Supporting Information

Total Synthesis of the Depsipeptide FR-901375

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Table of Contents

Title page	S1
TOC	S1
General Methods	S2
Synthesis and characterization of all compounds	S2-S31
Synthesis of 8	S2-S3
¹ H & ¹³ C NMR 8	S4
Synthesis of 9	S5
¹ H & ¹³ C NMR 9	S6
Synthesis of 10	S7
¹ H & ¹³ C NMR 10	S8
Synthesis of 4	S9
¹ H & ¹³ C NMR 4	S10
Synthesis of 13	S11
¹ H & ¹³ C NMR 13	S12
Synthesis of 17	S13-S14
¹ H & ¹³ C NMR 17	S15
Synthesis of 18	S16
¹ H & ¹³ C NMR 18	S17
Synthesis of 5	S18
¹ H & ¹³ C NMR 5	S19
Synthesis of 19	S20
¹ H & ¹³ C NMR 19	S21
Synthesis of 3	S22
¹ H & ¹³ C NMR 3	S23
Synthesis of 20	S24
¹ H & ¹³ C NMR 20	S25
HPLC trace of 20	S26
Synthesis of 1	S27-28
¹ H & ¹³ C NMR 1	S29
HPLC trace of 1 (synthetic)	S30
HPLC trace of 1 (authentic)	S31

Experimental Section

General Methods. All reagents and solvents were supplied by Aldrich Chemical Company unless otherwise stated. All reactions were carried out under an atmosphere of Ar or N₂ unless specified otherwise. Tetrahydrofuran (THF) was distilled from a deep blue ketyl and methylene chloride (CH₂Cl₂) was distilled from CaH₂. Dry DMF was obtained from Acros. MgSO₄ was used as drying agent. E. Merck silica gel 60 (230-400 mesh) was used for flash chromatography. Optical rotations were recorded on a Perkin-Elmer model 241 polarimeter using a 1 mL, 1 dm cell. Mass spectra were obtained on an IonSpec Ultima high-resolution FTMS instrument (HRMALDI-FTMS). NMR spectra were recorded on Bruker AMX-600 (600 MHz), AMX-500 (500 MHz), AMX-400 (400 MHz), or AC-250 (250 MHz) spectrometers. ¹³C NMR spectra were recorded on a Bruker AMX-500 (125.7 MHz) or AMX-400 (100.6 MHz) spectrometer. Chemical shifts are reported in parts per million (ppm) on the δ scale from an internal standard.

***N*- α -[(Fluorenylmethoxy)carbonyl]-D-cysteinyl-(*S*-triphenylmethyl)-L-threonine-(*O*-*t*-butyldimethylsilyl) methyl ester (8).** To a solution of L-threonine methyl ester **7** (665 mg, 5.0 mmol, 1.0 equiv.), *N*-Fmoc-*S*-triphenylmethyl-D-cysteine (2.92 g, 5.0 mmol, 1.0 equiv.) and 1-hydroxybenzotriazole (811 mg, 6.0 mmol, 1.2 equiv.) in CH₂Cl₂:DMF (1:1, 120 mL) at 0°C was added 1-[3-dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (1.15 g, 6.0 mmol, 1.2 equiv.). The solution was stirred at ambient temperature for 20 h. The reaction mixture was concentrated *in vacuo*, poured onto saturated aqueous NaHCO₃ (120 mL) and water (100 mL), and extracted with ethyl acetate (3 $\times$ 100 mL). The organic extracts were dried, filtered, and concentrated *in vacuo*. The crude product was used for next step without further purification.

A solution of the above crude product (5.0 mmol, 1.0 equiv.), imidazole (443 mg, 6.5 mmol, 1.3 equiv.) in DMF (15 mL) was treated with TBS-Cl (980 mg, 6.5 mmol, 1.3 equiv.) at 0°C. The solution was stirred at ambient temperature for 20 h. The reaction was then quenched by the addition of distilled water (80 mL). The mixture was extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were washed with brine, dried with MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (25% EtOAc:hexanes) to yield **8** (3.36 g, 84% over two steps). ¹H NMR (δ, ppm, CDCl₃): 7.80-7.84 (m, 2H), 7.64-7.67 (m, 2H), 7.52-7.44 (m, 8H), 7.36-7.29 (m, 8H), 7.27-7.29 (m, 3H), 6.71 (d, *J* = 9.0 Hz, 1H), 5.15 (d, *J* = 6.2 Hz, 1H), 4.48-4.54 (m, 3H), 4.35-4.39 (m, 1H), 4.26-4.29 (m, 1H), 3.99-4.00 (m, 1H), 3.73 (s, 3H), 2.70-2.81 (m, 2H), 1.20 (d, *J* = 5.5 Hz, 3H), 0.88 (s, 9H), 0.08 (s, 3H), 0.03 (s, 3H). ¹³C NMR (δ, ppm, CDCl₃): 170.5, 170.3, 155.8, 144.3, 143.5, 141.2, 129.5, 128.1, 127.7, 127.1, 126.8, 125.1, 119.9, 68.7, 67.1, 57.8, 53.9, 52.2, 47.0, 38.5, 33.7, 25.5, 20.7, 17.7, -4.4, -5.4. IR (neat, cm⁻¹): 3307, 3057, 2951, 2929, 2855, 1730, 1675, 1490, 1445, 1248, 1092, 1033. mp 53-55°C. [α]_D²⁵ = +8.8 (1.0, CH₂Cl₂).

Compound **8** (^1H NMR and ^{13}C NMR)

***N*- α -[(Fluorenylmethoxy)carbonyl]-D-valyl-D-cysteinyl-(*S*-triphenylmethyl)-L-threonine-(*O*-*t*-butyldimethyl) methyl ester (**9**).** A solution of **8** (3.36 g, 4.12 mmol) in CH₂Cl₂ : Et₂NH (1:1, 40 mL) was stirred at 0°C for 3h. The reaction was diluted with ethyl acetate and evaporated to dryness. The crude amine was dissolved in DMF (50 mL) and CH₂Cl₂ (20 mL) and treated with *N*-Fmoc-D-Valine (1.54 g, 4.53 mmol, 1.1 equiv.), EDC (1.027g, 5.3 mmol, 1.3 equiv.), HOBt (780 mg, 5.8 mmol, 1.4 equiv.) at 0°C. The resulting mixture was stirred at ambient temperature for 20 h. The reaction was quenched by addition of distilled water (150 mL) and saturated aqueous NaHCO₃ (70 mL). The mixture was extracted with EtOAc (4×60 mL). The organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated. The residue was purified by flash chromatography (30% EtOAc: hexanes) to yield **9** (3.65 g, 97%). ¹H NMR (δ , ppm, CDCl₃): 7.85 (d, *J* = 7.4 Hz, 2H), 7.65-7.68 (m, 2H), 7.49-7.50 (m, 8H), 7.32-7.38 (m, 8H), 7.24-7.27(m, 3H), 6.67 (d, *J* = 9.9 Hz, 1H), 6.18 (d, *J* = 7.4 Hz, 1H), 5.42 (d, *J* = 8.8 Hz, 1H), 4.48-4.54 (m, 3H), 4.39- 4.42(m, 2H), 4.04-4.07 (m, 1H), 3.74 (s, 3H), 2.67-2.76 (m, 2H), 2.12-2.14 (m, 1H), 1.20 (d, *J* = 6.2 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.6 Hz, 3H), 0.92 (s, 9H), 0.10 (s, 3H), 0.06 (s, 3H). ¹³C NMR (δ , ppm, CDCl₃): 170.9, 170.3, 169.9, 156.1, 144.2, 141.2, 129.4, 128.0, 127.6, 127.0, 126.8, 125.0, 119.9, 119.8, 68.7, 67.1, 66.9, 59.7, 57.9, 52.2, 52.0, 47.1, 33.5, 31.3, 25.5, 20.7, 19.1, 17.7, 17.4, -4.4, -5.3. IR (neat, cm⁻¹): 3293, 3058, 2950, 2926, 2894, 2853, 1732, 1706, 1649, 1519, 1446, 1280, 1236, 1029. MS (MALDI- FTMS) *m/z*: 936.4067 (Calc'd for C₅₃H₆₃N₃O₇SSi + Na = 936.4048). mp 143-145°C. [α]_D²⁵ = +15.5 (0.64, CH₂Cl₂).

Compound **9** (^1H NMR and ^{13}C NMR)

***N*- α -[(Fluorenylmethoxy)carbonyl]-D-valyl-D-valyl-D-cysteinyl-(*S*-triphenylmethyl)-L-threonine-(*O*-*t*-butyldimethylsilyl) methyl ester (**10**).** A solution of **9** (3.65 g, 4.0 mmol, 1.0 equiv.) was stirred in CH₂Cl₂: Et₂NH (1:1, 40 mL) at 0°C for 4 h. The reaction was diluted with ethyl acetate and evaporated to dryness. The crude amine was dissolved in DMF (50 mL) and CH₂Cl₂ (20 mL) and treated with *N*-Fmoc-D-Valine (1.494 g, 4.4 mmol, 1.1 equiv.), EDC (997 mg, 5.2 mmol, 1.3 equiv.) and HOBT (757 mg, 5.6 mmol, 1.4 equiv.) at 0°C. The resulting mixture was stirred at ambient temperature for 20 h. The reaction was quenched by the addition of distilled water (100 mL) and saturated aqueous NaHCO₃ (100 mL). The mixture was extracted with ethyl acetate (4× 60 mL). The organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (40% EtOAc: hexanes) to yield **10** (3.20 g, 71 % over two steps). ¹H NMR 500 MHz (δ , ppm, CDCl₃): 7.76 (d, *J* = 6.6 Hz, 2H), 7.58 (d, *J* = 6.3 Hz, 2H), 7.37-7.41 (m, 8H), 7.25-7.30 (m, 8H), 7.19-7.21 (m, 3H), 6.52 (d, *J* = 7.7 Hz, 1H), 6.33 (d, *J* = 6.2 Hz, 1H), 6.20-6.21 (m, 1H), 5.48 (d, *J* = 7.3 Hz, 1H), 4.39-4.45 (m, 3H), 4.34-4.36 (m, 1H), 4.19-4.25 (m, 2H), 4.10-4.13 (m, 1H), 3.96-3.98 (m, 1H), 3.63 (s, 3H), 2.66-2.69 (m, 1H), 2.56-2.59 (m, 1H), 2.01-2.04 (m, 2H), 1.10 (d, *J* = 5.5 Hz, 3H), 0.84- 0.88 (m, 12H), 0.81 (s, 9H), 0.00 (s, 3H), -0.04 (s, 3H). IR (neat, cm⁻¹): 3292, 2957, 2930, 2856, 1717, 1636, 1519, 1445, 1388, 1225, 1097. MS (MALDI- FTMS) *m/z*: 1035.4732 (Calc'd for C₅₈H₇₂N₄O₈SSi + Na = 1035.4732). mp 125-127°C. [α]_D²⁵ = +19.4 (0.64, CH₂Cl₂).

Compound **10** (¹H NMR and ¹³C NMR)

D-Valyl-D-valyl-D-cysteinyl-(S-triphenylmethyl)-L-threonine-(O-*t*-butyldimethylsilyl) methyl ester (4). To a solution of **10** (2.5 g, 2.47 mmol) in CH₂Cl₂ (25 mL) was added diethylamine (15 mL) at 0°C. The resulting solution was stirred at 0°C for three hours and at ambient temperature for an additional three hours. The reaction mixture was concentrated to dryness and the residue was purified by flash chromatography (50% EtOAc:hexanes, 1.5% MeOH + 1% Et₃N in CH₂Cl₂ and 3% MeOH + 1% Et₃N in CH₂Cl₂) to yield **4** as a white solid (2.03 g, 100 %). ¹H NMR 500 MHz (δ, ppm, CDCl₃): 7.92 (d, *J* = 9.2 Hz, 1H), 7.47-7.48 (m, 6H), 7.34 –7.37 (m, 6H), 7.27-7.30 (m, 3H), 6.72 (d, *J* = 9.2 Hz, 1H), 6.45 (d, *J* = 7.4 Hz, 1H), 4.46-4.52 (m, 2H), 4.27 (dd, *J* = 9.2 Hz, *J* = 6.6 Hz, 1H), 4.15-4.19 (m, 1H), 3.73 (s, 3H), 3.24 (d, *J* = 4.1 Hz, 1H), 2.19 –2.24 (m, 1H), 1.17 (d, *J* = 6.6 Hz, 3H), 1.03 (d, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.91 (s, 9H), 0.88 (d, *J* = 7.0 Hz, 3H), 0.09 (s, 3H), 0.05 (s, 3H). ¹³C NMR 125 MHz (δ, ppm, CDCl₃): 174.8, 171.4, 170.5, 170.1, 144.3, 129.5, 128.0, 126.8, 68.7, 67.0, 60.2, 57.9, 57.6, 52.2, 52.0, 33.6, 30.6, 29.9, 25.6, 20.7, 19.7, 19.6, 17.9, 17.8, 16.1, -4.5, -5.4. IR (neat, cm⁻¹): 3305, 2956, 2929, 2856, 1740, 1638, 1506, 1443, 1378, 1251, 1207, 1093. MS (MALDI- FTMS) *m/z*: 813.4040 (Calc'd for C₄₃H₆₂N₄O₆SSi + Na = 813.4051). mp 168-170°C. [α]_D²⁵ = +41.3 (1.0, CH₂Cl₂).

Compound **4** (^1H NMR and ^{13}C NMR) :

(2E)-5-[(Triphenylmethyl)thio]-2-pentenal (13). To a solution of triphenylmethyl mercaptan (1.382 g, 5 mmol, 1.0 equiv.) in CH₂Cl₂ (30 mL) was added acrolein (393 mg, 7 mmol, 1.4 equiv.) and triethylamine (0.98 mL, 7 mmol, 1.4 equiv.), the resulting mixture was stirred at room temperature for 1 h. Concentration *in vacuo* left aldehyde **12** as a white solid, spectroscopically pure form. A mixture of aldehyde **12** (5 mmol, 1.0 equiv.) and (triphenylphosphoranylidene) acetaldehyde (1.52 g, 5 mmol, 1.0 equiv.) in 30 mL of dry benzene was heated under reflux for 7h. Removal of solvent *in vacuo* left a crude residue. The crude product was purified by a two-step silica gel chromatography: the first was eluted with 20% EtOAc : hexanes to remove high polar impurity; the second one was eluted with 10% ether : hexane to afford the title aldehyde **13** as a yellow solid (1.06 g, 60% over two steps). ¹H NMR 500 MHz (δ, ppm, CDCl₃): 9.50 (d, *J* = 8.1 Hz, 1H), 7.49–7.50 (m, 6H), 7.30 (m, 9H), 6.70 (dt, *J* = 15.8 Hz, *J* = 6.6 Hz, 1H), 6.06 (dd, *J* = 15.8 Hz, *J* = 7.7 Hz, 1H), 2.37–2.45 (m, 4H). ¹³C NMR (δ, ppm, CDCl₃, 150 MHz): 193.6, 155.6, 144.5, 133.6, 129.5, 127.9, 126.7, 66.9, 31.6, 29.9.

Compound **13** (^1H NMR and ^{13}C NMR)

Chlorohydrin (17). To a solution of (4*R*)-3-(2-chloroacetyl)-4-benzyloxazolidinone **16** (380 mg, 1.5 mmol, 1.5 equiv.) in 5 mL of CH₂Cl₂ at -78 °C was added diisopropylethylamine (0.35 mL, 2.0 mmol, 2.0 equiv.), followed by di-*n*-butylboron trifluoromethanesulfonate (1.0 M solution in CH₂Cl₂, 1.8 mL, 1.8 equiv.). Removal of the cooling bath was followed by stirring for 1 h at ambient temperature. After recooling to -78°C, a solution of aldehyde **13** (358.5 mg, 1.0 mmol, 1.0 equiv.) in CH₂Cl₂ (4 mL plus 2 mL rinse) was added dropwise *via* cannula. The mixture was stirred for 2 h at -78°C followed by warming to -10°C over 6 h. The reaction was quenched by addition of aqueous NaHSO₄ (0.5 M, 7 mL). The mixture was extracted with EtOAc (3×20 mL) and the combined organic layers were concentrated *in vacuo*. After dissolving the residue in 10 mL ether, the solution was cooled to 0°C and treated with 5 mL of pH 7.0 phosphate buffer and 5 mL of 30% aqueous hydrogen peroxide. After stirring for 1 h at 0°C, the solution was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with saturated NaHCO₃ and brine, dried over MgSO₄, filtered, and concentrated. Purification *via* flash chromatography (gradient elution from 10 % to 25 % EtOAc: hexanes) afforded chlorohydrin **17** (421 mg, 69 %). ¹H NMR 400 MHz (δ, ppm, CDCl₃): 7.36- 7.14 (m, 20H), 5.66 (m, 1H), 5.65 (d, *J* = 2.0 Hz, 1H), 5.41 (dd, *J* = 15.2, 6.8 Hz, 1H), 4.54 (dd, *J* = 9.2, 4.8 Hz, 2H), 4.01 (dd, *J* = 9.2, 2.8 Hz, 1H), 3.87 (t, *J* = 9.2 Hz, 1H), 3.21 (dd, *J* = 13.6, 9.2 Hz, 1H), 2.69 (b, 1H), 2.24- 2.02 (m, 4H). ¹³C NMR 100 MHz (δ, ppm, CDCl₃): 167.1, 152.2, 144.3, 134.2, 133.9, 129.2, 129.1, 128.6, 127.5, 127.1, 126.3, 72.3, 66.3, 65.9, 58.6, 55.0, 36.7, 30.9, 30.8. MS (MALDI-FTMS) *m/z*, 634.1798 (Calc'd for C₃₆H₃₄ClNO₄S + Na = 634.1789). IR (neat, cm⁻¹): 3373, 3059,

2955, 2880, 1779, 1707, 1491, 1448, 1390, 1212, 1069. mp = 95-97°C. $[\alpha]_D^{25} = -34.5$
(3.1, CH₂Cl₂).

Compound 17 (¹H NMR and ¹³C NMR)

Alcohol (18). Aluminum powder (Acros, 100 mesh; 400 mg, 15 mmol, 20 equiv.) was added in one portion to an aqueous solution of HgCl_2 (1 %, 5 mL). The suspension was shaken for 15 s, and quickly filtered. The metal amalgam residue was washed once with distilled water and used immediately. To a solution of chlorohydrin **11** (466 mg, 0.75 mmol, 1.0 equiv.) in 10 mL THF: H_2O (9:1) was added aluminum amalgam prepared as described above at 0 °C. This suspension was stirred for 2 h at 0 °C. Filtration and concentration of the filtrate afforded alcohol **12** in spectroscopically pure form (430 mg, 100 %). ^1H NMR 400 MHz (δ , ppm, CDCl_3): 7.40- 7.18 (m, 20H), 5.60 (dt, J = 15.2, 6.8 Hz, 1H), 5.46 (dd, J = 15.2, 6.4 Hz, 1H), 4.65 (m, 1H), 4.54 (b, 1H), 4.13 (d, J = 4.4 Hz, 2H), 3.26- 3.04 (m, 3H), 2.98 (b, 1H), 2.76 (dd, J = 13.6, 9.6 Hz, 1H), 2.19 (t, J = 7.2 Hz, 2H), 2.08 (t, J = 6.8 Hz, 2H). ^{13}C NMR 100 MHz (δ , ppm, CDCl_3): 172.0, 153.3, 144.8, 134.9, 131.8, 130.1, 129.5, 129.3, 128.9, 127.8, 127.3, 126.5, 68.4, 66.5, 66.2, 54.9, 42.5, 37.7, 31.3, 31.2. IR (neat, cm^{-1}): 3497, 3056, 2917, 1776, 1693, 1594, 1487, 1443, 1385, 1196, 1112, 1032. MS (MALDI-FTMS): m/z : 600.2183 (Calc'd for $\text{C}_{36}\text{H}_{35}\text{NO}_4\text{S} + \text{Na} = 600.2179$). $[\alpha]_{\text{D}}^{25} = -21$ (4.0, CH_2Cl_2).

Compound **18** (^1H NMR and ^{13}C NMR)

(3*R*, 4*E*)-3-Hydroxy-7-mercapto-4-heptenoic acid (5). To a solution of compound **18** (300 mg, 0.52 mmol, 1.0 equiv.) in 7 mL THF was added an aqueous solution of LiOH (60 mg in 5 mL water, 1.42 mmol, 2.7 equiv.) and aqueous solution of H₂O₂ (30%, 1 mL) at 0°C. The reaction was stirred for 1 h, followed by slow addition of Na₂SO₃ (1.0 g). This mixture was stirred overnight. The reaction mixture was concentrated *in vacuo* and the aqueous layer was extracted with EtOAc (3× 20 mL). The combined organic extracts were washed with brine, dried over MgSO₄, filtered and concentrated. Purification by flash silicon gel chromatography (50% EtOAc: hexanes and 4% HOAc :EtOAc) afforded chiral acid **5** (171 mg, 78%). ¹H NMR 500 MHz (δ, ppm, CDCl₃): 7.48-7.49 (m, 6H), 7.34-7.37 (m, 6H), 7.29 –7.30 (m, 3H), 5.66 (dt, *J* = 15.4 Hz, *J* = 8.5 Hz, 1H), 5.50 (dd, *J* = 15.4 Hz, *J* = 6.3 Hz, 1H), 4.54 (dd, *J* = 11.8 Hz, *J* = 6.6 Hz, 1H), 2.61-2.63 (m, 2H), 2.27 (t, *J* = 8.0 Hz, 2H), 2.15-2.18 (m, 2H). ¹³C NMR (δ, ppm, CDCl₃, 150M Hz): 176.9, 144.8, 131.5, 130.6, 129.5, 127.8, 126.6, 68.4, 66.5, 41.2, 31.3, 31.2. HRMALDI-FTMS) *m/z*: 441.1505 (Calc'd for C₂₆H₂₆O₃S + Na = 441.1495); [α]²⁵_D = +4.52 (2.1, CH₂Cl₂). Published optical rotation data [α]²⁰_D = +4.6 (2.0, CH₂Cl₂). (Li, K. W.; Wu, J.; Xing, W. and Simon, J. A. *J. Am. Chem. Soc.* **1996**, *118*, 7237-7238).

^1H NMR and ^{13}C NMR of Compound **5**:

Hydroxy methyl ester (19). To a solution of **4** (171 mg, 0.215 mmol, 1.0 equiv.) in CH₂Cl₂ : CH₃CN (1:1, 10 mL) was added chiral acid **5** (90 mg, 0.215 mmol, 1.0 equiv.), BOP (144 mg, 0.325 mmol, 1.5 equiv.) and DIPEA (0.12 mL, 0.68 mmol, 3.0 equiv.). The solution was stirred at ambient temperature for one hour. The reaction mixture was concentrated to dryness and purified by flash chromatography (33%, 50% and 67% EtOAc: hexanes) to yield **19** as a white solid (260 mg, 100%). ¹H NMR 500 MHz (δ, ppm, CDCl₃): 7.46-7.48 (m, 12H), 7.24-7.36 (m, 18H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.80 (d, *J* = 13.4 Hz, 1H), 6.63 (d, *J* = 9.1 Hz, 1H), 5.60 (dt, *J* = 154.4 Hz, *J* = 6.6 Hz, 1H), 5.46 (dd, *J* = 15.4 Hz, *J* = 6.2 Hz, 1H), 4.45-4.54 (m, 4H), 4.37 (t, *J* = 7.4 Hz, 1H), 4.21 (m, 1H), 3.71 (s, 3H), 2.72 (dd, *J* = 12.9 Hz, *J* = 7.7 Hz, 1H), 2.64 (dd, *J* = 12.9 Hz, *J* = 6.2 Hz, 1H), 2.41-2.50 (m, 2H), 2.26 (t, *J* = 8.1 Hz, 2H), 2.10-2.15 (m, 2H), 1.98-2.06 (m, 2H), 1.16 (d, *J* = 6.3 Hz, 3H), 0.91 (d, *J* = 1.9 Hz, 6H), 0.90 (s, 9H), 0.86 (d, *J* = 7.0 Hz, 3H), 0.82 (d, *J* = 6.6 Hz, 3H), 0.09 (s, 3H), 0.05 (s, 3H). ¹³C NMR (δ, ppm, CDCl₃): 171.5, 171.0, 170.9, 170.5, 172.2, 144.8, 144.2, 132.8, 129.5, 129.4, 129.1, 127.9, 127.7, 126.7, 126.5, 68.9, 68.8, 66.9, 66.4, 58.2, 57.8, 53.4, 52.1, 51.7, 42.6, 34.3, 31.7, 31.4, 25.6, 20.5, 19.0, 18.9, 18.6, 18.5, 17.6, -4.5, -5.3. IR (neat, cm⁻¹): 3272, 2957, 2929, 2855, 1739, 1630, 1489, 1443, 1388, 1316, 1251, 1091, 1033. MS (MALDI- FTMS) *m/z*: 1213.5548 (Calc'd for C₆₉H₈₆N₄O₈S₂Si + Na = 1213.5548). mp 123-125°C. [α]²⁵_D = +16.2 (1.5, CH₂Cl₂).

Compound **19** (^1H NMR and ^{13}C NMR)

Hydroxy acid (3). To a solution of **19** (260 mg, 0.215 mmol, 1.0 equiv.) in THF (10 mL) was added aqueous LiOH solution (0.5 M, 7.2 mL) and the resulting solution was stirred overnight at ambient temperature. The reaction mixture was concentrated to remove most of THF and CH₂Cl₂ (20 mL) was added. Aqueous HCl solution (1.0 M) was added to adjust to pH = 5.0. The mixture was extracted with CH₂Cl₂ (3 × 20 mL). The organic extracts were dried, concentrated and the residue was purified by flash chromatography (3 %, 5 %, 8 % and 10 % MeOH: CH₂Cl₂) to yield hydroxy acid **3** as a white solid (200 mg, 79 %). ¹H NMR 500 MHz (δ, ppm, CDCl₃: CD₃OD = 9:1): 7.40 – 7.42 (m, 12H), 7.26-7.29 (m, 12H), 7.19-7.22 (m, 6H), 6.70 (br, 1H), 5.54 (dt, *J* = 15.1 Hz, *J* = 6.6 Hz, 1H), 5.42 (dd, *J* = 15.4 Hz, *J* = 6.3 Hz, 1H), 4.44 (br, 1H), 4.37 (br, 1H), 4.04 (br, 1H), 3.87 (br, 1H), 2.67 (m, 1H), 2.55 (dd, *J* = 12.8 Hz, *J* = 7.7 Hz, 1H), 2.35-2.45 (m, 2H), 2.21 (t, *J* = 7.3 Hz, 2H), 2.02-2.10 (m, 4H), 1.10 (d, *J* = 6.3 Hz, 3H), 0.82 – 0.88 (m, 12H), 0.86 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H). ¹³C NMR (δ, ppm, CDCl₃: CD₃OD = 9:1): 172.3, 172.1, 171.7, 170.3, 144.7, 144.2, 132.4, 129.4, 129.4, 129.3, 127.8, 127.7, 126.7, 68.8, 66.9, 66.4, 59.3, 52.6, 42.6, 31.3, 31.2, 30.3, 30.1, 29.5, 25.6, 25.6, 21.1, 19.1, 18.9, 18.1, 17.8, -4.9, -5.1. IR (neat, cm⁻¹): 3306, 3056, 2960, 2928, 2855, 1633, 1488, 1442, 1251, 1083, 1033. MS (MALDI- FTMS) *m/z*: 1199.5385 (Calc'd for C₆₈H₈₄N₄O₈S₂Si + Na = 1199.5392); mp 149-151°C. [α]_D²⁵ = +19.1 (1.0, CH₂Cl₂).

Compound **3** (^1H NMR and ^{13}C NMR):

Lactone (20). A solution of PPh₃ (315 mg, 1.2 mmol, 25 equiv.) and TsOH·H₂O (46 mg, 0.25 mmol, 5.0 equiv.) in THF (50 mL) at 25°C was treated with DIAD (0.19 mL, 1mmol, 20 equiv.). The cloudy mixture was stirred at room temperature for half an hour. The mixture was cooled to 0°C and a solution of the hydroxy acid **3** (50 mg, 0.05 mmol, 1.0 equiv.) in THF (5 mL) was added via syringe pump (2.5 mL/ h) and the resulting mixtute was stirred under a nitrogen atmosphere for additional two hours (0°C). The reaction was concentrated *in vacuo* and the residue was purified by flash chromatography (50 %, 70 %, and 100 % EtOAc: hexanes; 3%, 8% MeOH: CH₂Cl₂) to yield semi-pure lactone. Further purification by chromatotron (2mm plate, 1%, 8% MeOH: CH₂Cl₂) to yield lactone **20** (29.0 mg, 58%). ¹H NMR 500 MHz (δ, ppm, CDCl₃: CD₃OD = 9:1): 7.28-7.40 (m, 12H), 7.24-7.26 (m, 12H), 7.18-7.21 (m, 6H), 5.70 (dt, *J* = 15.1 Hz, *J* = 6.6 Hz, 1H), 5.40 (dd, *J* = 15.4 Hz, *J* = 9.2 Hz, 1H), 4.45 (m, 1H), 4.12 (m, 2H), 4.07 (d, *J* = 8.1 Hz, 1H), 3.90 (m, 1H), 3.63 (d, *J* = 11hz, 1H), 3.05 (dd, *J* = 15 Hz, *J* = 5.1 Hz, 1H), 2.62 (m, 2H), 2.51 (dd, *J* = 12.9 Hz, *J* = 8.1 hz, 1H), 2.17-2.22 (m, 3H), 1.97-2.09 (m, 4H), 1.09 (d, *J* = 6.3 Hz, 3H), 0.86-0.89 (m, 9H), 0.85 (s, 9H), 0.74 (d, *J* = 7.0 Hz, 3H), 0.04 (s, 3H), 0.02 (s, 3H). ¹³C NMR (δ, ppm, CDCl₃: CD₃OD = 9:1): 172.1, 171.4, 170.1, 169.3, 168.6, 144.6, 144.5, 144.2, 144.1, 129.4, 129.3, 127.8, 127.7, 126.6, 126.5, 68.8, 66.8, 66.5, 66.4, 54.7, 31.7, 31.1, 30.9, 29.5, 29.4, 29.1, 25.5, 22.5, 19.2, 19.0, 18.8, 17.8, 13.8, -5.0, -5.2. MS (MALDI- FTMS) *m/z*: 1181.5242 (Calc'd for C₆₈H₈₂N₄O₇S₂Si + Na = 1181.5286). IR (neat, cm⁻¹): 2924, 2852, 1734, 1648, 1442, 1257, 1084, 1033. mp 105-107°C. [α]_D²⁵ = +11.3 (1.0, CH₂Cl₂).

Compound **20** (^1H NMR and ^{13}C NMR)

HPLC analysis of Lactone **20**: (Vydac reversal phase C18 column)

Flow rate: 1 mL/min. Wavelength: 254 nm.

CH₃CN = H₂O (0.1% TFA)
= 50:50

Lactone (20)

254nm

FR-901375 (1). To a vigorously stirring solution of iodine (102 mg, 0.4 mmol, 20 equiv.) in MeOH (27 mL) was added lactone **18** (20 mg, 0.02 mmol) in MeOH (5 mL) at room temperature. After the deep brown solution was stirred for 10 min, the reaction was quenched by the addition of aqueous ascorbate (0.2 M) in pH 4.0 citrate (0.2M) buffer (4 mL). A colorless mixture was formed. After removal of most of methanol *in vacuo*, EtOAc (15 mL) and brine (5 mL) was added. The mixture was extracted with EtOAc (3 × 20 mL) and the combined extracts were concentrated to leave a residue. To the residue was added CH₃CN (10 mL) and aqueous HF (**Hazard:** hydrofluoric acid reacts rapidly with glass and quickly destroys tissue. New thick rubber or plastic gloves should be worn after carefully checking that no holes are present, skin burns must receive immediate and specialized attention) solution (48%, 0.5 mL). The mixture was stirred at ambient temperature for one hour before the reaction was quenched by the addition of EtOAc (20 mL) and brine (5 mL). Aqueous NaOH solution (6M, 1.6 mL) was added slowly to neutralize the excess HF. The mixture was extracted with EtOAc (3 × 20 mL). The combined extracts were concentrated and purified by flash chromatography (1% + 10 % MeOH: CH₂Cl₂ and 5% HOAc: EtOAc) to yield FR-901375 (**1**) (7.5 mg, 63% over two steps). ¹H NMR 500 MHz (δ, ppm, CD₃OD): 5.83-5.89 (m, 1H), 5.67 (d, *J* = 15.9 Hz, 1H), 5.60 (m, 1H), 4.70 (t, *J* = 5.5 Hz, 1H), 4.43 (dd, *J* = 9.8 Hz, *J* = 3.0 Hz, 1H), 4.31-4.35 (m, 1H), 4.03 (d, *J* = 8.6 Hz, 1H), 3.55 (d, *J* = 9.2 Hz, 1H), 3.37 (d, *J* = 5.5 Hz, 1H), 2.97-3.02 (m, 1H), 2.85-2.93 (m, 2H), 2.60-2.64 (m, 2H), 2.41-2.48 (m, 1H), 2.07-2.11(m, 1H), 1.17 (d, *J* = 6.7 Hz, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.98 (d, *J* = 6.7 Hz, 3H). ¹³C NMR 125 MHz (δ, ppm, CD₃OD): 175.5, 164.6, 172.5, 172.1, 170.5, 133.9, 127.5, 71.5, 68.6, 66.6, 62.1, 60.3, 55.2, 41.9,

40.6, 38.5, 34.9, 29.5, 20.4, 20.3, 20.1, 20.0, 19.3. IR (neat, cm^{-1}): 3349, 3269, 2968, 2925, 1734, 1665, 1625, 1529, 1408, 1263, 1240, 1022, 1002. MS (MALDI- FTMS) m/z : 581.2079 (Calc'd for $\text{C}_{24}\text{H}_{38}\text{N}_4\text{O}_7\text{S}_2 + \text{Na} = 581.2074$). $[\alpha]_{\text{D}}^{25} = -24.4$ (0.7, CHCl_3).

FR-901375 **1** (^1H NMR and ^{13}C NMR)

HPLC analysis of FR-901375 (synthesized):

Vydac reversal phase C18 column. Flow rate: 1 mL/min. Wavelength: 214 nm.

HPLC analysis of FR-901375 (Authentic):

Vydac reversal phase C18 column. Flow rate: 1 mL/min. Wavelength: 214 nm.