Supporting Information

Structure-Based Design of Conformationally Constrained, Cell-Permeable STAT3 Inhibitors

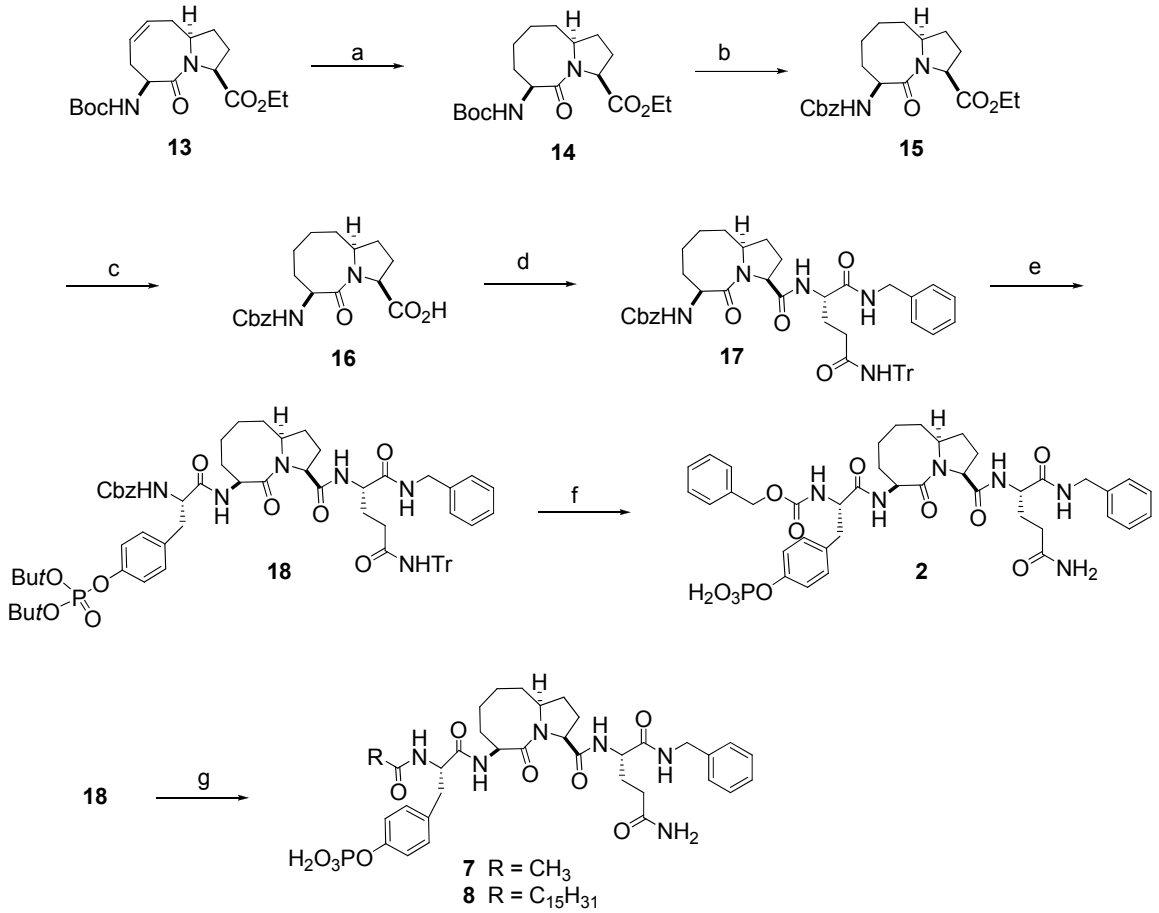
Jianyong Chen, Longchuan Bai, Denzil Bernard, Zaneta Nikolovska-Coleska, Cindy Gomez, Jian Zhang, Han Yi and Shaomeng Wang*

Comprehensive Cancer Center and Departments of Internal Medicine, Pharmacology and Medicinal Chemistry, University of Michigan, 1500, E. Medical Center Dr., Ann Arbor, MI 48109, USA

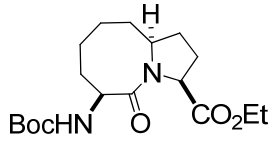
I. Chemistry

Solvents and reagents were obtained commercially and were used without further purification. Reactions were monitored by TLC carried out on 250 μm E. Merck silica gel plates (60F-254) using UV light as visualizing agent. E. Merck silica gel (60, particle size 15-40 μm) was used for flash column chromatography. NMR spectra were recorded on a Bruker Avance300 spectrometer (300 MHz). Chemical shifts (δ) are reported as δ values (ppm) downfield relative to TMS as an internal standard, with multiplicities reported in the usual manner. Electrospray ionization mass spectra (MS) were run on a Micromass AutoSpec Ultima mass spectrometer. Elemental analysis (EA) was performed by Atlantic Microlab, Inc. The final products were purified by HPLC on a Waters Sunfire C18 reverse phase semipreparative HPLC column (19 mm × 150 mm) using solvent A (0.1% of TFA in water) and solvent B (0.1% of TFA in CH3CN) as eluents with a flow rate of 10 mL/min.

Scheme I Synthesis of compounds **2, 7 and 8**



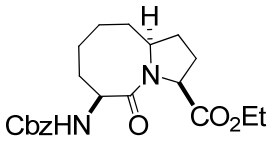
Reagents and conditions: (a) H_2, 10%Pd-C; (b) (i) 4M HCl in dioxane; (ii) Cbz-Cl, $NaHCO_3$, dioxane; (c) (i) 2N LiOH, dioxane; (ii) 1N HCl; (d) N^{δ}-trityl-*L*-glutamine benzylamide, EDC, HOBt, DIPEA, DCM; (e) (i) H_2, 10%Pd-C; (ii) Cbz-phospho-L-tyrisine di-*tert*-butyl ester, EDC, HOBt, DIPEA, DCM; (f) TFA, TES, DCM; (g) (i) H_2, 10%Pd-C; (ii) CH_3CO_2H or palmitic acid, EDC, HOBt, DIPEA, DCM; (iii) TFA, TES, DCM.



14

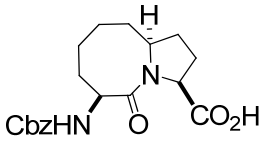
To a solution of compound **13** [1,2] (5.0 g, 14.2 mmol) in EtOH (50 mL) was added 10% Pd-C (0.5 g). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the ester **14** (4.8 g, 95%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 5.44 (d, *J* =

7.5 Hz, 1H), 4.75-4.55 (m, 1H), 4.50-4.30 (m, 1H), 4.27-4.15 (m, 3H), 2.35-1.50 (m, 12H), 1.40 (s, 9H), 1.29 (t, J = 7.1 Hz, 3H).



15

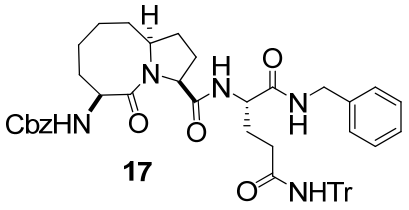
To a solution of compound **14** (2.5 g, 7.1 mmol) in dioxane (20 mL) was added 4M HCl in dioxane (5mL). The solution was stirred at room temperature for 4 h and the solvent was evaporated. The residue was dissolved in dioxane (20 mL) and benzyl chloroformate (1.2 mL, 8.4 mmol) and a solution of $NaHCO_3$ (1.41 g, 16.8 mmol) in water (20 mL) were added. The mixture was stirred at room temperature overnight and extracted with ethyl acetate (30mL x 3). The combined organic layer was dried over anhydrous Na_2SO_4 and evaporated. The residue was purified by flash chromatography (SiO_2, Hexane : Ethyl acetate = 40 : 60) to give compound **15** (2.6 g, 95% over two steps). ^{1}H NMR (300 MHz, CD_3OD), 7.29-7.36 (m, 5H), 5.69 (d, 1H, J = 8.1Hz), 5.08 (s, 2H), 4.67-4.73 (m, 1H), 4.34 (t, 1H, J = 9.3 Hz), 4.18-4.25 (m, 3H), 1.57-2.32 (m, 12H), δ1.29 (t, 3H, J = 7.1Hz),



16

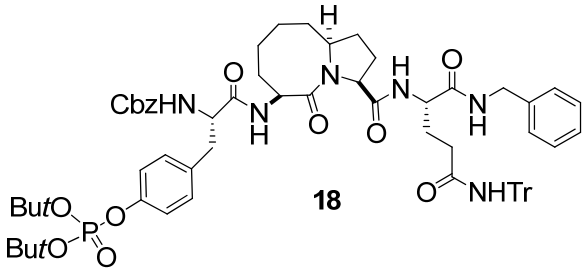
To a solution of **15** (2.7 g, 7.0 mmol) in dioxane (20 mL) was added 2N LiOH (9 mL). The mixture was stirred at room temperature for 2 h and then acidified with 1N HCl. After extraction of the mixture with ethyl acetate (30 mL x 3), the organic layer was

combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the acid **16** (1.9 g, 76%). This acid can be used in the next step without further purification. ^{1}H NMR (300 MHz, CD_3OD), δ7.30-7.40 (m, 5H), 5.78 (d, 1H, *J* = 8.1 Hz), 5.11 (s, 2H), 4.69-4.80 (m, 1H), 4.55 (t, 1H, *J* = 8.4 Hz), 4.23-4.29 (m, 1H), 1.95-2.33 (m, 4H), 1.52-1.83 (m, 8H).



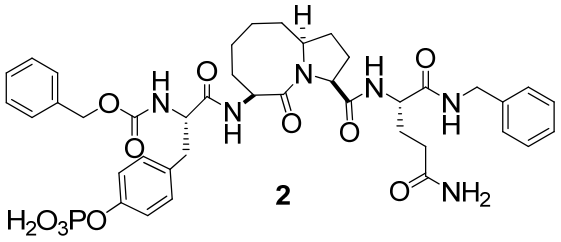
17

To a solution of **16** (1.28 g, 3.6 mmol) in DCM (20 mL) were added N^{δ}-trityl-*L*-glutamine benzylamide (1.77 g, 3.6 mmol), EDC (0.684 g, 3.6 mmol), HOBt (0.546 g, 3.6 mmol), and DIPEA (0.62 mL, 3.6 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, Hexane : Ethyl acetate = 40 : 60) gave compound **17** (2.8 g, 95%). ^{1}H NMR (300 MHz, CD_3OD), δ7.10-7.40 (m, 27H), 6.73 (t, 1H, *J* = 5.6 Hz), 5.45 (d, *J* = 8.1Hz), 5.10 (q of ABq, *J* = 12.3 Hz, Δν = 18.7 Hz, 2H), 4.55-4.70 (m, 1H), 4.08-4.42 (m, 5H), 2.50-2.65 (m, 1H), 2.30-2.45 (m, 1H), 1.40-2.22 (m, 13H), 1.18-1.30 (m, 1H).



18

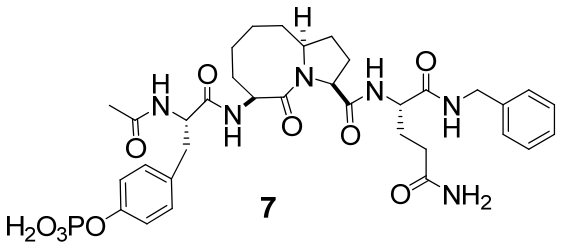
To a solution of compound **17** (400 mg, 0.49 mmol) in MeOH (10 mL) was added 10% Pd-C (60 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the crude amine which was used directly for the next step without further purification. To a solution of the crude amine in DCM (20 mL) were added Cbz-phospho-L-tyrisine di-*tert*-butyl ester (248 mg, 0.49 mmol), EDC (94 mg, 0.49 mmol), HOBt (75 mg, 0.49 mmol), and DIPEA (0.085 mL, 0.49 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave compound **18** (200 mg, 35% over two steps). ^{1}H NMR (300 MHz, CD_3OD), δ 7.68 (d, J = 6.8 Hz, 1H), 7.40-7.12 (m, 26H), 7.10-6.85 (m, 5H), 6.78 (t, J = 5.7 Hz, 1H), 5.40 (d, J = 8.3 Hz, 1H), 5.04 (q of ABq, J = 12.1 Hz, Δν = 17.9 Hz, 2H), 4.95-4.75 (m, 1H), 4.55-4.08 (m, 6H), 3.05-2.84 (m, 2H), 2.60-2.30 (m, 2H), 2.25-1.60 (m, 14H), 1.51 (s, 18H).



2

To a solution of compound **18** (80 mg, 0.068 mmol) in DCM (5 mL) were added TFA (5 mL) and triethylsilane (2 drops). The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **2** (33 mg, 59%). ^{1}H NMR (300 MHz, CD_3OD), δ7.30-7.10 (m, 10H), 7.11 (d, J = 8.6 Hz, 2H), 7.00 (d, J = 8.6 Hz, 2H), 4.90 (s, 2H), 4.75-4.65 (m, 1H), 4.42-4.16 (m, 6H), 2.99 (dd, J = 4.6, 13.6 Hz, 1H), 2.71 (dd, J = 9.6, 13.6 Hz), 2.08-1.35 (m, 16H); ^{13}C NMR (75 MHz, CD_3OD), δ176.65, 172.97, 172.22,

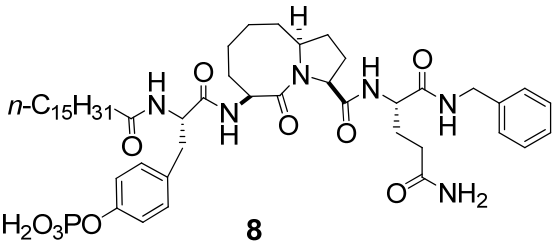
172.02, 171.09, 156.85, 150.25, 138.37, 136.67, 133.50, 130.11, 128.13, 128.07, 127.59, 127.32, 127.07, 126.81, 119.83, 119.77, 66.21, 61.52, 60.05, 56.15, 52.76, 49.78, 42.66, 36.89, 35.92, 34.03, 32.08, 31.19, 27.88, 27.22, 25.25, 21.98; Anal. Cacld for: $C_{40}H_{49}N_6O_{11}P{\cdot}2.5H_2O$: C, 55.49; H, 6.29; N, 9.71. Found: C, 55.61; H, 6.08; 9.71.



7

To a solution of compound **18** (90 mg, 0.077 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated and the residue was dissolved in DCM (5 mL). Acetic acid (9.2 mg, 0.154 mmol), EDC (15 mg, 0.077 mmol), HOBt (12 mg, 0.077 mmol), and DIPEA (10 mg, 0.077 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **7** (29 mg, 52% over three steps).^{1}H NMR (300 MHz, CD3OD), δ 7.35-7.25 (m, 5H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 4.83-4.75 (m, 1H), 4.60 (dd, *J* = 5.3, 9.3 Hz, 1H), 4.52-4.22 (m, 5H), 3.09 (dd, *J* = 5.3, 13.9 Hz, 1H), 2.82 (dd, *J* = 9.3, 13.9 Hz, 1H), 2.50-1.44 (m, 16H), 1.89 (s, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 178.08, 174.39, 173.65, 173.19, 173.02, 172.48, 151.68, 151.59, 139.79, 134.83, 131.41, 129.54, 128.48, 128.22, 121.22, 121.16, 62.93, 61.47,

55.92, 54.18, 51.17, 44.07, 38.12, 37.35, 35.45, 33.50, 32.60, 29.29, 28.64, 26.67, 23.40, 22.35; Anal. Cacld for: $C_{34}H_{45}N_6O_{10}P{\cdot}CF_3CO_2H{\cdot}0.5H_2O$: C, 50.76; H, 5.56; N, 9.87. Found: C, 50.87; H, 5.69; N, 9.98.



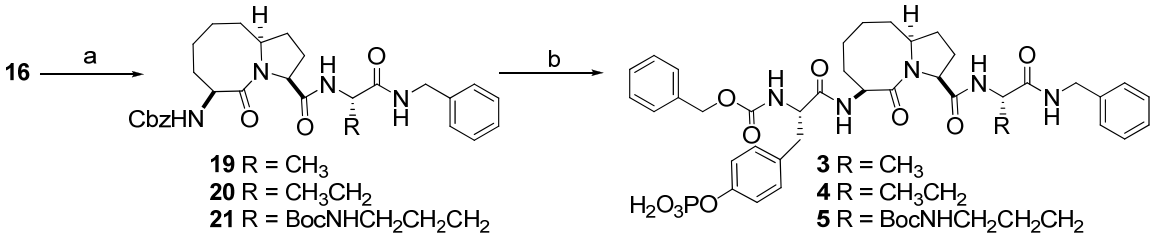
8

To a solution of compound **18** (45 mg, 0.038 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated and the residue was dissolved in DCM (5 mL). Palmitic acid (9.7 mg, 0.038 mmol), EDC (7.3 mg, 0.038 mmol), HOBt (5.8 mg, 0.038 mmol), and DIPEA (5 mg, 0.038 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **8** (17 mg, 48 % over three steps).

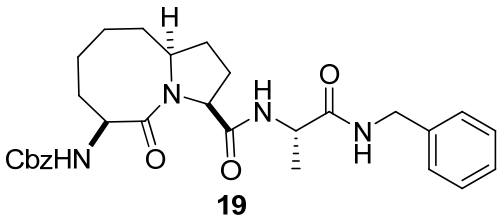
^{1}H NMR (300 MHz, CD3OD), δ 7.35-7.27 (m, 5H), 7.22 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 8.4 Hz, 2H), 4.83-4.80 (m, 1H), 4.64 (dd, J = 5.3, 9.3 Hz, 1H), 4.52-4.22 (m, 5H), 3.09 (dd, J = 5.2, 13.9 Hz, 1H), 2.83 (dd, J = 9.3, 13.9 Hz, 1H), 2.50-1.44 (m, 20H), 1.43-1.20 (m, 24H) , 0.92 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 178.06, 176.21, 174.41, 173.67, 173.09, 172.44, 151.77 (d, $J_{P,C}$ = 6.7 Hz) , 139.76, 134.67, 131.38,

129.53, 128.47, 128.21, 121.19 (d, $J_{P,C}$ = 4.6 Hz), 62.90, 61.47, 55.68, 54.18, 51.11, 44.05, 38.10, 37.32, 36.79, 35.46, 33.51, 33.08, 32.62, 30.82, 30.78, 30.63, 30.49, 30.43, 30.22, 29.28, 28.64, 26.89, 26.67, 23.75, 23.36, 14.47; Anal. Cacld for $C_{48}H_{73}N_6O_{10}P{\cdot}0.5CF_3CO_2H{\cdot}0.4H_2O$: C, 59.49; H, 7.57; N, 8.49. Found: C, 59.76; H, 7.87; N, 8.62.

Scheme II Synthesis of compounds **3-5**



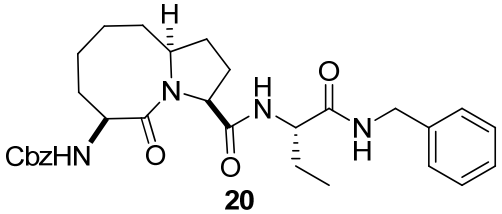
Reagents and conditions: (a) *L*-alanine-benzylamide, *L*-2-aminobutyric acid benzylamide, or *N*-δ-Boc-L-ornithine benzylamide, EDC, HOBt, DIPEA, DCM; (b) (i) H_2, 10%Pd-C; (ii) Cbz-phospho-L-tyrisine di-*tert*-butyl ester, EDC, HOBt, DIPEA, DCM; (iii) TFA, TES, DCM.



19

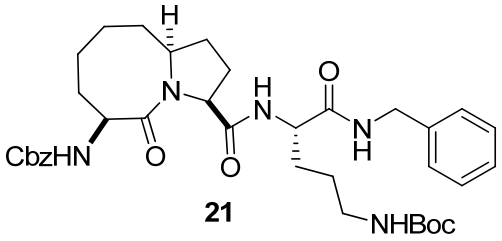
To a solution of **16** (358 mg, 1.0 mmol) in DCM (10 mL) were added *L*-alanine-benzylamide hydrochloride (214 mg, 1.0 mmol), EDC (192 mg, 1.0 mmol), HOBt (153 mg, 1.0 mmol), and DIPEA (129 mg, 1.0 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, Ethyl acetate) gave compound **19** (420 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.40-7.20 (m, 10H), 7.29 (d, J = 7.7 Hz, 1H), 6.71 (t, J = 5.5 Hz, 1H), 5.63 (d, J = 8.1 Hz, 1H),

5.10 (q of ABq, J = 8.1 Hz, Δν = 8.6 Hz, 2H), 4.75-4.57 (m, 1H), 4.52-4.30 (m, 4H), 4.25-4.10 (m, 1H), 2.40-1.50 (m, 12H), 1.44 (d, J = 7.1 Hz, 3H).



20

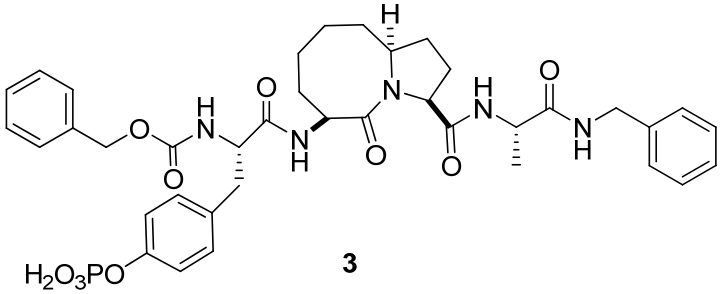
To a solution of **16** (179 mg, 0.5 mmol) in DCM (10 mL) were added *L*-2-aminobutyric acid benzylamide (96 mg, 0.5 mmol), EDC (96 mg, 0.5 mmol), HOBt (77 mg, 0.5 mmol), and DIPEA (65 mg, 0.5 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, Ethyl acetate) gave compound **20** (250 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.40-7.20 (m, 10H), 7.03 (d, J = 7.5 Hz, 1H), 6.61 (t, J = 5.5 Hz, 1H), 5.66 (d, J = 7.8 Hz, 1H), 5.15 (q of ABq, J = 8.1 Hz, Δν = 8.5 Hz, 2H), 4.75-4.60 (m, 1H), 4.50-4.10 (m, 5H), 2.40-1.40 (m, 14H), 0.96 (t, J = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 171.93, 171.31, 171.12, 155.56, 138.13, 136.42, 128.64, 128.51, 128.11, 128.00, 127.73, 127.44, 66.78, 60.98, 59.35, 55.15, 51.65, 43.50, 36.57, 36.18, 32.17, 25.69, 25.37, 25.08, 23.23, 10.16.



21

To a solution of **16** (358 mg, 1.0 mmol) in DCM (10 mL) were added *N*-δ-Boc-L-ornithine benzylamide (321 mg, 1.0 mmol), EDC (192 mg, 1.0 mmol), HOBt hydrate (153 mg, 1.0 mmol), and DIPEA (129 mg, 1.0 mmol). The mixture was stirred at room

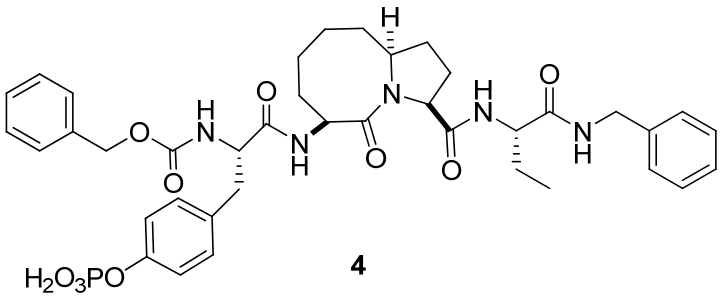
temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, Ethyl acetate) gave compound **21** (540 mg, 82%). ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.40-7.16 (m, 11H), 7.10 (t, *J* = 5.5 Hz, 1H), 5.80 (d, *J* = 8.0 Hz, 1H), 5.20-5.00 (m, 3H), 4.75-4.25 (m, 5H), 4.20-4.05 (m, 1H), 3.35-2.95 (m, 2H), 2.30-1.40 (m, 16H), 1.33 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 171.13, 172.02, 156.82, 156.16, 138.56, 136.84, 128.94, 128.85, 128.45, 127.93, 127.68, 79.48, 67.09, 61.69, 60.07, 52.96, 51.78, 43.71, 39.93, 36.61, 36.24, 32.76, 30.06, 28.80, 26.81, 26.11, 25.87, 23.04.



3

To a solution of compound **19** (161 mg, 0.31 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the crude amine which was used directly for the next step without further purification. To a solution of the crude amine in DCM (20 mL) were added Cbz-phospho-L-tyrisine di-*tert*-butyl ester (158 mg, 0.31 mmol), EDC (60 mg, 0.31 mmol), HOBt hydrate (47 mg, 0.31 mmol), and DIPEA (40 mg, 0.31 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a

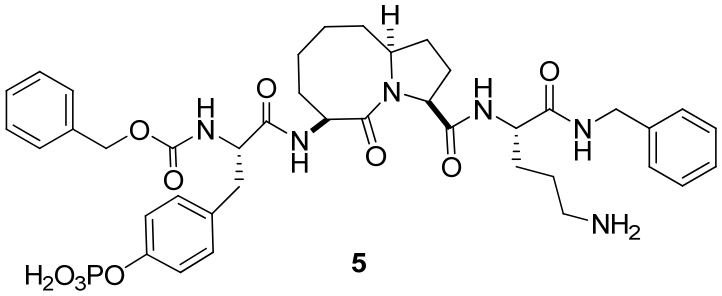
crude product which was purified by C18 reversed phase semipreparative HPLC to give **3** (80 mg, 34% over three steps). ^{1}H NMR (300 MHz, CD_3OD), δ 7.31-7.20 (m, 10H), 7.24 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.6 Hz, 2H), 5.01 (s, 2H), 4.92-4.80 (m, 1H), 4.48-4.20 (m, 6H), 3.10 (dd, *J* = 4.3, 13.8 Hz, 1H), 2.80 (dd, *J* = 10.1, 13.8 Hz), 2.30-1.40 (m, 12H), 1.42 (d, *J* = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 173.96, 173.04, 172.49, 171.47, 157.27, 150.60, 138.87, 137.08, 134.01, 130.53, 129.39, 128.49, 127.97, 127.71, 127.41, 127.16, 120.20, 66.59, 61.70, 60.39, 56.57, 50.17, 49.85, 43.01, 37.35, 36.38, 34.47, 32.48, 27.54, 25.66, 22.43, 16.99; Anal. Cacld for: $C_{38}H_{46}N_5O_{10}P{\cdot}2.25H_2O$: C, 56.75; H, 6.33; N, 8.71. Found: C, 56.58; H, 6.13; 8.59.



4

To a solution of compound **20** (120 mg, 0.225 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the crude amine which was used directly for the next step without further purification. To a solution of the crude amine in DCM (20 mL) were added Cbz-phospho-L-tyrisine di-*tert*-butyl ester (114 mg, 0.225 mmol), EDC (43 mg, 0.225 mmol), HOBt hydrate (34 mg, 0.225 mmol), and DIPEA (29 mg, 0.225 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was

stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **4** (42 mg, 24% over three steps). ^{1}H NMR (300 MHz, CD_3OD), δ 7.40-7.24 (m, 10H), 7.22 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.5 Hz, 2H), 5.02 (s, 2H), 4.90-4.80 (m, 1H), 4.55-4.13 (m, 6H), 3.12 (dd, *J* = 4.2, 14.0 Hz, 1H), 2.81 (dd, *J* = 10.0, 14.0 Hz, 1H), 2.30-1.50 (m, 14H), 0.99 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 172.76, 172.72, 172.03, 171.07, 156.86, 150.23, 150.14, 138.48, 136.69, 133.59, 130.12, 128.09, 127.57, 127.31, 127.11, 126.78, 119.85, 119.78, 66.20,61.26, 59.97, 56.18, 55.32, 49.80, 42.63, 36.92, 36.00, 34.16, 32.05, 27.05, 25.26, 24.91, 22.10, 9.42; Anal. Cacld for: $C_{39}H_{48}N_5O_{10}P\cdot1.5H_2O$: C, 58.20; H, 6.39; N, 8.70. Found: C, 58.37; H, 6.17; 8.71.

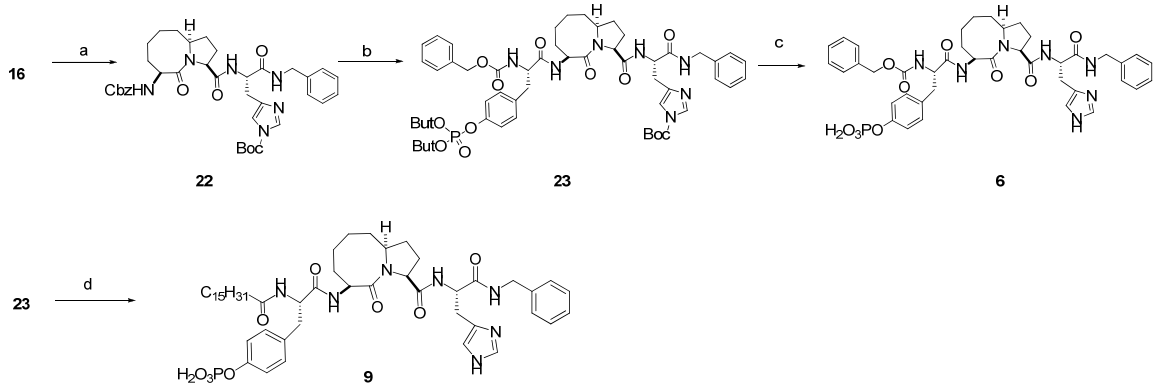


5

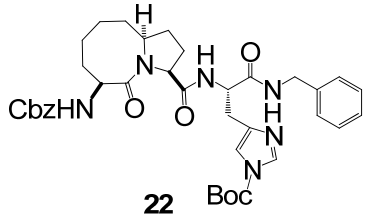
To a solution of compound **21** (66 mg, 0.1 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the crude amine which was used directly for the next step without further purification. To a solution of the crude amine in DCM (20 mL) were added Cbz-phospho-L-tyrisine di-*tert*-butyl ester (51 mg, 0.1 mmol), EDC (19 mg, 0.1 mmol), HOBt hydrate (14 mg, 0.1 mmol), and DIPEA (14 mg, 0.1 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL).

TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **5** (25 mg, 31% over three steps). ^{1}H NMR (300 MHz, CD_3OD), δ 7.40-7.00 (m, 14H), 5.07 (s, 2H), 4.85-4.74 (m, 1H), 4.50-4.18 (m, 6H), 3.00-2.78 (m, 4H), 2.40-1.46 (m, 16H); ^{13}C NMR (75 MHz, CD_3OD), δ 173.20, 172.41, 171.71, 170.71, 156.73, 151.19, 138.37, 136.71, 129.97, 128.14, 128.08, 127.63, 127.36, 127.02, 126.83, 119.68, 119.62, 66.24, 61.54, 60.09, 56.53, 52.54, 42.65, 38.68, 37.29, 35.94, 34.12, 32.08, 28.12, 27.37, 25.18, 23.43, 21.97; Anal. Cacld for: $C_{40}H_{51}N_6O_{10}P{\cdot}CF_3CO_2H{\cdot}1.5H_2O$: C, 53.22; H, 5.85; N, 8.87. Found: C, 53.17; H, 5.85; 8.87.

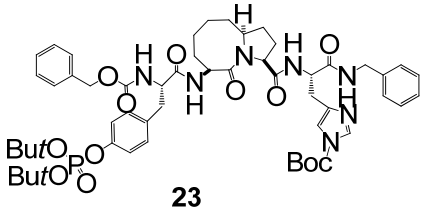
Scheme III Synthesis of compounds 6 and 9



Reagents and conditions: (a) *N*-Boc-*L*-hisdine benzylamide, EDC, HOBt, DIPEA, DCM; (b) (i) H_2, 10%Pd-C; (ii) Cbz-phospho-L-tyrisine di-*tert*-butyl ester, EDC, HOBt, DIPEA, DCM; (c) TFA, TES, DCM; (d) (i) H_2, 10%Pd-C; (ii) palmitic acid, EDC, HOBt, DIPEA, DCM; (iii) TFA, TES, DCM.



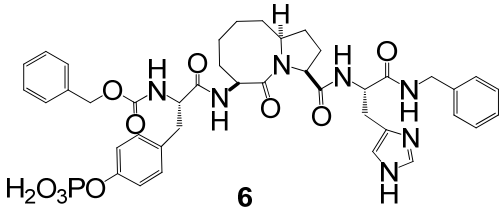
To a solution of **16** (448 mg, 1.25 mmol) in DCM (20 mL) were added *N*-Boc-*L*-hisdine benzylamide (430 mg, 1.25 mmol), EDC (240 mg, 1.25 mmol), HOBt (169 mg, 1.25 mmol), and DIPEA (163 mg, 1.25 mmol) and the reaction mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave compound **22** (750 mg, 87 % yield).^{1}H NMR (300 MHz, $CDCl_3$), δ 7.87 (s, 1H), 7.61 (d, J = 7.9 Hz, 1H), 7.50-7.28 (m, 6H), 7.22-7.05 (m, 4H), 7.03 (d, J = 8.1 Hz, 2H), 5.46 (d, J = 8.0 Hz, 1H), 5.06 (q of ABq, J_{AB} = 12.3 Hz, $\Delta\nu_{AB}$ = 9.8 Hz, 2H), 4.90-4.78 (m, 1H), 4.60-4.44 (m, 2H), 4.40-4.26 (m, 1H), 4.20-4.10 (m, 2H), 3.27 (dd, J = 5.5, 14.9 Hz, 1H), 2.91 (dd, J = 4.9, 14.9 Hz, 1H), 2.45-2.20 (m, 3H), 1.80-1.50 (m, 9H), 1.58 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 172.71, 171.19, 170.40, 155.53, 146.99, 139.42, 138.49, 136.51, 128.56, 128.35, 128.17, 128.07, 127.65, 127.21, 115.08, 85.81, 66.79, 63.09, 59.44, 52.99, 51.83, 43.38, 36.85, 35.75, 32.33, 29.23, 27.93, 27.22, 25.32, 23.36.



23

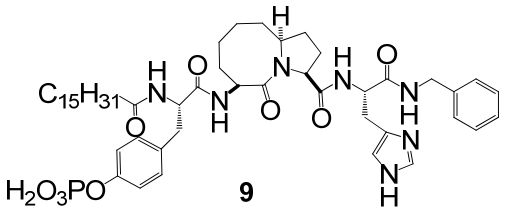
To a solution of compound **22** (123 mg, 0.18 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the crude amine which was used directly for the next step without further purification. To a solution of the crude amine in DCM (20 mL) were added Cbz-phospho-L-tyrisine di-*tert*-butyl ester (92 mg, 0.18 mmol), EDC (35 mg, 0.18 mmol), HOBt hydrate (28 mg, 0.18 mmol), and DIPEA (23 mg, 0.18 mmol). The mixture was

stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave **23** (140 mg, 87 % over two steps) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.92 (s, 1H), 7.79 (d, *J* = 7.7 Hz, 1H), 7.63 (t, *J* = 9.9 Hz, 1H), 7.40-7.30 (m, 5H), 7.25-7.15 (m, 4H), 7.10-7.00 (m, 6H), 6.65 (d, *J* = 6.95 Hz, 1H), 5.20 (d, *J* = 8.0 Hz, 1H), 5.70 (q of ABq, *J* = 12.3 Hz, Δν = 8.2 Hz, 2H), 4.82-4.65 (m, 2H), 4.52 (dd, *J* = 6.7, 15.0 Hz, 1H), 4.42-4.10 (m, 4H), 3.30 (dd, *J* = 5.7, 15.0 Hz, 1H), 3.05-2.90 (m, 3H), 2.30-1.55 (m, 12H), 1.61 (s, 9H), 1.48 (s, 18H).



6

To a solution of compound **23** (100 mg, 11.2 mmol) in DCM (5 mL) were added TFA (5 mL) and triethylsilane (2 drops). The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **6** (65 mg, 70 % yield). ^{1}H NMR (300 MHz, CD_3OD), δ 8.63 (s, 1H), 7.40-7.06 (m, 15H), 5.04 (s, 2H), 4.85-4.55 (m, 2H), 4.50-4.20 (m, 5H), 3.30-3.20 (m, 2H), 3.03 (dd, *J* = 6.5, 13.6 Hz, 1H), 2.88 (dd, *J* = 8.0, 13.6 Hz, 1H), 2.40-1.50 (m, 12H); ^{13}C NMR (75 MHz, CD_3OD), δ 174.39, 173.26, 172.02, 171.59, 158.19, 152.30 (d, $J_{P,C}$ = 7.1 Hz), 139.74, 138.08, 134.76, 133.91, 131.36, 130.91, 129.54, 129.46, 128.96, 128.56, 128.29, 121.08 (d, $J_{P,C}$ = 4.7 Hz); 118.39, 67.55, 63.01, 61.33, 57.84, 54.19, 51.07, 44.15, 38.40, 37.29, 35.59, 33.46, 28.55, 27.59, 26.49, 23.39; Anal. Cacld for: $C_{41}H_{48}N_7O_{10}P\cdot CF_3CO_2H$: C, 54.72; H, 5.23; N, 10.39. Found: C, 54.45; H, 5.64; N, 10.65.

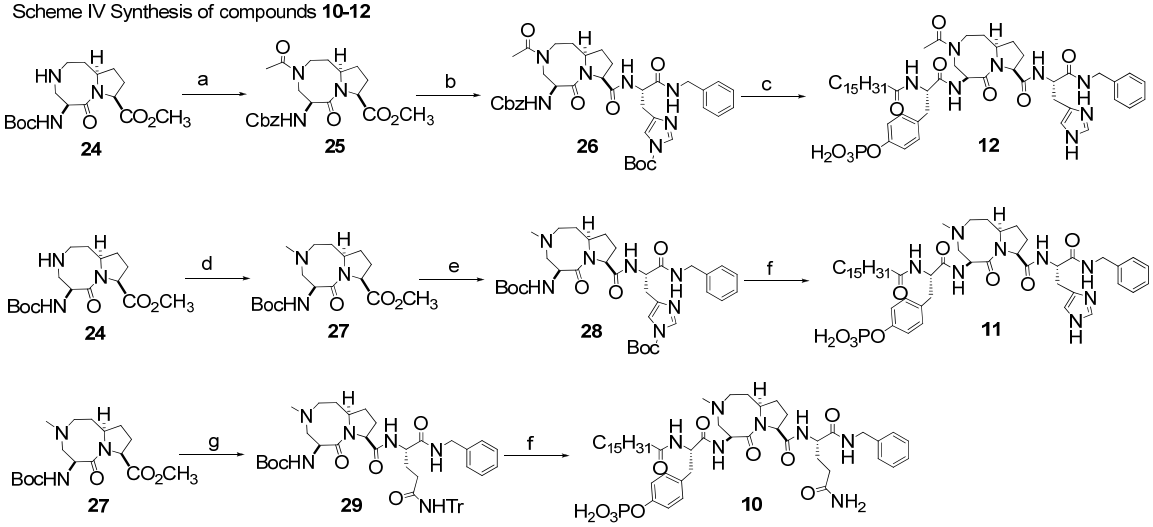


9

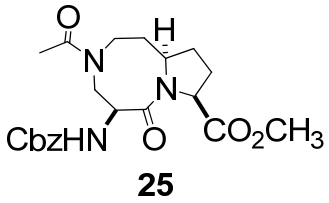
To a solution of compound **23** (66 mg, 0.074 mmol) in MeOH (10 mL) was added 10% Pd-C (20 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated and the residue was dissolved in DCM (5 mL). Palmitic acid (19 mg, 0.074 mmol), EDC (14 mg, 0.074 mmol), HOBt (11 mg, 0.074 mmol), and DIPEA (10 mg, 0.074 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **9** (32 mg, 46 % over three steps).

^{1}H NMR (300 MHz, CD_3OD), δ 8.63 (s, 1H), 7.35-7.20 (m, 6H), 7.13 (d, *J* = 8.8 Hz, 2H), 7.08 (d, *J* = 8.8 Hz, 2H), 4.85-4.70 (m, 1H), 4.52-4.20 (m, 6H), 3.35-3.20 (m, 2H), 2.91 (d, *J* = 7.8 Hz, 2H), 2.40-1.20 (m, 40H), 0.90 (t, *J* = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 176.10, 174.71, 172.54, 171.96, 171.52, 153.10, 139.79, 134.82, 132.87, 131.16, 129.56, 128.54, 128.28, 120.90, 120.84, 118.28, 62.96, 61.20, 56.52, 54.57, 50.82, 44.18, 38.56, 37.39, 36.75, 35.99, 33.49, 33.09, 30.83, 30.79, 30.76, 30.68, 30.51, 30.26, 28.61, 27.49, 26.95, 26.49, 23.76, 23.54, 14.47; Anal. Cacld for: $C_{49}H_{72}N_7O_9P{\cdot}CF_3CO_2H{\cdot}3H_2O$: C, 55.58; H, 7.22; N, 8.90. Found: C, 55.60; H, 7.08; N, 8.65.

Scheme IV Synthesis of compounds **10-12**



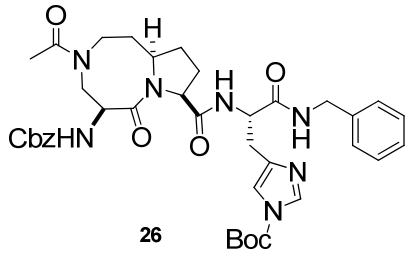
Reagents and conditions: (a) (i) Acetic anhydride, DIPEA, DCM; (ii) 4M HCl in dioxane; (iii) CbzCl, DIPEA, DCM; (b) (i) 2N LiOH, dioxane; (ii)*N*-Boc-*L*-hisdine benzylamide, EDC, HOBt, DIPEA, DCM; (c) (i) H_2, 10%Pd-C; (ii) Fmoc-phospho-L-tyrisine di-*tert*-butyl ester, EDC, HOBt, DIPEA, DCM; (iii) DEA, Acetontrile; (iv) palmitic acid, EDC, HOBt, DIPEA, DCM; (v) TFA, TES, DCM; (d) HCHO, triacetoxyborohydride, sodium acetic acid, DCM; (e) (i) 2N LiOH, dioxane; (ii) *N*-Boc-*L*-hisdine benzylamide, EDC, HOBt, DIPEA, DCM; (f) (i) 4 M HCl in dioxane; (ii) Fmoc-phospho-L-tyrisine di-*tert*-butyl ester, EDC, HOBt, DIPEA, DCM; (iii) DEA, Acetontrile; (iv) palmitic acid, EDC, HOBt, DIPEA, DCM; (v) TFA, TES, DCM; (g) (i) 2N LiOH, dioxane; (ii) N^{δ}-trityl-*L*-glutamine benzylamide, EDC, HOBt, DIPEA, DCM.



25

To a solution of **24** [3] (1.0 g, 2.93 mmol) in DCM (10 mL) were added acetic anhydride (0.60 g, 5.86 mmol) and DIPEA (0.76 g, 5.86 mmol), and the reaction mixture was stirred at room temperature for 4 hr. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a colorless oil. This oil was dissolved in methanol (10 mL) and 4M HCl in dioxane (3 mL) was added. The reaction mixture was stirred at room

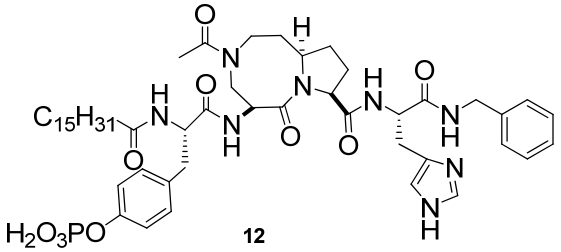
temperature for 12 h and evaporated. The residue was dissolved in DCM (20 mL). Benzyl chloroformate (600 mg, 3.5 mmol) and DIPEA (904 mg, 7.0 mmol) were then added and the mixture was stirred at room temperature for 12 h. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, Hexane : Ethyl acetate = 50: 50) gave compound **25** (450 mg, 37 % over three steps).^{1}H NMR (300 MHz, $CDCl_3$), δ 7.40-7.22 (m, 5H), 6.16 (d, J = 5.7 Hz, 1H), 5.12 (q of ABq, J_{AB} = 12.3 Hz, $\Delta\nu_{AB}$ = 25.0 Hz, 2H), 4.60-4.40 (m, 2H), 4.20-3.85 (m, 3H), 3.75 (s, 3H), 3.40-3.19 (m, 2H), 2.50-1.60 (m, 6H), 2.35 (s, 3H).



26

To a solution of **25** (62 mg, 0.14 mmol) in dioxane (10 mL) was added 2N LiOH (0.2 mL). The mixture was stirred at room temperature for 2 h and then acidified with 1N HCl. After extraction of the mixture with ethyl acetate (20 mL x 3), the organic layer was combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a colorless oil. The oil was dissolved in DCM (5 mL) and *N*-Boc-*L*-hisdine benzylamide (48 mg, 0.14 mmol), EDC (28 g, 0.14 mmol), HOBt (20 mg, 0.14 mmol), and DIPEA (19 mg, 0.14 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave compound **26** (70 mg, 67 % over two steps).^{1}H NMR (300 MHz,

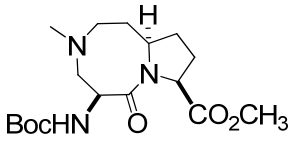
$CDCl_3$), δ 7.87 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.40-7.25 (m, 6H), 7.20-7.10 (m, 4H), 7.01 (d, J = 6.8 Hz, 2H), 5.93 (d, J = 5.9 Hz, 1H), 5.12 (q of ABq, J_{AB} = 12.2 Hz, $\Delta\nu_{AB}$ = 17.8 Hz, 2H), 4.80-4.70 (m, 1H), 4.55-4.30 (m, 3H), 4.20-3.80 (m, 4H), 3.30-3.00 (m, 3H), 2.89 (dd, J = 5.0, 15.0 Hz, 1H), 2.40-1.60 (m, 6H), 2.30 (s, 3H), 1.55 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 171.79, 171.29, 170.44, 170.32, 155.68, 147.13, 139.44, 138.53, 136.75, 136.34, 128.88, 128.64, 128.37, 127.79, 127.57, 115.49, 86.28, 67.36, 63.06, 56.72, 55.09, 54.84, 53.33, 46.47, 43.62, 32.88, 31.13, 29.61, 28.16, 27.13, 22.37.



12

To a solution of compound **26** (92 mg, 0.126 mmol) in MeOH (10 mL) was added 10% Pd-C (60 mg). The mixture was stirred under H_2 for 12 h and filtered. The filtrate was evaporated to give the crude amine which was used directly for the next step without further purification. To a solution of the crude amine in DCM (20 mL) were added Fmoc-phospho-L-tyrisine di-*tert*-butyl ester (77 mg, 0.126 mmol), EDC (24 mg, 0.126 mmol), HOBt (17 mg, 0.126 mmol), and DIPEA (16 mg, 0.126 mmol). The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave a colorless oil. The oil was dissolved in acetonitrile (5 mL) and diethylamine (1 mL) was added. The mixture was stirred at room temperature for 2 h and solvent was removed under vacuum. The residue was dissolved in DCM (10 mL). Palmitic acid (32 mg, 0.126 mmol), EDC (24 mg, 0.126

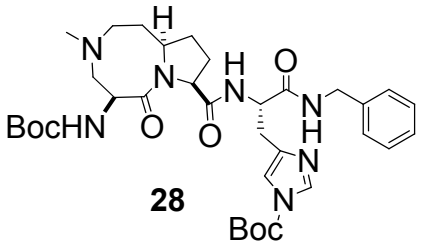
mmol), HOBt (17 mg, 0.126 mmol), and DIPEA (16 mg, 0.126 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **12** (45 mg, 37 % over five steps). ^{1}H NMR (300 MHz, CD_3OD), δ 8.67, 8.64 (two s, ratio ≈ 2:1, 1H), 7.35-7.05 (m, 10H), 4.70-4.10 (m, 8H), 3.75-3.65 (m, 2H), 3.25-3.10 (m, 2H), 3.05-2.75 (m, 3H), 2.40-1.50 (m, 13H), 1.48-1.20 (m, 24H), 0.90 (t, *J* = 6.3 Hz, 3H); Anal. Cacld for: $C_{50}H_{73}N_8O_{10}P{\cdot}CF_3CO_2H{\cdot}0.8H_2O$: C, 56.49; H, 6.89; N, 10.14. Found: C, 56.78; H, 7.15; N, 9.86.



27

To a solution of **24** (1.0 g, 2.93 mmol) in DCM (20 mL) were added 37% formaldehyde solution (0.36 g, 4.4 mmol), acetic acid (0.26 g, 4.4 mmol), and sodium triacetoxyborohydride (0.93 g, 4.4 mmol) and the reaction mixture was stirred at room temperature for 4 hr. The reaction was quenched with water and extracted with ethyl acetate. The organic layer was combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave **27** as a colorless oil (0.73 g, 76 % yield) . ^{1}H NMR (300 MHz, $CDCl_3$), δ 5.67

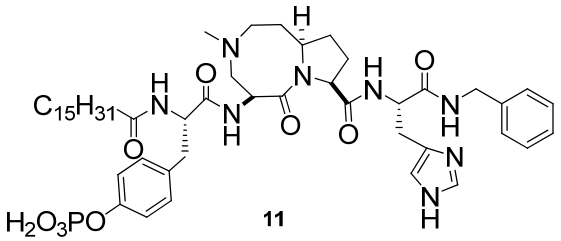
(d, J = 6.0 Hz, 1H) , 4.60-4.50 (m, 1H), 4.44 (t, J = 9.0 Hz, 1H), 4.40-4.25 (m, 1H), 3.76 (s, 3H), 3.05-2.67 (m, 4H), 2.54 (s, 3H), 2.40-1.50 (m, 6H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 172.92, 170.76, 154.90, 79.32, 63.19, 59.65, 56.24, 52.72, 52.29, 52.13, 43.57, 35.66, 31.41, 28.30, 26.84.



28

To a solution of **27** (151 mg, 0.41 mmol) in dioxane (10 mL) was added 2N LiOH (0.5 mL). The mixture was stirred at room temperature for 2 h and then acidified with 1N HCl. After extraction of the mixture with ethyl acetate (20 mL x 3), the organic layer was combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a colorless oil. The oil was dissolved in DCM (5 mL) and *N*-Boc-*L*-hisdine benzylamide (141 mg, 0.41 mmol), EDC (79 g, 0.41 mmol), HOBt (55 mg, 0.41 mmol), and DIPEA (53 mg, 0.41 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 10 : 90) gave compound **28** (200 mg, 73 % over two steps). ^{1}H NMR (300 MHz, $CDCl_3$), δ 8.05 (d, J = 9.7 Hz, 1H), 7.92 (s, 1H), 7.45-7.25 (m, 7H), 7.20 (s, 1H), 4.95-4.80 (m, 2H), 4.70-4.60 (m, 2H), 4.40-4.20 (m, 2H), 4.02 (dd, J = 2.7, 14.0 Hz, 1H), 3.63 (dd, J = 3.6, 15.3 Hz, 1H), 2.80-2.51 (m, 3H), 2.40-1.50 (m, 7H), 2.25 (s, 3H), 1.65 (s, 9H), 1.46 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 171.91, 171.63, 170.58, 154.87, 147.09,

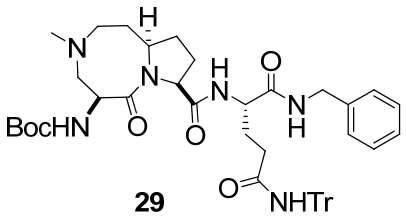
140.58, 138.65, 136.13, 129.34, 128.67, 127.79, 114.11, 85.50, 79.96, 62.79, 61.92, 59.94, 56.32, 52.60, 50.20, 48.14, 43.91, 34.45, 31.89, 29.82, 28.41, 27.89, 27.09.



11

To a solution of compound **28** (200 mg, 0.30 mmol) in MeOH (10 mL) was added 4M HCl in dioxane (2 mL). The mixture was stirred at room temperature for 12 h and evaporated. The residue was dissolved in DCM (20 mL), then Fmoc-phospho-L-tyrisine di-*tert*-butyl ester (183 mg, 0.30 mmol), EDC (58 mg, 0.30 mmol), HOBt (40 mg, 0.30 mmol), and DIPEA (77 mg, 0.60 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave a colorless oil. The oil was dissolved in acetonitrile (5 mL) and diethylamine (2 mL) was added. The mixture was stirred at room temperature for 2 h and solvent was removed under vacuum. The residue was dissolved in DCM (10 mL). Palmitic acid (77 mg, 0.30 mmol), EDC (58 mg, 0.30 mmol), HOBt (40 mg, 0.30mmol), and DIPEA (39 mg, 0.30 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **11** (85 mg,

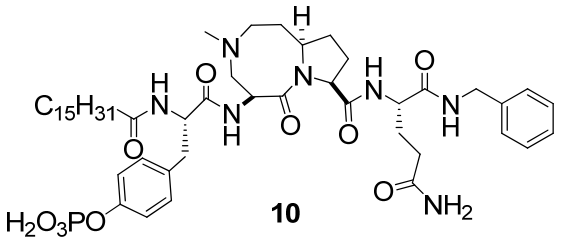
30 % over five steps). ^{1}H NMR (300 MHz, CD_3OD), δ 8.68 (s, 1H), 7.45-7.25 (m, 6H), 7.23-7.05 (m, 4H), 5.20-5.02 (m, 1H), 4.80-4.70 (m, 2H), 4.65-4.30 (m, 4H), 3.85-3.70 (m, 1H), 3.60-3.40 (m, 2H), 3.25-3.10 (m, 2H), 3.08-2.80 (m, 3H), 2.95 (s, 3H), 2.60-1.80 (m, 8H), 1.70-1.20 (m, 26H), 0.90 (t, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, CD_3OD), δ 176.24, 173.58, 171.27, 167.66, 153.19, 139.78, 134.86, 132.64, 131.17, 130.54, 129.63, 128.71, 128.45, 121.19, 118.58, 121.19, 118.58, 61.91, 60.53, 57.99, 56.76, 54.49, 46.66, 44.19, 38.36, 36.62, 33.08, 30.81, 30.77, 30.63, 30.49, 30.31, 27.90, 26.89, 23.74, 14.45; Anal. Cacld for: $C_{49}H_{73}N_8O_9P{\cdot}2CF_3CO_2H{\cdot}2H_2O$: C, 52.47; H, 6.56; N, 9.24. Found: C, 52.58; H, 6.57; N, 9.20.



29

To a solution of **27** (75 mg, 0.21 mmol) in dioxane (10 mL) was added 2N LiOH (0.3 mL). The mixture was stirred at room temperature for 2 h and then acidified with 1N HCl. After extraction of the mixture with ethyl acetate (20 mL x 3), the organic layer was combined and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave a colorless oil. The oil was dissolved in DCM (5 mL) and N^{δ}-trityl-*L*-glutamine benzylamide (104 mg, 0.21 mmol), EDC (40 mg, 0.21 mmol), HOBt (28 mg, 0.21 mmol), and DIPEA (27 mg, 0.21 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 10 : 90) gave compound **29** (110 mg, 65 % over two steps). ^{1}H NMR (300 MHz, $CDCl_3$), δ 7.93 (d, J = 9.5 Hz, 1H), 7.45-7.20 (m, 22H), 6.82 (s, 1H), 4.76 (dd, J = 7.8,

13.8 Hz, 1H), 4.70-4.4.30 (m, 3H), 4.20-4.15 (m, 1H), 4.02 (dd, J = 3.0, 13.8 Hz, 1H), 2.75-2.20 (m, 6H), 2.10 (s, 3H), 2.00-1.60 (m, 8H), 1.47 (s, 9H).



10

To a solution of compound **29** (110 mg, 0.138 mmol) in MeOH (10 mL) was added 4M HCl in dioxane (2 mL). The mixture was stirred at room temperature for 12 h and evaporated. The residue was dissolved in DCM (20 mL), then Fmoc-phospho-L-tyrisine di-*tert*-butyl ester (84 mg, 0.138 mmol), EDC (26 mg, 0.138 mmol), HOBt (19 mg, 0.138 mmol), and DIPEA (36 mg, 0.138 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave a colorless oil. The oil was dissolved in acetonitrile (5 mL) and diethylamine (2 mL) was added. The mixture was stirred at room temperature for 2 h and solvent was removed under vacuum. The residue was dissolved in DCM (10 mL). Palmitic acid (35 mg, 0.138 mmol), EDC (26 mg, 0.138 mmol), HOBt (19 mg, 0.138 mmol), and DIPEA (18 mg, 0.138 mmol) were added. The mixture was stirred at room temperature for 4 h. Water was added and the organic layer was separated and dried over anhydrous Na_2SO_4. Evaporation of the solvent and flash column chromatography (SiO_2, MeOH : Ethyl acetate = 5 : 95) gave an oil. The oil was dissolved in DCM (5 mL). TFA (5 mL) and triethylsilane (2 drops) were added. The mixture was stirred at room temperature for 30 min. Solvent was removed under vacuum to give a crude product which was purified by C18 reversed phase semipreparative HPLC to give **11** (30 mg,

23 % over five steps). ^{1}H NMR (300 MHz, CD_3OD), δ 7.40-7.10 (m, 9H), 5.20-5.10 (m, 1H), 4.60-4.30 (m, 6H), 3.90-3.40 (m, 3H), 3.15-2.90 (m, 6H), 2.60-1.80 (m, 12H), 1.70-1.30 (m, 26H), 0.92 (t, J = 6.4 Hz, 3H). ESI-MS: $[M+Na]^+$ *m/z* 962.

II. Biology

FP Binding Assay

FP experiments were performed in 96-well, black round-bottom plates (Microfluor 2, Fisher Scientific) using the Ultra plate reader (Tecan). To each well, 5 nM of fluorescein-labeled probe (GO300) and 50 nM of STAT3 (127-722 amino acid) protein were added to a final volume of 125 µl in the assay buffer (50 mM NaCl, 10 mM Hepes pH 7.5, 1 mM EDTA pH 8.0, 0.1% Nonidet, 2 mM DTT). The plate was mixed on a shaker for 15 min and incubated at room temperature for 3 h to reach equilibrium. The polarization values in millipolarization (mP) units were measured at an excitation wavelength of 485 nm and an emission wavelength of 530 nm. All experimental data were analyzed using Prism 3.0 software (GraphPad Software), and the inhibition constants were determined by nonlinear curve fitting as the concentration of the STAT3 at which 50% of the ligand is bound.

Cell Growth Assay

The effect of STAT3 compounds on cell growth was evaluated by a WST-8 [2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium, monosodium salt assay (Dojindo Molecular Technologies, Inc). Cells (3000−4000 cells in each well) were cultured in 96-well tissue culture plates in medium (200 µL)

containing various concentrations of STAT3 compounds for 4 days. At the end of incubation, WST-8 dye (20 μL) was added to each well and incubated for an additional 1−3 h, and then the absorbance was measured in a microplate reader (Molecular Devices) at 450 nm. Cell growth inhibition was evaluated as the ratio of the absorbance of the sample to that of the control.

Compound	Cellular Activity IC_{50} [μM]	
	MDA-231	MDA-468
1	>100	>100
2 (CJ-863)	>100	>100
3 (CJ-936)	36.0±11..2	52.5±32.3
4 (CJ-1418)	>100	>100
5 (CJ-1122)	>100	>100
6 (CJ-1131)	>100	>100
7 (CJ-887)	>100	>100
8 (CJ-1364)	25.6±7.4	35.0±6.8
9 (CJ-1367)	50.4±28.4	70.0±30.4
10 (CJ-1519)	42.6	20.6
11 (CJ-1383)	11.2±9.4	3.6±1.7
12 (CJ-1507)	41.5±3.1	36.1±2.2

Western Blot Analysis

Cells were lysed using radioimmunoprecipitation assay lysis buffer (PBS containing 1% NP40, 0.5% Na-deoxycholate, and 0.1% SDS) supplemented with 1 μmol/L phenylmethylsulfonyl fluoride and 1 protease inhibitor cocktail tablet per 10 mL on ice for 20 min, and lysates were then cleared by centrifugation before protein concentration determination using the Bio-Rad protein assay kit according to the manufacturer's instructions. Proteins were electrophoresed onto 4-20% SDS-PAGE gels (Invitrogen) and transferred onto polyvinylidene difluoride membranes. Following blocking in 5% milk, membranes were incubated with a specific primary antibody, washed, and incubated with horseradish peroxidase–linked secondary antibody (Amersham). The signals were visualized with the chemiluminescent horseradish peroxidase antibody detection reagent (Denville Scientific).

III. Computational method:

The crystal structure of the mouse STAT3 homodimer [4] was obtained from the PDB database (PDBID:1BG1). The C-terminal of one monomer, bearing the phosphotyrosine residue 705, binds to the SH2 domain of the other providing the target site for inhibitor design. A central residue in along the binding surface of the C-terminal tail, Val 637, was selected as the centre for docking with a radius of 13 Å defining the target site. Docking runs were performed using the GOLD program (v4.0.1) [5, 6], with the Goldscore fitness function used to evaluate the docked conformers. For each genetic algorithm (GA) run, a maximum number of 100,000 operations were performed on a population of five islands of 100 individuals. Operator weights for cross-over, mutation, and migration were set to 95, 95, and 10, respectively, and docking was terminated after 25 runs for each ligand. The conformations were evaluated with respect to the binding of the C-terminal

phosphotyrosine, and showed that the phosphate group could potentially interact with a number charged and polar groups involving residues such as K591, R609, S611, E612 and S613. Other residues likely to be involved in polar interactions with the current series of compounds include S636, E638, P639, and Y640.

References:

1. Sun, H.; Nikolovska-Coleska, Z.; Lu, J.; Meagher, J. L.; Yang, C.-Y.; Qiu, S.; Tomita, Y.; Ueda, Y.; Jiang, S.; Krajewski, K.; Roller, P. P.; Stuckey, J. A.; Wang, S. Design, synthesis, and characterization of a potent, nonpeptide, cell-Permeable, bivalent Smac mimetic that concurrently targets both the BIR2 and BIR3 Domains in XIAP. *J. Am. Chem. Soc.* **2007**, *129*, 15279–15294.

2. Duggan, H. M. E.; Hitchcock, P. B.; Young, D. W. Synthesis of 5/7-, 5/8- and 5/9-bicyclic lactam templates as constraints for external betaturns. *Org. Biomol. Chem.* **2005**, *3*, 2287–2295.

3. Peng, Y.; Sun, H.; Wang, S. Design and synthesis of a 1,5-diazabicyclo[6,3,0] dodecane amino acid derivative as a novel dipeptide reverse-turn mimetic. *Tetrahedron Lett.* **2006**, *47*, 4769–4770.

4. Becker, S.; Groner, B.; Müller, C. W. Three-dimensional structure of the Stat3β homodimer bound to DNA. *Nature,* **1998**, *394*, 145-151.

5. Jones, G.; Willett, P.; Glen, R. C.; Leach, A. R.; Taylor, R. Development and validation of a genetic algorithm for flexible docking. *J. Mol. Biol.* **1997**, *267*, 727-748.

6. Verdonk, M. L.; Cole, J. C.; Hartshorn, M. J.; Murray, C. W.; Taylor, R. D. Improved protein-ligand docking using GOLD. *Proteins*, **2003**, *52*, 609-623.