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SYNTHESIS OF DEUTERATED DEXANABINOL, A NONPSYCHOTROPIC CANNABINOID WITH NEUROPROTECTIVE PROPERTIES

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SUMMARY

The deuterium labeled form of dexanabinol, a potential neuroprotective agent, was synthesized by coupling the deuterated 5-(1,1-dimethylheptyl)resorcinol, obtained in a 5-step procedure, with 4-hydroxymyrtenyl pivalate, followed by deprotection.

Key words: dexanabinol, neuroprotection, nonpsychotropic cannabinoids

INTRODUCTION

Dexanabinol, [(6aS-trans)-6,6-dimethyl-3-(1,1-dimethylheptyl)-1-hydroxy-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b,d*]pyran-9-methanol], is a nonpsychotropic cannabinoid currently developed as a neuroprotective agent with potential use in the treatment of brain damage caused by stroke, cardiac arrest and brain injury [1 - 5].

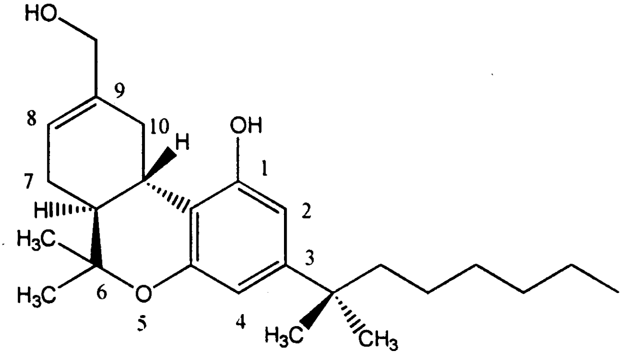

Fig. 1. Structure and atom numbering of dexanabinol

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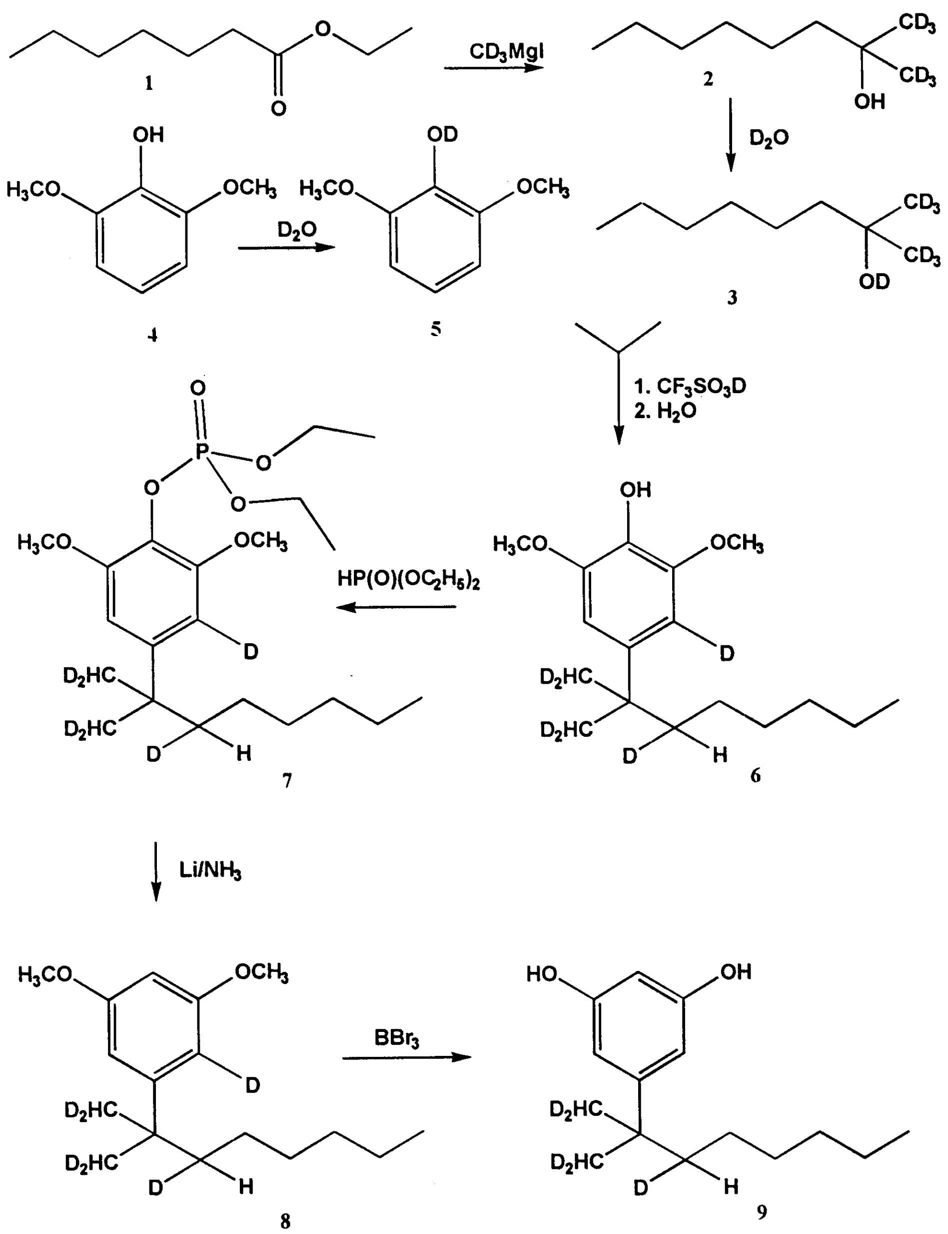
During the preclinical and clinical experiments, the concentration of dexanabinol in some biological materials such as blood (rat, human), urine (rat, human), brain (rat), has to be determined in order to establish the pharmacokinetic profile of the drug. An internal standard was needed for analytical determinations. Several closely related compounds including the 1 methoxy and 9 methoxymethyl derivatives [6], as well as the 3-*t*-butyl and 3-(1,1-dimethylbutyl) modified side- chain analogs [7] have been originally used in this purpose. However, the structural modifications also altered the solubility properties of the novel derivatives as compared to dexanabinol, leading to different recovery from biological materials, accompanied by errors in the quantitative determinations. The use of a compound labeled with stable isotopes has been considered.

The synthesis of dexanabinol in which five non-reactive hydrogen atoms are replaced by deuterium is presented herein. The labeled compound should have the same solubility as dexanabinol, while the difference in molecular weight should make possible its use for quantitative determinations by gas or liquid chromatographic- mass spectrometry (GC-MS or LC-MS) technique.

RESULTS AND DISCUSSION

The 3-side chain of dexanabinol appears to be a proper site for labeling with deuterium. Previously reported procedures for synthesis of dexanabinol could be followed [8 - 10], modifying only the segment involving the preparation of deuterated 5-(1,1-dimethylheptyl) resorcinol (**9**). This intermediate was obtained according to Scheme 1. By reacting ethyl heptanoate (**1**) with methyl-d_3 -magnesium iodide (obtained from iodomethane-d_3 and magnesium) in ethyl ether, deuterated (d_6) 2-methyloctan-2-ol **2** resulted. In contrast with nondeuterated 1,1-dimethylheptanol, the


1
CD3MgI
2
D2O
3
OH
H3CO
OCH3
D2O
OD
4
5
1. CF3SO3D
2. H2O
HP(O)(OC2H5)2
D
D2HC
D
H
7
6
Li/NH3
BBr3
HO
OH
8
9

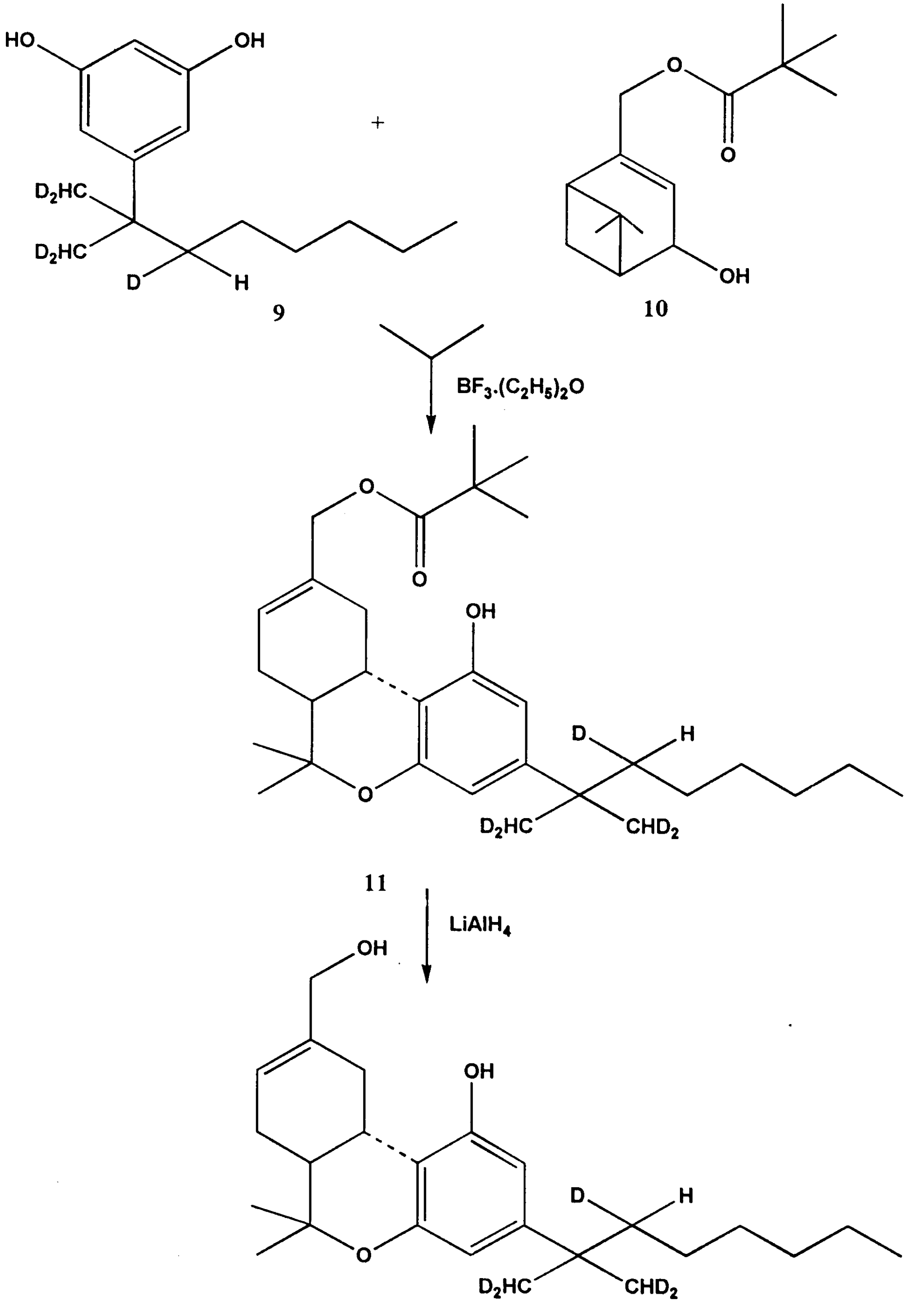
Scheme 1

NMR spectra of **2** showed no singlet at δ 1.19 (^1H) and no peak at δ 29.0 (^{13}C) corresponding to the methyl groups at C-1. In addition, the peak at δ 70.7 (C-1) became short and broad due to the long range coupling with six deuterium atoms. Alkylation of 2,6-dimethoxyphenol (**4**) with **2**, in methanesulfonic acid, followed by column chromatographic purification resulted in rather pure **6** (96.5%, based on HPLC peak area). However, during the reaction, deuterium was exchanged with hydrogen to a relatively high degree.

The synthesis was then repeated after taking the following precautions: (1) The hydrogen of the OH functionality of both the alcohol **2** and the phenol **4** were exchanged with deuterium ; this was accomplished by mixing vigorously **2** and **4** respectively with a large excess of deuterium oxide and toluene followed by distillation of the resulting azeotrope of toluene: water. This process was repeated several times, each time evaporating completely the solvent. (2) Methanesulfonic acid was replaced by trifluoromethanesulfonic acid-*d* . (3) Anhydrous conditions were enforced during the synthesis, in order to avoid any source of mobile hydrogen.

Since intramolecular $D \rightleftharpoons H$ exchange still occurred during the reaction, the product consisted mainly of **6**, rather than of the expected dimethyl-d_6 derivative. This exchange could not be avoided and was reproducible. In comparison with the nondeuterated reference, the ^1H NMR spectrum of **6** showed only a weak singlet at δ 1.25 (CD_2H groups) instead of a very strong singlet at δ 1.27 (CH_3 group). Integrations of the resonances of the C-2 methylene group of the aliphatic chain and those of the aromatic protons indicated only one proton each. In the ^{13}C NMR spectrum, resonances of the methyl groups and those of C-1, C-2, and C-3 of the aliphatic chain appeared as multiplets due to the C-D coupling. Substitution of one of the aliphatic C-2 hydrogens by a deuterium atom and $D \rightarrow H$ exchanges in the methyl groups indicate that the carbonium ion derived from 1,1-di(methyl- d_3) heptanol upon treatment with trifluoroacetic acid-*d* underwent several elimination-addition cycles before the final attack on the aromatic ring. Structure **6** represents an average, statistically most abundant form and obviously other structures with different distribution of deuterium atoms between the methyl and methylene groups are also possible. Whereas labeling of the aromatic ring was lost during the following transformations, attachment to the aromatic ring made the aliphatic chain inert and its deuterium distribution remained unchanged through all synthetic steps. The synthesis was then continued. By reacting **6** with diethyl phosphite in carbon tetrachloride in the presence of triethylamine, 4-(1,1-dimethylheptyl)-2,6-dimethoxyphenyldiethyl phosphate **7** resulted (98% purity, HPLC). Next, **7** was reacted with lithium in liquid ammonia at -70 °C, affording 5-(1,1-dimethylheptyl)-1,3-dimethoxybenzene (**8**) (purity 98%, HPLC) which, after deprotection with boron tribromide in methylene chloride, gave 5-(1,1-dimethylheptyl) resorcinol (**9**) (purity 98%, HPLC).

Resorcinol **9** was coupled with 4-hydroxymyrtenyl pivalate (**10**) (obtained by a reported procedure, 8), in methylene chloride in the presence of boron trifluoride etherate, to give dexanabinol pivalate **11** (Scheme 2) After purification by column chromatography, **11** was deprotected with lithium aluminum hydride in tetrahydrofuran, and the resulting dexanabinol **12** was purified by column chromatography and recrystallized from acetonitrile. The purity of **12** (HPLC) was 98%. Except for the side chain atoms C-1, C-2, C-3 , the methyl groups at C-1 and protons belonging to these, all features in the ^1H and ^{13}C NMR spectra of **12** including chemical shifts and coupling constants, were identical to those of the nondeuterated material. The average number of deuterium atoms incorporated into the molecule was 4.57 and 4.64 (after correcting for the contribution of the natural ^{13}C and ^{18}O isotopes [11]) based upon APCI and the low-energy EI mass spectrometric determinations, respectively. The distribution of the degree of deuteration in **12** also showed a good agreement between


HO
OH
D_2HC
D_2HC
D
H
9
+
O
O
OH
10
$BF_3.(C_2H_5)_2O$
O
O
OH
O
D
H
D_2HC
CHD_2
11
$LiAlH_4$
OH
OH
O
D
H
D_2HC
CHD_2
12

Scheme 2

the results obtained by the two independent methods, as illustrated in Fig. 1. Therefore, the structure depicted for **12** represents an average, the most abundant form.

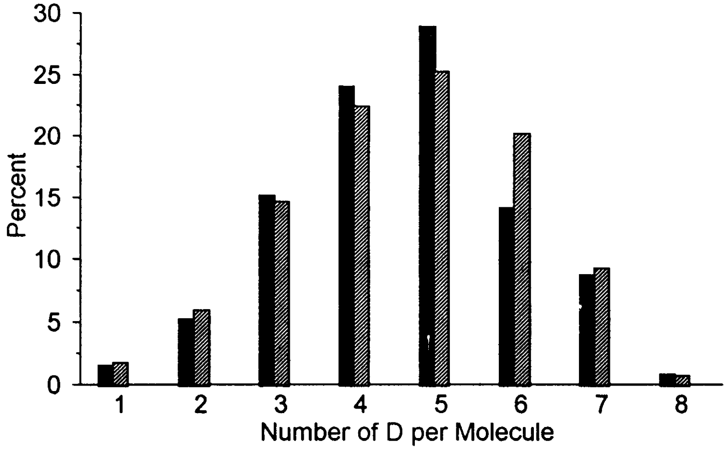


Fig. 1. Distribution of deuterium atoms in **12** (Solid bars: APCI, shaded bars: low-energy mass spectrometry).

EXPERIMENTAL

Melting points are uncorrected and were determined on an Electro-thermal melting point apparatus (Fisher Scientific). Elemental microcombustion analyses were performed by Atlantic Microlabs Inc., Atlanta, Georgia. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance spectra were recorded on a Varian XL-300 spectrometer. Samples were dissolved in an appropriate deuterated solvent and chemical shifts were reported as parts per million (δ) relative to tetramethylsilane (0.00) which served as an internal standard. Coupling constants (J) are reported in Hertz. Thin layer chromatography was performed on EM reagents DC-aluminum foil plates coated to a thickness of 0.2 mm with silica gel (60 mesh). Electron ionization (EI) mass spectra were recorded on a Kratos (Manchester, UK) MS80RFA instrument by using direct sample introduction. To reduce fragmentation of the molecular ion ($M^{+\bullet}$) that may cause interference upon determining the degree of deuteration of the products, the energy of the ionizing electrons was set to 20 eV. Atmospheric-pressure chemical ionization (APCI) mass spectra were recorded on a Finnigan MAT (San Jose, CA) ion trap mass spectrometer (LCQ®) by scanning a 10-amu wide range ("zoom-scan") with the expected protonated molecule (MH^+) as the center (APCI is a "soft" ionization method compared to EI). The samples were injected via a Rheodyne (Cotati, CA) 7125 injector with a 5-μl loop, while a flow of a solution containing 49.5% methanol, 49.5% water and 1% acetic acid to the APCI source was maintained at 1.0 ml/min by an high-pressure solvent delivery system (Kratos Spectroflow 400).

All solvents and chemicals were reagent grade. Ethyl heptanoate, iodomethane-d_3, 2,6-dimethoxyphenol, trifluoromethansulfonic acid, diethyl phosphite and (+) α-pinene were from Aldrich.

Deuterated (*d*$_6$) 2-methyl-2-octanol (2). To a stirred suspension of magnesium turnings (5.04 g, 0.21 mol) in dry ethyl ether (200 mL), was added iodine (6.35 g, 25 mmol) at 20 -25 °C. When the solution became colorless, deuterated iodomethane (d_3, Aldrich 99.5%, 25 g, 170 mmol) was added, in a rate which assured a gentle reflux. After the addition was accomplished, the solution was stirred for an additional hour, then decanted. To the stirred solution of the resulting deuterated methyl magnesium bromide, was added ethyl heptanoate (9.47 g, 10.9 mL, 60 mmol) at 20 - 25 °C. After the addition of the first 2 mL, the mixture was maintained under stirring for about 10 min required for initiation of the reaction. After the vigorous reaction (reflux) ceased, the rest of the alcohol was added and the mixture was stirred overnight at 20 - 25 °C, then poured into ice water (100 mL) and adjusted to pH 6 (acetic acid). The product was extracted with ether (100 mL), the organic layer was washed with water, 5% aqueous $KHCO_3$ and water and dried (anhydrous Na_2SO_4). Evaporation of the solvent gave **2** as a colorless oil (9.0 g, quantitative yield). ^{1}H NMR: δ 0.89 (t, J = 6.6 Hz, 3H), 1.23-1.40 (m, 10 H), 1.54 (bs, 1H); ^{13}C NMR: δ 14.), 22.6, 24.3, 29.9, 31.9, 43.9, 70.7 (broad).

Deuterated (d_6) 4-(1,1-dimethylheptyl)-2,6-dimethoxyphenol (6). To a solution of **2** (9.0 g, 60 mmol) in toluene (50 mL), was added deuterium oxide (10 mL) and stirred for 20 min at 20 -25 °C. The organic layer was separated and treated in the same manner twice with deuterium oxide. The solvent was then removed by distillation to yield **3** as an oil. In a separate flask, 2,6-dimethoxyphenol (10.0 g, 65 mmol) dissolved in toluene (50 mL) was stirred for 20 min with deuterium oxide (10 mL), followed by distillation of the solvents. The operation was repeated twice. The resulting **5** was dissolved in dry toluene (20 mL) and combined to a solution of **3** in dry toluene (20 mL) followed by distillation of the solvent. The residue was dissolved, under argon, in trifluoromethanesulfonic acid-*d* (10 g) and the reaction mixture was stirred for 72 h at 20 -25 °C, progress of the reaction being monitored by TLC. The reaction mixture was poured into ice water (150 g), stirred for 20 min and extracted with methylene chloride (2 x 100 mL). The combined organic solutions were washed with water (100 mL), 5% aqueous $KHCO_3$ (50 mL) and water (100 mL). After drying over anhydrous Na_2SO_4, the solvent was evaporated to afford **6** (11.5 g) as an oil. ^1H NMR: δ 0.86 (t, J = 6.9 Hz, 3H), 1.00-1.12 (m, 2H), 1.12-1.33 (m, 8H), 1.55 (m, 1H), 3.90 (s, 6H), 5.44 (bs, 1H), 6.55 (s, 1H): ^{13}C NMR: δ 14.0, 22.6, 24.6 (m), 28.7 (m, 2C), 29.9, 31.7, 37.3 (broad), 44.4 (m), 56.3 (2C), 103.0 (2C), 132.5, 141.0, 146.5 (2C).

Deuterated (d_6) 4-(1,1-dimethylheptyl)-2,6-dimethoxyphenyldiethyl phosphate (7). To a solution of **6** (11.0 g, 38 mmol) in carbon tetrachloride (20 mL), was added diethyl phosphite (7.5 mL), and the resulting solution was cooled to - 15 °C. Triethylamine (8.0 mL) was added dropwise at - 15 °C, and the temperature was maintained for an additional 15 min. Then the mixture was stored at 20-25 °C for 16 h. Water (200 mL) was added to the reaction mixture and the product was extracted with methylene chloride (200 mL). The organic layer was separated and washed with water, 0.1 M aqueous NaOH, water, 0.1 M aqueous HCl, and water (200 mL each). After drying over anhydrous Na_2SO_4, the solvent was removed by distillation. To the resulting oily residue was added hexane (70 mL), and the mixture was cooled at - 20 °C overnight. The white crystals were filtered off and dried to give 10.8 g (70 %) of **7**, m.p. 65-66 °C. ^1H NMR: δ 0.85 (t, J = 6.9 Hz, 3H), 1.05 (m, 2H), 1.13-1.27 (m, 8H), 1.39 (td, J = 6.9 and 0.9 Hz, 6H), 1.52 (m, 1H), 3.86 (s, 6H), 4.32 (m, 4H), 6.53 (s, 1H); ^{13}C NMR: δ 14.0, 16.0 (2C), 22.6, 24.5 (m), 28.6 (m, 2C), 29.9, 31.7, 37.7 (m), 44.2 (m), 56.1 (2C), 64.1 (2C), 103.2 (2C), 147.1, 151.2 (3C). Analysis calculated for $C_{21}H_{31}D_6O_6P$: C, 59.69; H, 8.82. Found: C, 59.90; H, 8.80.

Deuterated (*d*$_5$) 5- (1,1-dimethylheptyl) - 1,3-dimethoxybenzene (8). Lithium metal (ribbon, 1.2 g) was added to liquid ammonia (150 mL). To the blue coloured solution was added dropwise a solution of **7** (10.7 g, 26 mmol) in dry tetrahydrofuran (50 mL), maintaining the temperature below - 70 °C. The resulting mixture was stirred for 2 h at the same temperature, then added slowly to an ice-cold solution of ammonium chloride (30 g) in water (300 mL). The product was extracted with hexane (300 mL), and the organic solution was separated and washed with water (300 mL), then dried over anhydrous $MgSO_4$. The solvent was removed by distillation affording **8** (6.2 g, 88% yield) as an oil of 98% purity (HPLC).

Deuterated (d$_6$) 5-(1,1-dimethylheptyl) resorcinol (9). To a solution of **8** (6.2 g, 23 mmol) in methylene chloride (50 mL) was added boron tribromide (6.0 mL, 63 mmol), dropwise, mainaining the temperature at - 20 °C. The reaction mixture was then allowed to reach 20-25 °C overnight. Water (200 mL) was added to the mixture and the product was extracted with methylene chloride (200 mL). The organic layer was separated, washed with water (3 x 300 mL) and dried over anhydrous Na_2SO_4. After removing the solvent by distillation, an oily residue resulted which eventually solidified (24 h). By crystallization from hexane (50 mL) **9** (4.55 g, 81% yield) of 98.5% purity (HPLC) was obtained as white crystalls, m.p. 92 - 92.5 °C. ^{1}H NMR: δ 0.84 (t, J = 6.6 Hz, 3H), 1.04 (m, 2H), 1.11-1.26 (m, 8H), 1.49 (m, 1H), 6.21 (s, 1H), 6.32 (s, 2H), 8.37 (s, 2H); ^{13}C NMR: δ 13.8, 22.4, 24.4 (m), 28.3 (m, 2C), 29.8, 31.6, 37.2 (m), 43.9 (m), 100.1, 104.9 (2C), 152.4, 157.4 (2C). Analysis calculated for $C_{15}H_{19}D_5O_2$: C, 74.65; H, 10.02. Found: C, 75.00; H, 10.09.

Deuterated (*d$_5$*) dexanabinol pivalate (11). To a solution of **9** (4.30 g, 19 mmol) and **10** (5.30 g, 21 mmol) in methylene chloride (250 mL) was added dropwise boron trifluoride diethyl etherate (7.5 mL, 60 mmol), at - 15 °C. The mixture was stirred at - 10 to - 15 °C for 2 h, then another portion of boron trifluorate (7.5 mL) was added. After maintaining the stirring foran additional 2 h at the same temperature, the mixture was allowed to warm up to 0 - 5 °C, and it was poured into an ice - cold 5% aqueous solution of $NaHCO_3$ (400 mL). After 30 min of stirring, the layers were separated, the organic solution was washed with 5% aqueous $NaHCO_3$ (2 x 200 mL) and water (200 mL). After drying over anhydrous Na_2SO_4, the solvent was removed by distillation to afford 8.8.g of a glassy product (63% purity by HPLC). Column chromatography (silica gel\ toluene) gave **11** (3.2 g of 85% purity and 2.6 g of 62% purity, by HPLC).

Deuterated (*d$_5$*) dexanabinol (12). To a solution of **11** (3.2 g, 6.7 mmol) in dry tetrahydrofuran (40 mL) was added lithium aluminum hydride solution in tetrahydrofuran (30 mL, 30 mmol) at - 5 °C. After 30 min, the temperature was allowed to rise to 20 -25 °C, and the mixture was stirred for an additional 16 h. Ethyl acetate (10 mL) was added to the reaction mixture and, after 30 min, it was poured into ice-water (100 mL). The pH was adjusted to 5 (glacial acetic acid). The product was extracted with hexane (200 mL), and the extract was washed with water (2 x 100 mL), 5% aqueous $NaHCO_3$ (2 x 100 mL), and water (100 mL). After drying over anhydrous Na_2SO_4, the solvent was removed by distillation. The oily residue was purified by column chromatography (silica gel, ethyl acetate: toluene, 1: 19) to give a glassy material which was recrystallized from acetonitrile to give pure (98% by HPLC) **12** (1.74 g,. yield 66 %); m.p. 138-138.5 °C. M. S.[M$^+$] (m/z): 391. ^{1}H NMR: δ 0.84 (t, J = 6.9 Hz, 3H), 0.90-1.50 (m, 11H), 1.03 (s, 3H), 1.37 (s, 3H), 1.70-1.95 (m, 3H), 2.20 (m, 1H), 2.45 (bs, 1H), 2.68 (m, 1H), 3.50 (dd, J = 19.0 and 2.4 Hz, 1H), 4.06 (m, 2H), 5.73 (m, 1H), 6.25 (s, 1H), 6.28 (bs, 1H), 6.36 (s, 1H): ^{13}C NMR: δ 14.1, 18.4, 22.7, 24.5 (m), 27.6, 27.7, 28.1 (m, 2C), 30.0, 31.4, 31.5, 31.8, 37.0 (m), 44.0 (m), 45.1, 67.1, 76.4, 105.6, 107.8, 109.9, 121.7, 138.3, 150.2, 154.4, 154.7. Analysis calculated for $C_{25}H_{33}D_5O_3$: C, 76.69; H, 9.78. Found: C, 76.89; H, 9.83.

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