Expanding the Limits of Isonitrile-Mediated Amidations: On the Remarkable Stereosubtleties of Macrolactam Formation from Synthetic *Seco*-Cyclosporins

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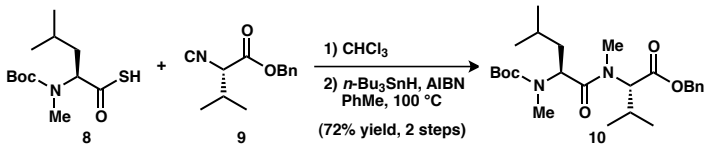
SUPPORTING INFORMATION

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, 77.16 ppm). Multiplicities are reported as follows: s = singlet, d = doublet, t = triplet, m = multiplet, comp. m = complex multiplet, app. = apparent, bs = broad singlet. All ^{13}C NMR spectra were recorded with complete proton decoupling. ***N*-Methylated peptides are typically observed as a mixture of rotamers. The data have been reported in order to provide the maximum amount of information regarding coupling constants, which has necessarily led to integrals reported *following* a group of peaks in some instances.** Variable temperature NMR data recorded on the DRX-600 MHz instrument in $CDCl_3$ at 10 ºC intervals ranging from 24 ºC to 54 ºC. In spectra below, the higher temperature NMR data is plotted above the lower temperature data. The data are presented to demonstrate the coalescence of the rotamers that begins to occur with increased temperature. The effects are clearest in the N-Me regions. 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.

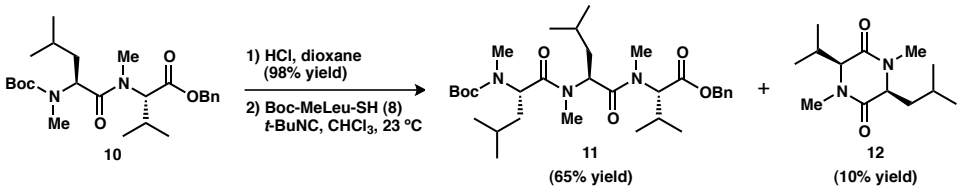
Preparation of Compounds.



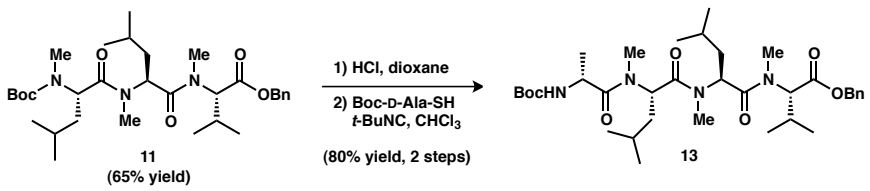
Boc-MeLeu-MeVal-OBn (10). To a solution of valine isonitrile **9**[1] (120 mg, 0.55 mmol) in chloroform (3.5 mL) was added *N*-methyl leucine thioacid **8**[2] (144 mg, 0.55 mmol) in chloroform (12 mL) via syringe pump over 3 hours. The reaction mixture was stirred at ambient temperature overnight then concentrated at low pressure to give a yellow oil. This residue was immediately dissolved in toluene (10 mL), and then *n*-Bu_3SnH (0.70 mL, 2.75 mmol) and AIBN (88 mg, 0.55 mmol) were added. The reaction mixture was heated to 100 ºC for 30 min. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (6:1 hexanes/ethyl acetate) to give dipeptide **10** (177 mg, 72% yield). $[\alpha]_D^{20} = -140.3°$ (c = 1.75, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers): δ 7.37 – 7.27 (m, 5H), [5.25 (d, J = 12.2 Hz), 5.19 (dd, J = 12.3, 1.3 Hz, 1H), 5.15 – 4.92 (m, 2H), 4.87 – 4.78 (m), 4.46 (d, J = 10.2 Hz), 4.22 (d, J = 10.4 Hz), *all sum to* 4H], [2.95 (s, 1H), 2.92 (s, 1H), 2.84 (s), 2.74 (s, 2H), 2.72 (s), 2.51 (s), 2.50 (s), *all sum to* 6H], 2.24 (ddq, J = 27.5, 10.6, 6.7 Hz, 1H), [1.70 (m), 1.63 – 1.47 (m), 1.47 – 1.37 (m), *all sum to* 12H], [1.03 (d, J = 6.4 Hz), 0.98 (app. dd, J = 6.5, 2.1 Hz), 0.95 – 0.86 (m), 0.83 (d, *J* = 6.7 Hz), 0.81 (d, *J* = 6.8 Hz), *all sum to* 12H]; ^{13}C NMR (151 MHz, $CDCl_3$, mixture of rotamers): δ 172.5, 171.8, 171.7, 171.0, 170.92, 170.9, 170.3, 170.1, 156.0, 155.5, 155.1, 154.2, 135.8, 135.8, 135.5, 135.4, 129.3, 128.7, 128.64, 128.61, 128.5, 128.42, 128.4, 128.3, 128.3, 80.6, 80.5, 80.0, 79.9, 67.3, 67.0, 66.6, 64.7, 64.5, 62.0, 61.9, 54.1, 53.9, 52.7, 52.4, 38.7, 38.4, 38.2, 38.0, 31.2, 30.9, 29.74, 29.71, 29.3, 29.2, 28.8, 28.53, 28.50, 28.46, 28.4, 28.30, 27.8, 27.6, 27.4, 24.8, 24.6, 24.5, 24.3, 23.2, 23.19, 23.15, 23.0, 22.9, 22.7, 22.44, 22.36, 20.1, 20.0, 19.9, 19.8, 19.4, 19.1, 18.84, 18.80; IR (thin film) 2962.1, 1738.5, 1687.4, 1655.6, 1455.0 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{25}H_{40}N_2O_5Na$): 471.2, $[M+K]^+$ ($C_{25}H_{40}N_2O_5K$): 487.2, found: 471.3, 487.3.

(1) Zhu, J.; Wu, X.; Danishefsky, S. J. *Tetrahedron Lett*. **2009**, *50*, 577–579.

(2) Wu, X.; Stockdill, J. L.; Wang, P.; Danishefsky, S. *J. Am. Chem. Soc*. **2010**, *132*, 4098–4100.

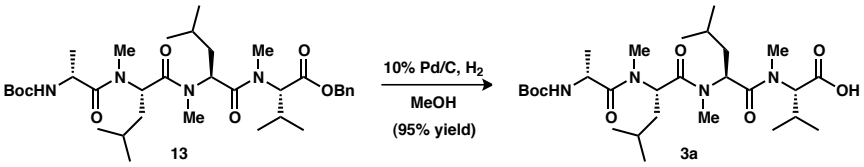


MeLeu-MeLeu-MeVal-OBn (11). Dipeptide **10** (45 mg, 0.10 mmol) was treated with 4.0 M HCl in dioxane (1.0 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was co-evaporated with toluene to give the corresponding deprotected dipeptide as the amine hydrochloride salt (38 mg, 98% yield). This material was carried directly into the next step without further purification. To a solution of **8** (52 mg, 0.2 mmol) in $CHCl_3$ (1 mL) was added *t*-butyl isonitrile (34 μL, 0.3 mmol), then the mixture was stirred for 5 min. Deprotected dipeptide (38 mg, 0.099 mmol) in $CHCl_3$ (2 mL) was added to the resulting mixture via syringe pump over 2 hours, then the reaction was stirred overnight at room temperature. The volatiles were removed at low pressure, and the residue was purified by flash chromatography using (4:1 hexanes/ethyl acetate) to give tripeptide **11** (37 mg, 65% yield) as an off-white oil. $[\alpha]_D^{20} = -142.1°$ (c = 1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers): δ 7.31 (td, *J* = 8.3, 7.5, 3.3 Hz, 5H), [5.55 – 5.48 (m), 5.46 (ddd, J = 12.2, 9.1, 5.9 Hz, 1H), 5.28 (dd, J = 12.3, 3.5 Hz), 5.19 (t, J = 11.9 Hz), 5.09 – 5.05 (m), 5.03 (dd, J = 9.9, 4.8 Hz), 4.98 (dd, J = 12.2, 1.9 Hz), 4.89 (d, J = 6.4 Hz), 4.87 (dd, J = 6.9, 2.8 Hz), 4.84 (s), 4.78 (dd, J = 9.4, 4.8 Hz), 4.34 (d, J = 10.1 Hz), 4.27 (d, J = 10.1 Hz) *all sum to* 5H], [3.00 (s), 2.96 (s), 2.92 (s), 2.89 (s), 2.81 (s), 2.76 (s), 2.73 (s), 2.72 (s), 2.69 (s), 2.67 (s) *all sum to* 9H], 2.21 (dddd, *J* = 17.6, 13.3, 11.3, 8.5 Hz, 1H), 1.72 – 1.59 (m, 1H), 1.59 – 1.49 (m, 3H), 1.45 (s, 4H), 1.44 – 0.78 (comp. m, 18H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.6, 171.9, 171.8, 171.6, 171.3, 171.1, 170.9, 170.8, 170.7, 170.4, 169.9, 169.8, 155.9, 155.7, 155.1, 155.0, 135.74, 135.73, 135.39, 135.36, 128.8, 128.7, 128.7, 128.6, 128.5, 128.43, 128.42, 128.40, 128.3, 128.0, 80.58, 80.55, 80.1, 80.0, 67.0, 66.8, 66.64, 66.60, 64.7, 64.5, 61.88, 61.87, 61.82, 54.12, 52.9, 52.7, 51.6, 51.4, 51.1, 51.0, 38.7, 38.6, 38.3, 38.1, 38.0, 37.9, 37.8, 37.7, 31.3, 31.2, 30.3, 30.2, 29.88, 29.86, 29.81, 29.7, 29.6, 29.4, 29.1, 28.49, 28.47, 28.44, 28.41, 27.7, 27.7, 27.5, 27.34, 27.31, 25.0, 24.9, 24.8, 24.57, 24.56, 24.52, 24.50, 24.2, 23.49, 23.46, 23.3, 23.14, 23.11, 23.05, 22.9, 22.8, 22.6, 22.5, 22.4, 22.35, 22.3, 21.9, 20.02, 20.01, 19.76, 19.74, 18.82, 18.80, 18.77; IR (thin film) 2958.3, 2871.5, 2359.5, 2341.2, 1739.5, 1690.3, 1644.0 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{32}H_{53}N_3O_6Na$): 598.2, found: 598.4.

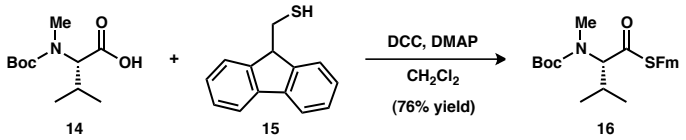


Boc-D-Ala-MeLeu-MeLeu-MeVal-OBn (13). Tripeptide **11** (29 mg, 0.05 mmol) was treated with 4.0 M HCl in dioxane (1.0 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was co-evaporated with toluene to give the corresponding deprotected tripeptide as the amine hydrochloride salt. This material was subsequently dissolved in chloroform (1.0 mL), then Boc-D-Ala-SH[3] (21 mg, 0.1 mmol) and *t*-butyl isonitrile (23 μL, 0.2 mmol) were added. The reaction was stirred at room temperature overnight. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (6:1 hexanes/ethyl acetate) to give tetrapeptide **13** (26 mg, 80% yield over two steps). $[\alpha]_D^{20}$ = –143.7° (c = 3.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers): δ 7.36 – 7.27 (m, 5H), [5.51 – 5.40 (m), 5.33 – 5.21 (m), 5.12 (dd, *J* = 72.2, 12.3 Hz), 4.98 (d, *J* = 12.2 Hz), 4.87 (d, *J* = 10.5 Hz), 4.56 (app. q, *J* = 7.4 Hz), 4.25 (d, *J* = 10.1 Hz), *all sum to* 6H], [2.95 (s), 2.93 (s), 2.91 (s), 2.90 (s), 2.68 (s), 2.67 (s), *all sum to* 9H], 2.21 (tdt, *J* = 13.2, 10.4, 6.6 Hz, 1H), [1.69 (dtd, *J* = 14.6, 10.3, 5.7 Hz), 1.64 – 1.58 (m), 1.57 – 1.46 (m), 1.40 (m), *all sum to* 6H] 1.40 (s, 9H), 1.26 (d, *J* = 6.9 Hz, 3H), [0.99 (d, *J* = 6.5 Hz), 0.97 (d, *J* = 6.3 Hz), 0.91 – 0.86 (m), 0.85 (d, *J* = 6.7 Hz), 0.77 (d, *J* = 6.7 Hz), *all sum to* 18H]; ^{13}C NMR (151 MHz, $CDCl_3$, mixture of rotamers): δ173.3, 173.2, 172.0, 171.0, 170.9, 170.7, 169.9, 169.8, 155.5, 155.4, 135.7, 135.3, 128.8, 128.7, 128.66, 128.6, 128.5, 128.44, 128.41, 128.35, 128.3, 79.8, 79.7, 67.0, 66.6, 64.6, 61.9, 51.7, 51.7, 51.6, 51.2, 46.7, 38.7, 38.1, 37.92, 37.90, 31.2, 30.5, 30.2, 30.0, 29.98, 29.8, 28.44, 28.41, 27.7, 27.3, 24.9, 24.86, 24.6, 24.5, 23.6, 23.11, 23.09, 23.0, 22.95, 22.7, 22.5, 22.3, 22.2, 20.0, 19.7, 18.80, 18.76, 18.4, 18.2; IR (thin film) 2959.2, 1737.6, 1642.1, 1456.9, 1367.3 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{35}H_{59}N_4O_7$): 647.4, found: 647.4.

(3) Synthesized by the procedure outlined in: Lehmann, J.; Linden, A.; Heimgartner, H. *Helv. Chim. Acta* **1999**, *82*, 888–908. Characterization data for Boc-L-Ala-SH are given in: Monfardini, I.; Huang, J.-W.; Beck, B.; Cellitti, J. F.; Pellecchia, M.; Dömling, A. *J. Med. Chem.* **2011**, *54*, 890–900.



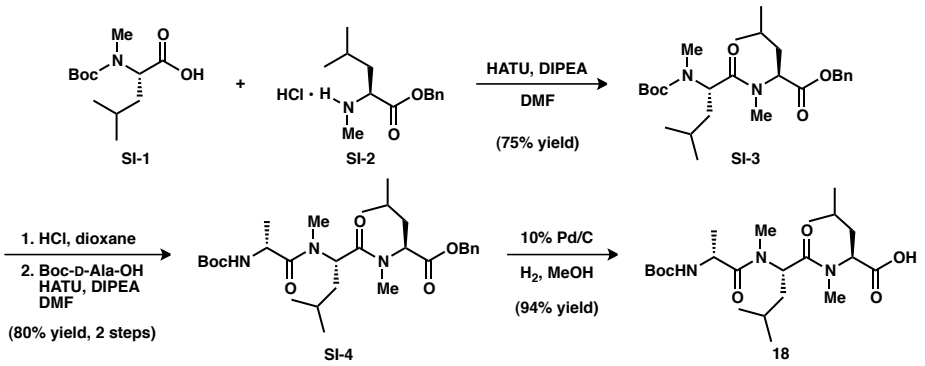
Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (3a). A suspension containing tetrapeptide **13** (64.5 mg, 0.1 mmol) and palladium on activated charcoal (10% Pd basis, 20 mg) in MeOH (10 mL) was placed under an atmosphere of hydrogen. The suspension was stirred overnight at room temperature. The reaction mixture was then filtered to remove the catalyst, and evaporated at low pressure. The resulting residue was dried under high vacuum to give acid **3a** as a white solid (52.8 mg, 95% yield). Selected characterization data: ^{1}H NMR consistent with Wenger's data; ^{13}C NMR (151 MHz, $CDCl_3$) δ 174.7, 173.7, 173.2, 172.7, 172.3, 170.9, 170.8, 169.9, 167.9, 155.9, 155.5, 132.6, 131.0, 128.9, 79.9, 79.8, 68.3, 65.0, 64.6, 52.8, 52.4, 51.6, 51.5, 46.8, 46.4, 38.9, 38.63, 38.58, 38.2, 37.8, 30.7, 30.50, 30.49, 30.3, 30.2, 29.2, 29.1, 28.5, 28.4, 27.2, 27.0, 25.0, 24.8, 24.7, 23.9, 23.3, 23.2, 23.12, 23.07, 23.02, 23.00, 22.6, 22.5, 22.2, 20.3, 19.7, 19.1, 18.5, 18.3, 17.3, 14.2, 11.1; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{28}H_{53}N_4O_7$): 557.4, found: 557.3.



Boc-MeVal-SFm (16). To a solution of Boc-MeVal-OH (**14**) (136 mg, 0.59 mmol) in CH_2Cl_2 (2 mL) was added (9*H*-fluoren-9-yl)methanethiol[4] (**15**) (150 mg, 0.71 mmol), DCC (134 mg, 0.65 mmol), and DMAP (7.0 mg, 0.057 mmol). The resulting mixture was stirred overnight, and passed through a filter to remove the precipitate. The filtrate was evaporated at low pressure. The crude residue was purified by flash chromatography (20:1 hexanes/ethyl acetate) to give thioester **16** (190 mg, 76% yield). $[\alpha]_D^{20} = -128.4°$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.72 (dt, *J* = 7.1, 3.3 Hz, 2H), 7.66 – 7.60 (m, 2H), 7.37 (td, *J* = 7.6, 2.4 Hz, 2H), 7.29 (qd, *J* = 7.3, 3.8 Hz, 2H), 4.28 (dd, *J* = 222.4, 10.4 Hz, 1H), 4.15 (dt, *J* = 11.5, 5.9 Hz, 1H), 3.59 – 3.46 (m, 2H), 2.63 (d, *J* = 57.6 Hz, 3H), 2.18 (dtt, *J* = 13.4, 9.9, 6.5 Hz, 1H), 1.44 (d, *J* = 21.9 Hz, 9H), 0.85 (app. dd, *J* = 21.9, 6.7 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$, mixture of rotamers) δ 197.8, 197.3, 156.1, 155.2, 145.5, 145.4, 145.32, 145.27, 141.3, 141.27, 141.2, 127.8,

(4) D. Crich, K. Sana, S. Guo, *Org. Lett.* **2007**, *9*, 4423–4426.

127.73, 127.7, 127.1, 127.05, 124.9, 124.8, 124.73, 124.68, 120.0, 119.9, 80.6, 80.3, 71.2, 69.4, 46.93, 46.88, 31.81, 31.75, 30.1, 30.0, 28.48, 28.44, 28.42, 27.3, 26.8, 19.9, 19.7, 19.1, 18.7; IR (thin film) 2970.8, 2931.3, 1690.3, 1450.2, 1156.1, 1128.2 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{25}H_{31}NO_3SNa$): 448.2, found: 448.2.



Boc-D-Ala-MeLeu-MeLeu-OH (18).[5] To a solution of Boc-MeLeu-OH (**SI-1**) (203 mg, 0.83 mmol) and H-MeLeu-OBn hydrochloride[6] (204 mg, 0.75 mmol) in DMF (4.0 mL) was added HATU (315 mg, 0.83 mmol) and DIPEA (0.4 mL, 2.25 mmol) at room temperature. The resulting mixture was stirred for 30 min. The reaction mixture was diluted with ethyl acetate, and then washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue was purified by flash chromatography (6:1 hexanes/ethyl acetate) to afford dipeptide **SI-3** (260 mg, 75% yield).

Dipeptide **SI-3** (346.5 mg, 0.75 mmol) was dissolved in a solution of HCl in dioxane (4 M, 4 mL) and stirred at room temperature for 10 min. Co-evaporation of the resulting mixture with toluene afforded the corresponding amine HCl salt, which was carried directly into the next step without further purification.

To a solution of Boc-D-Ala-OH (157 mg, 0.83 mmol) and H-MeLeu-MeLeu-OBn hydrochloride (300 mg, 0.75 mmol) in DMF (4.0 mL) was added HATU (315 mg, 0.83 mmol) and DIPEA (0.4 mL, 2.25 mmol) at room temperature. The resulting mixture was stirred for 30 min. The reaction mixture was diluted with ethyl acetate, and then washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue was purified by flash chromatography (6:1 hexanes/ethyl acetate) to afford tripeptide **SI-4** (320 mg, 80% yield over two steps).

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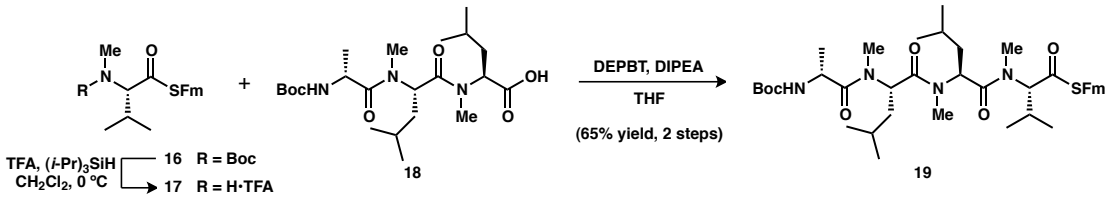
(5) This compound is known, but was synthesized by a method different from what was previously reported. The procedure we used is presented in brief, without characterization of intermediates. Full characterization data for **18** are found in: Wenger, R. M. *Helv. Chim. Acta* **1983**, *66*, 2672–2702.

(6) Bossler, H. G.; Seebach, D. *Helv. Chim. Acta* **1994**, *77*, 1124–1165.

A suspension containing tripeptide **SI-3** (320 mg, 0.6 mmol) and 10% palladium on activated charcoal (60 mg) in MeOH (30 mL) was placed under an atmosphere of hydrogen. The suspension was stirred overnight at room temperature. The reaction mixture was then filtered to remove the catalyst, and evaporated at low pressure. The resulting residue was dried under high vacuum to give acid **18** as a white solid (250 mg, 94% yield). ^{1}H NMR (600 MHz, $CDCl_3$, mixture of 2 rotamers) δ 5.55 – 5.47 (m, 1.5H), 5.42 (d, *J* = 8.1 Hz, 0.5H), 5.13 (t, *J* = 8.0 Hz, 0.5H), 4.68 (dd, *J* = 10.3, 4.3 Hz, 0.5H), 4.58 (tt, *J* = 15.8, 7.4 Hz, 1H), [2.96 (s), 2.92 (s), 2.84 (s), 2.83 (s), *sum to* 6H], 1.84 – 1.64 (m, 3H), [1.59 (ddd, *J* = 20.1, 10.0, 5.6 Hz), 1.50 (ddd, *J* = 13.3, 6.5, 2.4 Hz), 1.41 – 1.34 (m, 1H), *sum to* 2H], [1.43 (s, 4H), 1.41 (s, 5H), *sum to* 9H], 1.28 (dd, *J* = 7.0, 5.4 Hz, 3H), [1.00 (d, *J* = 6.5 Hz), 0.97 (d, *J* = 6.8 Hz), 0.95 (d, *J* = 6.7 Hz), 0.93 (app. t, *J* = 6.8 Hz), 0.91 (d, *J* = 6.6 Hz), 0.90 (d, *J* = 6.6 Hz), *all sum to* 12H]; ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.6, 173.6, 172.9, 171.7, 170.7, 156.4, 155.6, 105.1, 80.9, 79.8, 77.4, 57.4, 55.5, 51.8, 51.5, 46.8, 38.3, 37.8, 37.7, 37.2, 31.9, 30.3, 30.1, 29.9, 28.5, 28.4, 24.9, 24.8, 24.8, 24.7, 23.5, 23.4, 23.2, 23.1, 22.6, 22.4, 21.5, 21.3, 18.7, 18.2; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{22}H_{41}N_3O_6Na$): 466.3, found 466.3.



Boc-D-Ala-MeLeu-MeLeu-MeVal-SFm (19). To a cooled (0 °C) solution of Boc-MeVal-SFm (**16**) (60 mg, 0.14 mmol) in CH_2Cl_2 (1.0 mL) was added TFA (250 μL) and triisopropylsilane (20 μL). The reaction was stirred at 0 °C for 30 min. The resulting mixture was concentrated by rotary evaporation while being kept cold in a water bath containing ice. The crude amine TFA salt **17** was obtained in essentially quantitative yield, and carried directly into the next step without further purification.

To a solution of amine salt **17** (48 mg, 0.1 mmol) and tripeptide **18** (44 mg, 0.1 mmol) in THF (2.0 mL) was added DEBPT (45 mg, 0.15 mmol) and DIPEA (43 μL, 0.25 mmol) at room temperature. The reaction was stirred overnight at room temperature. Analysis of the reaction mixture by UPLC-MS showed the formation of two major products (~3:1), both possessing the desired mass (**Figure S-1**).[7] The solvent was evaporated at low pressure. The residue was purified by flash chromatography (6:1 hexanes/ethyl acetate) to give tetrapeptide thioester **19** (49 mg,

(7) The structure of the major isomer (retention time = 3.16 min) was assigned as **19** based on comparison to a sample of **19** that was independently synthesized by sequential HATU couplings (*vide infra*).

65% yield over two steps). $[\alpha]_D^{20}$ = –170.5° (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, 4:1 ratio of rotamers, *=both rotamers, $^+$ = major rotamer, $^\wedge$ = minor rotamer) δ 7.71* (d, *J* = 7.6 Hz, 2H), 7.58* (dd, *J* = 10.2, 7.6 Hz, 2H), 7.38 – 7.34* (m, 2H), 7.30 – 7.25* (m, 2H), 5.47* (dd, *J* = 8.4, 6.5 Hz, 1H), [5.39$^\wedge$ (dd, *J* = 9.9, 4.7 Hz), 5.35$^+$ (dd, *J* = 9.6, 4.9 Hz), 1H], [5.30$^+$ (d, *J* = 8.2 Hz), 5.27$^\wedge$ (d, *J* = 8.2 Hz), 1H], [4.86$^+$ (d, *J* = 10.6 Hz), 4.30$^\wedge$ (d, *J* = 9.7 Hz), 1H], 4.58* (dt, *J* = 16.8, 8.6 Hz, 1H), 4.17* (t, *J* = 5.3 Hz, 1H), [3.83$^\wedge$ (dd, *J* = 13.7, 4.7 Hz), 3.70$^+$ (dd, *J* = 13.7, 5.3 Hz,) 1H], [3.52$^+$ (dd, *J* = 13.7, 5.3 Hz), 3.32$^\wedge$ (dd, *J* = 13.7, 5.9 Hz), 1H], [3.08 – 3.00 (m), 2.96 (s), 2.93 (s), 2.89 (s), 2.87 – 2.80 (m), 2.60 (s), 2.59 (s), 2.58 (s), 2.28 (s), *all sum to* 9H], [2.23 – 2.18$^\wedge$ (m), 2.15$^+$ (dp, *J* = 10.6, 6.6 Hz), 1H], [1.71 – 1.66 (m), 1.66 – 1.58 (m), 1.56 (t, *J* = 9.2 Hz), 1.52 – 1.45 (m), 1.44$^\wedge$ (s, 2H), 1.42$^+$ (s, 7H), 1.40 – 1.29 (m), *all sum to* 15H], 1.28 (d, *J* = 6.8 Hz, 3H), [0.96$^\wedge$ (d, *J* = 6.4 Hz), 0.91 – 0.87* (m), 0.85$^+$ (d, *J* = 6.4 Hz), 0.81$^\wedge$ (d, *J* = 6.9 Hz), 0.68$^+$ (d, *J* = 6.7 Hz) *all sum to* 18H]; ^{13}C NMR (151 MHz, $CDCl_3$, mixture of rotamers) δ 197.6, 197.1, 196.2, 173.7, 172.1, 171.4, 171.0, 170.7, 169.9, 155.6, 155.55, 145.3, 145.1, 145.0, 144.6, 141.7, 141.6, 141.43, 141.39, 130.3, 130.2, 128.0, 128.0, 127.87, 127.85, 127.82, 127.76, 127.7, 127.3, 127.2, 127.14, 127.08, 125.5, 125.2, 125.1, 125.99, 124.95, 124.6, 124.4, 120.3, 120.2, 120.1, 120.09, 120.03, 120.02, 119.9, 91.5, 80.04, 79.96, 70.6, 67.5, 60.6, 51.84, 51.81, 51.4, 51.1, 46.9, 46.82, 46.79, 38.9, 38.1, 38.0, 37.6, 37.5, 32.2, 31.5, 31.3, 30.6, 30.3, 30.1, 30.0, 29.6, 28.5, 28.4, 27.5, 26.6, 26.5, 24.94, 24.85, 24.5, 24.4, 23.7, 23.23, 23.15, 23.0, 22.7, 22.5, 22.4, 22.1, 21.2, 19.81, 19.76, 19.72, 18.8, 18.5, 18.3, 18.1, 14.3; IR (thin film) 2960.2, 2871.5, 2360.4, 1692.2, 1639.2, 1169.6 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{42}H_{62}N_4O_6SNa$): 773.4, found: 773.6.

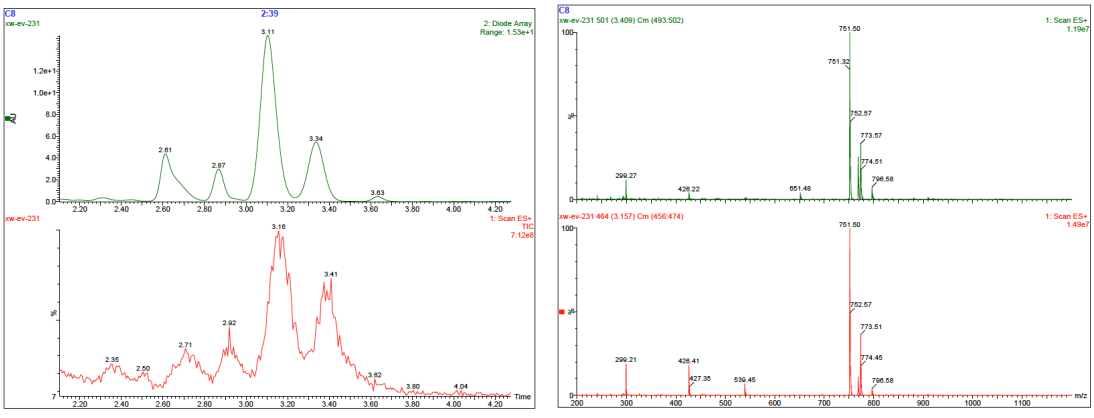


Figure S-1. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing tetrapeptide thioester **19**; major isomer retention time = 3.11 min, minor isomer retention time = 3.34 min, gradient 80–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of peak with retention time = 3.16 min (lower spectrum) and ESI-MS of peak with retention time = 3.41 min (upper spectrum); LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{42}H_{62}N_4O_6S$): 751.44, found: 751.50.

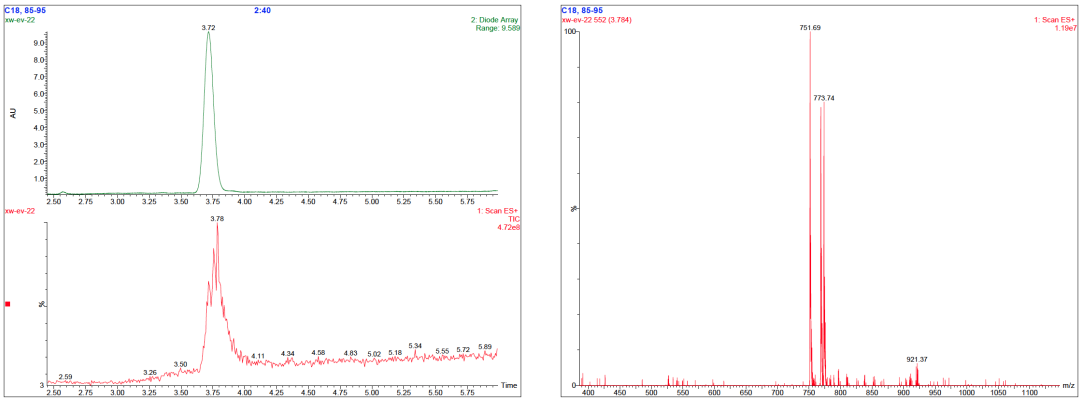
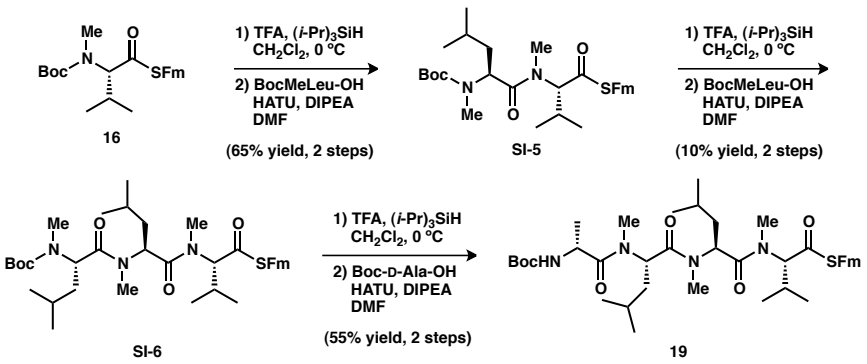


Figure S-2. (Left) UV and MS traces from UPLC-MS analysis of purified tetrapeptide thioester **19**; retention time = 3.72 min, gradient 85–95% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **19**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{42}H_{62}N_4O_6S$): 751.44, found: 751.69.



Boc-D-Ala-MeLeu-MeLeu-MeVal-SFm (19). ***Alternate route (C→N):***[8] To a cooled (0 °C) solution of Boc-MeVal-SFm (**16**) (60 mg, 0.14 mmol) in CH_2Cl_2 (1.0 mL) was added TFA (0.25 mL) and triisopropylsilane (20 μL). The reaction was stirred at 0 °C for 30 min. The resulting mixture was concentrated by rotary evaporation while being kept cold in a water bath containing ice. The crude amine TFA salt was obtained in essentially quantitative yield, and carried directly into the next step without further purification.

To a solution of Boc-MeLeu-OH (42 mg, 0.17 mmol) and TFA·H-MeVal-SFm (75 mg, 0.14 mmol) in DMF (1.0 mL) was added HATU (65 mg, 0.17 mmol) and DIPEA (54 μL, 0.31 mmol) at room temperature. The resulting mixture was stirred for 30 min. The reaction mixture was diluted with ethyl acetate, and then washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue was purified by flash chromatography (8:1 hexanes/ethyl acetate) to afford dipeptide thioester **SI-5** (50 mg, 65% yield over two steps).

To a cooled (0 °C) solution of dipeptide thioester **SI-5** (50 mg, 0.091 mmol) in CH_2Cl_2 (0.8 mL) was added TFA (0.20 mL) and triisopropylsilane (15 μL). The reaction was stirred at 0 °C for 30 min. The resulting mixture was concentrated by rotary evaporation while being kept cold in a water bath containing ice. The crude amine TFA salt was obtained in essentially quantitative yield, and carried directly into the next step without further purification.

To a solution of Boc-MeLeu-OH (11 mg, 0.044 mmol) and TFA·H-MeLeu-MeVal-SFm (16.4 mg, 0.029 mmol) in DMF (0.5 mL) was added HATU (17 mg, 0.044 mmol) and DIPEA (11 μL, 0.064 mmol) at room temperature. The resulting mixture was stirred for 30 min. The reaction mixture was diluted with ethyl acetate, and then washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue

(8) As noted in the manuscript, synthesis of **19** by sequential *C→N* extension proved to be less efficient than the route that involved coupling the thioester-bearing residue **17** to the *C*-terminus of tripeptide **18** (*vide supra*). The stepwise route is presented here in brief, however, because it enabled us to assign the major product of the coupling between **17** and **18** as the desired DLLL-tetrapeptide **19** (as opposed to the DLDL-tetrapeptide).

was purified by flash chromatography (8:1 hexanes/ethyl acetate) to afford tripeptide thioester **SI-6** (2 mg, 10% yield over two steps).

To a cooled (0 °C) solution of tripeptide thioester **SI-6** (2.7 mg, 0.004 mmol) in CH_2Cl_2 (0.4 mL) was added TFA (0.10 mL) and triisopropylsilane (5 µL). The reaction was stirred at 0 °C for 30 min. The resulting mixture was concentrated by rotary evaporation while being kept cold in a water bath containing ice. The crude amine TFA salt was obtained in essentially quantitative yield, and carried directly into the next step without further purification.

To a solution of Boc-D-Ala-OH (1.5 mg, 8 µmol) and TFA·H-MeLeu-MeLeu-MeVal-SFm (2.8 mg, 4 µmol) in DMF (0.2 mL) was added HATU (3 mg, 8 µmol) and DIPEA (2 µL, 12 µmol) at room temperature. The resulting mixture was stirred for 30 min. Analysis of the reaction mixture by UPLC-MS showed the formation of a product possessing the desired mass (**Figure S-3**). The reaction mixture was diluted with ethyl acetate, and then washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue was purified by flash chromatography (5:1 hexanes/ethyl acetate) to afford tetrapeptide thioester **19** (1.6 mg, 55% yield over two steps).

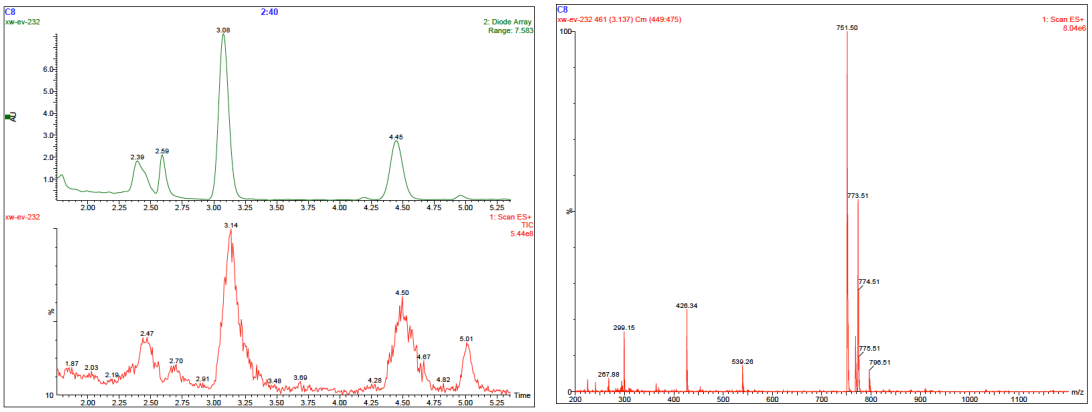
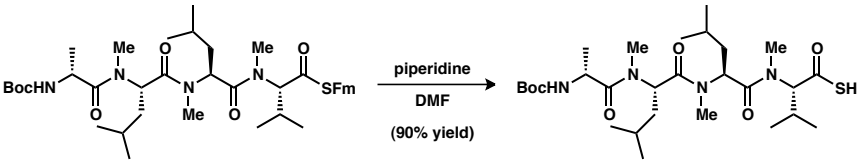


Figure S-3. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing tetrapeptide thioester **19**; retention time = 3.08 min, gradient 80–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of peak with retention time = 3.14 min; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{42}H_{62}N_4O_6S$): 751.44, found: 751.50.



19 3b

Boc-D-Ala-MeLeu-MeLeu-MeVal-SH (3b). To a solution of tetrapeptide thioester **19** (6 mg, 8 μmol) in DMF (0.2 mL) was added piperidine (0.02 mL). The reaction was stirred at room temperature for 15 min. Analysis of the reaction mixture by UPLC-MS indicated complete consumption of the starting material (**Figure S-4**). The reaction mixture was neutralized with acetic acid (0.02 mL). The product was purified by reversed-phase HPLC (C18 column, gradient 60–80% CH_3CN/H_2O with 0.05% TFA over 30 min) to afford the corresponding tetrapeptide thioacid **3b** (4.1 mg, 90% yield), which was used in the next step without further purification.

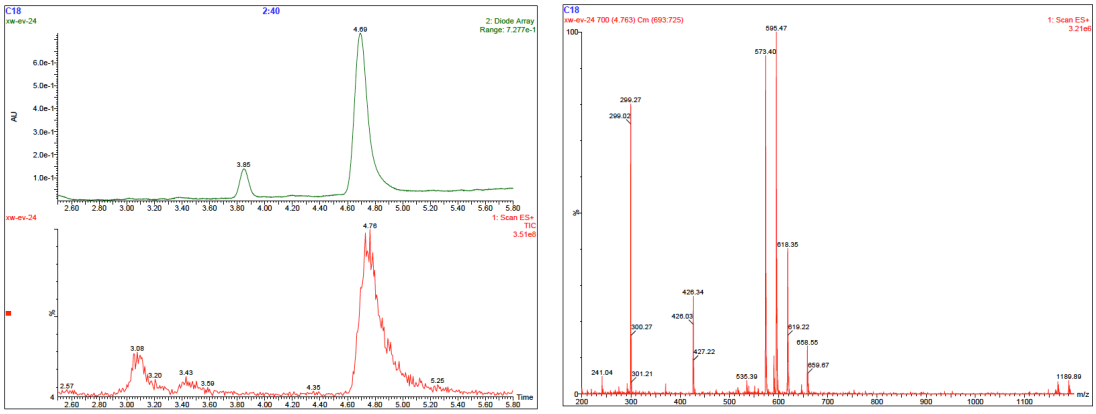
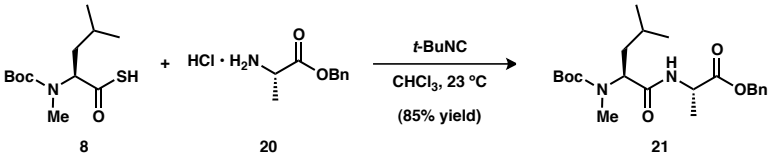


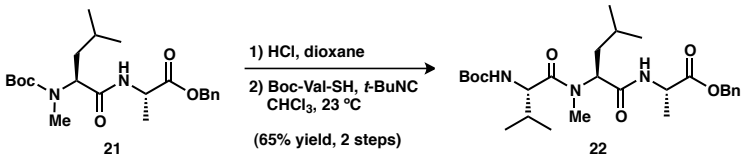
Figure S-4. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing tetrapeptide thioacid **3b**; retention time = 4.69 min, gradient 60–80% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **3b**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{28}H_{52}N_4O_6S$): 573.36, found: 573.40.



8 20 21

Boc-MeLeu-Ala-OBn (21). To a solution of Boc-MeLeu-SH (**8**) (130 mg, 0.5 mmol) was added H-Ala-OBn hydrochloride (**20**) (108 mg, 0.5 mmol) and *t*-butyl isonitrile (0.17 mL, 1.5 mmol). The resulting mixture was stirred overnight at room temperature. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (5:1 hexanes/ethyl acetate) to give dipeptide **21** (172 mg, 85% yield). $[\alpha]_D^{20}$ = –67.2° (c = 1.0, $CHCl_3$); ^1H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ 7.32 (q, *J* = 7.2 Hz, 5H), [6.70 (s), 6.43 (s), 1H], 5.14

(app. q, J = 12.3 Hz, 2H), [4.66 (s), 4.57 (br. d, J = 25.3 Hz), 2H], 2.73 (s, 1H), [1.66 (app. s), 1.62 (app. s), 1H], 1.45 (app. s, 10H), 1.37 (app. s, 3H), 0.91 (d, J = 7.0 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$); δ 172.5, 171.1, 170.8, 156.8, 155.5, 135.4, 128.6, 128.5, 128.4, 128.3, 128.2, 81.0, 80.4, 67.1, 57.6, 56.2, 48.1, 36.5, 30.0, 29.8, 28.4, 24.8, 24.7, 24.6, 23.3, 23.2, 23.15, 21.9, 21.5, 18.6; IR (thin film) 3321.8, 2959.2, 2872.5, 1745.3, 1688.4, 1663.3, 1367.3 cm^{-1}; LRMS (ESI+) m/z calc'd for $[M+Na]^+$ ($C_{22}H_{34}N_2O_5Na$): 429.3, found: 429.3.

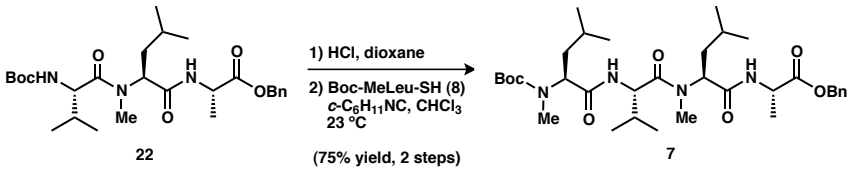


Boc-Val-MeLeu-Ala-OBn (22). Dipeptide **21** (101.5 mg, 0.25 mmol) was treated with 4 M HCl in dioxane (1.2 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was co-evaporated with toluene to give the corresponding deprotected dipeptide as the amine hydrochloride salt in essentially quantitative yield. This material was carried directly into the next step without further purification.

To a solution of Boc-Val-SH[9] (116 mg, 0.5 mmol) in $CHCl_3$ (3 mL) was added H-MeLeu-Ala-OBn hydrochloride (86 mg, 0.25 mmol) and *t*-butyl isonitrile (113 μL, 1.0 mmol). The resulting mixture was stirred overnight at room temperature. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (4:1 hexanes/ethyl acetate) to give tripeptide **22** (82 mg, 65% yield over two steps). $[\alpha]_D^{20}$ = –88.5° (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, 4:1 ratio of rotamers, *=both rotamers, $^+$ = major rotamer, $^\wedge$ = minor rotamer) δ 7.89$^\wedge$ (d, J = 7.2 Hz), 7.38 – 7.28* (m, 5H), 6.54$^+$ (d, J = 7.4 Hz, 1H), [5.24 – 5.11$^{*/+}$ (m, 3.8H) (includes 3H and major rotamer corresponding to dd at 4.68], 4.68$^\wedge$ (dd, J = 10.2, 4.0 Hz, 0.2H), 4.57 – 4.50* (m, 1H), [4.40$^+$ (dd, J = 9.4, 6.7 Hz), 4.25$^\wedge$ (t, J = 8.3 Hz), 1H], [2.99$^+$ (s), 2.79$^\wedge$ (s), 3H], [2.29$^\wedge$ (ddd, J = 13.3, 10.2, 5.0 Hz), 1.95* (dq, J = 13.5, 6.6 Hz, 1H), 1.65$^+$ (dd, J = 8.4, 6.3 Hz), 1.56$^\wedge$ (dt, J = 20.1, 5.9 Hz), 1.45* (m), 1.41$^+$ (s), 1.38$^\wedge$ (app. s), 1.37$^\wedge$ (app. s), 1.34$^+$ (d, J = 7.1 Hz) *all sum to* 16H], [1.06$^\wedge$ (d, J = 6.9 Hz), 1.01$^\wedge$ (d, J = 6.7 Hz), 0.99$^\wedge$ (d, J = 6.9 Hz), 0.94$^+$ (d, J = 6.8 Hz), 0.92$^\wedge$ (d, J = 6.5 Hz), 0.92$^+$ (d, J = 6.8 Hz), 0.90$^+$ (d, J = 6.6 Hz), 0.85$^+$ (d, J = 6.5 Hz), *all sum to* 12H]; ^{13}C NMR (151 MHz, $CDCl_3$, mixture of rotamers) δ 173.7, 172.6, 172.42, 172.35, 170.2, 168.7, 156.8,

(9) Le, H.-T. Gallard, J.-F. Mayer, M. Guittet, E.; Michelot, R. *Bioorg. Med. Chem.* **1996**, *4*, 2201–2209.

156.0, 135.8, 135.4, 128.7, 128.6, 128.5, 128.31, 128.29, 128.26, 128.2, 80.5, 79.7, 58.3, 55.6, 55.49, 54.46, 48.6, 48.2, 38.5, 36.2, 31.2, 31.1, 30.8, 29.0, 28.42, 28.40, 25.2, 24.7, 23.6, 23.2, 22.3, 22.1, 20.2, 19.6, 18.8, 18.3, 17.7, 17.6; IR (thin film) 3319.9, 2962.1, 2872.5, 1742.4, 1681.6, 1629.6, 1169.6 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{27}H_{43}N_3O_6Na$): 528.3, found: 528.3.

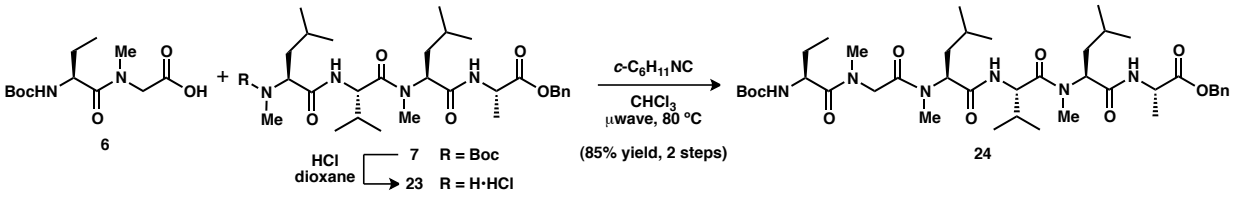


22 7

Boc-MeLeu-Val-MeLeu-Ala-OBn (7). Tripeptide **22** (50.5 mg, 0.1 mmol) was treated with 4 M HCl in dioxane (0.6 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was co-evaporated with toluene to give the corresponding deprotected tripeptide as the amine hydrochloride salt. This material was carried directly into the next step without further purification.

To a solution of Boc-MeLeu-SH (**8**) (40 mg, 0.15 mmol) in $CHCl_3$ (1.5 mL) was added H-Val-MeLeu-Ala-OBn hydrochloride (44 mg, 0.1 mmol) and cyclohexyl isonitrile (0.037 mL, 0.30 mmol). The resulting mixture was stirred overnight at room temperature. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (4:1 hexanes/ethyl acetate) to give tetrapeptide **7** (48 mg, 75% yield over two steps). $[\alpha]_D^{20} = -128.5°$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers)[10] δ 7.38 – 7.31 (m, 5H), [6.69 (app. s), 6.54 (app. s), 6.45 (app. s), 2H], [5.24 – 5.09 (m), 4.75 (br. m), 4.66 (app. s), 4.55 (p, *J* = 7.2 Hz), 4.48 (p, *J* = 7.2 Hz), *all sum to* 6H], [3.00 (s), 2.82 (s), 2.77 (s), 2.74 (s), *all sum to* 6H], [2.10 – 2.04 (m), 2.01 (dq, *J* = 13.2, 6.6, 6.2 Hz), 1.71 – 1.61 (m), 1.49 (br. s, 9H), 1.46 – 1.39 (m), 1.39 (s), 1.38 (d, *J* = 7.2 Hz), 1.35 (d, *J* = 7.2 Hz, 3H), 1.34 (s), 1.25 (s), *all sum to* 19H], [0.97 (br. d, *J* = 6.9 Hz, 1H), 0.94 (d, *J* = 6.2 Hz, 2H), 0.93 (d, *J* = 6.5 Hz, 8H), 0.91 (d, *J* = 6.3 Hz, 5H), 0.90 (d, *J* = 6.5 Hz, 11H), 0.88 (d, *J* = 6.8 Hz, 2H), 0.86 (d, *J* = 6.6 Hz, 3H) *all sum to* 18H]; ^{13}C NMR (151 MHz, $CDCl_3$) δ 172.7, 172.6, 172.4, 171.3, 170.1, 167.8, 135.44, 132.40, 131.1, 129.0, 128.8, 128.7, 128.6, 128.4, 128.2, 67.3, 67.0, 61.8, 58.3, 57.6, 56.2, 54.7, 54.6, 53.8, 48.7, 48.2, 38.7, 36.8, 36.7, 36.2, 32.1, 31.3, 30.8, 30.1, 29.9, 29.5, 29.1, 28.5, 25.2, 24.9, 24.8, 24.7, 23.6, 23.5, 23.2, 22.3, 22.1, 21.5, 20.1, 19.8, 19.0, 18.3, 17.7, 17.6, 17.31, 14.28, 14.27; IR (thin film) 2984.3, 1737.6, 1773.1, 1234.2, 1044.3 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{34}H_{56}N_4O_7Na$): 655.4, found: 655.6.

(10) Matches ^{1}H NMR data for **7** reported in: Wenger, R. M. *Helv. Chim. Acta* **1984**, *67*, 502–525.



Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (24). Tetrapeptide **7** (63.2 mg, 0.1 mmol) was treated with 4 M HCl in dioxane (0.6 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was co-evaporated with toluene to give the corresponding deprotected tetrapeptide **23** as the amine hydrochloride salt in essentially quantitative yield. This material was carried directly into the next step without further purification.

To a solution of dipeptide **6**[2] (13 mg, 0.046 mmol) in $CHCl_3$ (1 mL) was added compound **23** (13 mg, 0.023 mmol) and cyclohexyl isonitrile (8.6 μL, 0.069 mmol). The reaction mixture was stirred for 45 min at 80 °C under microwave irradiation. The resulting solution was purified directly by flash chromatography (3:1 hexanes/acetone) to give hexapeptide **24** (15.4 mg, 85% yield over two steps). $[\alpha]_D^{20} = -135.0°$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ [7.95 (d, *J* = 6.5 Hz), 7.45 (d, *J* = 7.9 Hz), 7.38 – 7.28 (m), 6.90 (d, *J* = 8.7 Hz), 6.80 (d, *J* = 8.7 Hz), 6.69 – 6.38 (m), 5.80 (d, *J* = 8.7 Hz), 5.57 (d, *J* = 8.6 Hz), 5.27 – 5.00 (m), 4.87 (d, *J* = 3.5 Hz), 4.71 – 4.63 (m), 4.60 (t, *J* = 6.7 Hz), 4.55 – 4.49 (m), 4.47 (td, *J* = 7.1, 3.3 Hz), 4.41 (d, *J* = 15.8 Hz), *all sum to* 14H], [3.90 (d, *J* = 15.8 Hz), 3.78 (d, *J* = 15.9 Hz), 3.61 (d, *J* = 15.5 Hz), *all sum to* 1H], [3.35 – 3.29 (m), 3.27 (d, *J* = 16.7 Hz), 3.22 – 3.14 (m), 3.11 – 3.05 (m), 3.03 (s), 2.96 (t, *J* = 8.6 Hz), 2.85 – 2.70 (m), *all sum to* 10H], [2.29 (ddd, *J* = 13.3, 10.6, 4.8 Hz), 2.10 – 2.00 (m), 1.81 (dq, *J* = 18.2, 6.0, 4.8 Hz), 1.78 – 1.70 (m), 1.68 – 1.56 (m), 1.42 (s), 1.38 (d, *J* = 7.2 Hz), 1.35 (d, *J* = 7.1 Hz), *all sum to* 21H], 1.03 – 0.77 (m, 24H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 173.7, 173.5, 173.4, 173.0, 172.5, 172.44, 172.39, 172.0, 171.8, 171.7, 170.7, 170.5, 170.2, 170.1, 169.8, 169.2, 169.1, 168.6, 168.4, 156.0, 155.9, 155.9, 135.78, 135.76, 135.4, 128.8, 128.74, 128.69, 128.68, 128.66, 128.65, 128.59, 128.41, 128.38, 128.37, 128.34, 128.32, 128.29, 128.27, 128.23, 128.22, 128.19, 128.11, 79.86, 79.81, 79.77, 67.34, 67.01, 67.00, 58.6, 58.5, 58.3, 55.3, 55.2, 55.1, 54.9, 54.4, 54.3, 52.2, 51.8, 51.6, 51.5, 50.2, 50.1, 50.0, 48.7, 48.3, 38.8, 37.6, 37.4, 37.2, 37.0, 36.5, 36.4, 36.3, 36.24, 36.19, 31.1, 31.0, 30.92, 30.88, 30.6, 30.5, 29.8, 29.4, 29.2, 28.49, 28.47, 28.45, 26.1, 25.8, 25.4, 25.2, 25.13, 25.10, 25.08, 24.90, 24.88, 24.84, 24.6, 23.63, 23.57, 23.4, 23.19, 23.16, 23.14, 23.11, 23.0, 22.5, 22.3, 22.2, 22.1, 22.04, 22.02, 22.0, 21.8, 21.61, 21.55, 20.1, 20.0, 19.53, 19.48, 19.4, 19.3, 18.22, 18.16, 18.14, 18.0, 17.9, 17.4, 17.30, 17.28, 10.1, 9.9, 9.79, 9.75, 9.70; IR (thin film) 3314.1, 2962.1, 2872.5, 2360.4, 1639.2 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{41}H_{68}N_6O_9Na$): 811.5, found 811.7.

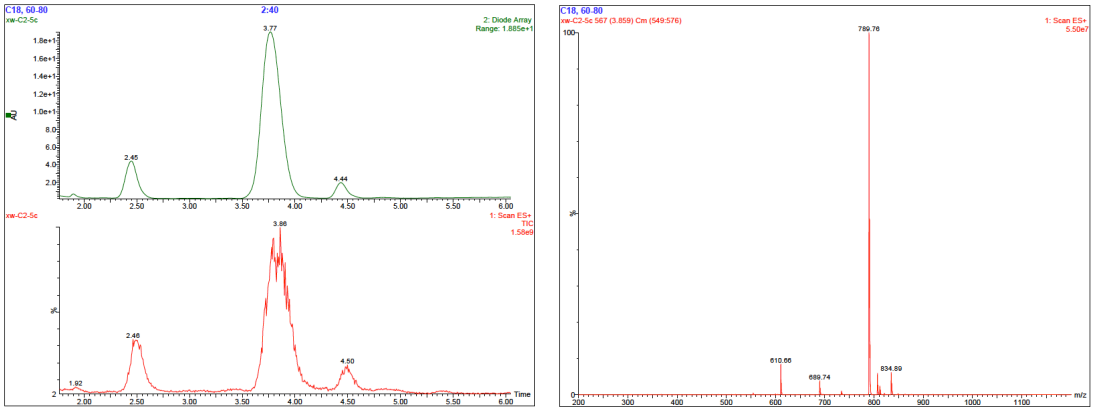


Figure S-5. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing hexapeptide **24**; retention time = 3.77 min, gradient 60–80% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **24**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{41}H_{68}N_6O_9$): 789.50, found: 789.76.

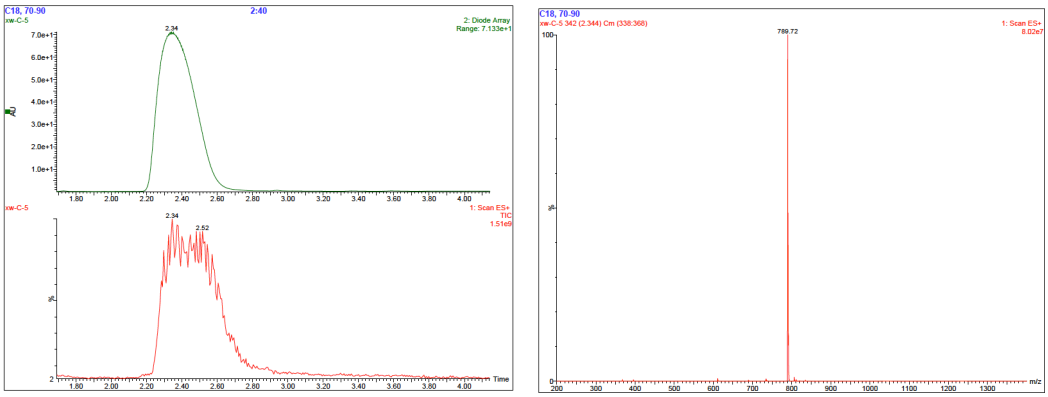
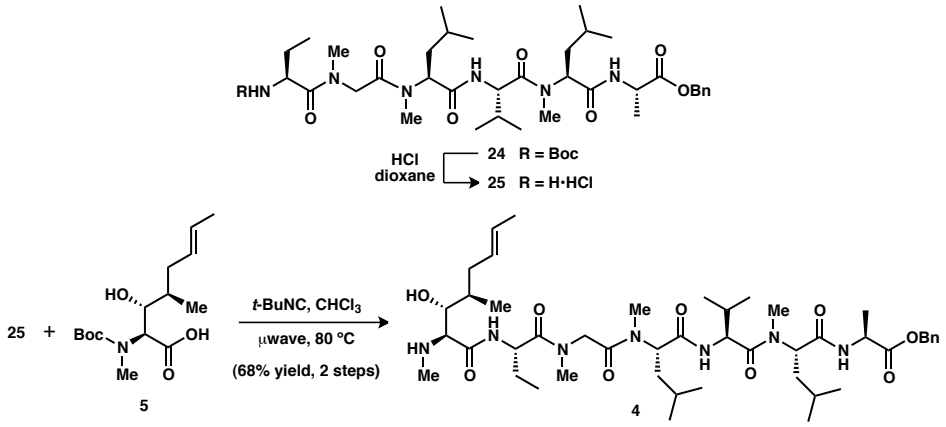


Figure S-6. (Left) UV and MS traces from UPLC-MS analysis of purified hexapeptide **24**; retention time = 2.34 min, gradient 70–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **24**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{41}H_{68}N_6O_9$): 789.50, found: 789.72.



H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (4). Hexapeptide **24** (78.9 mg, 0.1 mmol) was treated with 4 M HCl in dioxane (0.6 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was co-evaporated with toluene to give the corresponding deprotected hexapeptide **25** as the amine hydrochloride salt in essentially quantitative yield. This material was carried directly into the next step without further purification.

To a solution of Boc-MeBmt-OH (**5**) (36 mg, 0.12 mmol) in $CHCl_3$ (2 mL) was added compound **25** (55 mg, 0.076 mmol) and *t*-butyl isonitrile (34 μL, 0.3 mmol). The reaction mixture was stirred for 45 min at 80 °C under microwave irradiation. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (20:1 CH_2Cl_2/MeOH) to give heptapeptide **4** (45 mg, 68% yield over two steps). $[\alpha]_D^{20} = -139.0°$ (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of several rotamers): δ [8.07 (d, *J* = 6.4 Hz), 7.97 (s), 7.84 (d, *J* = 7.7 Hz), 2H], 7.33 (m, 5H), 5.50 – 5.25 (m, 1H), 5.20 (dd, *J* = 12.4, 6.5 Hz, 1H), 5.11 (dd, *J* = 25.2, 12.3 Hz, 1H), 5.06 – 4.91 (m, 1H), 4.81 (dd, *J* = 13.4, 6.4 Hz, 2H), [4.67 – 4.61 (m), 4.55 (q, *J* = 13.1, 11.2 Hz), 4.44 (tq, *J* = 19.0, 6.5, 5.6 Hz), *all sum to* 4H], [4.10 (s), 3.99 (s), 3.88 (app. d, *J* = 4.9 Hz), 3.85 – 3.81 (m), 3.77 – 3.71 (m), 3.54 (d, *J* = 14.5 Hz), *all sum to* 3H], [3.40 (s), 3.36 (s), 3.34 (s), 3.30 (s), 3.28 (s), 3.22 (s), 3.19 (s), 3.16 (s), 3.07 (s), 3.05 (s), 3.02 (s), 3.01 (s), 2.85 (s), 2.81 (s), 2.79 (s), 2.78 (s), 2.75 (s), 2.74 (s), *all sum to* 12H], [2.46 – 2.25 (m), 2.21 (dt, *J* = 21.4, 7.3 Hz), 2.06 (dt, *J* = 19.8, 5.4 Hz), *all sum to* 3H], 1.91 (ddd, *J* = 29.6, 14.6, 5.6 Hz), 1.83 (dt, *J* = 14.1, 7.1 Hz), 1.75 – 1.65 (m), 1.62 (d, *J* = 5.8 Hz), 1.59 (d, *J* = 6.1 Hz), 1.49 – 1.41 (m), 1.37 (dd, *J* = 7.2, 5.1 Hz), *all sum to* 13H], [1.25 (s), 1.05 (d, *J* = 6.5 Hz), 0.99 (d, *J* = 6.5 Hz), 0.97 – 0.90 (m), 0.90 – 0.80 (m), 0.76 (d, *J* = 6.5 Hz), *all sum to* 21H]; IR (thin film) 3303.5, 2963.1, 2359.5, 1672.9 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[C_{46}H_{77}N_7O_9+Na]^+$ ($C_{46}H_{77}N_7O_9Na$): 894.6, found: 894.9.

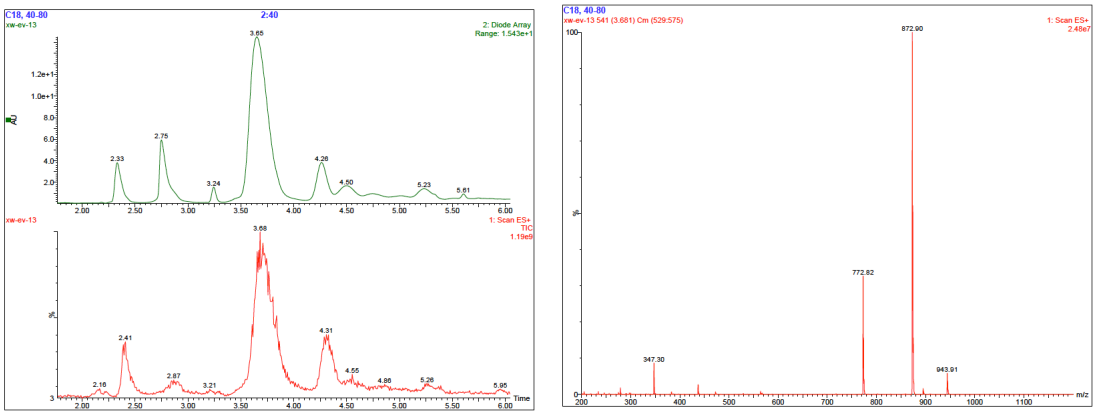


Figure S-7. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing heptapeptide **4**; retention time = 3.65 min, gradient 40–80% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **4**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{46}H_{77}N_7O_9$): 872.6, found: 872.9.

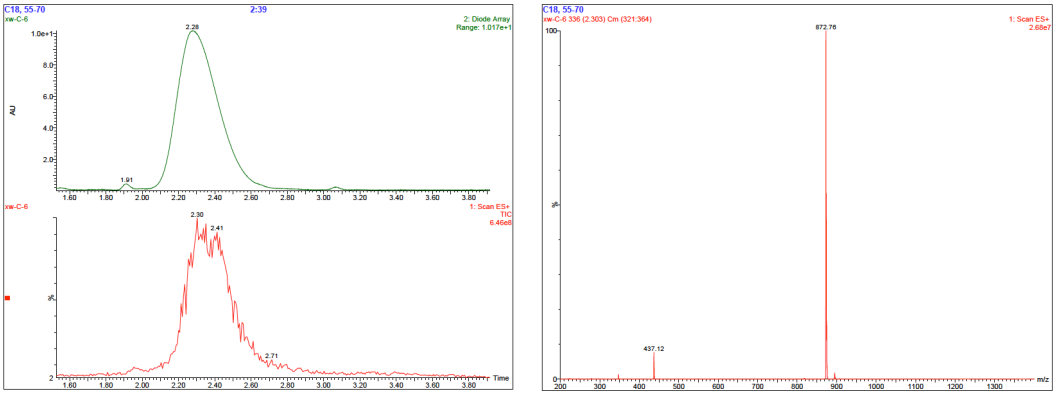
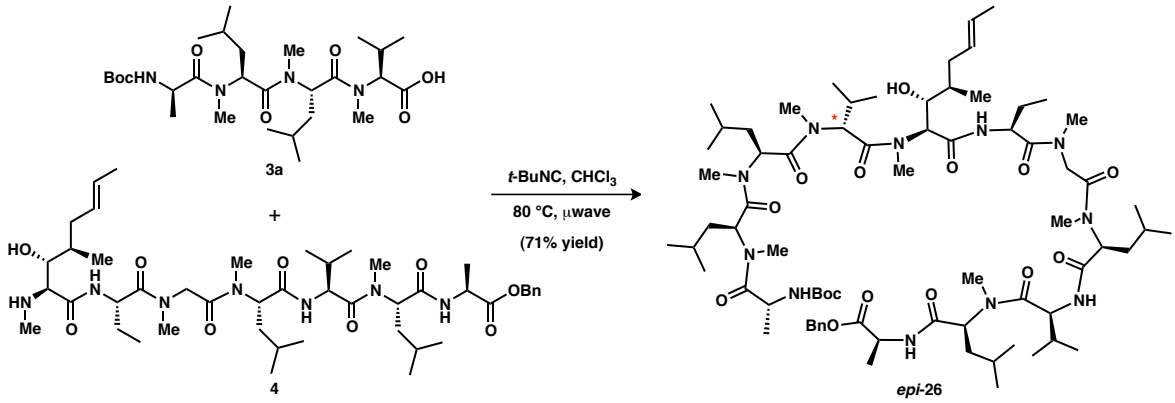


Figure S-8. (Left) UV and MS traces from UPLC-MS analysis of purified heptapeptide **4**; retention time = 2.28 min, gradient 55–70% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **4**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{46}H_{77}N_7O_9$): 872.6, found: 872.8.



Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (*epi*-26). *Type IIa method*: To a solution of tetrapeptide acid **3a** (11 mg, 0.02 mmol) in $CHCl_3$ (0.5 mL) was added heptapeptide **4** (10 mg, 0.012 mmol) and *t*-butyl isonitrile (6.8 μL, 0.06 mmol). The reaction mixture was stirred for 45 min at 80°C under microwave irradiation. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (15:1 CH_2Cl_2/MeOH) to give undecapeptide ***epi*-26** (12 mg, 71% yield). $[\alpha]_D^{20} = -112.0°$ (c = 0.8, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$), ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ [8.16 (s), 7.70 (s), 7.15 (s) various N–H], 7.39 – 7.27 (m, 5H), [5.51 (d, *J* = 7.4 Hz), 5.46 – 5.28 (m), 5.27 – 5.02 (m), 4.96 (s), 4.93 – 4.78 (m), 4.75 (s), 4.65 – 4.56 (m), 4.52 (q, *J* = 7.3 Hz), 4.50 – 4.37 (m), *all sum to* 15H], [3.71 (s), 3.65 – 3.53 (m), 3.53 – 3.43 (m), *sum to* 5H], [3.37 (app. dd, *J* = 27.2, 10.7 Hz), 3.31 – 3.17 (m), 3.13 (d, *J* = 22.3 Hz), 3.06 (d, *J* = 7.6 Hz), 3.02 – 2.87 (m), 2.87 – 2.73 (m), *all sum to* 21H], [2.52 (s), 2.44 – 2.21 (m), 1.89 (ddd, *J* = 14.2, 9.9, 4.5 Hz), 1.80 – 1.67 (m), 1.64 – 1.56 (m), 1.52 (ddd, *J* = 20.0, 12.7, 6.3 Hz), 1.42 (d, *J* = 1.6 Hz), 1.38 – 1.32 (m), 1.31 – 1.22 (m), *all sum to* 37H], [1.03 (d, *J* = 6.6 Hz), 0.98 (d, *J* = 6.6 Hz), 0.96 – 0.84 (m), 0.83 – 0.77 (m), 0.65 (dd, *J* = 24.3, 6.4 Hz) *all sum to* 42H]; IR (thin film) 3314.1, 2961.2, 1632.5 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{74}H_{127}N_{11}O_{15}Na$): 1432.9, found: 1433.2.

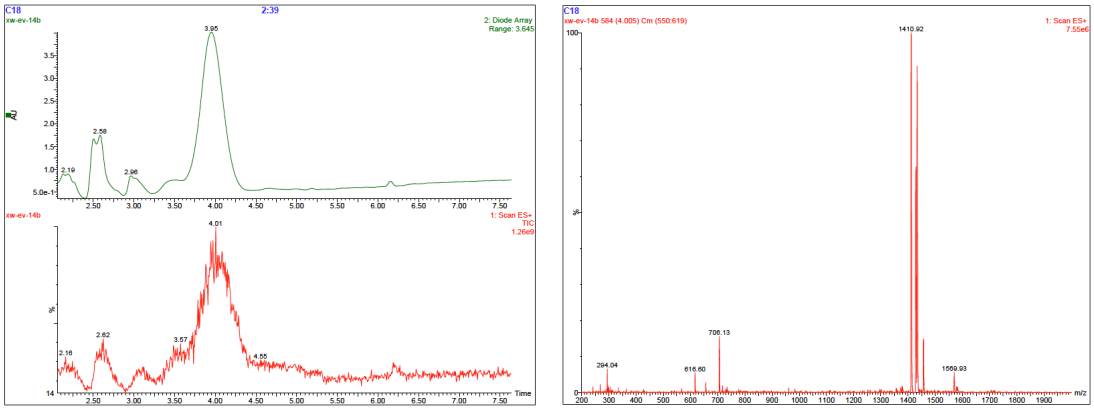


Figure S-9. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing undecapeptide *epi*-***26***; retention time = 3.95 min, gradient 70–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound *epi*-**26**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{74}H_{128}N_{11}O_{15}$): 1410.96, found: 1410.92.

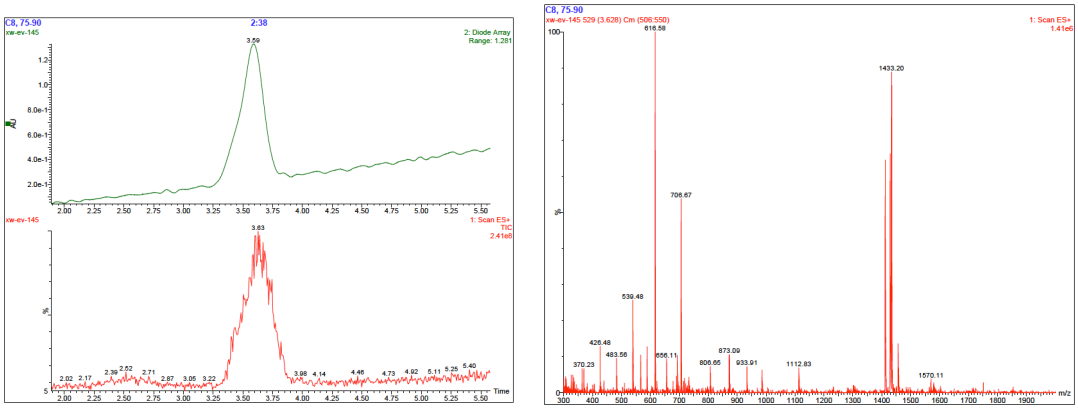
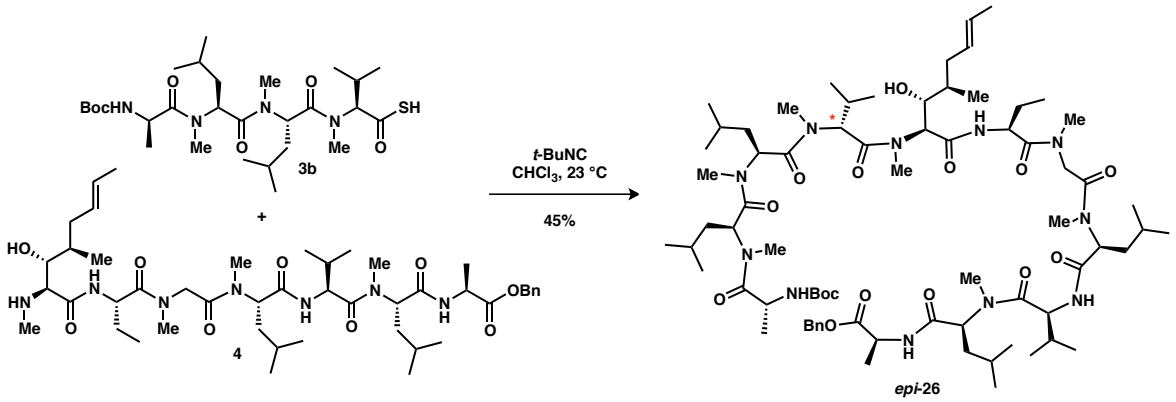
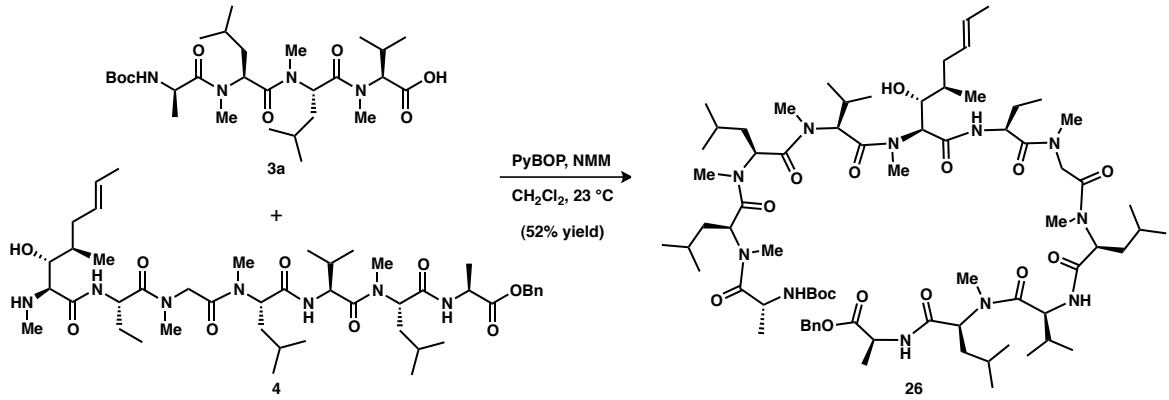


Figure S-10. (Left) UV and MS traces from UPLC-MS analysis of purified undecapeptide *epi*-***26***; retention time = 3.59 min, gradient 75–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound *epi*-**26**; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{74}H_{127}N_{11}O_{15}Na$): 1432.9, found: 1433.2.



Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (*epi*-26). ***Type IIb method*:** To a solution of tetrapeptide thioacid **3b** (2.9 mg, 5 μmol) in $CHCl_3$ (0.2 mL) was added heptapeptide **4** (3.5 mg, 4 μmol) and *t*-butyl isonitrile (2.3 μL, 0.02 mmol). The resulting mixture was stirred for 16 h at room temperature. The volatiles were removed at low pressure, and the residue was purified by flash chromatography (15:1 CH_2Cl_2/MeOH) to give undecapeptide *epi*-**26** (2.5 mg, 45% yield).



Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (26). To a solution of **3a** (42 mg, 75 μmol) in CH_2Cl_2 (3 mL) was added heptapeptide **4** (65 mg, 75 μmol), NMM (16 μL, 0.15 mmol), and PyBOP (78 mg, 0.15 mmol). The resulting solution was stirred for 24 hours at room temperature. The mixture was then diluted with CH_2Cl_2, and washed with water and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The resulting residue was purified by flash chromatography (15:1 CH_2Cl_2/MeOH) to give undecapeptide **26** (55 mg, 52% yield). $[\alpha]_D^{20}$ = –181.0° (c = 1.0, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ [8.13 (d, *J* = 6.6 Hz), 7.76 (d, *J* = 9.1 Hz), 7.56 (d, *J* = 6.9 Hz), 7.48 (d, *J* = 9.9 Hz), 7.04 (d, *J* = 9.0 Hz), 4H], 7.38 – 7.30 (m, 5H), 5.49 (ddd, *J* = 19.0, 8.3, 6.5 Hz, 2H), 5.41 – 5.29 (m, 3H), 5.26 (dd, *J* = 10.7, 5.3 Hz, 1H), 5.22 – 5.10 (m, 2H), 5.07 (d, *J* = 12.4 Hz, 1H), 5.05 – 5.00 (m, 1H), 4.96 (q, *J* = 7.6 Hz, 1H), 4.92 (d, *J* = 6.2

Hz, 1H), 4.89 – 4.83 (m, 1H), 4.77 – 4.66 (m, 1H), 4.60 (dt, *J* = 13.9, 6.8 Hz, 1H), [4.53 – 4.44 (m), 4.41 (d, *J* = 14.8 Hz), 3H], 3.72 – 3.65 (m, 1H), 3.61 (dd, *J* = 22.7, 14.6 Hz, 1H), [3.42 (s), 3.30 (s), 3.14 (s), 3.06 (s), 3.00 (s), 2.99 (s), 2.99 (s), (plus minor rotamer peaks) *all sum to* 21H], 2.55 (br. d, *J* = 13.5 Hz, 1H), 2.35 – 2.19 (m, 2H), [1.90 (ddd, *J* = 14.3, 9.6, 5.0 Hz), 1.78 (dt, *J* = 13.2, 7.4 Hz), 1.71 (td, *J* = 14.0, 7.1 Hz), 1.67 – 1.62 (m), 1.62 – 1.59 (m), 1.58 (s), 1.52 (dt, *J* = 14.0, 6.6 Hz), 1.42 (s), 1.35 (d, *J* = 7.3 Hz), 1.33 – 1.24 (m), 1.21 (d, *J* = 6.1 Hz), *all sum to* 37H], 1.03 (d, *J* = 6.5 Hz), 0.99 (d, *J* = 6.8 Hz), 0.96 (d, *J* = 6.9 Hz), 0.95 – 0.90 (m), 0.89 (d, *J* = 7.2 Hz), 0.86 (d, *J* = 6.5 Hz), 0.83 (d, *J* = 6.4 Hz), 0.78 (d, *J* = 6.7 Hz), 0.55 (d, *J* = 6.6 Hz), *all sum to* 42H]; ^{13}C NMR (151 MHz, $CDCl_3$, mixture of rotamers-*sample dilute*) δ 173.4, 173.1, 172.6, 172.3, 171.6, 170.0, 155.5, 135.7, 129.6, 128.8, 128.7, 128.66, 128.44, 128.35, 128.3, 128.24, 128.21, 126.7, 80.9, 79.8, 76.1, 67.0, 64.8, 57.3, 55.5, 54.9, 51.7, 51.3, 50.9, 50.4, 49.5, 48.2, 46.8, 38.7, 38.3, 38.2, 36.7, 36.5, 31.8, 31.2, 30.9, 30.6, 30.4, 30.3, 28.5, 28.47, 27.6, 25.3, 25.2, 25.1, 25.06, 25.0, 23.8, 23.4, 23.4, 23.1, 23.0, 22.9, 22.6, 22.4, 21.6, 19.5, 19.4, 18.5, 18.46, 18.3, 18.2, 17.6, 15.8, 10.1, 1.2; IR (thin film) 3316.0, 2961.2, 1635.3 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{74}H_{127}N_{11}O_{15}Na$): 1432.9, found: 1433.2.

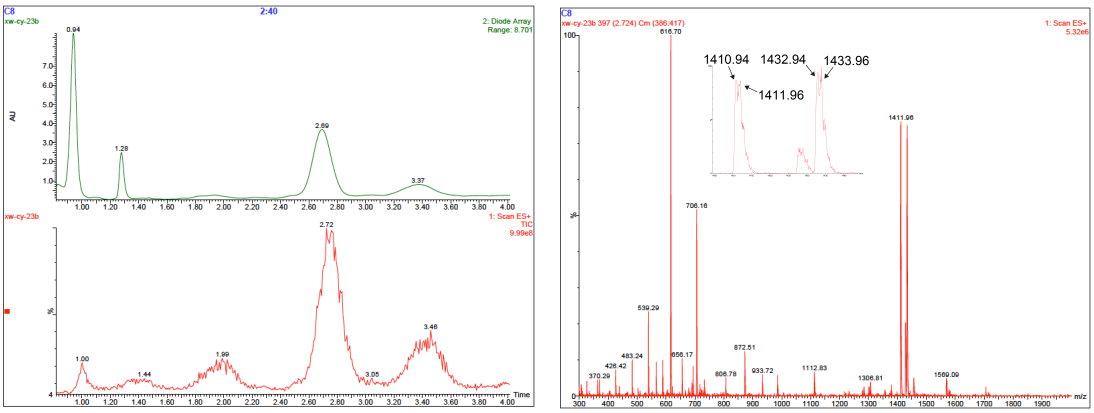


Figure S-11. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing undecapeptide **26**; retention time = 2.69 min, gradient 70–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **26**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{74}H_{128}N_{11}O_{15}$): 1410.96, found: 1410.94.

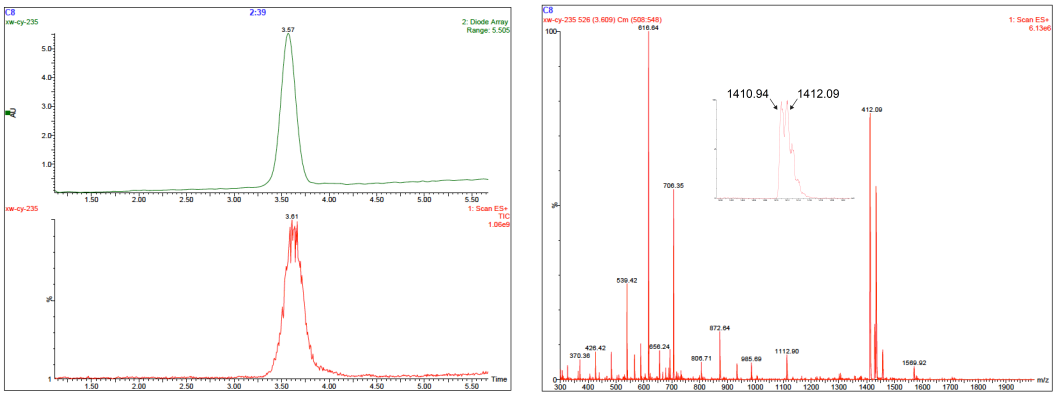
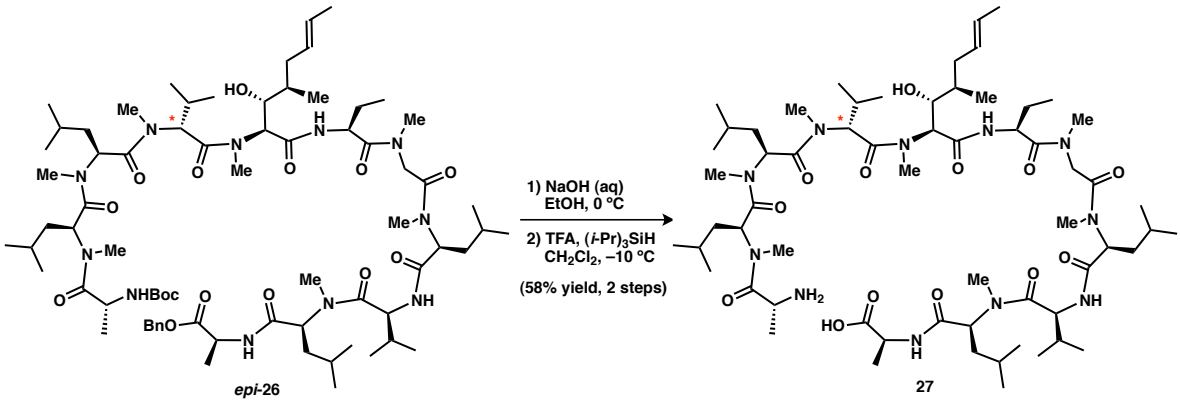


Figure S-12. (Left) UV and MS traces from UPLC-MS analysis of purified undecapeptide **26**; retention time = 3.57 min, gradient 75–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **26**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{74}H_{128}N_{11}O_{15}$): 1410.96, found: 1410.94.



H-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OH (27). Undecapeptide *epi*-**26** (5.0 mg, 3.5 μmol) was dissolved in pre-cooled (0 °C) ethanol (1.0 mL), then 0.2 N NaOH (0.15 mL) was added. The resulting mixture was stirred overnight at 0 °C. Analysis of the reaction mixture by LCMS indicated that the starting material was consumed. The pH of the reaction medium was adjusted to pH 4 with a few drops of acetic acid, then the volatiles were removed at low pressure. The remainder was taken up in CH_2Cl_2 (5 mL) and washed with brine. The organic phase was dried over $MgSO_4$, and evaporated at low pressure to give the crude free acid, which was carried directly into the next step without further purification.

To a cooled (–10 °C) solution of the free acid (from the previous step) in CH_2Cl_2 (1.0 mL) was added triisopropylsilane (5 μL) and TFA (0.25 mL). The resulting mixture was stirred at –10 °C until LCMS analysis of the reaction indicated that the starting material was consumed. The reaction mixture was then diluted with CH_2Cl_2 (5 mL) and washed with saturated $NaHCO_3$ (5 mL). The organic phase was dried over $MgSO_4$ and evaporated at low

pressure. The crude residue was purified by reversed-phase HPLC (C18 column, gradient 40–70% CH_3CN/H_2O with 0.05% TFA over 30 min) to give fully deprotected undecapeptide **27** (2.5 mg, 58% yield).

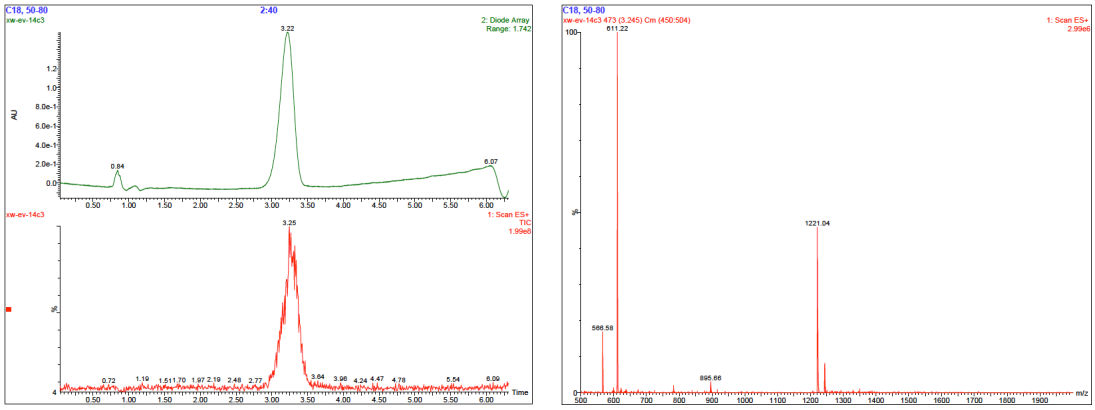
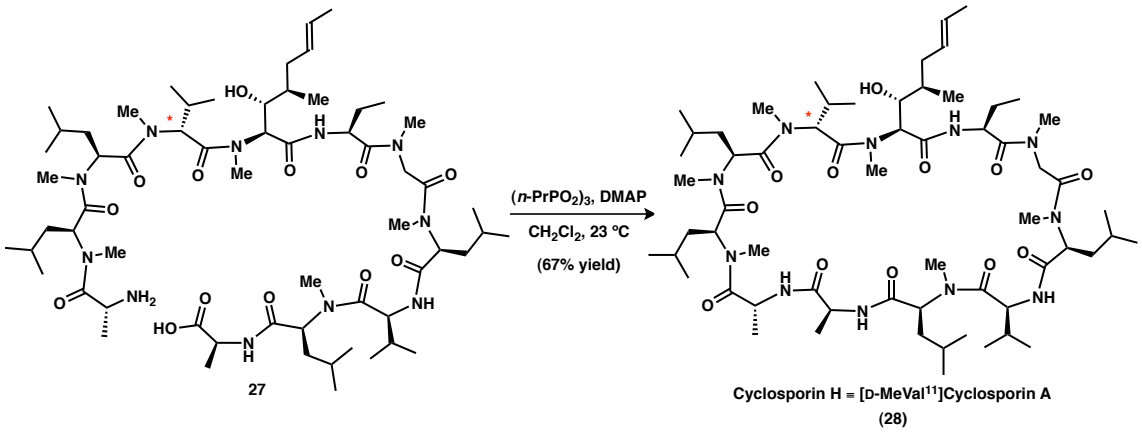


Figure S-13. (Left) UV and MS traces from UPLC-MS analysis of purified deprotected undecapeptide **27**; retention time = 3.22 min, gradient 50–80% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **27**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{62}H_{113}N_{11}O_{13}$): 1220.85, found: 1221.04.



Cyclosporin H (28). To a solution of fully deprotected undecapeptide **27** (6 mg, 4.9 μmol) in CH_2Cl_2 (20 mL) was added DMAP (3 mg, 24.6 μmol) and (n-PrO_2)$_3$ (50% w/w solution in ethyl acetate, 11.7 μL, 19.6 mmol). The reaction mixture was stirred for 30 hours at room temperature. The mixture was then was washed with water. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue was purified by flash chromatography (40:1 → 20:1 CH_2Cl_2/MeOH gradient) to give cyclosporin H (4 mg, 67% yield). $[\alpha]_D^{20}$ = –165.5°,

(c = 0.085, $CHCl_3$)[11]; ^1H NMR (600 MHz, $CDCl_3$): complicated (more than 8 conformers), but similar to natural cyclosporin H. IR (thin film) 3323.7, 2960.2, 2360.4, 1633.2, 1468.5 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{62}H_{111}N_{11}O_{12}Na$): 1224.8, found: 1225.0.

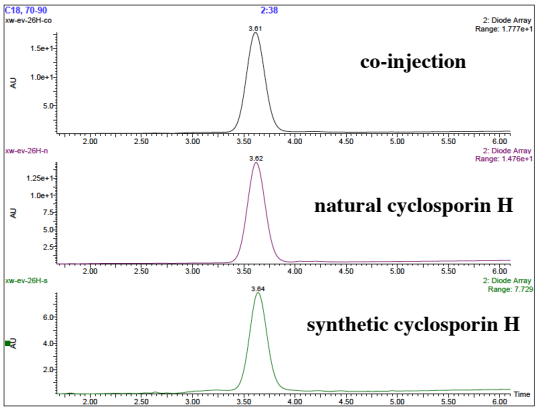
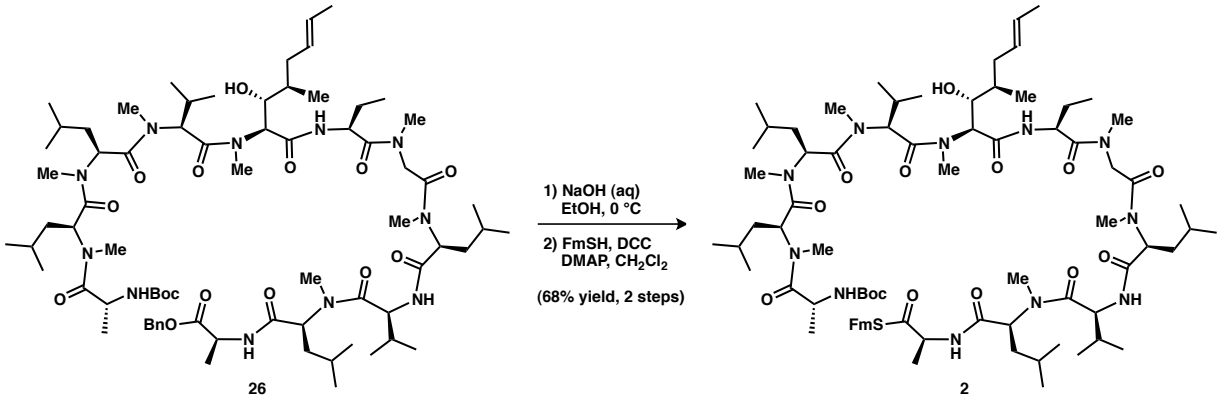


Figure S-14. UV traces from UPLC analysis of synthetic cyclosporin H (**28**) (bottom chromatogram, retention time = 3.61 min), natural cyclosporin H (middle chromatogram, retention time = 3.62 min), and co-injection of synthetic & natural cyclosporin H (top chromatogram, retention time = 3.64 min); gradient 70–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column.



Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-SFm (2). Undecapeptide **26** (16.4 mg, 11.7 μmol) was dissolved in pre-cooled (0 °C) ethanol (1.4 mL), then 0.2 N NaOH (0.2 mL) was added. The resulting mixture was stirred overnight at 0 °C. Analysis of the reaction mixture by LC-MS indicated that the starting material was consumed. The pH of the reaction medium was adjusted to pH 4 with a few drops of acetic acid, then the volatiles were removed at low pressure. The remainder was taken up in CH_2Cl_2 (5 mL) and washed with brine. The organic phase was dried over $MgSO_4$, and evaporated at low pressure to give the crude free acid,

(11) Literature value –177°, (c = 0.75, $CHCl_3$) (Traber, R.; Loosli, H.; Hofmann, H.; Kuhn, M.; Wartburg, A. V. *Helv. Chim. Acta* **1982**, *65*, 1655–1677). Purchased natural sample of cyclosporin H (AvaChem Scientific, catalog no. 1971) using our instrument: –167.5°, (c = 0.085, $CHCl_3$).

which was carried directly into the next step without further purification.

To a solution of the crude free acid in CH_2Cl_2 (1.5 mL) was added FmSH (12.5 mg, 58.5 μmol), DCC (12.0 mg, 58.5 μmol), and DMAP (0.7 mg, 5.85 μmol). The reaction mixture was stirred overnight at room temperature. Analysis of the reaction mixture by LC-MS indicated that the starting material was consumed. Purification by flash chromatography (40:1 CH_2Cl_2/MeOH) afforded undecapeptide thioester **2** (12 mg, 68% over two steps). Selected characterization data: $[\alpha]_D^{20} = -186.6°$ (c = 0.40, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$, mixture of rotamers) δ [7.83 (d, *J* = 9.2 Hz), 7.77 (d, *J* = 6.9 Hz), 1H], 7.72 (dd, *J* = 8.0, 3.4 Hz), 7.65 (dd, *J* = 14.4, 7.6 Hz), 7.63 – 7.55 (m), 7.37 (q, *J* = 7.3, 6.9 Hz), 7.32 – 7.27 (m), *all sum to* 8H], [7.11 (d, *J* = 8.8 Hz), 6.82 (d, *J* = 8.8 Hz), 6.76 (d, *J* = 7.2 Hz), 1H], [5.50 (q, *J* = 8.4 Hz), 5.46 – 5.37 (m), 5.33 (dd, *J* = 9.5, 5.3 Hz), 5.28 (dd, *J* = 10.5, 4.9 Hz), 5.24 (d, *J* = 11.2 Hz), 5.21 – 5.11 (m), 5.03 (t, *J* = 7.7 Hz), 4.95 (ddd, *J* = 14.4, 8.4, 6.3 Hz), 4.88 (dd, *J* = 8.9, 6.7 Hz), 4.65 (t, *J* = 7.8 Hz), 4.60 (t, *J* = 7.3 Hz), 4.54 – 4.48 (m), 4.48 – 4.41 (m), 4.21 (d, *J* = 15.1 Hz), 4.14 (q, *J* = 5.8 Hz), 4.02 (d, *J* = 15.5 Hz), 3.91 – 3.78 (m), 3.63 (dd, *J* = 9.7, 4.3 Hz), 3.53 (dd, *J* = 13.5, 5.8 Hz), *all sum to* 19H], [3.44 (s), 3.41 – 3.34 (m), 3.32 (s), 3.31 (s), 3.26 (s), 3.22 (s), 3.17 (s), 3.14 (s), 3.07 (s), 3.05 (s), 3.00 (s), 2.99 (s), 2.94 (s), 2.82 (s), 2.79 (s), 2.56 (s), *all sum to* 21H], [2.47 – 2.06 (m), 1.92 (s), 1.88 – 1.69 (m), 1.68 – 1.54 (m), 1.42 (s), 1.39 – 1.33 (m), 1.29 (d, *J* = 6.8 Hz), 1.25 (s), *all sum to* 37H], [1.22 (dd, *J* = 14.5, 7.4 Hz), 1.11 (d, *J* = 7.2 Hz), 1.05 (d, *J* = 6.5 Hz), 0.98 (dd, *J* = 6.6, 2.7 Hz), 0.97 – 0.87 (m), 0.88 – 0.81 (m), 0.79 (dd, *J* = 6.6, 3.7 Hz), 0.73 (d, *J* = 5.7 Hz), 0.68 (dd, *J* = 9.3, 6.4 Hz), 0.61 (d, *J* = 6.6 Hz), 0.52 (d, *J* = 6.6 Hz), *all sum to* 42H]; ^{13}C NMR (151 MHz, $CDCl_3$) δ 201.1, 173.4, 172.4, 172.1, 171.0, 170.0, 155.5, 151.0, 145.6, 145.6, 145.5, 141.3, 141.2, 129.6, 129.1, 128.3, 127.9, 127.8, 127.3, 127.21, 127.20, 127.18, 127.16, 127.1, 126.6, 124.9, 124.8, 120.0, 120.0, 119.0, 79.8, 57.3, 56.9, 55.5, 55.4, 55.0, 54.5, 51.7, 51.3, 50.4, 49.5, 46.9, 46.8, 46.7, 38.9, 38.2, 37.9, 37.3, 36.8, 36.49, 36.47, 36.24, 32.18, 31.9, 31.4, 31.2, 30.8, 30.6, 30.54, 30.51, 30.40, 30.35, 30.2, 29.9, 28.5, 27.7, 27.4; IR (thin film) 3313.1, 2963.1, 2359.5, 1640.2 cm^{-1}; LRMS (ESI+) *m/z* calc'd for $[M+K]^+$ ($C_{81}H_{131}N_{11}O_{14}SK$): 1552.96, found: 1553.20.

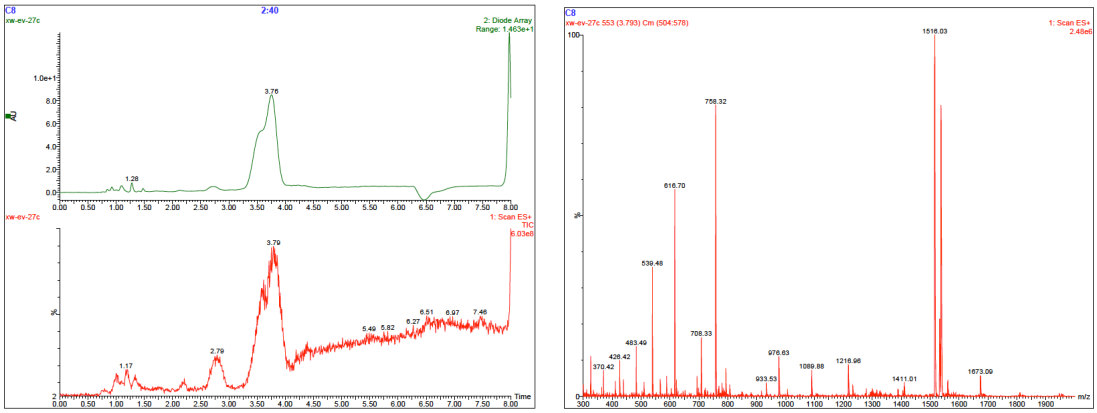
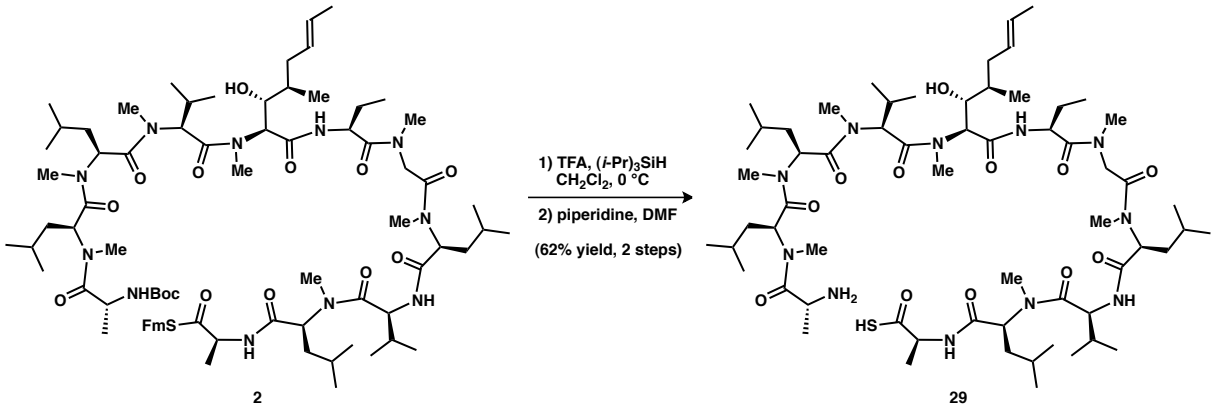


Figure S-15. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing undecapeptide thioester **2**; retention time = 3.76 min, gradient 70–85% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C8 column. (Right) ESI-MS of compound **2**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{81}H_{131}N_{11}O_{14}S$): 1515.96, found: 1516.03.



H-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-SH (29). To a cooled (0 °C) solution of undecapeptide thioester **2** (6 mg, 3.96 μmol) in CH_2Cl_2 (0.5 mL) was added triisopropylsilane (10 μL) and TFA (0.5 mL). The resulting mixture was stirred for 1 h at 0 °C. Analysis of the reaction mixture by LC-MS indicated that the starting material was consumed. The volatiles were removed by rotary evaporation while being kept cold in a water bath containing ice. The crude amine TFA salt was carried directly into the next step without further purification.

The crude residue from the preceding step was treated with 10% piperidine in DMF (0.3 mL) for 10 min at room temperature. Analysis of the reaction mixture by LCMS indicated that the starting material was consumed. The reaction medium was neutralized with acetic acid (0.1 mL), then purified by reversed-phase HPLC (C8 column, gradient 45–55% CH_3CN/H_2O with 0.05% TFA over 30 min) to give fully deprotected undecapeptide thioacid **29** (3.0 mg, 62% yield).

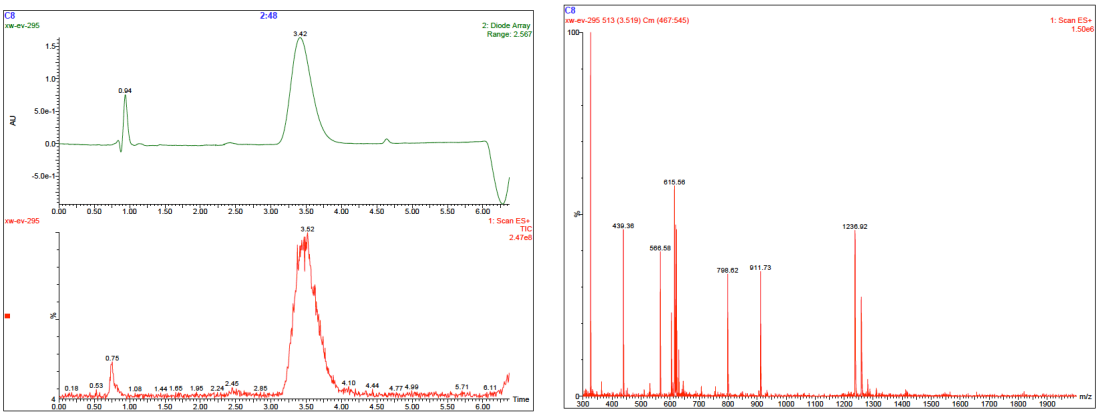
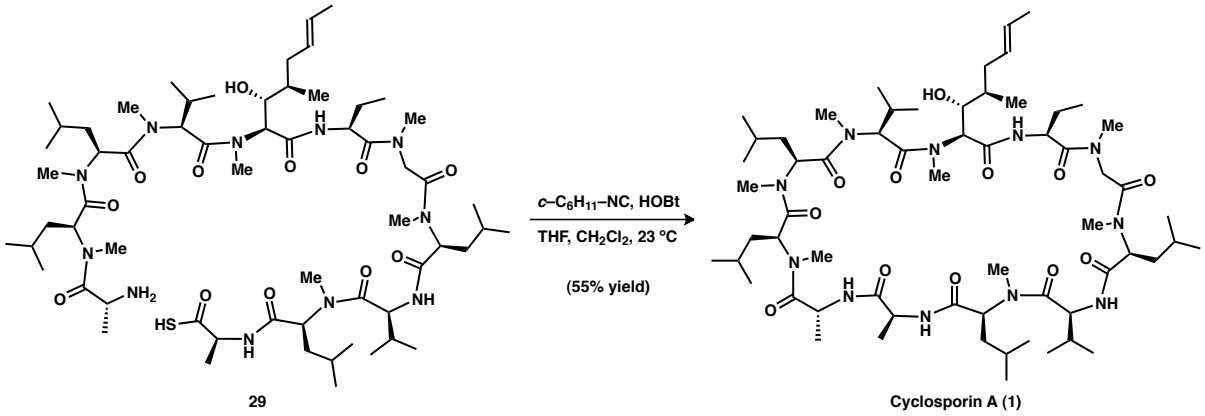
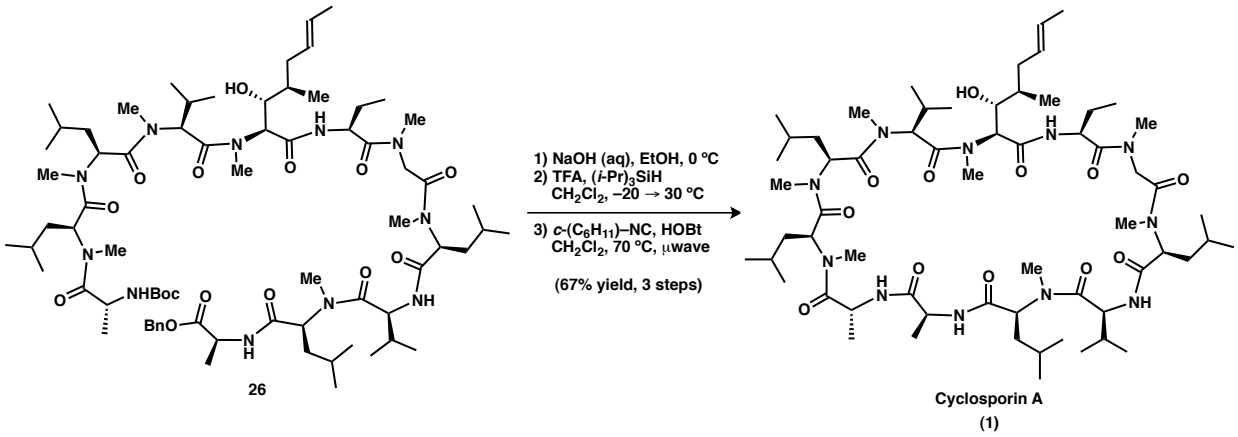


Figure S-16. (Left) UV and MS traces from UPLC-MS analysis of purified undecapeptide thioacid **29**; retention time = 3.42 min, gradient 55–65% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C8 column. (Right) ESI-MS of compound **29**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{62}H_{113}N_{11}O_{12}S$): 1236.83, found: 1236.92.



Cyclosporin A (1). ***Type IIb method*:** To a solution of **29** (3.0 mg, 2.43 μmol) in CH_2Cl_2 (3 mL) was added cyclohexyl isonitrile (3 μL, 24.3 μmol) and HOBt (0.65 mg, 4.86 mmol) in THF (0.2 mL). The resulting mixture was stirred for 24 h at room temperature. The volatiles were then removed at low pressure. The crude residue was purified by flash chromatography (40:1 CH_2Cl_2/MeOH) to give cyclosporin A (**1**) (1.6 mg, 55% yield). Spectral and analytical data were consistent with data for **1** obtained via the Type IIa method (*vide infra*).

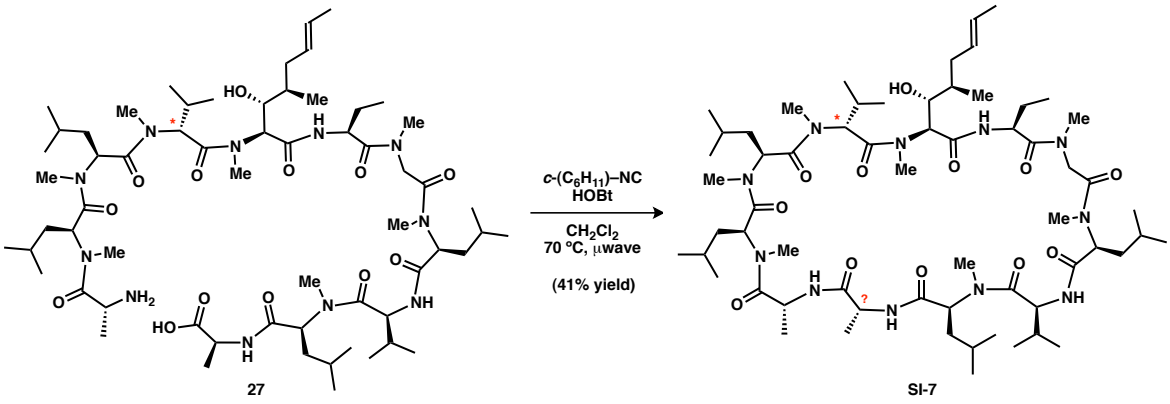


Cyclosporin A (1). ***Type IIa method*****:** Undecapeptide **26** (15 mg, 10.6 μmol) was dissolved in pre-cooled (0 °C) ethanol (1.4 mL), then 0.2 N NaOH (0.2 mL) was added. The resulting mixture was stirred overnight at 0 °C. Analysis of the reaction mixture by LC-MS indicated that the starting material was consumed. The pH of the reaction medium was adjusted to pH 4 with a few drops of acetic acid, then the volatiles were removed at low pressure. The remainder was taken up in CH_2Cl_2 (5 mL), then washed with water and brine. The organic phase was dried over $MgSO_4$, and evaporated at low pressure to give the crude free acid, which was carried directly into the next step without further purification.

The crude free acid from the preceding step was dissolved in 1:1 CH_2Cl_2/TFA (1 mL) at –20 °C, then triisopropylsilane (5 μL) was added. The resulting mixture was stirred overnight at –20 °C. Analysis of the reaction mixture by LC-MS indicated that the starting material was consumed. The reaction mixture was then diluted with CH_2Cl_2 (5 mL), then washed with saturated $NaHCO_3$ and brine. The organic phase was dried over $MgSO_4$ and evaporated at low pressure. The crude residue was rinsed by resuspending in ether, then centrifuging and decanting the supernatant. The resulting pellet was dissolved in CH_2Cl_2 and evaporated at low pressure. The crude fully deprotected undecapeptide was carried into the next step without further purification.

To a solution of crude fully deprotected undecapeptide in CH_2Cl_2 (10 mL) was added cyclohexyl isonitrile (6 μL, 48 μmol) and HOBt (2.8 mg, 21.2 μmol) in THF (0.2 mL). The resulting mixture was stirred for 20 h at 70 °C under microwave irradiation. The reaction mixture was purified directly by flash chromatography (40:1 CH_2Cl_2/MeOH) to give cyclosporin A (**1**) as a cloudy, white film (8.6 mg, 67% yield over three steps). Trituration of

this material in a mixed acetone/ether/hexanes solvent system resulted in the formation of a white amorphous solid,[12] m.p. 138–145 °C; $[\alpha]_D^{20}$ = –226.3° (c = 0.15, $CHCl_3$);[13] ^1H 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 (ESI+) *m/z* calc'd for $[M+Na]^+$ ($C_{62}H_{111}N_{11}O_{12}Na$): 1224.8311, found: 1224.8345.



(12) The melting point reported in the literature for crystalline cyclosporin A is 148–151 °C (Rüegger, A.; Kuhn, M.; Lichti, H.; Loosli, H.; Huguenin, R.; Quiquerez, C.; von Wartburg, A. *Helv. Chim. Acta* **1976**, *59*, 1075–1092). Attempts to crystallize our synthetic material at milligram scales were unsuccessful.

(13) Literature value: –244°, (c = 0.6, $CHCl_3$) (see citation in ref. 11). Purchased natural sample of cyclosporin A (Aldrich, catalog no. C3662) using our instrument: –227.8°, (c = 0.11, $CHCl_3$).

Type IIa cyclization of cyclosporin H precursor (SI-7). To a solution of fully deprotected undecapeptide **27** (2.5 mg, 2.0 μmol) in $CHCl_3$ (0.5 mL) was added cyclohexyl isonitrile (1.2 μL, 10.2 μmol) and HOBt (0.56 mg, 4.1 μmol) in THF (0.1 mL). The resulting mixture was stirred for 15 h at 70 °C under microwave irradiation. Analysis of the reaction mixture by LCMS indicated the presence of a product with a mass that is consistent with macrocyclization. The volatiles were removed at low pressure, and the residue was purified by reversed-phase HPLC (C18 column, gradient 65–80% CH_3CN/H_2O with 0.05% TFA over 30 min) to give **SI-7** (1.0 mg, 41% yield). Although this compound co-elutes with cyclosporin H when co-injected on the UPLC, its ^1H NMR spectrum does not match that of natural cyclosporin H or synthetic cyclosporin H (obtained by cyclizing **27** using $(n\text{-}PrPO_2)_3$, *vide supra*).

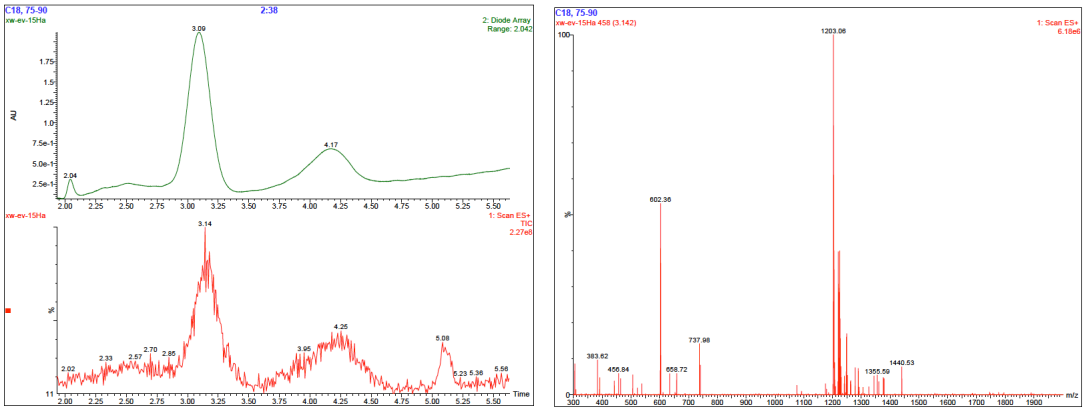


Figure S-17. (Left) UV and MS traces from UPLC-MS analysis of the crude reaction mixture containing compound **SI-7**; gradient 75–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **SI-7**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{62}H_{112}N_{11}O_{12}$): 1202.84, found: 1203.06.

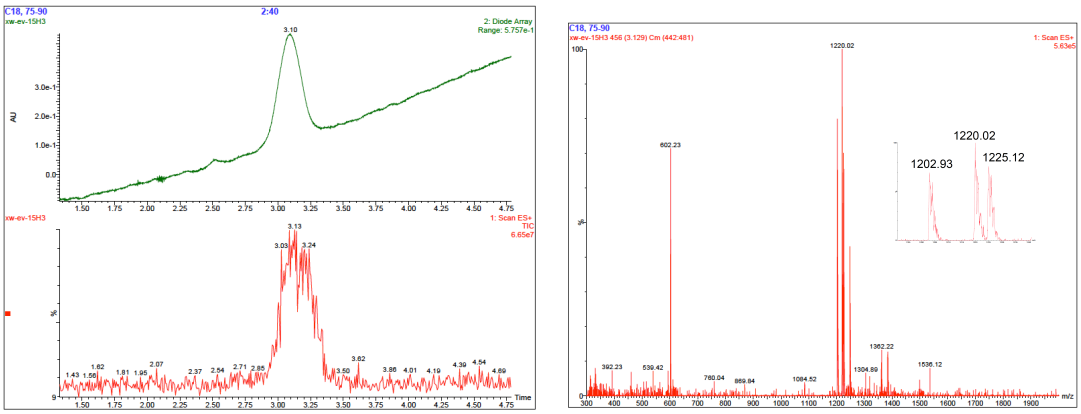
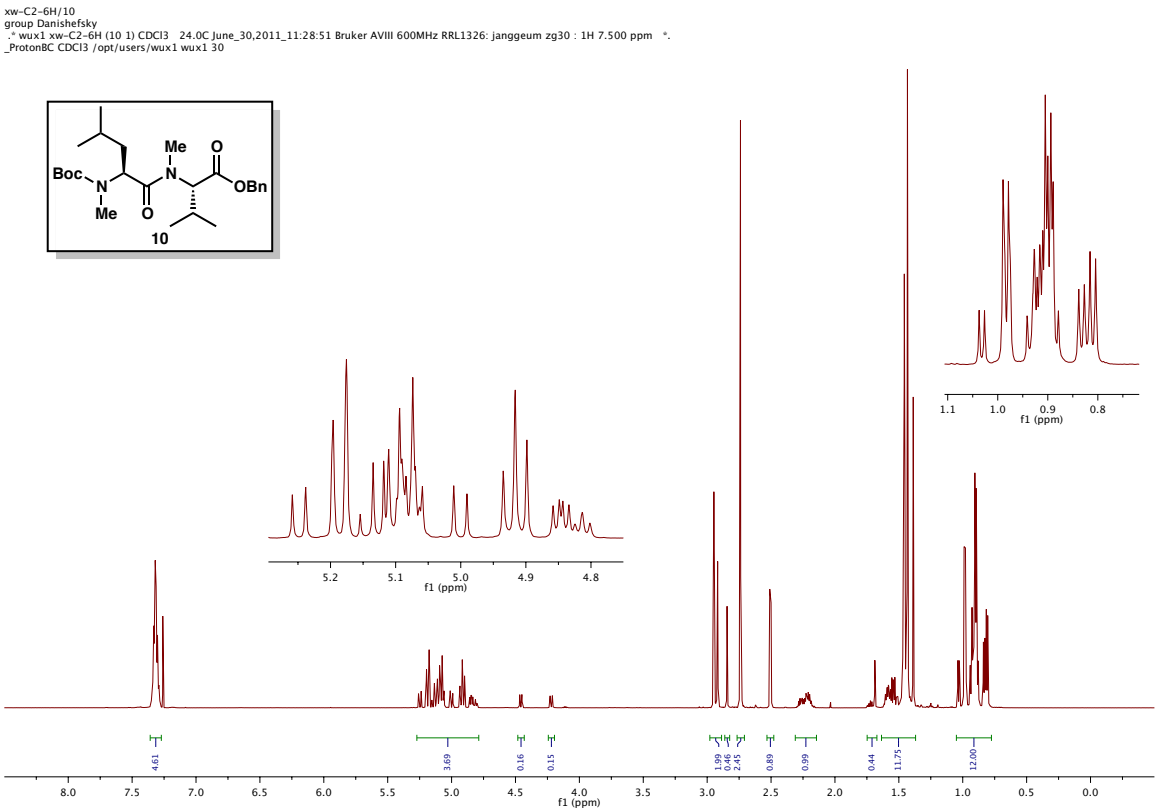
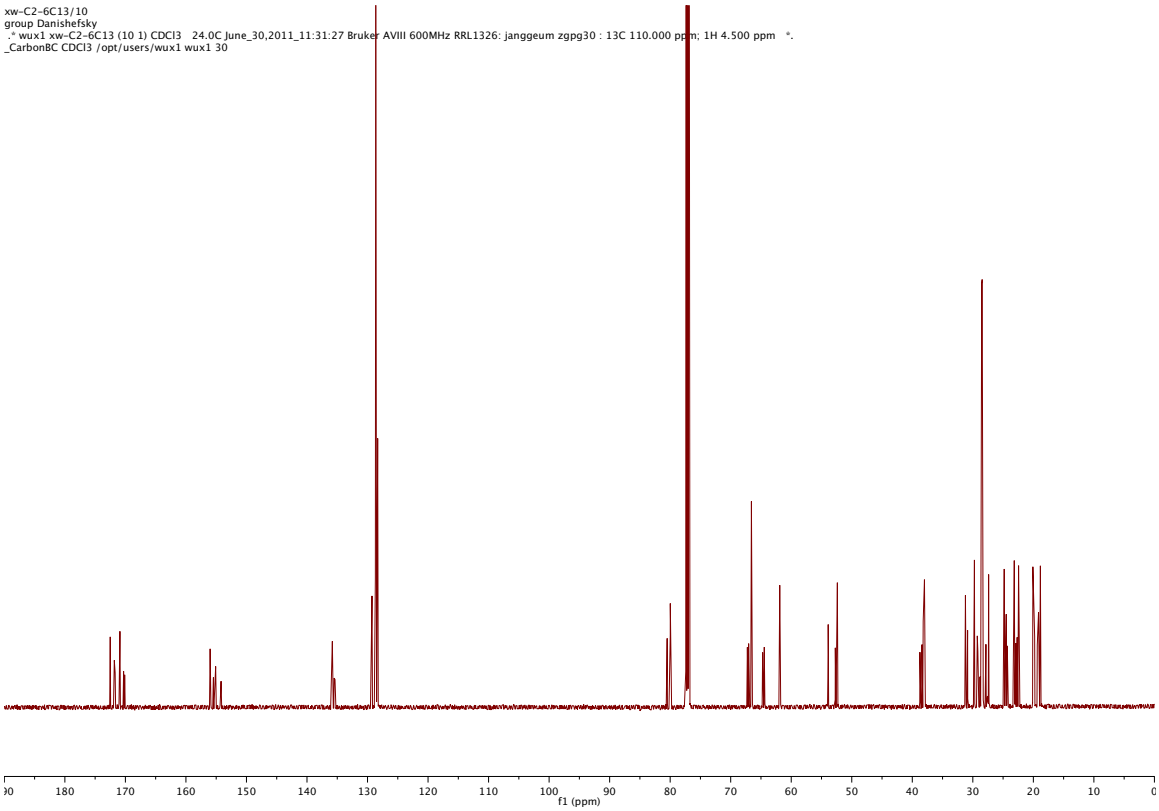


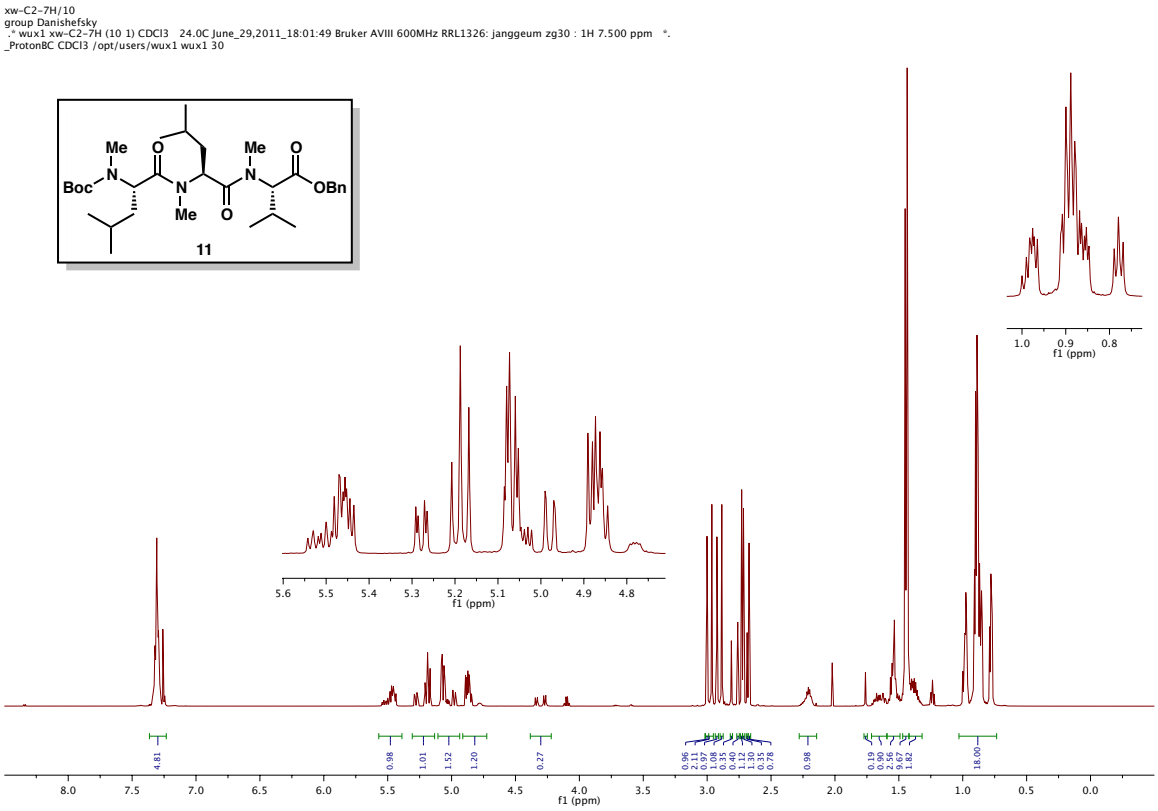
Figure S-18. (Left) UV and MS traces from UPLC-MS analysis of purified compound **SI-7**; gradient 75–90% CH_3CN/H_2O with 0.05% TFA over 6 min at a flow rate of 0.3 mL/min, C18 column. (Right) ESI-MS of compound **SI-7**; LRMS (ESI+) *m/z* calc'd for $[M+H]^+$ ($C_{62}H_{112}N_{11}O_{12}$): 1202.84, found: 1202.93.



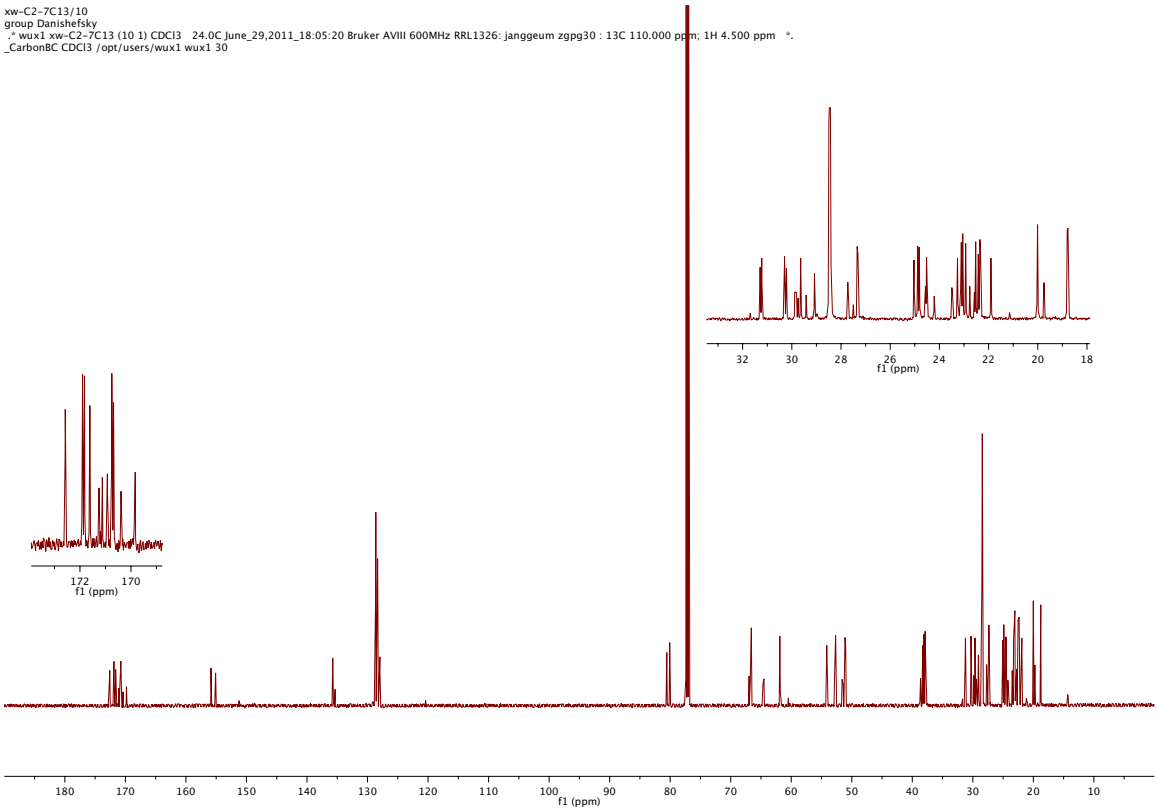
^{1}H NMR Spectrum of **Boc-MeLeu-MeVal-OBn (10)** in $CDCl_3$ at 600 MHz.



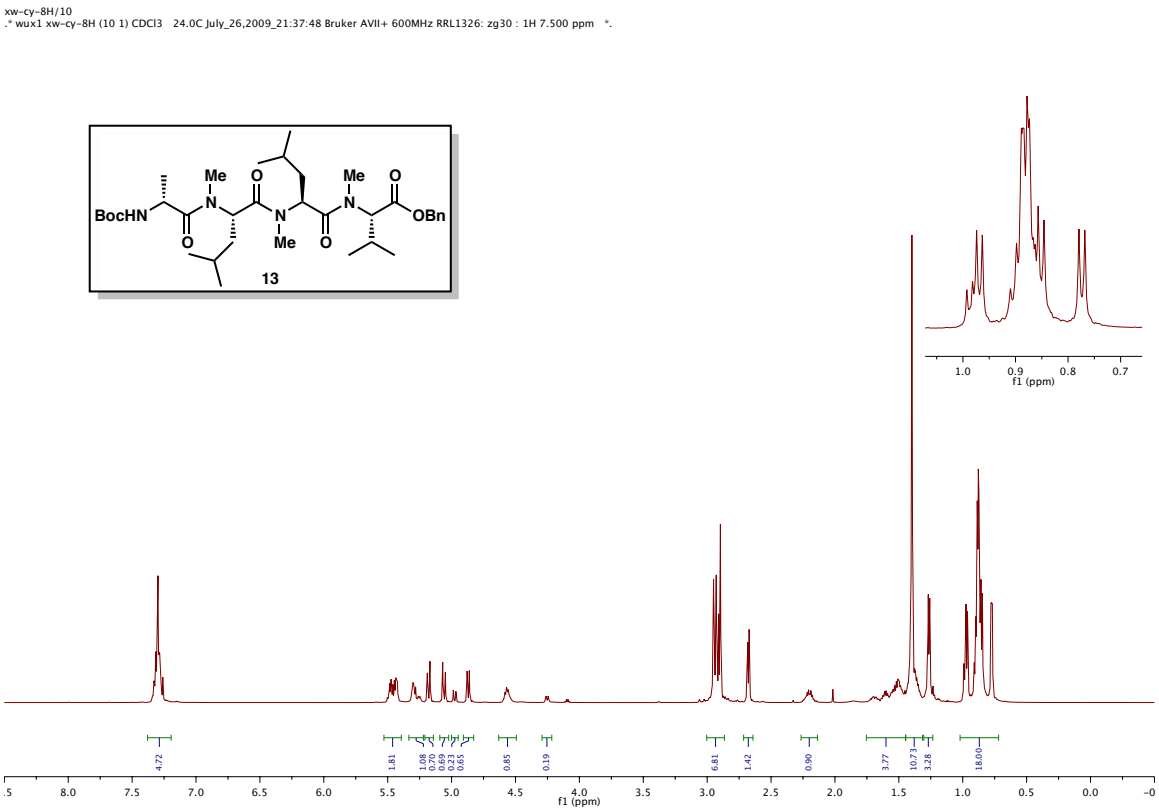
^{13}H NMR Spectrum of **Boc-MeLeu-MeVal-OBn (10)** in $CDCl_3$ at 151 MHz.



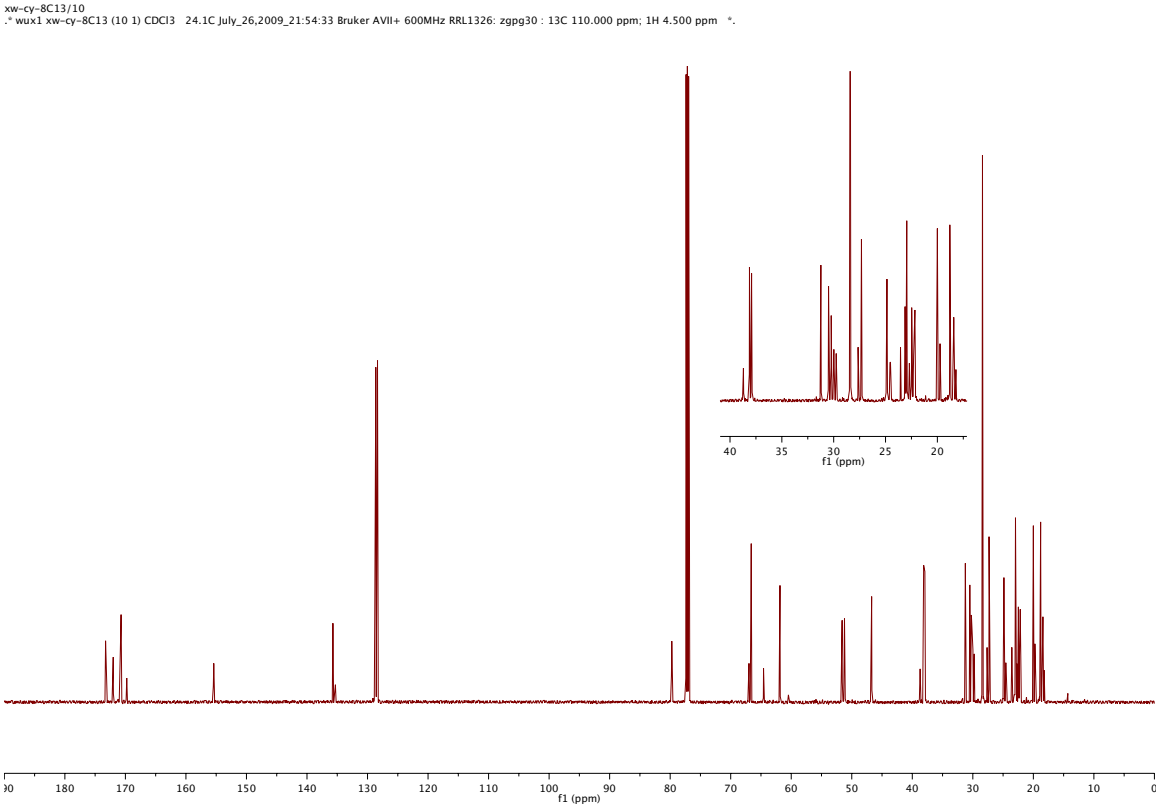
^{1}H NMR Spectrum of **MeLeu-MeLeu-MeVal-OBn (11)** in $CDCl_3$ at 600 MHz.



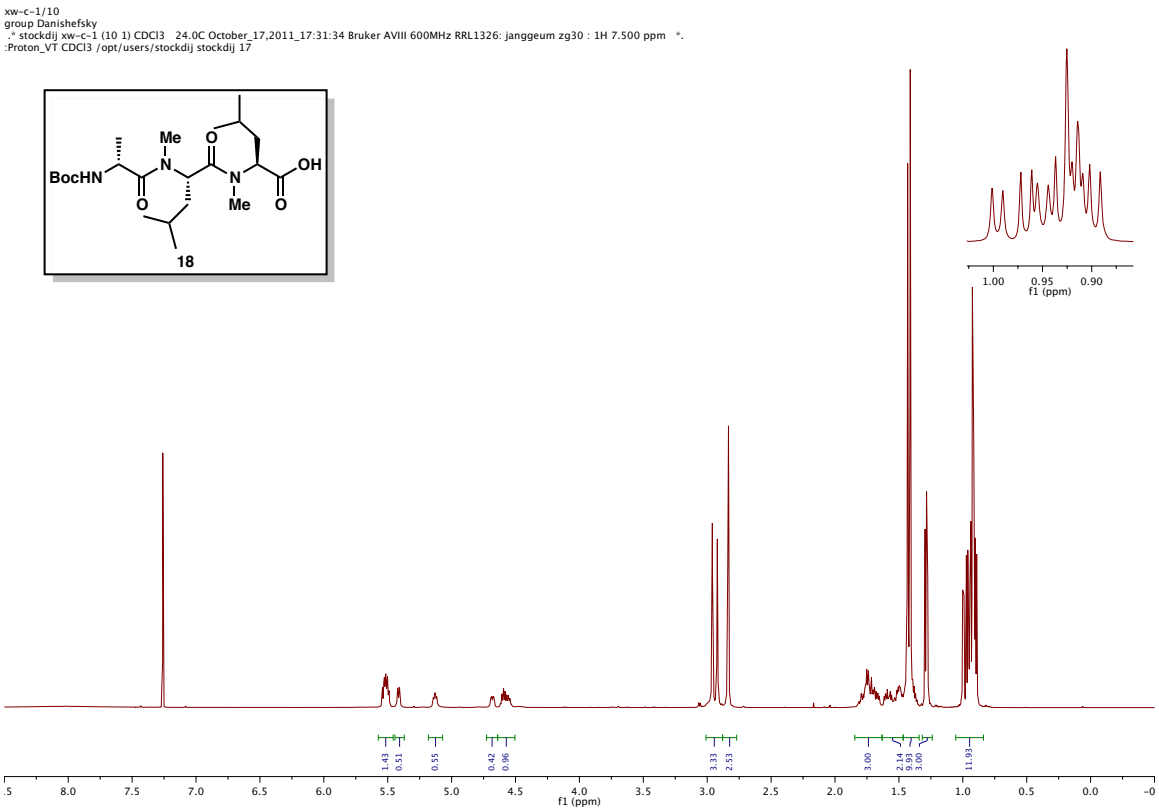
^{13}C NMR Spectrum of **MeLeu-MeLeu-MeVal-OBn (11)** in $CDCl_3$ at 151 MHz.



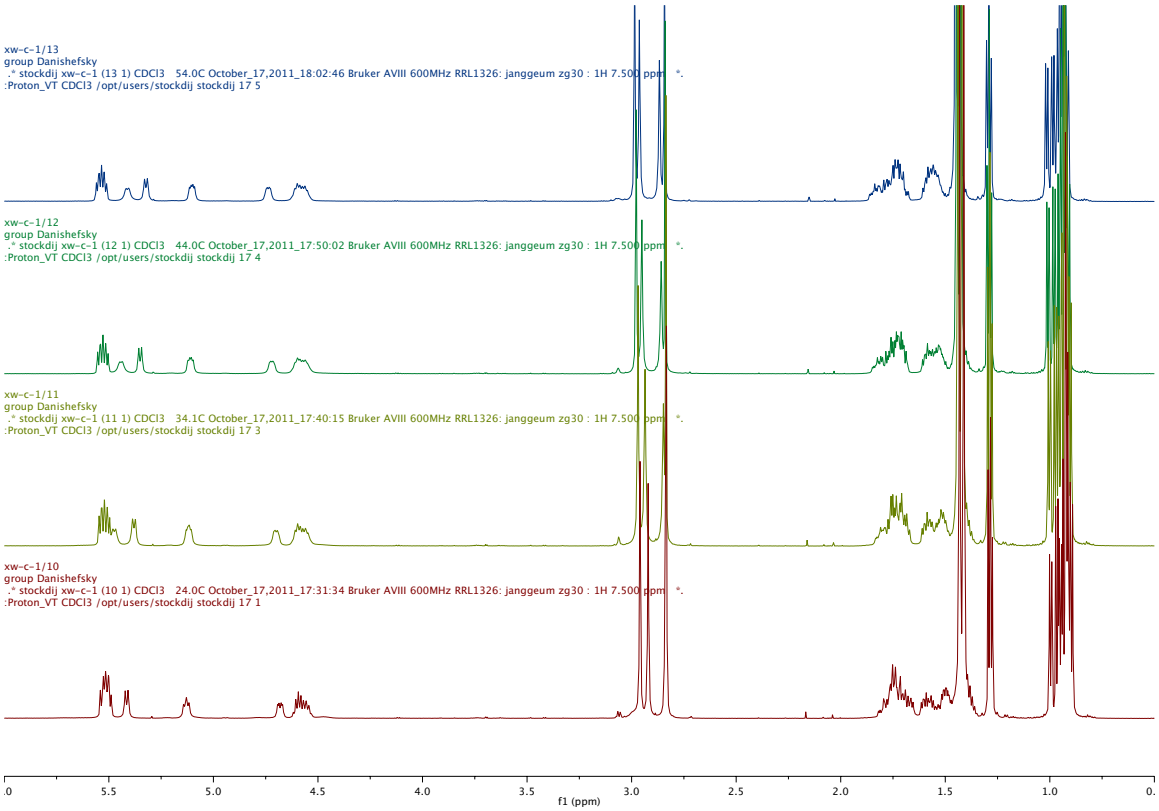
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-OBn (13)** in $CDCl_3$ at 600 MHz.



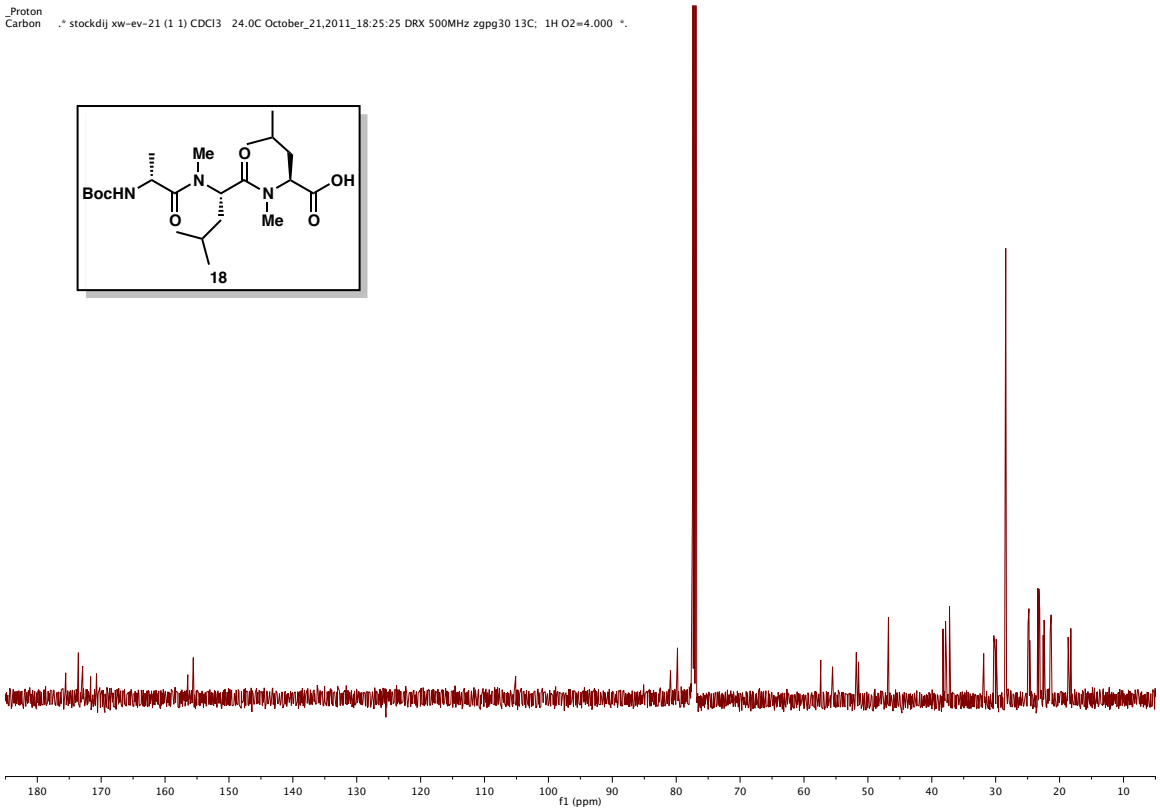
^{13}C NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-OBn (13)** in $CDCl_3$ at 151 MHz.



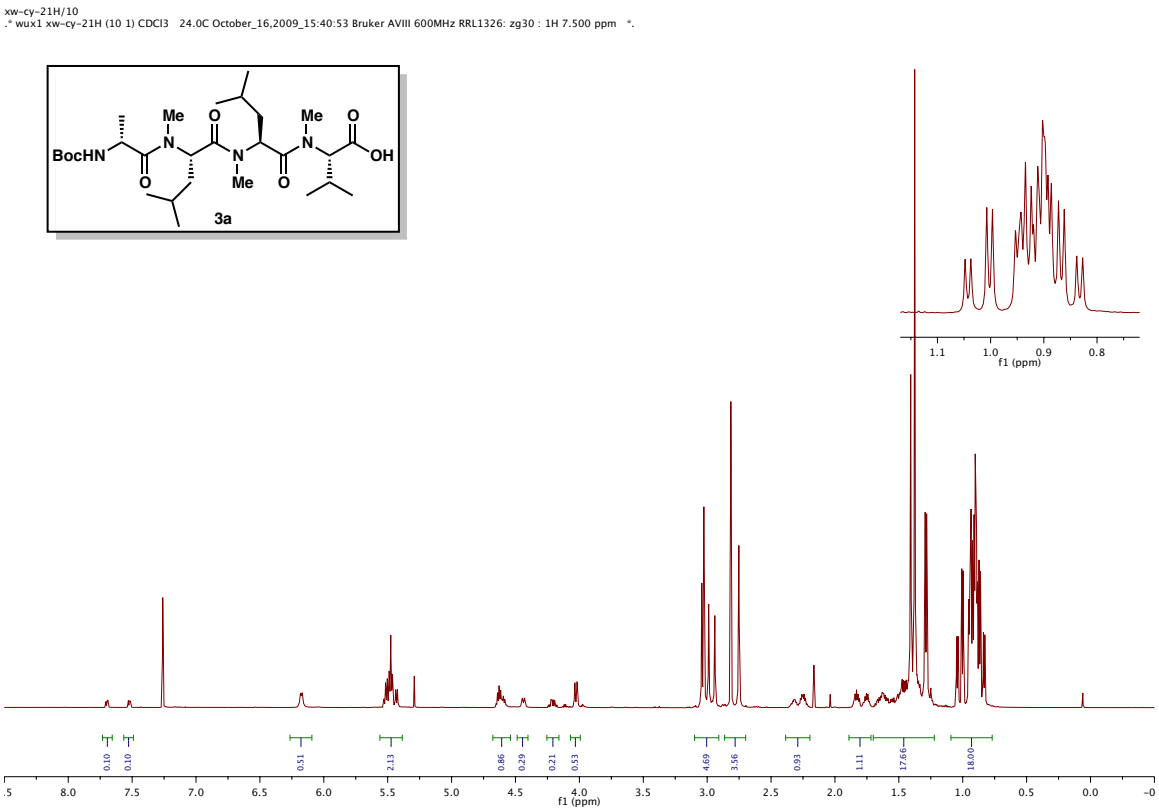
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-OH (18)** in $CDCl_3$ at 600 MHz.



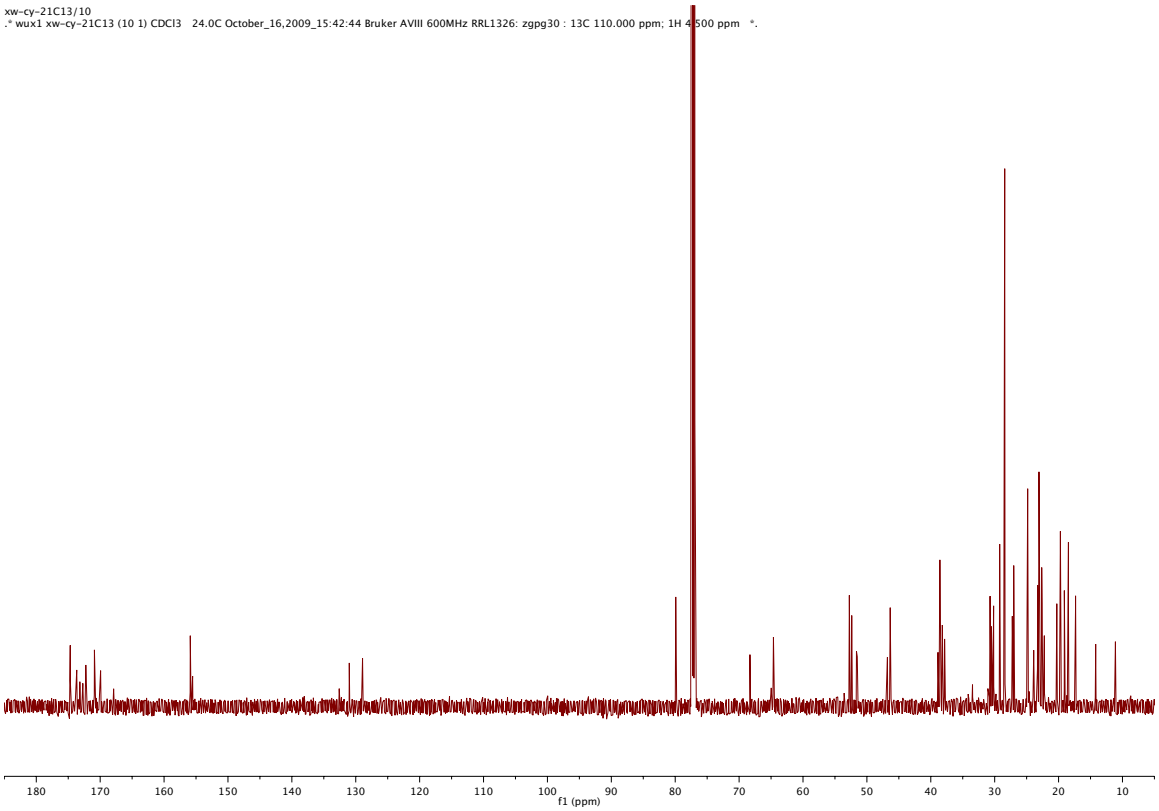
Variable Temperature ^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-OH (18)** in $CDCl_3$ at 600 MHz.



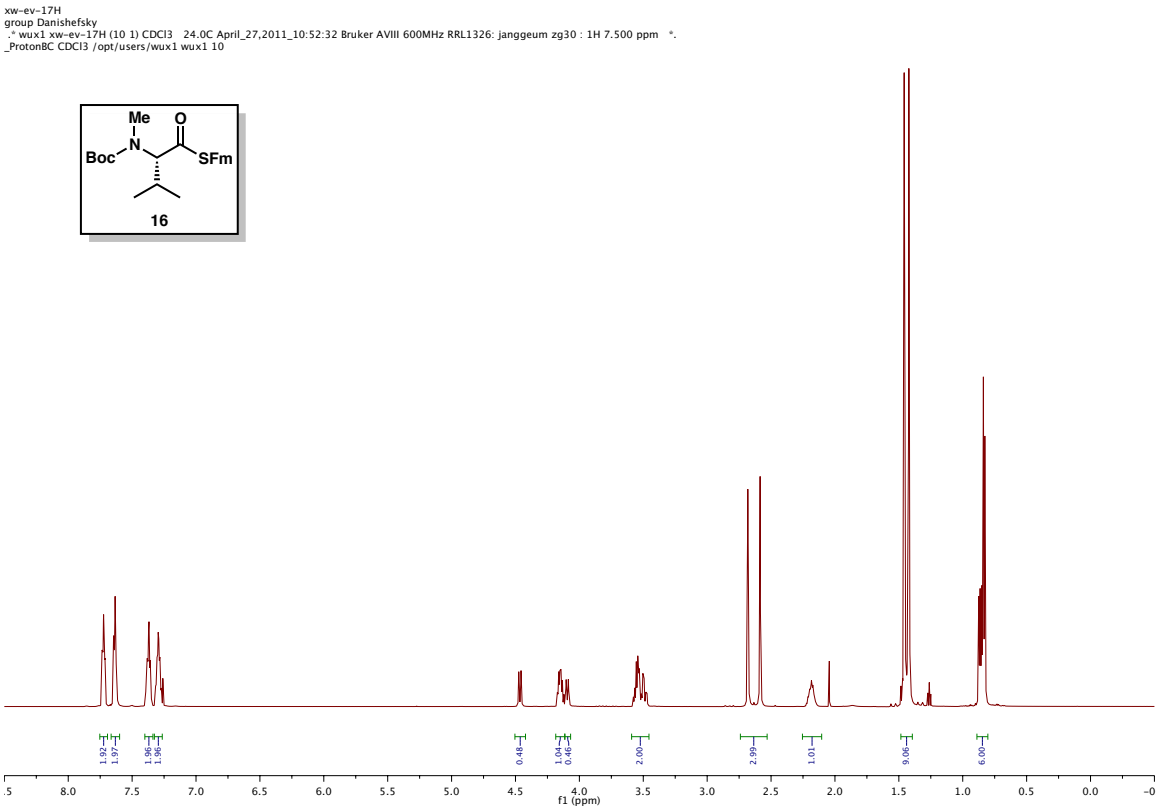
^{13}C NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-OBn (18)** in $CDCl_3$ at 151 MHz.



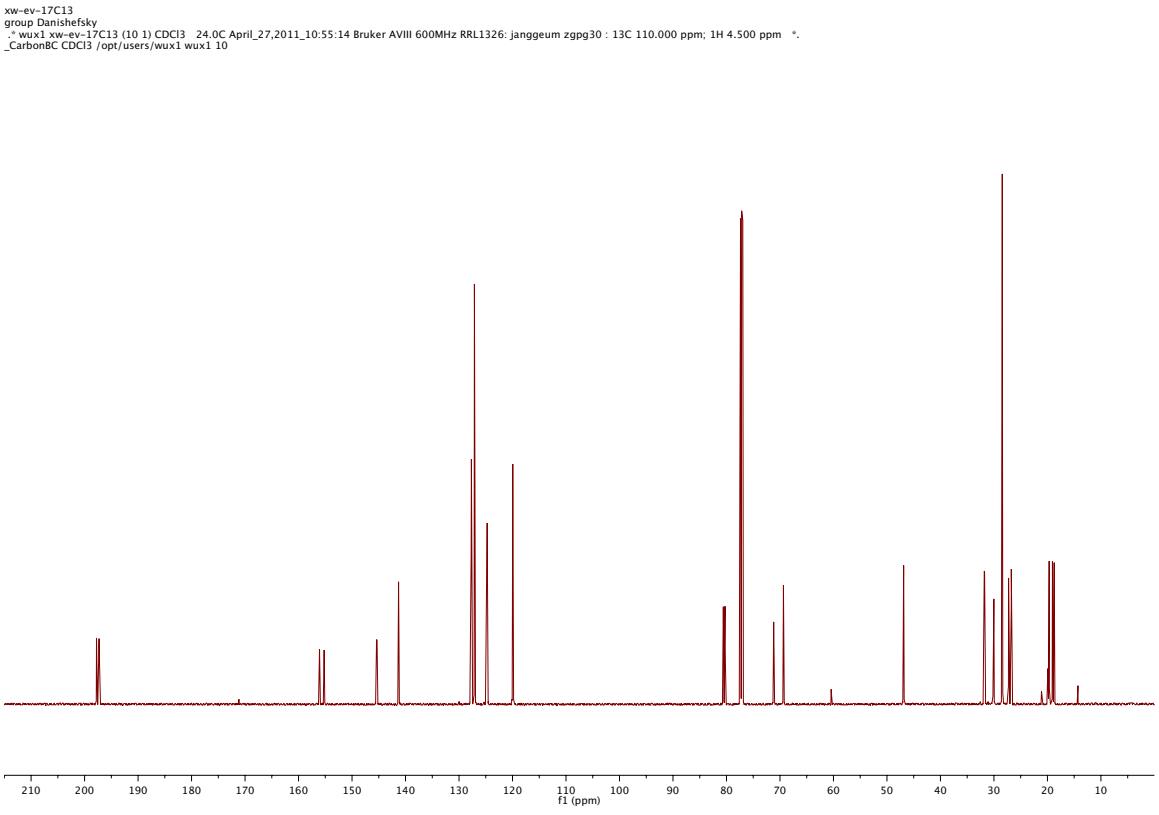
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (3a)** in $CDCl_3$ at 600 MHz.



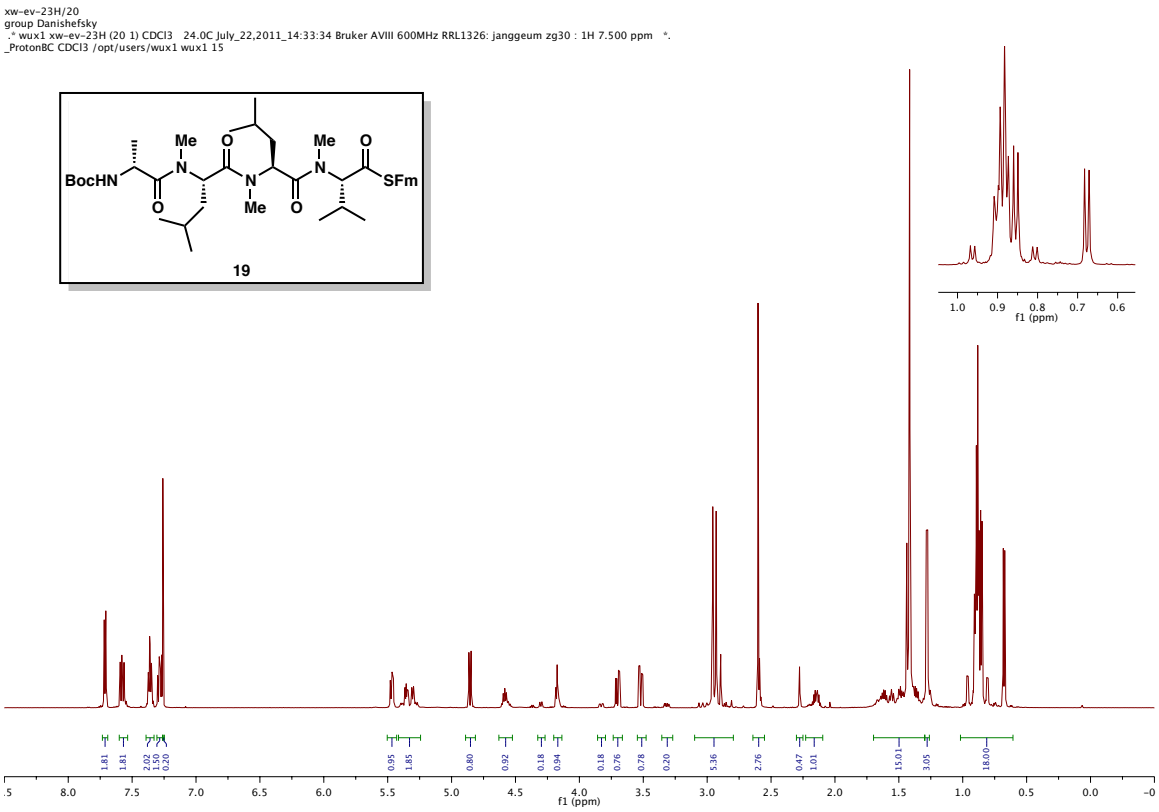
^{13}C NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-OH (3a)** in $CDCl_3$ at 151 MHz.



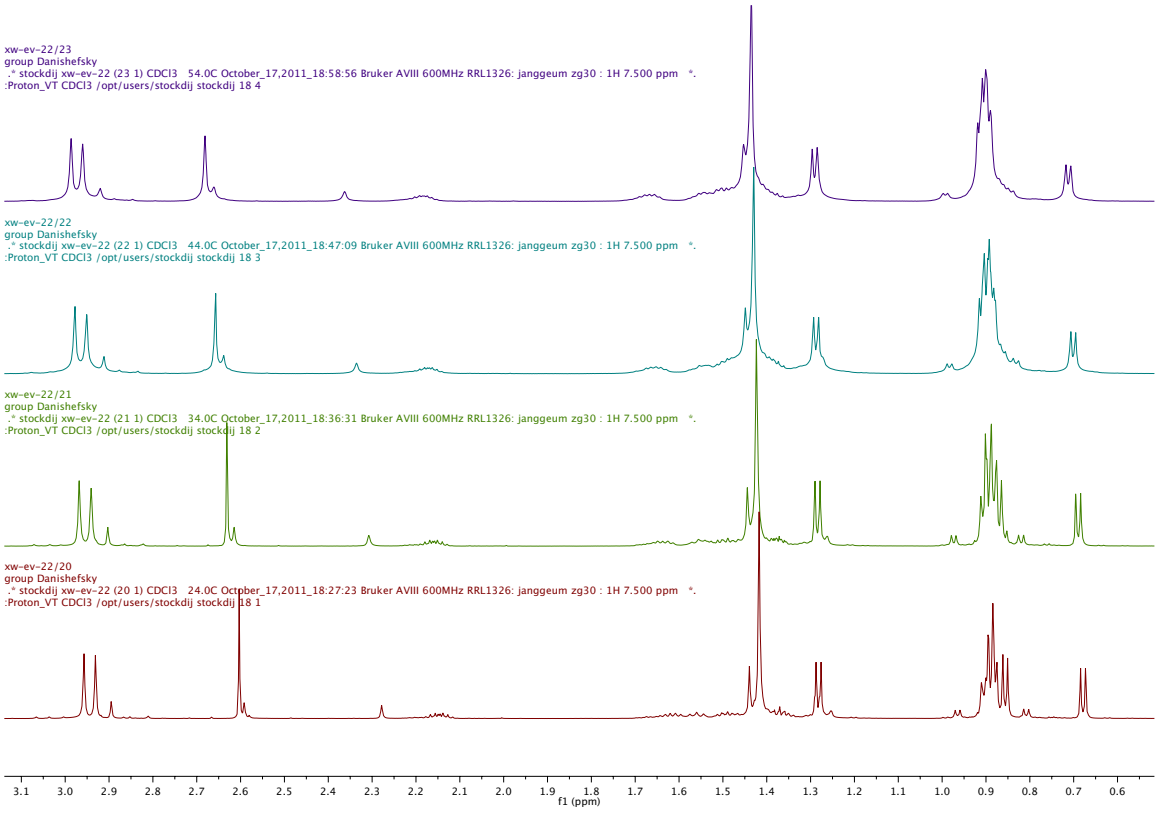
^{1}H NMR Spectrum of **Boc-MeVal-SFm (16)** in $CDCl_3$ at 600 MHz.



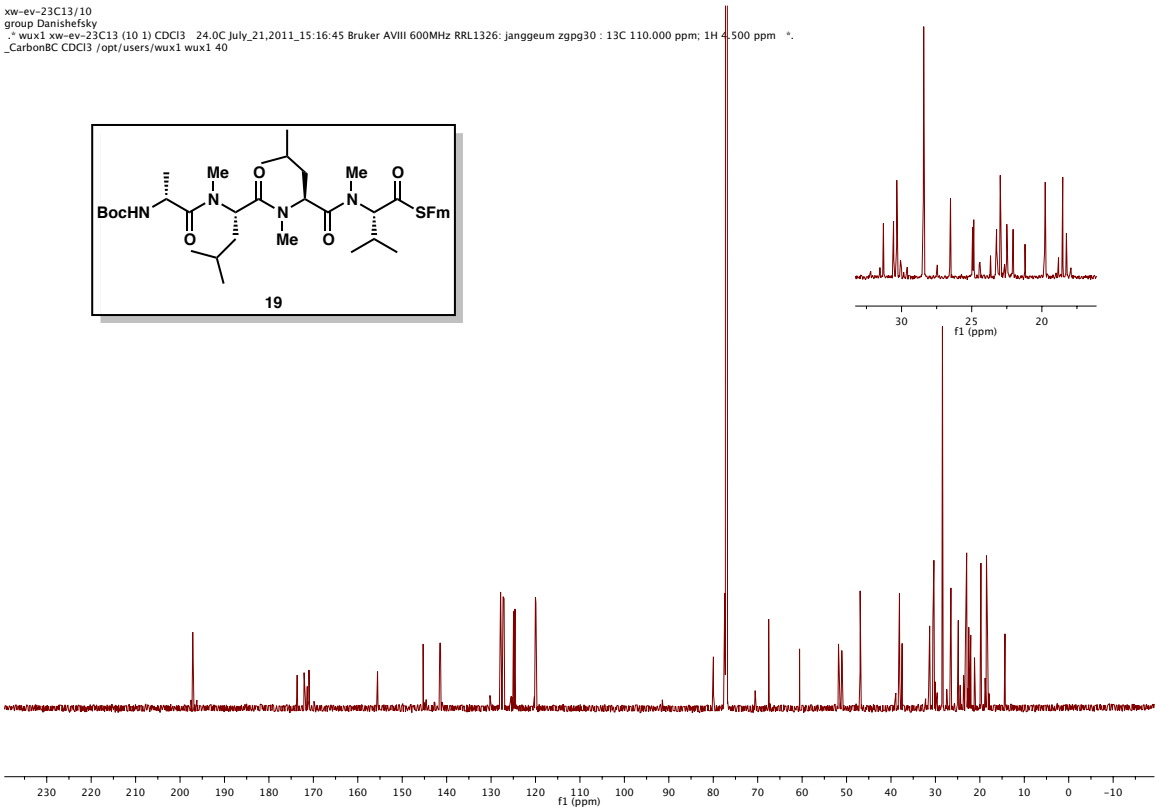
^{13}C NMR Spectrum of **Boc-MeVal-SFm (16)** in $CDCl_3$ at 151 MHz.



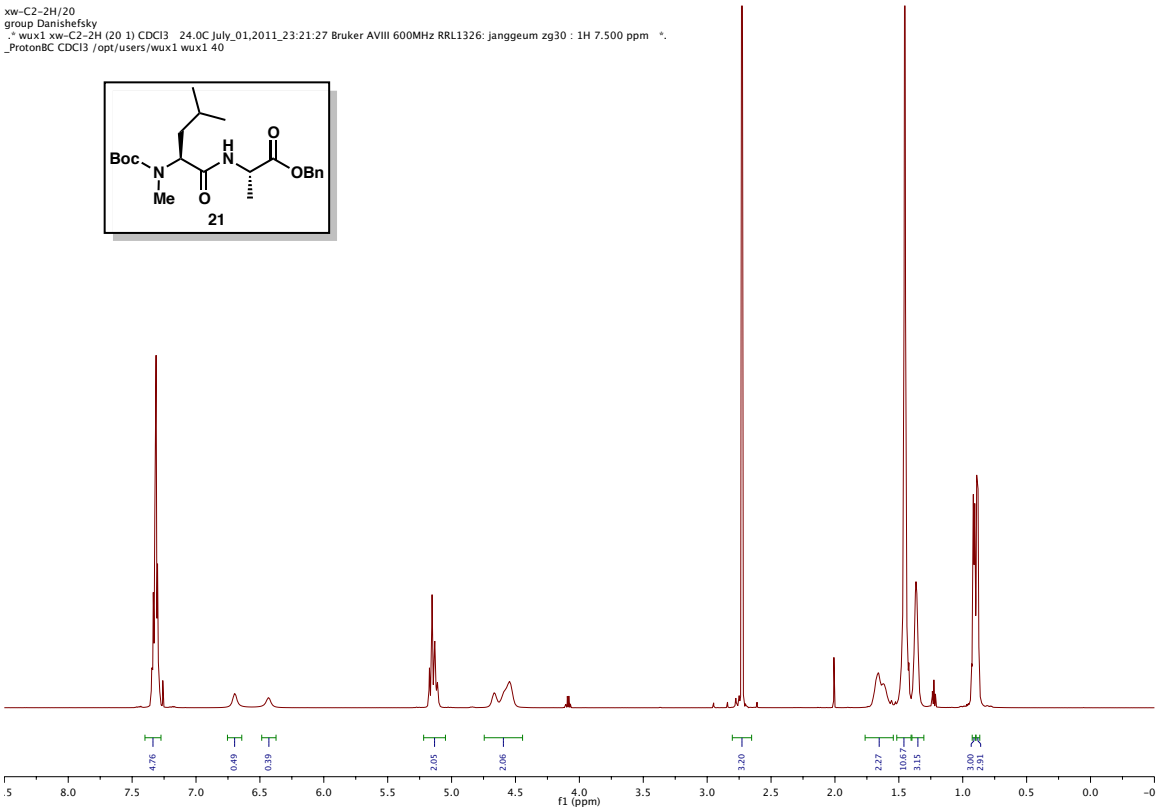
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-SFm (19)** in $CDCl_3$ at 600 MHz.



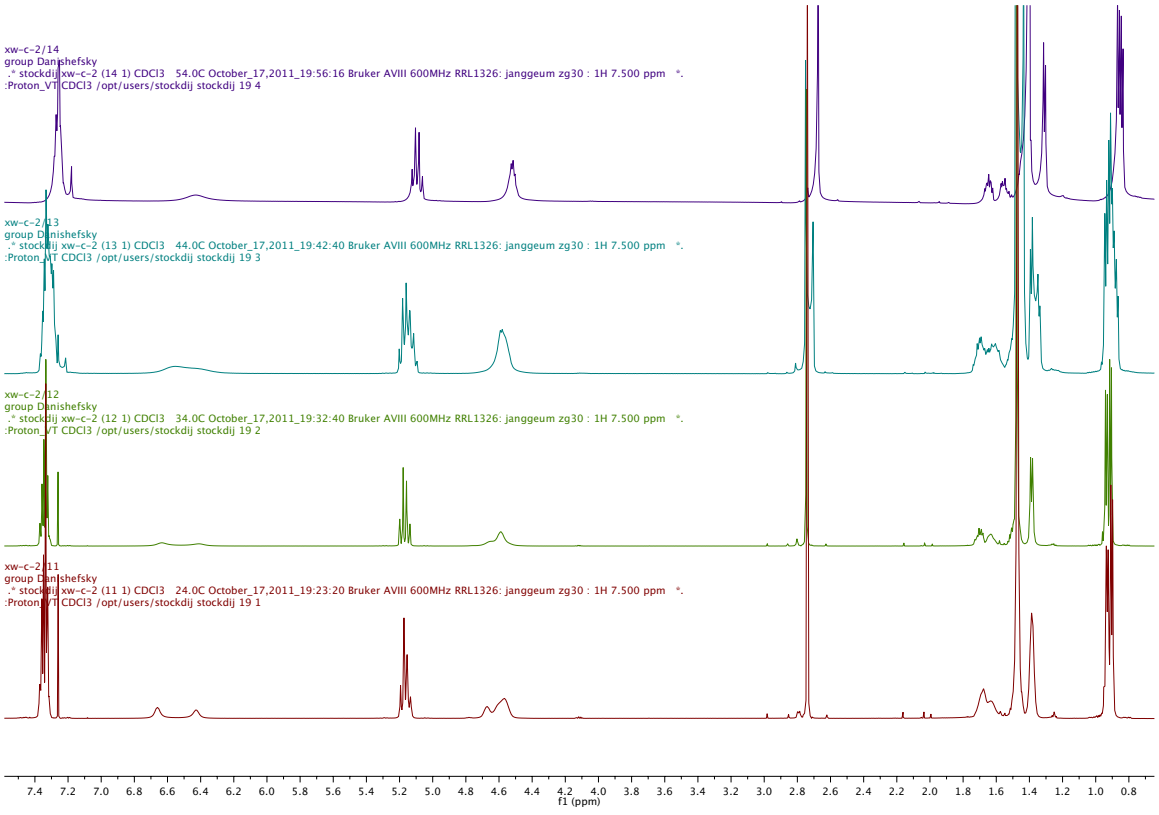
Variable Temperature ^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-SFm (19)** in $CDCl_3$ at 600 MHz.



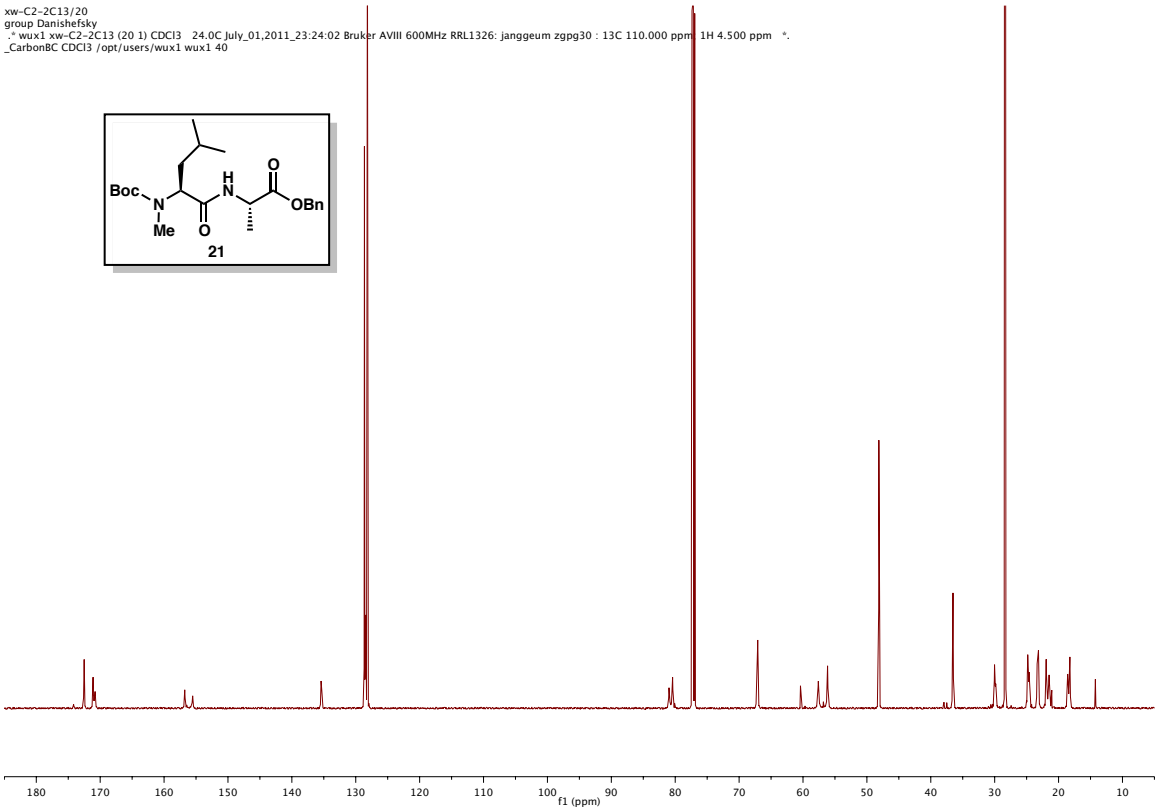
^{13}C NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-SFm (19)** in $CDCl_3$ at 151 MHz.



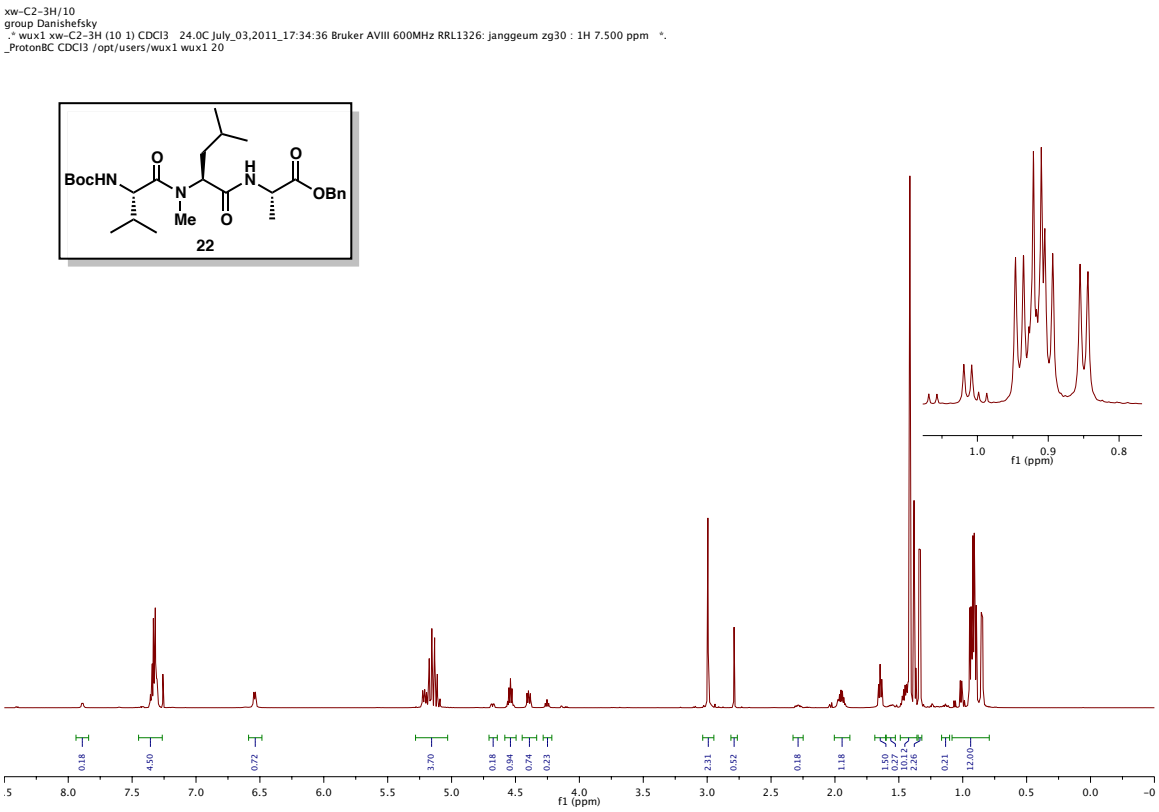
^{1}H NMR Spectrum of **Boc-MeLeu-Ala-OBn (21)** in $CDCl_3$ at 600 MHz.



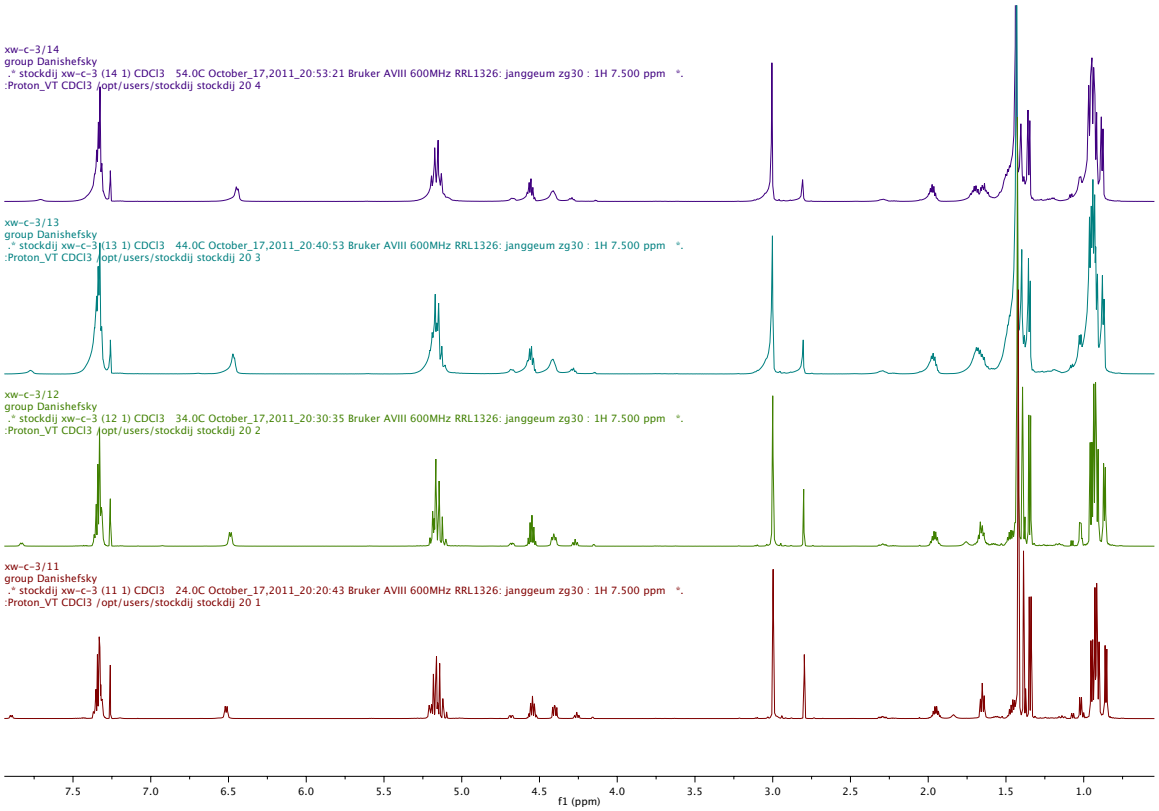
Variable Temperature ^{1}H NMR Spectrum of **Boc-MeLeu-Ala-OBn (21)** in $CDCl_3$ at 600 MHz.



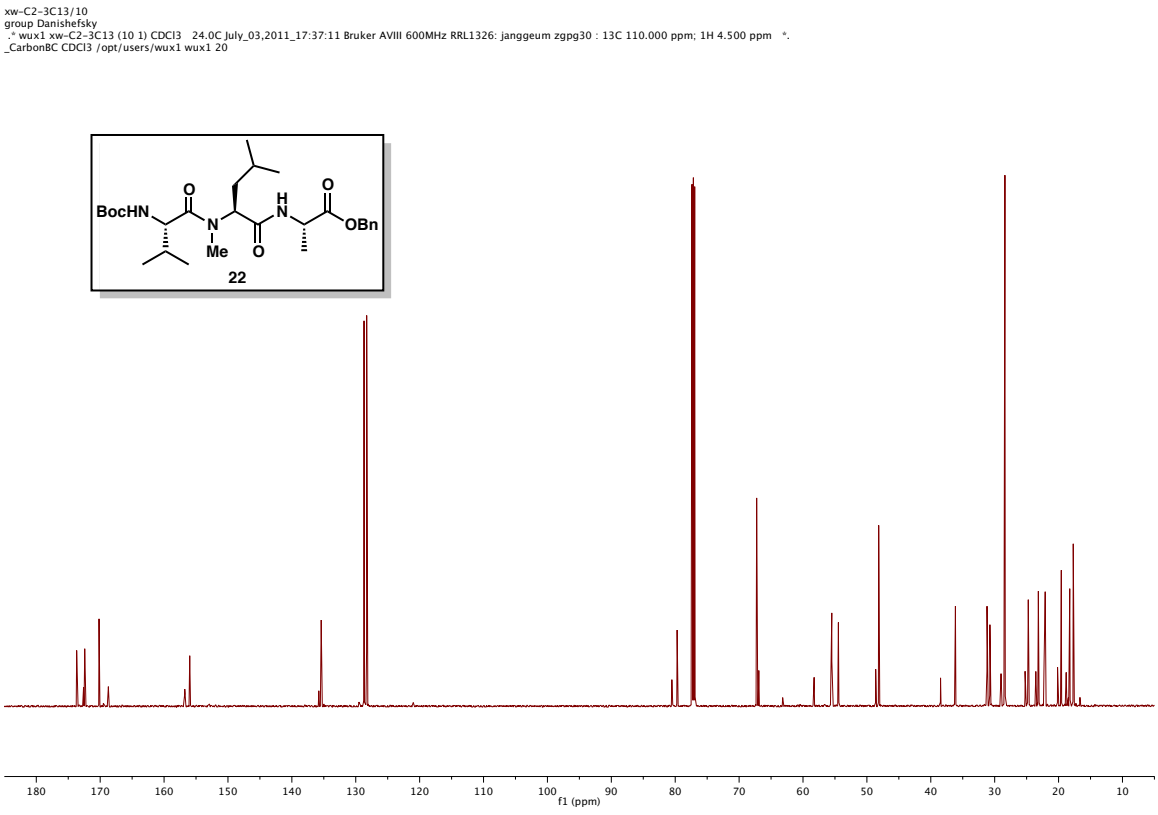
^{13}C NMR Spectrum of **Boc-MeLeu-Ala-OBn (21)** in $CDCl_3$ at 151 MHz.



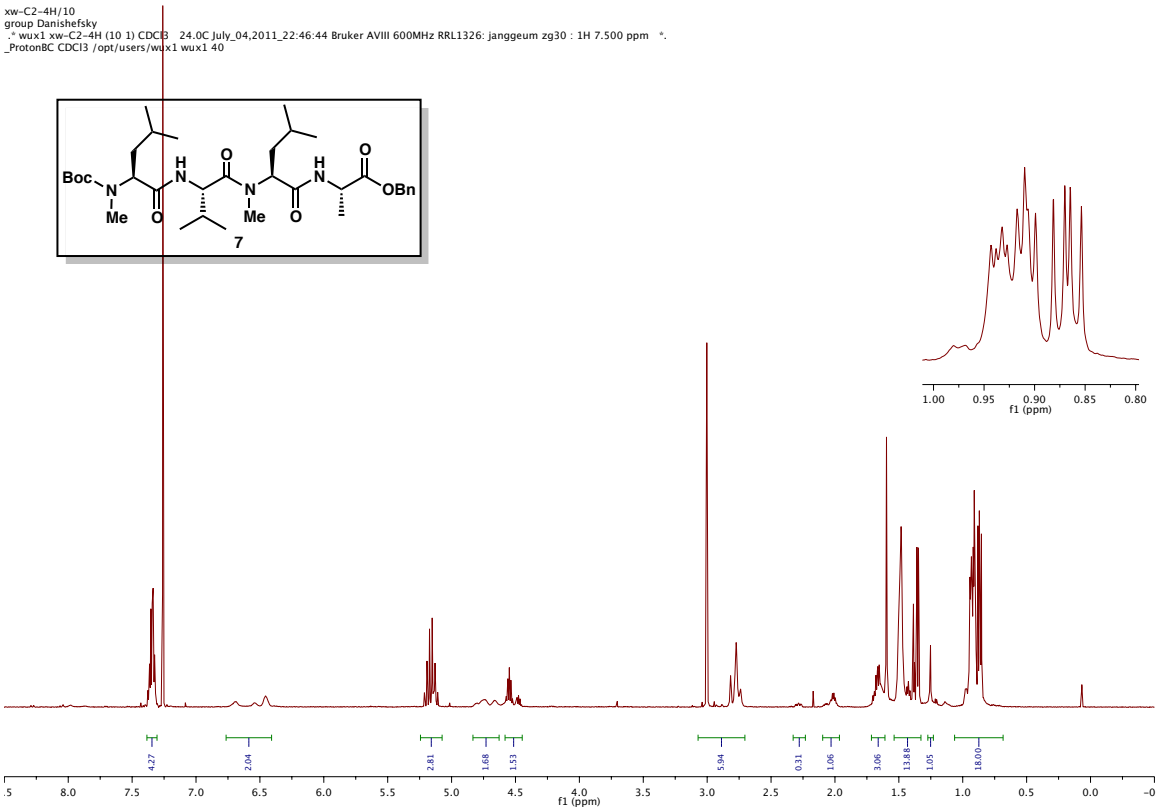
^{1}H NMR Spectrum of **Boc-Val-MeLeu-Ala-OBn (22)** in $CDCl_3$ at 600 MHz.



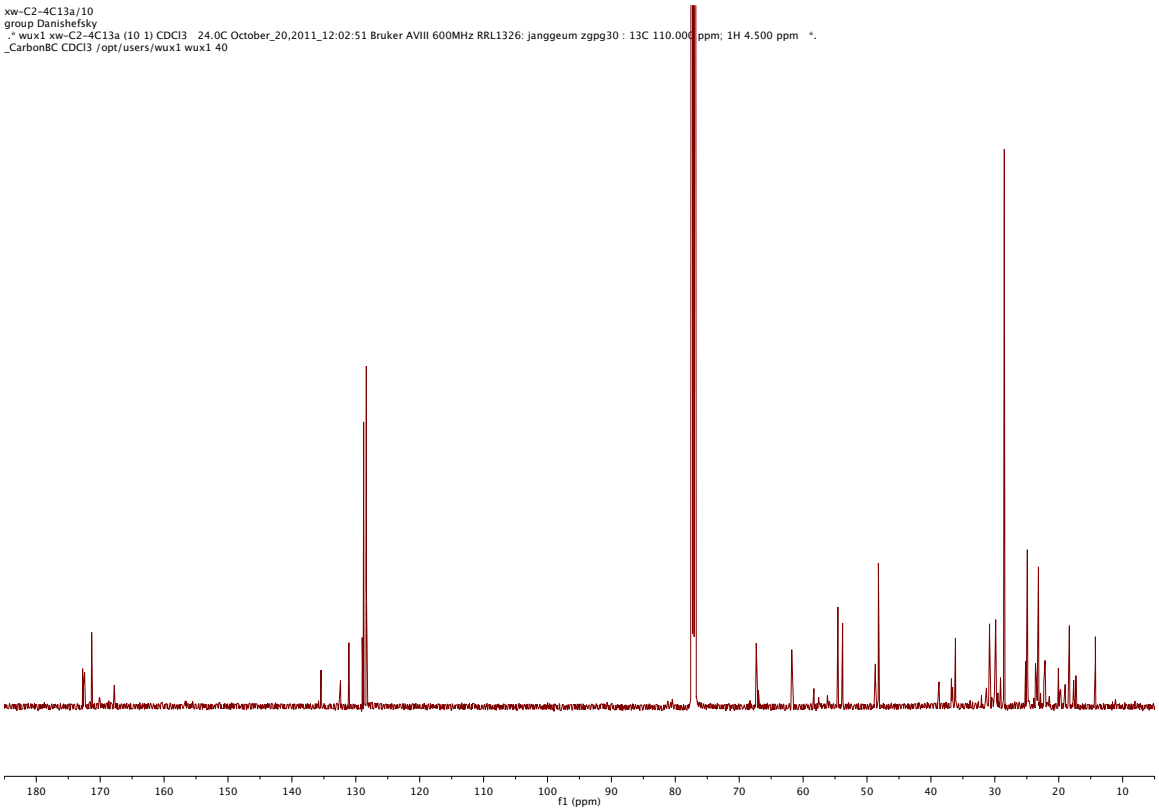
Variable Temperature ^{1}H NMR Spectrum of **Boc-Val-MeLeu-Ala-OBn (22)** in $CDCl_3$ at 600 MHz.



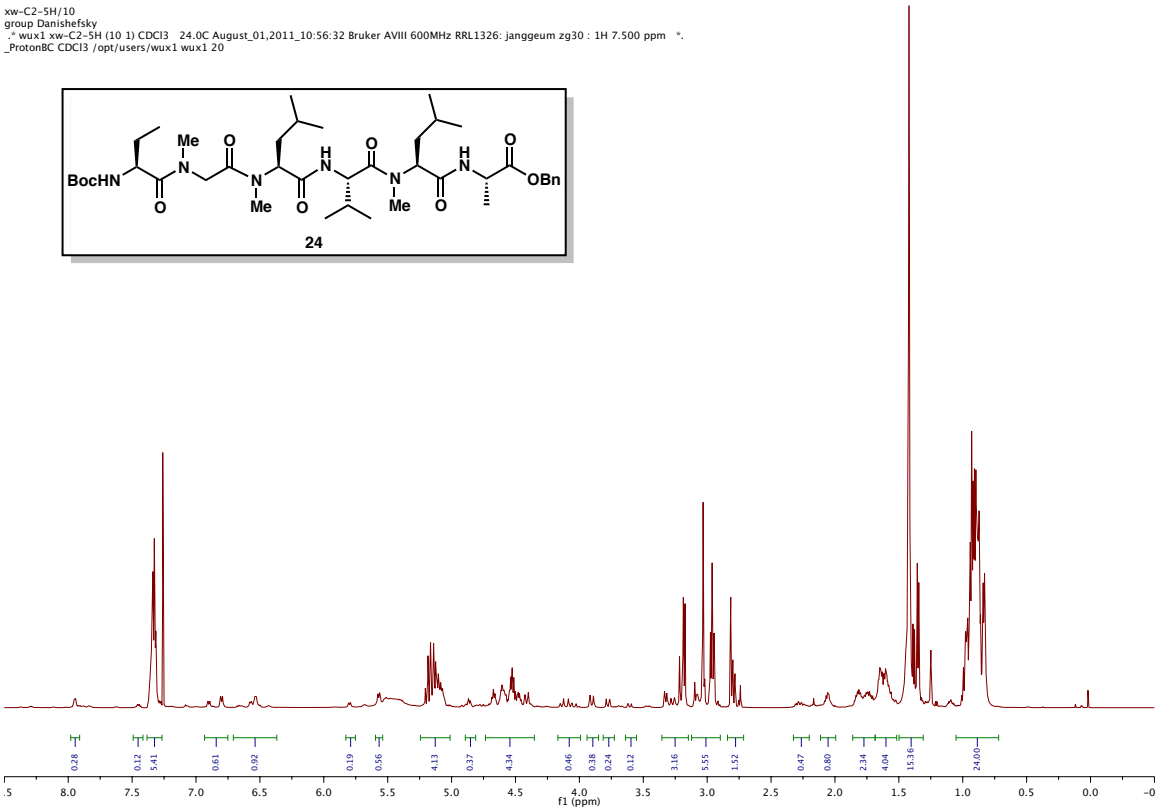
^{13}C NMR Spectrum of **Boc-Val-MeLeu-Ala-OBn (22)** in $CDCl_3$ at 151 MHz.



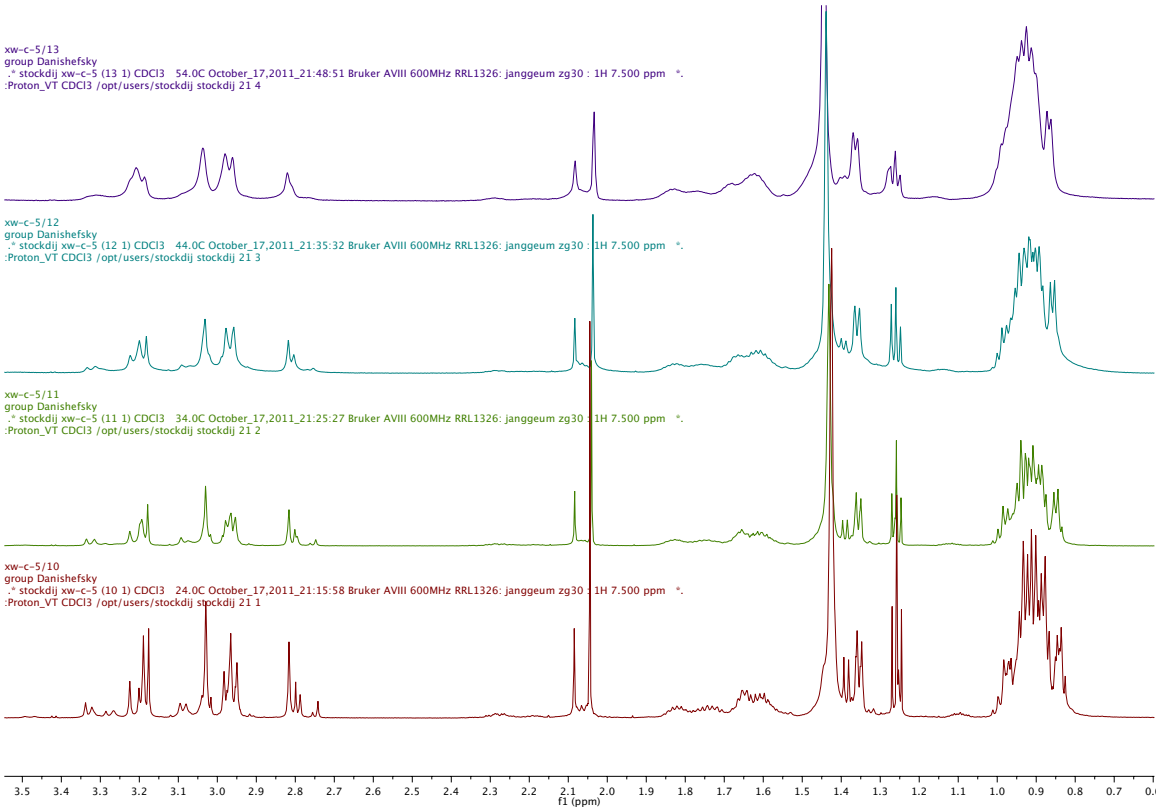
^{1}H NMR Spectrum of **Boc-MeLeu-Val-MeLeu-Ala-OBn (7)** in $CDCl_3$ at 600 MHz.



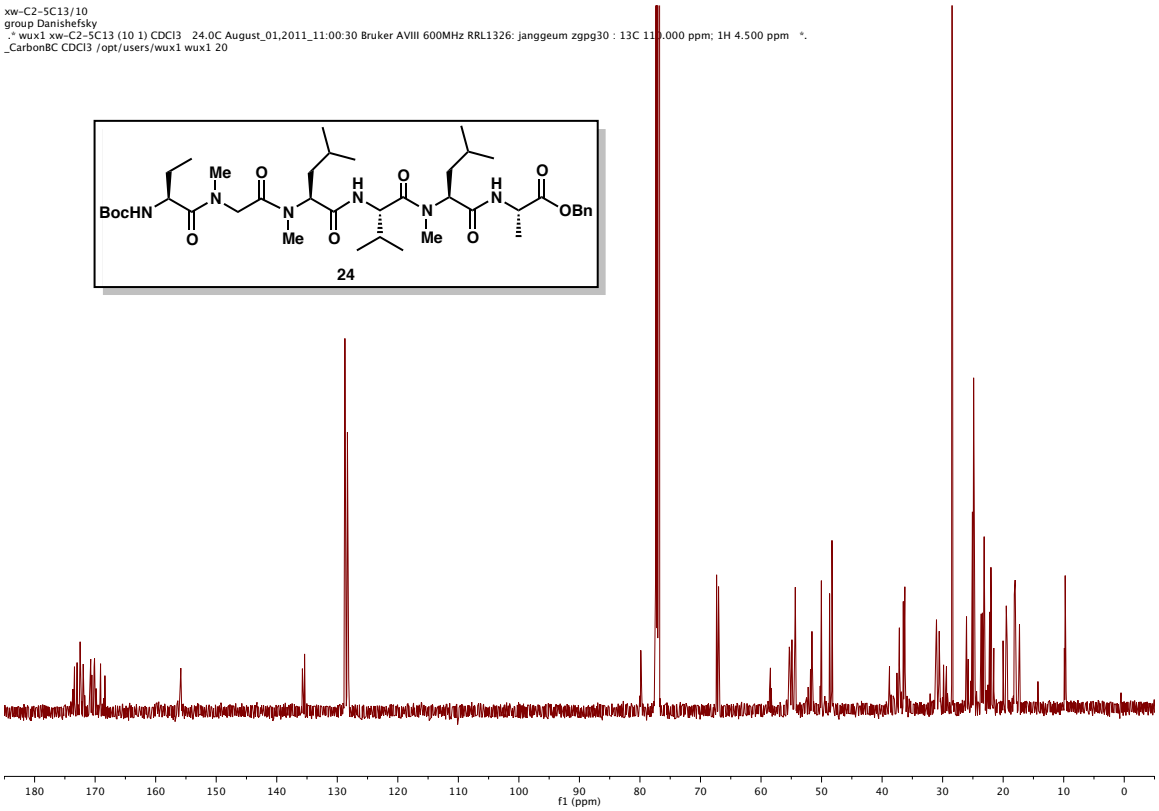
^{13}C NMR Spectrum of **Boc-MeLeu-Val-MeLeu-Ala-OBn (7)** in $CDCl_3$ at 151 MHz.



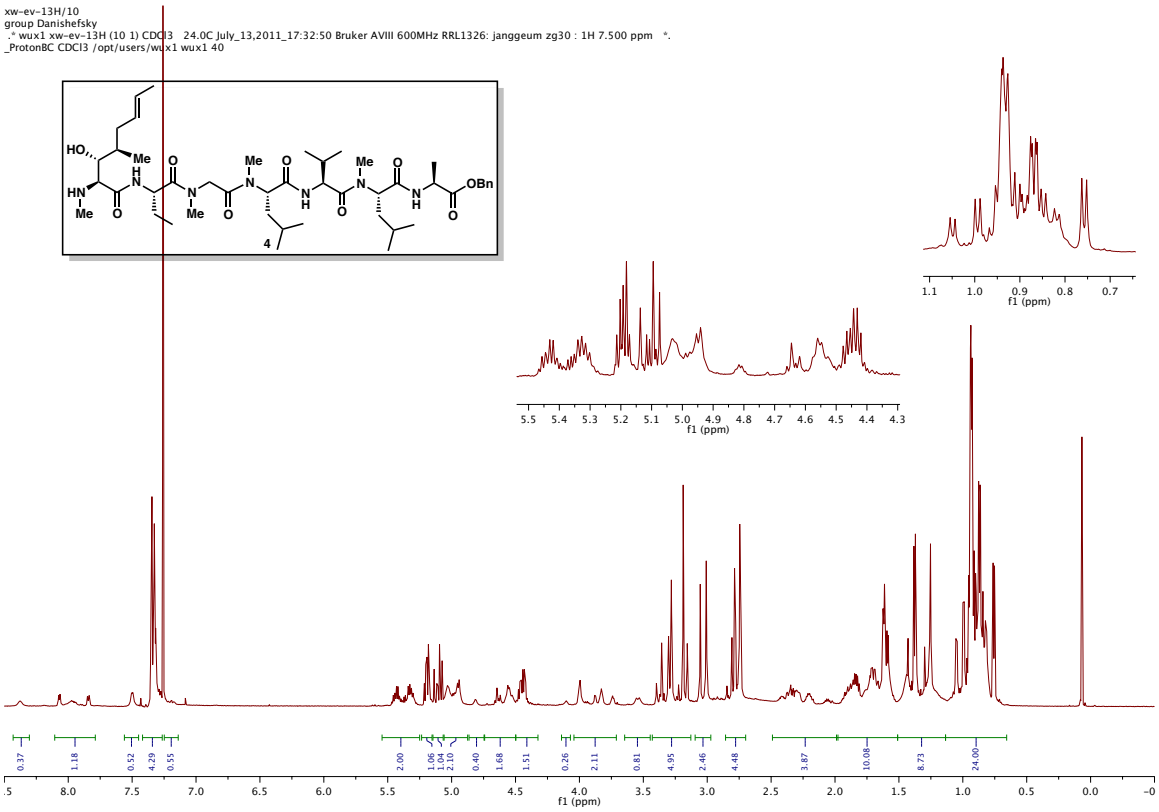
^{1}H NMR Spectrum of **Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (24)** in $CDCl_3$ at 600 MHz.



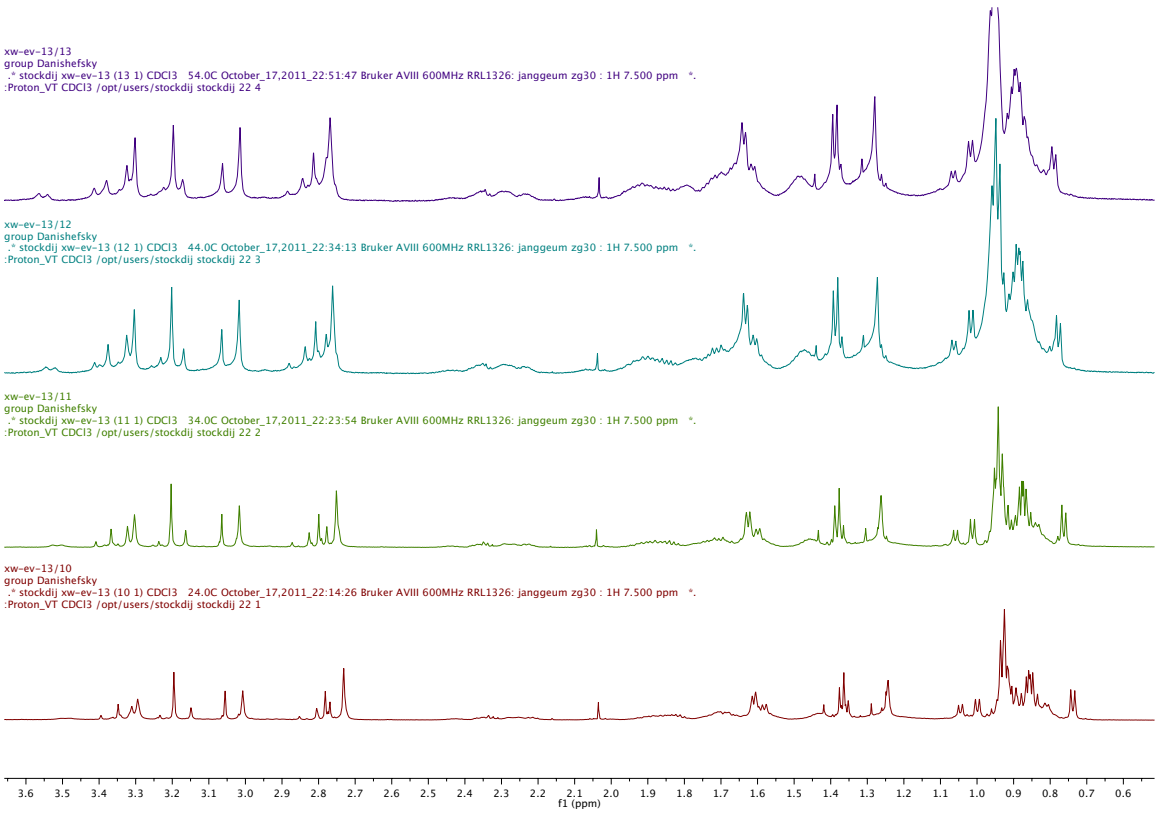
Variable Temperature ^{1}H NMR Spectrum of **Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (24)** in $CDCl_3$ at 600 MHz.



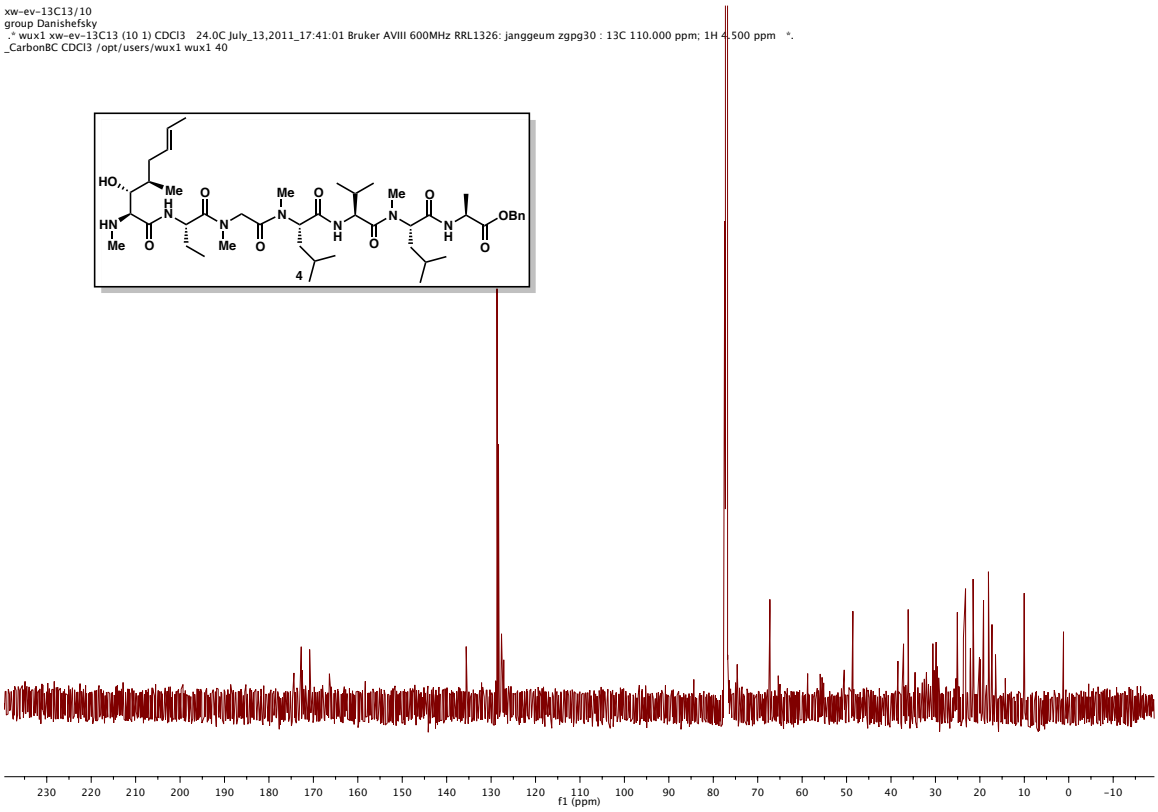
^{13}C NMR Spectrum of **Boc-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (24)** in $CDCl_3$ at 600 MHz.



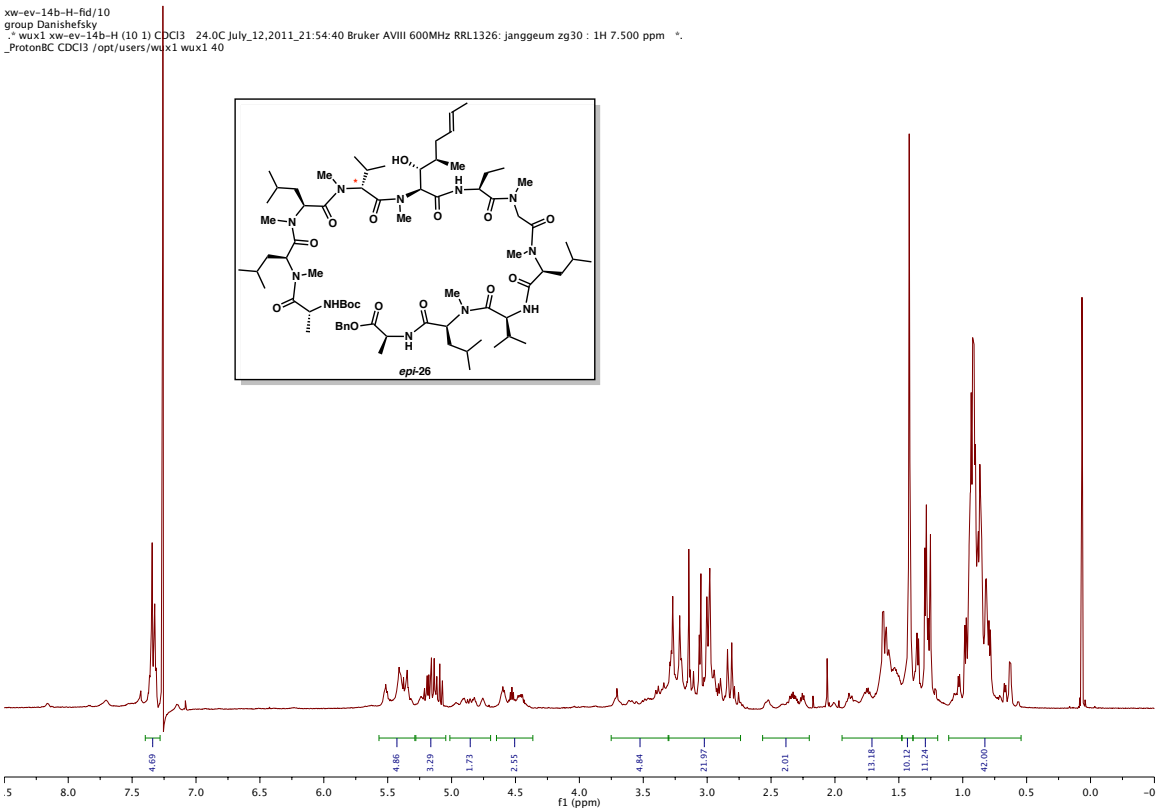
^{1}H NMR Spectrum of **H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (4)** in $CDCl_3$ at 600 MHz.



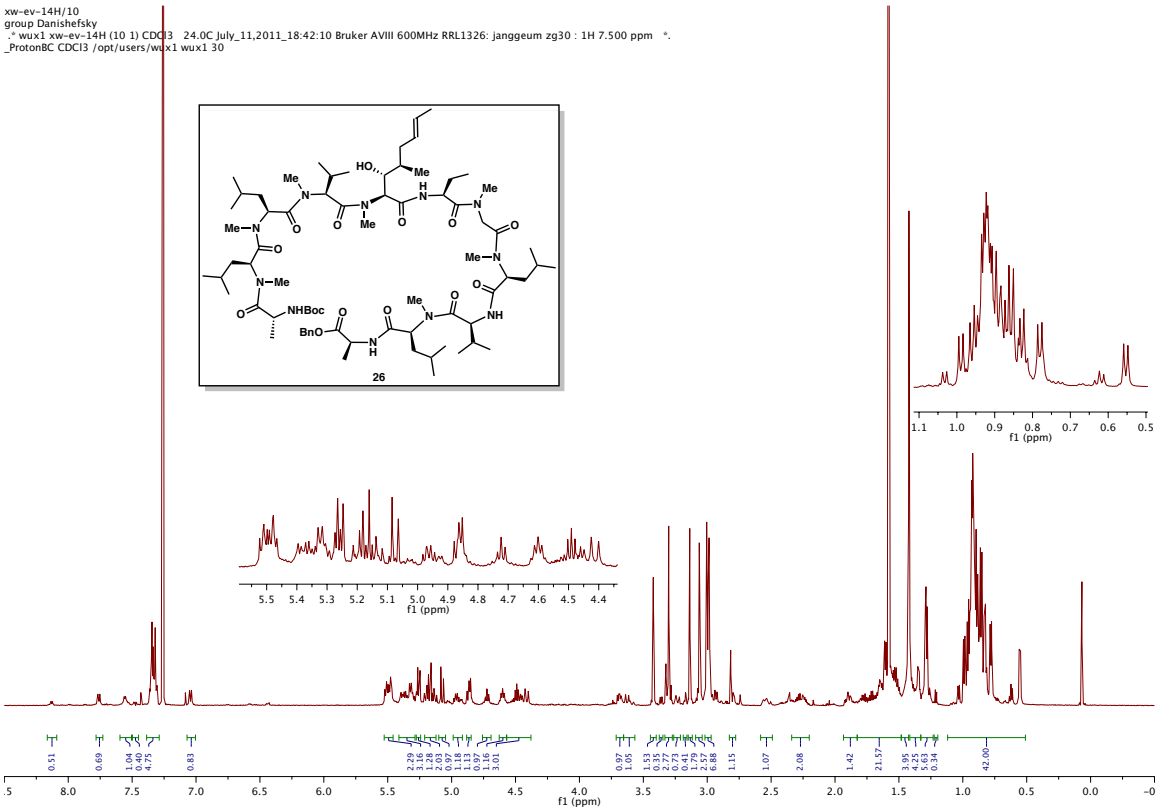
Variable Temperature ^{1}H NMR Spectrum of **H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (4)** in $CDCl_3$ at 600 MHz.



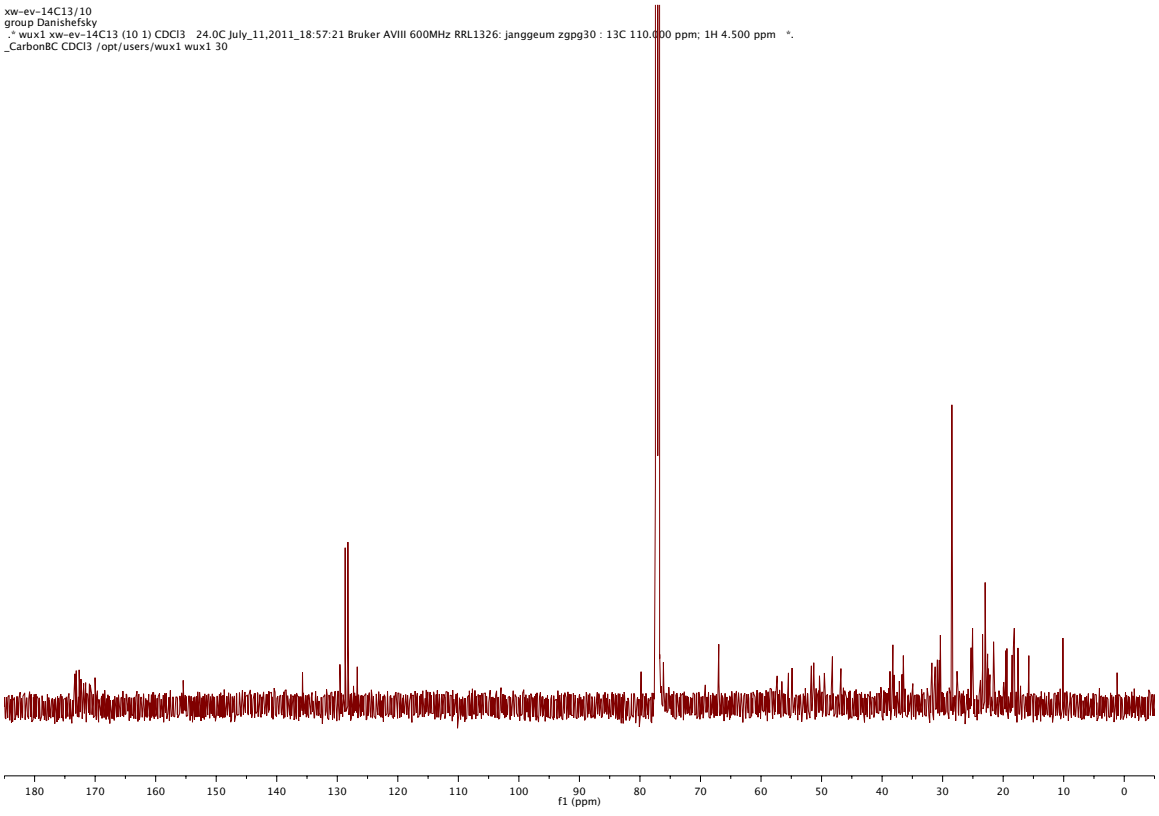
^{13}C NMR Spectrum of **H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (4)** in $CDCl_3$ at 151 MHz (dilute).



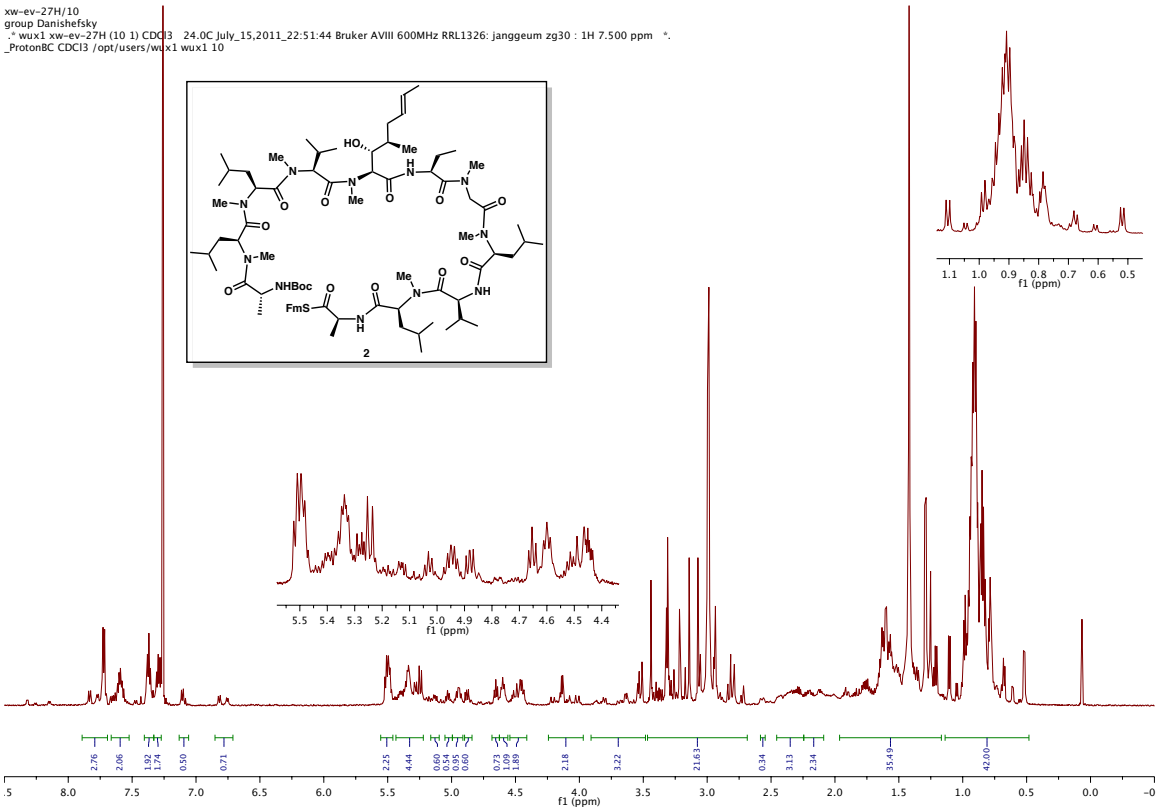
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (*epi*-26)** in $CDCl_3$ at 600 MHz.



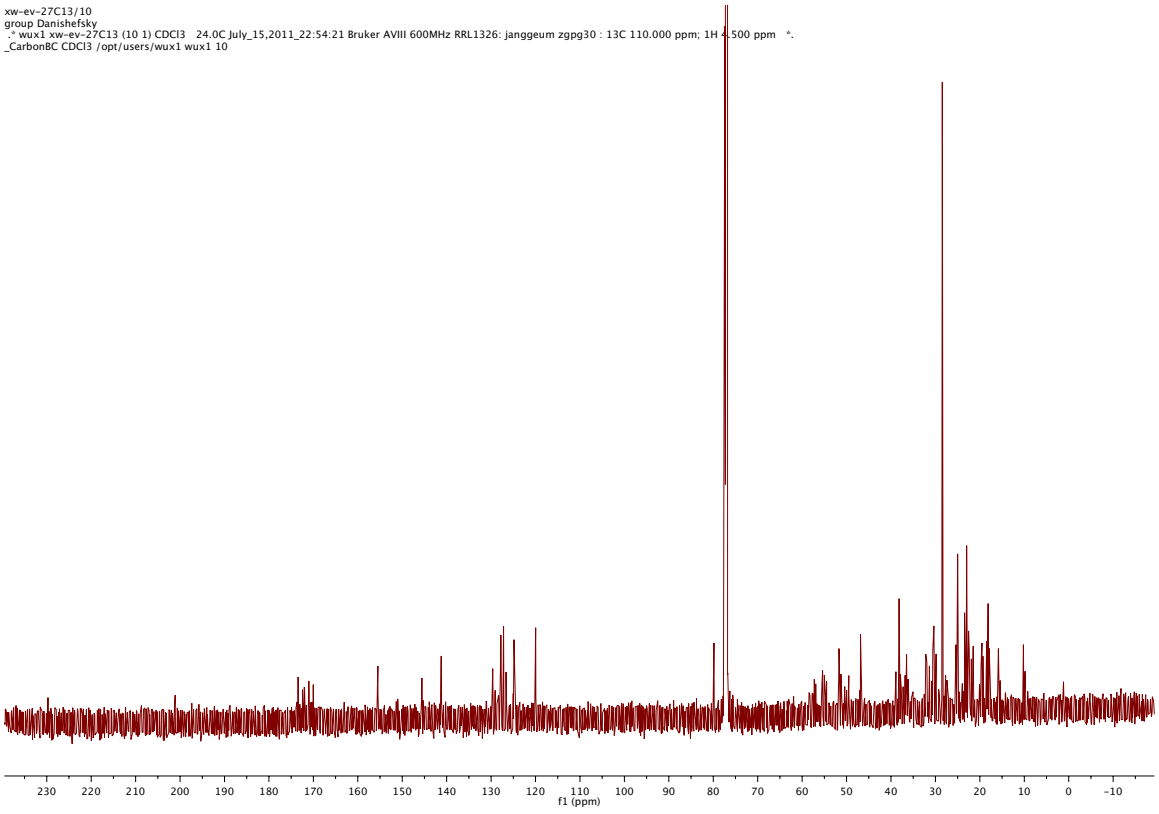
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (26)** in $CDCl_3$ at 600 MHz.



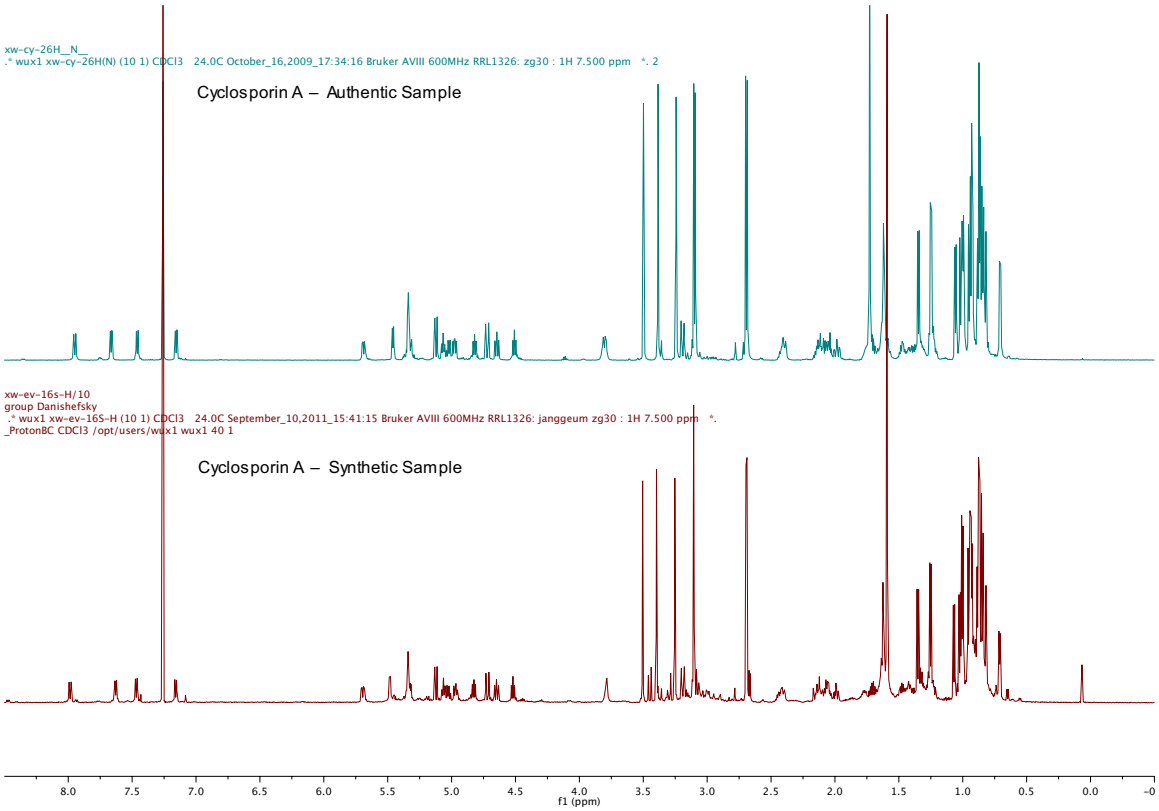
^{13}C NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBn (26)** in $CDCl_3$ at 600 MHz (dilute).



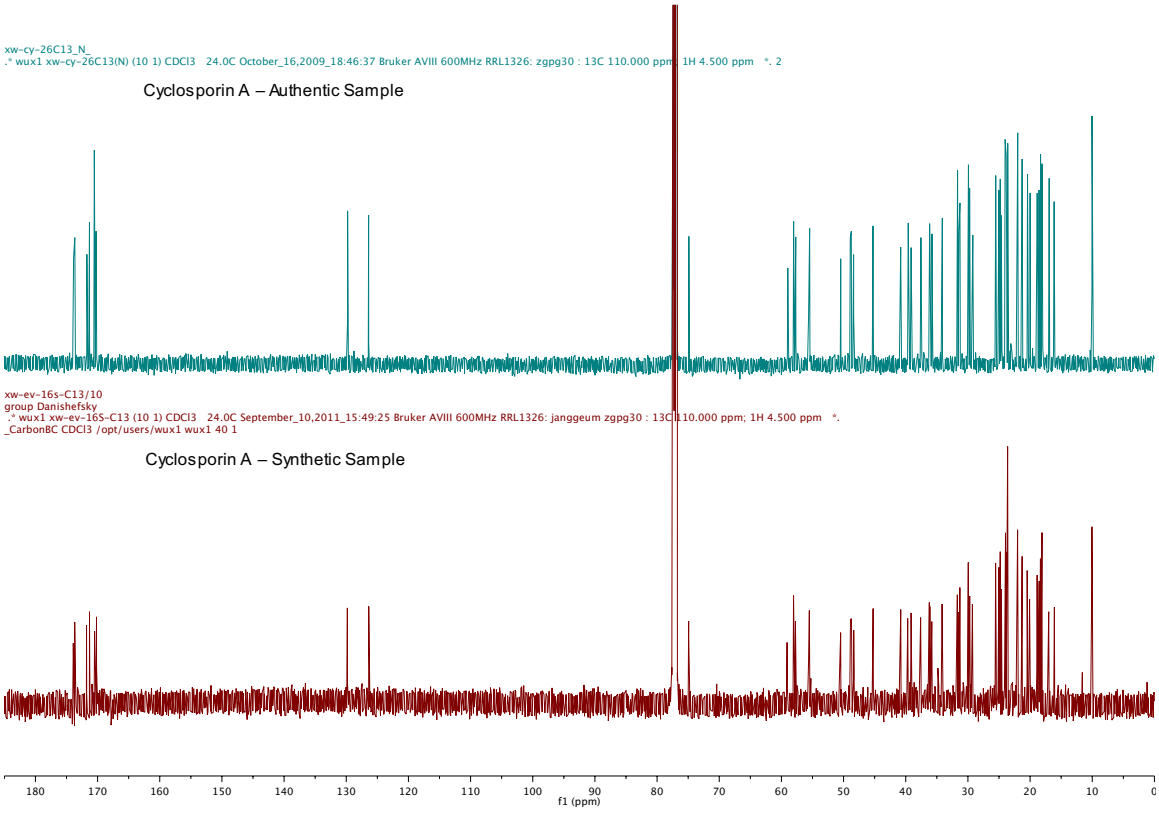
^{1}H NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-SFm (2)** in $CDCl_3$ at 600 MHz.



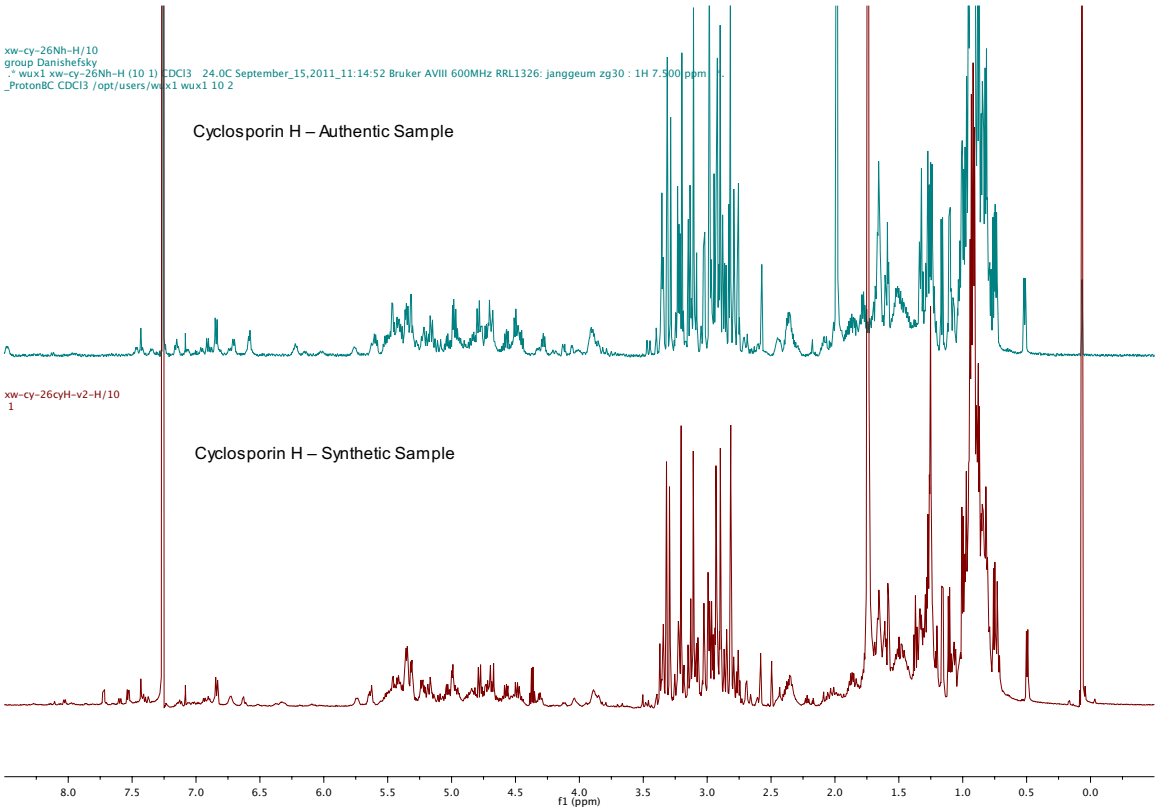
^{13}C NMR Spectrum of **Boc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-SFm (2)** in $CDCl_3$ at 151 MHz.



^{1}H NMR Spectrum of synthetic (bottom) and natural (top) **Cyclosporin A (1)** in $CDCl_3$ at 600 MHz.



^{13}C NMR Spectrum of synthetic (bottom) and natural (top) **Cyclosporin A (1)** in $CDCl_3$ at 151 MHz.



^{1}H NMR Spectrum of synthetic (bottom) and natural (top) **Cyclosporin H (28)** in $CDCl_3$ at 600 MHz.