Supporting Information

A Bivalent Ligand (KDN-21) Reveals Spinal δ- and κ-Opioid Receptors are Organized as Heterodimers that give rise to δ_1 and κ_2 Phenotypes. Selective Targeting of δ-κ Heterodimers.

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Experimental

All reactions involving moisture sensitive reagents were conducted in oven-dried glassware under nitrogen atmosphere. Solvents were dried when necessary. All other chemicals and solvents were reagent grade unless specified otherwise and were obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Naltrexone was obtained from Mallinckrodt & Co. ^{1}H NMR spectra were recorded on a Varian 300 MHz spectrometer and referenced to the solvent. Chemical shifts are expressed in ppm and coupling constants (J) are in hertz (Hz). Peak multiplicities are abbreviated: broad, br; singlet, s; doublet, d; triplet, t; quartet, q; pentet, p; and multiplet, m. Fast-atom bombardment (FAB) mass spectra (MS) were obtained on a VG 7070E-HF instrument. Flash column chromatography was performed on Merck Science silica gel 60 (230-400) mesh. Thin layer chromatography (TLC) was performed on analytical Uniplate silica gel GF glass plates (250 mm by 2.5 x 20 cm^2).

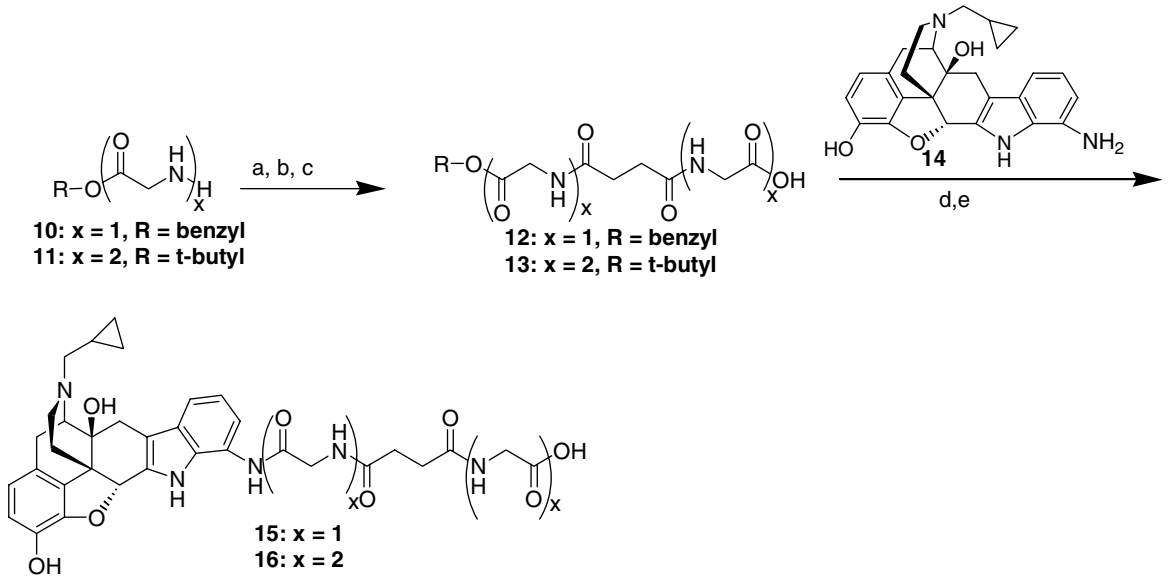
Preparative TLC was performed on 1.0 or 0.5 mm Analtech silica gel plates. Plates were visualized by UV light, iodine vapor or ninhydrin solution.

Chemistry

(a) Synthesis of bivalent ligands:

The key intermediates **15** and **16** were prepared as outlined in Scheme 1. Reacting either glycine-benzyl ester (**10**) or glycylglycine-*t*-butyl ester (**11**) with succinic anhydride and then coupling this intermediate with glycine-*t*-butyl ester or glycylglycine benzyl ester followed by deprotection of either the benzyl or the *t*-butyl group afforded the mono-protected oligoglycyl spacers (**12, 13**). These intermediates were then coupled with 7'-NH_2-NTI (**14**)[1] followed by removal of the benzyl or *t*-butyl ester group to give the key intermediates, **15** and **16.**

Scheme 1

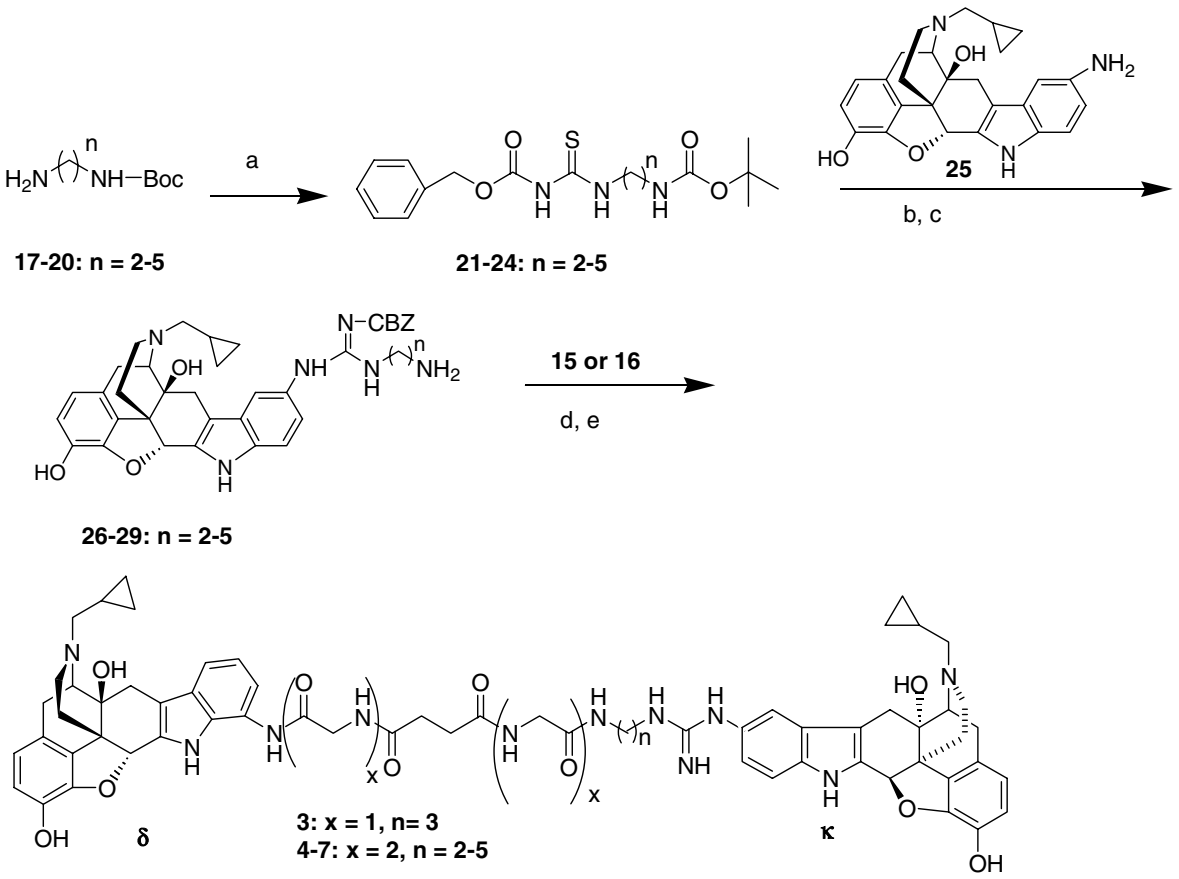


Reagents and Conditions : (a) Succinic anhydride, CH_2Cl_2, rt, 4h, (b) gly-t-butyl ester or gly-gly benzyl ester, DCC, HOBt, rt, 24h, (c) Pd/C, H_2, 70 psi or 1 M HCl in EtOAc, (d) DCC, HOBt, 24h, (e) 1M HCl in EtOAc, 24h, or Pd/C, H_2, 80 psi.

For the synthesis of the GNTI-alkyldiamine (**26-29**) portion of the ligand, mono-Boc-protected diamines[2] were reacted with benzylisothiocyanate[3] followed by coupling with 5'-

NH_2-NTI[1] and deprotection of the *N*-boc group. The final bivalent ligands were obtained by coupling of **15** or **16** with compounds **26-29** followed by deprotection of the carbobenzyloxy group to give bivalent ligands **3-7**.

Scheme 2



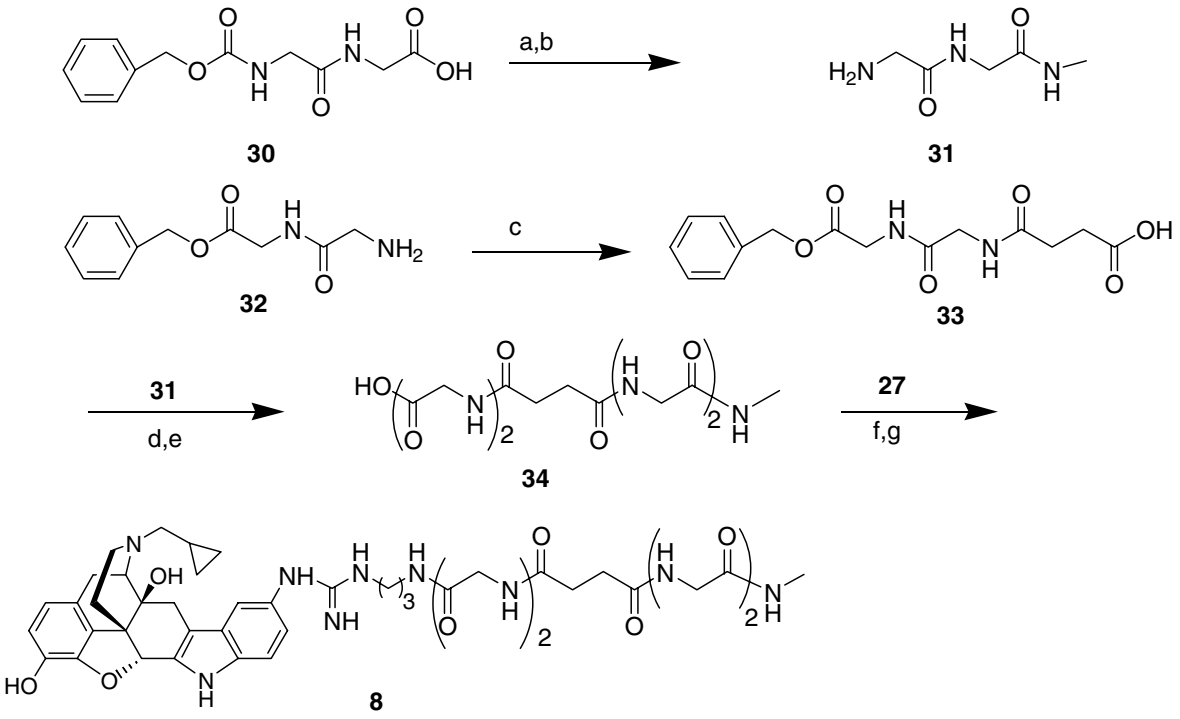
Reagents and Conditions : (a) Cbz-Cl, KSCN, 0-40°C, 30% (b) $HgCl_2$, Et_3N, rt, 24 h, 90 % (c) 1M HCl in EtOAc, 24h, 90%, (d) DCC, HOBt, 24 h, rt, 40%, (e) Pd/C, 85 psi, 24 h, 80%

(b) Synthesis of κ-monovalent:

The spacer for the κ-monovalent was prepared as outlined in Scheme 3. Cbz-glycylglycine was coupled with methylamine followed by catalytic hydrogenation to give **31**. Compound **31** was then coupled with the acid **33** (which was obtained by reacting glycly-glycyl benzyl ester with succinic anhydride) followed by catalytic hydrogenation to give spacer **34**.

Monovalent ligand **8** was obtained by coupling of compound **27** with the *N*-methyl capped spacer (**34**) followed by deprotection of the carbobenzyloxy group.

Scheme 3

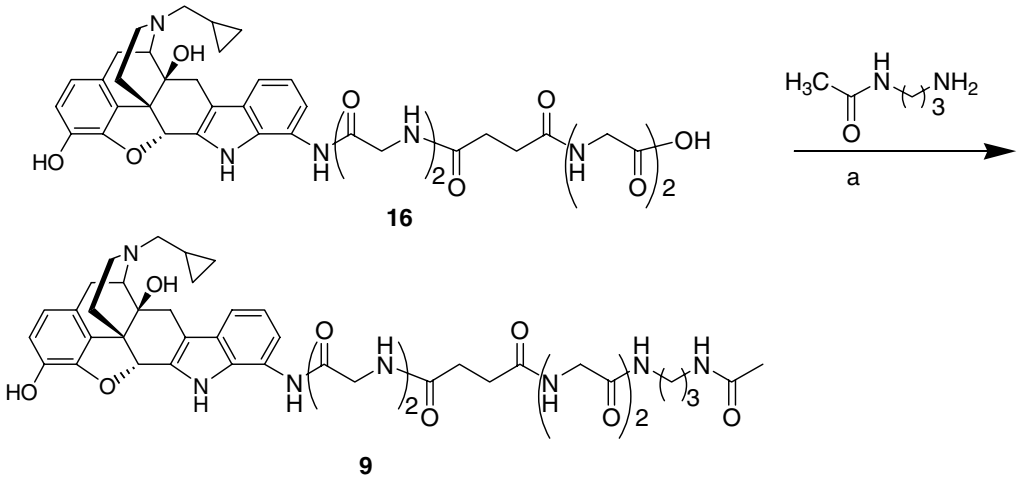


Reagents and Conditions : (a) EDCI, $MeNH_2$,Et_3N, 24h, 56 % (b) 10 % Pd/C, MeOH, 70 psi, 6h, 93%, (c) succinic anhydride, Et_3N, 24h, 84 %, (d) DCC, HOBt, 48 h, 88 %, (e) 10 % Pd/C, MeOH, 70 psi, 6h, 85 % (f) DCC, HOBt, 24 h, rt, 40%, (g) Pd/C, 85 psi, 24 h, 80%

(c) Synthesis of δ-monovalent ligands:

Monovalent **9** was obtained by coupling of compound **16** with *N*-(3-amino-propyl)-acetamide.

Scheme 4



Reagents and Conditions : (a) DCC, HOBt, 24h, 60%

Carbobenzyloxy-glycyl-succinyl-glycyine (12): Triethylamine (4.12 mL, 29.63 mmol) was added to a suspension of glycine-benzyl ester *p*-toluene sulfonate salt (10.0 g, 29.6 mmol) in dry CH_2Cl_2 (100 mL) and stirred at rt for 10 min. Succinic anhydride (2.97 g, 29.63 mmol) was added and the mixture was stirred for 3 h at rt under N_2. The reaction mixture was then concentrated and dissolved in anhydrous DMF (10 mL). To this solution DCC (4.8 g, 23.29 mmol) and HOBt (3.15 g, 23.29 mmol) were added and stirred at room temperature under nitrogen for 30 min. Glycine-*t*-butyl ester (3.9 g, 23.29 mmol) and triethylamine (3.2 mL) were added to the above reaction mixture and stirred at room temperature under nitrogen for 48 h. The reaction mixture was then filtered and the filtrate was concentrated under reduced pressure and purified by flash chromatography ($CHCl_3$: MeOH; 30:1) to give the bis-protected spacer (8.3 g, 66 %). Removal of the *t*-butyl ester group with 1 M HCl in EtOAc (3 eq) gave the spacer **12** in 88 % yield. ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H), 8.19 (s, 1H), 7.34 (m, 5H), 5.09 (s, 2H), 4.03 (m, 2H), 3.69 (m, 2H), 2.34 (m, 4H).

C-*tert*-butoxy-glycylglycyl-succinyl-glycylglycyine (13): To a cooled solution of glycylglycine-*t*-butyl ester (6.00 g, 31.895 mmol) in dry CH_2Cl_2 (75 mL) a solution of succinic anhydride (3.51 g, 35.084 mmol) in dry CH_2Cl_2 (70 mL) was added and stirred at room temperature under nitrogen for 4 h. The precipitated product (7.68 g, 26.6 mmol), was filtered, dissolved in anhydrous DMF (25 mL), and DCC (6.875 g, 33.33 mmol), HOBt (4.50 g, 33.33 mmol) were added and stirred at room temperature under nitrogen for 30 min. A solution of glycylglycine benzyl ester *p*-toluene sulfonate salt (10.517 g, 26.66 mmol) and triethylamine (5 mL) in anhydrous DMF (5mL) were then added to the above reaction mixture and stirred at room temperature under nitrogen for 48 h. The reaction mixture was t filtered and the filtrate was concentrated under reduced pressure and purified by flash chromatography ($CHCl_3$: MeOH; 5:1) to give the bis-protected spacer (8.84 g, 60 %). Catalytic hydrogenation with 10 % Pd/C for 24 followed by filtration over celite and removal of solvent gave the spacer **13** (6.04 g, 88 %). ^{1}H NMR (DMSO-d_6) δ 12.56 (b, 1H, OH), 8.22-8.06 (m, 4H, NH), 3.69-3.61 (m, 8H), 2.37 (s, 4H), 1.37 (s, 9H). HR-MS (FAB) m/z: 403.18 $(M + H)^+$ $C_{16}H_{26}N_4O_8$ requires 402.17.

Compound (15): To a solution of **12** (0.75g, 2.33 mmol) in anhydrous DMF (2 mL), DCC (0.53 g, 2.56 mmol) and HOBt (0.35 g, 2.56 mmol) were added and stirred at room temperature under nitrogen for 10 min. A solution of 7'-NH_2-NTI[1] (1.0 g, 2.33 mmol) in anhydrous DMF (1 mL) was added to the above reaction mixture and stirred at room temperature under nitrogen for 48 h. The reaction mixture was then filtered concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2: CH_3OH: NH_4OH;

94.5:5:0.5 to 89:10:1) to give the protected intermediate (0.62 g, 36 %) which was subjected to catalytic hydrogenation with 10% Pd/C at 85 psi for 48 h to give compound **15** (315 mg, 60 %). ^{1}H NMR (DMSO-d_6) δ 10.84 (s, 1H), 9.69 (s, 1H), 8.14 (m, 1H), 8.03 (m, 1H), 7.28 (d, 1H, *J* = 6.0 Hz), 6.90 (d. 1H, *J* = 6.0 Hz), 6.68 (t, 1H, *J* = 7.8 Hz), 6.25 (m, 2H) , 5.36 (s, 1H), 3.79 (m, 2H), 3.44 (m, 2H), 3.15 (m, 2H), 2.96 (m, 4H), 2.68 (m, 4H), 2.25 (m, 4H), 1.43 (d, 1H, *J* = 12 Hz), 0.71 (m, 1H), 0.34 (m, 2H), 0.15(m, 2H).

Compound (16): To a solution of **13** (2.0 g, 4.97 mmol) in anhydrous DMF (5 mL), DCC (1.86 g, 9.041 mmol) and HOBt (1.22 g, 9.04 mmol) were added and stirred at room temperature under nitrogen for 30 min. A solution of 7'-NH_2-NTI (1.94 g, 4.52 mmol) in anhydrous DMF (2 mL) was then added to the above reaction mixture and stirred at room temperature under nitrogen for 48 h. The reaction mixture was filtered, concentrated under reduced pressure, and purified by flash chromatography (CH_2Cl_2: CH_3OH: NH_4OH; 94.5:5:0.5 to 89:10:1) to give the protected intermediate (1.99 g, 54 %) which was then reacted with 1M HCl in EtOAc to give compound **16** (1.3 g, 70 %). ^{1}H NMR (DMSO-d_6) δ 11.03 (s, 1H), 9.78 (s, 1H), 8.40 (t, 1H, *J* = 5.7 Hz), 8.31-8.21 (m, 2H), 8.02 (t, 1H, *J* = 5.7 Hz), 7.50 (d, 1H, *J* = 7.5 Hz), 7.15 (d, 1H, *J* = 8.1 Hz), 6.92 (t, 1H, *J* = 7.8 Hz), 6.54 (dd, 2H, *J* = 8.1 and 6.0 Hz), 5.58 (s, 1H), 4.07 (m, 3H), 3.89-3.67 (m, 8H), 3.39 (m, 3H), 3.07 (br, s, 1H), 2.75-2.70 (m, 2H), 2.44 (m, 4H), 2.22-2.18 (m, 1H), 1.63 (d, 1H, *J* = 11.4 Hz), 0.919 (m, 1H), 0.55-0.46 (m, 2H), 0.19-0.18 (m, 2H). HR-MS (FAB) m/z: 758.31$(M + H)^+$ $C_{38}H_{43}N_7O_{10}$ requires 757.36.

General Procedure for the thioureas (21-24): CBZ-Cl (1 eq) was added to a suspension of potassium thiocyanate (1 eq) in dry acetone (40 mL) at 0°C. The reaction mixture was stirred in an ice-bath for 15 min and then heated at 40°C for 1h. The *N*-boc protected diamine (17-20, 1 eq) in 10 mL acetone was added to the above reaction and the temperature was maintained below 10°C by cooling. The reaction was then refluxed for 2h, cooled and stirred with ice-cold water for 15 min and then extracted with dichloromethane. The organic layer was dried over sodium sulfate, concentrated and purified by flash chromatography ($CHCl_3$) to give the thiourea (21-24) in ~30 % yield.

Compound 21: ^{1}H NMR (DMSO-d_6) δ 11.11 (s, 1H), 9.85 (s, 1H), 7.37-7.32 (m, 5H), 6.95 (s, 1H), 5.14 (s, 2H), 3.60 (m, 2H), 3.13 (m, 2H), 1.33 (s, 9H). HR-MS (FAB) m/z: 376.13 $(M + Na)^+$ $C_{16}H_{23}N_3O_4S$ requires 353.14.

Compound 22: ^{1}H NMR (DMSO-d_6) δ 11.04 (s, 1H), 9.84 (s, 1H), 7.38-7.35 (m, 5H), 6.85 (s, 1H), 5.14 (s, 2H), 3.55 (m, 2H), 2.91 (m, 2H), 1.65 (m, 2H), 1.34 (s, 9H). HR-MS (FAB) m/z: 390.145 $(M + Na)^+$ $C_{17}H_{25}N_3O_4S$ requires 367.156.

Compound 23: ^{1}H NMR ($CDCl_3$) δ 9.65 (s, 1H), 8.06 (s, 1H), 7.43-7.28 (m, 5H), 5.17 (s, 2H), 4.60 (s, 1H), 3.67 (m, 2H), 3.15(m, 2H), 1.75 (m, 2H), 1.5 (m, 2H), 1.4 (s, 9H). HR-MS (FAB) m/z: 382.1804 $(M + H)^+$ $C_{18}H_{27}N_3O_4S$ requires 381.1722

Compound 24: ^{1}H NMR (DMSO-d_6) δ 11.02 (s, 1H), 9.80 (s, 1H), 7.39-7.28 (m, 5H), 6.74 (s, 1H), 5.15 (s, 2H), 3.51 (m, 2H), 2.88-2.83 (m, 2H), 1.55 (m, 2H), 1.38 (m, 11H), 1.27 (m, 2H). HR-MS (FAB) m/z: 395.187 $(M + Na)^+$ $C_{19}H_{29}N_3O_4S$ requires 418.177.

General Procedure for intermediates 26-29: A mixture of 5'-NH_2-NTI[1] **25** (1 eq), thiourea (**21-24**, 1.3 eq), mercuric chloride (1.2 eq) and triethylamine (1 mL) in anhydrous DMF (5 mL) was stirred at room temperature under nitrogen atmosphere for 24 h. The reaction mixture was then filtered, concentrated under reduced pressure and purified by flash chromatography followed by removal of the *N*-boc group with 1 M HCl in EtOAc (3 eq) to give compounds **26-29** in ~90% yield.

Compound 26: ^{1}H NMR (DMSO-d_6) δ 11.60 (s, 2H), 11.15 (s, 1H), 10.8 (s, br, 1H), 9.4 (s, br, 1H), 9.0 (s, br, 1H), 8.4 (s, 3H), 7.35-7.29 (m, 7H), 7.02 (d, 1H, J = 7.5 Hz), 6.66 (d, 1H, J = 8.4 Hz), 6.56 (m, 2H), 5.69 (s, 1H), 5.12 (s, 2H), 4.18 (s, 1H), 3.16 (m, 1H), 3.08-3.03 (m, 8H), 2.47-2.43 (m, 4H), 2.15 (m, 2H), 1.79 (m, 1H), 1.05 (m, 1H), 0.69 (m, 2H), 0.47 (m, 2H).

Compound 27: ^{1}H NMR (DMSO-d_6) δ 11.58 (s, 1H), 11.08 (s, 1H), 10.72 (s, br, 1H), 9.49 (s, br, 1H), 9.30 (s, 1H), 8.98 (s, br, 1H), 8.17 (br, s, 3H), 7.34-7.22 (m, 7H), 6.97 (d, 1H, J = 7.5 Hz), 6.64-6.53 (m, 3H), 5.68 (s, 1H), 5.11 (s, 2H), 4.17 (s, 1H), 3.53 (m, 1H), 3.13-2.96 (m, 7H), 2.64 (m, 2H), 2.46 (m, 4H), 1.92 (m, 1H), 1.79 (m, 2H), 1.1 (m, 2H), 0.68 (m, 2H), 0.47 (m, 2H). HR-MS (FAB) m/z: 663.32 $(M + H)^+$ $C_{38}H_{42}N_6O_5$ requires 662.32.

Compound 28: ^{1}H NMR (DMSO-d_6) δ 11.61(s, 1H), 11.09 (s, 2H), 10.75 (s, br, 1H), 9.46 (s, br, 1H), 9.02 (s, br, 1H), 8.17 (br, s, 3H), 7.39-7.23 (m, 7H), 6.96 (d, 1H, J = 8.1 Hz), 6.66 (d, 1H), 6.59 (d, 1H), 5.71 (s, 1H), 5.24 (s, 2H), 4.20 (s, 1H), 3.46-3.29 (m, 6H), 3.09-2.90 (m, 4H), 2.75-2.60 (m, 4H), 1.80 (d, 1H), 1.65 (m, 2H), 1.49 (m, 2H), 1.10 (m, 1H), 0.71 (m, 2H), 0.50 (m, 2H). HR-MS (FAB) m/z: 677.3422 $(M + H)^+$ $C_{39}H_{44}N_6O_5$ requires 676.3372.

Compound 29: ^{1}H NMR (DMSO-d_6) δ 11.58 (s, 1H), 11.08 (s, 1H), 10.72 (s, br, 1H), 9.45 (s, br, 1H), 9.31 (s, 1H), 9.00 (s, br, 1H), 8.08 (br, s, 3H), 7.40-7.21 (m, 7H), 6.97 (d, 1H, J = 7.5 Hz), 6.66-6.56 (m, 3H), 5.71 (s, 1H), 5.12 (s, 2H), 4.20 (s, 1H), 3.53 (m, 1H), 3.13-2.96 (m, 7H), 2.64 (m, 2H), 2.46 (m, 4H), 1.92 (m, 1H), 1.79 (m, 2H), 1.39 (m, 4H), 1.02 (m, 2H), 0.66 (m, 2H), 0.50 (m, 2H). HR-MS (FAB) m/z: 691.1 $(M + H)^+$ $C_{40}H_{46}N_6O_5$ requires 690.805

General Procedure for Bivalent Ligands (3-7): A mixture of compounds **15** or **16** (1 eq), DCC (1 eq) and HOBt (1 eq) in anhydrous DMF (1-2 mL) was stirred at room temperature under nitrogen atmosphere for 10 min. The GNTI-alkyldiamine (**17-20**, 1 eq) dissolved in ~1 mL of anhydrous DMF was then added to the above reaction and the mixture was stirred at rt under nitrogen atmosphere for 24 h. The reaction mixture was filtered, concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2/ CH_3OH/ NH_4OH; 89:10:1 to 78:20:2) to give a solid (30 - 40 %). Catalytic hydrogenation with MeOH as a solvent and 10% Pd/C at 85 psi for 48h followed by filtration over celite and removal of solvent gave the final bivalent compounds (**3-7**) in 80-85 % yield.

KDN-15 (3): ^{1}H NMR (DMSO-d_6) δ 11.13 (s, 1H), 10.57 (s, 1H), 9.54 (br, 1H), 8.80 (s, 2H), 8.15 (br, 1H), 7.97 (br, 1H), 7.67 (br, 1H), 7.27 (m, 2H), 7.17 (m, 5H), 7.09 (m, 1H), 7.01(m, 1H), 6.76 (m, 1H), 5.40 (s, 1H), 5.36 (s, 1H), 4.84 (s, 2H), 4.60 (s, 2H), 3.85 (d, 2H), 3.43 (d, 2H), 3.12 (m, 2H), 3.01 (m, 3H), 2.87 (m, 2H), 2.57-2.52 (m, 3H), 2.35 (m, 6H), 2.24 (m, 6H), 1.98 (m, 2H), 1.45 (d, 2H, J = 10.5 Hz), 1.35 (m, 2H), 0.74 (m, 2H), 0.35 (m, 4H), 0.00 (m, 4H). HR-MS (FAB) m/z: 1154.55 $(M + H)^+$ $C_{64}H_{71}N_{11}O_{10}$ requires 1153.53.

KDN-20 (4): ^{1}H NMR (DMSO-d_6) δ 7.34 (m, 1H), 7.01 (m, 2H), 6.98 (m, 2H), 6.33 (m, 6H), 5.40 (s, 1H), 5.31 (s, 1H), 4.57 (s, br, 2H), 3.85 (m, 2H), 3.61 (m, 2H), 3.52 (m, 2H), 3.02 (m, 4H), 2.94 (m, 1H), 2.88 (m, 1H), 2.61-2.56 (m, 6H), 2.35 (m, 8H), 2.30 (m, 4H), 2.25 (m, 2H), 2.00 (m, 2H), 1.44 (m, 2H), 1.19 (m, 2H), 0.75 (m, 2H), 0.37 (m, 4H), 0.015 (m, 4H). LR-MS (MALDI) m/z: 1254.9 $(M + H)^+$ $C_{67}H_{75}N_{13}O_{12}$ requires 1254.3.

KDN-21 (5): ^{1}H NMR (DMSO-d_6) δ11.22 (s, 1H), 10.65 (s, br, 1H), 9.53 (s, 1H), 8.81 (s, br, 2H), 8.18 (s, 1H), 8.10-8.01 (m, 2H), 7.44 (s, br, 1H), 7.30 (m, 1H), 7.20 (m, 1H), 7.08 (m, 1H), 6.98 (m, 1H), 6.77 (m, 2H), 6.32 (m, 4H), 5.39 (s, 1H), 5.36 (s, 1H), 4.42 (s, br, 2H), 3.87 (m, 4H), 3.60 (m, 2H), 3.49 (m, 4H), 3.26 (m, 4H), 3.01-2.88 (m, 6H), 2.33 (m, 8H), 2.27 (m, 6H), 1.99 (m, 2H), 1.41 (m, 2H), 1.07 (m, 2H), 0.71 (m, 2H), 0.35 (m, 4H), 0.00 (m, 4H). LR-MS (MALDI) m/z: 1268.59 $(M + H)^+$ $C_{68}H_{77}N_{13}O_{12}$ requires 1267.58. Anal. ($C_{68}H_{77}N_{13}O_{12}$.5TFA.2H_2O) Calculated: C: 49.98; H: 4.62; N: 9.71. Found: C: 49.98; H: 4.43; N: 9.43.

KDN-22 (6): ^{1}H NMR (DMSO-d_6) δ11.32 (s, 1H), 10.68 (s, br, 1H), 9.57 (s, 1H), 8.87 (s, br, 2H), 8.16 (s, 1H), 8.10-8.06 (m, 2H), 7.93 (s, br, 1H), 7.57 (m, 1H), 7.51 (m, 1H), 7.03 (m, 1H), 6.93 (m, 1H), 6.80 (m, 2H), 6.32 (m, 4H), 5.37 (s, 1H), 5.32 (s, 1H), 3.81 (m, 2H), 3.69 (m, 1H), 3.56 (m, 2H), 3.43 (m, 6H), 3.27-3.07 (m, 8H), 2.88 (m, 2H), 2.71 (m, 1H), 2.61-2.44 (m, 4H), 2.31-2.03 (m, 8H), 1.43 (m, 2H), 1.27 (m, 4H), 0.71 (m, 2H), 0.38 (m, 4H), 0.04 (m, 4H). LR-MS (MALDI) m/z: 1282.4 $(M + H)^+$ $C_{69}H_{79}N_{13}O_{12}$ requires 1281.597

KDN-23 (7): ^{1}H NMR (DMSO-d_6) δ 7.51 (s, br, 1H), 7.32-7.30 (d, 1H, J = 7.5 Hz), 7.1 (m, 1H), 7.01 (d, 1H, J = 7.8 Hz), 1H), 6.78 (t, 2H, J = 7.8 Hz), 6.54 (s, br, 1H), 6.38-6.30 (m, 4H), 5.40 (s, 1H), 5.33 (s, 1H), 4.58 (s, br, 2H), 3.86 (m, 2H), 3.62 (m, 2H), 3.53-3.49 (m, 4H), 3.26 (m, 4H), 3.02 (m, 2H), 2.94 (m, 4H), 2.39 (m, 2H), 2.35 (m, 8H), 2.30 (m, 2H), 2.25 (m, 2H), 2.17 (m, 2H), 2.04 (m, 2H), 1.45 (m, 4H), 1.31 (m, 2H), 1.12 (m, 2H), 0.75 (m, 2H), 0.37 (m, 4H), 0.016 (m, 4H). LR-MS (MALDI) m/z: 1296.9 $(M + H)^+$ $C_{70}H_{81}N_{13}O_{12}$ requires 1296.4.

Compound 31: Cbz-glycyl-glycine (5.32 g, 20 mmol) was dissolved in 200 mL of dry THF and EDCI.HCl (4.08 g, 22 mmol), triethylamine (3.5 mL) and HOBt (2.85 g, 21 mmol) were added and stirred for 5 min. The reaction mixture was then cooled to 0^{o}C and methylamine (2 M solution in THF, 20 mL) was added and stirred overnight under N_2. The mixture was concentrated and the residue was dissolved in cold water. The insoluble product was filtered off, followed by washing with hexanes to give the *cbz*-protected intermediate (3.12 g, 56 %). Catalytic hydrogenation in MeOH (10% Pd/C at 70 psi)for 6h

followed by filtration over celite and removal of solvent gave **31** (1.4 g, 93 %). ^{1}H NMR (DMSO-d_6) δ 8.10 (br, 1H), 7.74 (br, 1H), 3.64 (s, 2H), 3.09 (s, 2H), 2.55 (d, 3H), 2.00 (br, 2H). HR-MS (FAB) m/z: 146.0938 $(M+H)^+$ $C_5H_{11}N_3O_2$ requires 145.0851.

Compound 33: Triethylamine (4.2 mL, 30.4 mmol) was added to a suspension of glycylglycine-benzyl ester *p*-toluene sulfonate salt (12.0 g, 30.4 mmol) in dry CH_2Cl_2 (250 mL) and stirred at rt for 10 min. Succinic anhydride (3.05 g, 30.5 mmol) was then added and the reaction was stirred overnight at rt under N_2. The precipitate that formed during this time was filtered and dried under vacuum to give **33** (8.31 g, 84 %). ^{1}H NMR (DMSO-d_6) δ 12.04 (s, 1H), 8.24 (t, 1H), 8.16 (t, 1H), 7.37-7.26 (m, 5H), 5.09 (s, 2H), 3.87 (d, 2H), 3.70 (d, 2H), 2.38(m, 4H). HR-MS (CI) m/z: 340.1509 $(M+NH_4)^+$ $C_{15}H_{18}N_2O_6$ requires 322.1164.

Compound 34: A mixture of **33** (3.22 g, 10 mmol), DCC (4.16 g, 20 mmol) and HOBt (2.7 g, 20 mmol) in anhydrous DMF (10 mL) was stirred at room temperature under nitrogen for 10 min. Compound **31** (1.45 g, 10 mmol) dissolved in 5 mL of anhydrous DMF was then added to the above reaction and the mixture was stirred at rt under nitrogen atmosphere for 24 h. The reaction mixture was then filtered, concentrated under reduced pressure, and purified by flash chromatography (CH_2Cl_2/ CH_3OH/ NH_4OH; 78:20:2) to give the benzyl protected intermediate in 88 % yield. Catalytic hydrogenation of 3.68 g (8.23 mmol) of this intermediate in MeOH: H_2O (2:1, 150 mL) with 10 % Pd/C at 70 psi for 24 h followed by filtration over celite and removal of solvent gave compound **34** (2.51g,

85 %). ^{1}H NMR (DMSO-d_6) δ 8.29-8.22 (m, 2H), 8.12 (m, 2H), 7.61 (q, 1H), 3.70-3.65 (m, 6H), 3.61 (d, 2H), 2.55(d, 3H), 2.38 (m, 4H).

KN-21 (8): A mixture of compound **34** (0.211 g, 0.588 mmol), DCC (0.121 g, 0.588 mmol) and HOBt (0.079 g, 0.588 mmol) in anhydrous DMSO (1 mL) was stirred at rt under nitrogen atmosphere for 10 min. Compound **27** (0.3 g, 0.452 mmol) dissolved in 1 mL of anhydrous DMSO, and triethylamine (0.02 mL) was added to the above reaction mixture which was stirred at rt under nitrogen atmosphere for 24 h. The mixture was filtered, concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2/ CH_3OH/ NH_4OH; 78:20:2) to give a solid in 40 % yield. Catalytic hydrogenation of 150 mg of this intermediate in 25 mL MeOH with 10 % Pd/C at 85 psi for 48 h followed by filtration over celite and removal of solvent gave compound **8** in 80 % yield. ^{1}H NMR (DMSO-d_6) δ 11.53 (s, 1H), 9.4, (s, 1H), 9.23 (s, 1H), 8.92 (s, 1H), 8.31 (m, 2H), 8.11 (m, 2H), 7.67 (m, 2H), 7.40 (m, 2H), 6.95 (m, 1H), 6.57 (m, 2H), 6.36 (m, 1H), 5.69 (s, 1H), 4.04 (m, 1H), 3.87-3.60 (m, 8H), 3.46-3.39 (m, 2H), 3.24-3.07 (m, 6H), 2.95-2.90 (m, 2H), 2.55 (m, 4H), 2.38 (d, 3H), 1.83-1.79 (m, 2H), 1.72-1.70 (m, 2H), 1.49 (m, 2H), 1.01 (m, 1H), 0.70 (m, 2H), 0.45 (m, 2H). HR-MS (ESI) m/z: 870.4252 $(M + H)^+$ $C_{43}H_{55}N_{11}O_9$ requires 869.418. Anal. ($C_{43}H_{55}N_{11}O_9$. 4TFA. $4H_2O$) Calculated: C: 43.81; H: 4.83; N: 11.02. Found: C: 43.78; H: 4.65; N: 11.22.

DN-21 (9): A mixture of compound **16** (0.2 g, 0.264 mmol), DCC (0.065 g, 0.264 mmol) and HOBt (0.035 g, 0.264 mmol) in anhydrous DMF (1 mL) was stirred at rt under nitrogen for 10 min. *N*-(3-Amino-propyl)-acetamide (0.03 g, 0.2864mmol) dissolved in 1 mL of

anhydrous DMF, and triethylamine (0.02 mL) was added to the above reaction and the reaction was stirred at rt under nitrogen atmosphere for 24 h. The reaction mixture was filtered, concentrated under reduced pressure and purified by flash chromatography (CH_2Cl_2/ CH_3OH/ NH_4OH; 89:10:1 to 78:20:2) to give a solid (**9**, 60 % mg). ^1H NMR (DMSO-d_6) δ 10.93 (s, 1H), 9.62 (s, 1H), 8.9 (m, 1H), 8.33-8.24 (m, 2H), 8.12 (s, 1H), 7.79 (m, 1H), 7.65 (m, 1H), 7.44 (m, 1H), 7.14 (m, 1H), 6.94 (m, 1H), 6.62 (m, 2H), 5.71 (s, 1H), 4.05-3.99 (m, 2H), 3.75-3.61 (m, 6H), 3.45-3.28 (m, 3H), 3.10 (m, 4H), 2.9 (m, 4H), 2.54 (m, 2H), 2.42 (m, 4H), 1.83 (m, 1H), 1.77 (s, 3H), 1.48 (m, 2H), 1.06 (m, 1H), 0.71 (m, 2H), 0.45 (m, 2H). HR-MS (ESI) m/z: 877.3796 $(M + Na)^+$ $C_{43}H_{53}N_9O_{10}$ requires 855.3915. Anal. ($C_{43}H_{53}N_9O_{10.}$ 2TFA. $2H_2O$) Calculated: C: 50.4; H: 5.31; N: 11.26. Found: C: 50.65; H: 5.34; N: 11.13.

Expression and co-expression of the DOR and KOR in HEK293 cells: cDNAs encoding the rat KOR and mouse DOR were inserted separately into the mammalian expression vector pcDNA3 and tagged with FLAG or c-Myc epitope respectively. HEK293 cells were cultured at 37°C in DMEM medium supplemented with 10% fetal bovine serum and P/S antibiotics. The cells, grown to about 50% confluence, were transfected with the expression vectors containing DOR or KOR cDNA using Calcium Phosphate Transfection Kit. For co-expression, the cells were first transfected with the pcDNA3 vector of KOR and then with the vector of DOR. An equal amount of pcDNA3 vector was co-transfected with each receptor construct to keep the total DNA used equivalent. Geneticin and hygromycin were used to obtain stable KOR and DOR cells.

Radioligand Binding Assays: HEK293 cells of each 100 mm plate, expressing single or combination of DOR and KOR, were suspended in 2.5 mL of 25 mM of HEPES Buffer (pH 7.4). Saturation binding was performed on whole cells using [^{3}H]diprenorphine (50 Ci/mmol) to determine *B*max and *Kd* of the receptors. Each concentration was examined in duplicate. The IC_{50} values for tested compounds were determined by competition binding in which whole cells were incubated at 25°C for 2 hours with [3H]diprenorphine, and 10 different concentrations (10^{-15}-10^{-6} M) of each ligand, in a final reaction volume of 500 µl. The concentration of the radioligand employed in the competition assay was approximately equivalent to its *Kd*. Nonspecific binding was determined as in the presence of 10 µM of naloxone. The samples were filtered and washed 3 times through GF/C filters (Whatman) presoaked in 0.25% PEI using a Brandel 48-well harvester. After filtration, the filters were incubated in 4 mL of Econo-Safe cocktail and counted in a LS3801 Beckman counter. The experiments were repeated three times in duplicate for each assay. IC50 values were determined from displacement curves using Kaleidgraph 3.1, and the Ki values were calculated according to the Cheng-Prussoff equation.[4]

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