Comparative Pharmacokinetic Properties and Antitumor Activity of the Marine HDACi Largazole and Largazole Peptide Isostere

Supporting Information

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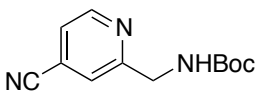
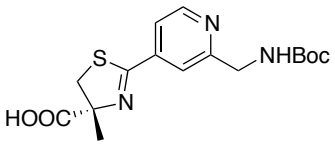
General experimental method. Unless otherwise noted, all reactions were run under an argon atmosphere in flame or oven dried glassware. Reactions were monitored using thin layer silica gel chromatography (TLC) using 0.25 mm silica gel 60F plates with fluorescent indicator from Merck. Plates were visualized by treatment with anisaldehyde stain with gentle heating. Products were purified via column chromatography using the solvent system(s) indicated. Silica gel 60, 230-400 mesh, was purchased from Sorbent Technologies. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN), triethylamine (Et_3N), toluene, diethyl ether (Et_2O), and *N,N*-dimethylformamide (DMF) were passed through an alumina drying column (Solv-Tek Inc.) using argon pressure. All other reagents were purchased from commercial sources and used as received without additional purification. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 300, 400, or 500 MHz NMR spectrometers. Chemical shifts are reported in ppm relative to $CHCl_3$ at δ = 7.27 (^{1}H NMR) and δ = 77.23 (^{13}C NMR) or tetramethylsilane (TMS) δ = 0.00, where noted. Mass spectra were obtained on Fisions VG Autospec. Optical rotations were collected at 589 nm on a Rudolph Research Automatic Polarimeter Autopol III.

Largazole[1], **largazole thiol**[1], **largazole peptide isostere**[2], and **largazole peptide isostere thiol**[2] were synthesized according to the published protocols.

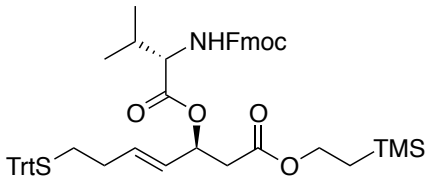


2-((((*tert*-Butoxy)carbonyl)amino)methyl)isonicotinonitrile (S1): To a solution of commercially available 2-(chloromethyl)isonicotinonitrile (5.84 g, 38.3 mmol) in DMF (250 mL) at ambient temperature was treated with potassium phthalimide (7.09 g, 38.3 mmol). After stirring for 5 h, the mixture was concentrated under vacuum. The remaining mixture was taken up in H_2O (100 mL) and was filtered to collect the solid. The solid was washed with H_2O (50 mL) and THF (50 mL) to obtain the desired phthalimide derivative (5.04 g, 50%) and was moved forward without further purification. To a solution of the crude phthalimide derivative (5.04 g, 19.1 mmol) in THF/MeOH (170 mL, 1:1, v/v) at ambient temperature was treated with hydrazine monohydrate (1.02 mL, 21.1 mmol). After 2 h, 1.0 M HCl (21.4 mL) was added to the mixture and was stirred for another 3 h before concentrating the reaction mixture under vacuum. The remaining residue was taken up in H_2O (200 mL) and the unwanted solid was removed through filtration. The filtrate was concentrated and placed under vacuum to remove the remaining H_2O. The crude solid was taken up in CH_2Cl_2 (150 mL) and triethylamine (8.00 mL, 57.4 mmol) and Boc_2O (4.59 g, 21.1 mmol) was added. After stirring for 12 h at room temperature, the reaction was quenched with a saturated solution of $NaHCO_3$ (200 mL), extracted with CH_2Cl_2 (3 x 150 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified using flash chromatography (15% to 50% ethyl acetate in hexanes) to provided the aryl pyridine "IN" fragement (3.74 g, 84%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.69 (d, *J* = 5.2 Hz, 1H), 7.51 (s, 1H), 7.39 (dd, *J* = 5.2, 0.8 Hz, 1H), 5.45 (s, 1H),

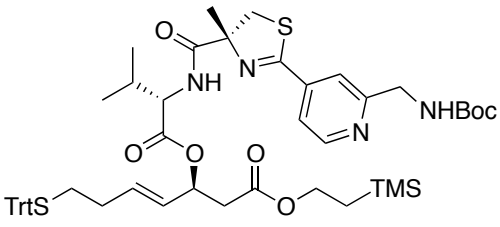
4.47 (d, *J* = 5.6 Hz, 2H), 1.44 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100 MHz) 159.9, 155.9, 150.0, 123.6, 123.1, 121.1, 116.4, 80.1, 45.6, 28.3; IR (neat) 3337, 2978, 2934, 2245, 1709, 1514, 1246, 1168, 949; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}N_3O_3$ $[M+H]^+$ 234.1243, found 234.1237.



(*R*)-2-(2-(((*tert*-Butoxycarbonyl)amino)methyl)pyridin-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxylic acid (S2): To a solution of nitrile **S1** (1.50 g, 6.43 mmol) in MeOH/pH 6.0 buffer (105 mL, 3:2, v/v) was added α-methyl-L-cysteine (1.32 g, 7.72 mmol) and $NaHCO_3$ (1.08 g, 12.9 mmol). The reaction mixture was heated to 70 °C for 48 h. The mixture was cooled to ambient temperature and the MeOH was removed under reduced pressure. The aqueous layer was extracted with diethyl ether (3 x 30 mL) before being acidified to a pH of ~2 using 1.0 M HCl. The aqueous layer was then extracted with ethyl acetate (3 x 30 mL) and the ethyl acetate layer was dried over $MgSO_4$. The solution was concentrated to give the desired acid (1.89 g, 85%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.61 (d, *J* = 4.8 Hz, 1H), 7.68 (s, 1H), 7.58 (dd, *J* = 5.2, 1.6 Hz, 1H), 5.64 (bs, 1H), 4.48 (d, *J* = 4.8 Hz, 2H) 3.94 (d, *J* = 11.6 Hz, 1H), 3.38 (d, *J* = 11.6 Hz, 1H), 1.66 (s, 3H), 1.43 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 175.5, 166.5, 158.5, 156.1, 148.7, 141.4, 121.2, 120.9, 85.0, 79.8, 45.1, 41.8, 28.3, 23.8; IR (neat) 3355, 2979, 2359, 1707, 1517, 1284, 1167, 754; HRMS (ESI) *m/z* calcd for $C_{16}H_{22}N_3O_4S$ $[M+H]^+$ 352.1331, found 352.1334; $[\alpha]_D$ = –39.3 (*c* 1.08, $CHCl_3$).

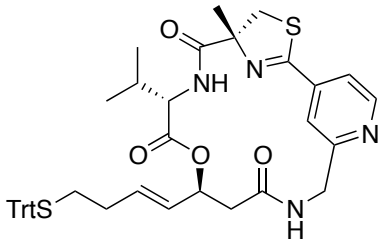


(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-(((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoyl)oxy)-7-(tritylthio)hept-4-enoate (S3): To a solution of 2-(trimethylsilyl)ethyl (*S*,*E*)-3-hydroxy-7-(tritylthio)hept-4-enoate[1] (300 mg, 0.578 mmol) in CH_2Cl_2 (15.0 mL) at ambient temperature was added *N*-Fmoc-L-valine (981 mg, 2.89 mmol), EDCI□HCl (665 mg, 3.47 mmol), DMAP (7.1 mg, 0.058 mmol), and DIPEA (0.60 mL, 3.47 mmol). After stirring for 18 h, the reaction mixture was concentrated. The crude residue was purified using flash chromatography (1% to 20% ethyl acetate in hexanes) to provide ester (375 mg, 77%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.71-7.75 (m, 2H), 7.56-7.58 (m, 2H), 7.18-7.38 (m, 19H), 5.58-5.69 (m, 2H), 5.34 (dd, *J* = 15.6, 7.6 Hz, 1H), 5.25 (d, *J* = 9.2 Hz, 1H), 4.30-4.40 (m, 2H), 4.25 (dd, *J* = 8.8, 4.4 Hz, 1H), 4.20 (t, *J* = 6.8 Hz, 1H), 4.12 (t, *J* = 8.8 Hz, 2H), 2.65 (dd, *J* = 16.0, 8.0 Hz, 1H), 2.52 (dd, *J* = 15.6, 5.2 Hz, 1H), 2.10-2.17 (m, 3H), 2.02 (q, *J* =6.8 Hz, 2H), 0.91-0.99 (m, 2H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.77 (d, *J* = 6.8 Hz, 3H), –0.01 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 170.9, 169.6, 156.1, 144.8, 143.9, 143.8, 141.3, 134.0, 129.6, 127.9, 127.7, 127.0, 126.6, 125.1, 120.0, 71.8, 67.0, 66.6, 63.1, 58.7, 47.2, 39.7, 31.4, 31.3, 31.1, 19.0, 17.3, –1.5; IR (neat) 3059, 2979, 1731, 1509, 1447, 1249, 1182, 1033, 858, 741; HRMS (ESI) *m/z* calcd for $C_{51}H_{57}NNaO_6SSi$ $[M+Na]^+$ 862.3574, found 862.3583; $[\alpha]_D$ = –15.0 (*c* 1.18, $CHCl_3$).

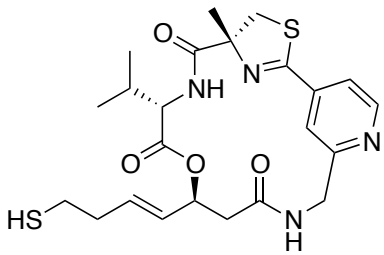


(*S*,*E*)-2-(Trimethylsilyl)ethyl 3-(((*S*)-2-((*R*)-2-(2-(((*tert*-butoxycarbonyl)amino)methyl)pyridin-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanoyl)oxy)-7-(tritylthio)hept-4-enoate (S4): To a solution of Fmoc protected amine **S3** (1.55 g, 1.84 mmol) in acetonitrile (125 mL) at ambient temperature was added diethylamine (10.0 mL). After 2 h, the reaction mixture was concentrated under reduced vacuum, taken back up in ethyl acetate (50 mL), and concentrated again. In a separate flask, acid **S2** (0.650 g, 1.84 mmol), PyBOP (1.92 g, 3.68 mmol), and DIPEA (0.96 mL, 5.52 mmol) was combined in CH_2Cl_2 (125 mL). The freshly deprotected amine was added via CH_2Cl_2 (10 mL) to the flask containing the activated acid at ambient temperature. After 3 h, the resulting mixture was concentrated under reduced pressure. The crude residue was purified using flash chromatography (5% to 40% ethyl acetate in hexanes) to obtain the desired amide (1.42 g, 81%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.51-8.52 (m, 1H), 7.72-7.73 (m, 1H), 7.15-7.38 (m, 15H), 7.11 (d, *J* = 8.8 Hz, 1H), 5.69 (dt, *J* = 15.2,

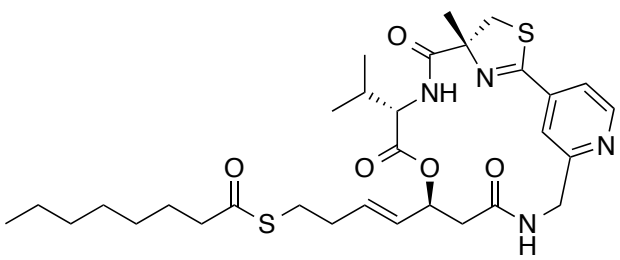
6.8 Hz, 1H), 5.60-5.65 (m, 1H), 5.36 (dd, *J* = 15.2, 7.6 Hz, 1H), 4.54-4.56 (m, 2H), 4.48 (dd, *J* = 8.8, 4.4 Hz, 1H), 4.14 (dd, *J* = 10.0, 8.0 Hz, 2H), 3.84 (d, *J* = 11.6 Hz, 1H), 3.41 (d, *J* = 11.6 Hz, 1H), 2.67 (dd, *J* = 15,6, 8.0 Hz, 1H), 2.54 (dd, *J* = 16.0, 5.6 Hz, 1H), 2.03-2.18 (m, 5H), 1.56 (s, 3H), 1.44 (s, 9H), 0.92-0.97 (m, 2H), 0.81 (d, *J* = 6.8 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H), 0.01 (s, 9H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.8, 170.3, 169.6, 167.0, 158.7, 155.9, 149.7, 144.8, 140.3, 133.9, 129.5, 127.8, 127.7, 126.6, 120.3, 119.9, 85.4, 79.6, 71.8, 66.6, 63.1, 56.8, 45.7, 41.7, 39.7, 31.3, 31.1, 31.0, 28.4, 24.5, 19.0, 17.4, 17.2, –1.5; IR (neat) 3390, 2960, 1737, 1681, 1593, 1504, 1444, 1248, 1170, 836, 744; HRMS (ESI) *m/z* calcd for $C_{52}H_{66}N_4NaO_7S_2Si$ $[M+Na]^+$ 973.4040, found 973.4058; $[\alpha]_D$ = –32.5 (*c* 1.03, $CHCl_3$).



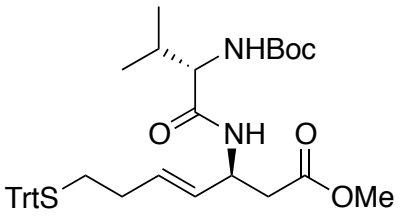
Trityl protected depsipeptide pyridine "OUT" macrocycle (S5): To a solution of depsipeptide **S4** (2.00 g, 2.10 mmol) in CH_2Cl_2 (100 mL) at 0 °C was treated with TFA (20.0 mL) dropwise. The reaction mixture was allowed to stir for 18 h. The mixture was concentrated under reduced pressure, taken back up in toluene (50 mL), and concentrated again. The remaining residue was taken up in acetonitrile (2100 mL) and was treated with HATU (1.60 g, 4.20 mmol), HOBt (0.570 g, 4.20 mmol), and DIPEA (2.19 mL, 12.6 mmol) at room temperature. After 18 h, the reaction mixture was concentrated under reduced pressure. The crude residue was passed through a silica plug using an eluent of 10% MeOH in CH_2Cl_2. The filtrate was concentrated and taken up in ethyl acetate (150 mL). The solution was washed with a saturated solution of $NaHCO_3$ (100 mL). The aqueous layer was extracted ethyl acetate (3 X 50 mL) and then the combined organic layers were washed with a saturated solution of NaCl (100 mL). After drying over Na_2SO_4 and concentrating under reduced vacuum, the crude mixture was purified using flash chromatography (1% to 6% MeOH in DCM) to obtain the macrocycle (0.350 g, 23%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.65 (d, *J* = 5.2 Hz, 1H), 7.77 (s, 1H), 7.15-7.40 (m, 16H), 6.97 (d, *J* = 9.2 Hz, 1H), 6.33 (dd, *J* = 8.0, 5.2 Hz, 1H), 5.78 (dtd, *J* = 15.6, 6.4, 0.8 Hz, 1H), 5.71 (q, *J* = 6.0 Hz, 1H), 5.50 (dd, *J* = 15.6, 6.0 Hz, 1H), 4.97 (dd, *J* = 17.6, 8.0 Hz, 1H), 4.52 (dd, *J* = 8.8, 4.0 Hz, 1H), 4.02 (dd, *J* = 18.0, 5.2 Hz, 1H), 3.99 (d, *J* = 11.6 Hz, 1H), 3.38 (d, *J* = 11.6 Hz, 1H), 2.74-2.78 (m, 2H), 2.01-2.29 (m, 5H), 1.74 (s, 3H), 0.76 (d, *J* = 6.8 Hz, 3H), 0.66 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.1, 169.9, 168.4, 168.2, 159.3, 150.5, 144.6, 140.0, 132.6, 129.4, 128.1, 127.9, 126.7, 120.8, 116.9, 84.5, 71.5, 66.7, 58.0, 44.5, 43.5, 40.8, 33.4, 31.2, 31.0, 24.9, 18.9, 17.2; IR (neat) 3405, 2962, 1739, 1683, 1552, 1508, 1404, 1255, 1183, 1029, 748; HRMS (ESI) *m/z* calcd for $C_{42}H_{44}N_4NaO_4S_2$ $[M+Na]^+$ 755.2702, found 755.2694; $[\alpha]_D$ = +27.6 (*c* 0.58, $CHCl_3$).



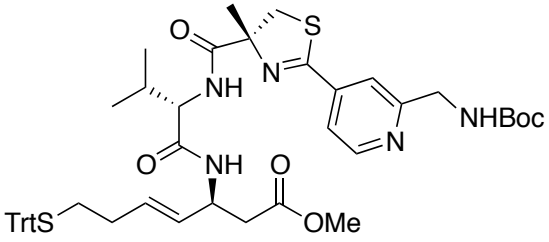
Depsipeptide pyridine "OUT" thiol (S6): To a solution of trityl protected thiol **S5** (320 mg, 0.437 mmol) in CH_2Cl_2 (60 mL) at 0 °C was treated with TFA (2.30 mL) and triisopropylsilane (0.18 mL, 0.873 mmol). The reaction mixture was warmed to ambient temperature and stirred for 2 h. The mixture was concentrated and purified using flash chromatography (1% to 6% MeOH in CH_2Cl_2) to obtain the desired thiol (188 mg, 89%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.70 (d, *J* = 5.2 Hz, 1H), 7.89 (s, 1H), 7.45 (dd, *J* = 5.2, 1.6 Hz, 1H), 6.92 (d, *J* = 9.2 Hz, 1H), 6.36-6.39 (m, 1H), 5.87 (dt, *J* = 15.6, 6.4 Hz, 1H), 5.73-5.78 (m, 1H), 5.67 (ddt, *J* = 15.6, 6.4, 1.2 Hz, 1H), 5.09 (dd, *J* = 17.6, 8.0 Hz, 1H), 4.57 (dd, *J* = 9.2, 4.0 Hz, 1H), 4.37 (dd, *J* = 17.6, 4.8 Hz, 1H), 4.05 (d, *J* = 11.6 Hz, 1H), 3.40 (d, *J* = 11.6 Hz, 1H), 2.76-2.89 (m, 2H), 2.50-2.64 (m, 2H), 2.30-2.44 (m, 2H), 2.11-2.20 (m, 1H), 1.78 (s, 3H), 1.38 (t, *J* = 7.6 Hz, 1H), 0.75 (d, *J* = 6.8 Hz, 3H), 0.66 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.1, 169.3, 168.7, 168.4, 158.7, 149.4, 141.1, 132.4, 128.8, 121.3, 117.8, 84.5, 72.2, 57.8, 43.6, 43.6, 40.3, 36.1, 33.6, 24.9, 23.7, 18.9, 17.0; IR (neat) 3319, 2962, 1736, 1670, 1551, 1508, 1242, 1184, 1027, 971, 730; HRMS (ESI) *m/z* calcd for $C_{23}H_{31}N_4O_4S_2$ $[M+H]^+$ 491.1787, found 491.1795; $[\alpha]_D$ = +68.3 (*c* 0.74, $CHCl_3$).



Depsipeptide pyridine "OUT" (S7): To a solution of depsipeptide pyridine "OUT" thiol **S6** (85.0 mg, 0.179 mmol) in CH_2Cl_2 (15.0 mL) at ambient temperature was treated with octanoyl chloride (.153 mL, 0.894 mmol) and triethylamine (0.050 mL, 0.357 mmol). The reaction mixture was stirred for 2 h before cooling to 0 °C and quenching with methanol (5.0 mL). The mixture was cooled and concentrated and purified using flash chromatography (0.5% to 4% MeOH in CH_2Cl_2) to obtain the desired octanoyl masked thiol (54.0 mg, 49%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.67 (d, *J* = 5.2 Hz, 1H), 7.91 (s, 1H), 7.45 (d, *J* = 4.8 Hz, 1H), 6.89 (d, *J* = 8.8 Hz, 1H), 6.57-6.60 (m, 1H), 5.85 (dt, *J* = 14.8, 7.2 Hz, 1H), 5.62-5.73 (m, 2H), 5.10 (dd, *J* = 18.0, 8.0 Hz, 1H), 4.56 (dd, *J* = 8.8, 3.6 Hz, 1H), 4.42 (dd, *J* = 17.6, 3.6 Hz, 1H), 4.05 (d, *J* = 11.6 Hz, 1H), 3.40 (d, *J* = 11.6 Hz, 1H) 2.74-2.90 (m, 3H), 2.49 (t, *J* = 7.2 Hz, 2H), 2.28-2.34 (m, 1H), 2.12-2.19 (m, 1H), 1.78 (s, 3H), 1.58-1.63 (m, 2H), 1.24-1.26 (m, 10H), 0.84 (t, *J* = 6.8 Hz, 3H), 0.74 (d, *J* = 6.8 Hz, 3H), 0.65 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (CD_3OD, 100 MHz) δ 199.4, 173.9, 170.2, 169.6, 168.2, 158.6, 149.0, 141.7, 132.0, 128.5, 121.5, 117.9, 84.3, 72.4, 57.7, 43.4, 42.7, 42.7, 39.1, 33.4, 32.0, 31.4, 28.6, 28.5, 27.4, 25.3, 23.6, 22.2, 18.1, 16.1, 13.0; IR (neat) 2959, 2929, 2856, 1740, 1684, 1553, 1420, 1234, 987; HRMS (ESI) *m/z* calcd for $C_{31}H_{45}N_4O_5S_2$ $[M+H]^+$ 617.2826, found 617.2827; $[\alpha]_D$ = +94.3 (*c* 0.62, $CHCl_3$).

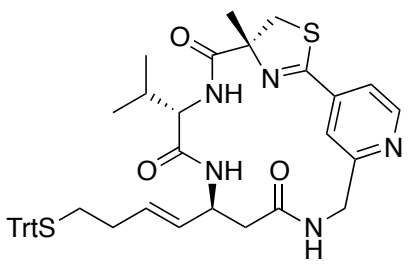


(*S*,*E*)-Methyl 3-((*S*)-2-((*tert*-butoxycarbonyl)amino)-3-methylbutanamido)-7-(tritylthio)hept-4-enoate (S8): To a solution of methyl (*S*,*E*)-3-((*tert*-butoxycarbonyl)amino)-7-(tritylthio)hept-4-enoate[2] (0.650 g, 1.22 mmol) in CH_2Cl_2 (37 mL) at 0 °C was treated with TFA (0.37 mL). After 2 h, the reaction mixture was concentrated under vacuum, taken back up in toluene (15 mL), and concentrated again. In another flask, Boc-L-valine (0.531 g, 2.44 mmol), PyBOP (1.27 g, 2.44 mmol), and DIPEA (0.65 mL, 3.66 mmol) were combined in CH_2Cl_2 (60 mL). The freshly deprotected amine was added via CH_2Cl_2 (10 mL) to the flask containing the activated acid at ambient temperature. After 3 h, the resulting mixture was concentrated under reduced pressure. The crude residue was purified using flash chromatography (5% to 50% ethyl acetate in hexanes) to obtain the desired amide (0.680 g, 88%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 7.15-7.37 (m, 15H), 6.49 (d, *J* = 8.0 Hz, 1H), 5.45 (dtd, *J* = 15.2, 6.4, 0.8 Hz, 1H), 5.34 (dd, *J* = 15.6, 6.0 Hz, 1H), 4.97-5.02 (m, 1H), 4.67-4.74 (m, 1H), 3.82-3.86 (m, 1H), 3.59 (s, 3H), 2.53 (d, *J* = 5.2 Hz, 2H), 2.05-2.16 (m, 3H), 2.01 (q, *J* = 6.8 Hz, 2H), 1.40 (s, 9H), 0.89 (d, *J* = 6.8 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 171.6, 170.5, 155.8, 144.8, 130.5, 129.5, 127.8, 126.5, 79.7, 66.5, 59.9, 51.7, 47.2, 38.7, 31.3, 31.0, 28.3, 19.3, 17.6; IR (neat) 3304, 2965, 1738, 1685, 1650, 1522, 1492, 1365, 1172, 751; HRMS (ESI) *m/z* calcd for $C_{37}H_{46}N_2NaO_5S$ $[M+Na]^+$ 653.3025, found 653.3032; $[\alpha]_D$ = –14.0 (*c* 1.21, $CHCl_3$).

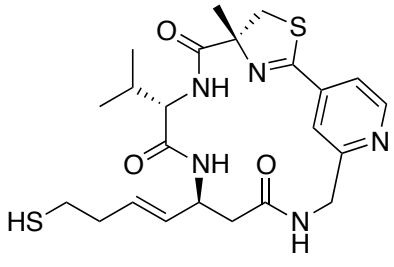


(*S*,*E*)-Methyl 3-((*S*)-2-((*R*)-2-(2-(((*tert*-butoxycarbonyl)amino)methyl)pyridin-4-yl)-4-methyl-4,5-dihydrothiazole-4-carboxamido)-3-methylbutanamido)-7-(tritylthio)hept-4-enoate (S9): To a solution of Boc protected amine **S8** (500 mg, 0.793 mmol) in CH_2Cl_2 (30 mL) at 0 °C was treated with TFA (3.0 mL). After 2 h, the reaction mixture was concentrated under vacuum, taken back up in toluene (15 mL), and concentrated again. In another flask, acid **S2** (278 mg, 0.793 mmol), PyBOP (822 mg, 1.58 mmol), and DIPEA (0.41 mL, 2.37 mmol) were combined in CH_2Cl_2 (35 mL). The freshly deprotected amine was added via CH_2Cl_2 (10 mL) to the flask containing the activated acid at ambient temperature. After 3 h,

the resulting mixture was concentrated under reduced pressure. The crude residue was purified using flash chromatography (20% to 100% ethyl acetate in hexanes) to obtain the desired amide (630 mg, 92%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.61 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.59-7.61 (m, 2H), 7.15-7.38 (m, 16H), 6.59 (d, *J* = 8.8 Hz, 1H), 5.47 (dtd, *J* = 15.6, 6.4, 0.8 Hz, 1H), 5.35 (dd, *J* = 15.2, 6.0 Hz, 1H), 4.69-4.75 (m, 1H), 4.48 (d, *J* = 5.2 Hz, 2H), 4.15 (dd, *J* = 8.8, 6.4 Hz, 1H), 3.79 (d, *J* = 11.6 Hz, 1H), 3.61 (s, 3H), 3.37 (d, *J* = 11.2 Hz, 1H), 2.57 (d, *J* = 5.2 Hz, 2H), 2.08-2.17 (m, 3H), 2.01 (q, *J* = 6.8 Hz, 2H), 1.55 (s, 3H), 1.45 (s, 9H), 0.82 (d, *J* = 6.4 Hz, 3H), 0.79 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 174.1, 171.6, 169.5, 167.2, 158.8, 155.9, 149.7, 144.8, 140.2, 130.6, 129.5, 129.5, 127.8, 126.5, 120.3, 120.0, 85.3, 79.6, 66.5, 58.4, 51.7, 47.3, 45.7, 41.7, 38.6, 31.4, 31.1, 31.0, 28.4, 24.4, 19.3, 17.9; IR (neat) 2969, 1654, 1511, 1443, 1390, 1366, 1168, 846, 750; HRMS (ESI) *m/z* calcd for $C_{48}H_{57}N_5NaO_6S_2$ $[M+Na]^+$ 886.3648, found 886.3661; $[\alpha]_D$ = –29.9 (*c* 0.64, $CHCl_3$).

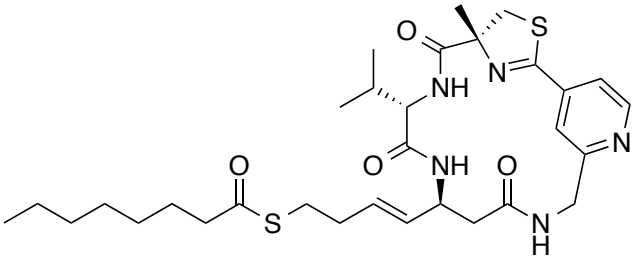


Trityl protected peptide pyridine "OUT" macrocycle (S10): To a solution of methyl ester **S9** (480 mg, 0.555 mmol) in THF/H_2O (15 mL, 2:1, v/v) at ambient temperature was added lithium hydroxide monohydrate (69.9 mg, 1.67 mmol). After 2 h, the reaction was cooled to 0 °C and acidified to a pH of ~3 using 1.0 M HCl. The mixture was extracted with CH_2Cl_2 (3 x 15 mL), dried over Na_2SO_4, and concentrated under vacuum. The freshly furnished acid was taken up in CH_2Cl_2 (20 mL) and cooled to 0 °C. The flask was treated with TFA (2.0 mL) and warmed to ambient temperature. After 2 h, the mixture was concentrated under reduced pressure, taken back up in toluene (25 mL), and concentrated again. The crude TFA salt was taken up in acetonitrile (20 mL) and was treated with DIPEA (0.61 mL, 3.52 mmol). The mixture was allowed to stir for 30 min. In another flask containing acetonitrile (550 mL) was added HATU (439 mg, 1.16 mmol), HOBt (157 mg, 1.16 mmol), and DIPEA (0.61 mL, 3.52 mmol) at room temperature. The flask containing the peptide was added to the flask containing the coupling reagents dropwise over 12 h using a syringe pump. After another 12 h, the reaction mixture was concentrated under reduced pressure. The crude residue was taken up in CH_2Cl_2 (15 mL) and the resulting solid was removed using filtration. The filtrate was concentrated and purified using flash chromatography (1% to 6% MeOH in DCM) to obtain the macrocycle (128 mg, 32%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.64 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.79 (d, *J* = 0.8 Hz, 1H), 7.16-7.32 (m, 16H), 6.29-6.35 (m, 2H), 6.12 (d, *J* = 10.0 Hz, 1H), 5.54-5.57 (m, 2H) 4.81-4.86 (m, 1H), 4.67 (dd, *J* = 17.2, 6.4 Hz, 1H), 4.40 (dd, *J* = 10.0, 4.4 Hz, 1H), 4.15 (dd, *J* = 17.2, 5.6 Hz, 1H), 3.80 (d, *J* = 11.6 Hz, 1H), 3.49 (d, *J* = 11.6 Hz, 1H), 2.72 (dd, *J* = 14.8, 4.8 Hz, 1H), 2.51 (dd, *J* = 14.8, 5.6 Hz, 1H), 2.34-2.41 (m, 1H), 2.23 (t, *J* = 7.2 Hz, 2H), 1.92-2.12 (m, 2H), 1.70 (s, 3H), 0.84 (d, *J* = 6.8 Hz, 3H), 0.60 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.1, 170.4, 170.1, 170.1, 159.1, 150.4, 144.6, 139.7, 130.3, 130.1, 129.4, 127.9, 126.7, 120.7, 117.9, 84.5, 66.7, 59.0, 47.9, 45.1, 44.1, 41.3, 31.3, 31.2, 23.8, 23.8, 19.4, 16.5; IR (neat) 2969, 2925, 1708, 1644, 1561, 1409, 1274, 1185, 1030, 842; HRMS (ESI) *m/z* calcd for $C_{42}H_{45}N_5NaO_3S_2$ $[M+Na]^+$ 754.2862, found 754.2867; $[\alpha]_D$ = +32.3 (*c* 0.13, $CHCl_3$).

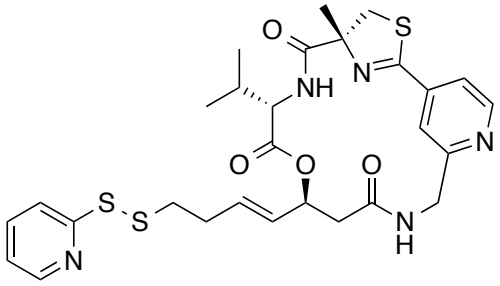


Peptide pyridine "OUT" thiol (S11): To a solution of trityl protected thiol **S10** (115 mg, 0.157 mmol) in CH_2Cl_2 (22.0 mL) at 0 °C was treated with TFA (0.90 mL) and triisopropylsilane (0.065 mL, 0.314 mmol). The reaction mixture was warmed to ambient temperature and stirred for 2 h. The mixture was concentrated and purified using flash chromatography (1% to 6% MeOH in CH_2Cl_2) to obtain the desired thiol (68.4 mg, 89%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.66 (d, *J* = 3.6 Hz, 1H), 7.76 (s, 1H), 7.35 (d, *J* = 4.4 Hz, 1H), 7.01 (d, *J* =8.4 Hz, 1H) 6.86-6.88 (m, 1H), 6.19 (d, *J* = 10.4 Hz, 1H), 5.52-5.53 (m, 2H), 4.89-4.91 (m, 1H), 4.85 (d, *J* = 17.6 Hz, 1H), 4.46 (dd, *J* = 10.4, 4.0 Hz, 1H), 4.36 (d, *J* = 17.2 Hz, 1H), 3.84 (d, *J* = 12.0 Hz, 1H), 3.48 (d, *J* = 12.0 Hz, 1H), 2.44-2.72 (m, 5H), 2.30 (q, *J* = 6.4 Hz, 2H), 1.78 (s, 3H), 1.31 (t, *J* = 7.2 Hz, 1H), 0.80 (d, *J* = 6.8

Hz, 3H), 0.49 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 173.3, 171.2, 159.6, 150.1, 140.3, 130.8, 128.9, 121.0, 117.8, 84.8, 58.6, 47.9, 44.0, 40.0, 36.2, 31.2, 24.0, 23.9, 23.7, 19.5, 16.1; IR (neat) 2965, 2930, 1655, 1534, 1410, 1201, 1142, 1026; HRMS (ESI) *m/z* calcd for $C_{23}H_{32}N_5O_3S_2$ $[M+H]^+$ 490.1947, found 490.1952; $[\alpha]_D$ = +129 (*c* 0.08, $CHCl_3$).



Peptide pyridine "OUT" (S12): To a solution of peptide pyridine "OUT" thiol **S11** (8.0 mg, 0.016 mmol) in CH_2Cl_2 (0.2 mL) at ambient temperature was treated with octanoyl chloride (.005 mL, 0.032 mmol), DIPEA (0.011 mL, 0.064 mmol), and DMAP (0.1 mg, 0.008 mmol). The reaction mixture was stirred for 2 h before cooling to 0 °C and quenching with methanol (5.0 mL). The mixture was cooled and concentrated and purified using flash chromatography (0.5% to 6% MeOH in CH_2Cl_2) to obtain the desired octanoyl masked thiol (5.4 mg, 55%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.65 (dd, *J* = 4.8, 0.8 Hz, 1H), 7.88 (d, *J* = 0.8 Hz, 1H), 7.33 (dd, *J* = 4.8, 1.6 Hz, 1H), 6.27-6.33 (m, 2H), 6.12 (d, *J* = 10.0 Hz, 1H), 5.61-5.72 (m, 2H), 4.90-4.95 (m, 1H), 4.75 (dd, *J* = 16.8, 6.8 Hz, 1H), 4.53 (dd, *J* = 17.2, 6.0 Hz, 1H), 4.46 (dd, *J* = 10.4, 4.0 Hz, 1H), 3.83 (d, *J* = 12.0, 1H), 3.48 (d, *J* = 11.6 Hz, 1H), 2.86 (t, *J* = 7.2 Hz, 2H), 2.71 (dd, *J* = 15.6, 4.4 Hz, 1H), 2.56 (dd, *J* = 15.6, 7.6 Hz, 1H), 2.43-2.51 (m, 2H), 2.26-2.33 (m, 1H), 1.77 (s, 3H), 1.56-1.64 (m, 2H), 1.24-1.29 (m, 10H), 0.85 (t, *J* = 6.8 Hz, 3H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.52 (d, *J* = 6.8 Hz, 3H); ^{13}C-NMR (CD_3OD, 100 MHz) δ 199.5, 173.4, 171.7, 170.9, 170.4, 158.3, 148.5, 142.1, 130.9, 128.5, 121.3, 118.9, 84.5, 58.1, 43.7, 43.4, 42.9, 39.2, 32.0, 31.4, 31.1, 28.6, 28.5, 27.7, 25.4, 22.3, 22.2, 18.9, 15.8, 12.9; IR (neat) 2956, 2927, 2855, 1679, 1649, 1519, 1403, 1031, 965; HRMS (ESI) *m/z* calcd for $C_{31}H_{46}N_5O_4S_2$ $[M+H]^+$ 616.2981, found 616.2981; $[\alpha]_D$ = +40.6 (*c* 0.35, $CHCl_3$).



Depsipeptide pyridine "OUT" pyridyl disulfide (S13): To a solution of thiol **S6** (20.0 mg, 0.040 mmol) in MeOH (0.2 mL) was added 1,2-di(pyridin-2-yl)disulfane (44.9 mg, 0.204 mmol). The reaction was stirred for 2 h before being concentrated and purified using flash chromatography (0.5% to 6% MeOH in CH_2Cl_2) to obtain the desired pyridyl disulfide inhibitor (17.3 mg, 71%): ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 8.67 (d, *J* = 5.2 Hz, 1H), 8.44 (d, *J* = 4.8 Hz, 1H), 7.79 (d, *J* = 0.4 Hz, 1H), 7.59-7.65 (m, 2H), 7.35 (dd, *J* = 4.8, 1.2 Hz, 1H), 7.07-7.10 (m, 1H), 6.95 (d, *J* = 9.2 Hz, 1H), 6.54 (dd, *J* = 8.0, 5.2 Hz, 1H), 5.92 (dt, *J* = 15.6, 6.4 Hz, 1H), 5.73-5.77 (m, 1H), 5.61 (dd, *J* = 15.6, 6.0 Hz, 1H), 5.06 (dd, *J* = 18.0, 8.4 Hz, 1H), 4.56 (dd, *J* = 9.2, 4.0 Hz, 1H), 4.27 (dd, *J* = 17.6, 4.8 Hz, 1H), 4.00 (d, *J* = 11.6 Hz, 1H), 3.37 (d, *J* = 11.6 Hz, 1H), 2.75-2.89 (m, 3H), 2.41-2.49 (m, 2H), 2.10-2.18 (m, 1H), 1.76 (s, 3H), 0.76 (d, *J* = 6.8 Hz, 3H), 0.66 (d, *J* = 6.8 Hz, 3H); HRMS (ESI) *m/z* calcd for $C_{28}H_{34}N_5O_4S_3$ $[M+H]^+$ 600.1773, found 600.1750.

References:

(1)Bowers, A.; West, N.; Taunton, J.; Schreiber, S. L.; Bradner, J. E.; Williams, R. M. Total synthesis and biological mode of action of largazole: a potent class I histone deacetylase inhibitor. *J. Am. Chem. Soc.* **2008**, *130*, 11219-11222.

(2) Bowers, A. A.; Greshock, T. J.; West, N.; Estiu, G.; Schreiber, S. L.; Wiest, O.; Williams, R. M.; Bradner, J. E. Synthesis and conformation-activity relationships of the peptide isosteres of FK228 and largazole. *J. Am. Chem. Soc.* **2009**, *131*, 2900-2905.