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Experimental Section

General. IR spectra were obtained using Nicolet 5DX or Magna spectrometers; ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300AC spectrometer. Mass spectral analyses were performed on a Hewlett-Packard 5890A gas chromatograph with a mass sensitive detector and HRMS data were obtained in the Mass Spectrometry Laboratory, School of Chemical Sciences, University of Illinois. Ether and THF were distilled from Na-benzophenone ketyl immediately before use, and other solvents were purified using standard procedures. Column chromatography was carried out on Universal silica gel (32-63μ) using the indicated solvents as eluents. All new compounds were homogeneous to TLC and ^{13}C NMR.

3-(1,1-Dimethylheptyl)methoxybenzene. To a stirred suspension of 20.0 g (667 mmol) of NaH (80%) in 100 mL of freshly distilled DMF was added dropwise at room temperature 81.0 mL (894 mmol) of 1-propanethiol. The resulting mixture was stirred until the solution became clear and 29.1 g (110 mmol) of 1,3-Dimethoxy-5-(1,1-dimethylheptyl)benzene (**4**) in 30 mL of dry DMF was added dropwise. The mixture was heated at 120 °C for 3 h, cooled to room temperature, poured into 10% aqueous HCl and extracted with ether. The ethereal layer was washed with successive portions of aqueous $NaHCO_3$, and brine and dried ($MgSO_4$). Concentration afforded 27.7 g (100%) of an oil which was used directly for the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.6 Hz, 3H), 0.97-1.11 (m, 2H), 1.11-1.33 (m, 6H), 1.22(s, 6H), 1.47-1.58 (m, 2H), 3.76 (s, 3H), 5.56 (s, 1H), 6.25 (dd, J=2.1, 2.1 Hz, 1H), 6.42 (dd, J=2.1, 1,8 Hz, 1H) 6.47 (dd, J=1.9, 1.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.6, 24.6, 28.8, 30.0, 31.7, 37.8, 44.5, 55.2, 97.9, 105.0, 105.7, 153.0, 156.3, 160.4. A solution of 26.6 g (106 mmol) of the phenol in 19 mL of CCl_4 was cooled to 0 °C with stirring, and 16.1 mL (125 mmol) of diethyl phosphite was added dropwise, followed by the dropwise addition of 17.1 mL of Et_3N. The mixture was stirred at 0 °C for 1 h, and at ambient temperature overnight, diluted with CH_2Cl_2, washed with water, 10% aqueous

NaOH, water, 10% HCl, water, dried ($MgSO_4$), and the solvent was removed *in vacuo*. The crude product was used in the next step without further purification: ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.6 Hz, 3H), 0.96-1.12 (m, 2H), 1.12-1.23 (m, 6H), 1.25 (s, 6H), 1.35 (dt, J=7.2, 0.8 Hz, 6H), 1.50-1.62 (m, 2H), 3.79 (s, 3H), 4.15-4.37 (m, 4H), 6.63 (s, 1H), 6.69 (s, 1H), 6.77 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 15.9, 16.0, 22.5, 24.5, 28.7, 29.8, 31.6, 37.8, 44.3, 55.1, 64.3, 64.4, 102.1, 109.3, 110.0, 151.2, 152.6, 160.0.

To 250 mL of liquid NH_3 at -78 °C was added 2.60 g (371 mg atoms) of Li. The reaction mixture was stirred for 10 min, and 45.4 g (110 mmol) of the phosphate ester in 90 mL of dry ether and 18 mL of dry THF was added dropwise. The dark blue solution was stirred at -78 °C for 1.5 h, then quenched by the addition of solid NH_4Cl. The NH_3 was evaporated and the solid residue was taken up into water and extracted into ether. The ethereal layers were washed with 10% HCl, and brine, dried ($MgSO_4$) and the solvent removed *in vacuo*, to afford an oil, which after distillation gave 17.5 g (68% from 1,3-dimethoxy-5-(1,1-dimethylheptyl)benzene) of pale yellow oil: b.p. 130 °C/0.05 mm Hg; ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.6 Hz, 3H), 0.98-1.12 (m, 2H), 1.12-1.26 (m, 6H), 1.31 (s, 6H), 1.52-1.62 (m, 2H), 3.80 (s, 3H), 6.71 (m, 1H), 6.88 (m, 1H), 6.92 (m, 1H), 7.21 (m, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 22.7, 24.7, 29.0, 30.0, 31.8, 37.7, 44.6, 55.0, 109.7, 112.6, 118.4, 128.8, 151.7, 159.3; Anal. Calcd for $C_{16}H_{26}O$: C, 81.99; H, 11.18; Found: C, 82.09; H, 11.16.

2-Bromo-5-(1,1-dimethylheptyl)methoxybenzene (5). To a stirred solution of 6.42g (27.5 mmol) of 3-(1,1-dimethylheptyl)methoxybenzene in 5 mL of glacial acetic acid at 0 °C was added dropwise 1.41 mL (27.4 mmol) of Br_2 in 5 mL of acetic acid over a period of 15 min. After stirring at 0 °C for 10 min, the mixture was warmed to ambient temperature and stirred for 2 h, diluted with water and extracted with ether. The ethereal extracts were washed successively with water, saturated $NaHCO_3$, brine, dried ($MgSO_4$) and the solvent removed *in vacuo* to afford an oil which after distillation gave 7.84 g (84%) of **5** as a pale yellow oil : b.p. 170°/0.05 mm Hg; ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.5 Hz, 3H), 0.99-1.11 (m, 2H), 1.11-1.25 (m, 6H),

1.27 (s, 6H), 1.51-1.68 (m, 2H), 3.87 (s, 3H), 6.79 (dd, J=8.3, 2.2 Hz, 2H), 7.41 (d, J=8.2 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.6, 24.6, 28.9, 29.9, 31.7, 37.9, 44.5, 56.0, 108.3, 110.1, 119.8, 132.5, 150.9, 155.4; Anal. Calcd for $C_{16}H_{25}BrO$: C, 61.34; H, 8.04; Found: C, 61.45; H, 8.05.

4-(2-Methoxy-4-[1,1-dimethylheptyl]phenyl)-6,6-dimethylbicyclo[3.1.1] hept-3-en-2-one (6). To a stirred solution of 4.85g (15.5 mmol) of 2-bromo-5-(1,1-dimethyl-heptyl)methoxybenzene (**5**) in 15 mL of dry THF at 0 °C was added dropwise 16 mL (15.7 mmol) of 0.98 M BuLi in hexane. The reaction mixture was stirred for 0.5 h at 0 °C, and 2.14 g (15.7 mmol) of apoverbenone in 5 mL of dry THF was added dropwise. The mixture was allowed to warm to room temperature and stirred overnight, then quenched with saturated NH_4Cl and extracted with ether. The ethereal extracts were washed with brine, dried ($MgSO_4$), and the solvent was removed *in vacuo*. The residue was dissolved in dry CH_2Cl_2, and the solution was added dropwise to a suspension of 18 g of PDC in 60 mL of dry CH_2Cl_2. The reaction mixture was stirred for 2 h at ambient temperature, diluted with ether, filtered, and the dark residue was washed thoroughly with ether. The combined ether extracts were washed successively with 10% NaOH, water, 10% HCl, water, saturated $NaHCO_3$, and water, dried ($MgSO_4$), and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography using petroleum ether/ether (3:1), to yield 2.2 g (40%) of pure **6** as a pale yellow oil : ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J=7.2 Hz, 3H), 1.12 (s, 3H), 1.33 (s, 6H), 1.51 (s, 3H), 1.00-1.16 (m, 2H), 1.16-1.27 (m, 6H), 1.50-1.68 (m, 2H), 2.25 (d, J=9.2 Hz, 1H), 2.72 (dt, J=5.9, 1.6 Hz, 1H), 2.84-2.97 (m, 1H), 3.07 (dt, J=6.0, 1.2 Hz, 1H), 3.83 (s, 3H), 6.09 (t, J=1.6 Hz, 1H), 6.87 (d, J=1.5 Hz, 1H), 6.93 (dd, J=8.0, 1.7 Hz, 1H), 7.15 (d, J=7.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.4, 22.6, 24.6, 26.7, 28.8, 29.9, 31.6, 38.0, 41.1, 44.4, 49.0, 53.9, 55.1, 57.8, 108.9, 118.3, 121.9, 125.3, 128.0, 153.4, 156.6, 168.0, 204.3; MS (EI) *m/z* 368 (53), 298 (39), 283 (100), 255 (53), 242 (28), 241 (48), 227 (38), 213 (67); HRMS Calcd for $C_{25}H_{36}O_2$: 368.2715, Found 368.2715.

4-(2-Methoxy-4-[1,1-dimethylheptyl]phenyl)-6,6-dimethylbicyclo[3.1.1] heptan-2-one (7). To a solution of 0.12g (17 mg atoms) of Li in 200 mL of liquid NH_3 at -78 °C was added dropwise 3.1 g (8.5 mmol) of enone **6** in 15 mL of dry ether. The reaction mixture was stirred at -78 °C for 1.5 h, then quenched by the addition of solid NH_4Cl. The NH_3 was evaporated at ambient temperature and the solid residue was taken up in ether. The ether solution was washed with successive portions of 10% HCl, water, saturated $NaHCO_3$, and water. After drying ($MgSO_4$), concentration afforded an oil which was purified by flash chromatography using petroleum ether/ether (3:1) to give 2.37 g (76%) of pure **7**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.9 Hz, 3H), 0.89 (s, 3H), 1.28 (s, 6H), 1.46 (s, 3H), 1.00-1.69 (m, 11H), 2.44-2.83 (m, 4H), 3.25-3.42 (m, 1H), 3.61-3.73 (m, 1H), 3.83 (s, 3H), 6.81 (s, 1H), 6.83 (d, J=8.3 Hz, 1H), 6.95 (d, J=7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.6, 24.5, 25.3, 26.6, 28.5, 28.9, 29.9, 31.6, 35.6, 37.6, 39.6, 40.7, 44.1, 44.5, 54.9, 58.3, 107.9, 116.8, 126.4, 129.2, 149.4, 157.2, 214.2; MS (EI) *m/z* 370 (4), 285 (8), 260 (10), 176 (14), 175 (100); HRMS Calcd for $C_{25}H_{38}O_2$: 370.2872, Found 370.2870.

(6a*S*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-6,6,-dimethyl-9H-dibenzo[b,d]pyran-9-one. To a stirred suspension of 0.96 g (32 mmol) of 80% NaH in 100 mL of dry DMF was added dropwise at room temperature 3.9 mL (43 mmol) of propanethiol , and the mixture was stirred until the solution turned clear. To this solution was added dropwise a solution of 2.37 g (6.41 mmol) of 4-(2-methoxy-4-[1,1-dimethylheptyl]phenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one (**7**) in 15 mL of DMF. The mixture was heated with stirring at 120 °C for 3 h. After cooling, the mixture was poured into 10% aqueous HCl and extracted with ether. The extracts were washed with brine, dried ($MgSO_4$), and concentrated to give an oil which was chromatographed using petroleum ether/ether (1:1) to give 0.45 g of recovered starting material and 1.50 g of phenol, (81% based on recovered starting material), which was used in the subsequent step without further purification : ^{1}H NMR (300 MHz, $CDCl_3$)

δ 0.82 (t, J=7.1 Hz, 3H), 0.87 (s, 3H), 1.21 (s, 6H), 1.50 (s, 3H), 0.95-1.80 (m, 11H), 2.60-2.90 (m, 4H), 3.50-3.75 (m, 2H), 6.73 (s, 1H), 6.74 (d, J=7.8 Hz, 1H), 6.89 (d, J=7.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.6, 24.6, 25.4, 26.6, 28.4, 28.8, 30.0, 31.7, 35.7, 37.2, 39.8, 40.8, 43.7, 44.5, 58.3, 113.0, 116.6, 126.7, 127.2, 149.6, 153.9, 217.4.

To a solution of 1.0 g (2.8 mmol) of the phenol in 10 mL of $CHCl_3$ was added 2.8 mL (2.8 mmol) of $SnCl_4$ (1 M solution in CH_2Cl_2). The resulting mixture was stirred at room temperature overnight, poured onto ice and extracted with ether. The extracts were combined and washed successively with 10% HCl, water, saturated $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield after chromatography using petroleum ether/ether (1:1) 0.733 g (73%) of ketone as an oil : ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.9 Hz, 3H), 1.22 (s, 6H), 1.37 (s, 3H), 1.46 (s, 3H), 0.96-1.75 (m, 11H), 2.00-2.40 (m, 4H), 2.65-2.80 (m, 1H), 3.00-3.15 (m, 1H), 3.60-3.70 (m, 1H), 6.72 (d, J=1.9 Hz, 1H), 6.84 (dd, J=8.2, 2.0 Hz, 1H), 7.14 (d, J=7.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.6, 23.1, 24.6, 26.6, 26.8, 28.8 30.0, 31.7, 34.4, 37.4, 39.8, 40.4, 42.9, 44.4, 75.8, 114.6, 117.0, 118.4, 126.6, 150.4, 152.4, 210.4.

(6a*R*,10a*R*)-(1,1-Dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-6,6,-dimethyl-9H-dibenzo[b,d]pyran-9-one (8). To a solution of 0.098 g (0.28 mmol) of (6a*S*,10a*R*)-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-6,6,-dimethyl-9H-dibenzo[b,d]-pyran-9-one in 2 mL of dry CH_2Cl_2 at room temperature was added 0.110 g (0.824 mmol) of $AlCl_3$ and the reaction mixture was stirred for 2 h. After pouring onto ice, the mixture was extracted with three portions of ether. The ethereal layers were washed with 10% HCl, brine, $NaHCO_3$, brine and dried ($MgSO_4$). Concentration yielded an oil which was chromatographed using petroleum ether/ether (1:1) to give 0.058 g (59%) of ketone **8** as a pale yellow oil: ^1H NMR (300 MHz, $CDCl_3$) δ 0.84. (t, J=6.9 Hz, 3H), 1.18 (s, 3H), 1.24 (s, 6H), 1.50 (s, 3H), 0.95-1.13 (m, 2H), 1.13-1.40 (m, 6H), 1.40-1.60 (m, 3H), 1.80-1.98 (m, 1H), 2.10-2.55 (m, 4H), 2.80-2.90 (m, 1H), 3.05-3.15 (m, 1H), 6.78 (d, J=1.8 Hz, 1H), 6.85 (dd, J=8.1, 1.9 Hz, 1H),

7.00 (d, J=8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.9, 22.6, 24.6, 27.2, 28.1, 28.8, 30.0, 31.7, 35.7, 37.4, 40.8, 44.4, 45.7, 45.8, 76.9, 114.8, 117.9, 120.2, 125.1, 150.4, 152.5, 209.0; MS (EI) *m/z* 356 (19), 273 (11), 272 (36), 271 (100), 161 (14); HRMS Calcd for $C_{24}H_{36}O_2$: 356.2715, Found 356.2714.

(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-9-carbomethoxy-6a,7,10,10a-tetrahaydro-6,6-dimethyl-6H-dibenzo[b,d]pyran (10). To a stirred solution of 0.398 g (1.94 mmol) of 2,6-di-tert-butyl-4-methylpyridine in 5 mL of dry CH_2Cl_2 was quickly added 0.250 mL (1.45 mmol) of trifluoromethanesulfonic anhydride. After stirring for 5 min a solution of 0.343 g (0.963 mmol) of (6a*R*,10a*R*)-(1,1-dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-6,6,-dimethyl-9H-dibenzo[b,d]pyran-9-one in 2 mL of dry CH_2Cl_2 was added dropwise and the reaction was stirred at reflux for 18 h. The solvent was removed *in vacuo*, and the residue was taken up in petroleum ether. The solid was filtered off, and the filtrate was washed with cold 10% HCl, brine, and the solvent was removed *in vacuo*. The product was purified by flash chromatography using petroleum ether/ether (10:1) to yield 0.452 g (93%) of triflate **9** as an oil: ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.8 Hz, 3H), 1.18 (s, 3H), 1.25 (s, 6H), 1.43 (s, 3H), 1.00-1.60 (m, 10H), 1.70-1.88 (m, 1H), 1.92-2.10 (m, 1H), 2.30-2.50 (m, 2H), 2.80-3.05 (m, 2H), 5.86 (m, 1H), 6.77 (d, J=1.9 Hz, 1H), 6.87 (dd, J=8.1, 1.9 Hz, 1H), 7.05 (d, J=8.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.0, 22.6, 24.6, 25.8, 27.7, 29.7, 30.0, 31.7, 32.5, 33.6, 37.4, 41.9, 44.5, 76.2, 114.9, 117.4, 118.2, 120.0, 125.8, 148.1, 150.5, 152.4.

To a solution of 0.173 g (0.343 mmol) of triflate **9** in 2 mL of dry DMF was added 0.096 mL (0.68 mmol) of Et_3N, 0.0077 g (0.034 mmol) of $Pd(OAc)_2$, 0.018 g (0.068 mmol) of triphenylphosphine and 0.56 mL of methanol. The reaction flask was purged for 10 min with carbon monoxide and then stirred at 45 °C for 18 h. The reaction mixture was poured into water, extracted with ether, the organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. The product was purified by flash chromatography using petroleum ether/ether to yield 0.103 g (75%) of methyl ester **10**: ^1H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J=6.3 Hz, 3H), 1.17

(s, 3H), 1.25 (s, 6H), 1.42 (s, 3H), 1.00-1.60 (m, 10H), 1.70-1.82 (m, 1H), 1.90-2.18 (m, 2H), 2.33-2.48 (m, 1H), 2.55-2.70 (m, 1H), 3.13-3.26 (m, 1H), 3.75 (s, 3H), 6.77 (d, J=1.8 Hz, 1H), 6.86 (dd, J=8.1, 1.8 Hz, 1H), 7.04 (m, 1H), 7.19 (d, J=8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 18.9, 22.6, 24.5, 27.6, 28.0, 28.6, 28.9, 29.9, 30.5, 31.7, 37.3, 42.2, 44.4, 51.5, 76.1, 114.6, 117.9, 121.2, 126.2, 129.8, 138.1, 149.7, 152.3, 167.3; MS (EI) *m/z* 398 (24), 314 (33), 313 (100), 147 (11); HRMS Calcd for $C_{26}H_{38}O_3$: 398.2821, Found 398.2819.

(6a*R*,10a*R*)-3-(1,1-dimethylheptyl)-9-hydroxymethyl-6a,7,10,10a-tetrahaydro-6,6-dimethyl-6H-dibenzo[b,d]pyran (3). To a stirred suspension of 0.060 g (1.58 mmol) of $LiAlH_4$ in dry ether was added dropwise at 0 °C 0.153 g (0.384 mmol) of ester **10** in 7 mL of dry ether . The reaction was stirred for 18 h at ambient temperature, quenched with water, acidified to pH 3, and extracted with ether. The ethereal layers were washed with brine, dried ($MgSO_4$), and the solvent was removed *in vacuo*. The crude product was purified by flash chromatography using petroleum ether/ether (1:1) to give 0.135 g (95%) of deoxy-HU 210 (**3**) as a colorless oil: R_F 0.61 (petroleum ether/ethyl acetate 1:1), 0.32 (petroleum ether/ether 1:1); ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.8 Hz, 3H), 1.16 (s, 3H), 1.24 (s, 6H), 1.40 (s, 3H), 1.00-1.49 (m, 7H), 1.49-1.66 (m, 3H), 1.66-2.11 (m, 3H), 2.11-2.33 (m, 1H), 2.60 - 2.90 (m, 2H), 4.06 (m, 2H), 5.74 (m, 1H), 6.76 (d, J=1.8 Hz, 1H), 6.84 (dd, J=8.1, 1.9 Hz, 1H), 7.13 (d, J=8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.0, 22.6, 24.5, 27.1, 27.6, 28.7, 28.8, 29.9, 31.7, 31.8, 32.0, 37.3, 43.0, 44.4, 66.7, 76.6, 114.6, 117.7, 121.4, 122.0, 126.0, 136.9, 149.5, 152.4; MS (EI) *m/z* ; 370 (37), 286 (30), 285 (100), 267 (21), 225 (14), 201 (13), 185 (10); $[\alpha]_D^{25}$ -74.1° (c=11.0, $CHCl_3$). HRMS Calcd for $C_{25}H_{38}O_2$: 370.2872, Found 370.2864.

1-Deoxy-3-(1',1'-dimethylheptyl)-Δ^8-tetrahydrocannabinol (12). To a stirred solution of 1.04 g (2.81 mmol) of Δ^8-THC-DMH (**11**) in 5mL of dry THF at 0 °C was added in

portions 0.127 g (4.23 mmol) of 80% NaH suspension in mineral oil, and the resulting mixture was stirred for 10 min. To this solution was added dropwise 0.820 mL (5.67 mmol) of diethyl chlorophosphate. The mixture was warmed to room temperature and stirred for 1 h. The solution was diluted with ether and the ethereal solution was washed with 10% aqueous NaOH and brine. After drying ($MgSO_4$), concentration *in vacuo* afforded an oil which was chromatographed using petroleum ether/ethyl acetate (3:1) to give 1.288 g (90%) of phosphate ester which was used in the next step without further purification: ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.8 Hz, 3H), 1.11 (s, 3H), 0.97-1.45 (m, 8H), 1.23 (s, 6H), 1.31 (t, J=7.7 Hz, 3H), 1.35 (t, J=7.3 Hz, 3H), 1.41 (s, 3H), 1.47-1.59 (m, 2H), 1.69 (s, 3H), 1.75-1.98 (m, 3H), 2.07-2.21 (m, 1H), 2.75-2.84 (m, 1H), 2.98-3.16 (m, 1H), 4.06-4.38 (m, 4H), 5.42 (d, J=4.3 Hz, 1H), 6.61 (d, J=1.2 Hz, 1H), 6.84 (d, J=1.3 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 16.0, 18.3, 22.5, 23.3, 24.5, 27.3, 27.7, 28.5, 29.8, 31.5, 31.6, 35.9, 37.4, 44.3, 44.7, 64.2, 76.7, 109.8, 111.0, 114.6, 119.3, 134.2, 149.6, 149.8, 154.2.

To a stirred solution of 0.033 g (4.6 mg atoms) of Li in 40 mL of liquid NH_3 at -78 °C was added dropwise a solution of 1.17 g (2.32 mmol) of phosphate ester in 5 mL of dry ether. After stirring at -78 °C for 45 min, the reaction was quenched with solid NH_4Cl, and the NH_3 was evaporated. The solid residue was taken up in water and extracted with ether. The ethereal layers were washed with 10% aqueous HCl and brine, dried ($MgSO_4$) and the solvent evaporated *in vacuo* to give the crude product which was purified by flash chromatography using petroleum ether/ethyl acetate (20:1) to give 0.85 g of starting material and 0.199 g (89% based on recovered starting material) of pure **12**: R_F 0.71 (petroleum ether/ethyl acetate 20:1), 0.31 (hexanes); ^1H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J=6.7 Hz, 3H), 1.15 (s, 3H), 1.25 (s, 6H), 1.00-1.34 (m, 8H), 1.39 (s, 3H), 1.47-1.61 (m, 2H), 1.72 (s, 3H), 1.61-2.04 (m, 3H), 2.07-2.22 (m, 1H), 2.52-2.75 (m, 2H), 5.43 (s, 1H), 6.76 (d, J=1.7 Hz, 1H), 6.84 (dd, J=8.1, 1.9 Hz, 1H), 7.10 (8.1, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 19.2, 22.7, 23.5, 24.6, 27.5, 27.7, 28.9, 30.0, 31.8, 32.1, 36.5, 37.3, 42.9, 44.6, 76.6, 114.7, 117.6, 120.0, 122.4, 126.0, 133.3, 149.4, 152.6; MS (EI) *m/z* 354 (23), 339 (13), 311 (20), 270 (31), 269 (100), 227 (15), 185

(19), 171 (13), 135 (26); $[\alpha]_D^{25}$ -104.7° (c=10.2, $CHCl_3$). HRMS Calcd for $C_{25}H_{38}O$: 354.2923, Found 354.2921.

CB1 Receptor Model Construction

Model construction was begun by aligning the amino acid sequence of the cannabinoid CB1 receptor with the sequences of fifty-eight other G-protein coupled receptors (GPCRs) including the cationic neurotransmitter GPCRs. Highly conserved residues in each transmembrane segment were used as alignment guides [1]. Gaps in the sequence were introduced in loop regions to facilitate the alignment. All transmembrane helices could be unambiguously aligned except Helix 5 which lacks the characteristically conserved proline of the cationic neurotransmitters. Consequently, a "structural alignment" of Helix 5 with the other GPCRs was performed using its hydrophobicity profile [2].

A sub-set alignment also was created in which the CB1 sequence was aligned with GPCR sequences that exhibit greater than 30% homology with CB1. These GPCRs were the cannabinoid CB2 receptor [3], the murine and human melanocyte-stimulating hormone (MSH) receptors [4], the adrenocorticotropic hormone (ACTH) receptor [4], the human endothelial differentiation protein, edg-1[5], the rat α-1B-adrenergic receptor [6], and the bovine α-1C- adrenergic receptor [7]. The variability at each position in the alignment was then determined. [See ref 8 for a discussion of the calculation of variability/conservancy.]

In order to be able to compare the CB1 sequence to those of other GPCRs, the generalized numbering scheme proposed by Ballesteros and Weinstein was used [8]. This scheme assigns the helix number as the first part of the label. The helix number is followed by a position locant in which a .50 value is assigned to the most highly conserved position in each transmembrane helix. Since Helix 5 lacks a highly conserved Proline, the Leucine which occupies its position in the sequence was used as the locant [2]. Amino acid locants in CB1 were N1.50(134), D2.50(163), R3.50(214), W4.50(241), L5.50(286), P6.50(358) and P7.50(394).

Fourier transform methods [9-12] were then used with the Cornette n-Prift hydrophobicity

scale [13] and with the variability profile described above to calculate the alpha-helical periodicity (AP) in the primary amino acid sequence of the CB1 receptor and of its alignment. A seven amino acid sliding window was used for these AP calculations. Plots of AP versus window were used to identify α helix segments present within the CB1 sequence. In addition to the expected seven α helix segments, the analysis detected an eighth helix segment. Variability and hydrophobicity moment vector calculations were used to show that the first seven helix segments were transmenbrane helices, while the eighth helix segment was an intracellular extension of Helix 7 [2]. Variability moment vectors for each amino acid window then were used to delineate the orientation of each helix in the membrane [2]. Based upon these vector calculations, an initial helix bundle arrangement was obtained. This arrangement was largely consistent with Baldwin's proposed transmembrane helix (TMH) bundle arrangement in rhodopsin, a GPCR [1]. For a more detailed discussion of the CB1 model building, please see ref 2.

Each helix of the CB1 receptor was then created using the ChemProtein module in the Chem-X suite of modeling programs [Chemical Design, Inc., Chipping Norton, England]. Only the transmembrane bundle was built as the initial model of the CB1 receptor. In order to refine the initial packing arrangement, the following criteria were used: (1) the length of loop regions; (2) data on proposed ligand interaction sites in homologous GPCRs; (3) mutagenesis data; (4) identified sites of covalent attachment in the GPCRs; and (5) NMR studies. The loop regions between Helices 1 and 2 and between Helices 2 and 3 are relatively short, necessitating the proximity of these helices in the bundle. The other four categories of information were used to adjust relative helix heights and to perform minor adjustments in helix orientations. For reasons detailed below, a structural change in Helix 7 had to be introduced. Helix 7 in the GPCRs has a highly conserved NPXXY motif [19]. Wong et al.'s study of the β-adrenergic receptor using an irreversible photoaffinity label revealed W7.40 as the site of the covalent attachment of the label [14]. This result implies that W7.40 must be accessible from within the TMH bundle. Double revertant mutagenesis studies of rhodopsin by Rao et al. [15] suggested that the retinal attachment site K7.43(296) is in proximity to G2.57(90) in addition to its wild-type counterion E3.28(113),

thus positioning Helix 7 adjacent to Helices 2 and 3. In addition, since K7.43(296) is the covalent site of attachment of cis-retinal, this residue must be accessible from the inside of the TMH bundle. Double revertant mutations D7.49N and N2.50D of the GnRH receptor performed by Zhou et al. [16] indicated that these two residues also must be in spatial proximity in the TMH bundle. Taken as a whole, these experimental results suggest that residues 7.40, 7.43 and 7.49 must be accessible from the interior of the TMH bundle of the receptor, i.e., these residues must be on the same face of Helix 7 in GPCRs. On a helical wheel (which represents a perfect alpha helix), residues 7.40,7.43 and 7.49 are not on the same face of the helix. Even a 3D model of Helix 7 with a normal proline kink does not place 7.40, 7.43, and 7.49 on the same face. Based on the results of a search of the Protein Data Bank, Konvicka and Weinstein have hypothesized that the NP motif in Helix 7 creates a break in the helix allowing N7.49 to flip to the other side of the helix (i.e. to the same face of Helix 7 occupied by 7.40 and 7.43) [17]. NMR studies of Helix 7 and the consequence of the S/TXXNPXXY motif [18] revealed Helix 7 to be kinked in a way similar to that predicted by Konvicka and Weinstein. Conseqently, we incorporated this hypothesized helix break into Helix 7 of the CB1 model.

Finally, after comparisons such as those detailed above, the amino acid side chain angles in the receptor bundle were set to typical representative values [see ref. 8 and references therein] and the CB1 TMH bundle was subjected to a Molecular Mechanics Minimization (ε=4) using the Amber force field [19].

References

1. J. Baldwin, "The Probable Arrangement of the Helices in G-Protein-Coupled Receptors", **EMBO J.**, *12*, 1693-1703, **1993.**

2. R.D. Bramblett, A.M. Panu, J.A. Ballesteros, and P.H. Reggio, "Construction of a 3D Model of the Cannabinoid CB1 Receptor: Determination of Helix Ends and Helix Orientation", **Life Sciences**, *56*, 1971-1982 **1995.**

3. S. Munro, K.L. Thomas and M. Abu-Shaar, "Molecular Characterization of a Peripheral Receptor for Cannabinoids", **Nature**, 365, 61-65, 1993.

4. K.G. Mountjoy, L.S. Robbins, M.T. Mortrud, and R.D. Cone, "The Cloning of a Family of Genes That Encode the Melanocortin Receptors", Science, *257*, 1248-1251, **1992**.

5. T. Hla and T. Maciag, "An Abundant Transcript Induced in Differentiating Human Endothelial Cells Encodes a Polypeptide with Structural Similarities to G Protein-Coupled Receptors", **J. Biol. Chem.**, *265*, 9308-9313, **1990**.

6. J.W. Lomasney, S. Cotecchia, W. Lorenz, W-Y. Leung, D.A. Schwinn, T.C. Yang-Feng, M. Brownstein, R.J. Lefkowitz, and M.G. Caron, "Molecular Cloning and Expression of the cDNA for the α_{1A}-Adrenergic Receptor", **J. Biol. Chem.**, *266*, 6365-6369, **1991**. (Note: This also contains the α_{1B} sequence.)

7. D.A. Schwinn, J.W. Lomasney, W. Lorenz, P.J. Szklut, R.T. Fremeau, T.L. Yang-Feng, M.G. Caron, R.J. Lefkowitz, and S. Cotecchia, "Molecular Cloning and Expression of the cDNA for a Novel α_1-Adrenergic Receptor Subtype", **J. Biol. Chem.**, *265*, 8183-8189, **1990**.

8. J.A. Ballesteros and H. Weinstein, "Integrated Methods for the Construction of Three Dimensional Models and Computational Probing of Structure Function Relations in G Protein-Coupled Receptors", **Methods in Neuroscience**, P.M. Conn and S.C. Sealfon (eds), Vol. 25, Chapter 19, 366-428, **1995**.

9. D. Eisenberg, R.M. Weiss and T.C. Terwilliger, "The Hydrophobic Moment Detects Periodicity in Protein Hydrophobicity", **Proc. Natl. Acad. Sci. USA**, *81*, 140-144, **1984**.

10. H. Komiya, T.O. Yeates, D.C. Rees, J.P. Allen, and G. Feher, "Structure of the Reaction Center from *Rhodobactersphaeroides* R-26 and 2.4.1: Symmetry Relations and Sequence Comparison Between Different Species", **Proc. Natl. Acad. Sci. USA**, *85*, 9012-9016, **1988**.

11. D. Donnelly, J.P. Overington, S.V. Ruffle, J.H.A. Nugent and T.L. Blundell, "Modeling α-Helical Transmembrane Domains: The Calculation and Use of Substitution Tables for Lipid-Facing Residues", **Prot. Sci.**, *2*, 55-70, **1993**.

12. D. Donnelly, M.S. Johnson, T.L. Blundell, and J. Saunders, "An Analysis of the Periodicity of Conserved Residues in Sequence Alignments of G-Protein Coupled Receptors. Implications for Three-Dimensional Stucture", **FEBS Letters**, *251*, 109-116, **1989**.

13. J.L. Cornette, K.B. Cease, H. Margalit, J.L. Spouge, J.A. Berzofsky and C. Delisi, "Hydrophobicity Scales and Computational Techniques for Detecting Amphipathic Structures in Proteins", **J. Mol. Biol.**, *195*, 659-685, **1987**.

14. S.K.-F. Wong, C. Slaughter, A.E. Ruoho, and E.M. Ross, "The Catecholamine Binding Site of the β-adrenergic Receptor is Formed by Juxtaposed Membrane Spanning Domains", **J. Biol. Chem.**, *263*, 7925-7928, **1988**.

15. V.R. Rao, G.B. Cohen, and D.D. Oprian, "Rhodopsin Mutation G90D and a Molecular Mechanism for Congenital Night Blindness", **Nature**, *367*, 639-642, **1994**.

16. W. Zhou, C. Flanagan, J.A.Ballesteros, K.Konvicka, J.S.Davidson, H. Weinstein, R.P.Millar, and S.C.Sealfon," A Reciprocal Mutation Supports Helix 2 and Helix 7 Proximity in the Gonadotropin-Releasing Hormone Receptor", *45*, 165-170, **1994**.

17. K. Konvicka and H. Weinstein, **Biophys. J.**, 68, Abstract, **1995**.

18. J.P. Berlose, O. Convert, A. Brunissen, G. Chassaing, and S. Lavielle, "Three-Dimensional Structure of the Highly Conserved Seventh Transmembrane Domain of G-Protein-Coupled Receptors", **Eur. J. Biochem.**, 225, 827-843, **1994**.

19. D.A. Pearlman, D.A. Case, J.C. Caldwell, G.L. Seibel, U.C. Singh, P. Weiner, and P.A. Kollman, Amber 4.0.1, **1991**.