Supporting information

Organolithium-Mediated Diversification of Peptide Thiazoles

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Materials obtained commercially were reagent grade and were used without further purification. Anhydrous solvents were collected from a commercial drying/purification system assembled at UCSF. All lithiation reactions were run under an argon atmosphere. ¹H NMR and ¹³C NMR spectra were recorded on a Varian 400 spectrometer, ¹H NMR spectra are referenced as TMS at 0.0 ppm; ¹³C NMR spectra are referenced as the middle peak of triplet CDCl₃ at 77.0 ppm. High-resolution mass spectra were recorded by the UCSF mass spectrometry facility (EI HRMS were recorded on a VG70 from Micro Mass, Manchester UK; ESI HRMS were recorded on a Mariner Biospectrometry work station from ABI/Perseptive Instruments). Reactions were monitored by thin layer chromatography (TLC) with Merck silica gel 60 F₂₅₄ glass plates (0.25 mm-thick).

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General procedure for the determination of optical purity of thiazoles:

To determine the optical purity of the thiazole adducts (Tables 1 and 2), we followed a modified Marfey protocol developed by Fujii *et al.* (Fujii, K.; Ikai, Y.; Oka, H.; Suzuki, M.; Harada, K. *Anal. Chem.* **1997**, *69*, 5146). The diastereomeric excess (d.e.) of the L-FDLA and D-FDLA derivatives, which we used to define the enantiomeric excess (e.e.) of the original thiazole adducts (Tables 1 and 2), was quantified by analytical HPLC.

The *N*-Boc or *N*-trityl peptide thiazole (~2 mg) was dissolved in 1:1 CH₂Cl₂/TFA (100 μL). The reaction was kept at rt for 1 h with occasional agitation before the solvents were removed. To the residue was added acetone (100 μL) and NaHCO₃ solution (40 μL, 1 M), and the mixture split into two halves; to each half was added L-FDLA and D-FDLA (100 μL, 1% concentration in acetone), respectively. The reactions were kept overnight at rt with occasional agitation before adding 1 M HCl (20 μL). The reactions were then diluted with methanol/water (1:1, v/v) to 1 mL and then filtered. An aliquot (20 μL) was injected onto an analytical C18 HPLC column (Phenomenex Luna 3 μ C18(2), 74 mm × 4.60 mm; linear MeOH/water gradient over 10 min, 1 mL/min). We experimentally determined the e.e. for 16 out of 27 of the electrophilic adducts and found no evidence for racemization. As all of the electrophilic addition reactions were performed under near-identical conditions (−78 °C, 1-5 min), this should be more than sufficient coverage. In every case, the L-FDLA and D-FDLA adducts were well-resolved (1-3 min) by analytical HPLC. We note that the e.e. of commercial L-FDLA and D-FDLA is approximately 98%.

General procedure for reactions of 5-thiazolyl lithium with electrophiles without additive:

To a solution of peptide thiazole (**1-3**: 50 mM, 1.0 equiv, 0.1 to 0.2 mmol scale; **5** and **7**: 4.0 mg scale, 25 mM) in dry THF at −78 °C was rapidly added LDA (1.05 equiv; 2.1 equiv used for substrate **3** and 4.2 equiv used for **5** and **7**); after stirring for 30 to 60 sec at −78 °C, the electrophile was added (2-2.5 equiv). Liquid electrophiles were added neat via syringe; solid electrophiles were added as a 1 M solution in dry THF. For **5** and **7**, 8.0 equiv of iodine in THF were used. The reaction was stirred at −78 °C for 1-5 min before quenching with 10% citrate buffer (pH = 4.0) at −78 °C. The reaction mixture was then brought to rt and partitioned between 10% citrate buffer (pH = 4.0) and EtOAc; the organic layer was washed with sat. NaCl solution, dried over Na₂SO₄, filtered and concentrated; the residue was purified by column chromatography or preparative TLC (for ceratospongamide or *epi*-ceratospongamide, reverse phase HPLC was used for the purification).

Procedure for reaction of 5-thiazolyl lithium with 1-iodobutane in the presence of HMPA:

To a solution of peptide thiazole **2** (50 mM, 1.0 equiv, 0.1 to 0.2 mmol scale) in dry THF and HMPA (5.0 equiv) at -78°C was rapidly added LDA (1.05 equiv). After stirring at -78°C for 1 min, 1-iodobutane (2.5 equiv) was added via syringe. The resulting mixture was stirred at -78°C for another 5 min before quenching with 10% citrate buffer (pH = 4.0) at -78°C . The reaction mixture was then brought to rt and partitioned between 10% citrate buffer (pH = 4.0) and EtOAc; the organic layer was washed with sat. NaCl solution, dried over Na_2SO_4 , filtered and concentrated; the residue was purified by preparative TLC.

Procedure for reaction of 5-thiazolyl lithium with allyl bromide in the presence of $\text{CuCN}\cdot 2\text{LiCl}_2$:

LDA (1.05 equiv.) was rapidly added to a solution of peptide thiazole **1** or **2** (50 mM, 1.0 equiv, 0.1 to 0.2 mmol scale) in dry THF at -78°C . After 1 min, a solution of $\text{CuCN}\cdot 2\text{LiCl}_2$ in THF (0.1 equiv, 1 M solution) was added. After another 30 sec, allyl bromide (2.5 equiv) was added. The resulting mixture was stirred at -78°C for another 5 min before quenching with 10% citrate buffer (pH = 4.0) at -78°C . The reaction mixture was then brought to rt and partitioned between 10% citrate buffer (pH = 4.0) and EtOAc. The organic layer was washed with sat. NaCl solution, dried over Na_2SO_4 , filtered and concentrated; the residue was purified by preparative TLC.

5-Iodo thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 5.3-5.2 (1H, m), 3.96 (s, 3H), 3.6-3.4 (m, 2H), 2.4-2.2 (m, 2H), 1.96-1.90 (m, 2H), 1.49 (s, 9H, minor rotamer), 1.35 (s, 9H, major rotamer); ESI-MS (m/z): 461 ($\text{M}+\text{Na}^+$), 477 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{14}\text{H}_{19}\text{IN}_2\text{O}_4\text{SH}$ [$\text{M}+\text{H}^+$], 439.0189; Found 439.0218; Exact mass calcd for $\text{C}_{14}\text{H}_{19}\text{IN}_2\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}^+$], 461.0008; Found 460.9984; e.e. $\geq 98\%$.

5-Bromo thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 5.2-5.1 (1H, m), 3.95 (s, 3H), 3.6-3.4 (m, 2H), 2.35-2.2 (m, 2H), 2.0-1.95 (m, 2H), 1.49 (s, 9H, minor rotamer), 1.36 (s, 9H, major rotamer); ESI-MS (m/z): 413 ($\text{M}+\text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{14}\text{H}_{19}\text{BrN}_2\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}^+$], 413.0147; Found 413.0123.

5-Chloro thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 5.2-5.1 (m, 1H), 3.96 (s, 3H), 3.6-3.4 (m, 2H), 2.4-2.1 (m, 2H), 2.0-1.9 (m, 2H), 1.47 (s, 9H); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamers are shown) δ 173.4, 161.0, 140.5, 80.8, 59.9, 59.4, 52.4, 46.8, 34.0, 32.4, 29.6, 28.3, 23.9, 23.1; ESI-MS (m/z): 369 ($\text{M}+\text{Na}^+$), 385 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{14}\text{H}_{19}\text{ClN}_2\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}$] $^+$, 347.0832; Found 347.0827; e.e. $\geq$ 98%.

5-Tributylstannyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 5.3 (s, minor rotamer 1H), 5.21 (d, major rotamer 1H, $J = 5.2$ Hz), 3.94 (s, 3H), 3.65 (m, 1H), 3.53 (m, 1H), 2.4-2.15 (m, 2H), 1.94 (m, 1H), 1.6-1.4 (m, 6H), 1.4-1.2 (m, 12H), 1.2-1.1 (m, 1H), 0.87 (t, 9H, $J = 7.2$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamers are shown) δ 181.1, 163.5, 154.2, 151.4, 143.7, 80.1, 59.6, 59.3, 52.3, 46.6, 34.3, 28.9, 28.2, 27.1, 23.2, 13.6, 11.8; ESI MS (m/z): 625 ($\text{M}+\text{Na}^+$), 641 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{26}\text{H}_{46}\text{N}_2\text{O}_4\text{SSnH}$ [$\text{M}+\text{H}$] $^+$, 603.2278; Found 603.2208; e.e. $\geq$ 98% (The stannyl group was cleaved during Boc removal for optical purity determination).

5-benzylmercapto thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 7.4-7.2 (m, 5H), 5.3-5.0 (m, 1H), 4.20 (s, 2H), 3.93 (s, 3H), 3.84-3.40 (m, 2H), 2.40-2.15 (m, 2H), 2.0-1.8 (m, 2H), 1.48 (s, br, minor rotamer 9H), 1.32 (s, br, major rotamer 9H); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamers are shown) δ 172.2, 162.5, 161.8, 154.1, 139.0, 135.0, 129.0, 128.7, 127.9, 126.9, 80.5, 59.7, 59.1, 52.3, 52.2, 46.7, 41.3, 34.1, 32.6, 28.2, 23.9, 23.1; ESI-MS (m/z): 457 (100%, $\text{M}+\text{Na}^+$), 435 (5%, $\text{M}+\text{H}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_4\text{S}_2\text{Na}$ [$\text{M}+\text{Na}$] $^+$, 457.1232; Found 457.1249; e.e. $\geq$ 98%.

5-BocNH(Boc)N thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, multiple rotamers, all peaks are broad) δ 7.6 (br), 5.2.-5.0 (br, 1H), 3.942 (s, 3H), 3.6-3.3 (br, m, 2H), 2.4-2.2 (br, m, 2H), 1.9 (br, m, 2H), 1.5-1.3 (m, 27); ESI MS (m/z): 565 ($\text{M}+\text{Na}^+$), 581 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{24}\text{H}_{38}\text{N}_4\text{O}_8\text{SH}$ [$\text{M}+\text{H}^+$], 543.2489; Found 543.2501.

5-Ethoxycarbonyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 5.19 (s, br, minor rotamer 1H), 5.17 (s, br, major rotamer 1H), 4.36 (q, 2H, $J = 6.8$ Hz), 3.97 (s, br, 3H), 3.85-3.40 (m, 2H), 2.40-2.20 (m, 2H), 2.0-1.90 (m, 2H), 1.49 (s, br, minor rotamer 9H), 1.36 (s, br, major rotamer 3H), 1.362 (t, 3H, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamers are shown) δ 179.7, 178.7, 162.9, 160.3, 154.0, 148.8, 129.8, 80.8, 62.2, 59.6, 59.2, 52.9, 47.0, 46.7, 33.9, 32.5, 28.3, 23.8, 23.0, 14.0; ESI MS (m/z): 407 ($\text{M}+\text{Na}^+$), 423 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_6\text{SNa}$ [$\text{M}+\text{Na}^+$], 407.1253; Found 407.1253; e.e. $\geq 98\%$.

5-Benzoyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 7.9-7.4 (m, 5H), 5.3-5.2 (m, 1H), 3.62 (br, s, 3H), 3.6-3.4 (br, m, 2H), 2.5-2.3 (br, m, 2H), 2.1-1.9 (br, m, 2H), 1.6-1.2 (br, m, 9H); ^{13}C NMR (CDCl_3 , 100 MHz) 161.3, 137.0, 133.9, 129.2, 128.7, 119.0, 80.7, 59.6, 52.3, 46.8, 34.1, 32.7, 28.3, 23.8, 23.1; ESI-MS (m/z): 439 (100%, $\text{M}+\text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{21}\text{H}_{24}\text{N}_2\text{O}_5\text{SNa}$ [$\text{M}+\text{Na}^+$], 439.1304; Found 439.1332; e.e. $\geq 98\%$.

5-Formyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 10.5 (1H), 5.2 (m, 1H), 3.64 (br, s, 3H), 3.6-3.4 (br, m, 2H), 2.5-2.3 (br, m, 2H), 2.1-1.9 (br, m, 2H), 1.6-1.2 (br, m, 9H); ESI-MS (m/z): 341 ($\text{M}+1$); HRMS (ESI): Exact mass calcd for $\text{C}_{15}\text{H}_{20}\text{N}_2\text{O}_5\text{SH}$ [$\text{M}+\text{H}$] $^+$, 341.1171; Found 341.1192.

5-(1-Hydroxybenzyl) thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two diastereomers) δ Higher R_f isomer (two rotamers): 7.5-7.3 (m, 5H), 6.47 (s, br, 1H), 5.16 (s, br, minor rotamer 1H), 5.06 (s, br, major rotamer 1H), 4.46 (s, br, major rotamer 1H), 3.97 (s, 3H), 3.55-3.3 (m, 2H), 2.4-2.1 (m, 2H), 1.9-1.8 (m, 2H), 1.45 (s, br, minor rotamer 9H), 1.31 (s, br, major rotamer 9H); Lower R_f isomer (two rotamers): 7.5-7.3 (m, 5H), 6.58 (s, br, major rotamer 1H), 6.48 (s, br, minor rotamer 1H), 5.15 (s, minor rotamer 1H), 5.04 (s, br, major rotamer 1H), 3.97 (s, br, 3H), 3.6-3.3 (m, 2H), 2.4-2.2 (m, 2H), 1.95-1.85 (m, 2H), 1.41 (s, br, minor rotamer 9H), 1.21 (s, br, major rotamer 9H); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamer are shown) δ Higher R_f isomer: 174.0, 163.6, 154.1, 141.8, 128.5, 126.3, 80.5, 69.0, 59.5, 52.7, 46.6, 34.1, 33.1, 28.2, 23.8, 23.2; Lower R_f isomer: 174.5, 163.5, 154.0, 141.9, 128.5, 128.3, 126.1, 80.4, 68.8, 59.6, 52.7, 46.6, 34.1, 28.1, 23.8, 23.1; ESI-MS (m/z): 441 (100%, $\text{M}+\text{Na}^+$), 419 ($\text{M}+\text{H}^+$); HRMS (EI): Exact mass calcd for $\text{C}_{21}\text{H}_{26}\text{N}_2\text{O}_5\text{S}$ [M] $^+$, 418.1562; Found 418.1549 (for higher R_f isomer) and 418.1566 (for lower R_f isomer); e.e. $\geq 98\%$ for both diastereomers.

5-[2-(1-Hydroxy)propyl] thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 6.21 (s, br, 1H), 5.18 (s, br, minor rotamer 1H), 5.10 (s, br, major rotamer 1H), 4.00 (s, 3H), 3.7-3.4 (m, 2H), 2.4-2.1 (m, 2H), 2.0-1.9 (m, 2H), 1.68 (s, 6H), 1.49 (s, br, minor rotamer 9H), 1.33 (s, br, major rotamer 9H); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamers are shown) δ 171.6, 165.0, 158.7, 154.0, 138.7, 80.4, 69.4, 59.4, 53.1, 46.7, 34.2, 33.0, 31.2, 28.2, 23.8, 23.1; ESI-MS (m/z): 393 (100%, $\text{M}+\text{Na}^+$), 293 ($\text{M}-\text{Boc}+\text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_5\text{SH}$ [$\text{M}+\text{H}^+$], 371.1641; Found 371.1615; Exact mass calcd for $\text{C}_{17}\text{H}_{26}\text{N}_2\text{O}_5\text{SNa}$ [$\text{M}+\text{Na}^+$], 393.1460; Found 393.1434; e.e. $\geq 98\%$.

5-Methyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 5.17 (m, 1H), 3.93 (s, 3H), 3.55 (m, 2H), 2.74 (s, 3H), 2.5-2.2 (m, 2H), 2.1-1.9 (m, 2H), 1.35 (s, 3H); ESI MS (m/z): 349 ($\text{M}+\text{Na}^+$), 365 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}^+$], 349.1198; Found 349.1194.

5-Allyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz, rotamers) δ 6.05-5.9 (m, 1H), 5.3-5.05 (m, 3H), 4.05-3.9 (m, 2H), 3.93 (s, br, 3H), 3.7-3.4 (m, 2H), 2.4-2.1 (m, 2H), 2.0-1.85 (m, 2H), 1.43 (s, br, minor rotamer 9H), 1.33 (s, br, major rotamer 9H); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers, only partial peaks from the minor rotamers are shown) δ 173.0, 162.7, 154.7, 154.2, 147.9, 135.0, 134.0, 118.6, 117.4, 80.4, 59.6, 52.4, 52.1, 47.0, 46.7, 34.2, 33.0, 31.7, 28.3, 23.8, 23.1; ESI-MS (m/z): 375 ($\text{M}+\text{Na}^+$), 391 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{17}\text{H}_{24}\text{N}_2\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}^+$], 375.1354; Found 375.1368.

5-Iodo thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz) δ 7.5-7.1 (m, 15H), 4.30 (q, 1H, $J = 6.4$ Hz), 3.91 (s, 3H), 2.46 (br, s, 1H), 0.96 (d, 3H, $J = 6.4$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 186.1, 146.8, 145.5, 128.7, 128.0, 126.8, 72.2, 52.5, 52.4, 24.8 ppm; ESI-MS (m/z): 577 ($\text{M}+\text{Na}^+$);

HRMS (ESI): Exact mass calcd for $C_{26}H_{23}IN_2O_2SNa$ $[M+Na]^+$, 577.0423; Found 577.0403.

5-Chloro thiazole adduct:

1H NMR ($CDCl_3$, 400 MHz) δ 7.5-7.1 (m, 15H), 4.20 (q, 1H, $J = 6.8$ Hz), 3.91 (s, 3H), 2.45 (s, br, 1H), 0.97 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.3, 161.2, 145.4, 140.0, 134.9, 128.7, 128.0, 126.8, 72.2, 52.5, 52.3, 24.6; ESI MS (m/z): 485 ($M+Na^+$), 501 ($M+K^+$); HRMS (ESI): Exact mass calcd for $C_{26}H_{23}ClN_2O_2SNa$ $[M+Na]^+$, 485.1066; Found 485.1081.

5-Tributylstannyl thiazole adduct:

1H NMR ($CDCl_3$, 400 MHz) δ 7.5-7.1 (m, 15 H), 4.29 (p, 1 H, $J = 6.8$ Hz), 3.89 (s, 3H), 2.50 (d, 1 H, $J = 6.8$ Hz), 1.6-1.0 (m, 18 H), 0.99-0.89 (m, 12 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 185.2, 163.7, 151.0, 145.9, 143.9, 128.8, 127.9, 127.8, 126.5, 72.1, 52.19, 52.17, 29.0, 27.2, 25.3, 13.7, 11.8; ESI MS (m/z): 720 ($M+1$) (for major tin isotope with a mass of 120); HRMS (ESI): Exact mass calcd for $C_{38}H_{50}N_2O_2SSnH$ $[M+H]^+$, 719.2693; Found 719.2745; e.e. $\geq 98\%$ (The stannyl group was cleaved during the Tr deprotection step).

5-Benzylmercapto thiazole adduct:

1H NMR ($CDCl_3$, 400 MHz) δ 7.5-7.1 (m, 20 H), 4.22 (s, 2H), 4.16 (q, 1H, $J = 6.8$ Hz), 3.88 (s, 3H), 2.37 (br, s, 1H), 0.92 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.1, 162.7, 145.6, 138.6, 135.4, 129.1, 128.7, 128.6, 128.0, 127.9, 126.6, 72.1, 52.2, 52.1, 41.1, 24.7; ESI MS (m/z): 551 ($M+1$), 573 ($M+Na^+$); HRMS (ESI): Exact mass calcd for $C_{33}H_{30}N_2O_2S_2Na$ $[M+Na]^+$, 573.1646; Found 573.1624; e.e. $\geq 98\%$.

5-BocHNBOcN thiazole adduct:

1H NMR ($CDCl_3$, 400 MHz, multiple rotamers, all peaks are broad) δ 7.72 (br, 1H), 7.5-7.1 (m, 15H), 4.15 (br, 1H), 3.90 (s, 3H), 2.35 (br, 3H), 1.55-1.46 (br, m, 18H), 0.85 (br, d, 3H); ESI-MS (m/z): 697 (7%, $M+K^+$), 681 (100%, $M+Na^+$); HRMS (ESI): Exact mass calcd for $C_{36}H_{42}N_4O_6SNa$ $[M+Na]^+$, 681.2723; Found 681.2722.

5-Ethoxycarbonyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz) δ 7.5-7.1 (m, 15 H), 4.38 (q, 2H, $J = 6.8$ Hz), 4.20 (q, 1H, $J = 6.8$ Hz), 3.93 (s, 3H), 2.48 (br, s, 1H), 1.39 (t, 3H, $J = 6.8$ Hz), 0.94 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 184.5, 163.0, 160.8, 148.4, 145.6, 130.1, 128.7, 128.0, 126.8, 72.1, 62.1, 52.8, 52.3, 24.6, 14.1; ESI MS (m/z): 523 ($\text{M} + \text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{29}\text{H}_{28}\text{N}_2\text{O}_4\text{SNa}$ [$\text{M} + \text{Na}$] $^+$, 523.1667; Found 523.1672; e.e. $\geq 98\%$.

5-Carbonyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz) δ 7.8-7.1 (m, 20 H), 4.37 (q, 1H, $J = 6.8$ Hz), 3.58 (s, 3H), 2.69 (br, s, 1H), 1.17 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 188.8, 182.7, 161.5, 145.4, 144.3, 141.2, 137.1, 133.7, 129.3, 128.9, 128.8, 128.6, 128.0, 126.8, 72.1, 52.2, 52.2, 25.1; ESI MS (m/z): 555 ($\text{M} + \text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{33}\text{H}_{28}\text{N}_2\text{O}_3\text{SNa}$ [$\text{M} + \text{Na}$] $^+$, 555.1718; Found 555.1697; e.e. $\geq 98\%$.

5-Formyl thiazole adduct:

^1H NMR (CDCl_3 , 400 MHz) δ 10.7 (s, 1H), 7.5-7.1 (m, 15H), 4.29 (q, 1H, $J = 6.6$ Hz), 3.99 (s, 3H), 2.55 (s, br, 1H), 1.01 (d, 3H, $J = 6.6$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 187.7, 184.9, 161.5, 148.0, 145.3, 143.8, 128.7, 128.0, 126.8, 72.2, 52.9, 52.7, 24.7; ESI MS (m/z): 479 ($\text{M} + 23$); HRMS (ESI): Exact mass calcd for $\text{C}_{27}\text{H}_{24}\text{N}_2\text{O}_3\text{SNa}$ [$\text{M} + \text{Na}$] $^+$, 479.1405; Found 479.1404.

5-(1-Hydroxybenzyl) thiazole adduct:

Higher R_f diastereomer: ^1H NMR (CDCl_3 , 400 MHz) δ 7.5-7.1 (m, 15H), 6.48 (s, 1H), 4.20 (q, 1H, $J = 6.8$ Hz), 3.91 (s, 3H), 2.48 (s, br, 1H), 1.00 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR

(CDCl₃, 100 MHz) δ 178.58, 163.68, 153.56, 145.62, 141.94, 139.65, 128.81, 128.42, 128.11, 127.88, 126.63, 126.39, 72.07, 69.02, 52.53, 52.10, 25.19; ESI MS: 557 (M+Na⁺), 573 (M+K⁺); HRMS (ESI): Exact mass calcd for C₃₃H₃₀N₂O₃SNa [M+Na]⁺, 557.1875; Found 557.1883; e.e. $\geq$ 98%.

Lower R_f diastereomer: ¹H NMR (CDCl₃, 400 MHz) δ 7.5-7.1 (m, 15H), 6.45 (s, 1H), 4.22 (q, 1H, *J* = 6.8 Hz), 3.91 (s, 3H), 2.50 (s, br, 1H), 1.07 (d, 3H, *J* = 6.8 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 178.76, 163.71, 153.62, 145.50, 141.80, 139.63, 128.85, 128.44, 128.19, 127.86, 126.66, 126.53, 72.05, 69.21, 52.52, 52.17, 25.32; ESI MS (m/z): 535 (M+1), 557 (M+Na⁺), 573 (M+K⁺); HRMS (ESI): Exact mass calcd for C₃₃H₃₀N₂O₃SNa [M+Na]⁺, 557.1875; Found 557.1893; e.e. $\geq$ 98%.

5-(2-Hydroxyl-2-propyl) thiazole adduct:

¹H NMR (CDCl₃, 400 MHz) δ 7.5-7.1 (m, 15H), 6.24 (s, 1H), 4.25 (q, 1H, *J* = 6.8 Hz), 3.94 (s, 3H), 2.50 (s, br, 1H), 1.647 (s, 3H), 1.643 (s, 3H), 1.07 (d, 3H, *J* = 6.8 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 175.9, 165.3, 159.0, 145.5, 138.2, 128.8, 127.9, 126.7, 72.1, 69.4, 52.9, 52.0, 31.24, 31.12, 25.3; ESI MS (m/z): 509 (M+23); HRMS (ESI): Exact mass calcd for C₂₉H₃₀N₂O₃SNa [M+Na]⁺, 509.1875; Found 509.1866.

5-(1-Hydroxyl-cyclohex-2-ene-yl) thiazole adduct:

¹H NMR (CDCl₃, 400 MHz, diastereomers) δ 7.5-7.1 (m, 30H), 6.39 (m, 1H), 6.1-5.9 (m, 3H), 4.12 (m, 2H), 3.94 (s, 6H), 2.44 (br, 2H), 2.05 (m), 1.05-0.97 (m); ¹³C NMR (CDCl₃, 100 MHz, diastereomers) δ 176.9, 176.6, 165.2, 158.7, 158.6, 145.7, 145.59, 145.55, 138.0, 128.9, 128.8, 128.1, 127.9, 127.8, 127.5, 126.7, 72.10, 72.06, 69.12, 69.07, 52.97, 52.20, 52.04, 37.4, 37.3, 25.4, 25.2, 27.8, 19.2; ESI-MS (m/z): 563 (5%, M+K⁺), 547 (100%, M+Na⁺); HRMS (ESI): Exact mass calcd for C₃₂H₃₂N₂O₃SNa [M+Na]⁺, 547.2031; Found 547.2007.

5-Methyl thiazole adduct:

¹H NMR (CDCl₃, 400 MHz) δ 7.5-7.1 (m, 15 H), 4.18 (q, 1H, *J* = 7.2 Hz), 3.88 (s, 3H), 2.71 (s, 3H), 2.33 (br, s, 1H), 0.90 (d, 3H, *J* = 7.2 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 176.5, 163.1, 145.8, 144.8, 140.2, 128.7, 128.0, 127.9, 126.5, 72.1, 52.0, 51.8, 24.9, 13.0;

ESI MS (m/z): 465 (M+Na⁺), 481 (M+K⁺); HRMS (ESI): Exact mass calcd for C₂₇H₂₆N₂O₂SNa [M+Na]⁺, 465.1613; Found 465.1613; e.e. ≥ 98%.

5-Allyl thiazole adduct:

¹H NMR (CDCl₃, 400 MHz) δ 7.5-7.1 (m, 15H), 5.97 (m, 1H), 5.17 (m, 1H), 5.14 (m, 1H), 4.21 (q, 1H, *J* = 6.8 Hz), 3.93 (m, 2H), 3.88 (s, 3H), 2.51 (br, 1H), 0.96 (d, 3H, *J* = 6.8 Hz); ¹³C NMR (CDCl₃, 100 MHz) δ 177.4, 162.9, 148.0, 145.7, 139.6, 135.3, 128.8, 127.9, 126.6, 117.0, 72.1, 52.1, 51.9, 31.7, 25.1; ESI-MS (m/z): 491 (100%, M+Na⁺), 507 (M+K⁺); HRMS (ESI): Exact mass calcd for C₂₉H₂₈N₂O₂SNa [M+Na]⁺, 491.1709; Found 491.1694; e.e. ≥ 98%.

5-*n*-Butyl thiazole adduct:

¹H NMR (CDCl₃, 400 MHz) δ 7.5-7.1 (m, 15H), 4.22 (q, 1H, *J* = 6.8 Hz), 3.88 (s, 3H), 3.17 (t, 2H, *J* = 7.8 Hz), 2.45 (br, 1H), 1.65 (m, 2H), 1.42 (m, 2H), 1.0-0.94 (m, 6H); ¹³C NMR (CDCl₃, 100 MHz) δ 176.53, 163.1, 151.1, 145.8, 128.8, 127.9, 126.6, 72.1, 52.1, 51.9, 33.7, 27.1, 25.1, 22.4, 13.8 ppm; ESI MS (m/z): 507 (M+Na⁺), 523 (M+K⁺); HRMS (ESI): Exact mass calcd for C₃₀H₃₂N₂O₂SNa [M+Na]⁺, 507.2082; Found 507.2075; e.e. ≥ 98%.

5-Iodo thiazole adduct:

¹H NMR (CDCl₃, 400 MHz) δ 7.5-7.1 (m, 15H), 7.09 (d, 1H, *J* = 8.0 Hz), 4.2-4.1 (m, 2H), 2.38 (br), 1.22 (t, 6 H, *J* = 6.0 Hz), 0.92 (d, 3H, *J* = 6.8 Hz); ESI-MS (m/z): 604 (M+Na⁺); HRMS (ESI): Exact mass calcd for C₂₈H₂₈IN₃OSNa [M+Na]⁺, 604.0896; Found 604.0899.

5-Iodo thiazole adduct of ceratospongamide:

^1H NMR (CDCl_3 , 400 MHz, two rotamers at a ratio of 1:6, peaks from minor rotamer shown partly) δ 9.06 (d, 1H, $J = 7.2$ Hz, from minor rotamer), 8.70 (d, 1H, $J = 8.0$ Hz, from minor rotamer), 8.32 (d, 1H, $J = 6.4$ Hz), 8.07 (d, 1H, $J = 9.2$ Hz, from minor rotamer), 7.5-7.2 (m, 10H), 6.60 (d, 1H, $J = 9.2$ Hz), 6.51 (d, 1H, $J = 9.6$ Hz), 5.54 (dd, 1H, $J = 3.8$ Hz, 8.6 Hz, from minor rotamer), 5.21 (m, 1H, from minor rotamer), 5.16 (dd, 1H, $J < 1.0$ Hz, $J = 6.6$ Hz), 5.00 (m, 1H, from minor rotamer), 4.84 (dd, 1H, $J = 2.4$ Hz, 7.6 Hz, from minor rotamer), 4.79-4.70 (m), 4.65-4.60 (m), 4.15 (dd, 1H, $J = 2.0$ Hz, 7.6 Hz, from minor rotamer), 4.03 (dd, 1H, $J = 6.4$ Hz, 9.6 Hz), 4.00-3.95 (m), 3.74-3.69 (m), 3.60-3.43 (m), 3.40 (d, 1H, $J = 7.6$ Hz), 3.18 (dd, 1H, $J = 2.0$ Hz, 9.2 Hz), 3.06 (dd, 1H, $J = 8.0$ Hz, 13.2 Hz), 2.90-2.81 (m, 2H), 2.52 (m, 1), 2.35-2.30 (m), 2.06-2.00 (m), 2.00-1.90 (m), 1.80-1.70 (m), 1.46 (d, 3H, $J = 6.4$ Hz, from minor rotamer), 1.36 (d, 3H, $J = 6.4$ Hz), 1.34-1.30 (m), 1.12-1.00 (m), 0.89 (t, 3H, $J = 7.2$ Hz), 0.83 (d, 3H, $J = 6.8$ Hz); ESI-MS (m/z): 916 ($\text{M}+\text{Na}^+$), 894 ($\text{M}+\text{H}^+$).

5-Iodo thiazole adduct of *epi*-ceratospongamide:

^1H NMR (CDCl_3 , 400 MHz) δ 8.52 (d, 1H, $J = 8.0$ Hz), 7.31-7.00 (m, 10H), 6.90 (br, 2H), 5.51 (dd, 1H, $J = 2.8$ Hz, 8.0 Hz), 5.06 (dt, 1H, $J = 1.6$ Hz, 11.2 Hz), 4.98 (dd, 1H, $J = 3.2$ Hz, 8.4 Hz), 4.96 (m, 1H), 4.94 (m, 1H), 4.31 (t, 1H, $J = 6.6$ Hz), 4.01 (m, 1H), 3.96 (d, 1H, $J = 6.4$ Hz), 3.9-3.8 (m, 2H), 3.47 (t, 1H, $J = 12.4$ Hz), 3.38-3.33 (m, 1H), 3.18 (dd, 1H, $J = 3.0$ Hz, 13.4 Hz), 2.97 (dd, 1H, $J = 6.4$ Hz, 13.6 Hz), 2.91 (dd, 1H, $J = 8.4$ Hz, 13.6 Hz), 2.61 (m, 1H), 2.50-2.44 (m, 2H), 2.21-2.09 (m), 1.95-1.90 (m), 1.51 (m, 1), 1.30 (d, 3H, $J = 6.0$ Hz), 1.17 (m, 1H), 1.00 (t, 3H, $J = 7.2$ Hz), 0.87 (d, 3H, $J = 6.8$ Hz); ESI-MS (m/z): 916 ($\text{M}+\text{Na}^+$), 894 ($\text{M}+\text{H}^+$).

Preparation of thiazole substrates 1-3:

Tr-L-Ala-Thiazole methyl ester (2):

a. EDC/HOBt/NMM, CH₂Cl₂; b. Deoxo-Fluor, -78 °C, CH₂Cl₂; c. H₂S, MeOH/Et₃N; d. Deoxo-Fluor, -78 °C, CH₂Cl₂; e. MnO₂, CH₂Cl₂, reflux.

To a suspension of *N*-trityl L-alanine (10.0 g, 30.2 mmol), L-serine methyl ester hydrochloride (4.7 g, 30.2 mmol), and HOBt (5.1 g, 33.2 mmol) in dry CH₂Cl₂ (300 mL), was added *N*-methyl morpholine (11.6 mL, 105.7 mmol) at rt, then cooled to 0 °C with an ice-water bath. EDC (6.4 g, 33.2 mmol) was then added to the above mixture portion-wise over 2 min. The resulting mixture was stirred overnight and allowed to warm to rt slowly. The reaction mixture was partitioned between CH₂Cl₂ and 10% citrate buffer (pH = 4.0); the organic layer was then washed with sat. NaHCO₃ and sat. NaCl, dried over anhydrous Na₂SO₄, and concentrated to give the crude dipeptide (12.75 g, 98%).

To a cold solution (-78 °C) of the crude dipeptide (12.5 g, 28.9 mmol) in dry CH₂Cl₂ (100 mL) was added Deoxo-Fluor (8.0 mL, 43.3 mmol) under argon. After stirring for 1 hr at -78 °C, the reaction mixture was quenched by slow addition of sat. NaHCO₃ solution, bringing the pH of reaction mixture to at least 7. The mixture was partitioned between CH₂Cl₂ and sat. NaHCO₃. The organic layer was washed with 10% citrate buffer (pH = 4.0) and sat. NaCl solution, dried over anhydrous Na₂SO₄, and concentrated to give the crude oxazoline (7.27 g, 60%).

The crude oxazoline (7.0 g) from the previous step was dissolved in MeOH (100 mL) and Et₃N (50 mL). Hydrogen sulfide was then bubbled through the solution (~10 g H₂S was absorbed). The reaction was stirred overnight at rt and the solvent was removed. The residue was partitioned between 10% citrate buffer (pH = 4.0) and EtOAc. The organic layer was washed with sat. NaCl solution, dried over anhydrous Na₂SO₄, and concentrated to give the crude thioamide (7.7 g, 100%).

The crude thioamide from the previous step (7.6 g, 16.9 mmol) was dissolved in dry CH₂Cl₂ (170 mL) and cooled to -78 °C under argon, followed by dropwise addition of Deoxo-Fluor (4.7 mL, 25.4 mmol). The reaction was stirred at -78 °C for one hour

before quenching with sat. NaHCO_3 solution. The organic layer was washed with sat. NaCl solution, dried over anhydrous Na_2SO_4 , and concentrated to give the crude thiazoline (7.1 g, 97%).

To the crude thiazoline (7.0 g, 16.3 mmol) in dry CH_2Cl_2 (100 mL) was added MnO_2 (8.5 g, 97.5 mmol). The mixture was refluxed for two days, filtered, and the filtrate was concentrated. The residue was purified by column chromatography to give the pure thiazole dipeptide **2** (5.8 g, 83%).

^1H NMR (CDCl_3 , 400 MHz) δ 8.01 (s, 1H), 7.5-7.1 (m, 15H), 4.28 (p, 1H, $J = 6.8$ Hz), 3.91 (s, 3H), 2.48 (d, 1H, $J = 6.8$ Hz), 0.99 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 181.6, 162.0, 146.2, 145.6, 128.7, 128.0, 127.8, 127.5, 126.6, 72.0, 52.1, 52.0, 25.0 ppm; ESI MS (m/z): 451 ($\text{M}+\text{Na}^+$), 467 ($\text{M}+\text{K}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{26}\text{H}_{24}\text{N}_2\text{O}_2\text{SH}$ [$\text{M}+\text{Na}^+$], 451.1456; Found 451.1441.

Boc-L-Pro-thiazole methyl ester (**1**)

The preparation of Boc-L-Pro-Thia-OMe (**1**) employed the same protocol as that of **2** shown above.

^1H NMR (CDCl_3 , 400 MHz, two rotamers, all peaks are broad) δ 8.07 (s, br, 1H), 5.23 (s, br, minor rotamer 1H), 5.17 (s, br, major rotamer 1H), 3.92 (s, 3H), 3.65-3.35 (m, br, 2H), 2.4-2.1 (m, br, 2H), 1.95-1.82 (m, 2H), 1.46 (s, br, minor rotamer 9H), 1.30 (s, br, major rotamer 9H); ^{13}C NMR (CDCl_3 , 100 MHz, two rotamers) δ 176.4, 175.5, 161.1, 153.9, 153.4, 146.1, 126.5, 79.6, 58.9, 58.5, 51.6, 46.4, 46.1, 33.6, 32.3, 27.6, 23.3, 22.5; ESI MS (m/z): 335 ($\text{M}+1$), 351 ($\text{M}+\text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{14}\text{H}_{20}\text{N}_2\text{O}_4\text{SNa}$ [$\text{M}+\text{Na}^+$], 335.1041; Found 335.1030.

Tr-L-Ala-Thia-NHPr-*i* (**3**)

Thiazole **2** (107 mg, 0.25 mmol) was dissolved in isopropylamine (1 mL) and treated with a catalytic amount of 25% NaOMe in MeOH (50 μL). The resulting clear solution was heated at 45 $^\circ\text{C}$ for 6 h, during which time solids precipitated from the reaction mixture. The solvents were removed to give a residue, which was partitioned between 10% citrate buffer (pH = 4.0) and EtOAc . The organic layer was washed with saturated NaCl aqueous solution, dried over anhydrous Na_2SO_4 , and concentrated under vacuum. The residue was then purified by column chromatography to give *N*-isopropyl carboxamide **3** (65 mg, 57%) along with recovered starting material **2** (40 mg, 37%).

^1H NMR (CDCl_3 , 400 MHz) δ 7.88 (s, 1H), 7.5-7.1 (m, 15H), 6.94 (d, $J = 7.6$ Hz), 4.3-4.1 (m, 2H), 2.44 (br, d, 1H), 1.25 (d, 3H, $J = 4.0$ Hz), 1.23 (d, 3H, $J = 4.0$ Hz), 0.99 (d, 3H, $J = 6.8$ Hz); ^{13}C NMR (CDCl_3 , 100 MHz) δ 179.8, 160.1, 149.6, 145.6, 129-126 (m), 71.7, 51.6, 40.8, 22.4; ESI-MS (m/z): 478 ($\text{M}+\text{Na}^+$); HRMS (ESI): Exact mass calcd for $\text{C}_{28}\text{H}_{29}\text{N}_3\text{OSH}$ $[\text{M}+\text{H}]^+$, 478.1929; Found 478.1928.

Selected NMR spectra for thiazoles

Higher R_f diastereomer

Lower R_f diastereomer