**Supporting Information for Manuscript "Naphthalene dicarboxaldehyde derivatives as opioid receptor reporter affinity labels with greatly improved fluorogenic properties.** McCurdy, C.R., et. al.

## Experimental

Naltrexone hydrochloride was obtained from Mallinkrodt and was used to synthesize β-naltrexamine as previously reported.[5] All other reagents used were obtained from Aldrich Chemical Company (Milwaukee, WI) unless otherwise noted. Reactions sensitive to air or moisture were performed under a nitrogen atmosphere, in oven-dried glassware. Dry tetrahydrofuran was obtained from a sodium metal still. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. $^{1}$H NMR spectra and $^{13}$C NMR spectra were taken on either a Varian Inova 300 MHz or Varian Unity 300 MHz instruments. Chemical shifts are expressed in ppm of the δ scale with TMS as an internal reference (δ=0.00).

All spectra were recorded at ambient temperature. Gravity and low-pressure column chromatrography were performed over silica gel (200-400 mesh, BET surface area 500 $m^2/g$, pore volume 0.75 $cm^3/g$, Adrich Chemical company) as the stationary phase under nitrogen. TLC was performed on Analtech silica gel GF plates. Infra-red (IR) spectra were recorded on a Perkin-Elmer PE-281 spectrophotometer on potassium bromide (KBr) discs. Low resolution (LRFABMS) and high resolution (HRFABMS) mass spectra were obtained on a VG-707EHF spectrometer using a m-nitro benzyl alcohol (MNOBA) matrix. High resolution CI mass spectra were obtained on a Finnigan MAT95 using a 5% ammonia in methane gas mixture.

### 6,7-Diformylnaphthalene-2-carboxylic acid (4).

3,4-diformyl benzoic acid **3** (2.0g, 0.0112 mol, 1 eq) was placed in a 25 mL round bottom flask. To this was added water (1.5 mL), glacial acetic acid (1 mL), 2,5-dimethoxytetrahydrofuran (1.48 g, 1.45 mL, 0.0112, 1 eq) and piperidine (3 drops). The mixture was the heated to reflux at which time everything was in a clear solution. Approximately, 3 hours into reflux an orange precipitate

began to form. The mixture continued to reflux for 20 hours. The precipitate was collected by filtration. The filtrated was then washed twice with water and 1 N HCl and then once with ether. It was left to dry on the vacuum for 30 minutes to yield 1.6 g of product (65%). Silica gel TLC $R_f$ 0.7 (7:3, dichloromethane: methanol). $^1$H NMR (DMSO-$d_6$): δ 8.20 (dd, 1H, H4); 8.23 (d, 1H, H3); 8.65 (s, 1H, H1); 8.79 (s, 1H, H5); 8.86 (s, 1H, H8); 10.49 (s, 1H, CHO); 10.51 (s, 1H, CHO); 13.51 (s, 1H, COOH). HRCI $[M+H]^+$ for $C_{13}H_8O_4$ Calcd. 229.0423 Found 229.0484 $[M+NH_4]^+$ 246.0764.

**6-carboxymethyl-1,3-dimethoxybenzylphthalan (5).**

6,7-diformylnaphthalene-2-carboxylic acid (0.90g, 0.004 mol, 1 eq) was dissolved in 125 mL of methanol and to this was added a catayltic amount of para-toluenesulfonic acid. The solution was refluxed overnight and water was removed from the reaction with the use of a dean-stark trap. The solution was cooled and concentrated under reduced pressure to afford an amber oil. The oil was dissolved in ethyl acetate and washed with water. The water layer was washed with ethyl acetate (2 x 50mL). The organic layers were combined and washed with water, aqueous 5% potassium carbonate and brine. The organic layer was dried over sodium sulfate and concentrated to an amber oil. This was purified over silica gel chromatography using 9:1, hexanes:ethyl acetate, to yield 0.35 g of a yellowish oil (30%). Silica gel TLC $R_f$ 0.6 (6:4, hexanes:ethyl acetate). $^1$H NMR (DMSO-$d_6$): δ 3.37 (s, 3H, $OCH_3$); 3.44 (s, 3H, $OCH_3$); 3.93 (s, 3H, $COOCH_3$); 6.22 (s, 1H, CHO); 6.47 (s, 1H, CHO); 8.03 (dd, 1H, H4); 8.07 (s, 1H, H1); 8.15 (d, 1H, H3); 8.20 (s, 1H, H8); 8.75 (s, 1H, H1); 10.49 (s, 1H, CHO); 10.51 (s, 1H, CHO); 13.51 (s, 1H, COOH).

**1,3-dimethoxybenzylphthalan-6-carboxylic acid (6).**

Compound **5** (0.6 g, 0.0021 mol, 1 eq) was dissolved in a 1:1 mixture of tetrahydrofuran and water (30 mL:30 mL). To this was added lithium hydroxide (0.06 g, 0.0025 mol, 1.2 eq). This yellow solution stirred at room temperature under nitrogen for 1 hour. The solvent was evaporated and the remaining water was washed with ether. Then the pH was adjusted very cautiously and

quickly to pH = 2 with 1 N HCl. The aqueous layer was quickly extracted with ethyl acetate. The organic layer was washed with brine and dried over sodium sulfate. The solvent was removed under reduced pressure to yield 0.56 g (99%) of a yellow powder. $^1$H NMR (DMSO-$d_6$): δ 3.37 (s, 3H, $OCH_3$); 3.43 (s, 3H, $OCH_3$); 6.21 (s, 1H, CHO); 6.47 (s, 1H, CHO); 8.15 (m, 3H, H4, H1, H3); 8.22 (s, 1H, H8); 8.71 (s, 1H, H1); 13.23 (bs, 1H, COOH exchangable in $D_2O$). HRCI $[M+H]^+$ for $C_{15}H_{14}O_5$ Calcd. 275.0841 Found $[M+Na]^+$ 297.0713 (weak signal at 275.1)

**β-1',3'-dimethoxybenzylphthalan naltrexamine (8).**

Compound **6** (1.5 mmol, 1.1 eq), 1-hydroxybenzotriazole (1.5 mmol, 1.1 eq), and β-naltrexamine (**7**, 0.45 g, 1.3 mmol, 1 eq) were dissolved in anhydrous DMF. The solution was cooled to 0° C at which time dicyclohexylcarbodiimide (1.6 mmol, 1.2 eq) was added. The solution was sealed under a nitrogen atmosphere and stirred at room temperature overnight (16 hrs). TLC indicated the formation of two products (n-acylated and both n- and o-acylated). The reaction mixture was filtered (to remove DCU) into water (10x initial volume of DMF) and extracted with ethyl acetate (4 times). The organic layers were combined, dried over sodium sulfate, filtered and concentrated under reduced pressure to a yellowish-orange oil. This was taken up in methanol and potassium carbonate (6.5 mmol, 5 eq) was added and the suspension was stirred for 1 hour at room temperature. The mixture was then concentrated under reduced pressure and the residue was dissolved in water and extracted with ethyl acetate, dried over sodium sulfate, filtered and concentrated to yield 0.49 g of desired product (63%). $^1$H NMR (DMSO-$d_6$): δ 0.13 (m, 2H); 0.48 (m, 2H); 0.86 (m, 1H); 1.32 (m, 2H); 1.50 (m, 1H); 1.63 (m, 1H); 1.93 (m, 2H); 2.20 (m, 1H); 2.35 (m, 2H); 2.59 (m, 2H); 3.01 (m, 2H); 3.41 (s, 3H, OCH3); 3.48 (s, 3H, OCH3); 3.74 (m, 1H); 4.76 (d, 1H, H-5, *j*= 7.5 Hz); 4.93 (bs, 1H, O<u>H</u>-14); 6.24 (m, 1H); 6.48 (m, 1H); 6.55 (d, 1H, H-1, *J*= 8.1 Hz); 6.61 (d, 1H, H-2, *J*= 8.1 Hz); 8.14 (m, 4H, Ar); 8.57 (s, 1H, Ar); 8.87 (d, 1H, amide, *J*= 8.4 Hz); 9.06 (bs, 1H, O<u>H</u>-3, exch D2O). ). FABHRMS $[M+H]^+$ for $C_{35}H_{38}N_2O_7$: Calcd. 599.2679 Found 599.2782.

**β-Naphthalene-6',7'-dicarboxaldehyde naltrexamine (2). [CM-II-183]**

Compound **8** (0.1 g, 0.16 mmol, 1eq) was dissolved in 10 mL of dry acetone (0.3% water). This produced a light yellow solution. To this was added 0.8 mL of 1N HCl dropwise. Initially, a white precipitate formed but went into solution. The solution turned a golden deep yellow and was sealed under nitrogen and stirred for 5 days. The solution was then filtered into a beaker containing ether and the compound precipitated out and was filtered to give 0.05 g (55%) of a pale yellow powder. $^{1}$H NMR (DMSO-$d_6$): δ 0.06 (m, 2H); 0.23 (m, 2H); 0.66 (m, 1H); 1.05 (m, 2H); 1.27 (m, 1H); 1.39 (m, 1H); 1.55 (m, 1H); 2.02 (m, 1H); 2.10 (m, 4H); 2.64 (m, 2H); 2.73 (m, 1H); 3.35 (m, 1H); 3.47 (bs, 1H, exch D2O); 4.47 (d, 1H, H-5, *J*= 7.8 Hz); 5.81 (m, 1H); 6.27 (d, 1H, H-1, *J*= 8.1 Hz); 6.33 (d, 1H, H-2, *J*=8.1 Hz); 7.83 (d, 1H, *j*= 8.7 Hz); 7.96 (d, 1H, *J*= 8.7 Hz); 8.27 (d, 1H, *J*= 6.6 Hz); 8.38 (s, 1H); 8.50 (bs, 1H); 8.33 (bs, 1H); 8.960 (bs, 1H); 10.14 (s, 1H); 10.15 (s, 1H). FABHRMS $[M+H]^+$ for $C_{33}H_{32}N_2O_6$: Calcd. 553.2260 Found 553.2302. Anal. Calcd. For $C_{33}H_{32}N_2O_6 \cdot 1\ HCl \cdot 1.5\ H_2O$: C, 64.54; H, 5.67; N, 4.43. Found: C, 64.26; H, 6.05; N, 4.54.