Supporting Information for

Synthesis of a Tripeptide Derivative Containing the Phe-Arg Hydroxyethylene Dipeptide Isostere

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General:

All reactions were carried out under an atmosphere of argon using flame-dried glassware. Tetrahydrofuran (THF) was distilled from sodium metal-benzophenone ketyl. Dichloromethane (CH_2Cl_2), DMPU, diisopropylamine, and toluene were distilled from calcium hydride. Allyl bromide and hexanes were distilled. All other solvents and reagents were used without further purification. N,N'-bis-Boc-1-guanylpyrazole was purchased from Advanced ChemTech. N-(9-Fluorenylmethoxycarbonyloxy)succinimide was purchased from Chem-Impex. All other reagents were purchased from Aldrich.

Flash column chromatography was performed using Merck grade 60 silica gel (230-400 mesh). ^{1}H NMR spectra were taken on a Bruker AC 300 or a Bruker AC 250 spectrometer in $CDCl_3$ at ambient temperature unless otherwise noted. Chemical shifts were reported in ppm (δ units) downfield from tetramethylsilane. Mass spectra were taken on a Micromass AutoSpec magnetic sector mass spectrometer using 3-nitrobenzyl alcohol as the matrix.

Experimental Procedures:

{(1S)-1-[(4R)-4-Allyl-5-oxo-tetrahydro-furan-(2S)-2-yl]-2-phenyl-ethyl} Carbamic Acid *tert*-Butyl Ester (3). A 1.48M solution of n-butyl lithium in THF (27.22 ml, 40.28 mmol) was add dropwise to a –78 °C solution of freshly distilled diisopropyl amine (6.61 ml, 47.16 mmol) in THF (48 ml), and the resulting mixture was stirred at this temperature for 20 min. To this solution was added lactone **2** (6.00 g, 19.65 mmol) in THF (20 ml) in a dropwise manner over a period of 30 min. The enolate was allowed to form for 20 min at this temperature, and then freshly distilled allyl bromide (1.87 ml, 21.6 mmol) was added dropwise in freshly distilled DMPU (19.7 ml) over a period of 15 min resulting in a thick mixture which was difficult to stir. This mixture was kept at –78 °C for 15 min and was then transferred to a –50 °C bath. The homogeneous solution was maintained between –50 and –40 °C for 1.5 h and the reaction was quenched with saturated aqueous NH_4Cl (14 ml). After warming to room temperature the reaction was concentrated in vacuo and the residue was partitioned between ethyl acetate and water. The organic layer was washed five times with water, once with brine, and was then dried over $MgSO_4$. The product was concentrated to an oil and purified by flash column chromatography eluting first with hexanes and then 80:20 hexanes : ethyl acetate to yield 3.93 g (58%) of **3** as a clear oil which solidified upon standing. Fractions contaminated with slower running diastereomer could be purified by flash column chromatography (80:20 hexanes : ethyl acetate) to yield an additional 0.27 g (4%) of product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33-7.18 (m, 5 H), 5.79-5.64 (m, 1 H), 5.11 (d, J = 5.3, 1 H), 5.06 (s, 1 H), 4.72 (d, J = 9.7, 1 H), 4.46 (ddd, J = 8.3, 5.3, 1.5, 1 H), 4.01 (q, J = 8.3, 1 H), 2.90 (d, J = 7.9, 2 H), 2.81-2.69 (m, 1 H), 2.58-2.45 (m, 1 H), 2.38-2.16 (m, 2 H), 2.05-1.90 (m, 1 H), 1.38 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.1, 155.9, 137.1, 134.0, 129.2, 128.5, 126.6, 117.9,

79.9, 78.3, 54.7, 39.2, 39.1, 35.3, 29.4, 28.2; FABMS: Calculated for $[C_{20}H_{27}NO_4Na]^+$: 368.1838. Found: 368.1856 .

{(1S)-1-[(4R)-4-[(3-Hydroxy-propyl]-5-oxo-tetrahydro-furan-(2S)-2-yl]-2-phenyl-ethyl} Carbamic Acid *tert*-Butyl Ester (4). A 1M solution of catecholborane (14.68 ml) was added dropwise to a 0 °C mixture of **3** (3.9 g, 11.3 mmol) and $Rh(PPh_3)_3Cl$ (0.21 g, 0.23 mmol) in anhydrous THF (50 ml). The reaction mixture was removed from the ice bath, stirred at room temperature for 40 min, and then cooled back to 0 °C. To this was added a 1:1 mixture of THF and EtOH (23 ml), phosphate buffer (pH 7.2, 23 ml) [gas evolution], and 30% H_2O_2 (23 ml) and the reaction was allowed to warm to room temperature overnight. The mixture was concentrated in vacuo and partitioned between ethyl acetate and brine. The aqueous layer was extracted with ethyl acetate and the organic layers were combined and washed with 10% Na_2CO_2 until the aqueous layer remained colorless. The organics were washed with brine, dried over $MgSO_4$, and concentrated to an oil which was purified by flash column chromatography (60 : 40 Hexane : Ethyl Acetate followed by 50 : 50 Hexane : Ethyl Acetate) to yield (2.87 g, 70%) alcohol **4**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.18 (m, 5 H), 4.68 (d, J = 9.7, 1 H), 4.49 (t, J = 6.4, 1 H), 4.01 (q, J = 8.3, 1 H), 3.65 (t, J = 5.7, 2 H), 2.90 (d, J = 8.3, 2 H), 2.75-2.60 (m, 1 H), 2.44-2.27 (m, 1 H), 2.00-1.78 (m, 2 H), 1.68-1.44 (m, 4 H), 1.38 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.7, 155.9, 137.1, 129.3, 128.6, 126.7, 80.0, 78.3, 62.0, 54.7, 39.3, 39.1, 30.4, 30.1, 28.2, 27.8; FABMS: Calculated for $[C_{20}H_{29}NO_5Na]^+$: 386.1943 Found: 386.1936.

{(1S)-1-[(4R)-4-(3-Azido-propyl)-5-oxo-tetrahydro-furan-(2S)-2-yl]-2-phenyl-ethyl} Carbamic Acid *tert*-Butyl Ester (6a). Methane sulfonyl chloride (0.73 ml, 9.48 mmol)

was added dropwise with cooling to a 0 °C mixture of **4** (2.87 g, 7.91 mmol) and triethylamine (1.32 ml, 9.48 mmol) in dry toluene (33 ml). The reaction mixture was warmed to room temperature and stirred until TLC showed total consumption of **4** (ca. 20 min). At this time *tetra*-butyl ammonium bromide (0.26 g, 0.79 mmol) was added to the reaction mixture followed by a solution of sodium azide (4.11 g, 63.20 mmol) in water (15 ml). The reaction mixture was warmed to reflux for 3 h, cooled to room temperature, and then partitioned between phosphate buffer (pH 5) and diethyl ether. The layers were separated and the aqueous layer was extracted with diethyl ether. The organic layers were combined, washed with brine, dried over Na_2SO_4, and concentrated to an oil which was purified by flash column chromatography (75:25 hexanes : ethyl acetate) to yield 2.92 g (7.52 mmol, 95%) of azide **6a** as a clear colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36-7.17 (m, 5 H), 4.59 (d, J = 9.7, 1H), 4.48 (ddd, J = 8.3, 5.2, 1.5, 1 H), 4.02 (q, J = 8.3, 1 H), 3.31 (t, J = 6.5, 2 H), 2.96-2.84 (m, 2 H), 2.70-2.53 (m, 1 H), 2.45-2.33 (m, 1 H), 1.97-1.78 (m, 2 H), 1.75-1.45 (m, 3 H), 1.38 (s, 9 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 179.0, 155.8, 137.0, 129.1, 128.5, 126.6, 79.8, 78.2, 78.0, 54.7, 50.7, 38.9, 30.2, 28.5, 28.1, 26.5; FABMS: Calculated for $[C_{20}H_{28}N_4O_4Na]^+$: 411.2008. Found: 411.2025.

[(1S)-1-{(1S)-1-[(4R)-4-(3-Azido-propyl)-5-oxo-tetrahydro-furan-(2S)-2-yl]-2-phenyl-ethylcarbamoyl}-2-(trityl-carbamoyl)-ethyl]-carbamic acid benzyl ester (8). Part A: To azide **6a** (1.00g, 2.58 mmol) dissolved in THF (1.5 ml) was added 4N HCl in dioxane (10 ml) at room temperature. The reaction mixture was stirred for 1 hr and then the solvents were removed in vacuo. The residue was then dissolved in ethyl acetate and the reaction mixture was concentrated in vacuo to provide the HCl salt of the corresponding amine.

Part B: To a 0 °C mixture of Z(Trt) diprotected asparagine and HOBt (0.58g, 4.26 mmol) in DMF (18 ml) was added EDCI (0.60g, 3.12 mmol). The solution was stirred at 0 °C for 0.5 h, and then a mixture of diisopropyl ethyl amine (0.45 ml, 2.58 mmol) and the amine from Part A (2.58 mmol) in DMF (8 ml) was added dropwise via cannula. The reaction mixture was allowed to warm to room temperature overnight, and was then partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organics were washed with 10% aqueous HCl, water, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo to a foam. The product was purified by flash column chromatography (50/50 hexanes/ethyl acetate) to yield a foam which was further purified by recrystallization (ethyl acetate/ hexanes) to give 1.54 g (77%) of asparagine derivative **8** as a white powder. The mother liquor was concentrated and the residue recrystallized (ethyl acetate/ hexanes) to yield another 0.25 g (12%) of product: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.40-7.05 (m, 25 H), 6.86 (s, 1 H), 6.67 (d, J = 9.5, 1 H), 6.15 (d, J = 5.5, 1 H), 5.10 (d, J = 12.1, 1 H), 5.01 (d, J = 12.1, 1 H), 4.35-4.20 (m, 3 H), 3.22 (t, J = 6.3, 2 H), 2.87-2.55 (m, 5 H), 2.25-2.10 (m, 1H), 1.85-1.25 (m, 5 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 178.5, 171.4, 169.5, 144.4, 137.1, 129.3, 128.7, 128.5, 128.4, 128.3, 128.2, 128.0, 127.2, 126.8, 77.5, 71.0, 67.5, 53.4, 52.3, 51.1, 38.4, 38.3, 38.1, 30.5, 28.8, 26.5; FABMS: Calculated for $[C_{46}H_{46}N_6O_6Na]^+$: 801.3377. Found 801.3393.

(2R)-2-(3-Azido-propyl)-(5S)-5-[(2S)-2-benzyloxycarbonylamino-3-(trityl-carbamoyl)-propionylamino]-(4S)-4-(*tert*-butyl-dimethyl-silanyloxy)-6-phenyl-hexanoic acid (9). To a room temperature solution of lactone **8** (0.100g, 0.128 mmol) in a mixture of dioxane (0.73 ml) and water (0.36 ml) was added in a dropwise manner a 1M aqueous solution of LiOH (0.154 ml). The reaction was monitored by TLC, and

when complete (ca. 1 h), the solvents were removed in vacuo and the residue was partitioned between Et_2O and a 10% aqueous citric acid solution. The aqueous layer was extracted two times with Et_2O and the organics were combined. The organics were washed with water and brine, dried over $MgSO_4$, and concentrated to a foam. The foam was dissolved in DMF (0.87 ml) and to this solution was added imidazole (0.192 g, 2.82 mmol) and t-butyl dimethylsilyl chloride (0.193 g, 1.28 mmol), and the reaction mixture was stirred at room temperature for 44 h. MeOH (8 ml) was added, the mixture was stirred for 3.5 h, and then concentrated in vacuo. The residue was dissolved in ethyl acetate, washed with 10% aqueous citric acid and brine, dried over $MgSO_4$, concentrated, and purified by flash column chromatography (96/4 CH_2Cl_2/MeOH) to yield 0.093 g (80%) of silyl ether **9** as a foam: ^{1}H NMR (250 MHz, $CDCl_3$, 325 K) δ 7.35-7.05 (m, 27 H), 6.33 (br d, 1 H), 5.03 (d, J = 12.5, 1 H), 4.97 (d, J = 12.5, 1 H), 4.45 (br q, J = 7.3, 1 H), 4.11 (br q, J = 7.6, 1 H), 3.78 (br t, J = 5.95, 1 H), 3.10 (t, J = 6.4, 2 H), 2.97-2.45 (m, 4 H), 2.36-2.24 (m, 1 H), 1.84 (p, J = 6.4, 1 H), 1.60-1.23 (m, 5 H), 0.90 (s, 9 H), 0.12 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$, 325 K) δ 177.7, 171.5, 170.7, 144.6, 138.3, 136.3, 129.0, 128.9, 128.8, 128.7, 128.6, 128.5, 128.1, 128.0, 127.9, 127.8, 126.9, 126.5, 126.4, 70.9, 70.0, 67.1, 54.2, 51.9, 51.1, 41.5, 36.1, 29.7, 26.6, 26.0, 18.0, -4.1, -4.2; FABMS: Calculated for $[C_{52}H_{62}N_6O_7SiNa]^+$: 933.4347. Found 933.4395.

(5S)-5-[(2S)-2-benzyloxycarbonylamino-3-(trityl-carbamoyl)-propionylamino]-(4S)-4-(*tert*-butyl-dimethyl-silanyloxy)-(2R)-2-(3-N,N'-bis-*t*-Boc-guanidino-propyl)-6-phenyl-hexanoic acid (12). To a solution of azide **9** (2.35g, 2.58 mmol), N,N'-bis-Boc-1-guanylpyrazole (1.2g, 3.87 mmol), and aqueous 1M LiOH (5.16 ml) in a mixture of THF (26 ml) and water (4.86 ml) was added triphenylphosphine (2.03g, 7.74 mmol). Gas evolution commenced, and the reaction was allowed to stir at room temperature for 48 h.

The THF was removed in vacuo, the residue was partitioned between aqueous 10% citric acid and ethyl acetate, and the layers were separated. The aqueous layer was extracted twice with ethyl acetate, the organics were combined, washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to an oil. The product was separated from excess triphenylphosphine by flash column chromatography (50 / 50 / 0.1 hexane / ethyl acetate / acetic acid) and then purified by flash column chromatography (97 / 3 CH_2Cl_2/MeOH) to yield 2.03g (70%) of a foam which could be recrystallized from petroleum ether to yield 1.57g (54%) of guanidine **12** as a white powder. The mother liquor was concentrated and the residue recrystallized to yield a further 0.098g (3%) of product as a white solid. The mother liquor was concentrated and purified by flash column chromatography (96 / 4 CH_2Cl_2 / MeOH) to yield 0.148g (5%) of product: ^{1}H NMR (250 MHz, $CDCl_3$, 325 K [Note: At this temperature slight decomposition was observed during course of data collection]): δ 11.40 (br s, 1H), 8.19 (t, J = 5.2, 1 H), 7.36-7.05 (m, 27 H), 6.34 (br d, J = 7.5, 1 H), 5.07 (d, J = 12.5, 1 H), 4.94 (d, J = 12.5, 1 H), 4.44 (br q, J = 7.5, 1 H), 4.10, (q, J = 7.9, 1 H), 3.77 (t, J = 4.9, 1 H), 3.35-3.17 (m, 2 H), 3.00-2.45 (m, 4 H), 2.35-2.20 (m, 1 H), 1.9-1.72 (m, 1 H), 1.60-1.20 (m, 23 H), 0.90 (s, 9 H), 0.12 (s, 3 H), 0.07 (s, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$, 325 K) δ 177.4, 171.4, 170.6, 163.7, 156.4, 156.2, 153.3, 144.6, 138.3, 136.3, 129.1, 128.9, 128.6, 128.5, 128.1, 128.0, 127.9, 127.0, 126.5, 83.0, 79.1, 70.9, 70.1, 67.1, 54.3, 51.9, 44.0, 41.9, 40.7, 36.3, 29.9, 28.4, 28.1, 28.0, 27.1, 26.0, 18.0, -4.1, -4.2; FABMS: Calculated for $[C_{63}H_{81}N_6O_{11}SiNa_2]^+$: 1171.5528. Found 1171.5523.

(4S)-4-(*tert*-butyl-dimethyl-silanyloxy)-(5S)-5-[(2S)-2-(9,9a-dihydro-4aH-flouren-9-ylmethoxycarbonylamino)-3-(trityl-carbamoyl)-propionylamino]-(2R)-2-(3-N,N'-

bis-*t*-Boc-guanidino-propyl)-6-phenyl-hexanoic acid (1). To a stirred solution of guanidine **12** (0.05 g, 0.044 mmol) in MeOH (2 ml) under a nitrogen atmosphere was added 20% $Pd(OH)_2$ on carbon (0.003 g). The reaction vessel was evacuated and purged with nitrogen three times, and then placed under an atmosphere of hydrogen using a balloon until TLC showed complete consumption of starting material (ca. 6 h). At this time the hydrogen gas was evacuated, the catalyst was removed by filtration, and the solvent was removed in vacuo. The residue was dissolved in dioxane (0.5 ml) and aqueous 10% sodium carbonate (0.5 ml) and cooled to 0 °C. To this mixture was added N-(9-Fluorenylmethoxycarbonyloxy)succinimide (0.018 g, 0.053 mmol) in dioxane (0.25 ml) and the reaction was allowed to warm to room temperature overnight, during which time it became very viscous. The reaction mixture was partitioned between aqueous 10% citric acid and diethyl ether and the layers were separated. The aqueous layer was extracted twice with diethyl ether, the organics were combined, washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The product was purified by flash column chromatography (98 / 2 CH_2Cl_2 / MeOH) to yield 0.038 g (71%) of the title compound **1**: ^{1}H NMR (250 MHz, $CDCl_3$, 325 K [Note: At this temperature slight decomposition was observed during course of data collection]) δ 11.42 (br s, 1 H), 8.18 (t, J = 5.5, 1 H), 7.72 (d, J = 7.5, 2 H), 7.5 (d, J = 7.5, 2 H), 7.42-7.03 (m, 28 H), 6.39 (d, J = 7.6, 1 H), 4.46 (br q, J = 6.1, 1 H), 4.37-4.05 (m, 4 H), 3.77 (t, J = 6.1, 1 H), 3.35-3.16 (m, 2 H), 3.05-2.47 (m, 4 H), 2.35-2.20 (m, 1 H), 1.92-1.76 (m, 1 H), 1.65-1.15 (m, 23 H), 0.88 (s, 9 H), 0.11 (s, 3 H), 0.06 (s, 3 H); ^{13}C NMR (63 MHz, $CDCl_3$, 325 K) δ 177.3, 171.3, 170.7, 163.7, 156.5, 156.2, 153.3, 144.6, 143.9, 141.4, 138.3, 129.0, 128.9, 128.6, 127.9, 127.7, 127.1, 127.0, 126.5, 125.2, 119.9, 83.0, 79.1, 70.9, 70.2, 67.6, 54.2, 52.0, 47.2, 41.8, 40.6, 38.4, 36.2, 31.3, 29.9, 28.4, 28.1, 27.0, 26.0, 18.0, -4.1, -4.2; FABMS: Calculated for $[C_{70}H_{85}N_6O_{11}SiNa_2]^+$: 1259.5841. Found 1259.5808.