Supporting Information

G-Protein Biased Opioid Agonists: 3-Hydroxy-*N*-Phenethyl-5-Phenylmorphans With Three-Carbon Chain Substituents at C9

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Experimental Section

Melting points were determined on a Mettler Toledo MP70 and are uncorrected. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were recorded on a Varian Gemini-400 spectrometer in $CDCl_3$ (unless otherwise noted) with the values given in ppm (TMS as internal standard) and J (Hz) assignments of ^{1}H resonance coupling. The analyses were performed on the free base, unless otherwise noted. Mass spectra (HRMS) were recorded on a VG 7070E spectrometer or a JEOL SX102a mass spectrometer. The optical rotation data were obtained on a PerkinElmer polarimeter model 341. Thin layer chromatography (TLC) analyses were carried out on Analtech silica gel GHLF 0.25 mm plates using various gradients of $CHCl_3$/MeOH containing 1% NH_4OH or gradients of EtOAc/*n*-hexane. Visualization was accomplished under UV light or by staining in an iodine chamber. Flash column chromatography was performed with Fluka silica gel 60 (mesh 220−400). Flash column chromatography was performed using RediSep Rf normal phase silica gel cartridges. Robertson Microlit Laboratories, Ledgewood, N.J., performed elemental analyses, and the results were within ±0.4% of the theoretical values.

(1*S*,5*S*)-5-(3-Methoxyphenyl)-2-azabicyclo[3.3.1]nonan-9-one (2). To a stirred solution of **(-)-1** (5.23 g, 20.2 mmol) in anhydrous acetonitrile (40 mL) was added K_2CO_3 (5.58 g, 40.4 mmol) and the reaction cooled to 0 °C. Cyanogen bromide (6.06 mL, 30.3 mmol, 5M in acetonitrile) was added dropwise over 30 min, and then stirred over 3 h, allowing the reaction to warm to room temperature. The reaction mixture was filtered and the filtrate concentrated under vacuum. The crude residue was dissolved in MeOH (5 mL) and 3N HCl (100 mL) and heated to reflux. After 16 h, the reaction was cooled to 0 °C, basified with saturated aq NH_4OH, extracted with $CHCl_3$ (3 x 50 mL), dried over Na_2SO_4 and concentrated under vacuum. Purification via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–15%) afforded **2** as a red oil (3.19 g, 13.0 mmol, 65%). ^{1}H-NMR (400 MHz; $CDCl_3$): δ7.26-7.24 (m, 1H), 6.84-6.76 (m, 3H), 3.78 (s, 3H), 3.54 (dq, *J* = 20.5, 5.9 Hz, 2H), 2.98 (dt, *J* = 13.7, 6.1 Hz, 1H), 2.51-2.48 (m, 2H), 2.45-2.42 (m, 2H), 2.30-2.21 (m, 3H), 2.10-2.01 (m, 1H), 1.80-1.73 (m, 1H). ^{13}C-NMR (101 MHz; $CDCl_3$): δ216.51, 159.13, 145.63, 128.84, 119.61, 113.65, 111.16, 61.71, 55.17, 53.54, 44.21, 41.90, 40.68, 35.70, 19.74.

(1*S*,5*S*)-5-(3-Methoxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-one (3). To a stirred solution of **2** (2.51 g, 8.57 mmol) in anhydrous acetonitrile (20 mL) was added K_2CO_3 (2.37 g, 17.14 mmol) and 2-phenylethyl bromide (1.75 mL, 12.86 mmol) and the reaction heated to reflux. After 18 h, the reaction was cooled, filtered through Celite and concentrated under vacuum. The residue was dissolved in H_2O and $CHCl_3$, the aq layer basified to pH 9 with saturated aq NH_4OH and the layers separated. The aq layer was extracted with $CHCl_3$ (3 x 25 mL), and the combined organic fractions dried over Na_2SO_4 and concentrated under vacuum. Purification via flash chromatography (EtOAc in hexanes, gradient 0–100%) afforded **3** as a clear oil (2.51 g, 7.19 mmol, 84%). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.30-7.18 (m, 6H), 6.81 (dd, *J* = 18.9, 10.0 Hz, 3H), 3.79 (s, 3H), 3.34 (s, 1H), 3.23 (dt, *J* = 11.5, 5.5 Hz, 1H), 2.90-2.73 (m, 5H), 2.45-2.34 (m, 4H), 2.18 (dd, *J* = 13.3, 3.6 Hz, 2H), 1.78-1.71 (m, 1H), 1.65 (t, *J* = 8.1 Hz, 1H). ^{13}C-NMR (101 MHz; $CDCl_3$): δ 214.36, 159.15, 146.05, 140.07, 128.84, 128.69, 128.35, 126.06, 119.69, 113.71, 111.18, 68.93, 58.89, 55.16, 52.84, 48.85, 40.58, 39.26, 34.46, 34.18, 18.95.

(1*S*,5*S*)-5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-one (4). To a cooled (-78 °C) solution of **3** (0.3 g, 0.86 mmol) in anhydrous CH_2Cl_2 (20 mL) was added BBr_3 (0.4 mL, 4.29 mmol) dropwise. The reaction was stirred at -78 °C for 15 min, then allowed to warm to room temperature over 2 h, at which time the reaction was quenched with MeOH, 1N HCl (10 mL) added and heated to reflux for 1 h. The reaction was cooled, basified with NH_4OH, extracted with $CHCl_3$ (3 x 25 mL), dried over Na_2SO_4, and concentrated. Purification via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–5%) afforded the product as a clear oil (270 mg, 94% yield). ^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.30-7.24 (m, 3H), 7.21-7.17 (m, 3H), 6.80 (d, *J* = 7.8 Hz, 1H), 6.70-6.68 (m, 2H), 5.06 (s, 1H), 3.35 (t, *J* = 3.1 Hz, 1H), 3.23 (dt, *J* = 11.8, 5.7 Hz, 1H), 2.91-2.72 (m, 5H), 2.47-2.32 (m, 4H), 2.22-2.14 (m, 2H), 1.79-1.71 (m, 1H), 1.65 (dtd, *J* = 10.7, 5.1, 2.0 Hz, 1H). ^{13}C-NMR (101 MHz; $CDCl_3$): δ 214.67, 155.15, 146.19, 139.99, 129.09, 128.69, 128.37, 126.09, 119.37, 114.54, 113.61, 68.82, 58.85, 52.78, 48.84, 40.57, 39.22, 34.41, 34.08, 18.98.

3-((1*S*,5*S*,*E*)-9-(Methoxymethylene)-2-phenethyl-2-azabicyclo[3.3.1]nonan-5-yl)phenol (7). A 25 mL flame-dried round-bottom flask was equipped with a magnetic stir bar and charged with

methoxymethyltriphenylphosphonium chloride (4.789 g, 13.97 mmol) and **4** (1.69g, 4.66 mmol) The flask was cooled to 0 °C in an ice/water bath and charged with 1M LiHMDS solution in THF (18.64 mL, 18.64 mmol) dropwise over 15 min. The color of the reaction changed from white to deep red over the course of the LiHMDS addition. The reaction was stirred for 1.5 h under argon and allowed to gradually warm to room temperature. The reaction mixture was cooled to 0 °C and quenched with MeOH (8 mL) and stirred for 10 min. The reaction was concentrated under vacuum and the residue taken up in H_2O (15 mL) and $CHCl_3$ (15 mL). The pH of the aqueous layer was adjusted to ~ 9 (litmus) with saturated aq NH_4OH. The aqueous phase was extracted with 9:1 $CHCl_3$/MeOH (3 x 15 mL) and the combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting residue was purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–5%) to afford methyl vinyl ether **7** (20:1 mixture of *E*/*Z* isomers) (1.26 g, 3.45 mmol, 74%) as tan foam. ^{1}H-NMR (400 MHz; $CDCl_3$ + MeOD): δ 7.22 (m, 5H), 7.11 (t, *J* = 7.9 Hz, 1H), 6.95 (d, *J* = 7.7 Hz, 1H), 6.86 (s, 1H), 6.64 (d, *J* = 7.8 Hz, 1H), 5.83 (s, 1H), 3.18 (s, 1H), 3.08 (s, 3H), 2.93-2.74 (m, 5H), 2.35 (dt, *J* = 13.6, 6.8 Hz, 1H), 2.13-2.05 (m, 3H), 2.05-1.96 (m, 1H), 1.91-1.88 (m, 1H), 1.76 (d, *J* = 13.8 Hz, 1H), 1.51-1.47 (m, 1H); ^{13}C NMR (101 MHz; $CDCl_3$ + MeOD): δ 155.2, 151.9, 140.4, 139.7, 128.7, 128.4, 127.9, 126.0, 120.3, 118.5, 114.3, 112.4, 60.5, 59.0, 58.6, 49.1, 41.1, 38.9, 37.7, 34.3, 31.2, 21.2; HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_{24}H_{30}NO_2$ 364.2277, found 364.2274.

Methyl (*E*)-3-((1*S*,5*R*,9*R*)-5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-yl)acrylate (11a). A 10 mL round-bottom flask was charged with **7** (0.500 g, 1.38 mmol) and dissolved in 3N aq HCl (2.1 mL) and THF (2.1 mL). The reaction was stirred for 16 h at room temperature before being cooled to 0 °C, basified with NH_4OH and concentrated. The residue and remaining aqueous phase was extracted with 9:1 $CHCl_3$/MeOH (3 x 10 mL), dried over $MgSO_4$, and concentrated under vacuum to afford the epimeric aldehydes as a teal foam. The mixture of aldehydes was used immediately in the subsequent step to avoid decomposition. In a separate 25 mL round-bottom flask, methyl diethylphosphonoacetate (1.26 mL, 6.89 mmol) was added slowly to a suspension of NaH (0.276 g, 6.89 mmol, 60% in oil) in anhydrous THF (3 mL) and stirred for 30 min. The mixture of aldehydes in THF (3 mL) was added and the reaction stirred for 16 h. The

reaction was then concentrated under vacuum, dissolved in $CHCl_3$ and H_2O, basified to pH 9 with NH_4OH and extracted with 9:1 $CHCl_3$/MeOH (3 x 15 mL), dried over $MgSO_4$, and concentrated under vacuum. The residue was purified via flash chromatography (EtOAc in hexanes, gradient 0–100%) to afford the enoate esters as 3.6:1 mixture of diastereomers favoring the (9*R*)-epimer (0.321 g, 0.79 mmol, 57%).

For (1*S*,5*R*,9*R*)-**11a** ^{1}H-NMR (400 MHz; $CDCl_3$ + MeOD): δ 7.27-7.09 (m, 7H), 6.79 (d, *J* = 8.0 Hz, 1H), 6.76 (s, 1H), 6.63 (d, *J* = 7.9 Hz, 1H), 5.72 (d, *J* = 15.9 Hz, 1H), 3.62 (s, 3H), 3.17-3.09 (m, 3H), 2.84 (d, *J* = 8.3 Hz, 1H), 2.80-2.69 (m, 4H), 2.42-2.25 (m, 1H), 2.06 (d, *J* = 12.7 Hz, 1H), 1.96-1.89 (m, 1H), 1.82 (t, *J* = 14.5 Hz, 2H), 1.69-1.67 (m, 1H), 1.54-1.50 (m, 1H); ^{13}C NMR (101 MHz; $CDCl_3$ + MeOD): δ 167.5, 156.2, 151.8, 150.7, 140.7, 129.1, 128.7, 128.2, 125.8, 121.4, 117.3, 113.0, 112.7, 57.7, 57.0, 51.3, 49.09, 48.97, 41.9, 38.5, 34.1, 30.3, 25.4, 23.0; HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_{26}H_{32}NO_3$ 406.2382 found 406.2384. For (1S,5R,9R)-**11** $[\alpha]^{20}_D$ = +13.3° (*c* 0.18, $CHCl_3$). HBr salt: mp: 200-202 °C; Anal. Calcd. For $C_{26}H_{32}BrNO_3 \bullet 0.3H_2O$: C, 63.49%; H, 6.68%; N, 2.85%. Found C, 63.74%; H, 6.57%; N, 2.53%.

3-((1*S*,5*R*,9*R*)-2-Phenethyl-9-propyl-2-azabicyclo[3.3.1]nonan-5-yl)phenol (12). A 10 mL round-bottom flask was charged with enol ether **7** (0.360 g, 0.99 mmol) and taken up in 3N aq HCl (1.5 mL) and THF (1.5 mL). The reaction was stirred for 16 h at room temperature before being cooled to 0 °C and made basic by addition of chilled saturated aq NH_4OH. The bulk of the THF was removed under vacuum and the aqueous residue extracted with 9:1 $CHCl_3$/MeOH (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum to afford the epimeric aldehydes as a blue oil. The mixtures of aldehydes were used immediately in the subsequent step. A flame-dried 10 mL round-bottom flask was charged with ethyltriphenylphosphonium iodide (1.24 g, 2.97 mmol) followed by a solution of the epimeric aldehydes from the first stage in THF (0.5 mL). The suspension was stirred for 5 min before adding LiHMDS (3.96 mL, 3.96 mmol, 1M solution in THF) dropwise over 15 min at 0 °C. The color of the reaction changed from amber to deep red over the course of the LiHMDS addition. The reaction was stirred for 1.5 h at 0 °C and then 16 h at room temperature before being quenched by the addition of MeOH (2 mL). The reaction was concentrated under vacuum and dissolved in H_2O and $CHCl_3$, then extracted with 9:1 $CHCl_3$:MeOH (3 x 10 mL). The

combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The crude material was purified via flash chromatography (EtOAc in hexanes, gradient 0–100%) to afford the intermediate olefinic material as a mixture of C9-epimers as well as *E/Z* isomers. This mixture of stereoisomers was dissolved in MeOH (10 mL) and transferred to a Parr shaker vessel, charged with Escat 103 (5% Pd/C, 0.050 g), and pressurized to 50 psi H_2 in a Parr shaker and shaken for 16 h at room temperature. The reaction mixture was filtered through Celite and concentrated under vacuum to afford a yellow oil. The residue was purified via flash chromatography (EtOAc in hexanes, gradient 0–100%) to afford (1*S*,5*R*,9*R*)-**12** as a teal foam (0.103g, 0.28 mmol, 28%). The HBr salt was obtained via crystallization from *i*-PrOH/Et_2O. The analyses were performed on the free base, unless otherwise noted. ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.09 (s, 1H), 7.23-7.17 (m, 4H), 7.15-7.09 (m, 1H), 7.03 (t, *J* = 7.9 Hz, 1H), 6.68 (d, *J* = 8.0 Hz, 1H), 6.65 (t, *J* = 1.8 Hz, 1H), 6.49 (dd, *J* = 7.9, 1.8 Hz, 1H), 2.97-2.94 (m, 2H), 2.84-2.82 (m, 1H), 2.74-2.62 (m, 4H), 2.20 (d, *J* = 14.6 Hz, 1H), 2.05 (q, *J* = 10.9 Hz, 1H), 1.86-1.76 (m, 3H), 1.75-1.64 (m, 1H), 1.59-1.52 (m, 2H), 1.51-1.42 (m, 1H), 1.41-1.30 (m, 1H), 1.14-1.04 (m, 1H), 0.96-0.86 (m, 1H), 0.61 (t, *J* = 7.3 Hz, 3H), 0.58-0.53 (m, 1H); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 157.47, 152.44, 141.24, 139.30, 129.24, 129.03, 128.38, 126.00, 116.32, 112.82, 112.49, 56.66, 53.01, 48.93, 45.28, 42.77, 33.87, 29.90, 28.87, 26.37, 23.47, 20.93, 14.72. HRMS-ESI (m/z): [M +H]+ calcd. for $C_{25}H_{34}NO$ 364.2640, found 364.2645. HBr salt: mp: 261-263 °C; Anal. Calcd. For $C_{25}H_{34}NOBr \bullet 0.2\ H_2O$: C, 67.56%; H, 7.71; N, 3.15%. Found C, 67.12%; H, 7.74%; N, 2.96%.

3-((1*S*,5*R*,9*R*)-9-((*E*)-3-Hydroxyprop-1-en-1-yl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-5-yl)phenol (13). To a cooled (0 °C) solution of **11** (0.1 g, 0.26 mmol) in anhydrous THF (3 mL) was added $LiAlH_4$ (0.77 mmol, 0.38 mL, 2.0M in THF) dropwise, producing a thick slurry, and the reaction was allowed to warm to room temperature over 4 h. The reaction was quenched with $Na_2SO_4 \bullet 10H_2O$, stirred for 15 min then filtered through Celite, and the filtrate concentrated under vacuum. The resulting residue was purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–10%) to give **13** as a foam (57 mg, 59%). The HBr salt was prepared from acetone and 48% HBr in H_2O. The analyses were performed on the free base, unless otherwise noted. ^{1}H NMR (400 MHz; $CDCl_3$) *δ* 7.25 (t, *J* = 7.3 Hz, 2H), 7.18 (t, *J* = 10.4 Hz, 3H), 7.10 (t, *J* =

7.8 Hz, 1H), 6.81—6.75 (m, 2H), 6.60 (t, *J* = 8.6 Hz, 1H), 5.75 (dd, *J* = 15.3, 7.9 Hz, 1H), 5.55 (dt, *J* = 14.5, 6.7 Hz, 1H), 3.80—3.70 (m, 2H), 3.10 (d, *J* = 9.7 Hz, 3H), 2.77 (d, *J* = 13.4 Hz, 5H), 2.36 (dt, *J* = 35.7, 12.4 Hz, 2H), 2.08 (d, *J* = 13.1 Hz, 1H), 1.89 (dt, *J* = 12.5, 6.2 Hz, 1H), 1.80 (t, *J* = 16.1 Hz, 2H), 1.67 (t, *J* = 6.4 Hz, 1H), 1.53 (s, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 156.03, 151.23, 140.37, 134.33, 129.91, 129.08, 128.77, 128.26, 125.98, 117.62, 113.63, 112.76, 63.37, 58.54, 57.10, 49.64, 48.12, 41.96, 38.09, 33.39, 30.24, 25.36, 23.14. HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_{25}H_{32}NO$ 378.2433, found 378.2438. $[\alpha]^{20}_D$ +15.06° (*c* 1.6, $CHCl_3$). HBr salt: mp 220-224 °C Anal. Calcd. For $C_{25}H_{32}NO_2Br \bullet 0.15\ H_2O$: C, 65.12%; H, 7.06; N, 3.04%. Found C, 65.15%; H, 6.97%; N, 2.90%.

Methyl 3-((1*S*,5*R*,9*R*)-5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-yl)propanoate (14). Compound **11** (0.25 g, 0.67 mmol) was dissolved in MeOH (7 mL) and transferred to a Parr shaker vessel, charged with aq AcOH (0.04 mL, 0.67 mmol) and Escat 103 (5% Pd/C, 0.03 g) and shaken for 8 h under an atmosphere of 50 psi H_2. The reaction mixture was filtered through Celite and concentrated under vacuum to afford a yellow oil. The residue was purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, isocratic 1%) to afford **14** as a white foam (0.19 g, 75%). The HCl salt was obtained from a warm solution of HCl in MeOH. The analyses were performed on the free base, unless otherwise noted. ^{1}H NMR (400 MHz; DMSO-d_6) δ 9.17 (s, 1H), 7.22 (d, *J* = 4.1 Hz, 4H), 7.15—7.12 (m, 1H), 7.08 (t, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 7.8 Hz, 1H), 6.68 (s, 1H), 6.55—6.53 (m, 1H), 3.51 (s, 3H), 3.00 (d, *J* = 8.4 Hz, 2H), 2.82 (s, 1H), 2.73—2.67 (m, 4H), 2.23 (d, *J* = 11.4 Hz, 1H), 2.12—2.00 (m, 2H), 1.91 (dt, *J* = 25.5, 10.6 Hz, 4H), 1.72 (t, *J* = 6.3 Hz, 2H), 1.60 (dd, *J* = 12.2, 4.8 Hz, 2H), 1.37 (t, *J* = 13.5 Hz, 1H), 1.01 (q, *J* = 8.6 Hz, 1H). ^{13}C-NMR (101 MHz, DMSO-d_6): δ 173.37, 151.51, 140.62, 128.81, 128.49, 127.86, 125.45, 115.77, 112.26, 112.08, 79.06, 56.24, 52.21, 50.92, 48.27, 43.90, 42.35, 38.44, 33.41, 31.44, 29.17, 25.60, 22.84, 21.67. HRMS-ESI (m/z): [M + H]+ cald for $C_{26}H_{34}NO_3$ 408.2539, found 408.2546. HCl salt: mp: 184-191 °C. Anal. Calcd for $C_{26}H_{34}ClNO_3 \bullet 0.25\ H_2O$: C, 69.63%; H, 7.75%; N, 3.12%. Found C, 69.95%, H, 7.54%, N, 3.10%.

3-((1*S*,5*R*,9*R*)-9-(3-Hydroxypropyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-5-yl)phenol (15a). A 50 mL single-neck round-bottom flask was charged with **14** (0.16 g, 0.4 mmol) and THF (3.4 mL). The flask was

cooled to 0 °C and $LiAlH_4$ (0.6 mL, 1.19 mmol, 2.0 M in THF) was added dropwise via syringe. The flask stirred for 30 min gradually warming to room temperature. The flask was equipped with a reflux condenser and the reaction was heated to reflux for 20 h under argon. The reaction was cooled to 0 °C and quenched by the drop-wise addition of 2M aq Rochelle salt (25 mL). The crude reaction mixture was stirred for 4 h affording a cloudy biphasic mixture. The organic layer was separated and the aqueous layer basified to pH 9 with NH_4OH. The aqueous layer was extracted with $CHCl_3$ (3 x 25 mL) and the combined organic layers, including the previously separated THF layer, were combined and dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting residue was purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–20%) to afford **15** (0.13 g, 0.34 mmol, 88%) as a white foam. The HBr salt was formed from 48% HBr in acetone. The analyses were performed on the free base, unless otherwise noted. ^{1}H NMR (400 MHz; DMSO-d_6): δ 9.14 (s, 1H), 7.27-7.20 (m, 4H), 7.20-7.12 (m, 1H), 7.06 (t, *J* = 7.9 Hz, 1H), 6.72 (d, *J* = 8.1 Hz, 1H), 6.68 (s, 1H), 6.53 (d, *J* = 8.1 Hz, 1H), 4.18 (t, *J* = 4.9 Hz, 1H), 3.20-3.08 (m, 2H), 3.04-2.94 (m, 2H), 2.91 (s, 1H), 2.80-2.64 (m, 4H), 2.24 (d, *J* = 14.5 Hz, 1H), 2.09 (q, *J* = 11.1 Hz, 1H), 1.94-1.77 (m, 3H), 1.78-1.66 (m, 1H), 1.66-1.54 (m, 2H), 1.54-1.25 (m, 3H), 1.22-1.05 (m, 1H), 0.85-0.63 (m, 1H). ^{13}C NMR (101 MHz; DMSO-d_6): δ 157.5, 152.4, 141.2, 129.3, 129.0, 128.5, 126.1, 116.3, 112.8, 112.5, 61.7, 56.8, 52.9, 49.0, 45.4, 42.7, 39.1, 34.0, 31.6, 29.8, 26.3, 23.5, 23.1. HRMS-ESI (*m/z*): $[M + H]^+$ calcd. for $C_{25}H_{34}NO_2$ 380.2590, found 380.2592. For (1*R*,5*S*,9*S*)-**15b**: $[\alpha]^{20}_D$ -23.2° (*c* 1.30, $CHCl_3$). HBr salt: mp: 259-262 °C. Anal. Calcd. For $C_{25}H_{34}BrNO_2 \bullet 0.05\ H_2O$: C, 65.09%; H, 7.45; N, 3.04%. Found C, 65.09%; H, 7.27%; N, 3.00%.

Ethyl 3-((1*S*,5*R*,9*R*)-5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-yl)propanoate (16). A flame-dried 5 mL round-bottom flask was charged with **14** (0.062 g, 0.14 mmol), anhydrous EtOH (2.8 mL), and cat. H_2SO_4 (3 drops). The suspension was refluxed under N_2 for 3 d. The reaction was quenched with 0.5M ammonia solution in dioxane (1 mL) and concentrated under vacuum. The residue was purified *via* flash chromatography eluting with $CHCl_3$/MeOH/NH_4OH (99:0.9:0.1 to 80:18:2) to afford **16** (0.043 g, 0.09 mmol, 63%) as an amber oil. ^{1}H-NMR (400 MHz; $CDCl_3$ + MeOD): δ 7.26-7.20 (m, 4H), 7.18-7.11 (m, 2H), 6.81-6.76 (m, 2H), 6.65 (d, *J* = 7.8 Hz, 1H), 4.13-4.01 (m, 2H), 3.11-3.01 (m, 2H), 2.89-2.85 (m, 1H), 2.84-2.69 (m, 4H),

2.32-2.24 (m, 2H), 2.16-1.82 (m, 7H), 1.82-1.70 (m, 2H), 1.67-1.59 (m, 1H), 1.48-1.35 (m, 1H), 1.23 (t, J = 7.2 Hz, 3H); ^{13}C-NMR (101 MHz; $CDCl_3$ + MeOD): δ 174.8, 156.4, 151.9, 141.0, 129.1, 128.8, 128.2, 125.8, 117.0, 112.69, 112.62, 60.3, 57.1, 52.9, 49.1, 44.3, 43.0, 39.1, 34.3, 32.1, 29.7, 26.3, 23.4, 21.9, 14.2; HRMS-ESI (*m/z*): $[M+H]^+$ calcd. For $C_{27}H_{36}NO_3$ 422.2695, found 422.2691. Oxalate salt: mp: 168-170 °C Anal. Calcd. For $C_{29}H_{37}NO_7 \bullet 0.4\ H_2O$ C, 67.14%; H, 7.34; N, 2.70%. Found C, 67.27%; H, 7.42%; N, 2.60%.

3-((1*S*,5*R*,9*R*)-5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-yl)propanoic acid (17). To a solution of **14** (0.11 g, 0.27 mmol) in MeOH (5 mL) was added 1N aq LiOH (0.54 mL, 0.54 mmol) and refluxed for 3 h. The crude reaction mixture was concentrated under vacuum and the residue was partitioned between H_2O (5 mL) and $CHCl_3$ (5 mL). The pH of the aqueous phase was adjusted to ~4 with 1N aq HCl and extracted with $CHCl_3$ (5 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and concentrated under vacuum. The resulting residue was purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 1–20%) to afford **17** (0.026 g, 0.03 mmol, 11%) as a tan solid; mp 137 °C (decomp.). ^{1}H-NMR (400 MHz; DMSO-d_6): δ 9.16 (s, 1H), 7.27-7.20 (m, 4H), 7.17-7.11 (m, 1H), 7.08 (t, J = 8.0 Hz, 1H), 6.74 (d, J = 7.6 Hz, 1H), 6.69 (s, 1H), 6.54 (d, J = 7.4 Hz, 1H), 3.00 (d, J = 9.8 Hz, 2H), 2.88-2.85 (m, 1H), 2.78-2.65 (m, 4H), 2.23 (d, J = 11.4 Hz, 1H), 2.18-1.98 (m, 2H), 1.94-1.80 (m, 4H), 1.79-1.66 (m, 2H), 1.65-1.54 (m, 2H), 1.44-1.33 (m, 1H), 1.07-0.98 (m, 1H); ^{13}C-NMR (101 MHz, DMSO-d_6): δ 175.21, 157.52, 152.09, 141.13, 129.33, 129.02, 128.44, 126.07, 116.32, 112.81, 112.57, 56.92, 52.74, 48.91, 44.51, 42.91, 39.00, 34.01, 32.39, 29.72, 26.10, 23.37, 22.24. HRMS-ESI (*m/z*): $[M+H]^+$ calcd. for $C_{25}H_{32}NO_3$ 394.2384, found 394.2384. Anal. Calcd. For $C_{25}H_{31}NO_3 \bullet 1.6\ H_2O$: C, 71.10%; H, 8.16; N, 3.32%. Found C, 71.18%; H, 7.74%; N, 3.20%.

Ethyl (*E*)-3-((1*S*,5*R*,9*S*)-5-(3-methoxyphenyl)-2-methyl-2-azabicyclo[3.3.1]nonan-9-yl)acrylate (18). To a cooled (0 °C) solution of **8** (20.46 g, 50 mmol) in anhydrous THF (150 mL) was added methoxymethyltriphenylphosphonium chloride (51.42 g, 150 mmol), followed by portion-wise potassium *tert*-butoxide (16.8 g, 150 mmol) over 20 min, and the reaction allowed to warm to room temperature over 18 h. The reaction was then concentrated under vacuum to a thick slurry which was suspended in EtOAc (200 mL) and

extracted with 1N HCl. The combined aqueous extracts were cooled to 0 °C, basified to pH 9 with NH_4OH and extracted with CH_2Cl_2 (3 x 100 mL), dried over Na_2SO_4 and concentrated. The crude mixture was filtered through a plug of silica to give **5** as a mixture of E/Z isomers, used without further purification. This mixture was dissolved in 3N HCl (180 mL) and stirred for 24 h, then basified with NH_4OH to pH 9.0, extracted with $CHCl_3$, dried over Na_2SO_4 and concentrated to give **8** as a 2:1 mixture of 9*R* and 9*S* epimers. The product was used without further purification (6.6 g, 48% yield from **1**). The mixture was then added to a solution of triethylphosphonoacetate (23.8 mL, 120.2 mmol) and NaH (4.8 g, 120.2 mmol) in anhydrous THF (320 mL) and stirred for 22 h. The reaction was concentrated under vacuum, suspended in NH_4OH and extracted with $CHCl_3$ (3 x 100 mL), dried over Na_2SO_4, and concentrated under vacuum. Purification via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, isocratic 2%) gave the desired 9*S*-epimer **18** as a dark oil (1.54 g, 18%). ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.18 (t, J = 8.0 Hz, 1H), 6.91—6.85 (m, 3H), 6.68 (dd, J = 8.1, 1.7 Hz, 1H), 5.79 (d, J = 15.8 Hz, 1H), 4.08 (q, J = 7.1 Hz, 2H), 3.75 (s, 3H), 3.22 (s, 1H), 3.07 (td, J = 12.1, 4.9 Hz, 1H), 2.90 (s, 1H), 2.83 (dd, J = 11.5, 7.7 Hz, 1H), 2.49 (s, 3H), 2.11 (t, J = 9.8 Hz, 3H), 1.98 (td, J = 16.3, 7.3 Hz, 2H), 1.79 (dd, J = 14.2, 4.3 Hz, 2H), 1.50 (t, J = 10.5 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 166.68, 159.48, 150.10, 148.48, 129.21, 122.98, 118.13, 112.14, 110.83, 59.94, 55.11, 51.39, 51.17, 42.79, 40.84, 37.61, 31.56, 22.63, 21.90, 18.77, 14.10. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{30}NO_3$ 344.2226, found 344.2224.

Methyl & Ethyl (*E*)-3-((1*S*,5*R*,9*R*)-5-(3-methoxyphenyl)-2-azabicyclo[3.3.1]nonan-9-yl)acrylate (19). To a solution of **18** (1.45 g, 4.22 mmol) in anhydrous DCE (11 mL) was added $NaHCO_3$ (2.48 g, 25.33 mmol) and 1-chloroethyl chloroformate (2.72 mL, 25.33 mmol) and the reaction refluxed for 18 h. The reaction was cooled, filtered over Celite and concentrated under vacuum. The residue was dissolved in MeOH and heated to reflux for 3 h, cooled and concentrated under vacuum. Purification via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0-10%) gave a mixture of methyl and ethyl esters as a yellow oil (1.27 g, 91%). The analyses were performed on the mixture of esters. ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.18 (t, J = 8.0 Hz, 1H), 6.91—6.84 (m, 2H), 6.82 (s, 1H), 6.67 (dd, J = 8.1, 2.4 Hz, 1H), 5.78 (d, J = 15.8 Hz, 1H), 4.07 (q, J =

7.1 Hz, 1H, ethyl ester), 3.75 (s, 3H), 3.61 (s, 1H, methyl ester), 3.59—3.52 (m, 1H), 3.15 (d, *J* = 2.7 Hz, 1H), 3.09 (d, *J* = 8.3 Hz, 1H), 2.98 (dd, *J* = 13.4, 6.1 Hz, 1H), 2.21—2.11 (m, 3H), 1.97 (s, 2H), 1.92—1.85 (m, 3H), 1.78—1.70 (m, 2H), 1.20 (t, *J* = 7.1 Hz, 1H, ethyl ester). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 166.67, 166.32, 159.45, 150.91, 150.88, 149.21, 148.92, 129.16, 122.89, 122.52, 118.01, 112.22, 110.45, 60.15, 55.09, 53.71, 53.68, 51.36, 49.08, 49.05, 42.66, 41.91, 38.46, 29.52, 26.98, 21.83, 14.18. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{28}NO_3$ 330.2069, found 330.2072 (ethyl ester)

Methyl & Ethyl (*E*)-3-((1*S*,5*R*,9*S*)-5-(3-methoxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-yl)acrylate (20). To a solution of **19** (0.32 g, 0.99 mmol) in anhydrous acetonitrile (10 mL) was added K_2CO_3 (0.27 g, 1.98 mmol) and 2-phenylethyl bromide (0.2 mL, 1.48 mmol) and heated to reflux. After 18 h the reaction was cooled, filtered through Celite, dried over Na_2SO_4 and concentrated. Purification via flash chromatography (EtOAc in hexanes, gradient 0–100%) yielded the product (a mixture of methyl and ethyl esters) as a yellow oil. The analyses were performed on the methyl ester. ^{1}H-NMR (400 MHz; $CDCl_3$): δ 7.33-7.28 (m, 2H), 7.25-7.18 (m, 4H), 6.95-6.87 (m, 3H), 6.71 (d, *J* = 6.1 Hz, 1H), 5.82 (d, *J* = 16.0 Hz, 1H), 3.78 (s, 3H), 3.64 (s, 3H), 3.23 (d, *J* = 7.6 Hz, 1H), 3.12-3.00 (m, 3H), 2.88-2.78 (m, 4H), 2.19-2.09 (m, 3H), 2.03-1.93 (m, 2H), 1.87-1.79 (m, 2H), 1.59-1.47 (m, 1H). ^{13}C NMR (101 MHz; $CDCl_3$): δ 166.8, 159.5, 150.6, 149.2, 140.5, 129.2, 128.7, 128.4, 126.1, 122.8, 118.2, 112.3, 110.7, 58.3, 57.8, 55.2, 51.4, 49.6, 48.6, 41.2, 38.2, 34.7, 29.7, 22.0, 19.9. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{27}H_{34}NO_3$ 420.2539, found 420.2544 (methyl ester).

Methyl 3-((1*S*,5*R*,9*S*)-5-(3-hydroxyphenyl)-2-phenethyl-2-azabicyclo[3.3.1]nonan-9-yl)propanoate (22). Compound **20** (0.29 g, 0.67 mmol) was dissolved in MeOH (7 mL) and transferred to a Parr shaker vessel, charged with aq AcOH (0.04 mL, 0.67 mmol) and Escat 103 (5% Pd/C, 0.030 g), and shaken for 8 h under 50 psi H_2. The reaction mixture was filtered through Celite and concentrated under vacuum to afford a yellow oil. The residue was purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–5%) to afford the reduced intermediate as a pale oil. The oil was dissolved in 48% aq HBr (10 mL) and toluene (3 mL) and refluxed under argon for 12 h. The reaction was cooled to room temperature and the reflux condenser was removed and replaced with a short-path distillation head. The bulk of the 48% aq HBr was removed by vacuum

distillation (40 mbar, 90 °C) affording a yellow oil. The oil was dissolved in MeOH (10 mL), trimethyl orthoformate (0.21 mL, 1.95 mmol) and a catalytic amount of H_2SO_4 (3 drops) was added and refluxed for 2.5 h. The reaction mixture was cooled to 0 °C and quenched by the addition of 7 N methanolic ammonia (0.4 mL). The crude reaction mixture was concentrated and purified via flash chromatography (10% NH_4OH/MeOH in $CHCl_3$, gradient 0–10%) to afford **22** (0.188 g, 0.43 mmol, 65%) as a yellow oil. ^{1}H NMR (400 MHz; DMSO-d_6): δ 9.19 (s, 1H), 7.30-7.23 (m, 4H), 7.18 (t, *J* = 6.8 Hz, 1H), 7.09 (t, *J* = 7.9 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 6.73 (s, 1H), 6.56 (d, *J* = 7.4 Hz, 1H), 3.52 (s, 3H), 2.94-2.85 (m, 3H), 2.78-2.66 (m, 4H), 2.29-2.21 (m, 1H), 2.10-2.02 (m, 2H), 1.97-1.60 (m, 7H), 1.47-1.36 (m, 2H), 1.24-1.16 (m, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$): δ 174.14, 155.75, 151.70, 140.54, 129.31, 128.74, 128.38, 126.04, 117.97, 113.28, 112.85, 58.25, 54.15, 51.55, 49.75, 44.76, 41.27, 38.66, 34.49, 32.35, 28.79, 22.62, 21.67, 18.21. HRMS-ESI (*m/z*): $[M+H]^+$ calcd for $C_{26}H_{34}NO_3$ 408.2539, found 408.2534. Oxalate salt: mp: 181-185 °C. Anal. Calcd. For $C_{28}H_{35}NO_7 \bullet 0.5\ H_2O$: C, 66.39%; H, 7.16%; N, 2.76%. Found C, 66.32%; H, 7.37%; N, 2.72%.

In Vitro Pharmacology

Detailed procedures are on page S29

Cell lines and cell culture. HitHunter Chinese hamster ovary cells (CHO-K1) that express human μ-opioid receptor (OPRM1), human κ-opioid receptor (OPRK1), and human δ-opioid receptor (OPRD1) were used for the forskolin-induced cAMP accumulation assay to determine potency and efficacy of the compounds. PathHunter CHO cells expressing human μ-opioid receptor β-arrestin-2 EFC cells were used for the β-arrestin-2 EFC recruitment assay. All cell lines were purchased from Eurofins DiscoverX (Fremont, CA). Cell culture was performed as previously described.[1]

Forskolin-induced cAMP accumulation assay. Assays were performed as previously described[2] Cytation 5 plate reader and Gen5 Software were used to quantify luminescence (BioTek, Winooski, VT).

β-Arrestin-2 EFC recruitment assay. Assays were performed as previously described[1] using the PathHunter Detection Kit from Eurofins DiscoverX (Fremont, CA) following manufacturer's instructions. Cytation 5 plate reader and Gen5 Software were used to quantify luminescence (BioTek, Winooski, VT).

Data analysis. Data were analyzed as previously described[1] using GraphPad Prism 6.0 software (GraphPad, La Jolla, CA). Bias factors were calculated using the equation shown below.

$$\text{Log(bias factor)} = \left(\text{Log}\left(\frac{EC50_{test} \times Emax_{DAMGO}}{Emax_{test} \times EC50_{DAMGO}}\right)\right)_{cAMP} - \left(\text{Log}\left(\frac{EC50_{test} \times Emax_{DAMGO}}{Emax_{test} \times EC50_{DAMGO}}\right)\right)_{\beta\text{-arrestin}}$$

X-ray Crystal Data. Single-crystal X-ray diffraction data on compound **14b** were collected using Mo Kα radiation and a Bruker APEX II area detector. The crystal was prepared for data collection by coating with high viscosity microscope oil. The oil-coated crystal was mounted on a micro-mesh mount (MiteGen, Inc.) and transferred to the diffractometer and a data set collected at 293K. The 0.193 x 0.159 x 0.114 mm^3 crystal was orthorhombic in space group $P2_12_12$, with unit cell dimensions a = 14.8241(8) Å, b = 17.2422(9) Å, c = 9.8923(5) Å, $\alpha = 90°$, $\beta = 90°$, and $\gamma = 90°$. Data was 100.0% complete to 25.252° θ (~0.83 Å) with an average redundancy of 6.78. The final anisotropic full matrix least-squares refinement on F^2 with 285 variables converged at R_1 = 4.86%, for the observed data and wR2 = 11.79% for all data. The structure was solved by direct methods and refined by full-matrix least squares on F^2 values using the programs found in the SHELXL suite (Bruker, SHELXL v2014.7, 2014, Bruker AXS Inc., Madison, WI). Corrections were applied for Lorentz, polarization, and absorption effects. Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. The H atoms were included using a riding model. Complete information on data collection and refinement is available in the supplemental material.

Atomic coordinates for **14b** have been deposited with the Cambridge Crystallographic Data Centre (deposition number CCDC 1914554). Copies of the data can be obtained, free of charge, on application to

CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail:

deposit@ccdc.cam.ac.uk.

Modeling and Simulations Fig. S1 and S2

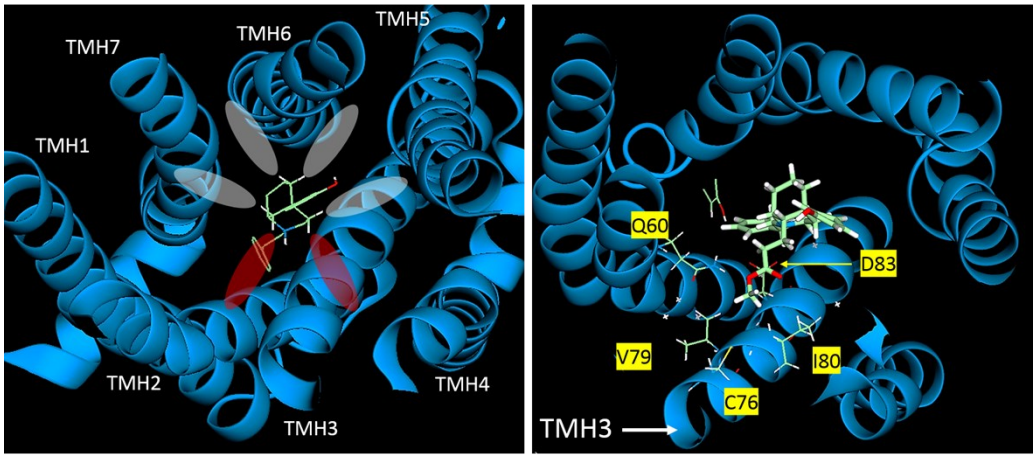


Fig. S1: Left panel: Extracellular view of the *N*-phenylethylmorphan (Reference 15, main text) in its putative binding mode with MOR, showing the regions targeted by the different substitutions at positions C4, C6, and C9. The series of compounds studied here (except **22**), regardless of chirality, span the regions indicated in red, and tend to target residues in helices 2, 3, and 5. Right: interactions of **14b** with TMH3 from a snapshot of the simulation; this mode is stabilized by hydrophobic interactions and an ester-Q60 H-bond. Sequence numbering follows the resolved residues in the X-ray structure (4DKL); for absolute numbering and a comparison between MOR and DOR, see ref. 9.

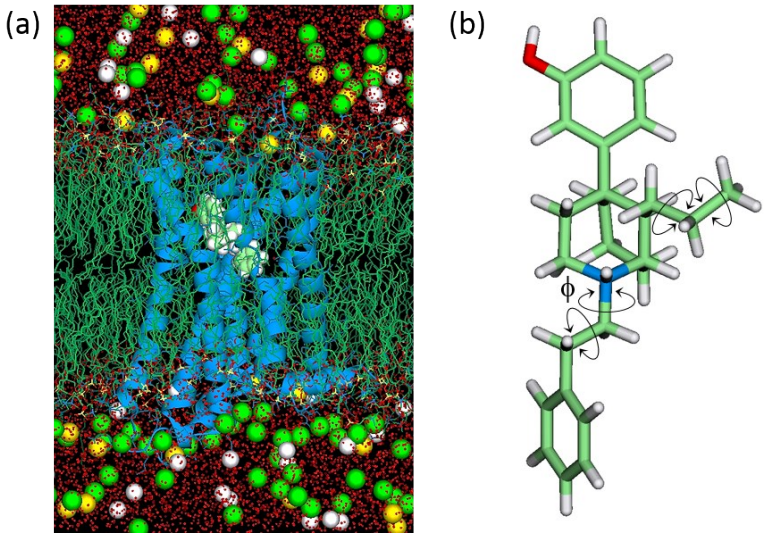


Fig. S2: a) snapshot of compound **12** bound to MOR. b) Relevant degrees of freedom (here for **12**) leading to multiple conformers with similar energies in aqueous solution; the two most relevant conformers involve rotations of the angle ϕ between N and C16 of the *N*-phenylethyl tail. The two conformers considered in this study (only one shown) can lead to slightly different interactions with the receptor and both were stable during the simulations. Unlike the other degrees of freedom indicated, which may rotate with little steric hindrance while docked, ϕ is constrained and remains close to its initial value once the ligand is in the constrained binding pocket

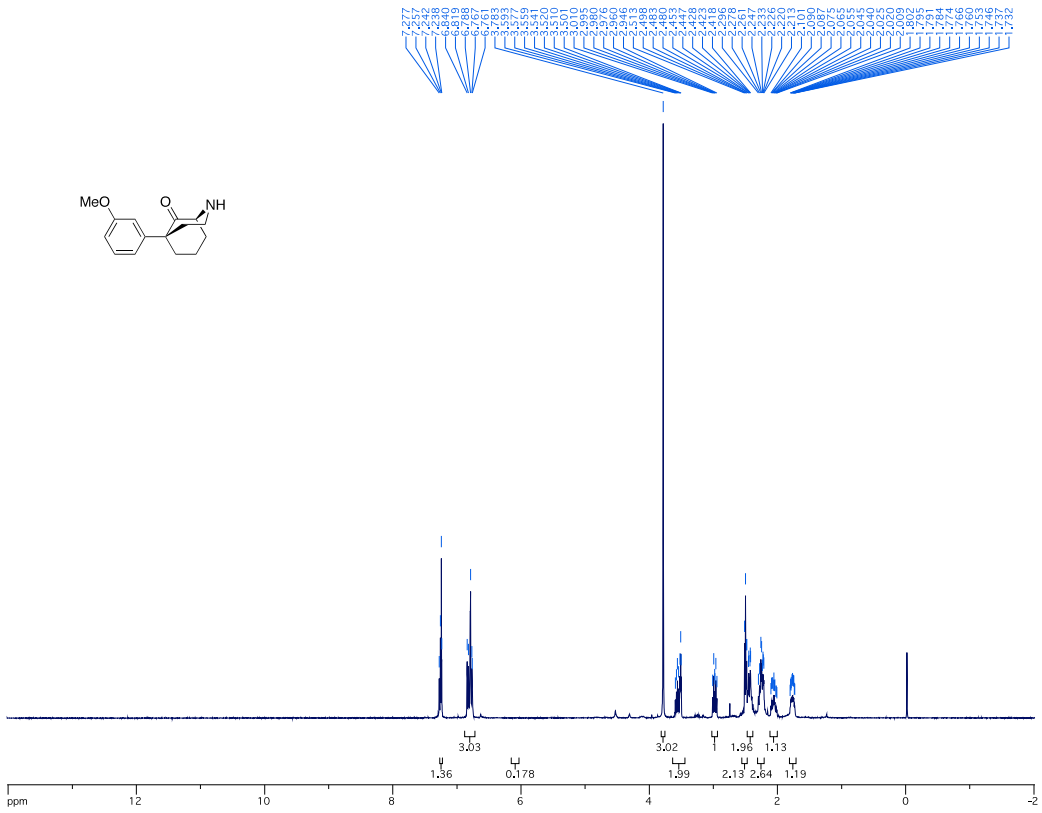
Modeling and Simulations. The initial structure of the μ receptor was taken from the corresponding chimera crystal bound to an antagonist (Protein Data Base (RCSB PDB) id: 4DKL). After removing the ligand and crystal water, the intracellular loop (ICL3) connecting TM5 and TM6 was first modeled using *ab initio* molecular mechanics methods, as discussed previously.[3] The receptor was then embedded in a membrane composed of 1-palmitoyl-2-oleoyl-phosphatidylcholine lipid molecules with initial concentrations of Na^+, K^+ and Cl^- ions in the EC and IC regions. The initial orientation and relative position of the receptor with respect to the membrane were obtained from the OPM database.[4] The receptor and the membrane were then solvated in TIP3P water, and the ligands docked as described below. Fig. S2 shows a snapshot of the compound **12**/MOR system. MD simulations were conducted at 37 °C and 1 atm, using periodic boundary conditions and particle mesh Ewald summations. Amino acids were assumed to be neutral at pH 7 except Asp^-, Glu^-, Lys^+ and Arg^+; chloride ions were used to neutralize the systems by placing them randomly in the aqueous phase. First, the water/membrane/ion system was heated and equilibrated thoroughly while the receptor and ligand were kept fixed; this was followed by extensive equilibration of the entire system without constraints. The production part of the simulation was extended for 50 ns, sufficient to ensure convergence of the quantities of interest, such as hydrophobic contacts, H-bonds, etc., and robust statistical analysis. See additional details on simulations and analysis in Ref. [9].

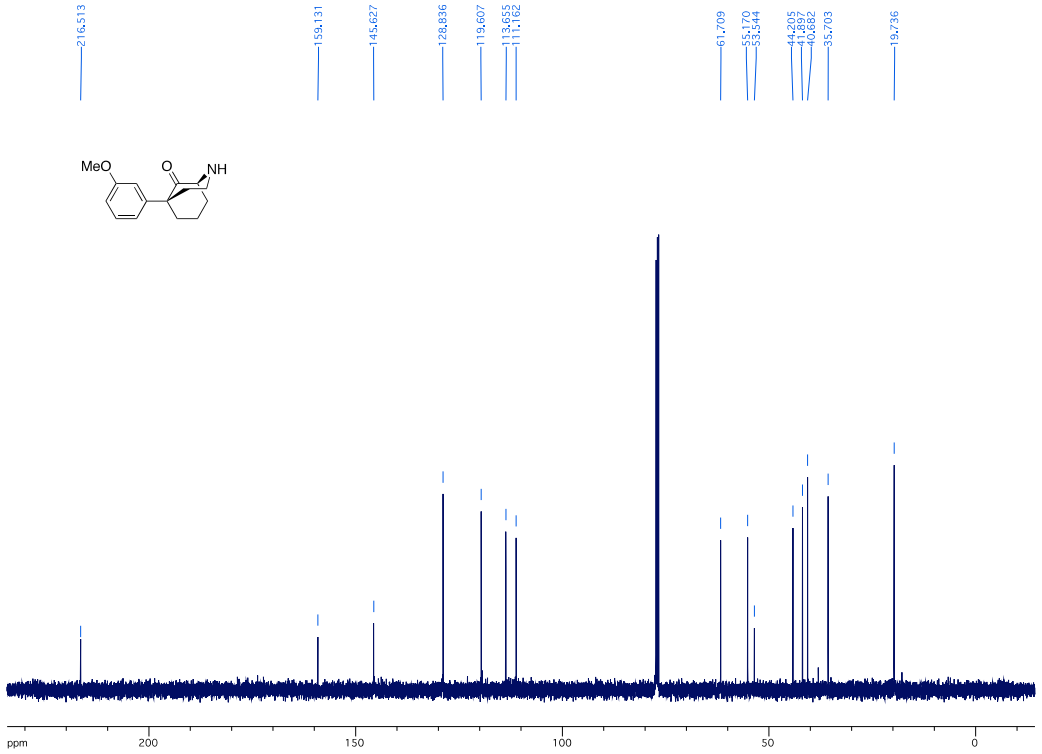
A simulation of the receptor containing only a crystallographic Na^+ ion in the binding pocket, and coordinated as in the DOR (RCSB PDB: 4N6H),[5] was taken as a reference for comparative analysis; the ion remained well coordinated throughout the simulation, including persistent interactions with D83 (residue numbers follow those in the crystal structure). A conformation of the receptor at the end of this simulations was used to dock all the ligands, with the requirement that the amine be H-bonded to D83 at the beginning of the simulations. See details in Ref. [9].

The structures of compounds **12**, **14** and **15** (Table 2) in their protonated forms were constructed based on the X-ray structure of **14b**. Geometry optimizations were carried out with density functional theory at the level of B3LYP/6-31G* in the gaseous phase using Gaussian.[6] The atomic polar tensor (APT)-derived charges were used to assign partial charges for the molecular dynamics (MD) simulations. All the other parameters were determined

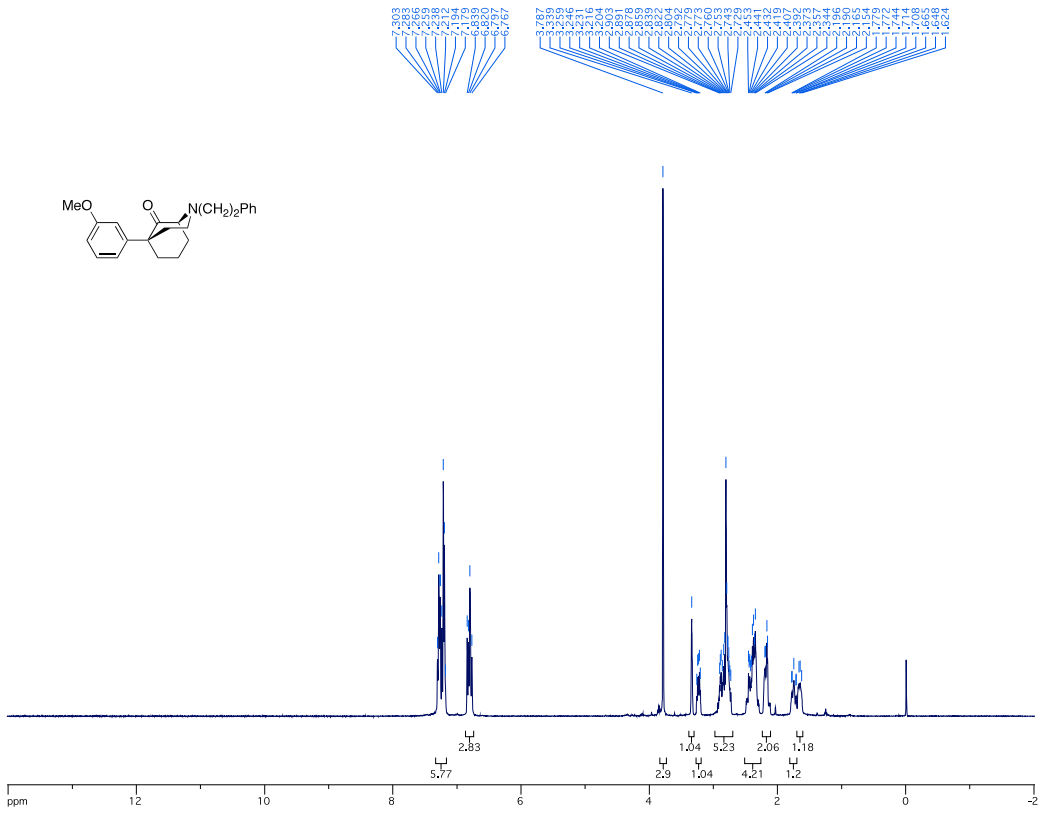
by chemical analogy from the topology and parameters files of the CHARMM all-atom force field (version c42b2) .[7] Several conformers were identified for each compound (Fig. S2), and quantum chemically optimized in the reaction field of water to select those of low energy in the unbound state in solution. All the ligands were initially docked into the MOR binding pocket based on the binding mode of β-FNA co-crystallized with the μ opioid receptor.[8] The most relevant conformers, i.e., those structurally compatible with the geometry of the pocket, were used in the simulations. There are two of these conformers for each ligand, differing only in the orientation of the N-phenylethyl group (Fig. S2). Both conformers were stable during the simulation, retaining the critical –NH-D83 H-bond, except for the enantiomer of **12** which we also examined, for which this interaction was lost due to conformational arrangement of the ligand in the pocket driven by the spacer.

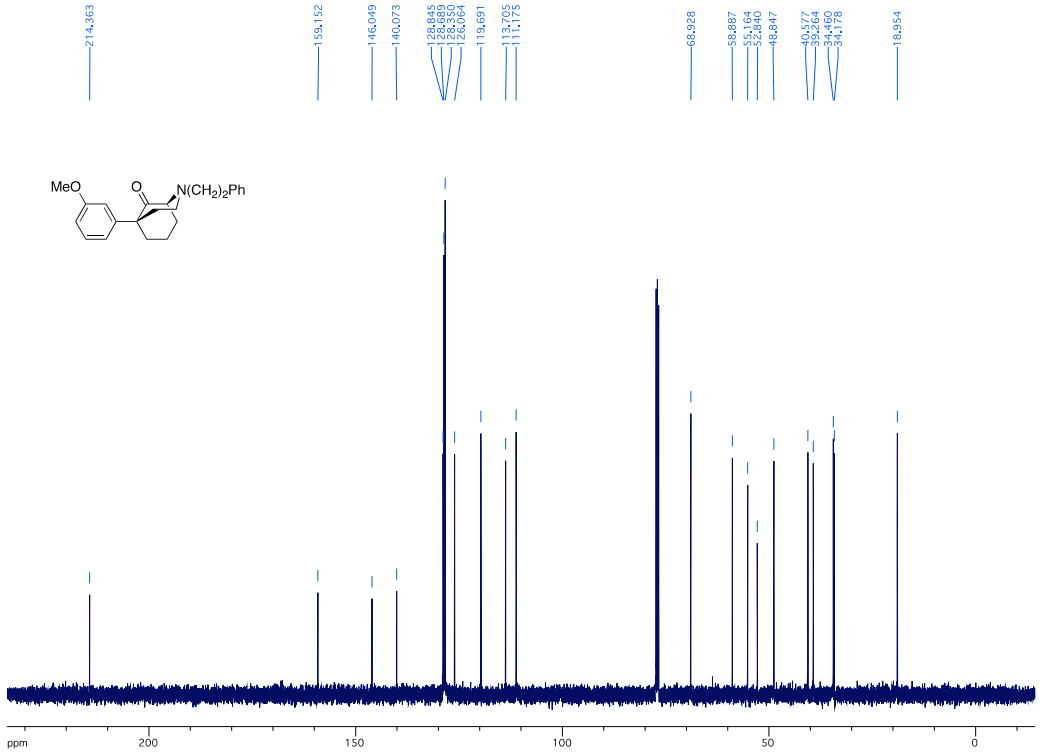
^{1}H and ^{13}C NMR spectra of **2**



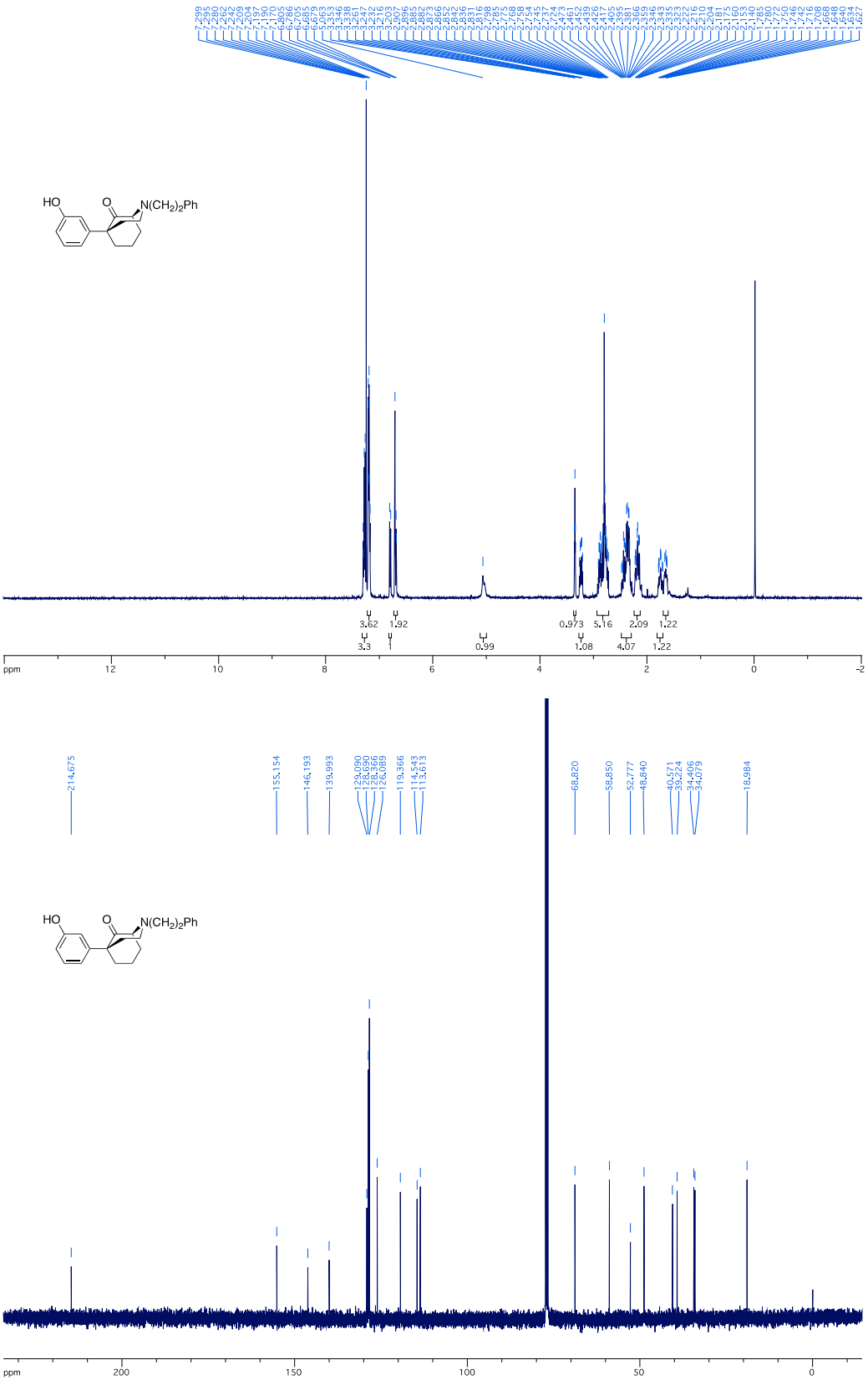


^{1}H and ^{13}C NMR spectra of **3**

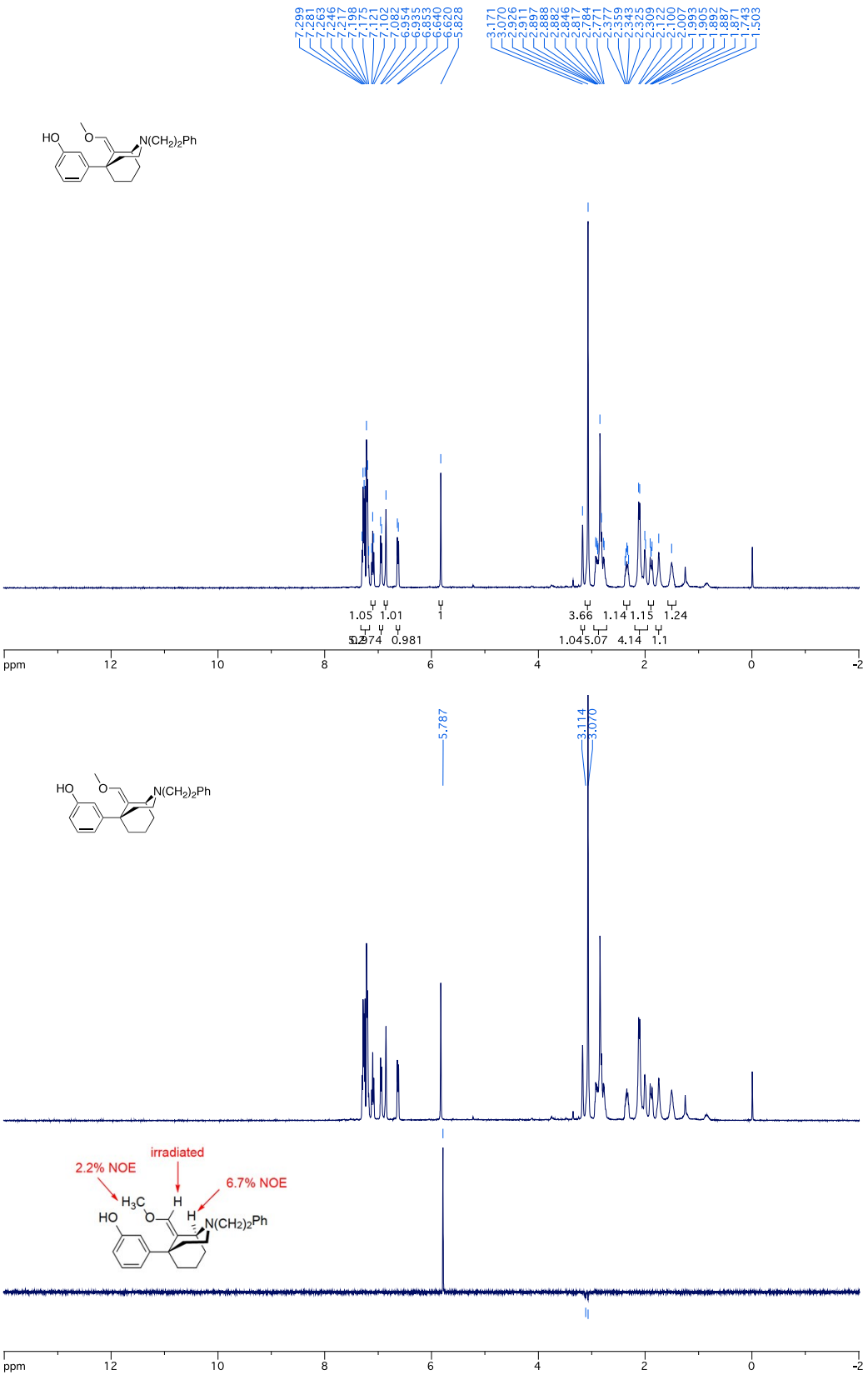




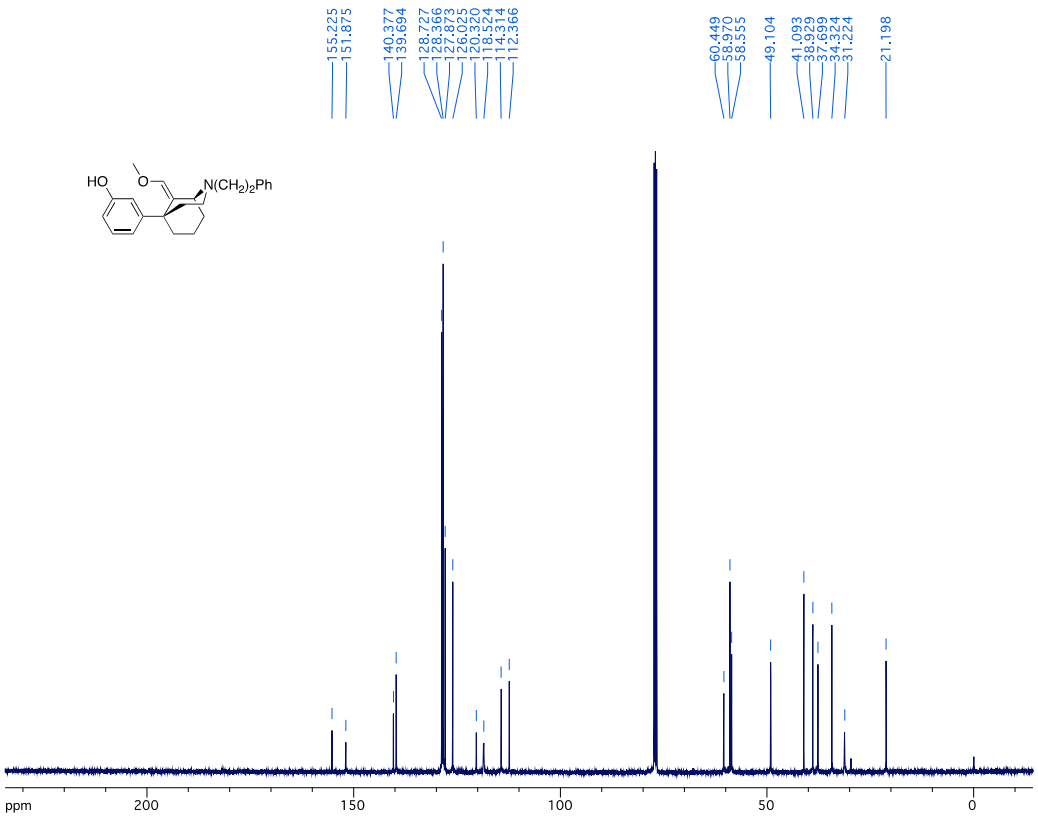
^{1}H and ^{13}C NMR spectra of **4**



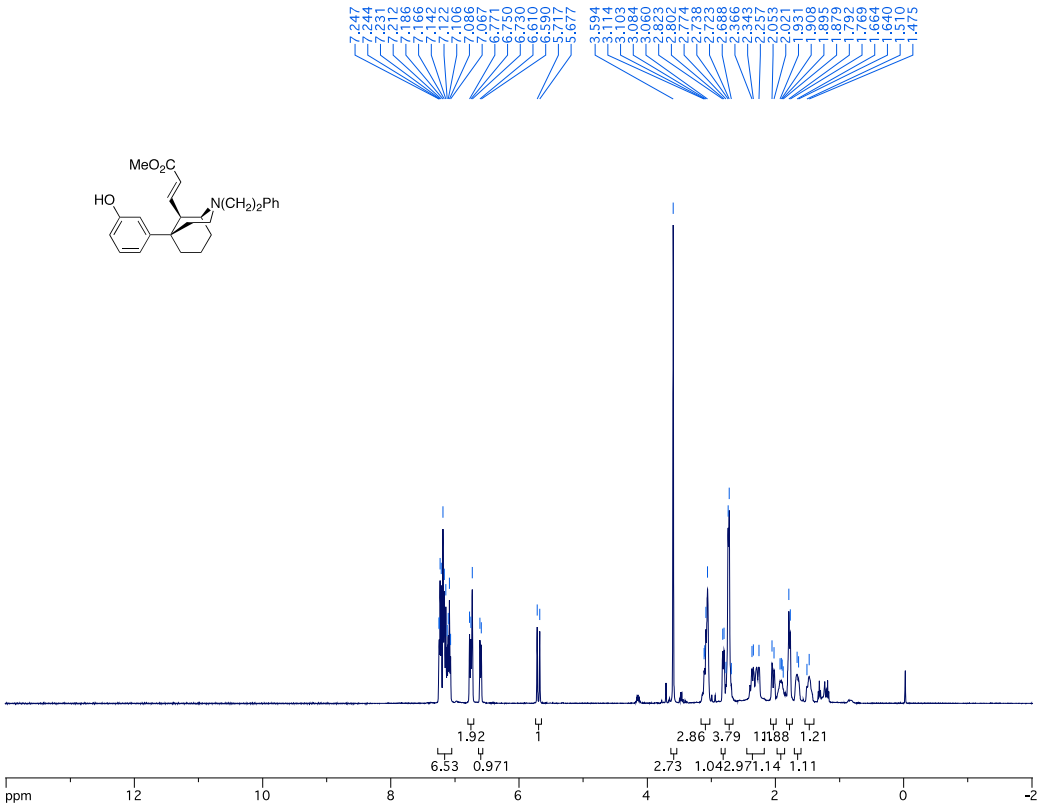
[1]H and 1D-NOESY NMR spectra of **7**

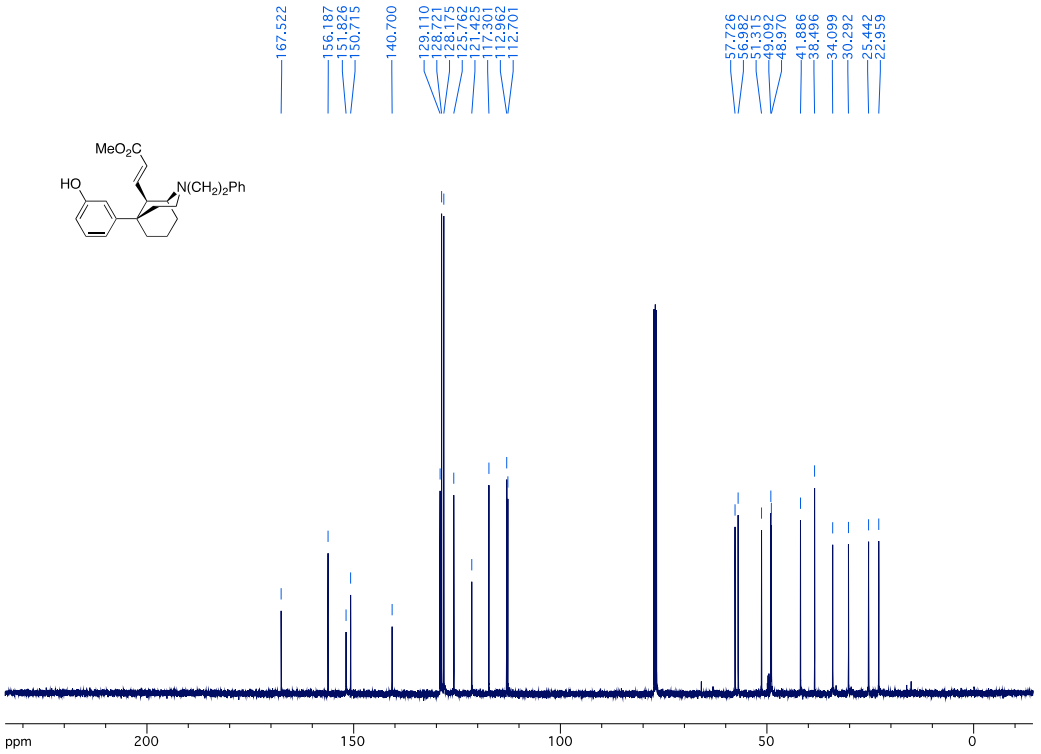


^{13}C NMR spectra of **7**

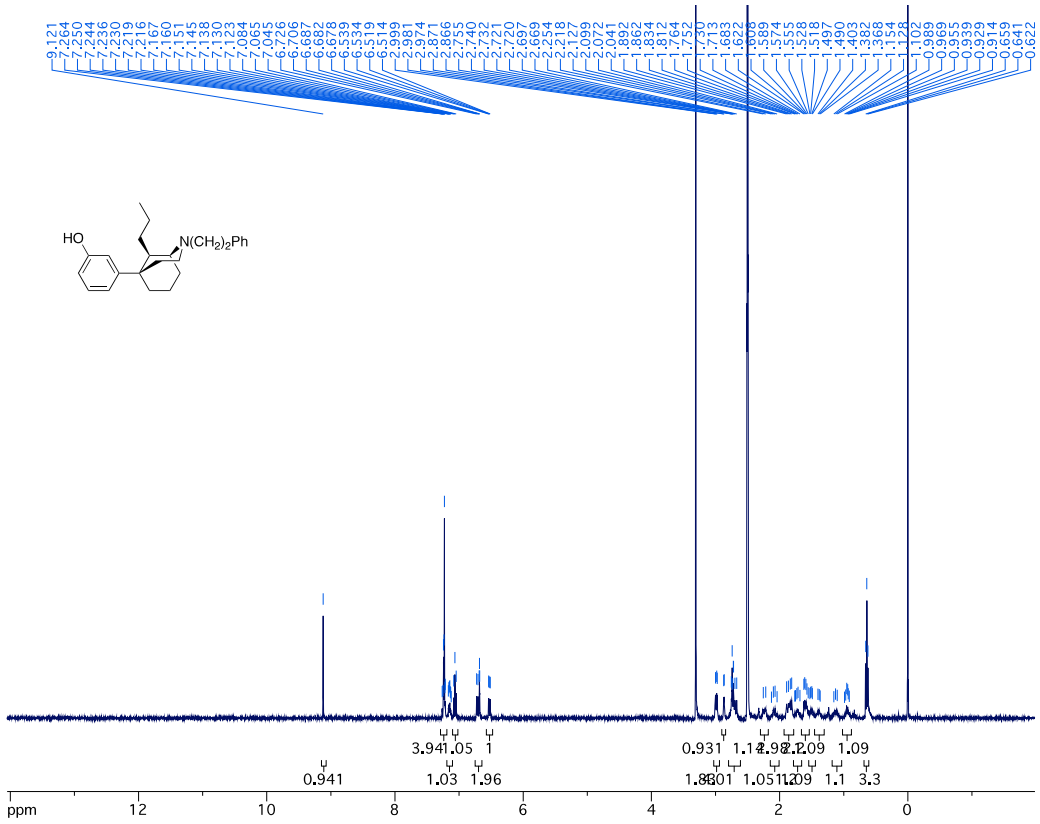


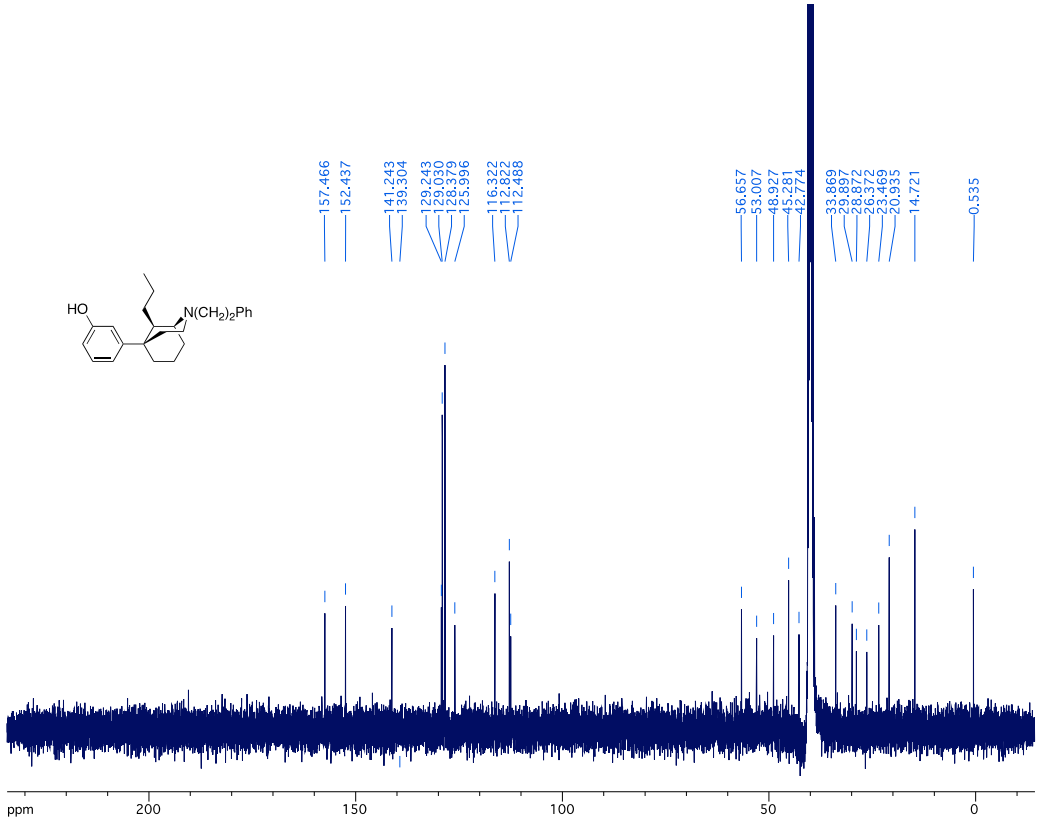
^{1}H and ^{13}C NMR spectra of **11**



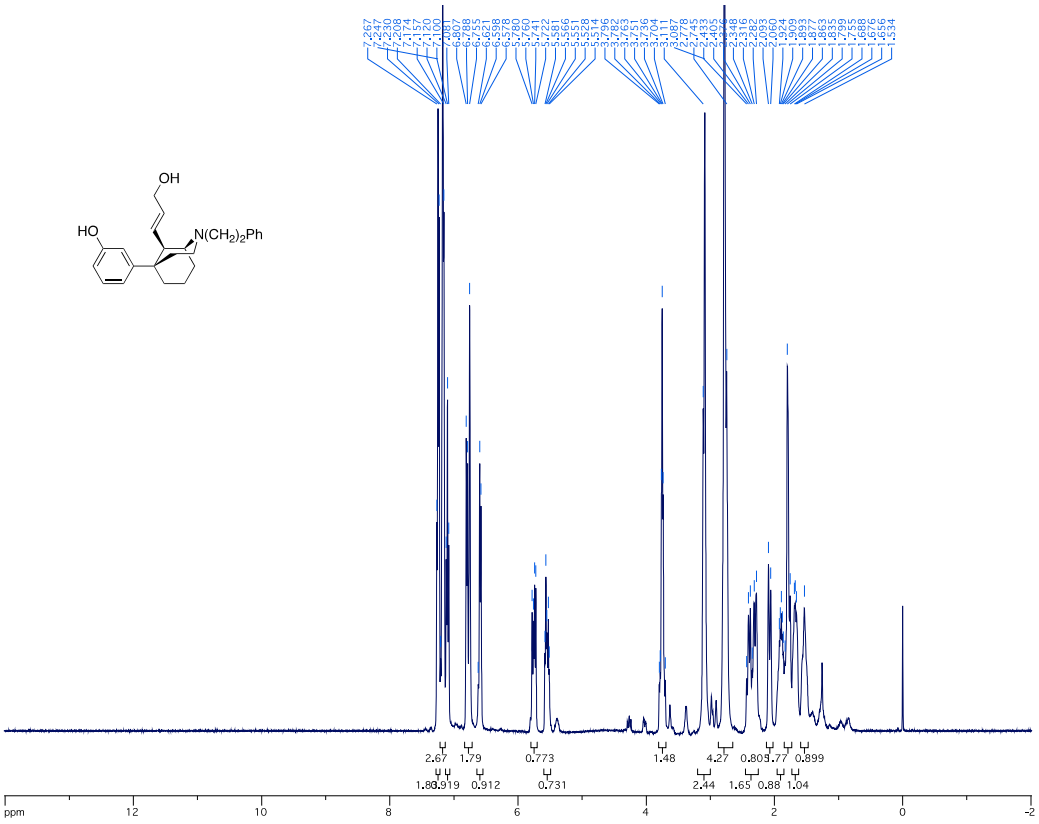


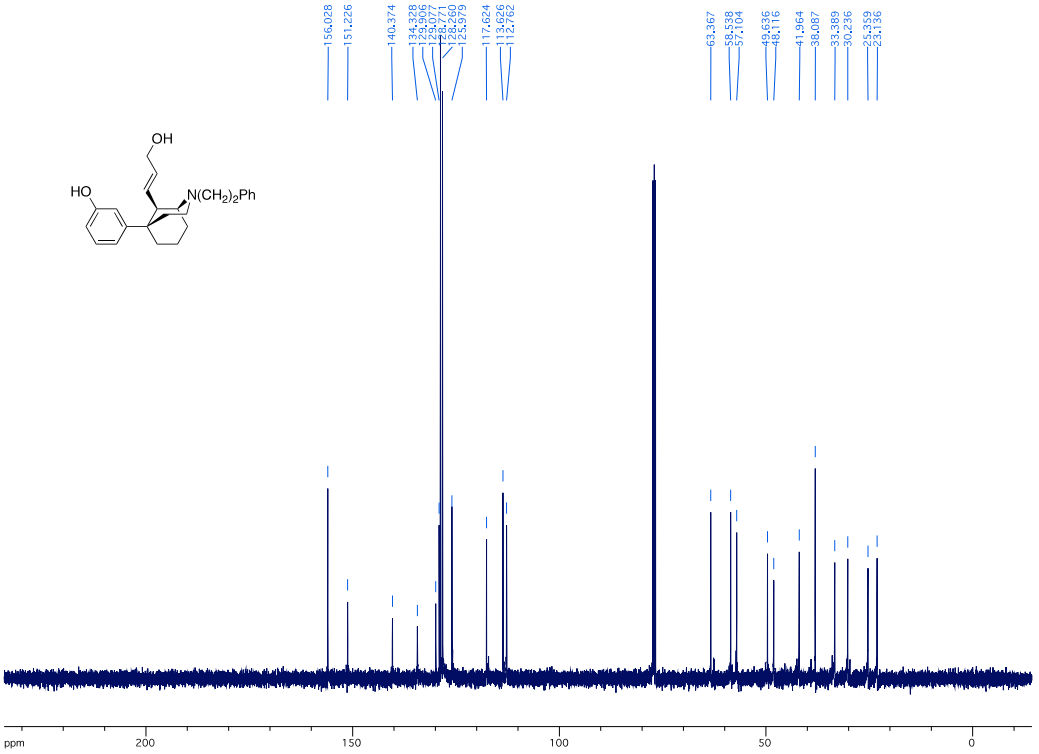
^{1}H and ^{13}C NMR spectra of **12**



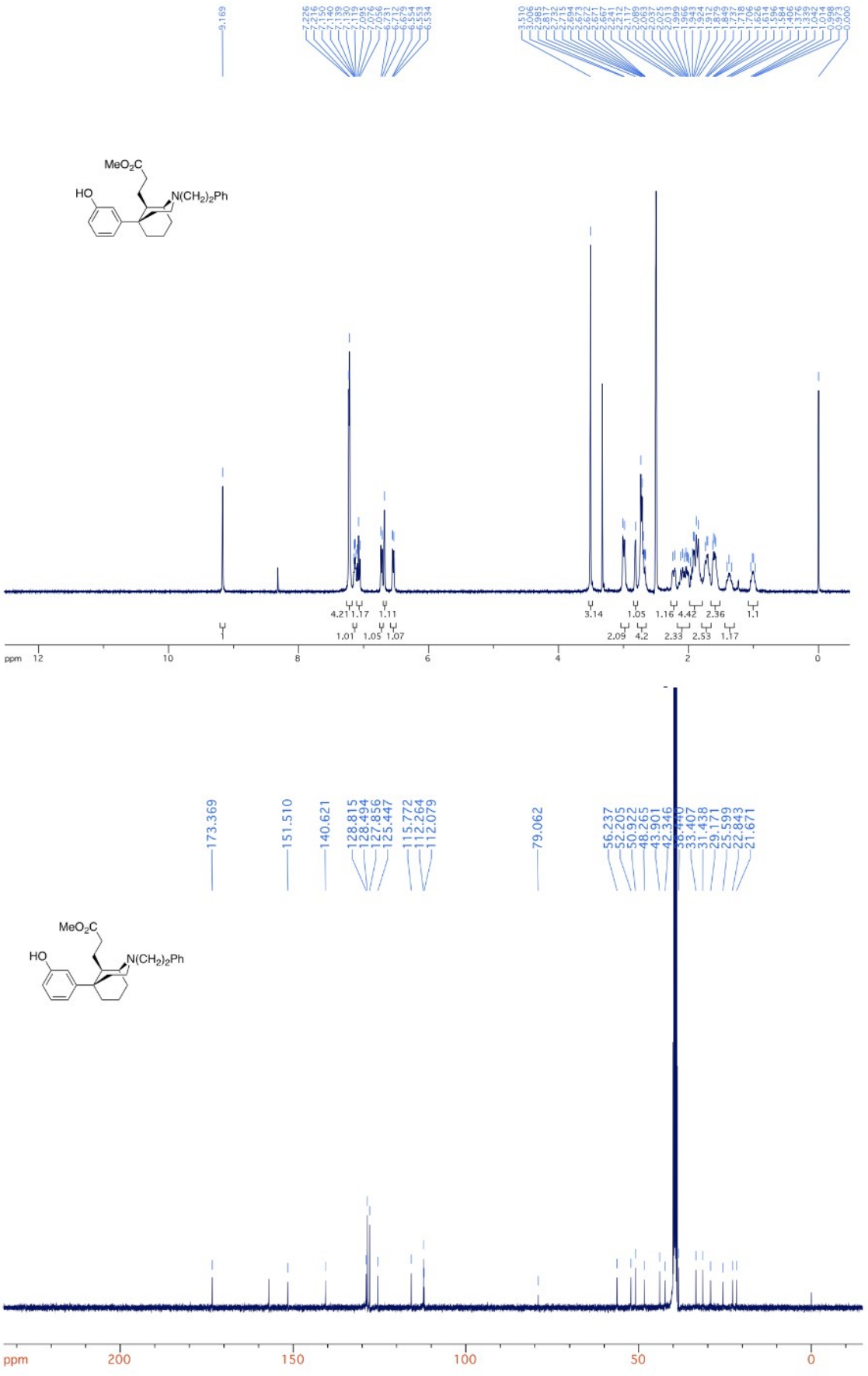


^{1}H and ^{13}C NMR spectra of **13**

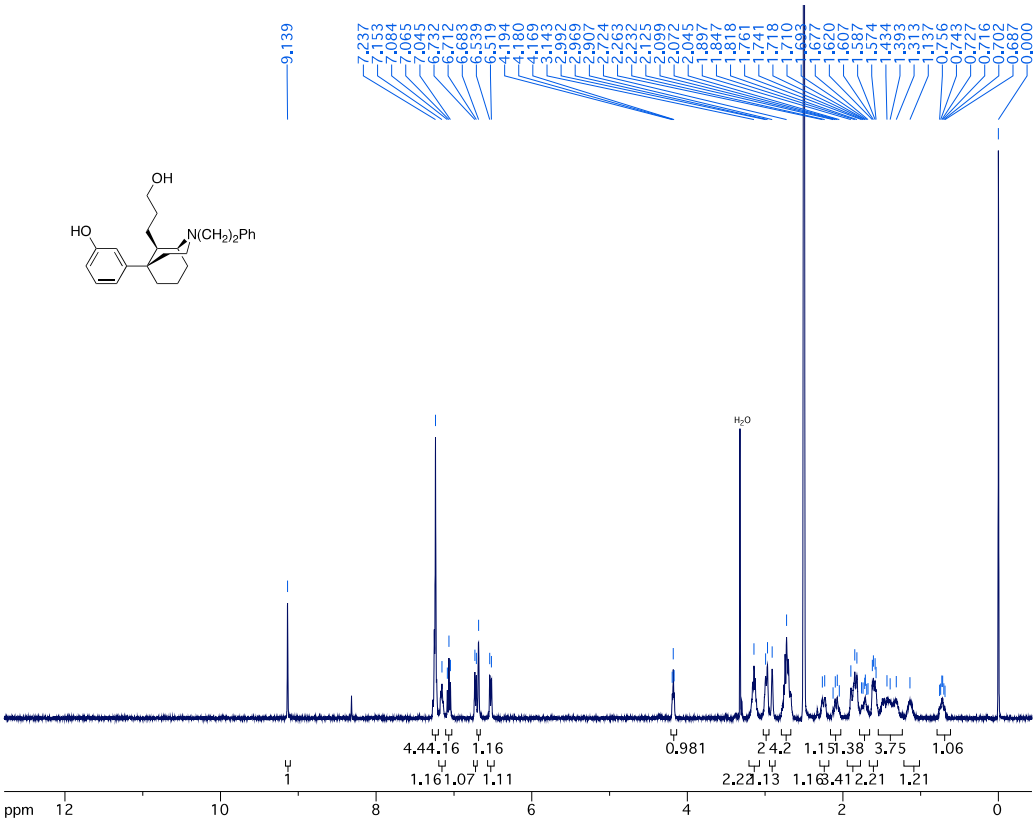


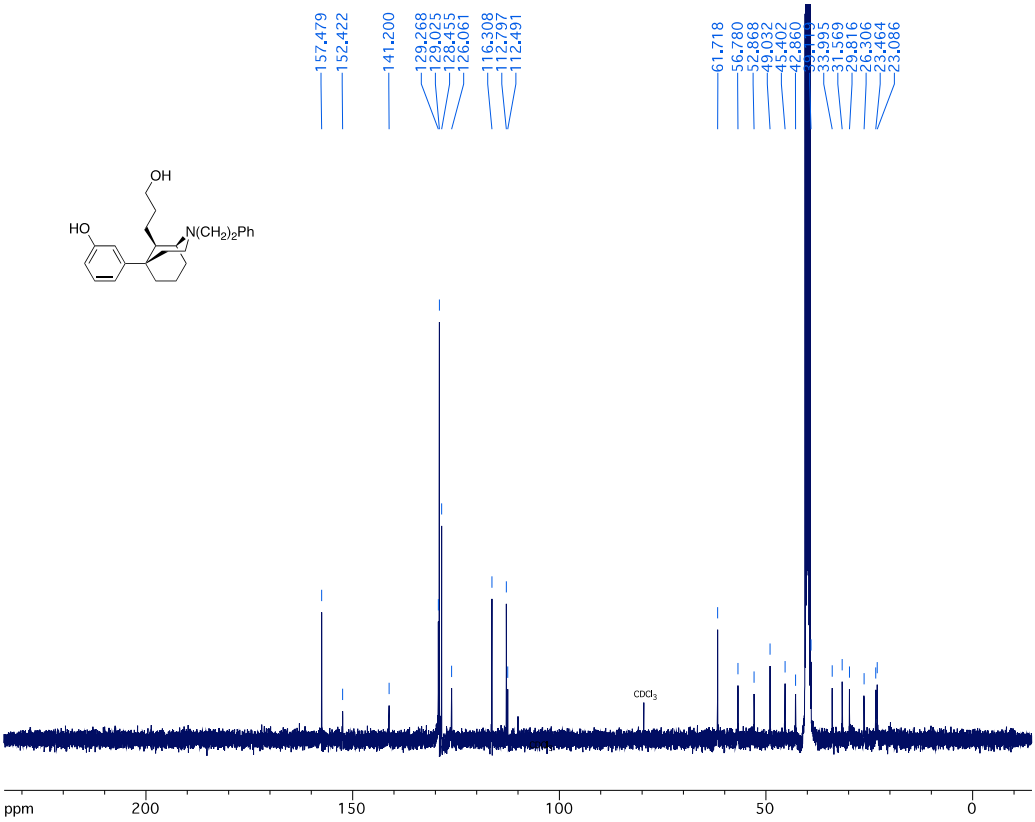


^{1}H and ^{13}C NMR spectra of **14**

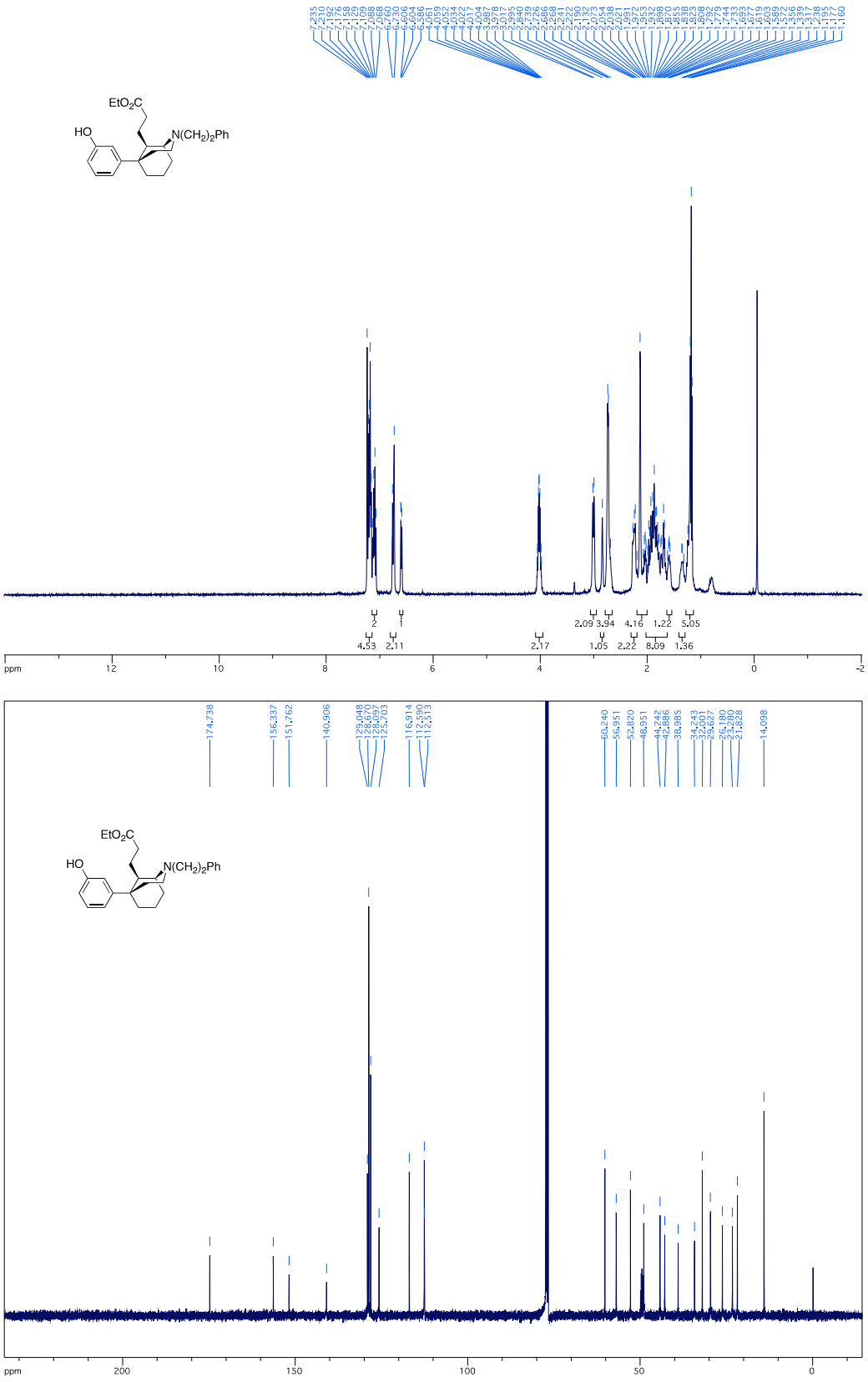


^{1}H and ^{13}C NMR spectra of **15**

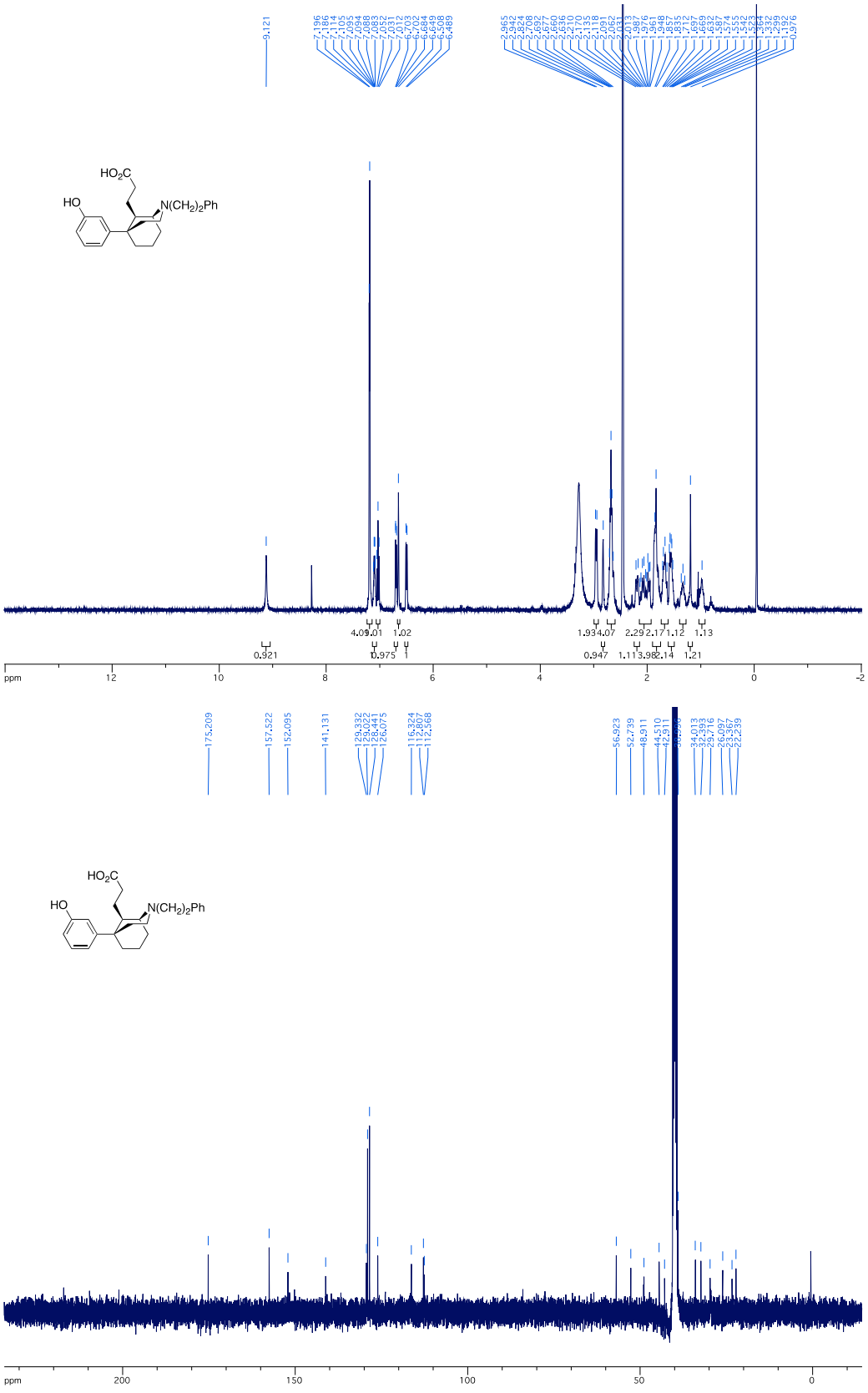




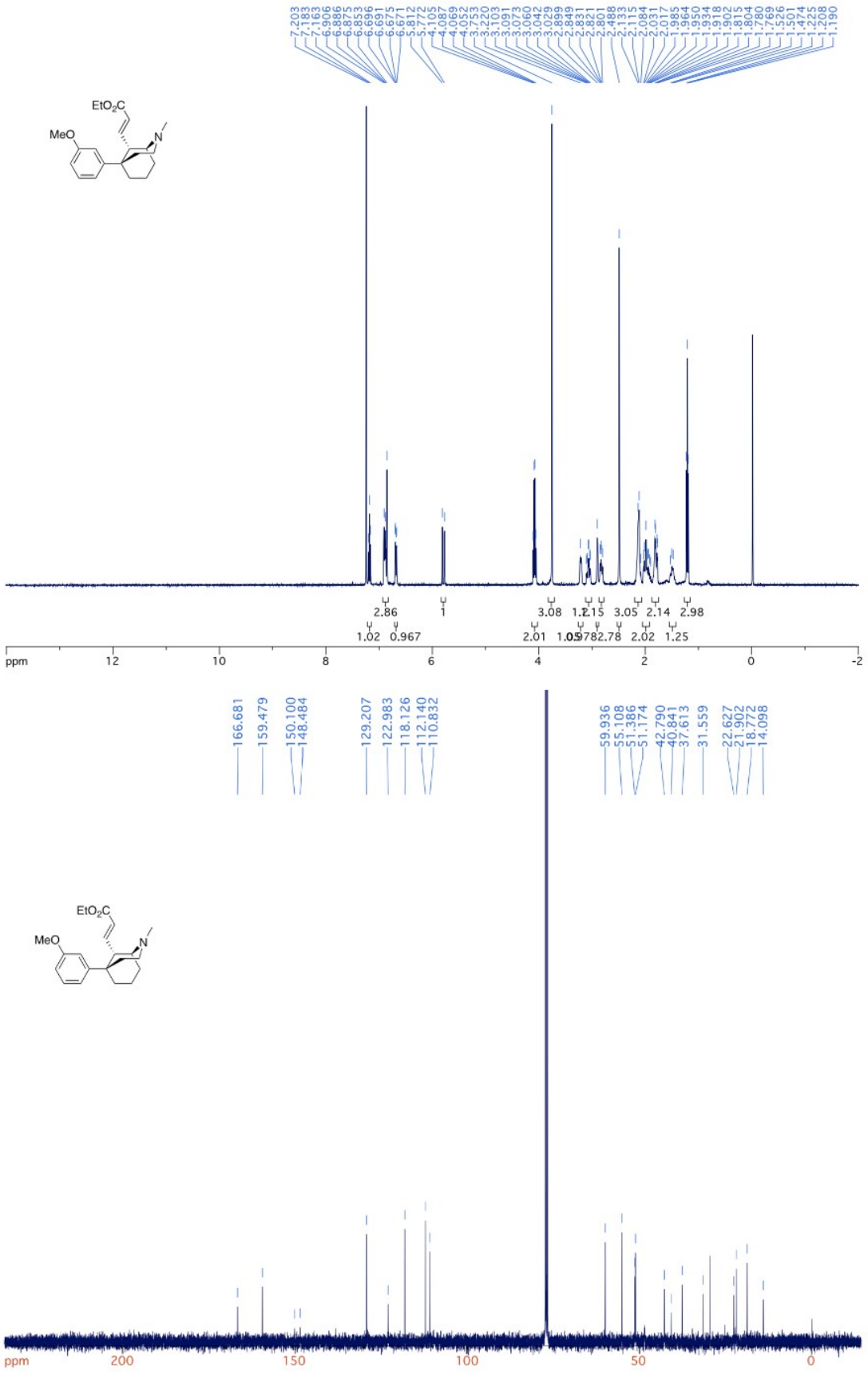
^{1}H and ^{13}C NMR spectra of **16**



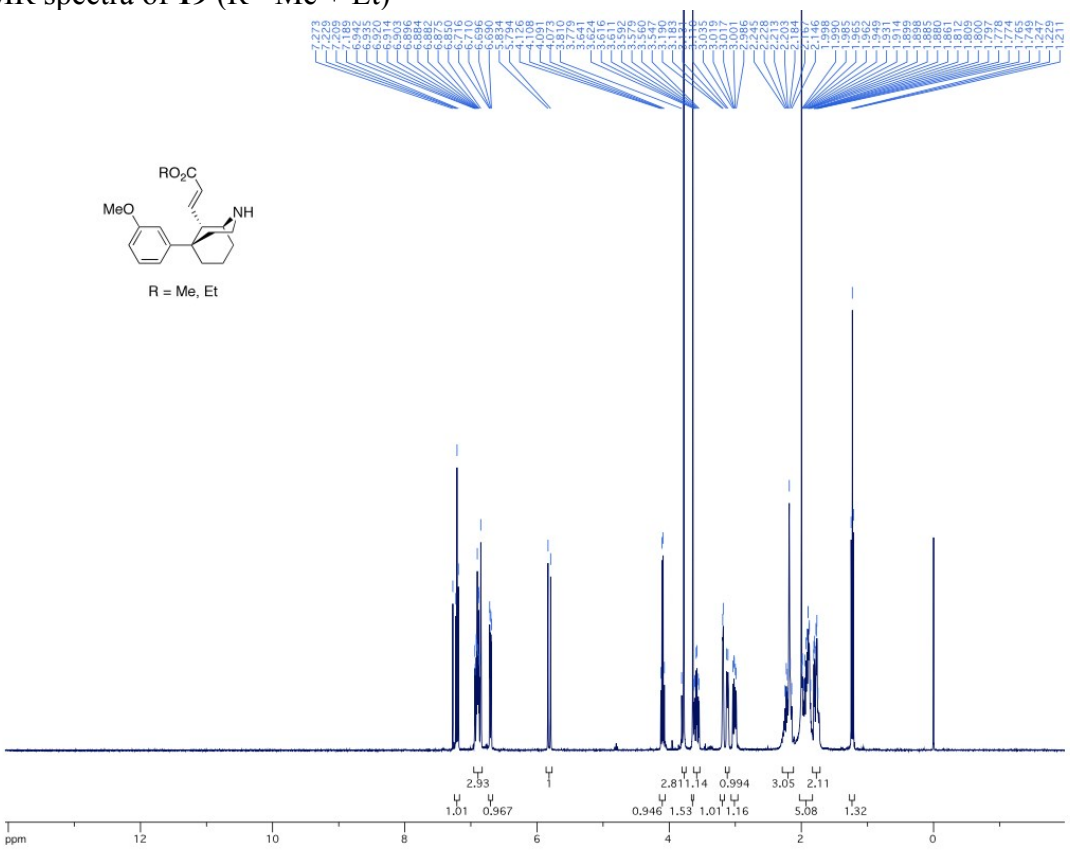
^{1}H and ^{13}C NMR spectra of **17**

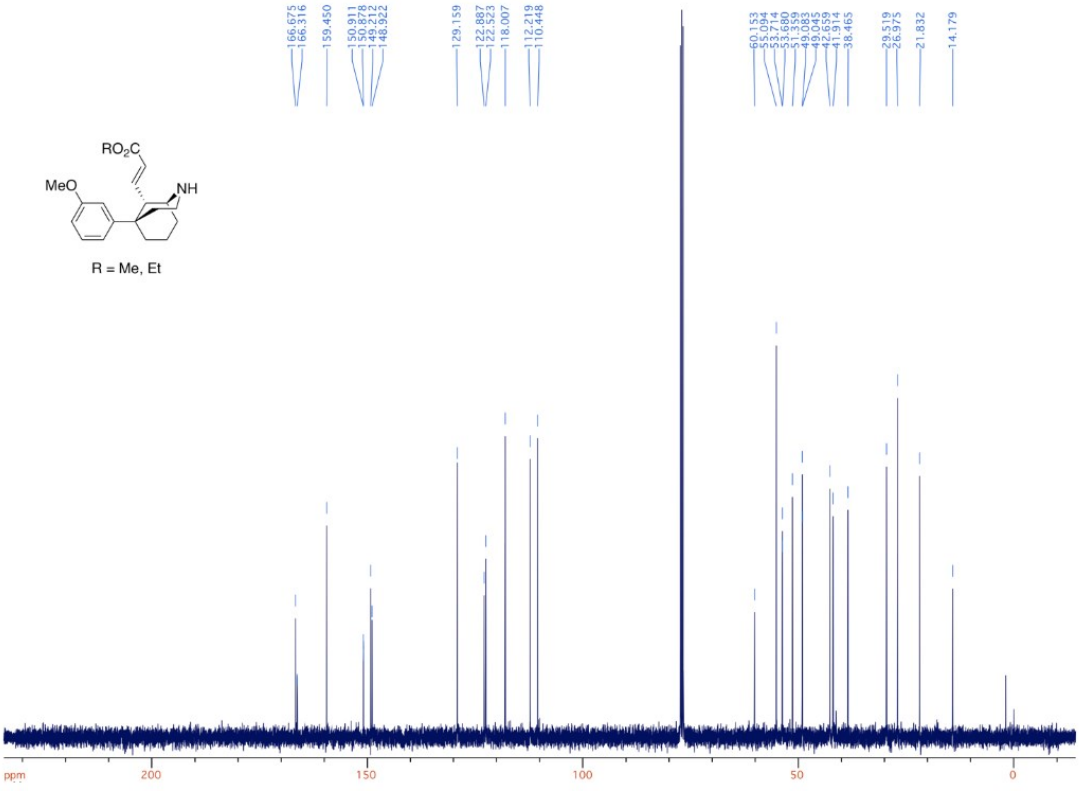


^{1}H and ^{13}C NMR spectra of **18**

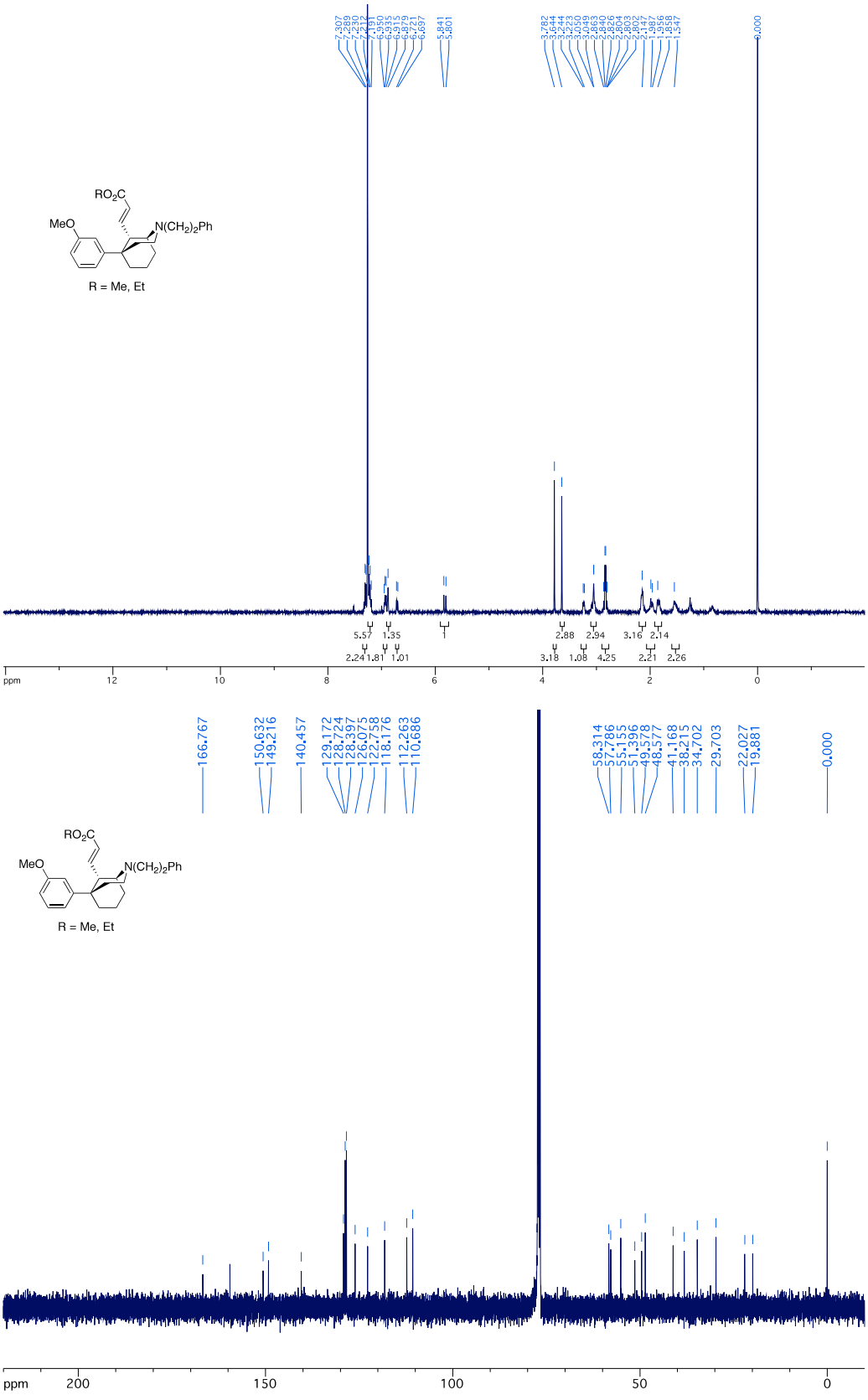


^{1}H and ^{13}C NMR spectra of **19** (R= Me + Et)





^{1}H and ^{13}C NMR spectra of the methyl ester in **20**



^{1}H and ^{13}C NMR spectra of **22**

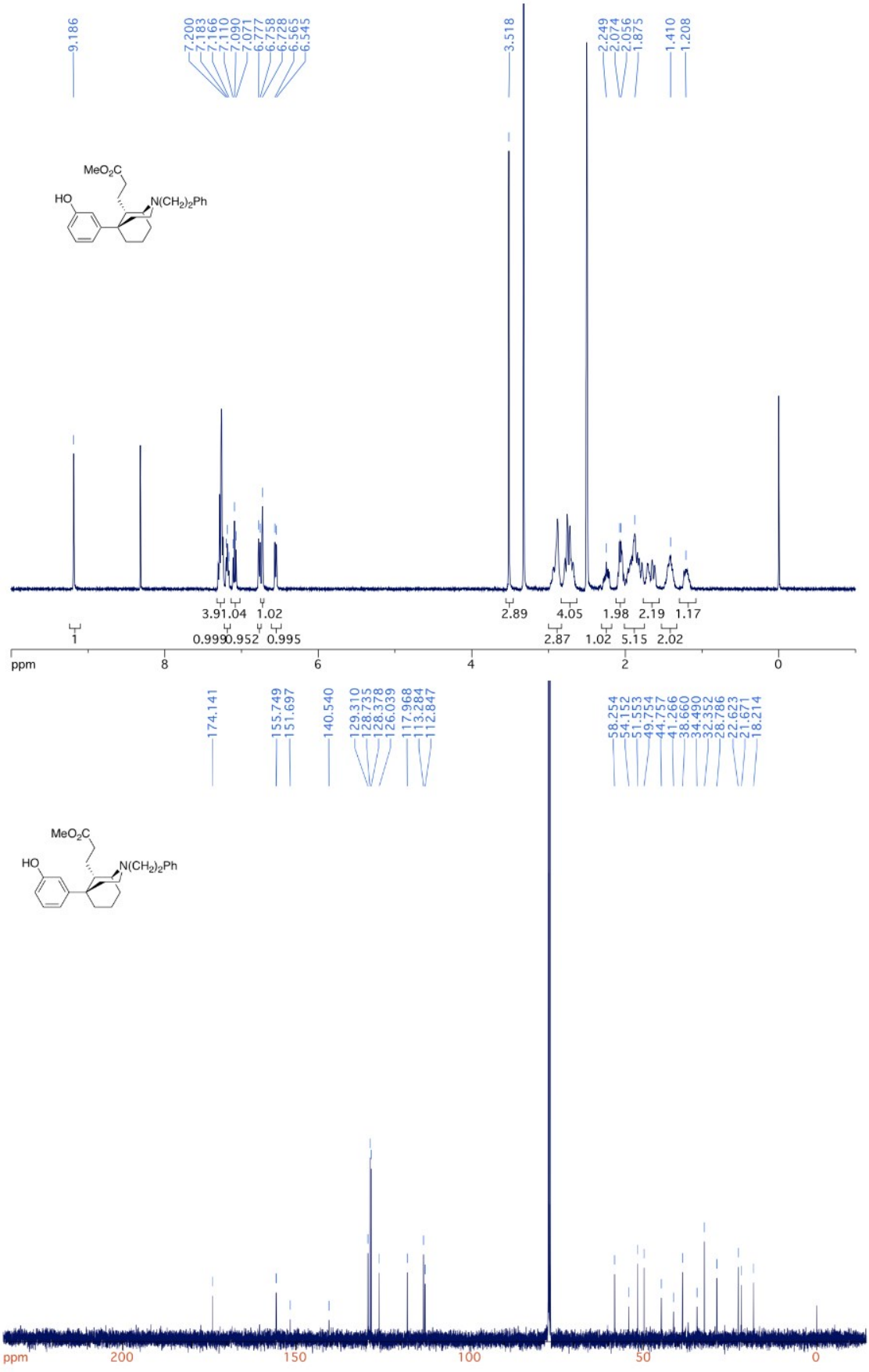


Table S1. Crystal data and structure refinement for **14b** (knih 121)

Identification code	knih121	
Empirical formula	$C_{26}H_{34}BrNO_3$	
Formula weight	488.45	
Temperature	296(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	$P2_12_12$	
Unit cell dimensions	a = 14.8241(8) Å	⟨= 90°.
	b = 17.2422(9) Å	®= 90°.
	c = 9.8923(5) Å	© = 90°.
Volume	2528.5(2) $Å^3$	
Z	4	
Density (20ºC)	1.283 Mg/m^3	
Absorption coefficient	1.651 mm^{-1}	
F(000)	1024	
Crystal size	0.193 x 0.159 x 0.114 mm^3	
θ range for data collection	1.812 to 29.540°.	
Index ranges	-20<=h<=20, -23<=k<=23, -13<=l<=13	
Reflections collected	27005	
Independent reflections	7026 [R(int) = 0.1118]	
Completeness to θ = 25.242°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	0.7459 and 0.6337	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7026 / 1 / 285	
Goodness-of-fit on F^2	0.960	
Final R indices [I>2σ(I)]	R1 = 0.0486, wR2 = 0.0882	
R indices (all data)	R1 = 0.1644, wR2 = 0.1179	
Absolute structure parameter	0.019(7)	
Largest diff. peak and hole	0.791 and -0.575 $e.Å^{-3}$	

Table S2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **14b**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	6452(3)	6855(3)	2766(6)	45(1)
N(2)	6625(3)	5984(2)	2704(5)	43(1)
C(3)	6909(4)	5635(3)	4020(6)	55(2)
C(4)	6376(4)	5921(3)	5224(6)	52(1)
C(5)	6192(3)	6799(3)	5270(5)	43(1)
C(6)	7088(4)	7269(4)	5487(6)	58(2)
C(7)	7758(4)	7245(4)	4312(7)	70(2)
C(8)	7315(4)	7324(3)	2931(7)	62(2)
C(9)	5782(3)	7029(3)	3904(5)	40(1)
C(10)	5580(3)	7014(3)	6453(5)	43(1)
C(11)	5273(3)	7771(3)	6590(5)	47(1)
O(12)	4429(3)	8728(2)	7858(5)	71(1)
C(12)	4746(3)	7998(3)	7687(6)	52(1)
C(13)	4517(4)	7461(3)	8660(6)	60(2)
C(14)	4812(4)	6714(3)	8539(6)	66(2)
C(15)	5333(4)	6487(3)	7455(6)	57(2)
C(16)	7254(3)	5731(3)	1604(6)	52(1)
C(17)	7030(4)	6057(3)	226(6)	59(2)
C(18)	7439(4)	5577(3)	-901(6)	56(2)
C(19)	8253(5)	5762(5)	-1475(9)	97(2)
C(20)	8604(6)	5320(6)	-2521(11)	118(4)
C(21)	8132(7)	4694(5)	-2984(9)	95(3)
C(22)	7326(6)	4504(4)	-2431(8)	80(2)
C(23)	6983(4)	4942(4)	-1398(6)	63(1)
C(24)	4833(3)	6691(3)	3687(5)	42(1)
C(25)	4323(4)	7035(3)	2521(6)	55(2)
O(26)	3087(3)	6208(2)	3032(5)	66(1)
C(26)	3412(4)	6684(3)	2330(6)	49(2)
O(27)	2991(2)	6973(2)	1238(4)	68(1)
C(27)	2097(4)	6682(4)	945(7)	79(2)
Br(1)	5000	5000	1327(1)	52(1)
Br(2)	0	5000	4303(1)	106(1)

Table S3. Bond lengths [Å] and angles [°] for **14b**.

C(1)-C(8)	1.520(7)	C(1)-N(2)	1.525(6)
C(1)-C(9)	1.532(7)	C(1)-H(1)	0.9800
N(2)-C(3)	1.495(7)	N(2)-C(16)	1.499(7)
N(2)-H(2)	0.9800	C(3)-C(4)	1.512(8)
C(3)-H(3A)	0.9700	C(3)-H(3B)	0.9700
C(4)-C(5)	1.540(7)	C(4)-H(4A)	0.9700
C(4)-H(4B)	0.9700	C(5)-C(10)	1.526(7)
C(5)-C(9)	1.534(7)	C(5)-C(6)	1.571(7)
C(6)-C(7)	1.530(8)	C(6)-H(6A)	0.9700
C(6)-H(6B)	0.9700	C(7)-C(8)	1.522(9)
C(7)-H(7A)	0.9700	C(7)-H(7B)	0.9700
C(8)-H(8A)	0.9700	C(8)-H(8B)	0.9700
C(9)-C(24)	1.537(7)	C(9)-H(9)	0.9800
C(10)-C(11)	1.388(7)	C(10)-C(15)	1.394(7)
C(11)-C(12)	1.393(7)	C(11)-H(11)	0.9300
O(12)-C(12)	1.353(6)	O(12)-H(12)	0.819(14)
C(12)-C(13)	1.378(8)	C(13)-C(14)	1.367(7)
C(13)-H(13)	0.9300	C(14)-C(15)	1.378(8)
C(14)-H(14)	0.9300	C(15)-H(15)	0.9300
C(16)-C(17)	1.511(8)	C(16)-H(16A)	0.9700
C(16)-H(16B)	0.9700	C(17)-C(18)	1.515(8)
C(17)-H(17A)	0.9700	C(17)-H(17B)	0.9700
C(18)-C(19)	1.370(9)	C(18)-C(23)	1.378(8)
C(19)-C(20)	1.386(11)	C(19)-H(19)	0.9300
C(20)-C(21)	1.366(10)	C(20)-H(20)	0.9300
C(21)-C(22)	1.355(11)	C(21)-H(21)	0.9300
C(22)-C(23)	1.369(8)	C(22)-H(22)	0.9300
C(23)-H(23)	0.9300	C(24)-C(25)	1.501(7)
C(24)-H(24A)	0.9700	C(24)-H(24B)	0.9700
C(25)-C(26)	1.492(8)	C(25)-H(25A)	0.9700
C(25)-H(25B)	0.9700	O(26)-C(26)	1.179(7)
C(26)-O(27)	1.344(7)	O(27)-C(27)	1.446(7)
C(27)-H(27A)	0.9600	C(27)-H(27B)	0.9600
C(27)-H(27C)	0.9600		

C(8)-C(1)-N(2)	112.7(4)	C(8)-C(1)-C(9)	111.3(5)
N(2)-C(1)-C(9)	109.3(4)	C(8)-C(1)-H(1)	107.8
N(2)-C(1)-H(1)	107.8	C(9)-C(1)-H(1)	107.8
C(3)-N(2)-C(16)	109.8(4)	C(3)-N(2)-C(1)	114.1(4)
C(16)-N(2)-C(1)	114.9(4)	C(3)-N(2)-H(2)	105.7
C(16)-N(2)-H(2)	105.7	C(1)-N(2)-H(2)	105.7
N(2)-C(3)-C(4)	114.0(4)	N(2)-C(3)-H(3A)	108.7
C(4)-C(3)-H(3A)	108.7	N(2)-C(3)-H(3B)	108.7
C(4)-C(3)-H(3B)	108.7	H(3A)-C(3)-H(3B)	107.6

Table 3. (continued).

C(3)-C(4)-C(5)	115.9(5)	C(3)-C(4)-H(4A)	108.3
C(5)-C(4)-H(4A)	108.3	C(3)-C(4)-H(4B)	108.3

C(5)-C(4)-H(4B)	108.3	H(4A)-C(4)-H(4B)	107.4
C(10)-C(5)-C(9)	112.1(4)	C(10)-C(5)-C(4)	111.6(4)
C(9)-C(5)-C(4)	107.4(4)	C(10)-C(5)-C(6)	105.8(4)
C(9)-C(5)-C(6)	108.8(4)	C(4)-C(5)-C(6)	111.2(4)
C(7)-C(6)-C(5)	115.5(5)	C(7)-C(6)-H(6A)	108.4
C(5)-C(6)-H(6A)	108.4	C(7)-C(6)-H(6B)	108.4
C(5)-C(6)-H(6B)	108.4	H(6A)-C(6)-H(6B)	107.5
C(8)-C(7)-C(6)	113.5(5)	C(8)-C(7)-H(7A)	108.9
C(6)-C(7)-H(7A)	108.9	C(8)-C(7)-H(7B)	108.9
C(6)-C(7)-H(7B)	108.9	H(7A)-C(7)-H(7B)	107.7
C(1)-C(8)-C(7)	114.4(5)	C(1)-C(8)-H(8A)	108.7
C(7)-C(8)-H(8A)	108.7	C(1)-C(8)-H(8B)	108.7
C(7)-C(8)-H(8B)	108.7	H(8A)-C(8)-H(8B)	107.6
C(1)-C(9)-C(5)	109.8(4)	C(1)-C(9)-C(24)	114.6(4)
C(5)-C(9)-C(24)	112.8(4)	C(1)-C(9)-H(9)	106.3
C(5)-C(9)-H(9)	106.3	C(24)-C(9)-H(9)	106.3
C(11)-C(10)-C(15)	117.2(5)	C(11)-C(10)-C(5)	119.9(5)
C(15)-C(10)-C(5)	122.8(5)	C(10)-C(11)-C(12)	121.6(5)
C(10)-C(11)-H(11)	119.2	C(12)-C(11)-H(11)	119.2
C(12)-O(12)-H(12)	109(4)	O(12)-C(12)-C(13)	116.8(5)
O(12)-C(12)-C(11)	123.6(5)	C(13)-C(12)-C(11)	119.5(5)
C(14)-C(13)-C(12)	119.6(5)	C(14)-C(13)-H(13)	120.2
C(12)-C(13)-H(13)	120.2	C(13)-C(14)-C(15)	121.1(5)
C(13)-C(14)-H(14)	119.5	C(15)-C(14)-H(14)	119.5
C(14)-C(15)-C(10)	121.0(5)	C(14)-C(15)-H(15)	119.5
C(10)-C(15)-H(15)	119.5	N(2)-C(16)-C(17)	114.2(4)
N(2)-C(16)-H(16A)	108.7	C(17)-C(16)-H(16A)	108.7
N(2)-C(16)-H(16B)	108.7	C(17)-C(16)-H(16B)	108.7
H(16A)-C(16)-H(16B)	107.6	C(16)-C(17)-C(18)	111.9(5)
C(16)-C(17)-H(17A)	109.2	C(18)-C(17)-H(17A)	109.2
C(16)-C(17)-H(17B)	109.2	C(18)-C(17)-H(17B)	109.2
H(17A)-C(17)-H(17B)	107.9	C(19)-C(18)-C(23)	118.0(6)
C(19)-C(18)-C(17)	122.0(6)	C(23)-C(18)-C(17)	120.0(5)
C(18)-C(19)-C(20)	120.8(7)	C(18)-C(19)-H(19)	119.6
C(20)-C(19)-H(19)	119.6	C(21)-C(20)-C(19)	119.5(8)
C(21)-C(20)-H(20)	120.2	C(19)-C(20)-H(20)	120.2
C(22)-C(21)-C(20)	120.5(8)	C(22)-C(21)-H(21)	119.7
C(20)-C(21)-H(21)	119.7	C(21)-C(22)-C(23)	119.7(7)
C(21)-C(22)-H(22)	120.2	C(23)-C(22)-H(22)	120.2
C(22)-C(23)-C(18)	121.5(6)	C(22)-C(23)-H(23)	119.2
C(18)-C(23)-H(23)	119.2	C(25)-C(24)-C(9)	114.7(4)

Table 3. (continued).

C(25)-C(24)-H(24A)	108.6	C(9)-C(24)-H(24A)	108.6
C(25)-C(24)-H(24B)	108.6	C(9)-C(24)-H(24B)	108.6
H(24A)-C(24)-H(24B)	107.6	C(26)-C(25)-C(24)	113.1(5)
C(26)-C(25)-H(25A)	109.0	C(24)-C(25)-H(25A)	109.0
C(26)-C(25)-H(25B)	109.0	C(24)-C(25)-H(25B)	109.0
H(25A)-C(25)-H(25B)	107.8	O(26)-C(26)-O(27)	122.8(6)
O(26)-C(26)-C(25)	125.4(6)	O(27)-C(26)-C(25)	111.8(5)

C(26)-O(27)-C(27)	117.3(5)	O(27)-C(27)-H(27A)	109.5
O(27)-C(27)-H(27B)	109.5	H(27A)-C(27)-H(27B)	109.5
O(27)-C(27)-H(27C)	109.5	H(27A)-C(27)-H(27C)	109.5
H(27B)-C(27)-H(27C)	109.5		

Table S4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **14b**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[h^2 a^{*2} U^{11} + ... + 2\ h\ k\ a^*\ b^*\ U^{12}]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	51(3)	34(3)	50(4)	-2(3)	8(3)	-4(2)
N(2)	45(3)	35(2)	47(3)	-6(2)	6(2)	-3(2)
C(3)	69(4)	44(3)	53(4)	-7(3)	-8(3)	10(3)
C(4)	64(4)	47(3)	45(4)	0(3)	-9(3)	5(3)
C(5)	52(3)	38(3)	40(3)	-7(2)	-2(2)	0(2)
C(6)	44(3)	68(4)	62(4)	-19(3)	-2(3)	-4(3)
C(7)	55(4)	74(4)	83(5)	-32(4)	12(4)	-23(3)
C(8)	60(4)	49(3)	77(5)	-19(3)	20(4)	-20(3)
C(9)	42(3)	30(2)	48(4)	-1(2)	6(2)	-4(2)
C(10)	46(3)	45(3)	37(3)	-1(3)	-4(2)	-3(2)
C(11)	51(3)	48(3)	40(3)	-3(2)	2(2)	-4(2)
O(12)	82(3)	61(3)	71(3)	-7(2)	27(2)	9(2)
C(12)	49(4)	58(3)	48(4)	-3(3)	0(3)	2(3)
C(13)	54(3)	80(4)	46(4)	-4(4)	6(3)	7(3)
C(14)	74(5)	73(4)	51(4)	14(3)	10(4)	-7(3)
C(15)	69(4)	54(3)	47(4)	2(3)	-1(3)	1(3)
C(16)	46(3)	52(3)	57(4)	-16(3)	5(3)	-2(2)
C(17)	67(4)	52(3)	58(4)	-7(3)	16(3)	-6(3)
C(18)	61(4)	50(3)	56(4)	0(3)	12(3)	3(3)
C(19)	85(5)	101(5)	104(6)	-27(5)	41(5)	-16(4)
C(20)	102(7)	126(8)	126(9)	-27(6)	55(6)	-4(6)
C(21)	129(8)	84(5)	73(6)	-10(4)	27(6)	42(5)
C(22)	116(6)	54(4)	70(5)	-10(4)	3(5)	14(4)
C(23)	80(3)	49(3)	61(3)	-5(4)	10(3)	10(4)
C(24)	50(4)	36(2)	39(3)	-1(2)	1(3)	-2(2)
C(25)	57(4)	53(3)	55(4)	9(3)	-5(3)	-1(3)
O(26)	56(3)	51(2)	90(4)	12(2)	-8(2)	0(2)
C(26)	50(3)	45(3)	50(4)	-4(3)	1(3)	10(3)
O(27)	55(2)	90(3)	58(3)	10(2)	-8(2)	-1(2)
C(27)	60(4)	101(5)	78(5)	-8(4)	-14(3)	6(4)
Br(1)	57(1)	47(1)	52(1)	0	0	-13(1)
Br(2)	176(1)	79(1)	64(1)	0	0	-6(1)

Table S5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (Å^{2}x 10^3) for **14b**.

	x	y	z	U(eq)
H(1)	6172	7011	1911	54
H(2)	6041	5748	2489	51
H(3A)	7542	5749	4167	66
H(3B)	6847	5076	3961	66
H(4A)	6698	5777	6039	62
H(4B)	5801	5652	5237	62
H(6A)	6932	7806	5661	70
H(6B)	7386	7071	6289	70
H(7A)	8192	7661	4424	84
H(7B)	8086	6758	4344	84
H(8A)	7179	7866	2774	74
H(8B)	7742	7161	2245	74
H(9)	5710	7594	3926	48
H(11)	5423	8135	5933	56
H(12)	4650(40)	9010(30)	7280(50)	78
H(13)	4164	7607	9395	72
H(14)	4660	6353	9198	79
H(15)	5523	5974	7391	68
H(16A)	7245	5169	1553	62
H(16B)	7862	5887	1843	62
H(17A)	7254	6584	166	70
H(17B)	6380	6072	116	70
H(19)	8573	6189	-1159	116
H(20)	9157	5450	-2905	142
H(21)	8366	4396	-3685	114
H(22)	7007	4077	-2752	96
H(23)	6430	4808	-1022	76
H(24A)	4888	6136	3544	50
H(24B)	4484	6768	4506	50
H(25A)	4256	7588	2668	66
H(25B)	4672	6964	1701	66
H(27A)	1890	6370	1686	119
H(27B)	2115	6374	138	119
H(27C)	1692	7110	816	119

Table S6. Torsion angles [°] for **14b**.

C(8)-C(1)-N(2)-C(3)	70.9(6)	C(9)-C(1)-N(2)-C(3)	-53.4(6)
C(8)-C(1)-N(2)-C(16)	-57.2(7)	C(9)-C(1)-N(2)-C(16)	178.5(4)
C(16)-N(2)-C(3)-C(4)	173.9(5)	C(1)-N(2)-C(3)-C(4)	43.3(6)
N(2)-C(3)-C(4)-C(5)	-43.3(7)	C(3)-C(4)-C(5)-C(10)	174.9(5)
C(3)-C(4)-C(5)-C(9)	51.6(6)	C(3)-C(4)-C(5)-C(6)	-67.3(6)
C(10)-C(5)-C(6)-C(7)	-171.1(5)	C(9)-C(5)-C(6)-C(7)	-50.5(6)
C(4)-C(5)-C(6)-C(7)	67.6(7)	C(5)-C(6)-C(7)-C(8)	42.1(7)
N(2)-C(1)-C(8)-C(7)	-70.3(7)	C(9)-C(1)-C(8)-C(7)	53.0(7)
C(6)-C(7)-C(8)-C(1)	-42.7(7)	C(8)-C(1)-C(9)-C(5)	-61.8(6)
N(2)-C(1)-C(9)-C(5)	63.3(5)	C(8)-C(1)-C(9)-C(24)	170.0(5)
N(2)-C(1)-C(9)-C(24)	-64.9(6)	C(10)-C(5)-C(9)-C(1)	175.8(4)
C(4)-C(5)-C(9)-C(1)	-61.3(5)	C(6)-C(5)-C(9)-C(1)	59.1(5)
C(10)-C(5)-C(9)-C(24)	-55.0(5)	C(4)-C(5)-C(9)-C(24)	67.9(5)
C(6)-C(5)-C(9)-C(24)	-171.7(4)	C(9)-C(5)-C(10)-C(11)	-53.7(6)
C(4)-C(5)-C(10)-C(11)	-174.2(4)	C(6)-C(5)-C(10)-C(11)	64.7(6)
C(9)-C(5)-C(10)-C(15)	128.7(5)	C(4)-C(5)-C(10)-C(15)	8.2(7)
C(6)-C(5)-C(10)-C(15)	-112.8(5)	C(15)-C(10)-C(11)-C(12)	0.5(7)
C(5)-C(10)-C(11)-C(12)	-177.2(4)	C(10)-C(11)-C(12)-O(12)	-179.7(5)
C(10)-C(11)-C(12)-C(13)	-0.3(8)	O(12)-C(12)-C(13)-C(14)	179.6(5)
C(11)-C(12)-C(13)-C(14)	0.2(8)	C(12)-C(13)-C(14)-C(15)	-0.2(9)
C(13)-C(14)-C(15)-C(10)	0.4(9)	C(11)-C(10)-C(15)-C(14)	-0.5(7)
C(5)-C(10)-C(15)-C(14)	177.1(5)	C(3)-N(2)-C(16)-C(17)	-179.6(4)
C(1)-N(2)-C(16)-C(17)	-49.4(6)	N(2)-C(16)-C(17)-C(18)	-159.0(5)
C(16)-C(17)-C(18)-C(19)	-95.0(7)	C(16)-C(17)-C(18)-C(23)	86.2(7)
C(23)-C(18)-C(19)-C(20)	-0.1(11)	C(17)-C(18)-C(19)-C(20)	-178.9(7)
C(18)-C(19)-C(20)-C(21)	0.0(14)	C(19)-C(20)-C(21)-C(22)	0.1(14)
C(20)-C(21)-C(22)-C(23)	-0.2(12)	C(21)-C(22)-C(23)-C(18)	0.1(10)
C(19)-C(18)-C(23)-C(22)	0.1(9)	C(17)-C(18)-C(23)-C(22)	178.9(6)
C(1)-C(9)-C(24)-C(25)	-66.1(6)	C(5)-C(9)-C(24)-C(25)	167.2(4)
C(9)-C(24)-C(25)-C(26)	179.1(4)	C(24)-C(25)-C(26)-O(26)	2.9(8)
C(24)-C(25)-C(26)-O(27)	-176.7(4)	O(26)-C(26)-O(27)-C(27)	0.7(8)
C(25)-C(26)-O(27)-C(27)	-179.7(5)		

Table S7. Hydrogen bonds for **14b** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(2)-H(2)...Br(1)	0.98	2.32	3.246(4)	158.1
C(3)-H(3B)...O(26)#1	0.97	2.40	3.324(7)	159.4
O(12)-H(12)...Br(2)#2	0.819(14)	2.373(19)	3.178(4)	168(6)
C(16)-H(16B)...O(12)#3	0.97	2.43	3.398(7)	172.8

Symmetry transformations used to generate equivalent atoms:
#1 -x+1,-y+1,z #2 -x+1/2,y+1/2,-z+1 #3 x+1/2,-y+3/2,-z+1

Cell lines and cell culture

The following HitHunter CHO-K1 cell lines stably expressing human opioid receptors were purchased from DiscoverX: human μ-opioid receptor (OPRM1), human κ-opioid receptor (OPRK1), and human δ-opioid receptor (OPRD1). HitHunter cells were maintained using Ham's F-12 media containing 10% fetal bovine serum (Life Technologies), 1% penicillin/streptomycin/L-glutamine (Life Technologies), and 800 μg/mL geneticin (Mirus Bio). PathHunter CHO-K1 cell line stably expressing the human μ-opioid receptor β-arrestin-2 EFC was also purchased from DiscoverX. PathHunter cells were maintained using Ham's F-12 media containing 10% fetal bovine serum (Life Technologies), 1% penicillin/streptomycin/L-glutamine (Life Technologies), 800 μg/mL geneticin, and 250 μg/mL hygromycin B (Mirus Bio). All cell lines were grown in a humidified incubator with 5% CO_2 at 37 °C and were passaged as needed once they reached 80-90% confluency using trypsin-EDTA (0.05%) with phenol red (Gibco).

Forskolin-induced cAMP accumulation assay

On day 1, human MOR, KOR, or DOR-expressing CHO-K1 cells were grown to approximately 80% confluency. Cells were detached from tissue culture plates using nonenzymatic dissociation buffer (Invitrogen) and incubated in a humidified incubator at 37 ^{o}C and 5% CO_2 until cells appeared to detach under microscope viewing. Cells were then counted using a hemocytometer and plated at 10000 cells/well in white 384-well plates in 20 uL/well Cell Plating 2 media (DiscoverX). Plates were placed in a humidified incubator at 37 ^{o}C and 5% CO_2 for at least 24 h.

On day 2, 5 mM stock solutions of compounds and controls were made using 100% DMSO (Alfa Aesar). A serial dilution of 10 concentrations was made for each compound at a 100X concentration using 100% DMSO. Assay buffer containing Hank's Balanced Salt Solution (Invitrogen), 10 mM HEPES (Invitrogen), and 100 uM forskolin were used to dilute the 100X stock to 5X. The HitHunter cAMP Accumulation Assay was then used according to manufacturer's instructions. An antibody solution from the

assay kit was made by diluting the provided antibody stock solution with assay buffer (Hank's Balanced Salt Solution and 10 mM HEPES). Plating media was removed from assay plate and cells were rinsed with 10 uL of assay buffer. 20 uL of antibody solution was added to each well. Cells were then treated with 5 uL of the 5X stock solutions and incubated in a humidified incubator for 30 min at 37 ^{o}C and 5% CO_2. Detection reagents were then mixed and added to the assay plate as described according to manufacturer's instructions. Assay plate was protected from light and incubated at room temperature overnight for approximately 16 h.

On day 3, luminescence was quantified using a BioTek Cytation 5 plate reader and Gen 5 software. Data were exported to an Excel sheet for processing and analysis.

MOR-mediated B-arrestin Recruitment Assay

On day 1, human μ-opioid receptor β-arrestin-2 EFC cells (DiscoverX) were grown to approximately 80% confluency. Cells were detached from tissue culture plates using nonenzymatic dissociation buffer (Invitrogen) and incubated in a humidified incubator at 37 ^{o}C and 5% CO_2 until cells appeared to detach under microscope viewing. Cells were then counted using a hemocytometer and plated at 10000 cells/well in white 384-well plates in 20 uL/well Cell Plating 2 media (DiscoverX). Plates were placed in a humidified incubator at 37 ^{o}C and 5% CO_2 for at least 24 h.

On day 2, 5 mM stock solutions of compounds and controls were made using 100% DMSO (Alfa Aesar). A serial dilution of 11 concentrations were made for each compound at a 100X concentration using 100% DMSO. Assay buffer containing Hank's Balanced Salt Solution (Invitrogen), 10 mM HEPES (Invitrogen) was used to dilute the 100X stock to 5X. The PathHunter Assay was then used according to manufacturer's instructions. An antibody solution from the assay kit was made by diluting the provided antibody stock solution with assay buffer. Plating media was removed from assay plate and cells were rinsed with 10 uL of assay buffer. 20 uL of antibody solution was added to each well. Cells were then treated with 5 uL of the 5X stock solutions and incubated in a humidified incubator for 90 min at 37 ^{o}C and 5% CO_2. Detection reagents were then mixed and added to the assay plate as described according to manufacturer's

instructions. Plate was incubated at room temperature for 1 h and protected from light. Luminescence was quantified using a BioTek Cytation 5 plate reader and Gen 5 software. Data were exported to an Excel sheet for processing and analysis.

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