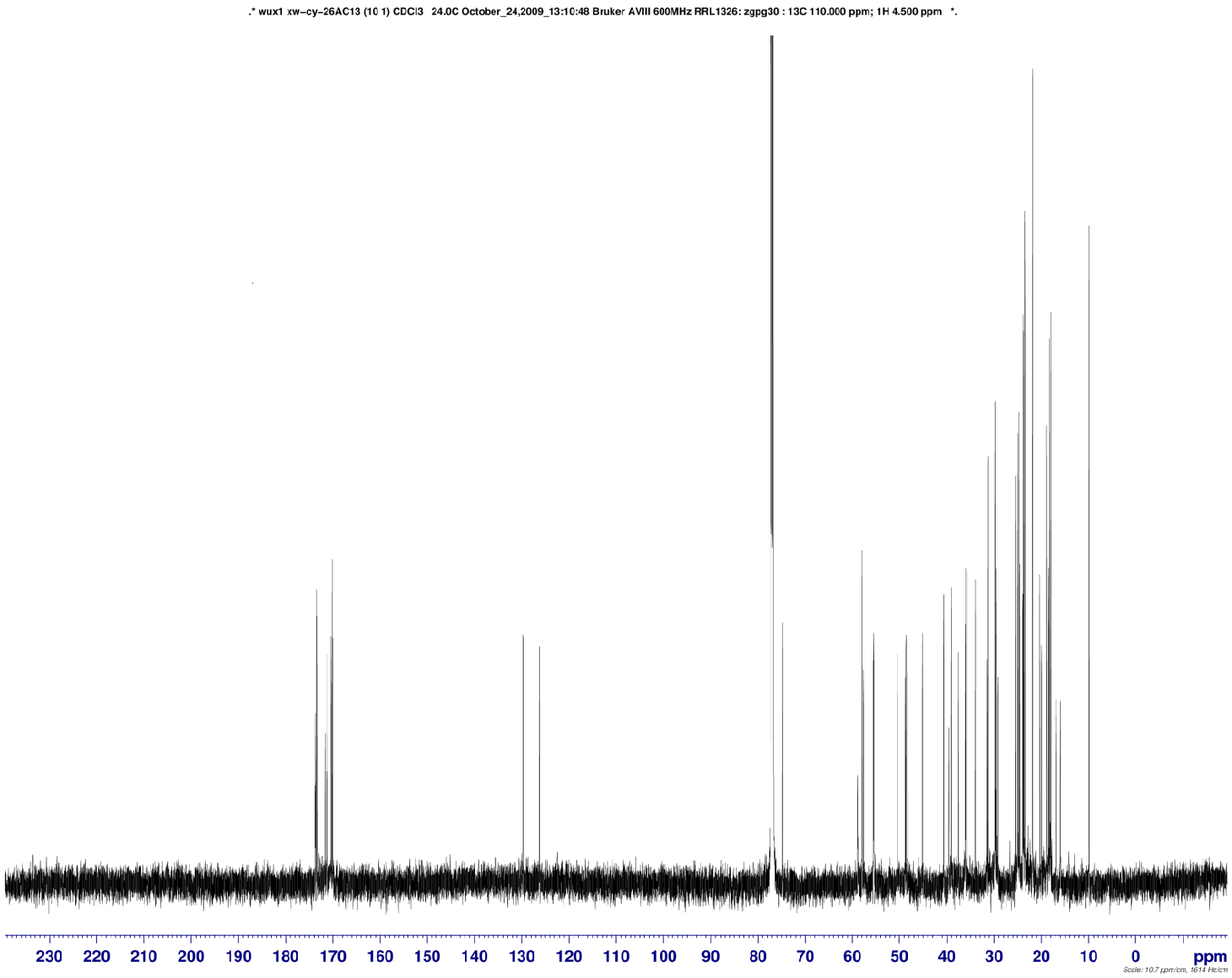
Variable Temperature ^{1}H NMR (600 MHz, DMSO-d_6) spectra of compound **16**.



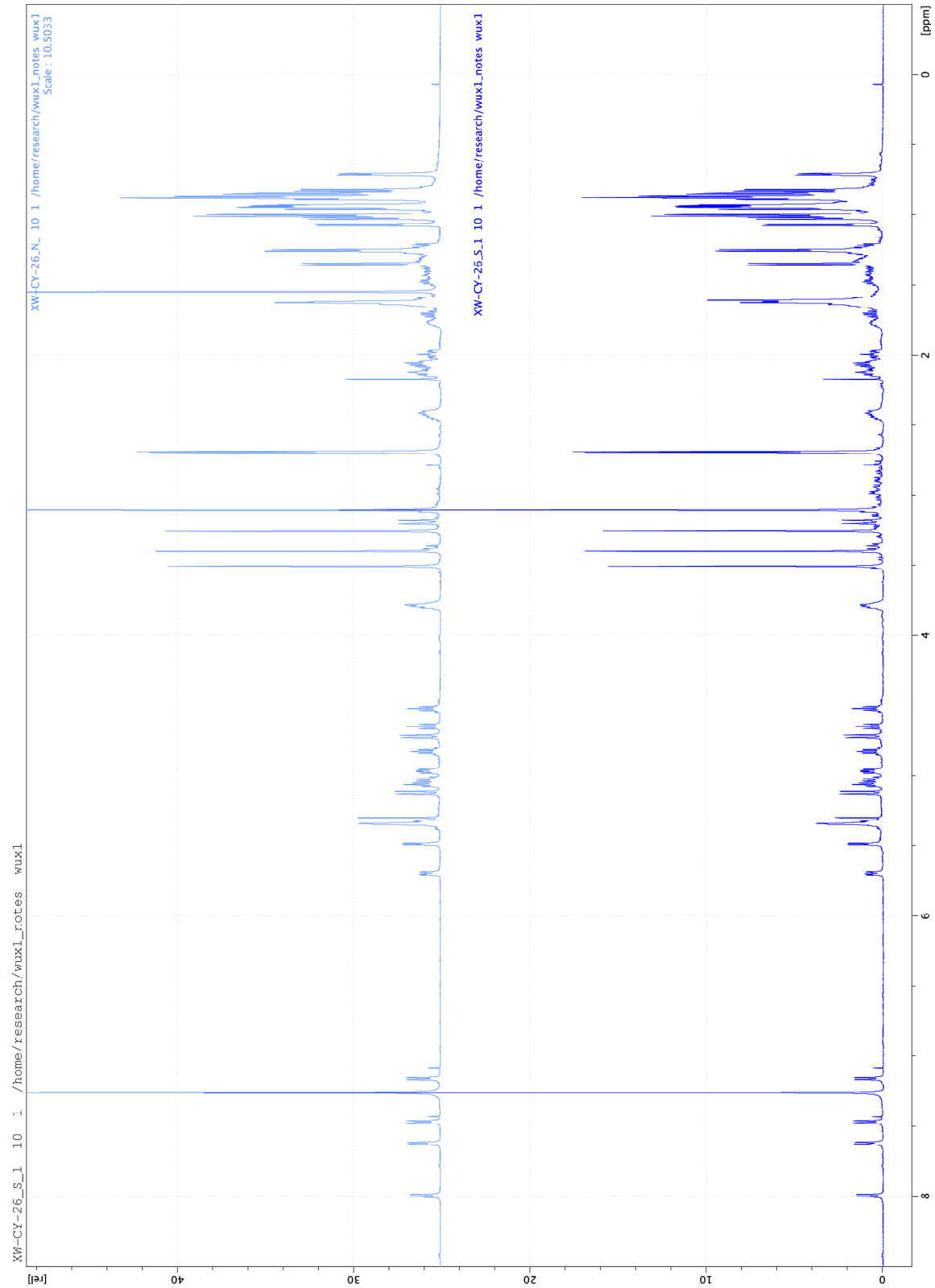
^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **23**.



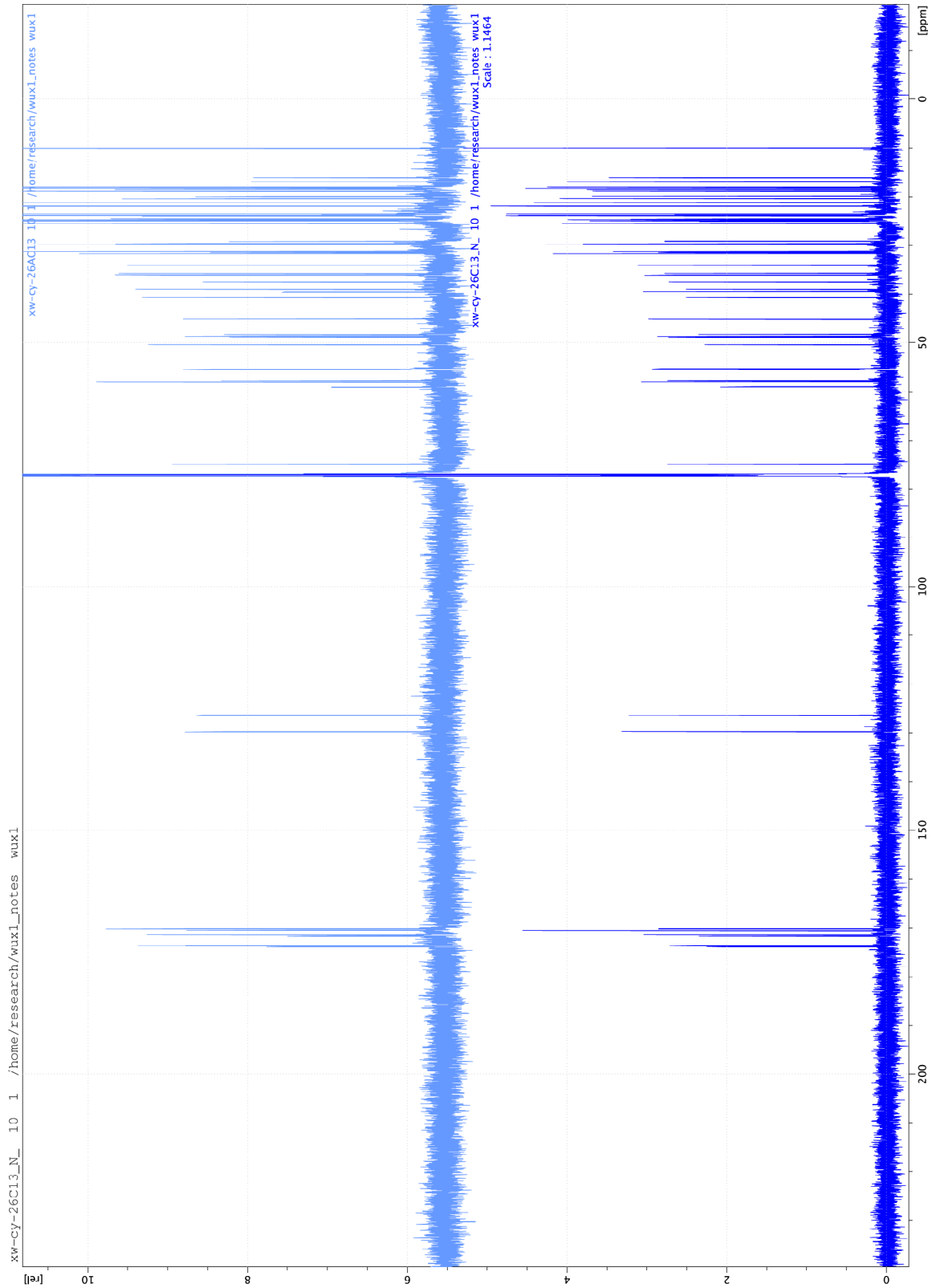
^{1}H NMR (600 MHz, $CDCl_3$) spectrum of synthetic compound **1**.



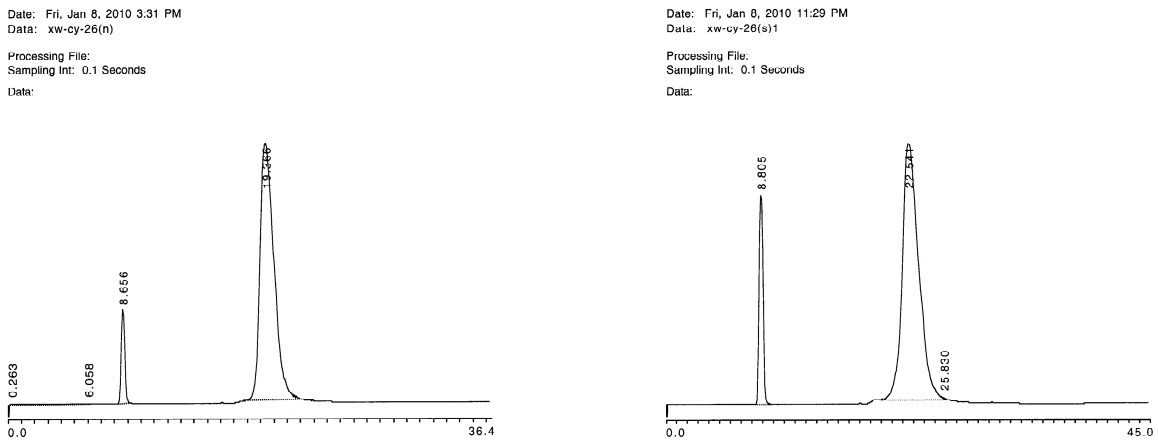
^{13}C NMR (150 MHz, $CDCl_3$) spectrum of compound **1**.



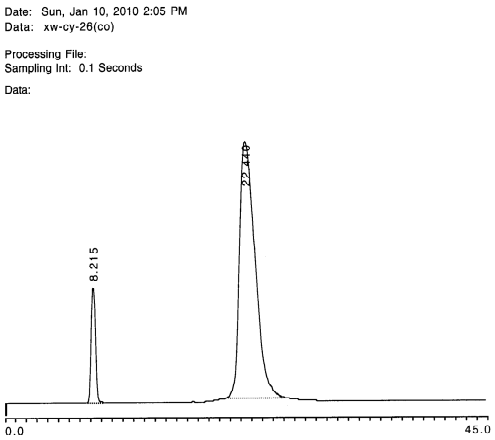
Overlay ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **1**, top = commercial, bottom = synthetic.



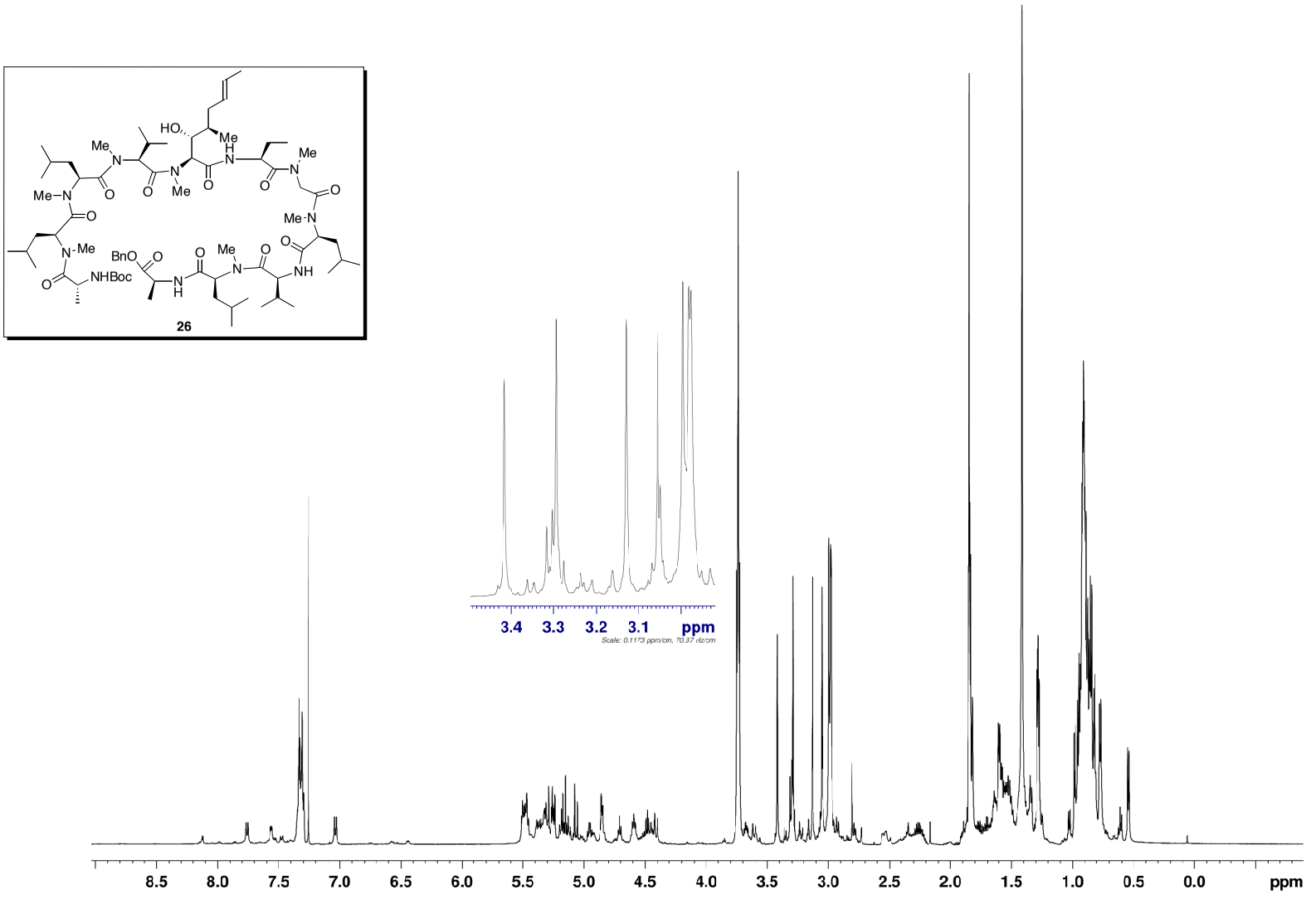
Overlay ^{13}C NMR (150 MHz, $CDCl_3$) spectrum of compound **1**, top = commercial, bottom = synthetic.



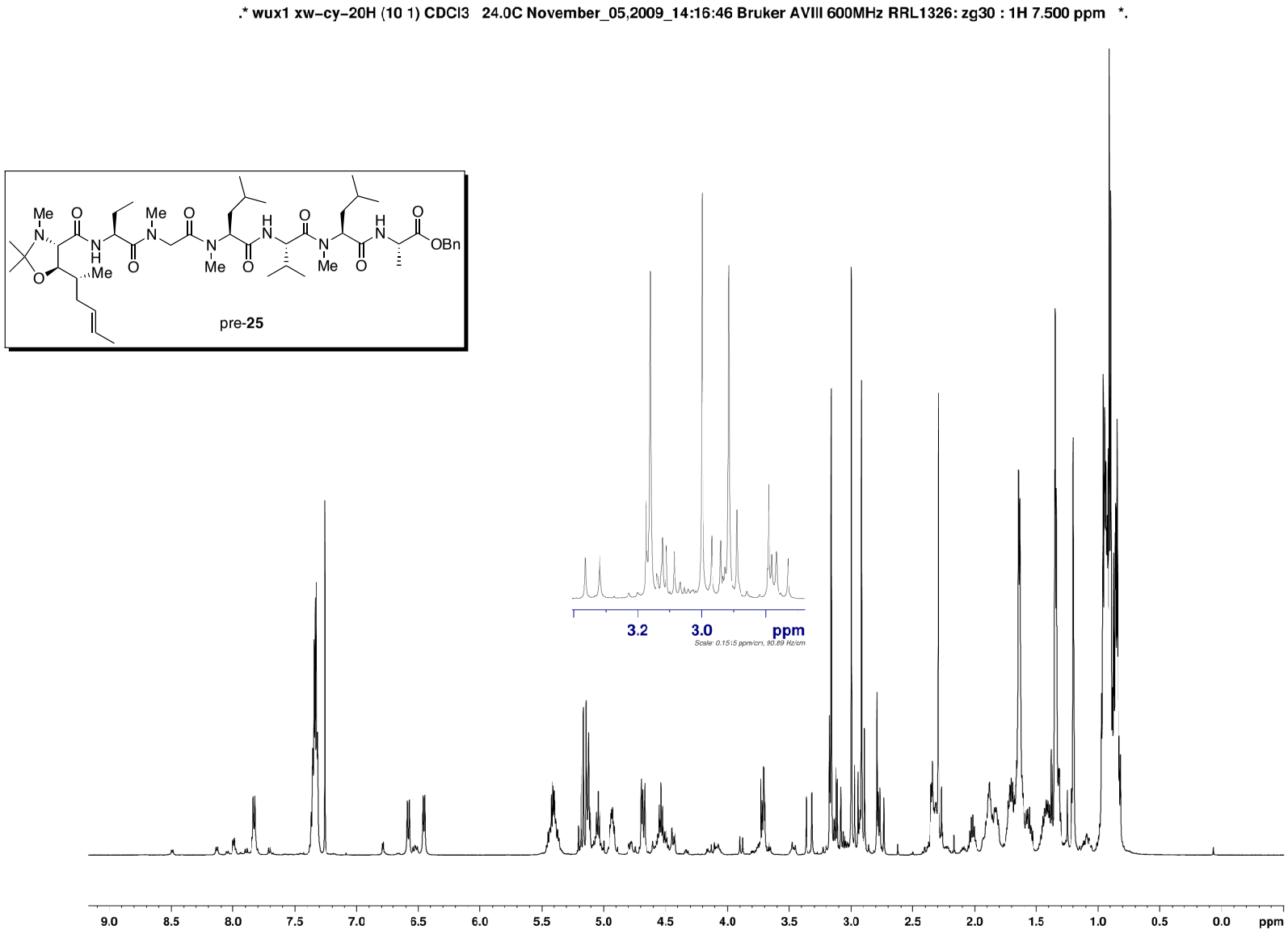
Chiral HPLC (analytical) trace for natural cyclosporine (left) and synthetic cyclosporine (right).



Chiral HPLC (analytical) trace for coinjection of natural cyclosporine with synthetic cyclosporine.



^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **26**, note clarity of N-Me groups.



^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound pre-**25**, note the appearance of rotamers by looking at the N-Me groups.