Total Syntheses of Depsipeptide Elastase Inhibitors YM-47141 and YM-47142 using Ylide Protection and Coupling Methods.

Supplementary Information

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N-(2-Bromoacetyl)-Leu-OBn (**2**).

A solution of leucine benzyl ester (15.047g, 68 mmol) in 400 mL of CH_2Cl_2 was cooled to 0 °C in an ice-water bath and sequentially treated with bromoacetic acid (10.421g, 75 mmol), DMAP (415 mg, 3.4 mmol) and DCC (14.475 g, 75 mmol). A white precipitate of dicyclohexylurea formed immediately upon the addition of DCC. The reaction mixture was stirred at 0 °C for 1 h and diluted with Et_2O (500 mL). The white precipitate was removed by filtration. The filtrate was concentrated *in vacuo*. Flash chromatography (SiO_2, 10 x 30 cm, 1:7 to 1:4 EtOAc-hexane gradient elution) gave the desired product which was further purified by recrystallization from EtOAc-hexane to provide product 21.429 g (92%) as white needles: mp 91.5-92.5 °C; $[\alpha]^{20}_D$ = -3.86 (C 10.0, $CHCl_3$); Rf 0.35 (SiO_2, EtOAc:hexane = 1:4); ^{1}H NMR ($CDCl_3$ 500 MHz) : δ 7.40-7.30 (m, 5 H), 6.81 (d, J = 7.6 Hz, 1 H), 5.19 (d, J = 12.3 Hz, 1 H), 5.16 (d, J = 12.3 Hz, 1 H), 4.66 (dt, J = 8.5, 5.2 Hz, 1 H), 3.88 (s, 2 H), 1.73-1.57 (m, 3 H), 0.93 (d, J = 6.3 Hz, 3 H), 0.92 (d, J = 6.3 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 172.1, 165.2, 135.1, 128.6, 128.5, 128.2, 67.2, 51.5, 41.3, 28.8, 24.8, 22.7, 21.9; IR (KBr) ν max 3069, 2964, 1742, 1649, 1550, 1189, 1153 cm^{-1}; HRMS: calcd for $(M+H)^+$, 342.0705, found 342.0715. Anal. calcd for $C_{15}H_{20}BrNO_3$: C, 52.64; H, 5.89; N, 4.09. Found: C, 52.84; H, 5.99; N, 4.07.

[*N*-(2-Triphenylphosphoniumacetyl)-Leu-OBn] bromide (**3**).

N-(2-Bromoacetyl)-Leu-OBn (**2**) (10.715 g, 31.3 mmol) was dissolved in a 1:3 ratio mixture of THF-Et_2O (160 mL) and 16.424 g of triphenylphosphine (62.6 mmol) was added to this solution in one portion. After 1 h, the clear reaction mixture started to turn cloudy. The reaction mixture was

stirred at 23 ° C and monitored by TLC. After 3 days, the reaction mixture became a thick white suspension, and TLC showed that the bromoacetyl compound (**2**) had been consumed. The white precipitate of the ylide salt (**3**) was collected by filtration. The white filtered cake was further rinsed with Et_2O (200 mL) and hexane (200 mL) to remove residual triphenylphosphine. The white precipitate was dried on a vacuum pump and provided the ylide salt (**3**) 18.320 g (97%).mp 150-151 °C; $[\alpha]^{20}_D$ = -29.72 (C 5.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 9.60 (d, J = 6.6 Hz, 1 H), 7.84-7.56 (m, 15 H), 7.33-7.27 (m, 5 H), 5.35 (dd, J = 14.3 Hz, J_{P-H} = 15.6 Hz, 1 H), 5.08 (d, J = 12.5 Hz, 1 H), 5.05 (dd, J = 15.0 Hz, J_{P-H} = 15.1 Hz, 1 H), 5.04 (d, J = 12.5 Hz, 1 H), 4.26 (dt, J = 10.3, 5.8 Hz, 1 H), 1.85-1.50 (m, 3H), 0.88 (d, J = 6.5 Hz, 3 H), 0.74 (d, J = 6.5 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 125 MHz), δ 171.3, 162.7 (d, J_{c-p} = 5.0 Hz), 135.8, 134.8, 134.0, 133.9, 130.0 (d, J_{c-p}=14.0 Hz), 128.1 (d, J_{c-p}=64.0 Hz), 127.9, 118.4 (d, J_{c-p} = 88.0 Hz), 66.4, 52.3, 39.3, 31.9 (d, J_{c-p} = 56.0 Hz), 24.6, 22.7, 21.4; IR (KBr) ν max 3168, 3016, 2955, 1749, 1735, 1667, 1549, 1535, 1439 cm^{-1}. HRMS calcd for ($M-Br^-$): 524.2355. Found: 524.2355. Anal. calcd for $C_{33}H_{35}BrNO_3P$: C, 65.56; H, 5.84; N, 2.32. Found: C, 65.37; H, 5.82; N, 2.33.

[(*4S*)-4-[(tert-Butoxylcarbonyl)amino]-6-methyl-3-oxo-2-(triphenylphosphoranylidene)-heptanoyl]-Leu-OBn (**4**).

In a 100 mL round-bottomed flask, 7.76 g of the ylide salt (**3**) (12.8 mmol) was dissolved in 30 mL of CH_2Cl_2 and subsequently treated with 18 mL of triethylamine (129 mmol). The resulting mixture was stirred at 23 °C under N_2 atmosphere for 1 h. In another 100 mL round-bottomed flask, a solution of *N*-Boc-L-Leu-OH (4.453 g, 19.3 mmol) in 30 mL of CH_2Cl_2 was treated with 3.20 g of CDI (19.3 mmol). This solution was stirred at 23 °C for 15 min. The *in situ*-generated ylide was then transferred into the CDI-activated *N*-Boc-L-Leu-OH solution through cannula. The combined mixture was stirred at 23 °C for an additional 24 h. The reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with water (150 mL) and saturated aqueous NaCl solution (150 mL). The organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography (SiO_2, 8 x 35 cm, Et_2O:hexane = 3:1 elution) gave product 9.083 g (96%) as a white foam: $[\alpha]^{20}_D$ = -13.85 (C 10.0, $CHCl_3$); Rf 0.24 (SiO_2, Et_2O:hexane = 3:1).

^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.29 (br, s, 1 H), 7.60-7.26 (m, 20 H), 6.91 (br, s, 1 H), 5.09 (d, J = 12.6 Hz, 1 H), 5.05 (d, J = 12.6 Hz, 1 H), 4.90 (br, s, 1 H), 4.19 (ddd, J = 10.5, 7.0, 4.5 Hz, 1 H), 1.72-1.69 (m, 1 H), 1.68-1.65 (m, 1 H), 1.47 (s, 9 H), 1.35-1.11 (m, 4 H), 0.88 (d, J = 6.5 Hz, 3 H), 0.75 (d, J = 6.5 Hz, 3 H), 0.70 (d, J = 6.5 Hz, 3 H), 0.61 (br, s, 3 H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 188.4, 172.8, 166.2, 156.5, 136.0, 132.7 (d, $J_{c\text{-}p}$ = 10.0 Hz), 131.6, 128.4, 128.3, 127.9, 127.6, 126.2 (d, $J_{c\text{-}p}$ = 92.3 Hz), 78.2, 73.2 (d, $J_{c\text{-}p}$ = 110.5 Hz), 65.6, 51.7, 50.8, 40.6, 40.2, 28.2, 24.3, 24.0, 22.8, 22.7, 22.5, 21.1; IR (KBr) ν max 3431, 3250, 2955, 1743, 1689, 1625, 1523, 1160 cm^{-1}; HRMS: calcd for $(M+H)^+$ 737.3720. Found: 737.3727. Anal. calcd for $C_{44}H_{53}N_2O_6P$: C, 71.72; H, 7.25, N, 3.80; Found: C, 71.75; H, 7.27; N, 3.86.

[(*4R*)-4-[(tert-Butoxylcarbonyl)amino]-6-methyl-3-oxo-2-(triphenylphosphoranylidene)-heptanoyl]-Leu-OBn (D-Leucine Isomer of **4**)

The ylide salt (**3**) (2.95 g, 4.88 mmol) was dissolved in 15 mL of CH_2Cl_2 and then treated with Et_3N (6.8 ml, 48.8 mmol). The mixture was stirred under N_2 for 1 h. In another flask, Boc-D-Leu-OH (1.75 g, 7.56 mmol) was dissolved in 10 ml of CH_2Cl_2 and was treated with CDI (1.25 g, 7.56 mmol). Then the ylide was added to CDI-activated Boc-D-Leu-OH solution. The resulting mixture was stirred for 24 h and then was washed with water. The organic layer was dried over Na_2SO_4. After the solvent was evaporated *in vacuo*, the residue was flash chromatographed ($CH_3CO_2Et/CHCl_3$ = 1/3) to give 3.2 g (89%) of expected product as a white foam. $[\alpha]^{20}_D$ = -22.33 (C 7.0, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.71 (br, s, 1 H), 7.70-7.29 (m, 20 H), 7.09 (d, J = 7.3 Hz, 1 H), 5.16 (m, 1 H), 5.09 (d, J = 12.7 Hz, 1 H), 5.03 (d, J = 12.7 Hz, 1 H), 4.41 (m, 1 H), 1.69 (m, 2 H), 1.55 (m, 2 H), 1.49 (s, 9 H), 1.34 (m, 2 H), 0.93 (d, J = 6.4, 3 H), 0.89 (d, J = 5.9 Hz, 3 H), 0.79 (d, J = 5.7 Hz, 3 H), 0.65 (br, s, 3 H). ^{13}C ($CDCl_3$, 75 Hz) δ 188.9, 173.8, 167.5, 156.8, 136.3, 133.3 (d, $J_{c\text{-}p}$ = 10.0 Hz), 131.5, 128.5, 128.4, 127.9, 127.7, 126.3 (d, $J_{c\text{-}p}$ = 93.6 Hz), 78.9, 73.9 (d, $J_{c\text{-}p}$ = 107.6 Hz), 66.1, 51.9, 50.9, 41.0, 40.6, 28.4, 25.1, 24.6, 23.2, 22.7, 22.0, 21.5. IR (KBr) ν max 3435, 3274, 2959, 1743, 1687, 1625, 1512 cm^{-1}; HRMS: calcd for $(M+H)^+$ 737.3719, found 737.3727. Anal. calcd for $C_{44}H_{53}N_2O_6P$: C, 71.72; H, 7.25; N, 3.80; Found: C, 71.50; H, 7.24; N, 3.88.

Ozonolysis of Ylide **4**:

The ylide **4** (0.51 g, 0.693 mmol) was dissolved in 20 mL of CH_2Cl_2, ozonized at -78 °C for 7 min, and the solution was then purged with N_2 for 10 min. After the solvent was evaporated *in vacuo*, the residue was flash-chromatographed (CH_3CO_2Et/hexanes = 1/2) to give 0.274 g (79%) of hemihydrate. $[\alpha]^{20}_D$ = -22.46 (C 5.0, $CHCl_3$).

^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.92 (d, J = 8.3 Hz, 1 H), 7.82 (s, 1 H), 7.34 (m, 10 H), 7.12 (d, J = 6.7 Hz, 2 H), 6.90 (d, J = 8.5 Hz, 1 H), 6.64 (s, 1 H), 5.10 (s, 4 H), 4.73 (m, 1 H), 4.34 (m, 3 H), 1.58 (m, 12 H), 1.33 (s, 18 H), 0.82 (m, 24 H). IR (KBr) max 3370, 2965, 1752, 1675, 1545 cm^{-1}; HRMS calcd for $(M+Na)^+$ 1021.5361. Found: 1021.5366.

Ozonolysis of D-Leucine Isomer of **4**:

The D-leucine isomer of **4** (0.28 g, 0.38 mmol) in 10 mL of CH_2Cl_2 was ozonized at -78 °C for 3 min and the solution was then purged with N_2 for 5 min. After the solvent was evaporated *in vacuo*, the residue was flash-chromatographed (CH_3CO_2Et: hexanes = 1:2 to give 0.135 g (71%) of hemihydrate. $[\alpha]^{20}_D$ = +3.9 (C 5.0, $CHCl_3$)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.37-7.10 (m, 14 H), 5.97 (s, 1 H), 5.84 (s, 1 H), 5.18 (m, 4 H), 4.63 (m, 4 H), 1.65 (m, 12 H), 0.92 (s, 18 H), 0.87 (m, 24 H). IR (KBr) ν max 3423, 3021, 2975, 1733, 1702, 1687, 1496 cm^{-1}. HRMS calcd for $(M+Na)^+$ 1021.5361. Found: 1021.5365.

N-Boc-Thr[*N*-Fmoc-D-Ala]-OBn (Benzyl Ester of **5**)

A suspension of N-Fmoc-D-Ala-OH (10.341 g, 33.2 mmol) in 200 mL of CH_2Cl_2 was added to 5.485 g of 1,1'-carbonyldiimidazole (33.2 mmol) in two portions. The resulting mixture immediately turned clear and CO_2 vigorously effervesced. After 15 min, a solution of *N*-Boc-Thr-OBn (25.450 g, 82.3 mmol) in 200 mL of CH_2Cl_2 was added through a cannula. The reaction mixture was stirred at 23 °C for 20 h under N_2 atmosphere and then was put through a short silica gel column (8 x 12 cm) directly and rinsed with 1.5 liter of 40% EtOAc-hexane. The fractions containing the product and unreacted *N*-Boc-Thr-OBn were concentrated. The residue was purified by flash chromatography

(SiO_2, 8 x 20 cm, 1:6 to 1:4 ratio of EtOAc-hexane gradient elution) to give the desired product 15.973 g (80%) along with recovered *N*-Boc-Thr-OBn (16.562 g). The product was further purified to yield white needles by recrystallization from Et_2O-hexane mixture: mp. 125.5 °C - 126.5 °C; $[\alpha]^{20}_D$ = +14.65 (C 11.0, $CHCl_3$); Rf 0.53 (EtOAc: hexane = 1:2); ^1H NMR (DMSO-D_6, 500 MHz) δ 7.88 (d, J = 7.6 Hz, 2 H), 7.73 - 7.68 (m, 3 H), 7.44-7.32 (m, 9 H), 7.25 (d, J = 9.2 Hz, 1 H), 5.24 (dq, J = 6.5, 3.2 Hz, 1 H), 5.10 (d, J = 12.5 Hz, 1 H), 5.06 (d, J = 12.5 Hz, 1 H), 4.39 (dd, J = 9.2, 3.2 Hz, 1 H), 4.29 (d, J = 6.8 Hz, 2 H), 4.21 (t, J = 6.8 Hz, 1 H), 4.03 (dq, J = 7.5, 7.2 Hz, 1 H), 1.37 (s, 9 H), 1.18 (d, 3 H, J = 7.5 Hz), 1.16 (d, 3 H, J = 6.5 Hz); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 171.9, 169.6, 155.9, 155.8, 143.8, 143.7, 140.7, 135.6, 128.3, 128.1, 127.8, 127.6, 127.0, 125.2, 120.1, 78.6, 70.2, 66.4, 65.6, 57.0, 49.4, 46.6, 28.1, 16.9, 16.4; IR (KBr) ν max 3396, 3339, 2797, 1750, 1714, 1513, 1216, 1165 cm^{-1}; HRMS calcd for $(M+H)^+$: 603.2706. Found: 603.2705. Anal. calcd for $C_{34}H_{38}N_2O_8$: C, 67.76; H, 6.35; N, 4.65. Found: C, 67.78; H, 6.33; N, 4.61.

N-Boc-Thr[*N*-Fmoc-D-Ala]-[(*4S*)-4-aminio-3-oxo-2-triphenylphosphoranylidene-heptanoyl]-Leu-OBn (**6**)

A solution of *N*-Boc-Thr[*N*-Fmoc-D-Ala]-OBn (benzyl ester of **5**) (4.632 g, 7.7 mmol) in 80 mL of CH_2Cl_2 was treated with 10% Pd-C (460 mg) and the resulting black suspension was stirred at 23 °C under H_2 atmosphere (balloon) for 14 h. The catalyst was removed by filtration through a short pad of celite and rinsed with additional 300 mL of CH_2Cl_2. The filtrate was concentrated to give crude acid **5** which was used directly in the next reaction without further purification.

In another 100 mL round-bottomed flask, 5.83 g of (**4**) (7.91 mmol) was treated with 15 mL of 3M HCl-EtOAc. The mixture was stirred at 23 °C for 30 min. EtOAc and excess HCl was removed *in vacuo*. The residual HCl was further removed by adding Et_2O (30 mL) to the hydrochloride salt, followed by its removal *in vacuo*. After this procedure was repeated twice, the hydrochloride salt thus obtained was used in the following reaction immediately without further purification.

A solution of acid and the hydrochloride salt in 60 mL of CH_2Cl_2 was cooled to 0 °C and sequentially treated with HOBt (1.029 g, 7.6 mmol), Et_3N (1.54 g, 15.2 mmol), and EDCI (1.460 g, 7.6 mmol). The reaction mixture was stirred at 0 °C for 2 h, slowly warmed to 23 °C and stirred for an additional

20 h. The mixture was poured into H_2O (150 mL) and extracted with EtOAc (3 x 200 mL). The combined organic phases were washed with saturated aqueous NaCl solution (200 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 8 x 18 cm, 40-60% EtOAc/hexane gradient elution) of the crude product afforded tetradepsipeptide (**6**) 6.809 g (78% as a white foam): : $[\alpha]^{20}_D$ = -1.38 (C 10.0, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.06 (br, s, 1 H), 8.11 (br, s, 1 H), 7.88 (d, J = 7.5 Hz, 2 H), 7.78-7.69 (m, 3 H), 7.59-7.27 (m, 24 H), 6.95 (d, J = 9.1 Hz, 1 H), 5.15-4.98 (m, 4 H), 4.32-4.21 (m, 5 H), 4.04 (t, J = 7.2 Hz, 1 H), 1.59-1.41 (m, 4 H), 1.36 (s, 9 H), 1.26 (d, J = 7.1 Hz, 3 H), 1.23-1.20 (m, 2 H), 1.12 (d, J = 5.4 Hz, 3 H), 0.85 (d, J = 6.4 Hz, 3 H), 0.71 (d, J = 5.9 Hz, 3 H), 0.62 (d, J = 6.1 Hz, 3 H), 0.58 (br, s, 3 H); ^{13}C (DMSO-d_6, 125 MHz) δ 186.6, 172.7, 172.3, 172.2, 169.6, 168.7, 166.0, 155.9, 155.2, 143.8, 143.7, 140.7, 135.9, 132.8 (d, $J_{c\text{-}p}$= 9.5 Hz), 131.6, 128.3, 127.9, 127.8, 127.6, 127.2, 125.9 (d, $J_{c\text{-}p}$ = 92.1 Hz), 125.2, 120.1, 78.4, 74.0 (d, $J_{c\text{-}p}$= 105.5 Hz), 71.2, 65.7, 65.6, 57.7, 51.4, 50.7, 49.4, 46.6, 40.9, 40.7, 28.1, 24.2, 23.8, 22.9, 22.7, 22.2, 22.0, 21.3, 16.9; IR (KBr) ν max 3270, 3061, 2956, 1722, 1659, 1515, 1163 cm^{-1}; HRMS calcd for $(M+H)^+$ 1131.5248. Found 1131.5251. Anal. calcd for $C_{66}H_{75}N_4O_{11}P$: C, 70.07; H, 6.68; N, 4.95. Found: C, 69.87; H, 6.88; N, 4.93.

N-Boc-Thr[(N^α-Cbz-N^γ-4,4-dimethoxylbenzhydryl)-Asn-D-Ala]-[(4S)-4-amino-3-oxo-2-triphenylphosphoranylidene-heptanoyl]-Leu-OBn (**7**)

A solution of **6** (4.24g, 3.75 mmol) in 80 ML of DMF was treated with 20 mL of piperidine. The resulting mixture was stirred at 23 °C for 1 h under N_2 atmosphere. The reaction mixture was poured onto 200 mL of water and extracted with EtOAc (3 x 200 mL). The combined organic layers were washed with 200 mL of saturated aqueous NaCl solution, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was chromatographed (SiO_2, 6 x 20 cm) by first eluting with EtOAc followed by 10% Et_3N/EtOAc elution to yield free amine as an off-white foam which was used immediately in the next reaction.

A solution of the free amine and N^α-Cbz-N^γ-Mbh-Asn-OH (1.84 g, 3.74 mmol) in 40 mL of DMF was sequentially treated with Et_3N (1.04 mL, 7.48 mmol), HOBt (0.505 g, 3.74 mmol), and EDCI (

0.718 g, 3.74 mmol). The reaction mixture was stirred at 0 °C for 2 h under N_2 atmosphere, and allowed to warm to 23 °C and stirred for additional 16 h. The reaction mixture was poured onto 200 mL of water and extracted with EtOAc (3 x 250 mL). The combined organic layers were washed with saturated aqueous NaCl solution (300 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography (SiO_2, 8 x 20 cm, 50-70% EtOAc/hexane gradient elution) of the crude product afforded pentadepsipeptide (**7**) 4.40 g (85 % as a white foam): $[\alpha]^{20}_D$ = -2.9 (C 20, $CHCl_3$); Rf 0.35 (SiO_2, EtOAc: hexane = 2:1); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.08 (br, s, 1 H), 8.59 (d, J = 8.4 Hz, 1 H), 8.20 (m, 2 H), 7.57-7.30 (m, 26 H), 7.16 (dd, J = 8.4, 3.3 Hz, 4 H), 6.95 (d, J = 8.9 Hz, 1 H), 6.85 (d, J = 8.4 Hz, 2 H), 6.82 (d, J = 8.4 Hz, 2 H), 6.00 (d, J = 8.4 Hz, 1 H), 5.05 (m, 6 H), 4.48 (m, 1 H), 4.34 (m, 1 H), 4.27 (m, 1 H), 4.22 (t, d = 7.1 Hz, 1 H), 3.70 (s, 3 H), 3.68 (s, 3 H), 2.58 (m, 2 H), 1.55 (m, 1 H), 1.47 (m, 1 H), 1.38 (s, 9 H), 1.23-1.15 (m, 10 H), 0.86 (d, J = 6.1 Hz, 3 H), 0.72 (d, J = 5.1 Hz, 3 H), 0.65 (d, J = 5.2 Hz, 3 H), 0.61 (br, s, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 186.7, 172.7, 171.7, 171.0, 168.1, 158.1, 158.0, 155.7, 155.2, 136.9, 135.9, 134.9, 134.7, 132.8 (d, $J_{c\text{-}p}$ = 9.6 Hz), 131.7, 128.6, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 125.9 (d, $J_{c\text{-}p}$ = 92.4 Hz), 113.6, 113.5, 78.4, 73.7 (d, $J_{c\text{-}p}$ = 104.0 Hz), 71.2, 65.7, 65.5, 57.6, 55.0, 54.9, 51.6, 50.7, 47.8, 40.6, 37.9, 28.1, 24.2, 23.8, 22.9, 22.7, 22.2, 21.3, 17.0, 16.8; IR (KBr) ν max 3300, 3070, 2958, 1742, 1717, 1659, 1518, 1250, 1182 cm^{-1}; HRMS calcd for $(M+H)^+$ 1383.6358. Found: 1383.6328.

[*N*-Boc-Thr-[(*4S*)-4-amino-3-oxo-2-triphenylphosphoranylidene-heptanoyl]-Leu-[(N^{γ}-4,4-dimethoxylbenzhydryl)-Asn]-D-Ala] (Threonine Hydroxyl) Lactone (**8**).

A solution of pentadepsipeptide **7** (3.50 g, 2.53 mmol) in 50 mL of CH_3OH was treated with 10% Pd-C (400 mg) and the resulting black suspension was stirred at 23 °C under H_2 (1 atm) for 24 h. The catalyst was removed by filtration through celite and the filtrate was concentrated *in vacuo* to give the crude amino acid which was used directly in the next reaction without further purification.

A solution of the above product and $NaHCO_3$ (217 mg, 2.58 mmol) in 500 mL of degassed DMF was cooled to 0 °C and treated with diphenyl phosphoryl azide (DPPA) (2.30 mL, 10.7 mmol). The reaction mixture was stirred at 0 °C for 72 h. The reaction mixture was concentrated *in vacuo*, and the

residue was diluted with 250 mL of EtOAc. The organic phase was washed with H_2O (150 mL) and saturated aqueous NaCl (150 mL), dried ($MgSO_4$), filtered and concentrated *in vacuo*. Flash chromatography (SiO_2, 5 x 20 cm, 3:1 to 4:1 EtOAc-hexane gradient eluent) afforded **8** (1.70 g, 59%) as a white powder: $[\alpha]^{20}_D$ = +48.0 (C 10.0, $CHCl_3$); Rf 0.28 (4:1 EtOAc-hexane); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.89-7.78 (m, 7 H), 7.52-7.36 (m, 10 H), 7.02 (m , 1 H), 6.98 (d, J = 8.3 Hz, 2 H), 6.89 (d, J = 8.3 Hz, 2 H), 6.75 (m, 4 H), 6.41 (d, J = 7.0 Hz, 1 H), 6.12 (m, 1 H), 5.67 (d, J = 8.2 Hz, 1 H), 5.61 (d, J = 8.3 Hz, 1 H), 5.38 (m, 1 H), 4.71 (m, 1 H), 4.62 (m, 1 H), 4.15 (m, 1 H), 4.10 (d, J = 8.1 Hz, 1 H), 3.97 (m, 1 H), 3.73 (s, 6 H), 3.04 (dd, J = 14.9, 3.8 Hz, 1 H), 2.32 (m, 1 H), 1.58 (m, 1 H), 1.41 (s, 9 H), 1.38-1.09 (m, 8 H), 0.95 (d, J = 6.2 Hz, 3 H), 0.88 (m, 6 H), 0.72 (d, J = 6.2 Hz, 3 H), 0.68 (d, J = 6.2 Hz, 3 H); ^{13}C ($CDCl_3$, 125 MHz) δ 196.6, 172.9, 170.7, 169.6, 167.6, 158.6, 158.5, 155.7, 134.3, 133.8 (d, $J_{c\text{-}p}$=9.9 Hz), 135.5, 132.1, 128.9 (d, $J_{c\text{-}p}$= 12.6 Hz), 128.6, 128.3, 125.9 (d, $J_{c\text{-}p}$=91.8 Hz), 113.8, 113.5, 79.4, 71.6, 71.5 (d, J = 101.5 Hz), 57.2, 55.1, 54.8, 53.9, 53.3, 49.6, 47.4, 44.6, 40.1, 35.9, 28.2, 25.2, 24.7, 23.2, 22.8, 22.6, 20.9, 18.8, 16.2; IR (KBr) ν max 3428, 3059, 2955, 1672, 1510, 1248, 1175 cm^{-1}; HRMS calcd for $(M+H)^+$ 1141.5415. Found: 1141.5402.

N-[*N*-(phenylacetyl)-Phe-Thr]-Thr-[(*4S*)-4-amino3-oxo-2-triphenylphosphoranylidene-heptanoyl]-Leu-Asn-D-Ala] (Threonine Hydroxyl) Lactone (**9a,** YM-47141 precursor).

A mixture of the cyclopentadepsipeptide **8** (245 mg, 0.215 mmol) and anisole (0.5 mL) was treated with trifluoroacetic acid (5 mL). The resulting mixture was stirred at 23 °C for 3 h. The volatiles were removed *in vacuo*. The residue was triturated with Et_2O (10 mL) and collected by filtration to give a white powder which was used directly in the following reaction without further purification.

A solution of the above salt in 5 mL of CH_2Cl_2 was sequentially treated with N-phenylacetyl-Phe-Thr-OH (165 mg, 0.43 mmol), HOBt (58 mg, 0.43 mmol), Et_3N (109 mg, 1.07 mmol), and EDCI (82.4 mg, 0.43 mmol). The resulting mixture was stirred at 23 °C for 36 h. The solution was then diluted with CH_2Cl_2 (30 mL) and washed with H_2O (15 mL) and saturated aqueous NaCl (15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The residue was purified on a

chromatotron plate (coated with 6 mm thickness silica gel-gypsum, 10 % EtOH-$CHCl_3$ eluent) to yield 222 mg of **9a** (87%) as a white powder: $[\alpha]^{20}_D$ = -40.1 (C 4.0, $CHCl_3$); Rf 0.4 (SiO_2, 10% CH_3OH-$CHCl_3$);

^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.39 (br, s, 1 H), 8.31 (m, 3 H), 7.80-7.03 (m, 25 H), 6.85 (br, s, 1 H), 5.64 (br, s, 1 H), 5.59 (m, 1 H), 5.44 (m, 1 H), 5.15 (d, J = 4.1 Hz, 1 H), 4.73 (m, 2 H), 4.46-4.40 (m, 3 H), 4.25 (m, 1 H), 4.10 (m, 1 H), 4.02 (m, 1 H), 3.91 (m, 1 H), 3.40 (d, J = 14.0 Hz, 1 H), 3.33 (d, J = 14.0 Hz, 1 H), 3.13 (d, J = 12.0 Hz, 1 H), 2.78 (m, 2 H), 2.55 (dd, J = 15.6, 4.6 Hz, 1 H), 2.44 (dd, J = 15.3, 6.4 Hz, 1 H), 1.47 (m, 3 H), 1.35 (d, J = 6.7 Hz, 3 H), 1.27 (m, 1 H), 1.18 (m, 4 H), 1.04 (d, J = 5.7 Hz, 3 H), 1.01 (m, 2 H), 0.83 (m, 5 H), 0.67 (d, J = 5.7 Hz, 3 H), 0.64 (d, J = 5.7 Hz, 3 H); ^{13}C (DMSO-d_6, 125 MHz) δ 192.7, 172.7, 172.0, 171.8, 170.3, 170.0, 169.9, 169.7, 168.2, 166.5, 138.0, 136.2, 133.5, 133.4 (d, $J_{c\text{-}p}$ = 9.2 Hz), 132.1, 129.4, 128.9, 128.8, 128.0, 127.9, 126.1, 125.8 (d, $J_{c\text{-}p}$ = 87.8 Hz), 79.2, 71.8, 70.9 (d, $J_{c\text{-}p}$ =104.0 Hz), 66.3, 57.9, 54.9, 53.9, 52.6, 49.5, 47.6, 43.4, 42.1, 40.2, 37.6, 35.6, 24.4, 23.9, 23.3, 22.8, 22.4, 21.9, 19.2, 18.2, 16.6;

IR (KBr) ν max 3308, 3061, 3032, 2955, 1743, 1662, 1522 cm^{-1}; HRMS calcd for $(M+H)^+$ 1181.5477, found 1181.5472.

YM-47141 (1a)

A solution of **9a** (0.20 g, 0.169 mmol) in 10 mL of CH_2Cl_2 was ozonized at -78 °C for 2 min. The light-blue reaction mixture was purged with O_2 and N_2 for 3 and 5 min, respectively, to remove excess O_3. During ozonolysis, the product precipitated out of the reaction solution. Filtration and washing the residue with CH_2Cl_2 provided 0.144 g (89%) of YM-47141 as a white powder identical in all respects with a sample of natural material. $[\alpha]^{20}_D$ = -8.6 (C 0. 3, CH_3CN). (lit. $[\alpha]^{25}_D$ = -10.1 (C 0.2, CH_3CN);

^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.49 (d, J = 8.8 Hz, 1 H), 8.38 (d, J = 8.3 Hz, 1 H), 8.11 (d, J = 7.3 Hz, 1 H), 7.99 (d, J = 7.8 Hz, 1 H), 7.90 (d, J = 6.8 Hz, 1 H), 7.57 (d, J = 9.3 Hz, 1 H), 7.54 (s, 1 H), 7.35 (s, 1 H), 7.33 (s, 1 H), 7.30 (m, 2 H), 7.21 (m, 2 H), 7.16 (m, 4 H), 7.02 (m,

2 H), 6.98 (d, J = 9.8 Hz, 1 H), 6.95 (s, 1), 5.42 (m, 1 H), 5.10 (d, J = 4.9 Hz, 1 H), 5.00 (m, 1 H), 4.69 (m, 1 H), 4.64 (dd, J = 9.3, 2.4 Hz, 1 H), 4.46 (m, 1 H), 4.41 (m, 1 H), 4.36 (dd, J = 8.8, 2.9 Hz, 1 H), 4.13 (m, 1 H), 4.02 (m, 1 H), 3.35 (d, J = 13.7 Hz, 1 H), 3.30 (d, J = 13.7 Hz, 1 H), 3.10 (dd, J = 13.7, 2.4 Hz, 1 H), 2.75 (dd, J = 13.7, 11.2 Hz, 1 H), 2.60 (dd, J = 15.6, 3.4 Hz, 1 H), 2.38 (dd, J = 15.6, 10.2 Hz, 1 H), 2.16 (m, 1 H), 1.61 (m, 2 H), 1.54 (m, 1 H), 1.43 (m, 1 H), 1.31 (m, 1 H), 1.13 (d, J = 6.3 Hz, 3 H), 1.03 (d, J = 6.3 Hz, 3 H), 1.00 (d, J = 6.8 Hz, 3 H), 0.89 (d, J = 6.3 Hz, 3 H), 0.87 (d, J = 6.4 Hz, 3 H), 0.85 (d, J = 6.3 Hz, 3 H), 0.81 (d, J = 6.4 Hz, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 206.3, 172.2, 170.9, 170.6, 170.5, 170.4, 170.1, 170.0, 167.6, 138.1, 136.3, 129.5, 129.0, 128.1, 127.9, 126.2, 94.7, 71.9, 66.3, 57.9, 54.6, 54.1, 53.7, 53.3, 50.4, 46.7, 42.1, 38.6, 37.8, 36.8, 24.9, 24.1, 23.6, 22.7, 22.0, 20.7, 20.0, 16.5, 16.1 IR (KBr) νmax 3400, 3067, 2960, 1737, 1662, 1528 cm^{-1}; HRMS calcd for $(M+Na)^+$ 975.4440, found 975.4435.

N-[*N*-(Isopropylacetyl)-Phe-Thr]-[(*4S*)-4-amino-3-oxo-2-triphenylphosphoranylidene-heptanoyl]-Leu-Asn-D-Ala] (Threonine Hydroxyl) Lactone (**9b,** YM-47142 precursor)

A mixture of the cyclopentadepsipeptide **8** (0.49 g, 0.429 mmol) and anisole (1 mL) was treated with trifluoroacetic acid (8 mL). The resulting solution was stirred for 2 h at rt and then was evaporated *in vacuo*. The residue was washed with ethyl ether (15 x 2 mL) to give a white powder which was used directly in the following reaction without further purification.

A solution of the above salt in 10 mL of CH_2Cl_2 was treated with *N*-isopropylacetyl-Phe-Thr-OH (0.30 g, 0.857 mmol), HOBt (0.12 g, 0.888 mmol), Et_3N (O.30 mL, 2.15 mmol) and EDCI (0.16 g, 0.835 mmol). The resulting mixture was stirred for 40 h at rt and then was diluted with CH_2Cl_2 (20 mL) and washed with water (20 x 2 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified on a chromatotron (EtOH/$CHCl_3$ = 1:9) to give 0.35 g (72%) of expected product **9b** as a white powder. $[\alpha]^{20}_D$ = -32.4 (C 5.0, $CHCl_3$); ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.38 (br, s, 1 H), 8.14 (d, J = 8.3 Hz, 1 H), 8.02 (d, J = 7.8 Hz, 1 H), 7.78-7.14 (m, 23 H), 6.82 (br, s, 1 H), 5.67 (m, 1 H), 5.49 (m, 1 H), 5.41 (m, 1 H), 5.13 (d, J = 3.9 Hz, 1 H), 5.02 (m, 1 H), 4.67 (m, 2 H), 4.42 (m, 3 H), 4.09 (m, 1 H), 3.93 (m, 1 H),

3.08 (d, J = 13.2, Hz, 1 H), 2.72 (m, 2 H), 2.54 (m, 1 H), 2.40 (m, 1 H), 1.85 (m, 2 H), 1.79 (m, 1 H), 1.44 (m, 3 H), 1.32 (d, J = 6.3 Hz, 3 H), 1.16 (m, 6 H), 1.03 (d, J = 5.4 Hz, 3 H), 1.00 (m, 1 H), 0.93 (m, 1 H), 0.80 (m, 6 H), 0.71 (d, J = 6.3 Hz, 3 H), 0.65 (d, J = 5.9 Hz, 3 H), 0.63 (d, J = 6.3 Hz, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 192.5, 172.6, 172.1, 171.4, 170.2, 169.9, 169.8, 169.6, 168.2, 166.4, 138.2, 133.4 (d, J_{c-p} = 9.4 Hz), 132.0, 129.2, 128.8, 127.8, 126.0, 125.7 (d, J_{c-p} = 81.0 Hz), 71.7, 70.9 (d, J_{c-p} = 106.1 Hz), 66.2, 57.7., 54.9, 53.6, 52.5, 52.2, 49.5, 47.6, 44.5, 39.9, 38.9, 37.3, 35.6, 25.4, 24.4, 23.8, 23.3, 22.8, 22.2, 22.1, 22.0, 20.8, 19.1, 18.1, 16.5. IR (KBr) ν max 3289, 3016, 2952, 1661, 1519 cm $^{-1}$; HRMS calcd for $(M+H)^+$ 1147.5633, found 1147.5641.

YM-47142 (1b)

A solution of YM-47142 precursor **9b** (0.30 g, 0.262 mmol) in 10 mL of CH_2Cl_2 was ozonized at -78 °C for 2 min and then was purged with N_2. During ozonolysis, the product precipitated out of the reaction solution. Filtration and washing the residue with CH_2Cl_2 provided 0.22 g (92%) of YM-47142 (**1b**) as a white powder identical with the authentic natural product. $[\alpha]^{20}_D$ = -2.0 (C 0.2, CH_3CN) (lit. $[\alpha]^{25}_D$ = -1.8 (0.5, CH_3CN));

^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.35 (d, J = 8.8 Hz, 1 H), 8.10 (d, J = 7.8 Hz, 1 H), 8.07 (d, J = 8.3 Hz, 1 H), 7.99 (d, J = 8.3 Hz, 1 H), 7.90 (d, J = 6.8 Hz, 1 H), 7.57 (d, J = 8.8 Hz, 1 H), 7.53 (s, 1 H), 7.35 (s, 1 H), 7.32 (m, 2 H), 7.30 (s, 1 H), 7.21 (m, 2 H), 7.13 (m, 1 H), 6.98 (d, J = 9.3 Hz, 1 H), 6.94 (s, 1 H), 5.41 (m, 1 H), 5.09 (d, J = 4.9 Hz, 1 H), 5.00 (m, 1 H), 4.69 (m, 1 H), 4.62 (dd, J = 8.8, 2.0 Hz, 1 H), 4.46 (m, 1 H), 4.41 (m, 1 H), 4.35 (dd, J = 8.3, 2.9 Hz, 1 H), 4.14 (m, 1 H), 4.02 (m, 1 H), 3.06 (dd, J = 13.2, 2.4 Hz, 1 H), 2.71 (dd, J = 13.2, 11.7 Hz, 1 H), 2.60 (dd, J = 15.6, 3.4 Hz, 1 H), 2.38 (dd, J = 15.6, 10.2 Hz, 1 H), 2.15 (m, 1 H), 1.85 (m, 2 H), 1.80 (m, 1 H), 1.60 (m, 1 H), 1.59 (m, 1 H), 1.53 (m, 1 H), 1.44 (m, 1 H), 1.30 (m, 1 H), 1.13 (d, J = 6.3 Hz, 3 H), 1.03 (d, J = 5.9 Hz, 3 H), 1.00 (d, J = 7.3 Hz, 3 H), 0.88 (d, J = 6.8 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 0.85 (d, J = 6.8 Hz, 3 H), 0.81 (d, J = 6.3 Hz, 3 H), 0.71 (d, J = 6.4 Hz, 3 H), 0.62 (d, J = 6.8 Hz, 3 H); ^{13}C NMR (DMSO-d_6, 125 MHz), δ 206.4, 172.5, 172.4, 171.7, 171.1, 170.7, 170.6, 170.5, 170.2, 167.6, 138.4, 129.5, 128.1, 126.3, 94.7, 72.0, 66.4,

57.9, 54.7, 54.0, 53.8, 53.4, 50.5, 46.8, 44.6, 40.0, 38.7, 37.6, 36.8, 25.7, 25.0, 24.2, 23.7, 22.8, 22.3, 22.2, 22.0, 20.8, 19.7, 16.6, 16.2. IR (KBr) ν max 3299, 2959, 1651, 1532 cm^{-1}; HRMS calcd for $(M+H)^+$ 919.4776, found 919.4779.