CHEMISTRY
A EUROPEAN JOURNAL

Supporting Information

Design, Synthesis, and Biological Properties of Highly Potent Tubulysin D Analogues

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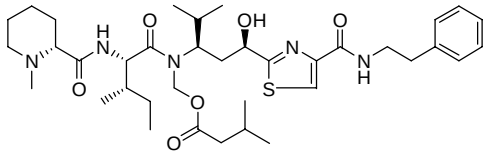
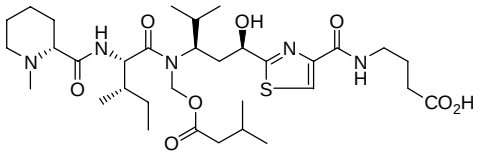
General Methods. Compounds **9**, **12**, **14**, **17**, **20**, and **22** were prepared as previously described.[1] Unless otherwise noted, all starting materials were obtained from commercial suppliers and used without further purification. Methyl iodide was filtered through a plug of basic alumina (Brockman activity 1) immediately prior to use. Toluene, THF, ether, dioxane, and CH_2Cl_2 were dried over alumina under a nitrogen atmosphere. Methanol, *i*-Pr_2NH, *i*-Pr_2EtN, dichloroethane, and pyridine were distilled from CaH_2 immediately prior to use. Where noted, water and acetic acid were degassed using three consecutive freeze pump thaw cycles. Reactions were carried out in flame or oven-dried glassware under a N_2 atmosphere. Extracts were dried over Na_2SO_4. Products were concentrated using a Büchi rotary evaporator under reduced pressure. Chromatography was carried out either with Merck 60 Å 230-400 mesh silica gel or via HPFC purification on a Biotage SP1 instrument (Charlottesville, VA) equipped with a normal-phase Biotage Si flash column or reverse-phase Biotage C18 column. Where noted, water was removed from samples by lyophilization using a Labconco Corp. freeze-dry system (Kansas City, MO). Optical rotation measurements were performed on a Perkin-Elmer 241 polarimeter. Optical rotations ([α]) are measured in deg cm^3 g^{-1} dm^{-1}. Concentration (*c*) is measured in g dL^{-1}. IR spectra were recorded on a Nicolet Avatar 360 FTIR spectrometer equipped with an attenuated total reflectance accessory and only partial data are listed. ^1H NMR and ^{13}C NMR spectra were obtained at room temperature with Bruker AV-400 and DRX-500 spectrometers. Chemical shifts are expressed in ppm relative to internal solvent. High-resolution mass spectra were performed by the University of California at Berkeley Micro-Mass Facility.

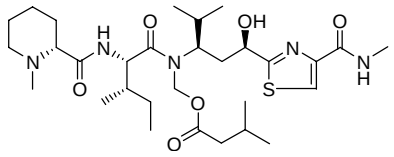


3-Methyl-butyric acid ({1-[2-hydroxy-2-(4-phenethylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-methyl ester (13a). Acid **12** (40.0 mg, 0.0670 mmol) was added to a solution of pentafluorophenol (19.0 mg, 0.101 mmol) and 1,3-diisopropylcarbodiimide (11.5 μL, 0.0737 mmol) in 0.51 mL CH_2Cl_2 at 0 °C. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated. EtOAc (10 mL) was added, and the crude product was filtered, with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated, and the crude material was used without further purification. DMF (0.270 mL, 0.25 M) was added to the crude product at 0 ºC, followed by phenethylamine (10.2

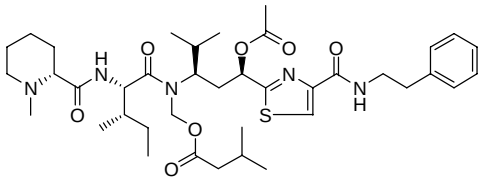
μL, 0.0804 mmol) and *i*-Pr_2EtN (23.0 μL, 0.134 mmol). The reaction mixture was allowed to warm to rt, stirred for 24 h, and concentrated. Normal-phase HPFC purification (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 23.0 mg (49%) of **13a**. $[\alpha]_D^{23}$ = +15.0 (*c* = 1.5, MeOH). IR: 1497, 1544, 1661, 1738, 2793, 2874, 2934, 2961, 3301, 3370 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.80-0.92 (m, 12H), 0.97 (d, 3H, *J* = 6.9 Hz), 1.00 (d, 3H, *J* = 6.7 Hz), 1.19-1.30 (m, 3H), 1.49-1.65 (m, 5H), 1.69-1.75 (m, 2H), 1.98-2.18 (m, 7H), 2.14 (s, 3H), 2.58 (app d, 1H, *J* = 10.4 Hz), 2.91 (m, 1H), 2.92 (app t, 2H, *J* = 7.5 Hz), 3.55-3.66 (m, 2H), 4.58 (d, 1H, *J* = 9.8 Hz), 4.75 (d, 1H, *J* = 10.2 Hz), 5.51 (d, 1H, *J* = 12.2 Hz), 6.17 (d, 1H, *J* = 12.2 Hz), 7.18-7.21 (m, 1H), 7.24-7.30 (m, 4H), 8.09 (s, 1H). ^{13}C NMR (125 MHz, MeOD): δ 10.7, 16.2, 20.7, 22.77, 22,81, 24.3, 25.96, 25.97, 26.1, 26.7, 31.5, 36.71, 36.73, 37.6, 38.8, 42.0, 44.4, 44.7, 55.4, 56.6, 69.5, 70.3, 124.7, 127.5, 129.6, 129.8, 140.3, 150.7, 163.5, 173.4, 175.5, 178.2, 179.1. HRMS (FAB) calcd for $C_{37}H_{58}N_5O_6S$ (*M*+H): 700.4108. Found: 700.4105.



3-Methyl-butyric acid ((1-{2-[4-(3-carboxy-propylcarbamoyl)-thiazol-2-yl]-2-hydroxy-ethyl}-2-methyl-propyl)-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-methyl ester (13b). Acid **12** (25.0 mg, 0.0419 mmol) was added to a solution of pentafluorophenol (12.0 mg, 0.0628 mmol) and 1,3-diisopropylcarbodiimide (7.22 μL, 0.0461 mmol) in 0.31 mL of CH_2Cl_2 at 0 °C. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated. EtOAc (10 mL) was added, and the crude product was filtered, with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated and the crude material was used without further purification. DMF (0.170 mL, 0.25 M) was added to the crude product at 0 ºC, followed by the 4-aminobutyric acid (5.18 mg, 0.0503 mmol) and *i*-Pr_2EtN (18.2 μL, 0.105 mmol). The reaction mixture was allowed to warm to rt, stirred for 24 h, and concentrated. Normal-phase HPFC purification (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 20.0 mg (70%) of **13b**. $[\alpha]_D^{23}$ = +10.3 (*c* = 1.0, MeOH). IR: 1451, 1550, 1643, 1658, 1736, 2795, 2872, 2925, 2961, 3365 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.81-0.85 (m, 3H), 0.88-0.91 (m, 9H), 0.97 (d, 3H, *J* = 7.0 Hz), 1.00 (d, 3H, *J* = 6.1 Hz), 1.19-1.26 (m, 1H), 1.27-1.34 (m, 1H), 1.53-1.66 (m, 5H), 1.70-1.77 (m, 2H), 1.91 (m, 3H), 1.99-2.06 (m, 4H), 2.13-2.19 (m, 3H), 2.19 (s, 3H), 2.28 (app t, 2H, *J* = 7.3 Hz), 2.70 (app d, 1H, *J* = 11.4 Hz), 2.96 (app d, 1H, *J* = 11.1 Hz), 3.42 (app t, 2H, *J* = 7.2 Hz), 4.59 (d, 1H, *J* = 9.2 Hz), 4.76 (d, 1H, *J* = 9.0 Hz), 5.51 (d, 1H, *J* = 11.9 Hz), 6.16 (d, 1H, *J* = 12.2 Hz), 8.08 (s, 1H). ^{13}C NMR (125 MHz, MeOD): δ 10.7, 16.2, 20.7, 22.77, 22.81, 24.1, 25.9, 26.7, 27.3, 31.4, 35.6, 37.6, 38.89, 38.90, 40.4, 43.0, 44.44, 44.48, 55.4, 56.6, 58.7, 69.9, 70.1, 124.5, 150.9, 163.6, 173.4, 174.92, 174.95, 174.96, 180.9. HRMS (FAB) calcd for $C_{33}H_{56}N_5O_8S$ (*M*+H): 682.3850. Found: 682.3861.

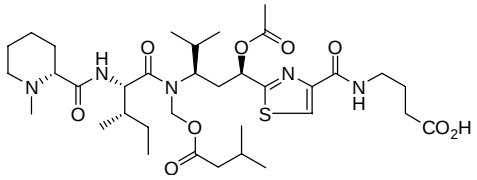


3-Methyl-butyric acid ({1-[2-hydroxy-2-(4-methylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-methyl ester (13c). Acid **12** (30.0 mg, 0.0503 mmol) was added to a solution of pentafluorophenol (14.0 mg, 0.0754 mmol) and 1,3-diisopropylcarbodiimide (8.70 μL, 0.0553 mmol) in 0.38 mL of CH_2Cl_2 at 0 °C. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated. EtOAc (10 mL) was added, and the crude product was filtered, with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated, and the crude material was used without further purification. DMF (0.201 mL, 0.25 M) was added to the crude product at 0 ºC, followed by the hydrochloride salt of methylamine (18.0 mg, 0.151 mmol) and *i*-Pr_2EtN (44.0 μL, 0.251 mmol). The reaction mixture was allowed to warm to rt, stirred for 24 h at rt, and concentrated. Normal-phase HPFC purification (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 31.0 mg (68%) of **13c**. $[\alpha]_D^{23}$ = +15.5 (*c* = 1.0, MeOH). IR: 1420, 1498, 1654, 1737, 2791, 2872, 2934, 2961, 3322, 3367 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.83 (d, 3H, *J* = 5.9 Hz), 0.90 (app t, 9H, *J* = 7.5 Hz), 0.98 (app t, 6H, *J* = 7.5 Hz), 1.20-1.30 (m, 3H), 1.49-1.63 (m, 5H), 1.69-1.72 (m, 2H), 1.98-2.06 (m, 5H), 2.13 (s, 3H), 2.14-2.17 (m, 2H), 2.55 (app d, 1H, *J* = 11.5 Hz), 2.88-2.91 (m, 1H), 2.93 (s, 3H), 4.59 (d, 1H, *J* = 9.9 Hz), 4.75 (d, 1H, *J* = 10.8 Hz), 5.51 (d, 1H, *J* = 12.1 Hz), 6.18 (d, 1H, *J* = 12.1 Hz), 8.08 (s, 1H). ^{13}C NMR (125 MHz, MeOD): δ 10.6, 16.2, 20.7, 22.7, 22.8, 24.3, 25.9, 26.1, 26.3, 26.6, 31.56, 31.64, 36.9, 37.6, 38.8, 44.4, 44.7, 55.3, 56.6, 69.5, 70.4, 124.4, 150.7, 164.2, 164.9, 173.4, 175.7, 179.1. HRMS (FAB) calcd for $C_{30}H_{52}N_5O_6S$ (*M*+H): 610.3638. Found: 610.3634.

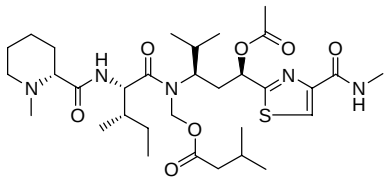


3-Methyl-butyric acid ({1-[2-acetoxy-2-(4-phenethylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-methyl ester (2). A 0.10 M solution of **13a** (15.5 mg, 0.0221 mmol) in pyridine (0.221 mL) was cooled to 0 °C, and acetic anhydride (10.0 μL, 0.111 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 24 h. The solvent was removed under reduced pressure. Column chromatography (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 16.4 mg (99%) of **2** as an amorphous solid. $[\alpha]_D^{23}$ = +50.0 (*c* = 0.4, MeOH). IR: 1229, 1370, 1426, 1455, 1497, 1544, 1667, 1742, 2848, 2874, 2934, 2961, 3306, 3383 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.79 (d, 3H, *J* = 6.3 Hz), 0.83 (d, 3H, *J* = 6.5 Hz), 0.86 (d, 3H, *J* = 6.6 Hz), 0.90 (app t, 3H, *J* = 7.3 Hz), 0.96 (d, 3H, *J* = 7.17 Hz), 1.05 (d, 3H, *J* = 6.4 Hz), 1.16-1.22 (m, 1H), 1.28-1.36 (m, 2H), 1.53-1.68 (m, 4H), 1.75-1.86 (m, 3H), 1.91-2.08 (m, 3H), 2.10-2.14 (m, 1H), 2.13 (s, 3H), 2.17-2.26 (m, 1H), 2.24 (s, 3H), 2.48 (app t, 1H, *J* = 13.1 Hz), 2.72 (app t, 1H, *J* =

10.8 Hz), 2.91 (app t, 2H, *J* = 7.5 Hz), 2.99 (app d, 1H, *J* = 11.7 Hz), 3.62 (m, 2H), 4.43 (br s, 1H), 4.60 (d, 1H, *J* = 9.3 Hz), 5.40 (d, 1H, *J* = 12.7 Hz), 5.85 (d, 1H, *J* = 11.3 Hz), 6.16 (d, 1H, *J* = 12.5 Hz), 7.18-7.21 (m, 1H), 7.25-7.30 (m, 4H), 8.17 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 10.7, 16.4, 20.3, 20.7, 20.8, 22.71, 22.73, 24.0, 25.6, 25.8, 26.7, 31.4, 32.2, 35.7, 36.7, 37.3, 42.0, 44.2, 44.4, 55.1, 56.5, 70.0, 70.6, 125.7, 127.5, 129.6, 129.9, 140.3, 150.7, 163.1, 170.6, 171.9, 173.2, 174.6, 176.5. HRMS (FAB) calcd for $C_{39}H_{60}N_5O_7S$ (*M*+H): 742.4213. Found: 742.4206.

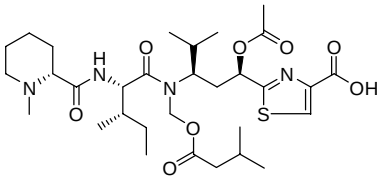


3-Methyl-butyric acid ((1-{2-acetoxy-2-[4-(3-carboxy-propylcarbamoyl)-thiazol-2-yl]-ethyl}-2-methyl-propyl)-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-methyl ester (3). A 0.10 M solution of **13b** (15.0 mg, 0.0220 mmol) in pyridine (0.220 mL) was cooled to 0 °C, and acetic anhydride (10.4 μL, 0.110 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 24 h. The reaction mixture was then cooled to 0 °C, and a 1:1 mixture of dioxane/water (0.630 mL) was added. The mixture was allowed to warm to rt and was stirred for 12 h at rt. The solvent was removed under reduced pressure. Column chromatography (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 13.0 mg (81%) of **3** as an amorphous solid. $[\alpha]_D^{23}$ = +24.3 (*c* = 0.7, MeOH). IR: 1371, 1420, 1497, 1544, 1667, 1741, 2342, 2360, 2840, 2872, 2929, 2961, 3293, 3368 cm^{-1}. ^{1}H NMR (500 MHz, MeOD): δ 0.79 (d, 3H, *J* = 6.7 Hz), 0.85 (d, 3H, *J* = 6.9 Hz), 0.87-0.92 (m, 6H), 0.96 (d, 3H, *J* = 6.6 Hz), 1.05 (d, 3H, *J* = 6.3 Hz), 1.15-1.23 (m, 1H), 1.35-1.42 (m, 1H), 1.57-1.65 (m, 3H), 1.69-1.72 (m, 1H), 1.77-1.81 (m, 1H), 1.84-1.93 (m, 4H), 1.95-2.01 (m, 2H), 2.08-2.18 (m, 2H), 2.13 (s, 3H), 2.28-2.39 (m, 4H), 2.33 (s, 3H), 2.48-2.53 (m, 1H), 2.94 (app d, 1H, *J* = 10.1 Hz), 3.08 (app d, 1H, *J* = 11.3 Hz), 3.40-3.43 (m, 2H), 4.41 (br s, 1H), 4.60 (d, 1H, *J* = 9.5 Hz), 5.40 (d, 1H, *J* = 12.4 Hz), 5.87 (d, 1H, *J* = 10.8 Hz), 6.13 (d, 1H, *J* = 10.8 Hz), 8.18 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 10.7, 16.4, 20.3, 20.70, 20.76, 22.73, 22.74, 23.7, 25.54, 22.56, 26.7, 26.9, 31.1, 32.2, 34.7, 35.9, 37.4, 40.2, 44.18, 44.25, 55.2, 56.5, 69.7, 70.7, 125.6, 150.8, 163.2, 170.7, 171.9, 173.3, 173.8, 176.4, 180.5. HRMS (FAB) calcd for $C_{35}H_{58}N_5O_9S$ (*M*+H): 724.3955. Found: 724.3937.

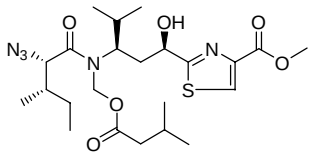


3-Methyl-butyric acid ({1-[2-acetoxy-2-(4-methylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-methyl ester (4). A 0.10 M solution of **13c** (10.5 mg, 0.0172 mmol) in pyridine

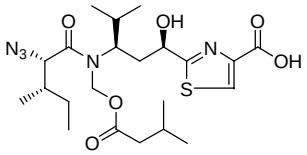
(0.172 mL) was cooled to 0 °C, and acetic anhydride (8.10 μL, 0.0861 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 24 h. The solvent was removed under reduced pressure. Column chromatography (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 10.1 mg (90%) of **4** as an amorphous solid. $[\alpha]_D^{23}$ = +60.5 (*c* = 0.6, MeOH). IR: 1229, 1371, 1420, 1466, 1499, 1549, 1665, 1742, 2792, 2848, 2875, 2934, 2961, 3305, 3380 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.78 (d, 3H, *J* = 7.0 Hz), 0.84 (d, 3H, *J* = 6.7 Hz), 0.87-0.91 (m, 6H), 0.96 (d, 3H, *J* = 6.7 Hz), 1.04 (d, 3H, *J* = 6.3 Hz), 1.25-1.15 (m, 1H), 1.24-132 (m, 1H), 1.48-1.65 (m, 4H), 1.72-1.75 (m, 2H), 1.79-1.86 (m, 1H), 1.93-2.18 (m, 5H), 2.13 (s, 3H), 2.15 (s, 3H), 2.22-2.29 (m, 1H), 2.47-2.57 (m, 2H), 2.89-2.94 (m, 1H), 2.92 (s, 3H), 4.43 (br s, 1H), 4.60 (d, 1H, *J* = 10.1 Hz), 5.39 (d, 1H, *J* = 12.2 Hz), 5.87 (d, 1H, *J* = 12.0 Hz), 6.18 (d, 1H, *J* = 12.5 Hz), 8.16 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 10.7, 16.4, 20.3, 20.69, 20.74, 22.68, 22.69, 24.3, 25.6, 26.1, 26.3, 26.6, 31.5, 32.2, 35.9, 37.3, 44.2, 44.7, 55.0, 56.6, 70.4, 70.7, 125.5, 150.7, 163.8, 170.8, 171.9, 173.3, 175.5, 176.7. HRMS (FAB) calcd for $C_{32}H_{54}N_5O_7S$ (*M*+H): 652.3744. Found: 652.3719.



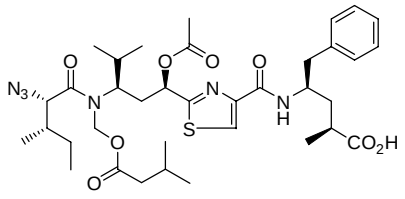
2-[1-Acetoxy-4-methyl-3-((3-methyl-butyryloxymethyl)-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazole-4-carboxylic acid (5). A 0.10 M solution of **12** (25.0 mg, 0.0419 mmol) in pyridine (0.420 mL) was cooled to 0 °C, and acetic anhydride (19.8 μL, 0.209 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 24 h. The reaction mixture was then cooled to 0 °C, and a 1:1 mixture of dioxane/water (1.50 mL) was added. The mixture was allowed to warm to rt and was stirred for 12 h at rt. The solvent was removed under reduced pressure. Column chromatography (100:0 to 90:10 CH_2Cl_2:MeOH) afforded 26.0 mg (97%) of **5** as an amorphous solid. $[\alpha]_D^{23}$ = +12.0 (*c* = 2.6, MeOH). IR: 1371, 1422, 1471, 1499, 1597, 1666, 1743, 2874, 2934, 2962, 3384 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.82-0.84 (m, 3H), 0.88-0.92 (m, 9H), 0.96 (d, 3H, *J* = 6.7 Hz), 1.01 (d, 3H, *J* = 6.3 Hz), 1.15-1.22 (m 1H), 1.52-1.70 (m, 4H), 1.75-1.82 (m, 2H), 1.93-2.05 (m, 4H), 2.12 (s, 3H), 2.20-2.23 (m, 4H), 2.39-2.55 (m, 6H), 3.15-3.22 (m, 1H), 4.64 (d, 1H, *J* = 9.6 Hz), 5.39 (d, 1H, *J* = 12.1 Hz), 5.83 (d, 1H, *J* = 11.7 Hz), 5.98 (br s 1H), 8.02 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 10.9, 16.3, 20.6, 20.8, 20.9, 22.8, 23.2, 25.1, 25.4, 26.6, 30.9, 31.8, 36.1, 37.5, 44.0, 44.1, 54.8, 55.5, 56.3, 69.1, 71.0, 125.3, 155.1, 169.0, 171.8, 172.0, 173.5, 175.7, 178.4. HRMS (FAB) calcd for $C_{31}H_{51}N_4O_8S$ (*M*+H): 639.3428. Found: 639.3439.



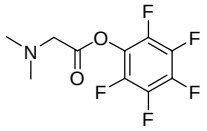
2-{3-[(2-Azido-3-methyl-pentanoyl)-(3-methyl-butyryloxymethyl)-amino]-1-hydroxy-4-methyl-pentyl}-thiazole-4-carboxylic acid methyl ester (15). A 0.02 M solution of **14** (475 mg, 0.759 mmol) in deoxygenated AcOH/H_2O/THF (38.0 mL, 3:1:1, v/v/v) was stirred at rt for 27 h. Addition of 400 mL of toluene followed by concentration and normal-phase HPFC purification (95:5 to 60:40 hexanes:EtOAc) afforded 283 mg (73%) of **15** as an amorphous solid. $[\alpha]_D^{23}$ = +55.1 (*c* = 1.0, MeOH). IR: 1095, 1212, 1652, 1735, 2099, 2963 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.88-0.99 (m, 15H), 1.02 (d, 3H, *J* = 6.5 Hz), 1.25-1.35 (m, 1H), 1.72-1.79 (m, 1H), 1.80-1.90 (m, 1H), 1.98-2.09 (m, 2H), 2.10-2.25 (m, 2H), 2.34 (d, 2H, *J* = 7.0 Hz), 3.74 (d, 1H, *J* = 9.5 Hz), 3.89 (s, 3H), 4.47-4.70 (br s, 1H), 4.79 (d, 1H, *J* = 10.5 Hz), 5.48 (d, 1H, *J* = 12.5 Hz), 5.58 (d, 1H, *J* = 10.5 Hz), 8.30 (s, 1H). ^{13}C NMR (125 MHz, MeOD): δ 11.0, 16.1, 20.4, 20.8, 22.88, 22.91, 26.1, 26.7, 32.0, 36.7, 39.3, 44.2, 52.8, 64.6, 69.6, 129.3, 147.7, 163.3, 173.6, 173.7, 180.2. HRMS (FAB) calcd for $C_{23}H_{37}N_5O_6SNa$ (*M*+Na): 534.2362. Found: 534.2367.



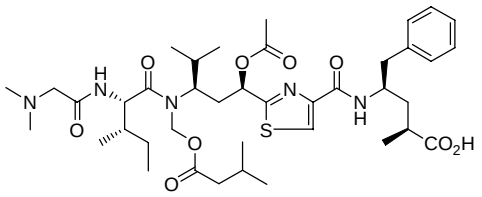
2-{3-[(2-Azido-3-methyl-pentanoyl)-(3-methyl-butyryloxymethyl)-amino]-1-hydroxy-4-methyl-pentyl}-thiazole-4-carboxylic acid (16). Me_3SnOH (736 mg, 4.07 mmol) was added to a 0.020 M solution of methyl ester **15** (260 mg, 0.509 mmol) in dichloroethane (25.0 mL). The reaction mixture was heated to 55 °C for 22 h and then concentrated. Normal-phase HPFC (100:0 to 90:10:1 CH_2Cl_2:MeOH:AcOH) followed by reverse-phase HPFC (20:80 to 100:0 MeCN:H_2O) and lyophilization afforded 90.0 mg (36%) of **16** as an amorphous solid. $[\alpha]_D^{23}$ = +51.5 (*c* = 1.0, MeOH). IR: 1088, 1217, 1651, 1735, 2100, 2964 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.88-0.94 (m, 9H), 0.97 (app t, 6H, *J* = 6.8), 1.03 (d, 3H, *J* = 6.5), 1.31 (sept, 1H, *J* = 7.4), 1.72-1.81 (m, 1H), 1.82-1.90 (br s, 1H), 2.00-2.09 (m, 2H), 2.10-2.17 (m, 1H), 2.18-2.28 (m, 1H), 2.31 (app t, 2H, *J* = 7.5), 3.75 (d, 1H, *J* = 9.5), 4.44-4.68 (br s, 1H), 4.77 (d, 1H, *J* = 9.5), 5.48 (d, 1H, *J* = 12.5), 5.58 (d, 1H, *J* = 12.5), 8.24 (s, 1H). ^{13}C NMR (125 MHz, MeOD): δ 10.8, 16.0, 20.3, 20.6, 22.71, 22.73, 26.0, 26.5, 32.0, 36.7, 39.1, 44.1, 64.6, 69.5, 128.5, 149.2, 164.7, 173.58, 173.61, 179.5. HRMS (FAB) calcd for $C_{22}H_{35}N_5O_6SNa$ (*M*+Na): 520.2206. Found: 520.2200.



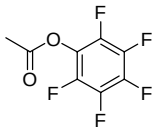
4-[(2-{1-Acetoxy-3-[(2-azido-3-methyl-pentanoyl)-(3-methyl-butyryloxymethyl)-amino]-4-methyl-pentyl}-thiazole-4-carbonyl)-amino]-2-methyl-5-phenyl-pentanoic acid (8). Acid **16** (39.0 mg, 0.0784 mmol) was added to a 0.070 M solution of pentafluorophenol (2.0 mg, 0.118 mmol) and 1,3-diisopropylcarbodiimide (13.4 μL, 0.0862 mmol) in CH_2Cl_2 at 0 °C. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated. EtOAc (10 mL) was added, and the crude product was filtered with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated, and the crude material was used without further purification. DMF (1.00 mL, 0.080 M) was added to the crude product at 0 ºC, followed by **17** (57.0 mg, 0.235 mmol) and *i*-Pr_2EtN (68.0 μL, 0.392 mmol). The reaction mixture was allowed to warm to rt, stirred for 24 h at rt, and concentrated. Normal-phase HPFC purification (100:0 to 95:5 EtOAc:MeOH) afforded 32.0 mg of product containing trace amounts of *i*-Pr_2EtN. The product mixture (0.181 mmol) was dissolved in pyridine (1.80 mL), cooled to 0 °C, and acetic anhydride (0.140 mL, 1.45 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 22 h. The reaction mixture was then cooled to 0 °C, and a 1:1 mixture of deoxygenated H_2O/dioxane (0.5 mL) was added. The mixture was allowed to warm to rt and was stirred for 14 h at rt. The solvent was removed under reduced pressure. Reverse-phase HPFC (20:80 to 100:0 MeCN:H_2O) followed by lyophilization afforded 51.0 mg (39%, over three steps) of **8** as an amorphous solid. $[\alpha]_D^{23}$ = +61.4 (*c* = 1, MeOH). IR: 1218, 1669, 1739, 2099, 2964 cm^{-1}. ^{1}H NMR (500 MHz, MeOD): δ 0.87 (app t, 6H, *J* = 6.8 Hz), 0.92 (d, 3H, *J* = 6.5 Hz), 0.93 (d, 3H, *J* = 6.5 Hz), 0.98 (t, 3H, *J* = 7.3 Hz), 1.10 (d, 3H, *J* = 6.5 Hz), 1.17 (d, 3H, *J* = 7.0 Hz), 1.25-1.35 (m, 1H), 1.62-1.69 (m, 1H), 1.72-1.80 (m, 1H), 1.86-1.94 (m, 1H), 1.94-2.04 (m, 2H), 2.06-2.16 (m, 3H), 2.17 (s, 3H), 2.28-2.40 (m, 1H), 2.48-2.58 (m, 2H), 2.91 (d, 2H, *J* = 6.5 Hz), 3.72 (d, 1H, *J* = 9.5 Hz), 4.32-4.41 (m, 1H), 4.41-4.54 (br s, 1H), 5.46 (d, 1H, *J* = 12.5 Hz), 5.59 (d, 1H, *J* = 12.5 Hz), 5.90 (dd, 1H, *J* = 2.0, 11.0 Hz), 7.16 (app sextet, 1H, *J* = 4.5 Hz), 7.23 (app d, 4H, *J* = 4.5 Hz), 8.10 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 10.9, 16.2, 18.7, 20.1, 20.8, 20.9, 22.8, 22.9, 26.1, 26.8, 32.2, 35.8, 36.3, 38.0, 39.4, 42.3, 44.3, 50.9, 64.6, 70.7, 125.6, 127.5, 129.4, 130.6, 139.5, 150.9, 162.8, 170.9, 172.0, 173.21, 173.24, 180.0. HRMS (FAB) calcd for $C_{36}H_{52}N_6O_8NaS$ (*M*+Na): 751.3465. Found: 751.3456.



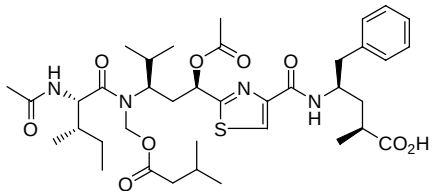
Dimethylamino-acetic acid pentafluorophenyl ester (18). To a 0.40 M solution of *N*,*N*-dimethylglycine (82.0 mg, 0.800 mmol) in EtOAc (2.00 mL, filtered through activated alumina) were added pentafluorophenol (162 mg, 0.880 mmol) and 1,3-dicyclohexylcarbodiimide (182 mg, 0.88 mmol). The reaction mixture was stirred for 12 h at rt at which time it was filtered (washing with EtOAc) and concentrated. Ester **18** was used immediately without further purification.



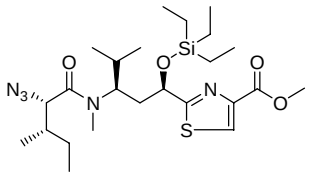
4-[(2-{1-Acetoxy-3-[[2-(2-dimethylamino-acetylamino)-3-methyl-pentanoyl]-(3-methyl-butyryloxymethyl)-amino]-4-methyl-pentyl}-thiazole-4-carbonyl)-amino]-2-methyl-5-phenyl-pentanoic acid (6). Pd/C (10 wt%, 8.7 μg) and azide **8** (18.0 mg, 0.0247 mmol) were added to a 0.20 M solution of **18** (0.0988 mmol) in 0.40 mL of EtOAc (filtered through activated alumina). The reaction mixture was stirred under a hydrogen atmosphere for 26 h and then filtered through a plug of Celite with washing of the filter pad with EtOAc. The filtrate was concentrated, and a 1:1 mixture of deoxygenated H_2O/dioxane (4.0 mL) was added. The mixture was stirred for 20 h at rt and concentrated. Reverse-phase HPFC (20:80 to 100:0 MeCN:H_2O) followed by lyophilization afforded 9.3 mg (48%) of **6** as an amorphous solid. $[\alpha]_D^{23}$ = -2.0 (*c* = 0.6, MeOH). IR: 1226, 1496, 1542, 1741, 2964 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.81 (d, 3H, *J* = 6.5 Hz), 0.87 (d, 3H, *J* = 6.5 Hz), 0.89 (d, 3H, *J* = 7.0 Hz), 0.92 (t, 3H, *J* = 7.5 Hz), 0.98 (d, 3H, *J* = 6.5 Hz), 1.06 (d, 3H, *J* = 6.5 Hz), 1.12-1.21 (m, 1H), 1.17 (d, 3H, *J* = 7.0 Hz), 1.58-1.70 (m, 2H), 1.77-1.91 (m, 2H), 1.92-2.05 (m, 3H), 2.06-2.17 (m, 2H), 2.16 (s, 3H), 2.35 (s, 6H), 2.46-2.57 (m, 2H), 2.92 (d, 2H, *J* = 5.5 Hz), 3.11 (q, 2H, *J* = 16.2 Hz), 4.28-4.50 (br s, 1H), 4.32-4.38 (m, 1H), 4.70 (d, 1H, *J* = 8.5 Hz), 5.46 (d, 1H, *J* = 12.0 Hz), 5.87 (d, 1H, *J* = 11.0 Hz), 6.05 (d, 1H, *J* = 12.0 Hz), 7.12-7.18 (m, 1H), 7.19-7.26 (m, 4H), 8.09 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 11.1, 16.6, 19.0, 20.3, 20.8, 20.9, 22.9, 25.5, 26.2, 26.9, 32.4, 35.9, 37.6, 39.1, 39.7, 42.1, 44.5, 45.8, 51.2, 55.2, 62.7, 70.8, 125.6, 127.5, 129.4, 130.6, 139.7, 150.9, 162.7, 170.8, 171.7, 172.0, 173.3, 176.5, 181.8. HRMS (FAB) calcd for $C_{40}H_{62}N_5O_9S$ (*M*+H): 788.4268. Found: 788.4256.



Acetic acid pentafluorophenyl ester (19). To a 0.40 M solution of acetic acid (46 μL, 0.800 mmol) in EtOAc (2.00 mL, filtered through activated alumina) were added pentafluorophenol (162 mg, 0.880 mmol) and 1,3-dicyclohexylcarbodiimide (182 mg, 0.88 mmol). The reaction mixture was stirred for 12 h at rt at which time it was filtered (washing with EtOAc) and concentrated. Ester **19** was used immediately without further purification.

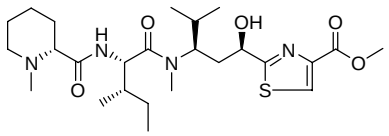


4-[(2-{1-Acetoxy-3-[(2-acetylamino-3-methyl-pentanoyl)-(3-methyl-butyryloxymethyl)-amino]-4-methyl-pentyl}-thiazole-4-carbonyl)-amino]-2-methyl-5-phenyl-pentanoic acid (7). Pd/C (10 wt%, 7.0 μg) and azide **8** (15.0 mg, 0.021 mmol) were added to a 0.20 M solution of **19** (0.082 mmol) in 0.40 mL of EtOAc (filtered through activated alumina). The reaction mixture was stirred under a hydrogen atmosphere for 21 h and then filtered through a plug of Celite with washing of the filter pad with EtOAc. The filtrate was concentrated, and a 1:1 mixture of deoxygenated H_2O/dioxane (2.0 mL) was added. The mixture was stirred for 7 h at rt and concentrated. Normal-phase HPFC (99:1 to 90:10 CH_2Cl_2:MeOH) afforded 12.0 mg (77%) of **7** as an amorphous solid. $[\alpha]_D^{23}$ = -7.0 (*c* = 0.4, MeOH). IR: 1225, 1554, 1647, 1740, 2876, 2963 cm^{-1}. ^1H NMR (500 MHz, MeOD): δ 0.81 (d, 3H, *J* = 7.0 Hz), 0.86 (d, 3H, *J* = 7.0 Hz), 0.88 (d, 3H, *J* = 7.0 Hz), 0.92 (t, 3H, *J* = 7.5 Hz), 0.95 (d, 3H, *J* = 7.0 Hz), 1.07 (d, 3H, *J* = 6.5 Hz), 1.17 (d, 3H, *J* = 7.5 Hz), 1.12-1.22 (m, 1H), 1.26-1.36 (m, 1H), 1.57-1.69 (m, 2H), 1.80-1.91 (m, 1H), 1.95 (s, 3H), 1.96-2.08 (m, 4H), 2.09-2.16 (m, 1H), 2.16 (s, 3H), 2.25-2.34 (m, 1H), 2.45-2.57 (m, 2H), 2.91 (d, 2H, *J* = 7.0 Hz), 4.32-4.50 (br s, 1H), 4.34-4.42 (m, 1H), 4.62 (d, 1H, *J* = 9.5 Hz), 5.42 (d, 1H, *J* = 12.0 Hz), 5.89 (d, 1H, *J* = 9.5 Hz), 6.13 (d, 1H, *J* = 12.0 Hz), 7.12-7.18 (m, 1H), 7.19-7.25 (m, 4H), 8.10 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 11.0, 16.4, 18.8, 20.0, 20.8, 20.9, 22.2, 22.87, 22.88, 25.6, 26.9, 32.5, 35.8, 37.2, 38.1, 39.5, 42.3, 44.5, 50.9, 55.6, 70.8, 125.7, 127.5, 129.4, 130.6, 139.5, 150.7, 162.7, 170.9, 172.0, 173.0, 173.3, 176.6, 180.3. HRMS (FAB) calcd for $C_{38}H_{56}N_4O_9NaS$ (*M*+Na): 767.3666. Found: 767.3648.

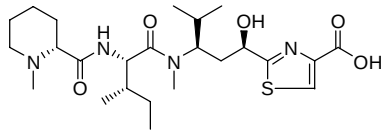


2-{3-[(2-Azido-3-methyl-pentanoyl)-methyl-amino]-4-methyl-1-triethylsilanyloxy-pentyl}-thiazole-4-carboxylic acid methyl ester (21). A 0.30 M solution of amide **20** (905 mg, 1.77 mmol) in THF (6.0 mL) was cooled to -45 °C and KHMDS (6.02 mL, 3.01 mmol, 0.50 M in toluene) was added. The resulting mixture was stirred for 20 minutes at -45 °C. Methyl iodide (754 mg, 5.31 mmol, filtered through activated alumina) was added, and the reaction mixture was allowed to warm to rt over 4.5 h at which time the reaction was quenched with MeOH (5.0 mL). The crude product was diluted with EtOAc (250 mL) and washed with brine (100 mL). The aqueous layer was extracted with EtOAc (2 x 100 mL). The organic portions were dried, filtered, and concentrated. Normal-phase HPFC (95:5 to 60:40 hexanes:EtOAc) yielded 761 mg of **21** (82%) as an amorphous solid. The ^1H NMR corresponds to a 10:1 mixture of rotamers, with the major isomer reported. $[\alpha]_D^{23}$ = +67.5 (*c* = 1.0, $CHCl_3$). IR: 1094, 1210, 1238, 1645, 1736, 2098, 2877, 2960 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.55-0.70 (m, 6H), 0.84 (d, 3H, *J* = 6.8 Hz), 0.85-0.93 (m, 15H), 0.95 (d, 3H, *J* = 6.8 Hz), 1.17-1.29 (m, 1H), 1.60-1.79 (m, 2H), 2.00-2.20 (m, 3H), 2.92 (s, 3H), 3.50 (d, 1H, *J* = 9.6 Hz), 3.89 (s, 3H), 4.37-4.45 (m, 1H), 4.90 (dd, 1H, *J* = 3.6, 6.4 Hz), 8.07 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 4.6, 6.7, 10.6,

15.9, 19.1, 20.0, 25.0, 30.2, 34.9, 40.1, 52.2, 57.3, 63.9, 71.0, 127.4, 146.4, 161.8, 169.5, 178.3. HRMS (FAB) calcd for $C_{24}H_{44}N_5O_4SiS$ (*M*+H): 526.2883. Found: 526.2877.

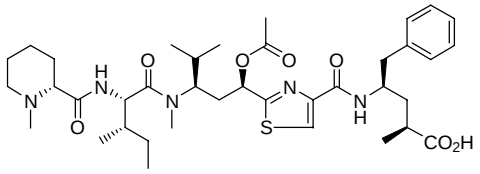


2-[1-Hydroxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazole-4-carboxylic acid methyl ester (23). Pd/C (10 wt%, 242 mg) and azide **21** (359 mg, 0.683 mmol) were added to a 0.32 M solution of **22** (2.17 mmol) in 6.80 mL of EtOAc (filtered through activated alumina). The reaction mixture was stirred under a hydrogen atmosphere for 26 h and then filtered through a plug of Celite, with washing of the filter pad with EtOAc. Normal-phase HPFC purification (99:1 to 95:5 EtOAc:MeOH) provided 483 mg of Mep-coupled product. The product was dissolved in 35.0 mL of deoxygenated AcOH/H_2O/THF (3:1:1, v/v/v, 0.02 M) and stirred at rt for 28 h. Concentration followed by normal-phase HPFC purification (98:2 to 85:15 EtOAc:MeOH) afforded 302 mg (87%, over two steps) of **23** as an amorphous solid. The ^{1}H NMR corresponds to a 7.5:1 mixture of rotamers, with the major isomer reported. $[\alpha]_D^{23}$ = -4.8 (*c* = 1.0, MeOH). IR: 1095, 1212, 1238, 1495, 1622, 1722, 2936 cm^{-1}. ^{1}H NMR (500 MHz, MeOD): δ 0.81 (d, 3H, *J* = 6.5 Hz), 0.91 (t, 3H, *J* = 7.5 Hz), 0.97 (d, 3H, *J* = 6.5 Hz), 0.99 (d, 3H, *J* = 6.5 Hz), 1.16-1.34 (m, 2H), 1.49-1.66 (m, 4H), 1.75 (d, 2H, *J* = 10.5 Hz), 1.77-1.86 (br s, 1H), 1.89-2.01 (m, 2H), 2.02-2.09 (m, 1H), 2.17 (s, 3H), 2.18-2.26 (m, 1H), 2.57 (d, 1H, *J* = 9.0 Hz), 2.85-2.95 (m, 1H), 3.17 (s, 3H), 3.91 (s, 3H), 4.40-4.55 (br s, 1H), 4.68 (d, 1H, *J* = 9.5 Hz), 4.75 (d, 1H, *J* = 9.0 Hz), 8.32 (s, 1H). ^{13}C NMR (125 MHz, MeOD): δ 11.2, 16.2, 20.5, 20.7, 24.4, 25.9, 26.3, 31.1, 31.6, 32.1, 37.7, 38.8, 44.9, 52.8, 55.0, 56.7, 69.9, 70.6, 129.3, 147.5, 163.2, 175.4, 175.7, 180.6. HRMS (FAB) calcd for $C_{25}H_{43}N_4O_5S$ (*M*+H): 511.2954. Found: 511.2947.

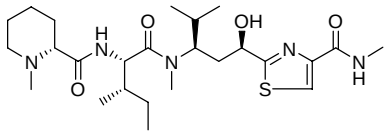


2-[1-Hydroxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazole-4-carboxylic acid (24). Me_3SnOH (496 mg, 2.74 mmol) was added to a 0.020 M solution of methyl ester **23** (175 mg, 0.343 mmol) in dichloroethane (17.0 mL). The reaction mixture was heated to 60 °C for 20 h and then concentrated. Column chromatography (100% CH_2Cl_2 to elute tin containing materials followed by 80:20:1 CH_2Cl_2:MeOH:NH_4OH to elute the product) afforded 150 mg (88%) of **24** as an amorphous solid. The ^{1}H NMR corresponds to a 6:1 mixture of rotamers, with the major isomer reported. $[\alpha]_D^{23}$ = -17.4 (*c* = 1.0, MeOH). IR: 1276, 1368, 1471, 1616, 2874, 2961 cm^{-1}. ^{1}H NMR (500 MHz, d_6-DMSO): δ 0.70 (m, 3H), 0.75-0.82 (m, 3H), 0.83-0.90 (m, 6H), 1.04-1.16 (m 1H), 1.17-1.28 (m, 2H), 1.37-1.55 (m, 3H), 1.56-1.72 (m, 3H), 1.73-1.91 (m, 3H), 2.00-2.24 (m, 2H), 2.22 (s, 3H), 2.84 (br

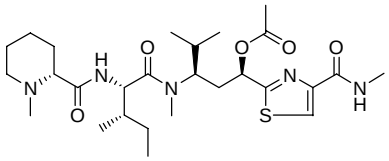
s, 1H), 2.94-3.00 (m, 1H), 3.04 (s, 3H), 4.16-4.60 (br s, 1H), 4.49 (d, 1H, J = 10.5), 4.56 (app t, 1H, J = 9.0), 5.93-6.40 (br s, 1H), 8.05 (s, 1H), 8.25 (s, 1H). ^{13}C NMR (125 MHz, d_6-DMSO): δ 10.0, 14.7, 19.1, 19.5, 19.6, 21.8, 23.58, 23.62, 28.75, 28.8, 35.2, 36.7, 42.6, 52.3, 54.1, 67.1, 67.5, 126.7, 147.8, 162.2, 170.7, 172.0, 177.6. HRMS (FAB) calcd for $C_{24}H_{41}N_4O_5S$ (M+H): 497.2798. Found: 497.2793.



4-({2-[1-Acetoxy-4-methyl-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl]-thiazole-4-carbonyl}-amino)-2-methyl-5-phenyl-pentanoic acid (10). Acid **24** (34.0 mg, 0.0684 mmol) was added to a solution of pentafluorophenol (19.0 mg, 0.103 mmol) and 1,3-diisopropylcarbodiimide (12.0 μL, 0.0752 mmol) in 0.52 mL of CH_2Cl_2 at 0 °C. The reaction mixture was warmed to rt, stirred for 24 h, and concentrated. EtOAc (10 mL) was added, and the crude product was filtered with rinsing of the reaction vessel with EtOAc. The filtrate was concentrated, and the crude material was used without further purification. DMF (0.270 mL, 0.25 M) was added to the crude product at 0 ºC, followed by **17** (50.0 mg, 0.205 mmol) and i-Pr_2EtN (60.0 μL, 0.342 mmol). The reaction mixture was allowed to warm to rt, stirred for 24 h at rt, and concentrated. Normal-phase HPFC purification (98:2 to 80:20 CH_2Cl_2:MeOH) followed by reverse-phase HPFC (20:80 to 100:0 MeCN:H_2O) afforded 34.0 mg of product containing trace amounts of i-Pr_2EtN. The product mixture (34.0 mg, 0.496 mmol) was dissolved in pyridine (0.50 mL), cooled to 0 °C, and acetic anhydride (38.0 μL, 0.397 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 22 h. The reaction mixture was then cooled to 0 °C, and a 1:1 mixture of deoxygenated H_2O/dioxane (1.6 mL) was added. The mixture was allowed to warm to rt and was stirred for 20 h at rt. The solvent was removed under reduced pressure. Normal-phase HPFC (95:5 to 80:20 CH_2Cl_2:MeOH) followed by lyophilization afforded 28.0 mg (56%, over three steps) of **10** as an amorphous solid. The ^{1}H NMR corresponds to a 16:1 mixture of rotamers, with the major isomer reported. $[\alpha]_D^{23}$ = -19.2 (c = 0.9, MeOH). IR: 1220, 1495, 1541, 1643, 1712, 2964 cm^{-1}. ^{1}H NMR (500 MHz, MeOD): δ 0.81 (d, 3H, J = 6.5 Hz), 0.92 (t, 3H, J = 7.3 Hz), 0.98 (d, 3H, J = 6.5 Hz), 1.03 (d, 3H, J = 6.5 Hz), 1.16 (d, 3H, J = 7.0 Hz), 1.09-1.23 (m, 1H), 1.37-1.41 (m, 1H), 1.56-1.74 (m, 5H), 1.75-1.92 (m, 4H), 1.96-2.05 (m, 1H), 2.15 (s, 3H), 2.31 (s, 3H), 2.23-2.41 (m, 3H), 2.51 (br s, 1H), 2.85 (d, 1H, J = 10.5 Hz), 2.92 (d, 2H, J = 6.5 Hz), 3.05 (d, 1H, J = 11.5 Hz), 3.10 (s, 3H), 4.30-4.50 (m, 2H), 4.73 (d, 1H, J = 8.0 Hz), 5.71 (dd, 1H, J = 2.5, 11.0 Hz), 7.13-7.18 (m, 1H), 7.19-7.25 (m, 4H), 8.08 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 11.3, 16.4, 19.1, 20.4, 20.6, 20.9, 23.7, 25.5, 25.5, 30.9, 31.0, 31.1, 35.6, 37.6, 39.5, 39.6, 42.0, 44.2, 51.2, 55.2, 56.4, 69.7, 71.2, 125.1, 127.4, 129.3, 130.6, 139.8, 151.1, 162.7, 171.6, 171.8, 173.6, 175.0, 182.5. HRMS (FAB) calcd for $C_{38}H_{57}N_5O_7S$ (M+H): 728.4057. Found: 728.4053.



1-Methyl-piperidine-2-carboxylic acid [1-({1-[2-hydroxy-2-(4-methylcarbamoyl-thiazol-2-yl)-ethyl]-2-methyl-propyl}-methyl-carbamoyl)-2-methyl-butyl]-amide (25). In a sealed tube, 10.0 mL of a 2.0 M solution of methylamine (5.00 mmol) in THF was added to a 0.02 M solution of **23** (27.0 mg, 0.0529 mmol) in MeOH (2.50 mL). The reaction solution was heated to 100 °C for 21 h. After the solution cooled to rt, the solvent was removed under reduced pressure. Reverse-phase HPFC (20:80 to 100:0 MeCN/H_2O) followed by lyophilization provided compound **25** (14.0 mg, 52%) as an amorphous solid. The ^{1}H NMR corresponds to a 6:1 mixture of rotamers, with the major isomer reported. $[\alpha]_D^{23}$ = -2.9 (*c* = 1.0, MeOH). IR: 1070, 1499, 1551, 1646, 2876, 2961 cm^{-1}. ^{1}H NMR (500 MHz, MeOD): δ 0.83 (d, 3H, *J* = 6.5 Hz), 0.90 (t, 3H, *J* = 7.5 Hz), 0.966 (d, 3H, *J* = 6.5 Hz), 0.974 (d, 3H, *J* = 6.5 Hz), 1.15-1.25 (m, 1H), 1.25-1.32 (m, 1H), 1.48-1.66 (m, 4H), 1.72 (d, 2H, *J* = 10.5 Hz), 1.81-1.99 (m, 3H), 2.00-2.10 (m, 1H), 2.16 (s, 3H), 2.19-2.37 (m, 1H), 2.53-2.58 (m, 1H), 2.87-2.94 (m, 1H), 2.92 (s, 3H), 3.17 (s, 3H), 4.16-4.58 (br s, 1H), 4.64 (dd, 1H, *J* = 2.3, 10.3 Hz), 4.72 (d, 1H, *J* = 9.0 Hz), 8.06 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 11.1, 16.1, 20.5, 20.6, 24.4, 25.9, 26.3, 26.4, 31.3, 31.7, 37.8, 38.8, 44.8, 55.4, 56.7, 70.0, 70.6, 124.2, 151.0, 164.4, 175.3, 175.7, 179.4. HRMS (FAB) calcd for $C_{25}H_{44}N_5O_4S$ (*M*+H): 510.3114. Found: 510.3098.



Acetic acid 4-methyl-1-(4-methylcarbamoyl-thiazol-2-yl)-3-(methyl-{3-methyl-2-[(1-methyl-piperidine-2-carbonyl)-amino]-pentanoyl}-amino)-pentyl ester (11). A 0.050 M solution of **25** (12.0 mg, 0.0235 mmol) in pyridine (0.500 mL) was cooled to 0 °C, and acetic anhydride (18.0 μL, 0.188 mmol) was added. The reaction mixture was allowed to warm to rt over 2 h and was stirred at rt for 21 h. The solvent was removed under reduced pressure. Reverse-phase HPFC (20:80 to 100:0 MeCN:H_2O) followed by lyophilization afforded 9.3 mg (72%) of **11** as an amorphous solid. The ^{1}H NMR corresponds to a 23:1 mixture of rotamers, with the major isomer reported. $[\alpha]_D^{23}$ = -2.2 (*c* = 0.6, MeOH). IR: 1221, 1498, 1549, 1643, 1755, 2937 cm^{-1}. ^{1}H NMR (500 MHz, MeOD): δ 0.80 (d, 3H, *J* = 7.0 Hz), 0.92 (t, 3H, *J* = 7.5 Hz), 0.98 (d, 3H, *J* = 7.0 Hz), 1.02 (d, 3H, *J* = 6.5 Hz), 1.13-1.22 (m, 1H), 1.24-1.34 (m, 1H), 1.49-1.67 (m, 4H), 1.72-1.78 (m, 2H), 1.79-1.91 (m, 2H), 2.07 (dt, 1, *J* = 3.0, 11.5 Hz), 2.15 (s, 3H), 2.18 (s, 3H), 2.22-2.31 (m, 1H), 2.34-2.41 (m, 1H), 2.56 (dd, 1H, *J* = 2.5, 11.0 Hz), 2.90-2.95 (m, 1H), 2.94 (s, 3H), 3.11 (s, 3H), 4.40-4.51 (br s, 1H), 4.74 (d, 1H, *J* = 8.0 Hz), 5.70 (dd, 1H, *J* = 2.5, 11.5 Hz), 8.14 (s, 1H). ^{13}C NMR (125 MHz, MeOD) δ 11.2, 16.4, 20.4, 20.6, 20.9, 24.4, 25.6, 26.3, 26.4, 31.1, 31.7, 35.7, 37.7, 44.9, 54.9, 56.7, 70.6, 71.2, 125.0, 150.9, 163.9, 171.82, 171.83, 175.3, 175.6. HRMS (FAB) calcd for $C_{27}H_{46}N_5O_5S$ (*M*+H): 552.3220. Found: 552.3218.

Biological Assays.

Cell culture and growth inhibition assay. Cell lines were obtained from the American Type Culture Collection (ATCC) and the German Collection of Microorganisms and Cell Cultures (DSMZ). All cell lines were cultivated under conditions recommended by their respective depositors. Growth inhibition was measured in microtiter plates. Aliquots of 120 µl of the suspended cells (50,000/mL) were given to 60 µL of a serial dilution of the inhibitor and incubated at 37 °C and 10% CO_2. After 5 days, when control cells had grown to confluence state, the metabolic activity in each well was determined using an MTT assay.[2] IC_{50} values were defined as the analogue concentration that showed only 50% of the activity of the control wells..

Fluorescence staining. PtK_2 cells (ATCC CCL-56) were grown on glass coverslips (13 mm diameter) in four-well plates. Exponentially growing cells were incubated with the analogues for 18 hours. Cells were then fixed with cold (–20 °C) acetone/methanol (1:1) for 10 minutes. For labeling the microtubules, cells were incubated with a primary monoclonal antibody against α-tubulin (1:500; Sigma), then with a secondary goat anti-mouse IgG antibody conjugated with Alexa Fluor 488 (1:200; Molecular Probes) at 37 °C for 45 minutes. Nuclei and chromosomes were stained with DAPI (1 µg/mL). The cells were washed with PBS between all incubations. The coverslips were mounted using Prolong Antifade Gold (Molecular Probes), and viewed with a Zeiss Axiophot fluorescence microscope using appropriate filter sets.

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[1] H. M. Peltier, J. M. McMahon, A. W. Patterson, J. A. Ellman, *J. Am. Chem. Soc.* **2006**, *128*, 16018-16019.

[2] T. Mosmann, *J. Immunol. Methods* **1983**, *65*, 55-63.