Supporting Information

Synthetic Fermentation of β-Peptide Macrocycles by Thiadiazole-Forming Ring-Closing Reactions

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General Methods

Reactions and Purifications
Reactions were carried out under air unless otherwise stated. Thin layer chromatography (TLC) was performed on Merck TLC plates (0.25 mm) pre-coated with silica gel 60 F254 and visualized by UV quenching and/or staining with potassium permanganate stain and warming with a heat gun. Flash column chromatography was performed under a forced-flow of air using Silicycle SiliaFlash F60 (40-63 mm particle size). Macrocycle mixtures were analyzed and purified by reversed phase high performance liquid chromatography (RP-HPLC) on Jasco analytical and preparative instruments with dual pumps, mixer and in-line degasser, a variable wavelength UV detector (simultaneous monitoring of the eluent at 220 nm, 254 nm, 301 nm) and a Rheodyne 7725i injector fitted with a 20 μL injection loop. The mobile phase for analytical and preparative HPLC were Millipore-H₂O with 0.1% TFA (Buffer A) and HPLC grade CH₃CN with 0.1% TFA (Buffer B). Analytical HPLC was performed on Shiseido C18 (5 μm, 4.6 mm I.D. x 250 mm) column at a flow rate of 1 mL/min. Preparative HPLC was performed on YMC C18 (5 μm, 20 mm I.D. x 250 mm) column at a flow rate of 10 mL/min. LCMS analysis was performed on Dionex UltiMate 3000 RSLC connected to a Surveyor MSQ Plus mass spectrometer; a reversed-phase RESTEK Pinnacle DB C18 (4.6 x 50 mm) column was used, running a gradient of 5 to 100% CH₃CN in H₂O over 6.5 min, 100% CH₃CN for 2.5 min.

Characterization
NMR spectra were recorded on Bruker AV-400 or AV-III-600 instruments. Chemical shifts (δ) are given in ppm relative to residual solvent peaks. Data for 1H NMR are reported as follows: chemical shift (multiplicity, coupling constants where applicable, number of hydrogens). Abbreviations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), dd (doublet of doublet), m (multiplet), br (broad), ABq (AB quartet). IR spectra were recorded on a Jasco FT/IR-4100 spectrometer and major peaks are reported in frequency of absorption (cm⁻¹). Optical rotations were measured on a Jasco P-2000 operating at the sodium D line with a 100 mm path length cell. High-resolution mass spectra were obtained by the mass spectrometry service of the ETH Zürich Laboratorium für Organische Chemie on a Bruker Daltonics maXis ESI-QTOF spectrometer (ESI).
**Solvents and Reagents**

All organic solvents (CH$_3$CN, DMF, t-BuOH, Et$_2$O, MeOH) were used as supplied (ACS or HPLC grade) unless otherwise stated. THF was purified by distillation over sodium benzophenone ketyl prior to use. CH$_2$Cl$_2$ was purified by distillation over calcium hydride. H$_2$O used for reactions was obtained from a Millipore purification system. All other starting materials were used as supplied by commercial vendors or prepared by the method described in the corresponding reference.

**Synthesis of Building Blocks**

1.1 **Synthesis of Initiator 16**

1.1.1 3-Iodophenylhydrazide 19

EDCI·HCl (0.95 g, 4.98 mmol, 1.1 equiv) was added to a suspension of 3-iodobenzoic acid (1.20 g, 4.98 mmol, 1.1 equiv) and HOBt (0.67 g, 4.98 mmol, 1.1 equiv) in CH$_2$Cl$_2$ (23 mL) at rt. The mixture was stirred for 10 min to give a yellow solution. t-Butylcarbazate (0.60 g, 4.52 mmol, 1.0 equiv) and EtNPr$_2$ (3.2 mL, 18.1 mmol, 4.0 equiv) were added and the mixture was stirred for 16 h. The resulting suspension was cooled to 0 °C, filtered and washed with cold CH$_2$Cl$_2$, to afford the desired product 19 (1.05 g, 64%) as a white solid.

**MP** 198–199 °C; **$^1$H NMR** (400 MHz, DMSO-$d_6$) $\delta$ 10.29 (s, 1H), 8.96 (s, 1H), 8.19 (s, 1H), 7.94 (d, $J = 7.8$ Hz, 1H), 7.86 (d, $J = 7.8$ Hz, 1H), 7.31 (t, $J = 7.8$ Hz, 1H), 1.43 (s, 9H); **$^{13}$C NMR** (101 MHz, DMSO-$d_6$) $\delta$ 164.6, 155.4, 140.3, 135.8, 134.5, 130.7, 126.7, 94.7, 79.3, 28.1; **IR** ($\nu$/cm$^{-1}$, thin film): 3232 (br), 2980, 1718, 1659, 1425, 1394, 1366, 1250, 1152, 1066, 699; **HRMS** (ESI): calculated for C$_{12}$H$_{15}$IN$_2$NaO$_3$ [M+Na]$^+$: 385.0020, found: 385.0025.
1.1.2 Aldehyde 21

3-Iodophenylhydrazide 19 (1.00 g, 2.76 mmol, 1.0 equiv), Pd(OAc)$_2$ (37 mg, 0.17 mmol, 6 mol%), tetrabutylammonium bromide (0.89 g, 2.76 mmol, 1.0 equiv), NaHCO$_3$ (0.58 g, 6.90 mmol, 2.5 equiv) and MS 4Å were placed under a N$_2$ atmosphere in a flame dried flask. DMF (8.3 mL) was added, followed by allyl alcohol (0.28 mL, 4.14 mmol, 1.5 equiv) and the mixture was heated to 70 ºC for 4 h. The resulting mixture was diluted with EtOAc, washed with H$_2$O and brine, dried over Na$_2$SO$_4$, and concentrated in vacuo. The crude mixture was purified by flash chromatography (hexanes/EtOAc, 4:1→1:1) to afford the desired product 21 (0.66 g, 81%) as a white solid.

**MP** 60–61 ºC; **$^1$H NMR** (400 MHz, CDCl$_3$) $\delta$ 9.79 (s, 1H), 8.95 (br. s, 1H), 7.67 (s, 1H), 7.61 (d, J = 7.4 Hz, 1H), 7.38 – 7.17 (m, 2H), 6.96 (br. s, 1H), 2.91 (t, J = 7.5 Hz, 2H), 2.76 (t, J = 7.5 Hz, 2H), 1.48 (s, 9H); **$^{13}$C NMR** (101 MHz, CDCl$_3$) $\delta$ 201.3, 166.9, 156.4, 141.1, 132.4, 132.0, 128.9, 127.3, 125.4, 82.2, 45.0, 28.2, 27.9; **IR** ($\upsilon$/cm$^{-1}$, thin film): 3281 (br), 2978, 2932, 1713, 1661, 1524, 1480, 1393, 1367, 1252, 1156, 730; **HRMS** (ESI): calculated for C$_{15}$H$_{20}$N$_2$NaO$_4$ [M+Na]$^+$: 315.1315, found: 315.1316.

1.1.3 Enol ester 23

Tetramethylguanidine (0.25 mL, 2.05 mmol, 1.2 equiv) was added dropwise to a mixture of phosphonate 22$^{1,2}$ (0.55 g, 1.88 mmol, 1.1 equiv) and LiCl (86 mg, 2.05 mmol, 1.2 equiv) in THF (9 mL) at −10 ºC under an atmosphere of N$_2$. After stirring for 30 min a solution of aldehyde 21 (0.50 g, 1.71 mmol, 1.0 equiv) in THF (2 mL) was added. The resulting mixture was stirred for 10 min, diluted with sat. aq. NH$_4$Cl and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$ and concentrated in vacuo. The crude mixture was purified
by flash chromatography (hexanes/EtOAc, 4:1→2:1) to afford the desired product 23 (0.53 g, 72%, 2:1 mixture of isomers) as a white solid.

**MP** 46–48 °C; **1H NMR** (400 MHz, CDCl₃) δ 8.26 (br. s, 1H), 7.68 – 7.58 (m, 2H), 7.36 – 7.28 (m, 2H), 6.80 (br. s, 1H), 5.56* (t, J = 7.7 Hz, 0.33H), 5.47 (t, J = 8.3 Hz, 0.66H), 2.92 – 2.81 (m, 1H), 2.79-2.72 (m, 2H), 2.35 – 2.48* (m, 0.66H), 1.79 – 1.61 (m, 8H), 1.49 – 1.39 (m, 11H). (*signals from minor isomer); **13C NMR** (101 MHz, CDCl₃) δ 167.1, 167.0, 163.0, 162.6, 156.0, 141.7, 141.6, 139.0, 138.1, 132.5, 132.4, 132.0, 128.7, 128.7, 127.6, 127.6, 125.2, 125.1, 113.2, 111.9, 111.0, 108.8, 82.0, 82.0, 36.2, 36.1, 35.7, 34.5, 28.2, 27.0, 25.7, 24.3, 24.3, 22.9, 22.8 (mixture of isomers); **IR** (υ/cm⁻¹, thin film): 3283 (br), 2939, 2855, 1780, 1721, 1667, 1480, 1452, 1368, 1250, 1158, 940, 909, 730; **HRMS** (ESI): calculated for C₂₃H₃₀N₂NaO₆ [M+Na]+: 453.1996, found: 453.1996.

1.1.4 *Thiohydrazide* 24

A suspension of enol ester 23 (0.28 g, 0.65 mmol, 1.0 equiv) and Lawesson’s reagent (0.26 g, 0.65 mmol, 1.0 equiv) in THF (1.2 mL) was stirred at 45 °C for 6 h under an atmosphere of N₂. The mixture was filtered through a silica plug, washing hexanes/EtOAc (4:1), and the filtrate was concentrated in vacuo. The crude mixture was purified by flash chromatography (hexanes/EtOAc, 9:1) to afford the desired product 24 (0.29 g, 70%, single isomer) as a yellow oil.

**1H NMR** (400 MHz, CDCl₃) δ 9.86 (br. s, 1H), 8.86 (br. s, 1H), 7.62 (s, 1H), 7.58 – 7.56 (m, 1H), 7.32 – 7.30 (m, 2H), 5.56 (t, J = 7.7 Hz, 1H), 2.79 (t, J = 7.7 Hz, 2H), 2.52 (dt, J = 7.7, 7.7 Hz, 2H), 1.79 – 1.63 (m, 8H), 1.51 (s, 9H), 1.49 – 1.38 (m, 2H); **13C NMR** (101 MHz, CDCl₃) δ 163.0, 153.6, 141.9, 139.1, 139.1, 138.2, 131.9, 128.8, 127.3, 124.7, 111.9, 108.6, 83.5, 36.2, 34.5, 28.3, 27.0, 24.3, 22.9. (C=S carbon not observed); **IR** (υ/cm⁻¹, thin film): 3263 (br), 2939, 2855, 1780, 1721, 1667, 1480, 1452, 1368, 1250, 1158, 940, 909, 730; **HRMS** (ESI): calculated for C₂₃H₃₀N₂NaO₅S [M+Na]+: 469.1768, found: 469.1774.
1.1.5 α-Ketoacid 16

Aqueous NaOH (2 M, 0.22 mL, 0.44 mmol, 2.0 equiv) was added to a solution of thiohydrazide 24 (0.10 g, 0.22 mmol, 1.0 equiv) in methanol (1.1 mL) and the reaction was stirred at rt for 15 min. The mixture was diluted with H₂O and washed with Et₂O. The aqueous phase was acidified to pH 1 with 3 M HCl and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄, and concentrated in vacuo to afford the desired product 16 (80 mg, 99%) as a yellow oil that was used without further purification.

\[ \text{δ}^{1}H \text{ NMR (400 MHz, CDCl₃)} \delta 10.04 (\text{br. s, 1H}), 8.85 (\text{br. s, 1H}), 7.62 (d, J = 7.3 \text{ Hz, 1H}), 7.53 (s, 1H), 7.35 – 7.27 (m, 2H), 2.89 (t, J = 7.2 \text{ Hz, 2H}), 2.70 (t, J = 7.2 \text{ Hz, 2H}), 2.01 (tt, J = 7.2, 7.2 \text{ Hz, 2H}), 1.53 (s, 9H); \text{δ}^{13}C \text{ NMR (101 MHz, CDCl₃)} \delta 195.5, 160.3, 154.2, 141.5, 138.4, 132.0, 129.1, 127.1, 125.5, 84.0, 36.7, 34.7, 28.3, 24.8. (C=S carbon not observed); \text{IR} (\text{υ/cm}^{-1}, \text{thin film}): 3258 (\text{br}), 2979, 2934, 1713, 1427, 1368, 1250, 1152, 735, 698; \text{HRMS (ESI)}: \text{calculated for C}_{17}H_{22}N_{2}NaO_{5}S [M+Na]^+: 389.1142, \text{found: 389.1144.}

1.2 Synthesis of Monomers

Monomers were prepared using a previously described three-step procedure from 2,3:5,6-O-diisopropylidene-D-gulose oxime (46), 5-chloromethyl-2,2-pentamethylene-1,3-dioxolan-4-one (47) and commercially available or known aldehydes 48.²,³

**General procedure:**

1) A solution of NEt₃ (2.0 equiv) and dioxolanone 47 (1.0 equiv) in ⁰PrOAc (0.5 M) was heated to reflux for 18 h. Oxime 46 (1.0 equiv) and aldehyde 48 (1.0 equiv) were added and the mixture was heated to reflux for 24 h. The reaction mixture was
diluted with EtOAc, washed with 1 M HCl and brine, dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by flash chromatography and/or recrystallization to afford the desired cycloaddition products 49.

2) HClO₄ (70%, 3.0 equiv) was added dropwise to a solution of cycloaddition product 49 (1.0 equiv) in CH₃CN (0.1 M) at rt. The reaction was stirred for 6 h, sat. aq. NaHCO₃ was added and the mixture was extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by flash chromatography to afford the unprotected isoxazolidine product.

3) HCl in dioxane (4 M, 1.1 equiv) was added dropwise to a solution of unprotected isoxazolidine (1.0 equiv) in Et₂O (0.1 M). After stirring at rt for 30 min a precipitate was formed. The precipitate was collected by filtration, washed with Et₂O and dried under vacuum to afford the desired monomer HCl salt 50.

For monomers previously reported (25, 30 and 37), the syntheses were performed according to the literature procedures.²,³

1.2.1 Characterization Data for New Monomers and Related Intermediates

**n-Propyl monomer intermediate 51**

\[
\begin{align*}
[a]_D^{28} &= (c = 0.53, \text{CHCl}_3) = +19.4; \text{MP} 90^-91^\circ\text{C}; \quad \text{¹H NMR (400 MHz, CDCl}_3) \delta 4.87 (d, J = 6.1 \text{ Hz}, 1\text{H}), 4.69 – 4.64 (m, 2\text{H}), 4.36 (dt, J = 8.5, 6.7 \text{ Hz}, 1\text{H}), 4.19 (dd, J = 8.6, 6.8 \text{ Hz}, 1\text{H}), 4.03 (dd, J = 8.5, 3.9 \text{ Hz}, 1\text{H}), 3.85 – 3.78 (m, 1\text{H}), 3.71 (dd, J = 8.6, 6.6 \text{ Hz}, 1\text{H}), 2.92 (dd, J = 13.8, 7.7 \text{ Hz}, 1\text{H}), 2.11 (dd, J = 13.8, 1.9 \text{ Hz}, 1\text{H}), 1.92 – 1.58 (m, 9\text{H}), 1.50 – 1.33 (m, 14\text{H}), 1.28 (s, 3\text{H}), 0.94 (t, J = 7.1 \text{ Hz}, 3\text{H}); \quad \text{¹³C NMR (101 MHz, CDCl}_3) \delta 169.6, 113.0, 111.8, 109.9, 105.8, 96.9, 84.5, 84.4, 80.3, 75.7, 66.2, 60.5, 41.1, 37.7, 36.5, 35.6, 26.8, 26.2, 25.4, 25.1, 24.4, 23.1, 23.0, 20.3, 13.9; \quad \text{IR (υ/cm}^{-1}, \text{thin film): 2988, 2939, 1754, 1380, 1372, 1216, 1093, 1060, 1031, 844; HRMS (ESI): calculated for C_{25}H_{46}NO_{9} [M+H]^+: 498.2698, found: 498.2701.\end{align*}
\]
**n-Propyl monomer 26**

\[
\text{[\(\alpha\)]D}_{28}^{28} (c = 0.52, \text{MeOH}) = +34.9; \text{ MP } 113-114 \degree \text{C;} \text{ } ^{1}H \text{ NMR (400 MHz, MeOD)} \delta 4.19 (ddt, J = 7.6, 7.6, 7.6 Hz, 1H), 3.14 (dd, J = 14.3, 7.6 Hz, 1H), 2.56 (dd, J = 14.3, 7.6 Hz, 1H), 1.95 – 1.83 (m, 6H), 1.80 – 1.65 (m, 4H), 1.56 – 1.43 (m, 4H), 1.03 (t, J = 7.3 Hz, 3H); ^{13}C \text{ NMR (101 MHz, MeOD)} \delta 166.4, 114.6, 108.1, 62.9, 41.0, 38.2, 36.6, 32.6, 25.1, 24.0, 23.9, 20.8, 14.0; \text{ IR (}\nu/\text{cm}^{-1}, \text{ thin film): } 2959, 2859, 1807, 1377, 1292, 1258, 1191, 1139, 915, 718; \text{ HRMS (ESI): calculated for C}_{13}\text{H}_{22}\text{NO}_{4} [\text{M-Cl}]^{+} 256.1543, \text{ found: 256.1550.}\]

**Pyran monomer intermediate 52**

\[
\text{[\(\alpha\)]D}_{25}^{25} (c = 0.53, \text{CHCl}_{3}) = -15.3; \text{ } ^{1}H \text{ NMR (400 MHz, CDCl}_{3}\text{)} \delta 4.85 (d, J = 6.1 Hz, 1H), 4.69 (s, 1H), 4.65 (dd, J = 6.1, 3.9 Hz, 1H), 4.35 (dt, J = 8.5, 6.7 Hz, 1H), 4.20 (dd, J = 8.6, 6.9 Hz, 1H), 4.02 – 3.94 (m, 3H), 3.72 (dd, J = 8.6, 6.5 Hz, 1H), 3.58 – 3.53 (m, 1H), 3.43 – 3.33 (m, 2H), 2.79 (dd, J = 14.1, 8.0 Hz, 1H), 2.31 (dd, J = 14.1, 1.1 Hz, 1H), 1.92 – 1.56 (m, 11H), 1.47 – 1.25 (m, 16H); \text{ } ^{13}C \text{ NMR (101 MHz, CDCl}_{3}\text{)} \delta 169.5, 113.1, 111.8, 109.9, 106.0, 96.7, 84.7, 84.3, 80.3, 75.7, 67.8, 67.6, 66.1, 65.4, 37.7, 36.7, 36.6, 36.5, 31.3, 30.2, 26.9, 26.2, 25.3, 25.0, 24.4, 23.1, 23.1; \text{ IR (}\nu/\text{cm}^{-1}, \text{ thin film): } 2986, 2939, 2856, 1800, 1450, 1372, 1249, 1237, 1210, 1086, 1032, 847, 732; \text{ HRMS (ESI): calculated for C}_{27}\text{H}_{41}\text{NNaO}_{10} [\text{M+Na}]^{+} 562.2623, \text{ found: 562.2620.}\]
Pyran monomer 27

[a]_D^28 (c = 0.51, MeOH) = +28.1; MP 129–132 °C; 'H NMR (400 MHz, MeOD) δ 4.01–3.89 (m, 3H), 3.50–3.39 (m, 2H), 3.17 (dd, J = 14.4, 7.5 Hz, 1H), 2.60 (dd, J = 14.4, 9.9 Hz, 1H), 2.12-2.02 (m, 1H), 1.94–1.83 (m, 4H), 1.81–1.60 (m, 6H), 1.57–1.46 (m, 4H); ^13C NMR (101 MHz, MeOD) δ 165.3, 113.2, 106.9, 66.6, 66.2, 38.2, 36.8, 35.8, 35.3, 30.0, 29.0, 23.7, 22.6, 22.5; IR (υ/cm⁻¹, thin film): 2929, 2863, 2834, 1799, 1279, 1250, 1244, 1199, 1088, 943, 711; HRMS (ESI): calculated for C_{15}H_{24}NO_{5} [M-Cl]^+: 298.1649, found: 298.1650

4-Benzylxybenzyl monomer intermediate 53

[a]_D^26 (c = 0.53, CHCl₃) = −24.3; MP 123–124 °C; 'H NMR (400 MHz, CDCl₃) δ 7.44–7.30 (m, 5H), 7.13 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 8.6 Hz, 2H), 5.03 (s, 2H), 4.85 (d, J = 6.1 Hz, 1H), 4.69 (s, 1H), 4.57 (dd, J = 6.0, 4.1 Hz, 1H), 4.32–4.26 (m, 1H), 4.13 (dd, J = 8.4, 6.6 Hz, 1H), 4.10–4.04 (m, 1H), 3.64 (dd, J = 8.4, 4.1 Hz, 1H), 3.56 (dd, J = 8.4, 6.9 Hz, 1H), 3.01 (dd, J = 13.7, 7.7 Hz, 1H), 2.82–2.73 (m, 2H), 2.20 (dd, J = 14.0, 1.7 Hz, 1H), 1.94–1.88 (m, 2H), 1.81–1.67 (m, 6H), 1.50–1.42 (m, 8H), 1.37 (s, 3H), 1.28 (s, 3H). ^13C NMR (101 MHz, CDCl₃) δ 169.4, 157.6, 137.2, 130.9, 130.6, 128.7, 128.1, 127.6, 114.9, 113.0, 111.8, 109.8, 105.9, 96.4, 84.5, 84.1, 80.2, 75.7, 70.1, 66.1, 61.7, 39.8, 38.5, 37.7, 36.5, 27.1, 26.2, 25.5, 25.0, 24.4, 23.2, 23.1; IR (υ/cm⁻¹, thin film): 2990, 2940, 2998, 1512, 1376, 1239, 1209, 1156, 1089, 1066, 1037, 847; HRMS (ESI): calculated for C_{36}H_{48}NO_{10} [M+H]^+: 652.3116, found: 652.3104.
4-Hydroxybenzyl monomer intermediate 54

\[
\begin{align*}
\text{[a]}_D^{28} (c = 0.52, \text{CHCl}_3) &= -26.2; \quad \text{MP} \ 83-85 \ ^\circ\text{C}; \\
^1H \text{ NMR} (400 MHz, \text{CDCl}_3) &\delta 7.07 (d, J = 8.5 \text{ Hz}, 2H), 6.75 (d, J = 8.5 \text{ Hz}, 2H), 5.45 (s, 1H), 4.84 (d, J = 6.0 \text{ Hz}, 1H), 4.68 (s, 1H), 4.56 (dd, J = 6.1, 4.0 \text{ Hz}, 1H), 4.32 - 4.27 \text{ (m, 1H)}, 4.13 (dd, J = 8.4, 8.0 \text{ Hz}, 1H), 2.98 (dd, J = 13.8, 8.0 \text{ Hz}, 1H), 2.80 - 2.70 \text{ (m, 2H)}, 2.18 (dd, J = 13.9, 1.7 \text{ Hz}, 1H), 1.93 - 1.87 \text{ (m, 2H)}, 1.82 - 1.65 \text{ (m, 6H)}, 1.48 - 1.40 \text{ (m, 8H)}, 1.37 (s, 3H), 1.27 (s, 3H); \\
^13C \text{ NMR} (101 MHz, \text{CDCl}_3) &\delta 169.4, 154.4, 130.8, 130.6, 115.5, 113.0, 111.9, 109.8, 106.0, 96.4, 84.5, 84.0, 80.2, 75.7, 66.1, 61.7, 39.8, 38.5, 37.7, 36.5, 26.9, 26.1, 25.3, 24.9, 24.4, 23.2, 23.0; \\
\text{IR (u/cm}^{-1}, \text{thin film}) &= 2987, 2938, 1799, 1516, 1228, 1516, 1372, 1228, 1210, 1085, 1066, 1038, 844; \\
\text{HRMS (ESI)}: &\text{calculated for C}_{29}H_{40}NO_{10} [M+H]^+; 562.2647, \text{found: 562.2648.}
\end{align*}
\]

4-Hydroxybenzyl monomer 28

\[
\begin{align*}
\text{[a]}_D^{28} (c = 0.53, \text{MeOH}) &= +47.9; \quad \text{MP} \ 120-122^\circ\text{C}; \\
^1H \text{ NMR} (400 MHz, \text{MeOD}) &\delta 7.14 (d, J = 8.5 \text{ Hz}, 2H), 6.80 (d, J = 8.5 \text{ Hz}, 2H), 4.43 - 4.35 \text{ (m, 1H)}, 3.12 (d, J = 8.5 \text{ Hz}, 2H), 3.03 (dd, J = 14.4, 7.5 \text{ Hz}, 1H), 2.59 (dd, J = 14.4, 6.7 \text{ Hz}, 1H), 1.95 - 1.87 \text{ (m, 4H)}, 1.82 - 1.65 \text{ (m, 4H)}, 1.57 - 1.48 \text{ (m, 2H)}; \\
^13C \text{ NMR} (101 MHz, \text{MeOD}) &\delta 166.4, 158.3, 131.2, 126.9, 117.0, 114.6, 108.0, 64.3, 40.4, 38.3, 36.6, 35.5, 25.1, 24.0, 24.0; \text{IR (u/cm}^{-1}, \text{thin film}) = 3318, 2940, 2875, 1807, 1519, 1269, 1213, 1190, 1153, 1116, 920, 872, 839, 720; \text{HRMS (ESI)}: &\text{calculated for C}_{17}H_{22}NO_{5} [M-Cl]^+; 320.1492, \text{found: 320.1493.}
\end{align*}
\]
**Amide monomer intermediate 55**

\[
\begin{align*}
[a]_D^{28} & \text{ (c = 0.52, CHCl}_3) = +31.6; \\
& \text{MP 144–145°C; } ^1H \text{ NMR (400 MHz, CDCl}_3) \delta 6.53 \\
& \text{(t, } J = 5.5 \text{ Hz, 1H), 4.87 (d, } J = 6.0 \text{ Hz, 1H), 4.67 – 4.61 (m, 2H), 4.35 (dt, } J = 8.2, 7.0 \\
& \text{Hz, 1H), 4.22 (dd, } J = 8.6, 6.7 \text{ Hz, 1H), 4.09 (dd, } J = 8.2, 3.7 \text{ Hz, 1H), 3.74 – 3.67 (m, 2H), 3.61 – 3.47 (m, 7H), 3.40 – 3.34 (m, 4H), 2.95 (dd, } J = 13.9, 8.1 \text{ Hz, 1H), 2.37 – 2.30 (m, 1H), 2.17 – 2.10 (m, 2H), 2.08 – 1.98 (m, 1H), 1.92 – 1.59 (m, 9H), 1.47 – 1.36 (m, 11H), 1.28 (s, 3H); } ^{13}C \text{ NMR (101 MHz, CDCl}_3) \delta 172.6, 169.2, 113.2, 111.9, \\
& 110.0, 106.0, 97.5, 84.6, 83.7, 80.3, 75.8, 72.0, 70.3, 69.9, 66.1, 61.1, 59.1, 41.0, \\
& 39.5, 37.7, 36.5, 34.5, 30.0, 26.8, 26.2, 25.5, 25.1, 24.4, 23.1, 22.9; } \text{IR (u/cm}^{-1}, \text{ thin film): 2988, 2939, 1755, 1379, 1372, 1217, 1060, 1031, 845; } \text{HRMS (ESI): calculated for C}_{30}H_{49}N_{2}O_{12} \text{ [M+H]}^+: 629.3280, \text{ found: 629.3281.}
\end{align*}
\]

**Amide monomer 29**

\[
\begin{align*}
[a]_D^{24} & \text{ (c = 0.49, MeOH) = −17.5; } ^1H \text{ NMR (400 MHz, MeOD) } \delta 4.33 – 4.26 (m, 1H), \\
& 3.62 – 3.60 (m, 2H), 3.57 – 3.54 (m, 4H), 3.41 – 3.37 (m, 5H), 3.15 (dd, } J = 14.4, 7.9 \\
& \text{Hz, 1H), 2.66 – 2.49 (m, 3H), 2.28 – 2.09 (m, 2H), 1.93 – 1.85 (m, 4H), 1.76-1.66 (m, 4H), 1.54 – 1.47 (m, 2H); } ^{13}C \text{ NMR (101 MHz, MeOD) } \delta 175.0, 166.2, 114.5, 107.8, \\
& 73.0, 71.0, 70.3, 62.8, 59.2, 41.3, 40.6, 38.2, 36.6, 32.9, 26.4, 25.0, 24.0, 23.9; \text{IR (u/cm}^{-1}, \text{ thin film): 2938, 2857, 1798, 1651, 1269, 1238, 1177, 1139, 1090, 929; } \text{HRMS (ESI): calculated for C}_{18}H_{39}N_{2}O_{7} \text{ [M-Cl]}^+: 387.2122, \text{ found: 387.2126.}
\end{align*}
\]
1.3 Synthesis of Thiohydrazides

**General procedure:**

1) EDCI·HCl (1.1 equiv) was added to a suspension of carboxylic acid (1.1 equiv) and HOBt (1.1 equiv) in CH₂Cl₂ (0.2 M) at rt. The mixture was stirred for 10 min, t-butylcarbazate (1.0 equiv) and DIPEA (4 equiv) were added and the mixture was stirred for 16 h. The reaction mixture was diluted with CH₂Cl₂, washed with sat. aq. NaHCO₃, 1 M HCl and brine, dried over Na₂SO₄ and concentrated *in vacuo*. The crude mixture was purified by flash chromatography (hexanes/EtOAc) to afford the desired hydrazide product.

2) A suspension of hydrazide (1.0 equiv) and Lawesson’s reagent (1.0 equiv) in THF (0.5 M) was stirred at 45 °C for 24 h under an atmosphere of N₂. The mixture was filtered through a silica plug, washing with hexanes/EtOAc (2:1), and the filtrate was concentrated *in vacuo*. The crude mixture was purified by flash chromatography (hexanes/EtOAc) to afford the desired Boc-thiohydrazide product.

3) Boc-thiohydrazide (1.0 equiv) was dissolved in HCl in dioxane (4 M, 10 equiv) and the solution was stirred for 1-24 h (depending on the substrate) at rt. The reaction mixture was diluted with Et₂O and the precipitate was collected by filtration, washed with Et₂O and dried under vacuum to afford the desired thiohydrazide-HCl salt.

1.3.1 3-Methoxyphenyl thiohydrazide 10

![Chemical structure of 3-Methoxyphenyl thiohydrazide 10](image)

Prepared according to the general procedure. 1) 2.27 mmol scale, quant. yield. 2) 2.03 mmol scale, 68% yield. 3) 1.27 mmol scale, 2 h, 70% yield, white solid.

**MP** 141–143 °C; **¹H NMR** (400 MHz, MeOD) δ 7.40 – 7.37 (m, 3H), 7.17 – 7.13 (m, 1H), 3.86 (s, 3H); **¹³C NMR** (101 MHz, MeOD) δ 198.0, 159.7, 129.3, 119.3, 117.7, 113.0, 54.6; **IR** (υ/cm⁻¹, thin film): 3200-2500 (br), 3129, 3082, 2942, 1607, 1580,
1483, 1459, 1433, 1288, 989, 786, 778; **HRMS** (EI): calculated for C$_8$H$_{10}$N$_2$OS [M-HCl]$^+$: 182.0508, found: 182.0509

1.3.2 *Pyridine thiohydrazide 12*

![Pyridine thiohydrazide 12](image)

Prepared according to the general procedure. 1) 4.55 mmol scale, 61% yield. 2) 2.53 mmol scale, 22% yield. 3) 0.55 mmol scale, 24 h, 79% yield, yellow solid. **MP** 133–134 °C; $^1$H NMR (400 MHz, MeOD) δ 8.64 (ddd, J = 5.5, 1.6, 1.0 Hz, 1H), 8.56 (ddd, J = 7.9, 1.0, 1.0 Hz, 1H), 8.41 (ddd, J = 7.9, 7.9, 1.6 Hz, 1H), 7.90 (ddd, J = 7.9, 5.5, 1.0 Hz, 1H); $^{13}$C NMR (101 MHz, MeOD) δ 184.4, 150.0, 146.0, 143.7, 128.7, 126.1; IR (υ/cm$^{-1}$, thin film): 3382 (br), 3073, 3038, 2993, 2531(br), 1578, 1557, 1513, 1456, 1351, 1250, 1219, 1081, 979, 942, 773; **HRMS** (ESI): calculated for C$_6$H$_8$N$_3$S [M–Cl]$^+$: 154.0433, found: 154.0437

1.3.3 *Alkyl thiohydrazide 15*

![Alkyl thiohydrazide 15](image)

Prepared according to the general procedure. 1) 4.66 mmol scale, 60% yield. 2) 2.92 mmol scale, 27% yield. 3) 0.80 mmol scale, 1 h, 67% yield, white solid. **MP** 121-123 °C; $^1$H NMR (400 MHz, MeOD) δ 7.29 – 7.16 (m, 5H), 3.12 (t, J = 7.3 Hz, 2H), 2.99 (t, J = 7.3 Hz, 2H); $^{13}$C NMR (101 MHz, MeOD) δ 205.0, 141.3, 129.6, 127.5, 45.1, 36.2; IR (υ/cm$^{-1}$, thin film): 3200-2600 (br), 1581, 1548, 1465, 1372, 1209, 1110, 1065, 752, 695; **HRMS** (ESI): calculated for C$_9$H$_{13}$N$_2$S [M-Cl]$^+$: 181.0794, found: 181.0799
1.3.4 Phenyl thiohydrazide 2

Phenyl thiohydrazide 2 was prepared according to a literature procedure. S-(thiobenzoyl)-thioglycolic acid (1.30 g, 6.12 mmol, 1.0 equiv) was dissolved in 1 M NaOH (6 mL) and H₂O (6 mL) was added. The solution was cooled to 0 °C and hydrazide hydrate (1.1 mL, 12.2 mmol, 2.0 equiv) was added dropwise. After stirring for 10 min the pH of the reaction mixture was adjusted to pH 5-6 using 1 M HCl and the resulting suspension was stirred for a further 1 h. The precipitate was collected by filtration, washing with cold H₂O, and dried under vacuum. The crude material was recrystallized (CH₂Cl₂/hexanes) to provide desired product 2 (0.61 g, 66%) as a pale yellow solid. ¹H NMR (400 MHz, DMSO-d₆) δ 7.72 – 7.69 (m, 2H), 7.47 – 7.38 (m, 3H). The spectroscopic data were in agreement with literature values.

Synthesis of 1,3,4-Thiadiazoles

2.1 Intermolecular Reaction of Thiohydrazides and α-Ketoacids

General procedure:
A solution of thiohydrazide (1.5 equiv) in tBuOH/1M HCl (5:1, 0.1 M) was added to a solution of α-ketoacid (1.0 equiv) in tBuOH/1M HCl (5:1, 0.1 M) and the mixture was heated at 70 °C for 16 h. The reaction mixture was diluted with water and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by flash chromatography to afford the desired 1,3,4-thiadiazole products.
2.1.1 *Phenyl thiadiazole 5*

![Phenyl Thiadiazole](image)

Thiadiazole 5 was prepared following the general procedure with thiohydrazide 2 (50 mg, 0.33 mmol, 1.5 equiv) and α-ketoacid 3 (36 mg, 0.22 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 9:1 → 4:1) as a white solid (39 mg, 71%).

**MP** 71–72 °C; **1H NMR** (400 MHz, CDCl₃) δ 7.91 – 7.87 (m, 2H), 7.47 – 7.41 (m, 3H), 7.38 – 7.33 (m, 4H), 7.32 – 7.28 (m, 1H), 4.45 (s, 2H); **13C NMR** (101 MHz, CDCl₃) δ 169.8, 169.4, 137.3, 131.1, 130.3, 129.2, 129.1, 129.0, 127.9, 127.6, 36.7; **IR** (υ/cm⁻¹, thin film): 3066, 3030, 1494, 1455, 1427, 1225, 1124, 1058, 981, 920, 763, 702, 690; **HRMS** (ESI): calculated for C₁₅H₁₃N₂S [M+H]⁺: 253.0794, found: 253.0793

2.1.2 *Propanoic acid thiadiazole 7*

![Propanoic Acid Thiadiazole](image)

Thiadiazole 7 was prepared following the general procedure with thiohydrazide 2 (50 mg, 0.33 mmol, 1.5 equiv) and α-ketoacid 6 (32 mg, 0.22 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 4:1 + 1% formic acid) as a white solid (42 mg, 73%).

**MP** 168–170 °C; **1H NMR** (400 MHz, MeOD) δ 7.95–7.91 (m, 2H), 7.55 – 7.49 (m, 3H), 3.42 (t, J = 7.0 Hz, 2H), 2.86 (t, J = 7.0 Hz, 2H); **13C NMR** (101 MHz, MeOD) δ 177.1, 171.5, 170.7, 132.5, 131.1, 130.4, 128.8, 34.7, 26.6; **IR** (υ/cm⁻¹, thin film): 3376 (br), 3062, 2934, 1693, 1536, 1456, 1419, 1239, 1048, 982, 758, 690; **HRMS** (ESI): calculated for C₁₁H₁₁N₂O₂S [M+H]⁺: 235.0536, found: 235.0535
2.1.3 *Fmoc-amine thiadiazole* 9

![Structure of Fmoc-amine thiadiazole](image)

Thiadiazole 9 was prepared following the general procedure with thiohydrazide 2 (58 mg, 0.39 mmol, 1.5 equiv) and \( \alpha \)-ketoacid 8\(^5,6\) (94 mg, 0.26 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 4:1) followed by recrystallization (hexanes/EtOAc) as a white solid (64 mg, 54%).

\[ \alpha \]\text{D}^2_4 (c = 0.25, CHCl3) = -41.7; MP 154-156 °C; \(^1\text{H NMR} \) (400 MHz, CDCl3) \( \delta \) 7.98 – 7.92 (m, 2H), 7.76 (d, \( J = 7.6 \) Hz, 2H), 7.64 – 7.58 (m, 2H), 7.52 – 7.46 (m, 3H), 7.42 – 7.36 (m, 2H), 7.34 – 7.27 (m, 2H), 5.67 (d, \( J = 9.1 \) Hz, 1H), 5.13 – 5.07 (m, 1H), 4.47 (d, \( J = 7.0 \) Hz, 2H), 4.23 (t, \( J = 7.0 \) Hz, 1H), 2.48 – 2.37 (m, 1H), 1.03 (d, \( J = 6.8 \) Hz, 6H); \(^13\text{C NMR} \) (101 MHz, CDCl3) \( \delta \) 169.9, 168.9, 156.2, 143.8, 141.5, 131.3, 130.1, 129.3, 128.1, 127.9, 127.2, 125.2, 120.1, 67.2, 56.5, 47.4, 33.6, 19.4, 18.0; \( \text{IR} \) (\( \nu \)/cm\(^{-1} \), thin film): 3306, 2968, 2950, 1690, 1532, 1452, 1425, 1301, 1261, 1236, 1021; \( \text{HRMS} \) (ESI): calculated for C\(_{27}\)H\(_{25}\)N\(_3\)NaO\(_2\)S [M+Na]^+: 478.1560, found: 478.1556

2.1.4 *3-Methoxyphenyl thiadiazole* 11

![Structure of 3-Methoxyphenyl thiadiazole](image)

Thiadiazole 11 was prepared following the general procedure with thiohydrazide 10 (46 mg, 0.21 mmol, 1.5 equiv) and \( \alpha \)-ketoacid 3 (23 mg, 0.14 mmol, 1.0 equiv). The desired product was isolated by flash chromatography (hexanes/EtOAc, 9:1→4:1) as a yellow oil (32 mg, 80%).

\(^1\text{H NMR} \) (400 MHz, CDCl3) \( \delta \) 7.52 (dd, \( J = 2.6, 1.6 \) Hz, 1H), 7.41 – 7.28 (m, 7H), 7.00 (ddd, \( J = 8.2, 2.6, 1.0 \) Hz, 1H), 4.46 (s, 2H), 3.86 (s, 3H); \(^13\text{C NMR} \) (101 MHz, CDCl3) \( \delta \) 167.0, 169.4, 160.2, 137.3, 131.5, 130.3, 129.2, 129.0, 127.7, 120.7, 117.6, 112.2, 55.3, 36.8; \( \text{IR} \) (\( \nu \)/cm\(^{-1} \), thin film): 3028, 2964, 2935, 1598, 1581, 1455, 1426, 1206, 1043, 1002, 843, 781; \( \text{HRMS} \) (ESI): calculated for C\(_{16}\)H\(_{15}\)N\(_2\)OS [M+H]^+: 283.0900, found: 283.0899
2.1.5 2,3-Dihydrothiadiazole carboxylic acid 4

A solution of thiohydrazide 2 (50 mg, 0.33 mmol, 1.0 equiv) and α-ketoacid 3 (54 mg, 0.33 mmol, 1.0 equiv) in tBuOH/H₂O (5:1, 6.6 mL) was heated at 45 °C for 14 h. The reaction mixture was diluted with water and extracted with EtOAc (3×). The combined organic extracts were washed with brine, dried over Na₂SO₄ and concentrated in vacuo. The crude mixture was purified by preparative HPLC (35-95% CH₃CN with 0.1% TFA over 28 min) to obtain a sample of desired product 4 for characterization. Retention time: 16.9 min.

¹H NMR (400 MHz, CDCl₃) δ 7.60 – 7.56 (m, 2H), 7.40 – 7.34 (m, 3H), 7.31 – 7.23 (m, 5H), 3.56 (ABq, 2H); ¹³C NMR (101 MHz, CDCl₃) δ 174.8, 147.4, 134.2, 130.6, 130.3, 130.2, 128.8, 128.8, 127.9, 127.2, 84.1, 44.5: IR (υ/cm⁻¹, thin film): 3299, 2923, 2853, 1724, 1494, 1447, 1267, 1193, 973, 759, 688; HRMS (ESI): calculated for C₁₆H₁₆N₂O₂S [M+H]⁺: 299.0849, found: 299.0844
Synthesis of β-Peptide Macrocycle Mixtures

3.1 One-Pot Elongation/Macrocyclization with One Monomer

**General procedure:**
Reactions were prepared using 0.1 M stock solutions of all reagents in tBuOH/H2O (5:1).
Solutions of initiator 16 (10 μL, 1.0 equiv) and a monomer (20 μL, 2.0 equiv) were mixed and heated at 45 °C for 14 h. The mixture was diluted with tBuOH/1M HCl (5:1) (90 μL) and heated to 70 °C for 4 h. The resulting mixture of cyclic compounds was analyzed by HPLC and/or LCMS.
This procedure was performed on a larger scale (e.g. 0.90 mL of 16, 1.80 mL monomer) for purification by preparative HPLC. For cyclization on larger scale the mixtures were stirred in an oversized flask (e.g. 50 mL flask for 9 mL solvent) to enhance O2 exchange.
3.1.1 HPLC spectra with mass traces of the major peaks

**Monomer 25**

HPLC: Gradient 30 to 90% CH$_3$CN with 0.1% TFA in 17 min
**Monomer 26**

HPLC: Gradient 30 to 90% CH$_3$CN with 0.1% TFA in 17 min
Monomer 27

HPLC: Gradient 20 to 70% CH$_3$CN with 0.1% TFA in 17 min
Monomer 28

HPLC: Gradient 30 to 90% CH$_3$CN with 0.1% TFA in 17 min
**Monomer 29**

HPLC: Gradient 20 to 70% CH₃CN with 0.1% TFA in 17 min
**Monomer 30**

HPLC: Gradient 30 to 90% CH$_3$CN with 0.1% TFA in 17 min
3.2 Cyclization of Purified Tri-β-Peptide

A solution of tripeptide **56** (0.26 mg, 0.37 μmol) was dissolved in tBuOH/1M HCl (5:1, 37 μL) was heated to 70 °C for 4 h. The crude reaction mixture was directly analyzed by HPLC (Gradient 30 to 90% CH₃CN with 0.1% TFA in 17 min).

**HRMS (ESI)** Tripeptide ketoacid **56**: calculated for C₃₅H₅₆N₅O₈S [M+H]⁺: 706.3844, found: 706.3837. Calculated for C₃₅H₅₄N₅O₈S [M−H]⁻: 704.3699, found: 704.3696.

**HRMS (ESI)** Macrocycle **32b**: calculated for C₂₉H₄₄N₅O₃S [M+H]⁺: 542.3159, found: 542.3154.

**HPLC: Before reaction**

**HPLC: Crude reaction mixture**
3.3 One-Pot Elongation/Macrocyclization Reaction with Two Monomers

**General procedure:**

Reactions were prepared using 0.1 M stock solutions of all reagents in tBuOH/H2O (5:1).

Solutions of initiator 16 (10 μL, 1.0 equiv) and the first monomer (10 μL, 1.0 equiv) were mixed and heated at 45 °C for 6 h. A solution of the second monomer (10 μL, 1.0 equiv) was added and the mixture heated at 45 °C for 14 h. The reaction mixture were diluted with tBuOH/1 M HCl (5:1) (90 μL) and heated to 70 °C for 4 h. The resulting mixture of cyclic compounds was analyzed by HPLC and/or LCMS.

This procedure was performed on a larger scale (e.g. 0.90 mL of 16, 0.90 mL of each monomer) for purification by preparative HPLC. For large-scale cyclization the mixtures were stirred in an oversized flask (e.g. 50 mL flask for 9 mL solvent) to enhance O2 exchange.
3.3.1 HPLC spectra with mass traces of the major peaks

Monomers 28 and 27

HPLC: 25 to 70% CH$_3$CN with 0.1% TFA over 17 min
Monomers 28 and 25

HPLC: 25 to 70% CH₃CN with 0.1% TFA over 17 min
**Monomers 25 and 27**

HPLC: 25 to 70% CH₃CN with 0.1% TFA over 17 min
Monomers 27 and 25

HPLC: 25 to 70% CH$_3$CN with 0.1% TFA over 17 min
Monomers 27 and 28

HPLC: 25 to 70% CH$_3$CN with 0.1% TFA over 17 min
Monomers 28 and 30

HPLC: 30 to 90% CH$_3$CN with 0.1% TFA over 17 min

m/z values:
- 43a: m/z=601.27
- 43b: m/z=667.28
- 43c: m/z=490.20
- 43d: m/z=844.36
- 43e: m/z=778.35
- 43f: m/z=889.42
- 43g: m/z=712.34
Monomers 28 and ent-27

HPLC: 25 to 70% CH$_3$CN with 0.1% TFA over 17 min
Monomers 37 and 27

HPLC: 30 to 90% CH$_3$CN with 0.1% TFA over 17 min
3.4 Isolation and Characterization of Macroyclic Compounds

**Macrocycle 34a**

Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator 16 (0.09 mmol, 1.0 equiv) and monomer 28 (0.18 mmol, 2.0 equiv). The crude reaction mixture was diluted with CH$_3$CN:H$_2$O (1:1) and purified directly by preparative HPLC (20 to 95% CH$_3$CN with 0.1% TFA over 28 min). Macrocycle 34a was isolated as a white powder after lyophilization. Retention time: 15.8 minutes.

$^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.22 (s, 1H), 9.17 (s, 1H), 8.02 (d, $J = 9.5$ Hz, 1H), 7.83 – 7.72 (m, 1H), 7.63 (d, $J = 7.9$ Hz, 1H), 7.46 (t, $J = 7.7$ Hz, 1H), 7.37 – 7.29 (m, 1H), 7.15 (s, 1H), 7.00 (d, $J = 8.5$ Hz, 2H), 6.93 (d, $J = 8.5$ Hz, 2H), 6.69 – 6.66 (m, 4H), 4.34 – 4.30 (m, 1H), 4.21-4.15 (m, 1H), 3.25 (dd, $J = 15.8$, 2.6 Hz, 1H), 2.95 (dd, $J = 15.8$, 11.9 Hz, 1H), 2.81 – 2.75 (m, 1H), 2.74 – 2.61 (m, 3H), 2.48 – 2.43 (m, 1H), 2.26 (dd, $J = 16.5$, 10.1 Hz, 1H), 2.15 (dd, $J = 16.5$, 2.2 Hz, 1H), 2.09 – 1.95 (m, 3H), 1.72 – 1.65 (m, 1H); $^{13}$C NMR (151 MHz, DMSO-$d_6$) δ 171.1, 169.9, 168.5, 167.4, 155.7, 155.6, 141.5, 131.2, 130.3, 130.2, 130.0, 129.5, 129.3, 128.6, 128.0, 122.3, 115.0, 114.9, 49.9, 47.0, 40.7, 39.3, 37.7, 33.7, 31.4, 31.4, 22.6; HRMS (ESI): calculated for C$_{31}$H$_{32}$N$_4$NaO$_4$S [M+Na]$^+$: 579.2036, found: 579.2039.
Macrocycle 31b

Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator 16 (0.10 mmol, 1.0 equiv) and monomer 25 (0.20 mmol, 2.0 equiv). The crude reaction mixture was diluted with CH$_3$CN:H$_2$O (1:1) and purified directly by preparative HPLC (30 to 65% CH$_3$CN with 0.1% TFA over 28 min). Macrocycle 31b was isolated as a white powder after lyophilization. Retention time: 17.0 minutes.

$^1$H NMR (600 MHz, DMSO-$d_6$) $\delta$ 8.05 (d, $J$ = 9.3 Hz, 1H), 7.90 (d, $J$ = 8.2 Hz, 1H), 7.63 (s, 1H), 7.48 – 7.45 (m, 3H), 7.39 (d, $J$ = 7.7 Hz, 1H), 4.16 – 4.11 (m, 1H), 3.91 – 3.82 (m, 2H), 3.37 (dd, $J$ = 14.8, 3.0 Hz, 1H), 3.03 (dd, $J$ = 14.8, 11.4 Hz, 1H), 2.76 – 2.71 (m, 1H), 2.67 – 2.63 (m, 1H), 2.30 (dd, $J$ = 14.1, 9.7 Hz, 1H), 2.22 (dd, $J$ = 14.0, 4.0 Hz, 1H), 2.13 – 1.98 (m, 4H), 1.85 – 1.75 (m, 3H), 1.73 – 1.65 (m, 2H), 0.94 (d, $J$ = 6.8 Hz, 3H), 0.91 (d, $J$ = 6.8 Hz, 3H), 0.82 – 0.80 (m, 6H), 0.77 (d, $J$ = 6.8 Hz, 3H), 0.69 (d, $J$ = 6.9 Hz, 3H)$; ^{13}$C NMR (151 MHz, DMSO-$d_6$) $\delta$ 171.0, 170.2, 169.9, 168.3, 167.2, 143.1, 131.2, 129.5, 129.4, 129.2, 123.4, 53.4, 51.3, 49.7, 39.9, 39.8, 39.7, 39.5, 39.4, 39.2, 39.1, 37.3, 34.8, 34.0, 32.3, 32.3, 31.8, 30.0, 27.1, 19.5, 19.2, 18.8, 18.3, 18.2, 16.4; HRMS (ESI): calculated for C$_{29}$H$_{44}$N$_{5}$O$_{3}$S [M+H]$^+$: 542.3159, found: 542.3154.

Macrocycle 36a

Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator 16 (0.08 mmol, 1.0 equiv) and monomer 30 (0.12 mmol, 1.5 equiv). The crude reaction mixture was diluted with CH$_3$CN:H$_2$O (1:1) and purified
directly by preparative HPLC (25 to 55% CH₃CN with 0.1% TFA over 38 min). Macrocycle 36a was isolated as a white powder after lyophilization. Retention time: 22.5 minutes.

**1H NMR** (600 MHz, DMSO-**d**₆) δ 7.95 (d, *J* = 9.4 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.52 – 7.46 (m, 2H), 7.36 (d, *J* = 7.6 Hz, 1H), 7.22 (s, 1H), 4.22 – 4.14 (m, 2H), 3.46 – 3.39 (m, 1H), 2.81 – 2.75 (m, 1H), 2.74 – 2.68 (m, 1H), 2.53 – 2.51 (m, 1H), 2.29 – 2.24 (m, 2H), 2.20 – 2.16 (m, 1H), 2.12 – 2.02 (m, 3H), 1.95 – 1.64 (m, 8H), 1.62 – 1.53 (m, 2H); **13C NMR** (151 MHz, DMSO-**d**₆) δ 172.1, 171.0, 170.1, 168.2, 141.6, 131.3, 131.2, 129.9, 129.2, 122.4, 56.7, 53.0, 47.0, 45.7, 31.8, 31.7, 31.2, 30.7, 27.3, 24.3, 24.2, 21.2, 20.7; **HRMS** (ESI): calculated for C₂₃H₂₈N₄NaO₂S [M+Na]**⁺**: 477.1825, found: 447.1831.

**Macrocycle 38d**

![Macrocycle 38d](image)

Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator 16 (0.09 mmol, 1.0 equiv), monomer 28 (0.09 mmol, 1.0 equiv) and monomer 27 (0.09 mmol, 1.0 equiv). The crude reaction mixture was diluted with CH₃CN:H₂O (1:1) and purified directly by preparative HPLC (25 to 60% CH₃CN with 0.1% TFA over 40 min). Macrocycle 38d was isolated as a white powder after lyophilization. Retention time: 32.5 minutes.

**1H NMR** (600 MHz, DMSO-**d**₆) δ 9.17 (s, 1H), 7.89 (d, *J* = 9.7 Hz, 1H), 7.81 (d, *J* = 7.6 Hz, 1H), 7.61 (d, *J* = 7.9 Hz, 1H), 7.47 (t, *J* = 7.6 Hz, 1H), 7.34 (d, *J* = 7.6 Hz, 1H), 7.16 (s, 1H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.66 (d, *J* = 8.5 Hz, 2H), 4.42 – 4.36 (m, 1H), 3.99 – 3.91 (m, 1H), 3.88 – 3.83 (m, 2H), 3.40 – 3.35 (m, 1H), 3.26 – 3.19 (m, 2H), 2.97 (dd, *J* = 15.9, 12.2 Hz, 1H), 2.80 – 2.71 (m, 2H), 2.67 (dd, *J* = 13.3, 4.3 Hz, 1H), 2.47 (dd, *J* = 12.3, 7.0 Hz, 2H), 2.28 – 2.25 (m, 2H), 2.06 – 1.98 (m, 3H), 1.71 – 1.60 (m, 3H), 1.51 – 1.42 (m, 1H), 1.28 – 1.19 (m, 2H); **13C NMR** (151 MHz, DMSO-**d**₆) δ 171.3, 170.6, 168.6, 168.1, 155.7, 141.6, 131.3, 130.5, 130.3, 129.6, 129.4, 128.7, 122.3, 115.0, 67.0, 66.7, 52.2, 47.0, 40.0, 39.3, 37.6, 31.9, 31.5, 31.5, 29.5,
28.3, 22.7; HRMS (ESI): calculated for C$_{29}$H$_{35}$N$_4$O$_4$S [M+H]$^+$: 535.2374, found: 535.2381.

**Macrocyle 45b**

![Macrocyle 45b](image)

Synthetic Fermentation/Macrocyclization reaction performed according to the general procedure using initiator 16 (0.08 mmol, 1.0 equiv), monomer 38 (0.07 mmol, 0.9 equiv) and monomer 27 (0.07 mmol, 0.9 equiv). The crude reaction mixture was diluted with CH$_3$CN:H$_2$O (1:1) and purified directly by preparative HPLC (30 to 60% CH$_3$CN with 0.1% TFA over 38 min). Macrocycle 45b was isolated as a white powder after lyophilization. Retention time: 18.5 minutes.

$^1$H NMR (600 MHz, DMSO-d$_6$) δ 7.90 (d, $J = 9.7$ Hz, 1H), 7.81 (dt, $J = 7.7, 1.4$ Hz, 1H), 7.66 (d, $J = 7.9$ Hz, 1H), 7.47 (t, $J = 7.7$ Hz, 1H), 7.34 (dt, $J = 7.7, 1.4$ Hz, 1H), 7.29-7.27 (m, 2H), 7.22 – 7.13 (m, 4H), 4.49 – 4.44 (m, 1H), 3.99 – 3.92 (m, 1H), 3.87-3.83 (m, 2H), 3.40 – 3.36 (m, 1H), 3.28 – 3.19 (m, 2H), 2.97 (dd, $J = 15.9, 12.3$ Hz, 1H), 2.81 – 2.71 (m, 3H), 2.62 (dd, $J = 13.2, 7.8$ Hz, 1H), 2.31 – 2.29 (m, 2H), 2.07 – 1.99 (m, 3H), 1.70 – 1.64 (m, 2H), 1.64 – 1.58 (m, 1H), 1.51 – 1.46 (m, 1H), 1.27 – 1.20 (m, 2H); $^{13}$C NMR (151 MHz, DMSO-d$_6$) δ 171.4, 170.5, 168.6, 168.1, 141.6, 138.6, 131.3, 130.5, 129.6, 129.5, 129.4, 128.2, 126.1, 122.3, 66.9, 66.7, 52.2, 46.7, 40.1, 40.0 37.7, 31.9, 31.5, 31.4, 29.6, 28.3, 22.7; HRMS (ESI): calculated for C$_{29}$H$_{34}$N$_4$NaO$_3$S [M+Na]$^+$: 541.2244, found: 541.2237.

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NMR spectra
Macrocycle 34a

$^1$H NMR (600 MHz, DMSO-$d_6$)

$^{13}$C NMR (150 MHz, DMSO-$d_6$)
$^1$H-$^1$H DQF-COSY (600 MHz, DMSO-$d_6$)

$^1$H-$^{13}$C HSQC (600 MHz, DMSO-$d_6$)
$^1$H-$^{13}$C HMBC (600 MHz, DMSO-$d_6$)
Macrocycle 31b

$^1$H NMR (600 MHz, DMSO-$d_6$)

$^{13}$C NMR (150 MHz, DMSO-$d_6$)
$^1$H-$^1$H DQF-COSY (600 MHz, DMSO-$d_6$)

$^1$H-$^{13}$C HSQC (600 MHz, DMSO-$d_6$)
$^{1}H\text{-}^{13}C$ HMBC (600 MHz, DMSO-$d_{6}$)
Macrocycle 36a

$^1$H NMR (600 MHz, DMSO-$d_6$)

$^{13}$C NMR (150 MHz, DMSO-$d_6$)
$^1$H-$^1$H DQF-COSY (600 MHz, DMSO-$d_6$)

$^1$H-$^{13}$C HSQC (600 MHz, DMSO-$d_6$)
$^1$H-$^{13}$C HMBC (600 MHz, DMSO-$d_6$)
Macrocycle 38d

$^1$H NMR (600 MHz, DMSO-$d_6$)

$^{13}$C NMR (150 MHz, DMSO-$d_6$)
$^1$H-$^1$H DQF-COSY (600 MHz, DMSO-$d_6$)

$^1$H-$^{13}$C HSQC (600 MHz, DMSO-$d_6$)
$^{1}H-^{13}C$ HMBC (600 MHz, DMSO-$d_{6}$)
Macrocycle 45b

$^1$H NMR (600 MHz, DMSO-$d_6$)

$^{13}$C NMR (150 MHz, DMSO-$d_6$)
$^1$H-$^1$H DQF-COSY (600 MHz, DMSO-\textit{d}_6)

$^1$H-$^13$C HSQC (600 MHz, DMSO-\textit{d}_6)
$^1$H-$^{13}$C HMBC (600 MHz, DMSO-$d_6$)
3-Iodophenylhydrazide 19

$^1$H NMR (400 MHz, DMSO-$d_6$)

$^{13}$C NMR (101 MHz, DMSO-$d_6$)
Aldehyde 21

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
Enol ester 23

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
Thiohydrazide 24

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
Ketoacid 16

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
3-Methoxyphenyl thiohydrazide 10

$^1$H NMR (400 MHz, MeOD)

$^{13}$C NMR (101 MHz, MeOD)
Pyridine thiohydrazide 12

\( ^1H \text{NMR (400 MHz, MeOD)} \)

\( ^{13}C \text{NMR (101 MHz, MeOD)} \)
Alkyl thiohydrazide 15

$^1$H NMR (400 MHz, MeOD)

$^{13}$C NMR (101 MHz, MeOD)
Phenyl thiadiazole 5

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
Propanoic acid thiadiazole 7

$^{1}H$ NMR (400 MHz, CDCl$_3$)

$^{13}C$ NMR (101 MHz, CDCl$_3$)
Fmoc-aminothiadiazole 9

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
3-Methoxyphenyl thiadiazole 11

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
2,3-Dihydrothiadiazole carboxylic acid 4

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
\textit{n-Propyl monomer intermediate 51}

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
**n-Propyl monomer 26**

**$^1$H NMR (400 MHz, MeOD)**

![1H NMR spectrum](image)

**$^{13}$C NMR (101 MHz, MeOD)**

![13C NMR spectrum](image)
Pyran monomer intermediate 52

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
Pyran monomer 27

$^1$H NMR (400 MHz, MeOD)

$^{13}$C NMR (101 MHz, MeOD)
4-Benzylxoybenzyl intermediate 53

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
4-Hydroxybenzyl monomer intermediate 54

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
4-Hydroxybenzyl monomer 28

$^1$H NMR (400 MHz, MeOD)

$^{13}$C NMR (101 MHz, MeOD)
Amide monomer intermediate 55

$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (101 MHz, CDCl$_3$)
Amide monomer 29

$^1$H NMR (400 MHz, MeOD)

$^{13}$C NMR (101 MHz, MeOD)