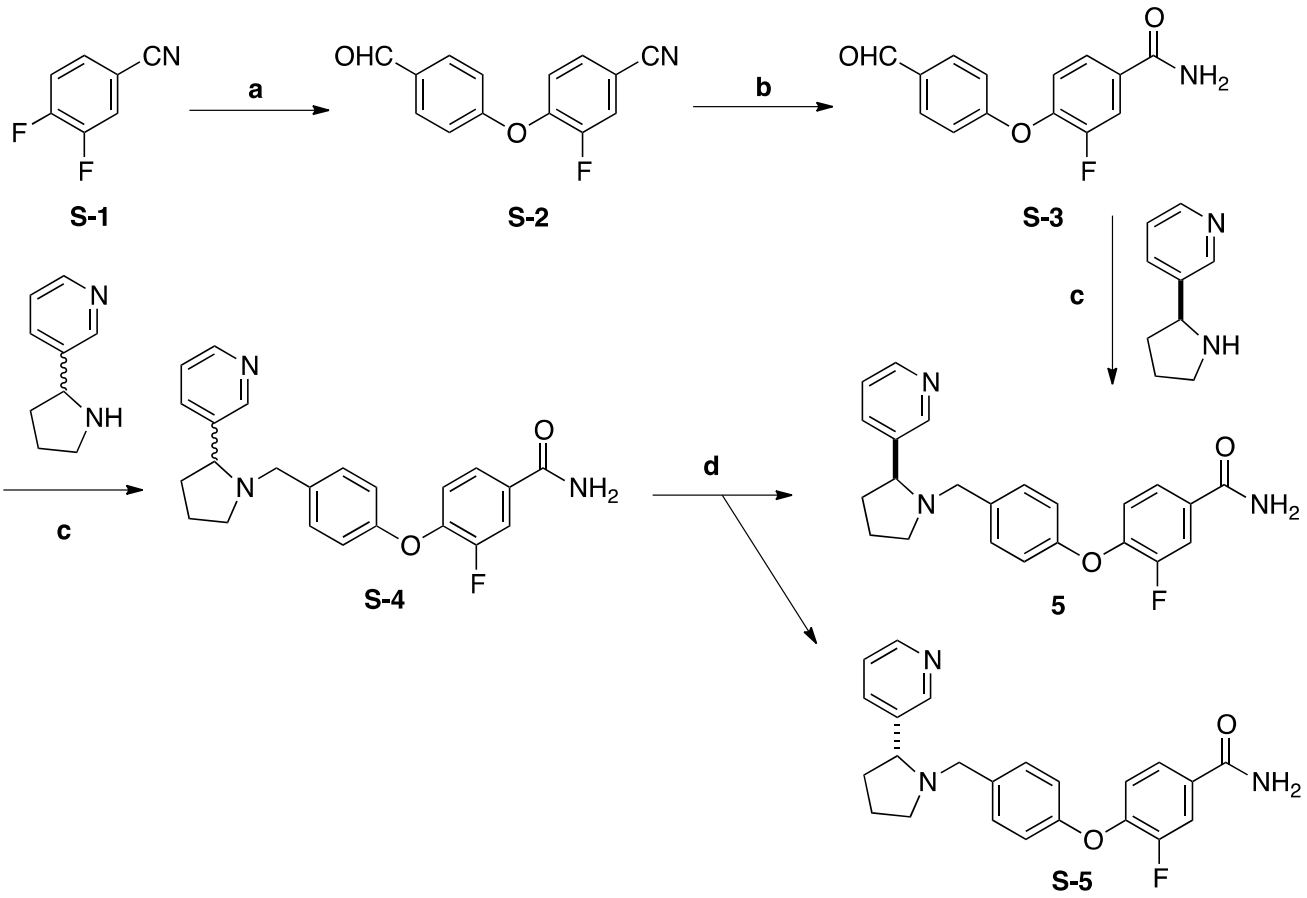
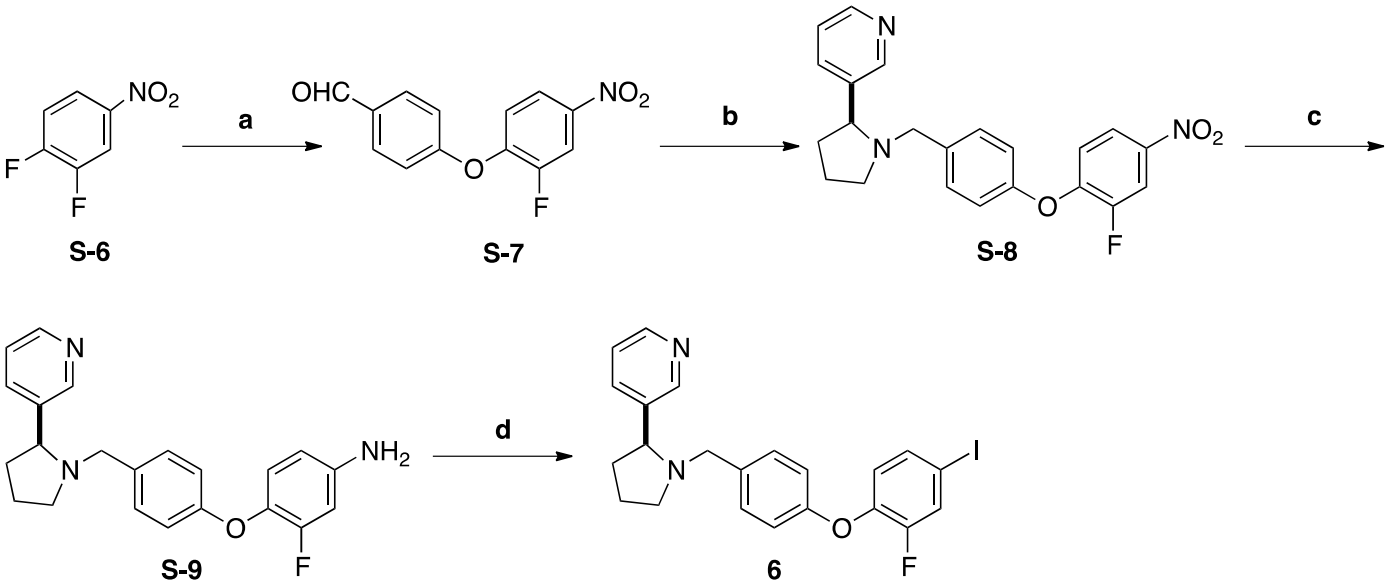
Supplemental Materials

Supplemental Figure 1. Synthesis of LY2459989



Reagents and conditions: **a**. 4-hydroxybenzaldehyde, K_2CO_3, DMF, reflux; **b**. H_2O_2, K_2CO_3, DMSO, rt; **c**. $NaBH(OAc)_3$, HOAc, $ClCH_2CH_2Cl$, rt; **d**. chiral HPLC.

Supplemental Figure 2. Synthesis of the radiolabeling precursor for ^{11}C-LY2459989



Reagents and conditions: **a**. 4-hydroxybenzaldehyde, K_2CO_3, DMF, rt; **b**. (-)-nornicotine, $NaBH(OAc)_3$, $ClCH_2CH_2Cl$, rt; **c**. H_2, Pd/C, rt; **d**. 1. $NaNO_2$, H_2SO_4, 0 °C; 2. KI, 2 N NaOH, 40-45 °C.

Materials and Methods: Synthesis

All reagents and anhydrous solvents were obtained from commercial sources and used without further purification unless noted otherwise.

^{1}H NMR spectra were recorded on a Varian 400 MHz or on an AVANCE III 400 MHz UltraShield-PI spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CDCl_3$: 7.26 ppm; DMSO-d_6: 2.49 ppm). HPLC-MS was performed with an 1100 or 1200 Series LC-MSD single quadrupole instrument (Agilent; Santa Clara, CA) with an ESI interface. The samples were analyzed by the following described methods.

HPLC Method 1: Phenomenex Gemini C18 column (2.0 x 50 mm, 3.0 μm) eluting with 5 to 100% MeCN in water (both containing 0.1% formic acid), 7.0 min gradient with a 1 min hold at a flow rate of 1.0 mL/min. API-ES (Atmospheric Pressure Ionization-Electrospray) mass spectra data was acquired with an Agilent Technologies MSD single quadrupole coupled to an HP1100 LC system.

HPLC Method 2: Waters Sunfire C18 column (4.6 x 30 mm, 2.5 μm; Waters, Milford, MA) eluting at 1.5 mL/min with a gradient of A (0.025% TFA in water) and B (0.025% TFA in MeCN), with B increased linearly from 10% to 95% (v/v) over 3 min and then hold for 2 min. Ions between m/z 100-750 were captured after electrospray ionization of the eluted test sample.

Chiral HPLC Method C1: Chiralpak AS-H column (4.6 x 150 mm) eluting with 0.2% *N*,*N*-dimethylethylamine in MeOH at a flow rate of 1.0 mL/min; UV detector at λ = 225 nm.

Chiral HPLC Method C2: Chiralpak AD (4.6 mm x 250 mm, 10 μm) eluting with 0.2% *N*,*N*-dimethylethylamine in EtOH at a flow rate of 0.75 mL/min; UV detector at λ= 254 and 325 nM.

*3-Fluoro-4-(4-formylphenoxy)benzonitrile (**S-2**).* Potassium carbonate (55.3 g, 400 mmol) was added to a solution of 4-hydroxybenzaldehyde (24.4 g, 200 mmol), 3,4-difluorobenzonitrile (**S-1**) (27.8

g, 200 mmol) in dimethylacetamide (475 mL). The reaction mixture was heated at 100 °C for 2 h, cooled to room temperature and poured into ice water (1.4 L). The resulting tan solid was collected by filtration, washed with water (2 x 150 mL), and dried in a vacuum oven to afford **S-2** in 72% yield. ^{1}H NMR (400 MHz, DMSO-d_6): 9.92 (s, 1H), 7.98-7.89 (m, 2H), 7.76 (dd, *J* = 1.8, 10.3 Hz, 1H), 7.65-7.56 (m, 1H), 7.34 (d, *J* = 8.1 Hz, 1H), 7.22-7.12 (m, 2H).

*3-Fluoro-4-(4-formylphenoxy)benzamide (**S-3**).* Potassium carbonate (13.3 g, 96 mmol) was added to a solution of **S-2** (46.3 g, 192 mmol) in DMSO (170 mL). The mixture was cooled to 10 °C and 30% aq. H_2O_2 (21.5 mL, 211 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature, stirred for 2 h, and poured into ice water. The tan precipitate was filtered, washed with water (3 x 100 mL), and dried in a vacuum oven at 50°C to afford **S-3** in 100% yield. HPLC = 100% @ 3.19 min by HPLC Method 1, mass spectrum (m/z): 260 (M+1). ^{1}H NMR (400 MHz, DMSO-d_6): 9.94 (s, 1H), 8.11 (bs, 1H), 7.98-7.88 (m, 3H), 7.74-7.78 (m, 1H), 7.58 (bs, 1H), 7.41 (t, *J* = 8.2 Hz, 1H), 7.22-7.15 (m, 2H).

*3-Fluoro-4-[4-[[2-(3-pyridyl)pyrrolidin-1-yl]methyl]phenoxy]benzamide (**S-4**).* To the solution of (±)-nornicotine (0.50 g, 3.4 mmol), **S-3** (0.73 g, 2.8 mmol) in 1, 2-dichloroethane (15 mL) was added powdered 3 Å molecular sieves. The mixture was stirred at room temperature overnight whereupon $NaBH(OAc)_3$ (1.48 g, 7.0 mmol) and AcOH (0.40 mL, 7.0 mmol) were added in portions. After stirring at room temperature for 3 h, the reaction mixture was filtered through a layer of celite. The filtrate was quenched with saturated $NaHCO_3$ solution (20 mL) and extracted with EtOAc (3 x 25 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated on a rotary evaporator. The resulting crude product was purified by flash chromatography on silica gel eluting with 1 to 25% of 10% aq. NH_4OH in EtOH in CH_2Cl_2:hexanes (1:1) to afford compound **S-4** (0.47 g, 43% yield). HPLC = 99% @ 3.098 min, mass spectrum (m/z): 392 (M+1) by HPLC-MS Method 1. Chiral HPLC = 49% @ 3.01 min, 51% @ 3.69 min by Chiral HPLC Method C1. ^{1}H NMR (400 MHz,

$CDCl_3$): 8.62 (d, *J* = 1.5 Hz, 1H), 8.49 (dd, *J* = 1.1, 4.8 Hz, 1H), 7.78 (m, 1H), 7.67 (dd, *J* = 2.2, 10.9 Hz, 1H), 7.50 (m, 1H), 7.30-7.21 (m, 3H), 6.98-6.90 (m, 3H), 6.20-5.64 (bs, 2H), 3.74 (d, *J* = 12.4 Hz, 1H), 3.42 (t, *J* = 7.7 Hz, 1H), 3.20-3.09 (m, 2H), 2.33-2.17 (m, 2H), 2.00-1.72 (m, 3H).

*3-Fluoro-4-[4-[[(2S)-2-(3-pyridyl)pyrrolidin-1-yl]methyl]phenoxy]benzamide (**5**) and 3-Fluoro-4-[4-[[(2R)-2-(3-pyridyl)pyrrolidin-1-yl]methyl]phenoxy]benzamide (**S-5**).* Enantiomers of compound **S-4** were separated by chiral chromatography utilizing a Chiralpak AD (20 mm x 250 mm, 10 μm) eluting with 0.2% DMEA in ethanol at 8 mL/min monitoring at λ= 254/325 nM to afford **5**, chiral HPLC = 98% @ 4.90 min, and **S-5**, chiral HPLC = 98% @ 6.52 min by chiral HPLC method C2.

*3-Fluoro-4-[4-[[(2S)-2-(3-pyridyl)pyrrolidin-1-yl]methyl]phenoxy]benzamide (**5**).* Compound **5** was prepared in 46% yield in a procedure same as that for **S-4** but using 3-[(2S)-pyrrolidin-2-yl]pyridine ((-)-nornicotine). HPLC = 100% @ 1.02 min by HPLC Method 1, mass spectrum (m/z): 392.2 (M+1). Chiral HPLC = 99% @ 2.988 minutes by chiral HPLC Method C1. The retention time of this compound matched that of the first eluting peak from enantiomers previously separated by preparative chiral HPLC. ^{1}H NMR (400 MHz, DMSO-d_6): 8.55 (d, *J* = 1.9 Hz, 1H), 8.44-8.42 (m, 1H), 8.01-8.00 (m, 1H), 7.84-7.78 (m, 2H), 7.70-7.67 (m, 1H), 7.47-7.45 (m, 1H), 7.35-7.31 (m, 1H), 7.25-7.22 (m, 2H), 7.03 (t, *J* = 8.4 Hz, 1H), 6.96-6.93 (m, 2H), 3.60-3.55 (m, 1H), 3.46-3.43 (m, 1H), 3.17-3.13 (m, 1H), 3.02-2.99 (m, 1H), 2.25-2.21 (m, 2H), 1.87-1.85 (m, 3H).

*4-(2-Fluoro-4-nitrophenoxy)benzaldehyde (**S-7**).* To a solution of 4-hydroxybenzaldehyde (5.0 g, 40.94 mmol) and **S-6** (6.51 g, 40.94 mmol) in DMF (50 mL) was added K_2CO_3 (8.49 g, 61.41 mmol). The mixture was stirred at room temperature for 20 h and water (500 mL) was added. The precipitate was filtered, washed with water and dried in air to afford **S-7** as a white solid (10.3 g, 96% yield). Purity = 100% @ 2.77 min by HPLC-MS (HPLC Method 2), mass spectrum (m/z): 262.1 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$): 10.00 (s, 1H), 8.17 (dd, J_1 = 2.8 Hz, J_2 = 9.6 Hz, 1H), 8.11 (m, 1H), 7.97 (t,

J = 2.0 Hz, 1H), 7.95 (t, *J* = 2.0 Hz, 1H), 7.26 (d, *J* = 16 Hz, 1H), 7.24 (t, *J* = 8.8 Hz, 1H), 7.18 (d, *J* = 8.8 Hz, 1H).

*(S)-3-(1-(4-(2-Fluoro-4-nitrophenoxy)benzyl)pyrrolidin-2-yl)pyridine (**S-8**).* To a solution of **S-7** (9.3 g, 35.6 mmol) and (-)-nornicotine (5.8 g, 39.2 mmol) in dichloroethane (200 mL) was added $NaBH(OAc)_3$ (11.32 g, 53.4 mmol) in portions and the resulting mixture was stirred at room temperature for 4 h. Saturated aq. $NaHCO_3$ was added and the mixture extracted with CH_2Cl_2 (3 x 200 mL). The combined organic phases were washed with brine (2 x 200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting crude product was purified by flash chromatography on silica gel eluting with 15 to 35% EtOAc in hexanes to afford **S-8** (12.0 g, 87% yield) as a yellowish oil. Purity = 98.6% @ 1.70 min by HPLC-MS (HPLC Method 2), mass spectrum (m/z): 394.2 (M+1). ^{1}H NMR (400 MHz, $CDCl_3$): 8.65 (d, *J* = 1.6 Hz, 1H), 8.53 (dd, J_1 = 1.6 Hz, J_2 = 4.8 Hz, 1H), 8.10 (dd, J_1 = 2.8 Hz, J_2 = 10.0 Hz, 1H), 7.98 (dm, *J* = 8.8 Hz, 1H), 7.80 (br d, *J* = 6.4 Hz, 1H), 7.30 (m, 3H), 7.02 (d, *J* = 8.4 Hz, 2H), 6.93 (t, *J* = 8.4 Hz, 1H), 3.78 (d, *J* = 12.8 Hz, 1H), 3.47 (t, *J* = 8.0 Hz, 1H), 3.20 (m, 2H), 2.28 (m, 2H), 1.92-1.77 (m, 3H).

*(S)-3-Fluoro-4-(4-((2-(pyridine-3-yl)pyrrolidin-1-yl)methyl)phenoxy)benzenamine (**S-9**).* Compound **S-8** (12.0 g , 30.5 mmol) was dissolved in THF (150 mL) under nitrogen in an autoclave. Palladium on carbon (1.3 g, 1.22 mmol) was added, the nitrogen was replaced with hydrogen, and the reaction was carried out at 320 psi hydrogen atmosphere for 20 h. The gas was removed by bubbling nitrogen into the reaction mixture, which was then filtered through a layer of Celite and the filtrate concentrated *in vacuo* to give compound **S-9** as a brown solid (11.0 g, 99% yield), which was used directly in the next steps of reaction without further purification. Purity = 98.6% @ 1.14 min by HPLC-MS (HPLC Method 2), mass spectrum (m/z): 364.2 (M+1).

*(S)-3-(1-(4-(2-fluoro-4-iodophenoxy)benzyl)pyrrolidin-2-yl)pyridine (**6**):* Concentrated H_2SO_4 (10 mL) was added dropwise to water (40 mL) in a round bottom flask cooled with ice water. Compound **S-9** (11.0 g, 30.3 mmol) was added and the mixture stirred at 4 to 15 °C for 30 min to form a suspension. To this suspension cooled with ice water was then added dropwise a solution of $NaNO_2$ (3.3 g, 47.8 mmol) in water (100 mL). The resulting mixture was stirred at 0 °C for 40 min to form the diazonium salt. The suspension was then added dropwise over 1.5 h to a solution of KI (21.8 g, 131.3 mmol) in water (100 mL) and maintained at 40-45 °C. After complete addition the reaction mixture was stirred at 45 °C for 3 h, cooled to room temperature, adjusted to pH 10 with 2 N NaOH, and extracted with EtOAC. The organic phase was dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was purified by flash column chromatography on silica gel eluting with 25 to 35% EtOAc in hexanes to give **6** as a yellow oil. Further purification on preparative HPLC afforded 4.5 g of the product (31% yield). Purity = 100% @ 1.93 min by HPLC-MS (HPLC Method 2), mass spectrum (m/z): 476.1 (M+1). ^{1}H NMR (400 MHz; $CDCl_3$): 8.65 (s, 1H), 8.52 (d, J = 4.0 Hz, 1H), 7.79 (d, J = 7.2 Hz, 1H), 7.51 (d, J = 9.2 Hz, 1H), 7.40 (d, J = 8.4 Hz, 1H), 7.28 (t, J = 6 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 6.75 (t, J = 8.8 Hz, 1H), 3.75 (d, J = 12.8 Hz, 1H), 3.44 (t, J = 8.0 Hz, 1H), 3.13 (m, 2H), 2.29-2.19 (m, 2H), 1.82-1.64 (m, 3H). ^{19}F NMR (400 MHz; $CDCl_3$): 128.1.

Chemistry

The synthesis of the racemic compound **S-4** and its resolution into the two enantiomers **5** and **S-5** are described in Supplemental Figure 1. 3,4-Difluorobenzonitrile (**S-1**) was reacted with 4-hydroxybenzaldehyde to afford compound **S-2** in 72% yield. Partial hydrolysis of the cyano group in **S-2** then led to formation of the amide **S-3** in quantitative yield. Reductive amination of **S-3** with (±)-nornicotine gave the racemic compound **S-4** in 47% yield, which underwent chiral preparative HPLC separation into its two respective enantiomers **5** and **S-5**. Alternatively, compound **5** was also prepared

in 46% yield from reaction of **S-3** with (-)-nornicotine. Chiral HPLC analysis indicated an enantiomeric purity of >98% for both **5** and **S-5**.

Preparation of the radiolabeling precursor **6** is depicted in Supplemental Figure 2. Briefly, coupling of 1,2-difluoro-4-nitrobenzene (**S-6**) with 4-hydroxybenzaldehyde afforded **S-7** in 96% yield. Reductive amination with (-)-nornicotine then gave rise to compound **S-8** in 87% yield. Reduction of the nitro group in **S-9** with palladium-carbon, followed by diazonium salt formation and Sandmeyer reaction with potassium iodide, provided compound **6** in 31%. Chiral HPLC analysis indicated that compound **6** was obtained in >99% chemical purity, and >99% enantiomeric excess.

In vitro binding assays

Supplemental Table 1 lists the *in vitro* binding affinity, selectivity and functional activity of compound **5**, the racemic compound **S-4** and the (*R*)-enantiomer **S-5** for the opioid receptors. Note that the (*S*)-enantiomer **5** displays high affinity (K_i = 0.18 nM) for the KOR and over 42-fold selectivity for KOR over other opioid receptors. Further, it displays the property of a full antagonist at all three ORs. KOR binding affinities of the racemic compound **S-4** and the (*R*)-enantiomer (**S-5**) are lower, although their selectivity over MOR and DOR is largely retained. Compared with the previously reported ligand LY2795050 (*1*), ligand **5** displays 4 times higher KOR affinity and similar selectivity.

Supplemental Table 1. *In vitro* binding affinities, selectivity and agonist activities at cloned human opioid receptors[1]

Compound	Binding Affinity (K_i, nM)			Selectivity			Agonist activity (EC_{50}, nM)		
	κ	μ	δ	κ/μ	κ/δ	μ/δ	κ	μ	δ
5	0.18	7.68	91.3	42.7	507	11.9	>10,000	>10,000	>10,000
S-4	0.45	27.5	97.0	61.1	216	3.53	>10,000	>10,000	>10,000
S-5	9.02	>500	>500	>55.4	>55.4	-	>10,000	>10,000	>10,000
GR103545[2]	0.44	33.5	423	76.1	961	12.6	0.09	>10,000	34.7
LY2795050[2]	0.72	25.8	153	35.8	213	5.93	>10,000	>10,000	>10,000

[1]Data presented are the mean of three independent experiments. [2]Taken from reference (*1*)

1. Zheng MQ, Nabulsi N, Kim SJ, et al. Synthesis and evaluation of 11C-LY2795050 as a kappa-opioid receptor antagonist radiotracer for PET imaging. *J Nucl Med.* Mar 2013;54(3):455-463.