Supporting information

Synthesis of constrained analogues of cholecystokinin/opioid chimeric peptides

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Experimental

General procedure for alkylation of aspartic acid

In a 100-mL flask, α-benzyl β-methyl N^{α}-tert-butoxycarbonylamino-(*S*)-aspartate **4** (5.0 g, 14.82 mmol) was dissolved in THF (10 mL) and cooled to -42 ^{o}C. Lithium bis(trimethylsilyl)amide LHMDS (1M solution in THF, 37.0 mL, 37.0 mmol) followed by hexamethylphosphoramide HMPA (1.7 mL, 9.8 mmol) were added and the mixture stirred at -42 ^{o}C for 30 min before adding allyl bromide (1.6 mL, 18.49 mmol). The mixture was stirred for a further 4 h and saturated aqueous NH_4Cl (20 mL) was added. The mixture was warmed up to room temperature and the THF evaporated off. The product was redissolved in DCM (100 mL) and washed with aqueous NH_4Cl (2 x 50 mL) and brine (60 mL). The organic layer was dried over $MgSO_4$ and the solvent evaporated off. After purification on a silica gel column with hexanes-ethyl acetate (10:1 v/v), two colorless liquids (2.8 g of **5a** and 0.7g of **5b**) were obtained in a total yield of 62% and a ratio of 4:1 in favor of **5a**.

Methyl 2-*tert*-butoxycarbonylamino-3-carbobenzyloxy-(2*S*, 3*R*)-hex-5-enoate (5a): $[\alpha]^{25}_{D}$ = +28.7^{o} (c = 1.52, $CHCl_3$); ^{1}H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.37-7.29 (5H, m), 5.84-5.69 (1H, m), 5.49 (1H, d, *J* = 9.92 Hz), 5.23-5.06 (4H, m), 4.60 (1H, dd, *J* = 3.63, 9.92 Hz), 3.56 (3H, s), 3.22-3.13 (1H, m), 2.56-2.45 (1H, m), 2.36-2.26 (1H, m), 1.44 (9H, s); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 173.3, 171.2, 155.8, 135.3, 134.1, 128.5, 128.3, 128.2, 118.2, 80.0, 67.2, 53.5, 51.9, 46.4, 32.8, 28.2; HRMS (FAB) MH^{+} calculated for 378.1917, found 378.1921.

Methyl 2-*tert*-butoxycarbonylamino-3-carbobenzyloxy-(2*S*, 3*S*)-hex-5-enoate (5b): $[\alpha]^{25}_{D}$ = +25.5^{o} (c = 1.33, $CHCl_3$); ^{1}H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.39-7.31 (5H,

m), 5.74-5.69 (1H, m), 5.29 (1H, d, *J* = 9.92 Hz), 5.21-5.13 (4H, m), 4.68 (1H, dd, *J* = 4.78, 8.6 Hz), 3.62 (3H, s), 2.95-2.92 (1H, m), 2.55-2.49 (1H, m), 2.28-2.25 (1H, m), 1.44 (9H, s); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 172.5, 170.5, 155.2, 135.1, 134.5, 128.6, 128.5, 117.8, 80.2, 67.5, 54.1, 52.0, 47.9, 32.2, 28.2; HRMS (FAB) MH^+ calculated for 378.1917, found 378.1927.

General procedure for the synthesis of 5-allyl substituted proline analogues

In 500-mL flask, **5a** was dissolved in dichloromethane (300 mL and cooled to -78 ^{o}C. The solution was exposed to O_3 until a persistent blue color appeared. Dimethylsulfide (50 mL) was added and the mixture stirred at room temperature for 60 h. The solvent was evaporated off and the product dried under high vacuum. The dried product was dissolved in methanol (100 mL) and *para*-toluenesulfonic acid (220 mg, 1.16 mmol) was added. The mixture was stirred at room temperature for 12 h and the solvent evaporated off. The product was redissolved in ethyl acetate (150 mL) and the organic layer washed with aqueous NH_4Cl (2 x 50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated. The product was dried under high vacuum to give 8.16 g of crude product, a 91.5 % yield. The crude product was dissolved in ether (120 mL) and cooled to -78 ^{o}C. BF_3OEt_2 (8.0 mL, 63.13 mmol) and allyl trimethyl silane (10.0 mL, 62.92 mmol) were added and the mixture stirred at -78 ^{o}C for 20 min. The cold bath was removed and the mixture stirred for a further 40 min before cooling to -78 ^{o}C and slowly adding concentrated aqueous $NaHCO_3$. The solution was slowly warmed to room temperature and the aqueous phase separated. The organic phase was washed with concentrated aqueous $NaHCO_3$ (2 x 50 mL) and brine (50 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated off. The product was purified by column to

give 2.45 g of a white semi solid **8a** and 2.59 g of a pale yellow liquid **8b** in a total yield of 55%. When the reaction was repeated with **5b**, only 5-allyl proline analogue **8c,** was obtained in 50% yield.

Benzyl (2*S*, 3*R*, 5*R*)-5-allyl-1-(*tert*-butoxycarbonyl)-3-methoxycarbonyl prolinate (8a): $[\alpha]^{25}_{D}$ = -0.016^{o} (c = 0.62, $CHCl_3$); ^{1}H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.44-7.27 (5H, br), 5.81-5.67 (1H, m), 5.19-4.99 (4H, m), 4.59 (0.4H, d, *J* = 8.0 Hz), 4.50 (0.6H, d, *J* = 8.5 Hz), 4.22-4.14 (0.6H, td, *J* = 3.0, 9.0 Hz), 4.10-4.02 (0.4H, td, *J* = 3.0, 9.0 Hz), 3.54 (2H, s), 3.46 (1H, s), 3.42-3.28 (1H, m), 2.63-2.55 (0.6H, br), 2.54-2.40 (1.4H, m), 2.20-2.11 (1H, m), 1.91 (1H, q, *J* = 6.5 Hz), 1.46 (3.6H, s), 1.32 (5.4H, s); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 170.63, 170.54, 170.40, 170.1, 154.1, 153.3, 135.42, 135.19, 134.48, 134.37, 128.72, 128.51, 128.40, 128.36, 128.12, 117.87, 117.78, 80.51, 80.43, 67.18, 67.07, 61.87, 61.66, 56.86, 56.79, 52.02, 51.97, 45.1, 44.1, 39.3, 38.4, 31.0, 30.0, 28.3, 28.1; HRMS (FAB) MH^+ calculated for 404.2073, found 404.2066.

Benzyl (2*S*, 3*R*, 5*S*)-5-allyl-1-(*tert*-butoxycarbonyl)-3-methoxycarbonyl prolinate (8b): $[\alpha]^{25}_{D}$ = +0.17^{o} (c = 1.39, $CHCl_3$); ^{1}H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.44-7.28 (5H, br), 5.80-5.65 (1H, br), 5.22-4.96 (4H, m), 4.83 (0.4H, d, *J* = 6.5 Hz), 4.68 (0.6H, d, *J* = 8.5 Hz), 3.90-3.71 (1H, br), 3.63-3.41 (3H, br), 3.25-3.12 (1H, br), 3.09-2.97 (0.6H, br), 2.87-2.76 (0.4H, br), 2.36-2.11 (3H, m); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 170.7, 170.3, 153.9, 153.2, 135.3, 134.4, 128.6, 128.45, 128.38, 128.2, 117.3, 80.4, 67.0, 61.7, 61.4, 57.5, 52.0, 45.4, 44.9, 39.7, 38.4, 33.4, 32.7, 28.3, 28.2; HRMS (FAB) MH^+ calculated for 404.2073, found 404.2061.

Benzyl (2*S*, 3*S*, 5*S*)-5-allyl-1-(*tert*-butoxycarbonyl)-3-methoxycarbonyl prolinate (8c): $[\alpha]^{25}_{D}$ = + 0.117^{o} (c = 1.05, $CHCl_3$); ^{1}H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.40-7.30

(5H, br), 5.84-5.69 (1H, br), 5.36-4.98 (4H, m), 4.72-4.65 (0.3H, br), 4.57 (0.5H, d, *J* = 6.5 Hz), 4.07 (0.65H, br), 3.97 (0.35H, br), 3.67 (3H, s), 3.25-3.13 (1H, br), 2.76-2.66 (0.6H, br), 2.63-2.53 (0.4H, br), 2.32-2.13 (2H, br), 2.10-1.96 (1H, br), 1.55-1.28 (9H, br); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 172.1, 171.6, 171.3, 170.6, 153.8, 153.2, 135.6, 135.3, 134.6, 134.4, 134.3, 128.4, 128.3, 128.2, 117.0, 80.4, 77.2, 66.8, 62.3, 57.5, 52.3, 46.5, 45.6, 39.1, 38.2, 33.4, 32.5, 28.3, 28.0; HRMS (FAB) MH^+ calculated for 404.2073, found 404.2063.

General procedure for the synthesis of dehydroamino acids

Compound **8a** (1.83 g, 4.54 mmol) was dissolved in THF (60 mL)/H_2O (30 mL) mixture. OsO_4 (60 mg, 0.24 mmol), was added and the mixture stirred at room temperature for 10 min before adding $NaIO_4$ (2.7 g, 12.62 mmol) and stirring the mixture for a further 4 h. The mixture was filtered and the THF evaporated. Ethyl acetate (50 mL) was added and the aqueous layer separated. The organic phase was washed with saturated NH_4Cl (2 x 30 mL) and brine (30 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated off. The crude product was dried under high vacuum and used in the next step without purification. In another 100-mL flask, N-(benzyloxycarbonyl)-α-phosphonoglycine trimethyl ester (1.7 g, 5.13 mmol) was dissolved in CH_2Cl_2 (30 mL). 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU) (750 μL, 5.02 mmol) was added and the mixture stirred for 10 min. The crude product from above, dissolved in CH_2Cl_2 (10 mL) was added and the mixture stirred at room temperature for 8 h. The CH_2Cl_2 layer was diluted to 60 mL and washed with aqueous 1 N HCl (2 x 30 mL) and brine (30 mL). The organic phase was dried over $MgSO_4$ and evaporated off. The product was then purified

by column to give 1. 91 g of a pale yellow liquid **9a** in 71% yield. The compound was not characterized since it existed as a rotamer.

General method for the synthesis of Nle-Asp bicyclic dipeptide mimetics

The dehydroamino acid **9a** (2.4g, 3.92 mmol) was dissolved in degassed methanol (50 mL). [Rh-(S,S)-EtDuPHOS]-OTf (6 mg, 0.008 mmol) was added and the mixture exposed to hydrogen at 70 psi for 20 h. The solvent was evaporated and the product purified by passing it through a short silica column using ethyl acetate. The solvent was evaporated off and the product, obtained in quantitative yield, dried under high vacuum. The product was then dissolved in CH_2Cl_2 (30 mL) and the mixture cooled to 0 ^oC. TFA (10 mL) was added and the mixture stirred at 0 ^oC for 10 min and at room temperature for 40 min. The solvent was evaporated off and the product dissolved in ethyl acetate (50 mL). The organic layer was washed with $NaHCO_3$ (2 x 30 mL) and brine (30 mL). The organic phase was dried over $MgSO_4$ and the solvent evaporated off. The crude product was dried under high vacuum and dissolved in pyridine (250 mL). The mixture was then heated at 50 ^oC for 4 days. The solvent was then evaporated off and the product dissolved in ethyl acetate (50 mL). The organic phase was washed with aqueous I N HCl (2 x 20 mL) and brine (30 mL). The product was purified by column to give 1.0 g of **3a** in 53 % yield.

(3*S*, 6*R*, 8*R*, 9*S*) Benzyl 2-oxo-3-N-(benzyloxycarbonyl)amino-8-methylester-1-azabicyclo[4.3.0]nonane-9-carboxylate (3a): $[\alpha]^{25}_D$ = -15.9^o (c = 0.6, $CHCl_3$); ^{1}H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.58-7.22 (11H, m), 6.5 (1H, br), 5.11 (4H, d, *J* = 8.5 Hz), 4.04 (1H, d, *J* = 7.0 Hz), 3.78-3.68 (3H, m), 3.46 (3H, s), 3.34-3.26 (1H, m), 2.38-2.22 (3H, m), 1.81-1.71 (1H, m); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 172.7, 171.8, 164.9,

154, 136.1, 135.2, 131.4, 128.58, 128.54, 128.48, 128.44, 128.17, 128.14, 128.03, 67.23, 67.17, 62.5, 57.3, 52.3, 51.8, 47.3, 35.0; HRMS (FAB) MH^+ calculated for 481.1975, found 481.1958.

(3*S*, 6*S*, 8*R*, 9*S*) Benzyl 2-oxo-3-N-(benzyloxycarbonyl)amino-8-methylester-1-azabicyclo[4.3.0]nonane-9-carboxylate (3b): $[\alpha]^{25}_D = +5.2^o$ (c = 0.6, $CHCl_3$); ^1H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.40-7.32 (10H, m), 5.79 (2H, d, *J* = 5.5 Hz), 5.13 (4H, s), 4.78 (1H, d, *J* = 8.5 Hz), 4.23-4.15 (1H, m), 3.83-3.71 (1H, m), 3.48 (3H, s), 3.35-3.26 (1H, m), 2.56-2.45 (1H, m), 2.38 (1H, p, *J* = 6.28 Hz), 2.24-2.05 (2H, m), 1.83-1.71 (1H, m), 1.68-1.56 (1H, m); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 169.9, 169.0, 156.0, 136.4, 135.0, 128.6, 128.5, 128.4, 128.02, 127.94, 67.6, 66.8, 60.1, 55.7, 52.1, 50.2, 45.7, 34.9, 26.91, 26.67; HRMS (FAB) MH^+ calculated for 481.1975, found 481.1995.

(3*S*, 6*S*, 8*S*, 9*S*) Benzyl 2-oxo-3-N-(benzyloxycarbonyl)amino-8-methylester-1-azabicyclo[4.3.0]nonane-9-carboxylate (3c): $[\alpha]^{25}_D = +34.9^o$ (c = 1.2, $CHCl_3$); ^1H NMR, 500 MHz, $CDCl_3$, δ (ppm): 7.41-7.27 (10H, m), 5.77 (1H, d, *J* = 4.5 Hz), 5.18 (2H, q, *J* = 12.0 Hz), 5.11 (2H, s), 4.88 (1H, s), 4.26-4.14 (1H, br), 3.90-3.77 (1H, br), 3.73 (3H, s), 3.12 (1H, d, *J* = 7.5 Hz), 2.58-2.42 (2H, m), 2.14-2.01 (1H, br), 1.91-1.81 (1H, m), 1.70-1.58 (2H, m); ^{13}C NMR, 125 MHz, $CDCl_3$, δ (ppm): 171.8, 170.0, 168.8, 156.0, 136.4, 135.1, 128.6, 128.5, 128.4, 128.2, 128.01, 127.96, 67.5, 66.7, 61.0, 55.1, 52.7, 50.3, 46.1, 34.9, 26.9, 26.6; HRMS (FAB) MH^+ calculated for 481.1975, found 481.1958.

Synthesis of peptides

All the peptides were synthesized using the N^α-Fmoc/t-butyl chemistry and Rink-amide AM resin. The N^α-Boc protected BTD's were first hydrolyzed using $LiOH/H_2O$/MeOH

system. The Boc group was then removed using TFA (30%) in CH_2Cl_2. The amino group was Fmoc protected and the crude BTD used in peptide synthesis. For bicyclic dipeptide **3b**, the benzyl carboxylate and carbobenzyloxyamino groups were deprotected by hydrogenation. The amino terminus was then Fmoc protected, and the product used in peptide synthesis without purification. Rink amide resin (0.5g) was swollen overnight in DMF in a coarse sintered filter fitted vessel. The resin was deprotected with piperidine (20% v/v in DMF) first for 5 min and then for 20 min. The resin was then washed with DMF (3 times) and CH_2Cl_2 (3 times). The first amino acid was then coupled to the resin using O-benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate (HBTU), 1-hydroxybenzotriazole hydrate (HOBt) and diisopropylethylamine (DIPEA). In all the couplings, 3 equivalents of each reagent (amino acid, HBTU, HOBt, and DIPEA) were used. The coupling was allowed to proceed for 1 h before coupling the next amino acid. This protocol was followed until the last amino acid was coupled and N-terminal deprotected. The completeness of the coupling was monitored by a negative Kaiser test. The resin was then dried and transferred to a 20-mL borosilicate scintillation vial. The peptide was cleaved from the resin and other side chain protecting groups removed using a cleavage cocktail (10 mL per gram of resin). The cleavage cocktail was made of 95% TFA, 2.5% triisopropylsilane (TIS) and 2.5% water. The cleavage was allowed to proceed for 2 h before the resin was filtered through a cotton plugged glass pipette. The resin was then washed with an additional TFA (2-3 mL). The TFA-peptide solution was then transferred to a propylethylene conical centrifuge tube and the solvent gently evaporated to about 3 mL using argon. The peptide was then precipitated by addition of ether (10 mL). The precipitate was isolated by centrifugation. The organic layer was then

decanted and the precipitate washed two more times with ether. The product was then dried in air to give 50-90% crude peptide. In case of peptide **JMN6** the peptide was synthesized and cleaved from the solid support using the same protocol. The crude product was then dissolved in a H_2O/MeOH system and 2.0 eq. of LiOH added. The mixture was stirred for 30 min and MeOH evaporated. The crude product was then obtained by lyophilization. Low yields were obtained during the synthesis of **JMN5** while all the other peptides were obtained in reasonable yields.

The crude peptides were purified using a Vydac C18 semi-preparative column. The crude peptides were dissolved in a mixture of aqueous 0.1% TFA, MeOH and acetonitrile, with the percentage of MeOH and acetonitrile not exceeding 20%. The solution was then filtered through a 0.45 micron cellulose acetate filter (Aerodisc). The loading used was 10 mg crude product per injection and the gradient depended on peptide. The purified fractions were combined and the acetonitrile removed by rotary evaporation. The pure peptide was then obtained by lyophilization.

R_f Values for the CCK/opioid chimeric peptides

Peptide	A[a]	B	C
JMN1	0.75	0.84	0.57
JMN2	0.67	0.75	0.43
JMN3	0.72	0.76	0.51
JMN4	0.73	0.80	0.57
JMN5	0.73	0.78	0.55
JMN6	0.52	0.76	0.40

[a]R_f values on thin layer chromatograms of silica gel in the following solvents: (A) 1-butanol/water/acetic acid (4:1:1), (B) chloroform/methanol/water (4:4:1), (C) methanol/ethylacetate/hexanes/water (6:4:4:4:1)

Retention times for the CCK/opioid chimeric peptides

Peptide	HPLC Retention Time[a]	k'
JMN1	22.9	5.5
JMN2	21.7	5.4
JMN3	22.0	5.3
JMN4	23.6	6.0
JMN5	23.7	6.1
JMN6	17.0	5.7

[a]HPLC retention time; k' = [(peptide retention time – solvent retention time)/solvent retention time] in a solvent of 10% ACN in 0.1 TFA and a gradient of 10-90% ACN over 40 min. An analytical Vydac C18 column was used with a flow rate of 1 mL/min

High resolution mass spectrometry for the CCK/opioid chimeric peptides

		HR-MS	
Peptide	molecular formula	Calculated	Observed
JMN 1	$C_{49}H_{61}N_9O_{10}S$	968.4340	968.4348
JMN2	$C_{49}H_{61}N_9O_{10}S$	968.4340	968.4348
JMN 3	$C_{49}H_{61}N_9O_{10}S$	968.4340	968.4348
JMN 4	$C_{53}H_{61}N_9O_{10}S$	1016.4340	1016.4348
JMN5	$C_{53}H_{61}N_9O_{10}S$	1016.4340	1016.4348
JMN6	$C_{47}H_{57}N_9O_{10}$	908.4278	908.4286