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3-(1′,1′-Dimethylbutyl)-1-deoxy-Δ^8-THC and Related Compounds: Synthesis of Selective Ligands for the CB_2 Receptor

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Abstract—The synthesis and pharmacology of 15 1-deoxy-Δ^8-THC analogues, several of which have high affinity for the CB_2 receptor, are described. The deoxy cannabinoids include 1-deoxy-11-hydroxy-Δ^8-THC (**5**), 1-deoxy-Δ^8-THC (**6**), 1-deoxy-3-butyl-Δ^8-THC (**7**), 1-deoxy-3-hexyl-Δ^8-THC (**8**) and a series of 3-(1′,1′-dimethylalkyl)-1-deoxy-Δ^8-THC analogues (**2**, $n=0$–4, 6, 7, where $n=$ the number of carbon atoms in the side chain−2). Three derivatives (**17**–**19**) of deoxynabilone (**16**) were also prepared. The affinities of each compound for the CB_1 and CB_2 receptors were determined employing previously described procedures. Five of the 3-(1′,1′-dimethylalkyl)-1-deoxy-Δ^8-THC analogues (**2**, $n=1$–5) have high affinity ($K_i = <20$ nM) for the CB_2 receptor. Four of them (**2**, $n=1$–4) also have little affinity for the CB_1 receptor ($K_i = >295$ nM). 3-(1′,1′-Dimethylbutyl)-1-deoxy-Δ^8-THC (**2**, $n=2$) has very high affinity for the CB_2 receptor ($K_i = 3.4 \pm 1.0$ nM) and little affinity for the CB_1 receptor ($K_i = 677 \pm 132$ nM).

Introduction

Traditional cannabinoid structure–activity relationships (SAR)[1–3] state that a phenolic hydroxyl at C-1 of the cannabinoid skeleton is necessary for interaction with the CB_1 receptor. However, we reported recently that 3-(1′,1′-dimethylheptyl)-1-deoxy-11-hydroxy-Δ^8-tetrahydrocannabinol (1-deoxy-11-hydroxy-Δ^8-THC-DMH, deoxy-HU-210, **1**), a traditional cannabinoid lacking the 1-hydroxyl, has very high affinity for the CB_1 receptor ($K_i = 1.2 \pm 0.1$ nM), and exhibits characteristic cannabinoid in vivo pharmacology. Cannabinoid **1** also has exceptionally high affinity for the CB_2 receptor ($K_i = 0.032 \pm 0.019$ nM).[4] A second 1-deoxy-cannabinoid, 3-(1′,1′-dimethylheptyl)-1-deoxy-Δ^8-THC (1-deoxy-Δ^8-THC-DMH, **2**, $n=5$, where $n=$ the number of carbon atoms in the side chain−2), is also potent in vivo, has significant affinity for the CB_1 receptor ($K_i = 23 \pm 7$ nM), and nearly 10 times higher affinity for the CB_2 receptor ($K_i = 2.9 \pm 1.6$ nM).[4] A group at Merck Frosst also described 1-deoxy-Δ^8-THC-DMH; however, they found that it had an order of magnitude lower affinity for each receptor than we reported.[5] This group also reported that two 1-methoxy cannabinoids, 1-methoxy-Δ^8-THC-DMH (**3**) and 1-methoxy-$\Delta^{9(11)}$-THC-DMH (**4**), had affinities for the CB_2 receptor in the 20 nM range, and virtually no affinity for the CB_1 receptor.[5] This is in contrast to traditional cannabinoids with a phenolic hydroxyl at C-1 which have similar affinities for both cannabinoid receptors.[5,6]

The unexpected potency of 11-hydroxy-1-deoxy-Δ^8-THC-DMH (**1**) was explained in terms of a possible interaction of the 11-hydroxyl with Lys 192 of the CB_1 receptor.[4] It is thought that this amino acid hydrogen bonds to the phenolic hydroxyl of traditional cannabinoids, and in a mutant receptor which lacks Lys 192, the affinities of the potent cannabinoids CP 55,940 and 11-hydroxy-Δ^8-THC-DMH were greatly attenuated.[7,8] The potency of 1-deoxy-Δ^8-THC-DMH (**2**, $n=5$) was rationalized on the basis of molecular modeling studies which suggested that this compound docked with the receptor in an orientation such that Lys 192 hydrogen bonds to the pyran oxygen.[4] However, no rationalization was presented for the very high affinities of cannabinoids **1** and **2**, $n=5$, for the CB_2 receptor.[4]

In view of the selective affinity for the CB_2 receptor reported for cannabinoids **1**–**4**, the synthesis of a number

Key words: Cannabinoid; deoxy-cannabinoid; structure–activity relationships; CB_2 receptor.
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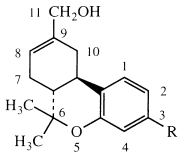
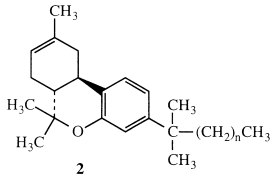
of additional 1-deoxy-cannabinoids has been carried out to obtain selective ligands for the CB_2 receptor, and to explore the SAR of the 1-deoxy-cannabinoids at both cannabinoid receptors. Three series of compounds were synthesized which included simple 1-deoxy analogues of Δ^8-THC, 1-deoxy-1′,1′-dimethylalkyl-Δ^8-THCs, and 1-deoxynabilone analogues.



1 R = C_9H_{19}

5 R = C_5H_{11}



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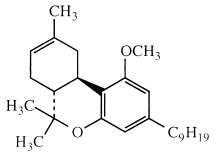

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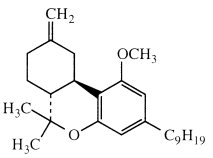

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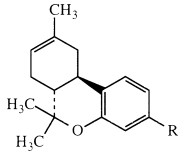
Results

Analogues of Δ^8-THC included 11-hydroxy-1-deoxy-Δ^8-THC (**5**), 1-deoxy-Δ^8-THC (**6**), its 3-butyl (**7**), and 3-hexyl analogues (**8**). As outlined in Scheme 1, alcohol **5** was prepared from 2-bromo-5-pentylmethoxybenzene (**9**) and apoverbenone following the protocol employed in the synthesis of the dimethylheptyl analogue (**1**).[4,9,10] The aryllithium derived from **9** was added to apoverbenone to give, after oxidative rearrangement, enone **10**, dissolving metal reduction of which proceeded stereoselectively to provide saturated ketone **11**. Ether cleavage, followed by treatment with $SnCl_4$, gave *cis*-fused ketone **12**. Isomerization of ketone **12** with $AlCl_3$ provided the 1-deoxynabilone analogue (**13**) in mediocre yield, plus a mixture of several other products from which flourene derivative **14** was isolated. Conversion of ketone **13** to the vinyl triflate, followed by palladium mediated carbonylation in the presence of methanol, gave ester **15** which provided alcohol **5** after reduction. 1-Deoxycannabinoids **6**–**8** were prepared from the corresponding Δ^8-THC analogue by conversion to the phosphate ester, followed by dissolving metal reduction as outlined in Scheme 2.[4,5]

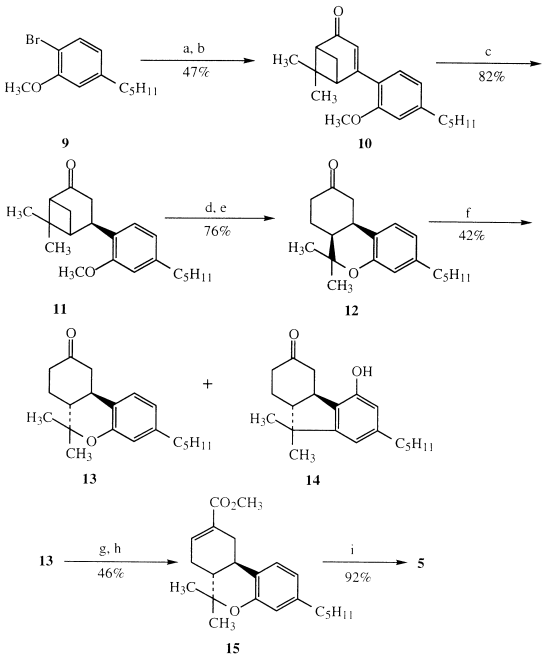


6 R = C_5H_{11}

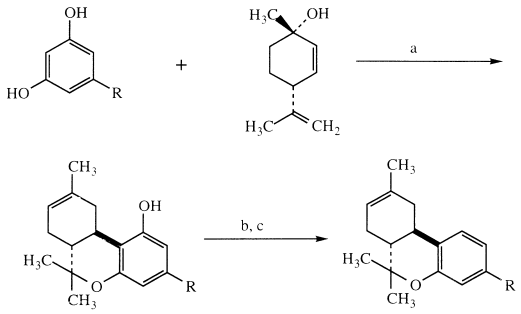
7 R = C_4H_9

8 R = C_6H_{13}

Modeling studies had indicated that in the proposed orientation of 1-deoxy-Δ^8-THC-DMH (**2**, $n=5$) which interacts with the CB_1 receptor, the dimethylheptyl side chain has sufficient length to reach the hydrophobic binding pocket of the receptor.[4] In designing additional 1-deoxy-cannabinoids it was assumed that decreasing the length of the alkyl side chain would attenuate the affinity for the CB_1 receptor. However, no prediction could be made concerning the effect of this structural modification on the affinity for the CB_2 receptor. A series of 1-deoxy-1′,1′-dimethylalkyl-Δ^8-THCs (**2**, $n=0$–7, where $n=$ the number of carbon atoms in the side chain−2) was synthesized employing the method used for the preparation of deoxycannabinoids **2**, $n=5$, and **6**–**8**. The resorcinol precursors were synthesized in four steps from 2,6-dimethoxyphenol using modifications of the procedure described by Dominianni et al. for the synthesis of 1,3-dimethoxy-5-(1,1-dimethylheptyl)benzene.[10,11] Ether cleavage to the resorcinol employing boron tribromide, followed by acid catalyzed reaction with *trans-para*-menthadienol, provided Δ^8-THC analogues,[12] which were converted to the 1-deoxycannabinoids by reduction of the phosphate ester as described above for the preparation of **2**, $n=5$, and **6**–**8** (Scheme 2).

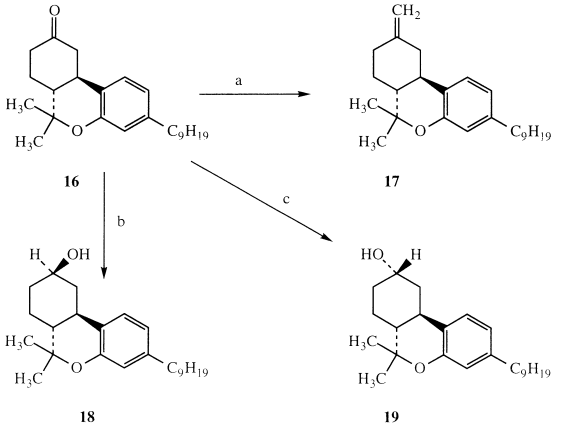


Scheme 1. (a) BuLi/THF, 0°C then apoverbenone; (b) PDC/CH_2Cl_2, 25°C; (c) Li/NH_3, THF, −78°C; (d) NaSPr/DMF, 120°C; (e) $SnCl_4$/CH_2Cl_2, 25°C; (f) $AlCl_3$/CH_2Cl_2, 25°C; (g) Tf_2O, 2,6-di-*tert*-butyl-4-methyl-pyridine/CH_2Cl_2, 40°C; (h) Et_3N, $Pd(OAc)_2$, Ph_3P, CO, CH_3OH/DMF, 45°C; (i) $LiAlH_4$/Et_2O, 0–25°C.



Scheme 2. (a) HOTs/C_6H_6, 80°C; (b) NaH/THF, 0°C then $(C_2H_5O)_2P(O)Cl$; (c) Li/NH_3, THF, −78°C.

Gareau et al. reported that nabilone methyl ether showed modest selectivity for the CB_2 receptor, and that the $\Delta^{9(11)}$-olefin (**4**) derived from it had high affinity for the CB_2 receptor ($K_i = 19.4$ nM), but effectively no affinity for the CB_1 receptor ($K_i = > 20{,}000$).[5] Based on the premise that the lack of the phenolic hydroxyl would have an effect on receptor affinity similar to that of converting the hydroxyl to a methyl ether, 1-deoxynabilone (**16**) and the derived $\Delta^{9(11)}$-olefin (**17**) were prepared (Scheme 3). We had earlier employed deoxynabilone (**16**) as an intermediate in the synthesis of alcohol **1**, and the corresponding $\Delta^{9(11)}$-olefin (**17**) was prepared by Wittig reaction of **16** with methylenetriphenylphosphorane. Epimeric alcohols **18** and **19** were obtained by reduction of **16**. Lithium aluminum hydride provided the equatorial β-isomer (**18**) as the major product, and K-Selectride (potassium tri-*sec*-butyl borohydride) gave predominantly the axial α-isomer (**19**).



Scheme 3. (a) $(C_6H_5)_3PCH_3^+$ Br^-, *n*-BuLi/THF, 65°C; (b) $LiAlH_4$/THF, 25°C; (c) KBH(*sec*-Bu)$_3$/THF, −78 to 25°C then H_2O_2/NaOH.

The affinities of deoxycannabinoids **2**, **5**, **6**–**8** and **16**–**19** for the CB_1 receptor were determined by measuring their ability to displace the potent cannabinoid [^{3}H]CP 55,940 from its binding site in a membrane preparation from rat brain as described by Compton et al.[13] Affinities for the CB_2 receptor were determined by measuring the ability of the compounds to displace [^{3}H]CP 55,940 from a cloned human receptor preparation using the procedure described by Showalter et al.[6] The results of these determinations are summarized in Table 1. Also included in Table 1 are the receptor affinities for cannabinoids **1** and Δ^9-THC.

With the exception of 1-deoxy-Δ^8-THC-DMH (**2**, $n = 5$) and its dimethyloctyl homologue (**2**, $n = 6$), 1-deoxycannabinoids lacking an oxygen function at C-9 or C-11 have little affinity for the CB_1 receptor. However, 1-deoxy-1′,1′-dimethylalkyl-Δ^8-THCs with side chains of three to seven carbon atoms (**2**, $n = 1$–5) have high affinity for the CB_2 receptor, ranging from $K_i = 2.9 \pm 1.6$ nM for **2**, $n = 5$, to $K_i = 19 \pm 4$ nM for 1-deoxy-1′,1′-dimethylhexyl-Δ^8-THC (**2**, $n = 4$).

1-Deoxy-1′,1′-dimethylbutyl-Δ^8-THC (**2**, $n = 2$) has only slight affinity for the CB_1 receptor ($K_i = 677 \pm 132$ nM), but has 200-fold selectivity for the CB_2 receptor ($K_i = 3.4 \pm 1.0$ nM). 1-Deoxy-Δ^8-THC (**6**) has virtually no affinity for the CB_1 receptor ($K_i > 10{,}000$ nM), but has significant affinity for the CB_2 receptor ($K_i = 32 \pm 9$ nM).

Of the 9- and 11-oxygenated cannabinoids, deoxynabilone (**16**) and the derived alcohols (**18** and **19**) show significant affinity for the CB_1 receptor (Table 1). However, the affinity of these compounds for the CB_2 receptor are approximately the same as their affinities for the CB_1 receptor. In accord with generalizations

Table 1. Receptor affinities (mean ± SEM) of 1-deoxy-cannabinoids and related compounds

Compound	K_i (nM)	
	CB_1	CB_2
Δ^9-THC	41 ± 2[a]	36 ± 10[b]
Δ^8-THC	44 ± 12	44 ± 17
1-Deoxy-11-hydroxy-Δ^8-THC-DMH (**1**)	1.2 ± 0.1[c]	0.032 ± 0.019[c]
3-(1′,1′-Dimethylethyl)-1-deoxy-Δ^8-THC (**2**, $n = 0$)	2150 ± 231	58 ± 13
3-(1′,1′-Dimethylpropyl)-1-deoxy-Δ^8-THC (**2**, $n = 1$)	2290 ± 505	14 ± 10
3-(1′,1′-Dimethylbutyl)-1-deoxy-Δ^8-THC (**2**, $n = 2$)	677 ± 132	3.4 ± 1.0
3-(1′,1′-Dimethylpentyl)-1-deoxy-Δ^8-THC (**2**, $n = 3$)	399 ± 76	10 ± 2
3-(1′,1′-Dimethylhexyl)-1-deoxy-Δ^8-THC (**2**, $n = 4$)	295 ± 52	19 ± 4
Deoxy-Δ^8-THC-DMH (**2**, $n = 5$)	23 ± 7[c]	2.91 ± 1.6[c]
3-(1′,1′-Dimethyloctyl)-1-deoxy-Δ^8-THC (**2**, $n = 6$)	51 ± 11	76 ± 4
3-(1′,1′-Dimethylnonyl)-1-deoxy-Δ^8-THC (**2**, $n = 7$)	178 ± 2	449 ± 98
1-Deoxy-11-hydroxy-Δ^8-THC (**5**)	161 ± 26	16 ± 3
1-Deoxy-Δ^8-THC (**6**)	> 10,000	32 ± 9
3-Butyl-1-deoxy-Δ^8-THC (**7**)	2790 ± 820	54 ± 8
3-Hexyl-1-deoxy-Δ^8-THC (**8**)	1610 ± 302	273 ± 63
Deoxynabilone (**16**)	44 ± 2	25 ± 7
Deoxy-$\Delta^{9(11)}$-THC-DMH (**17**)	909 ± 121	137 ± 6
β-Deoxynabilol (**18**)	7.9 ± 0.9	5.2 ± 2.0
α-Deoxynabilol (**19**)	28 ± 3	23 ± 7
Δ^8-THC-DMH methyl ether (**3**)	924 ± 104, 15,850 ± 2960[d]	65 ± 8, 20 ± 12[d]
$\Delta^{9(11)}$-THC-DMH methyl ether (**4**)	529 ± 49, > 20,000[d]	35 ± 14, 19 ± 4[d]

[a] ref. 13.
[b] ref. 6.
[c] ref. 4.
[d] ref. 5.

regarding the steric requirements in the region of C-9,[14,15] 1-deoxy-$\Delta^{9(11)}$-THC-DMH (**17**) shows little affinity for the CB_1 receptor ($K_i = 909 \pm 121$ nM), and only modest affinity for the CB_2 receptor ($K_i = 137 \pm 6$ nM).

In order to independently evaluate the affinities of 1-methoxy cannabinoids **3** and **4**, an effort was made to obtain samples of these ligands. However, samples were unavailable and an independent synthesis of these compounds was carried out. Methyl ether **3** was prepared by routine *O*-methylation of Δ^8-THC-DMH (K_2CO_3/dimethyl sulfate), and olefin **4** was obtained from nabilone methyl ether by a Wittig reaction with methylentriphenylphosphorane. Nabilone was prepared as described by Tius et al.[16] and was converted to the methyl ether by the procedure employed for the preparation of **3**. The affinity of each compound was determined as described above, and the data are included in Table 1. Neither compound had appreciable affinity for the CB_1 receptor; for **3**, $K_i = 924 \pm 104$ nM, and for **4**, $K_i = 529 \pm 49$ nM. These data are considerably different from those reported by Gareau et al. who found $K_i = > 10{,}000$ for both compounds.[5] However, it has been our experience that for compounds with very low receptor affinity, in which K_i approaches or exceeds the micromolar range, there is considerable variability in affinity data. Also, there are possible differences in methodology; our data were obtained using rat brain homogenates as described above,[13] while the Merck Frosst group employed a human CB_1 preparation of unspecified origin.[5] Both compounds **3** and **4** have moderate affinity ($K_i = 65 \pm 8$ and 35 ± 14 nM, respectively) for the CB_2 receptor. These data are in the same general range as those reported by Gareau et al., who found that **3** had $K_i = 20 \pm 12$ nM, and that **4** had $K_i = 19 \pm 4$ nM. As in the case of the CB_1 receptor, these differences may be due to differences in methodology in determining the receptor affinities.

From the data summarized in Table 1, it is possible to develop preliminary SAR for the CB_2 receptor based upon the affinities of 1-deoxy-Δ^8-THC analogues. First, it is quite apparent that a 1′,1′-dimethyl group leads to enhanced affinity for the CB_2 receptor. A comparison of the affinities of 3-(1′,1′-dimethylbutyl)-1-deoxy-Δ^8-THC (**2**, $n = 2$), its pentyl (**2**, $n = 3$) and hexyl (**2**, $n = 4$) homologues with 1-deoxy-cannabinoids with the same length side chain but lacking the gem-dimethyl group (**6**–**8**) shows that the 1′,1′-dimethylbutyl and dimethylhexyl cannabinoids have approximately 15-fold greater affinity for the CB_2 receptor than the unsubstituted analogues. Although 3-butyl-1-deoxy-Δ^8-THC (**7**) has moderate affinity for the CB_2 receptor ($K_i = 54 \pm 8$ nM), the 3-hexyl analogue (**8**) has low affinity ($K_i = 273 \pm 63$ nM). With a pentyl side chain the 1′,1′-dimethyl-deoxy-cannabinoid (**2**, $n = 3$) has threefold greater affinity for the CB_2 receptor than 1-deoxy-Δ^8-THC (**6**).

In the 1′,1′-dimethyl-1-deoxy-Δ^8-THC series, those compounds with a three to seven carbon side chain (**2**, $n = 1$–5) all have high affinity for the CB_2 receptor ($K_i = < 20$ nM). The greatest affinities are 1′,1′-dimethylbutyl- (**2**, $n = 2$) and 1′,1′-dimethylheptyl-Δ^8-THC (**2**, $n = 5$)[4] which have comparable affinities for the CB_2 receptor. The 1′,1′-dimethylethyl (**2**, $n = 0$) and 1′,1′-dimethyloctyl (**2**, $n = 6$) have moderate affinity for the CB_2 receptor, while 1′,1′-dimethylnonyl-1-deoxy-Δ^8-THC (**2**, $n = 7$) has very low affinity for the CB_2 receptor.

For all of the 1-deoxy-cannabinoids listed in Table 1, with the exception of 1-deoxy-Δ^8-THC-DMH[4] (**2**, $n = 5$) and the next higher homologue, 3-(1′,1′-dimethyloctyl)-1-deoxy-Δ^8-THC (**2**, $n = 6$), only those 1-deoxy-cannabinoids with an oxygen functionality at C-9 or C-11, and a 3-(1′,1′-dimethylheptyl) side chain (**1**, **16**, **18** and **19**), show significant affinity for the CB_1 receptor. These data are in accord with the hypothesis reached on the basis of molecular modeling studies reported previously, which suggested that the 11-hydroxyl of cannabinoid **1** interacts with Lys 192 of the CB_1 receptor in a manner similar to that of the 1-hydroxyl of traditional cannabinoids, and that 1-deoxy-Δ^8-THC-DMH adopts an orientation with respect to the receptor in which the pyran oxygen interacts with Lys 192.[4] The modeling studies indicated that the heptyl side chain of **2**, $n = 5$, has sufficient length to reach the lipophilic portion of the receptor, and presumably the octyl side chain of **2**, $n = 6$, also is of the correct length to interact with the lipophilic portion of the CB_1 receptor. 1-Deoxy-Δ^8-THC analogues with a 1,1-dimethylalkyl side chain of less than seven or more than eight carbon atoms have relatively little affinity for the CB_1 receptor; however, three of the lower homologues (**2**, $n = 1$–3) which have little affinity for the CB_1 receptor ($K_i = > 650$ nM) have high affinity for the CB_2 receptor. In particular, 3-(1′,1′-dimethylbutyl)-1-deoxy-Δ^8-THC (**2**, $n = 2$) has exceptionally high affinity ($K_i = 3.4 \pm 1.0$ nM) for the CB_2 receptor and low affinity for the CB_1 receptor ($K_i = 677 \pm 32$ nM).

In conclusion, 3-(1′,1′-dimethylbutyl)-1-deoxy-Δ^8-THC (**2**, $n = 2$) and several other 1-deoxy-Δ^8-THC analogues have high affinity for the CB_2 receptor, but little affinity for the CB_1 receptor. These compounds are readily available and should prove to be useful selective ligands for investigating the role of the CB_2 receptor.

Experimental

General

IR spectra were obtained using Nicolet 5DX or Magna spectrometers; ^1H 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 (32–63 μm) using the indicated solvents as eluents. All new compounds were homogeneous to TLC and ^{13}C

NMR. All target compounds were homogeneous to TLC in two different solvent systems.

2-Bromo-5-pentylmethoxybenzene (9). To a solution of 5.30 g (29.7 mmol) of 3-pentylmethoxybenzene[17] in 120 mL of freshly distilled cyclohexane at 25°C was added 23.7 mL (59.3 mmol) of 2.5 M *n*-butyllithium in hexanes. The mixture was stirred at reflux temperature for 18 h, cooled and diluted with 60 mL of dry THF. After cooling to −78°C, 7.10 mL (59.4 mmol) of 1,2-dibromoethane was added dropwise, and the mixture was stirred at −78°C for 0.5 h, allowed to warm to ambient temperature and stirred for an additional 1 h. The reaction was quenched with water, the phases were separated, and the aqueous phase extracted with two portions of ether. The combined organic layers were washed with brine, dried ($MgSO_4$) and the solvent removed in vacuo. Chromatography (petroleum ether:ethyl acetate 50:1) gave 5.50 g (72%) of aryl bromide as a pale yellow oil: bp 160°C/0.5 mm Hg; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (t, J = 6.6 Hz, 3H), 1.22–1.41 (m, 4H), 1.52–1.68 (m, 2H), 2.55 (t, J = 8.0 Hz, 2H), 3.86 (s, 3H), 6.64 (dd, J = 1.6, 7.9 Hz, 1H), 6.71 (d, J = 1.7 Hz, 1H), 7.39 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.4, 30.9, 31.3, 35.9, 55.9, 108.3, 112.2, 121.7, 132.7, 143.8, 155.5; HRMS: calcd for $C_{12}H_{17}BrO$ 256.0463, found 256.0464.

4-(2-Methoxy-4-pentylphenyl)-6,6-dimethylbicyclo[3.1.1]-hept-3-en-2-one (10). To a stirred solution of 7.80 mL (19.5 mmol) of 2.5 M *n*-butyllithium in hexanes and 3.20 mL of freshly distilled TMEDA in 10 mL of dry THF at −78°C was added dropwise a solution of 4.48 g (17.4 mmol) of 2-bromo-5-pentylmethoxybenzene (**9**) in 5 mL of dry THF. The reaction mixture was stirred for 0.5 h at −78°C, and 2.18 g (16.0 mmol) of apoverbenone in 5 mL of dry THF was added dropwise. The mixture was allowed to warm to room temperature, stirred for 18 h, quenched with saturated aqueous 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 25 mL of dry CH_2Cl_2 and the solution was added dropwise to a suspension of 3.3 g of PDC in 30 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% aqueous NaOH, water, 10% aqueous HCl, water, saturated aqueous $NaHCO_3$ and water. After drying ($MgSO_4$), the solvent was removed in vacuo to give the crude enone which was purified by flash chromatography (petroleum ether: ether 3:1) to give 2.57 g (47%) of pure enone: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 6.7 Hz, 3H), 1.11 (s, 3H), 1.21–1.43 (m, 4H), 1.54 (s, 3H), 1.57–1.68 (m, 2H), 2.26 (d, J = 9.2 Hz, 1H), 2.61 (t, J = 7.9 Hz, 2H), 2.68–2.77 (m, 1H), 2.83–2.95 (m, 1H), 3.00–3.07 (m, 1H), 3.81 (s, 3H), 6.06 (s, 1H), 6.73 (s, 1H), 6.79 (d, J = 7.8 Hz, 1H), 7.11 (d, J = 7.7 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.4, 26.8, 31.0, 31.5, 36.1, 41.2, 49.2, 54.0, 55.2, 57.8, 111.3, 120.7, 121.9, 125.7, 128.3, 146.4, 156.8, 168.2, 204.4; MS (EI) m/z 312 (78), 269 (44), 242 (100), 213 (25), 185 (36), 115 (29); HRMS: calcd for $C_{21}H_{28}O_2$ 312.2089, found 312.2090.

(2-Methoxy-4-pentylphenyl)-6,6-dimethylbicyclo[3.1.1]-heptan-2-one (11). To a solution of 0.50 g (71 mg atoms) of Li in 200 mL of liquid NH_3 at −78°C was added dropwise 2.57 g (8.5 mmol) of enone **10** in 25 mL of dry ether and 5 mL of dry THF. The reaction mixture was stirred at −78°C for 1.5 h, quenched by the addition of solid NH_4Cl, and the NH_3 evaporated at ambient temperature. The solid residue was taken up in ether, and the ether solution was washed with successive portions of 10% aqueous HCl, water, saturated aqueous $NaHCO_3$ and water. After drying ($MgSO_4$), the solvent was removed in vacuo to afford an oil which was purified by flash chromatography (petroleum ether:ether 3:1) to give 2.41 g (82%) of pure ketone: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82–0.96 (m, 6H), 1.21–1.41 (m, 4H), 1.46 (s, 3H), 1.51–1.69 (m, 3H), 2.39–2.65 (m, 4H), 2.70–2.82 (m, 2H), 3.25–3.41 (m, 1H), 3.60–3.70 (m, 1H), 3.82 (s, 3H), 6.68 (s, 1H), 6.70 (d, J = 8.9 Hz, 1H), 6.93 (d, J = 7.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 22.5, 25.3, 26.6, 28.5, 31.1, 31.6, 35.7, 35.8, 39.6, 40.8, 44.1, 55.0, 58.3, 110.3, 119.2, 126.8, 129.6, 142.4, 157.4, 214.2; MS (EI) m/z 314 (31), 243 (34), 204 (100), 147 (29), 115 (21), 83 (70); HRMS: calcd for $C_{21}H_{30}O_2$ 314.2246, found 314.2246.

(6a*S*,10a*R*)-3-Pentyl-6,6a,7,8,10,10a-hexahydro-6,6-dimethyl-9*H*-dibenzo[b,d]pyran-9-one (12). To a stirred solution of 2.01 g (67.0 mmol) of 80% NaH in 80 mL of dry DMF was added dropwise at ambient temperature 6.80 mL (74.5 mmol) of 1-propanethiol. The mixture was stirred until it became clear, and a solution of 2.34 g (7.45 mmol) of 4-(2-methoxy-4-pentylphenyl)-6,6-dimethylbicyclo[3.1.1]heptan-2-one **11** in 5 mL of DMF was added dropwise. The mixture was heated with stirring at 120°C for 5 h, cooled, 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 (petroleum ether:ethyl acetate 5:2) to give 2.10 g (94%) of phenol which was used in the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.78–0.96 (m, 6H), 1.17–1.40 (m, 4H), 1.40–1.72 (m, 6H), 2.46 (t, J = 8.1 Hz, 2H), 2.58–2.90 (m, 4H), 3.51–3.78 (m, 2H), 6.60 (d, J = 8.2 Hz, 1H), 6.62 (s, 1H), 6.85 (d, J = 7.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 22.5, 25.4, 26.5, 28.4, 28.8, 31.0, 31.5, 35.3, 35.7, 39.8, 41.0, 43.6, 58.3, 115.2, 118.8, 126.8, 127.6, 142.4, 154.4, 218.0.

To a solution of 1.63 g (8.30 mmol) of phenol in 8 mL of $CHCl_3$ was added 8.30 mL (8.30 mmol) of 1 M $SnCl_4$ in CH_2Cl_2. The reaction was stirred at ambient temperature for 18 h, poured onto ice and extracted with ether. The ether extracts were washed with successive portions of 10% aqueous HCl, water, saturated aqueous $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was removed in vacuo to afford, after chromatography (petroleum ether:ethyl acetate 2:1), 1.55 g (81%) of ketone as an oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.18–1.40 (m, 5H), 1.34 (s, 3H), 1.43 (s, 3H), 1.50–1.73 (m, 3H), 1.96–2.43 (m, 4H), 2.49 (t, J = 8.0 Hz, 2H), 2.64–2.84 (m, 1H), 3.58–3.77 (m, 1H), 6.60 (d, J = 1.2 Hz, 1H), 6.70 (dd, J = 8.0, 1.4 Hz, 1H), 7.13 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.8, 22.4, 23.0, 26.3, 26.6, 30.6, 31.4, 34.3, 35.3, 39.6, 40.1, 43.9, 75.6, 116.6, 117.2, 120.7, 126.9, 143.0, 152.6, 210.1; MS (EI)

m/*z* 300 (100), 285 (51), 257 (39), 217 (85), 134 (51); HRMS: calcd for $C_{20}H_{28}O_2$ 300.2089, found 300.2090.

(6a*R*,10a*R*)-3-Pentyl-6,6a,7,8,10,10a-hexahydro-6,6-dimethyl-9*H*-dibenzo[b,d] pyran-9-one (13). To a solution of 1.47 g (4.89 mmol) of *cis*-ketone **12** in 10 mL of dry CH_2Cl_2 at ambient temperature was added 1.95 g (14.7 mmol) of $AlCl_3$, and the reaction mixture was stirred for 2 h. The mixture was poured onto ice, extracted with three portions of ether, and the combined extracts were washed with 10% aqueous HCl, brine, saturated aqueous $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was removed in vacuo to give an oil which was chromatographed (petroleum ether:ethyl acetate 4:1) to give 0.066 g of recovered *cis*-ketone and 0.590 g (42% based on recovered starting material) of *trans*-ketone as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.16 (s, 3H), 1.49 (s, 3H), 1.23–1.43 (m, 4H), 1.43–1.67 (m, 3H), 1.82–1.96 (m, 1H), 2.09–2.63 (m, 6H), 2.78–2.92 (m, 1H), 3.03–3.17 (m, 1H), 6.66 (s, 1H), 6.70 (d, J = 8.0 Hz, 1H), 6.98 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.8, 22.5, 27.2, 28.1, 30.8, 31.5, 35.4, 35.8, 40.8, 45.7, 45.9, 76.9, 116.9, 120.4, 120.6, 125.4, 143.2, 152.7, 210.0; MS (EI) *m*/*z* 300 (100), 217 (96), 190 (63), 177 (52), 161 (66); HRMS: calcd for $C_{20}H_{28}O_2$ 300.2089, found 300.2090.

Further elution with the same solvent pair afforded 0.65 g of a mixture of several compounds with very close polarities. Repeated chromatography provided 0.042 g of the major component of the mixture, flourene derivative **14**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, J = 6.8 Hz, 3H), 0.96 (s, 3H), 1.36 (s, 3H), 1.22–1.41 (m, 4H), 1.48–1.66 (m, 2H), 1.66–1.84 (m, 1H), 1.88–2.11 (m, 2H), 2.31–2.57 (m, 4H), 2.57–2.71 (m, 1H), 2.98–3.16 (m, 1H), 3.50–3.65 (m, 1H), 6.11 (s, 1H, disappears when shaken with D_2O), 6.44 (s, 1H), 6.58 (s, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 32;14.0, 22.3, 22.5, 23.2, 26.0, 31.3, 31.7, 35.9, 41.4, 43.5, 44.8, 46.3, 57.4, 114.0, 114.5, 124.9, 144.0, 152.3, 155.6, 213.6; MS (EI) *m*/*z*: 300 (60), 285 (48), 244 (37), 243 (42), 229 (100), 173 (22), 145 (15), 55 (62).

(6a*R*,10a*R*)-9-Carbomethoxy-3-pentyl-6a,7,10,10a-tetrahydro-6,6-dimethyl-6*H*-dibenzo[b,d]pyran (15). To a stirred solution of 0.602 g (2.93 mmol) of 2,6-di-*tert*-butyl-4-methylpyridine in 3 mL of dry CH_2Cl_2 was quickly added 0.370 g (2.20 mmol) of trifluoromethanesulfonic anhydride. After stirring for 5 min, a solution of 0.440 g (1.46 mmol) of ketone **13** in 2.5 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 precipitated solid was filtered off, and the filtrate was washed with cold 10% aqueous HCl, and brine. After drying ($MgSO_4$) the solvent was removed in vacuo to give an oil which was purified by flash chromatography (petroleum ether:ether 10:1) to afford 0.514 g (81%) of triflate as an oil which was used in the subsequent step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.0 Hz, 3H), 1.16 (s, 3H), 1.42 (s, 3H), 1.12–1.38 (m, 3H), 1.48–1.68 (m, 3H), 1.74–1.88 (m, 1H), 1.90–2.19 (m, 1H), 2.28–2.46 (m, 2H), 2.52 (t, J = 7.6 Hz, 2H), 2.80–3.05 (m, 2H), 5.80–5.90 (m, 1H), 6.65 (d, J = 1.3 Hz, 1H), 6.72 (dd, J = 8.0, 1.5 Hz, 1H), 7.03 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 18.9, 22.5, 25.8, 27.6, 30.9, 31.5, 32.5, 33.7, 35.5, 41.9, 76.2, 117.2, 117.4, 120.4, 120.7, 126.2, 143.3, 148.0, 152.6.

To a solution of 0.490 g (1.13 mmol) of triflate in 4 mL of dry DMF were added 0.320 mL (2.26 mmol) of Et_3N, 0.0255 g (0.11 mmol) of $Pd(OAc)_2$, 0.0595 g (0.226 mmol) of triphenylphosphine and 1.90 mL of methanol. The reaction flask was purged for 10 min with CO, then stirred under an atmosphere of CO at 45°C for 18 h. The reaction mixture was poured into water, and extracted with ether. After drying ($MgSO_4$), the solvent was removed in vacuo and the residue was purified by flash chromatography (petroleum ether:ether 10:1) to give 0.219 g (57%) of methyl ester: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.7 Hz, 3H), 1.16 (s, 3H), 1.40 (s, 3H), 1.20–1.82 (m, 7H), 1.90–2.16 (m, 2H), 2.32–2.47 (m, 1H), 2.52 (t, J = 7.9 Hz, 2H), 2.58–2.73 (m, 1H), 3.10–3.28 (m, 1H), 3.76 (s, 3H), 6.64 (s, 1H), 6.72 (d, J = 7.7 Hz, 1H), 7.04 (m, 1H), 7.18 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 18.9, 22.5, 27.6, 28.0, 30.6, 30.8, 31.5, 31.7, 35.5, 42.2, 51.6, 76.2, 116.8, 120.4, 12.7, 126.6, 129.8, 138.2, 142.6, 152.6, 167.4; MS (EI) *m*/*z* 342 (95), 230 (27), 215 (100), 177 (63), 159 (27), 119 (32); HRMS: calcd for $C_{22}H_{30}O_3$ 342.2195, found 342.2196.

(6a*R*,10a*R*)-9-Hydroxymethyl-3-pentyl-6a,7,10,10a-tetrahydro-6,6-dimethyl-6*H*-dibenzo[b,d]pyran (5). To a stirred suspension of 0.090 g (2.37 mmol) of $LiAlH_4$ in 10 mL of dry ether was added dropwise at 0°C a solution of 0.201 g (0.587 mmol) of ester **15** in 7 mL of dry ether. The reaction mixture was stirred at ambient temperature for 18 h, quenched with water, and acidified to pH 3. The phases were separated, and the aqueous phase was extracted with ether. The combined organic extracts were washed with brine, dried ($MgSO_4$), and the solvent was removed in vacuo. The crude product was purified by flash chromatography (petroleum ether:ether 1:1) to give 0.172 g (92%) of alcohol as a colorless oil: R_f 0.32 (petroleum ether:ether 1:1), 0.61 (petroleum ether:ethyl acetate 1:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.15 (s, 3H), 1.39 (s, 3H), 1.23–1.45 (m, 4H), 1.50–1.67 (m, 2H), 1.67–2.31 (m, 5H), 2.51 (t, J = 7.6 Hz, 2H), 2.68–2.86 (m, 2H), 4.05 (m, 2H), 5.69–5.78 (m, 1H), 6.63 (d, J = 1.4 Hz, 1H), 6.69 (dd, J = 7.9, 1.5 Hz, 1H), 7.11 (d, J = 7.9 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 19.0, 22.5, 27.1, 27.6, 30.8, 31.5, 31.9, 32.1, 35.4, 43.0, 66.8, 76.6, 116.8, 120.3, 121.5, 122.4, 126.4, 136.9, 142.4, 152.7; MS (EI) *m*/*z* 314 (100), 281 (28), 253 (35), 215 (95), 177 (63); HRMS: calcd for $C_{21}H_{30}O_2$ 314.2246, found 314.2246.

1-Deoxy-Δ^8-tetrahydrocannabinol (6). To a stirred solution of 0.495 g (1.58 mmol) of Δ^8-THC in 2 mL of dry THF at 0°C was added, in portions, 0.071 g (2.4 mmol) of 80% NaH (suspension in mineral oil). The resulting mixture was stirred for 10 min at 0°C and 0.460 mL (3.18 mmol) of diethyl chlorophosphate was added dropwise. The mixture was allowed to warm to room temperature and stirred for 1 h. The reaction mixture was diluted with ether and the ethereal solution was washed with 10% NaOH, brine, and dried ($MgSO_4$). Concentration afforded an oil which was chromatographed using

petroleum ether:ethyl acetate (3:1) to give 0.558 g (79%) of pure (TLC) phosphate ester which was used in the subsequent step without further purification. The phosphate ester was reduced as described above for the preparation of ketone **11**. From 0.301 g (0.669 mmol) of ester there was obtained 0.173 g (88%) of pure deoxy-Δ^8-THC (**6**) after purification by flash chromatography (petroleum ether: ethyl acetate 20:1). R_f 0.50 (hexanes:ethyl acetate 50:1), 0.61 (hexanes:ethyl acetate 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.12 (s, 3H), 1.36 (s, 3H), 1.19–1.47. (m, 4H), 1.51–2.02 (m, 5H), 1.71 (s, 3H), 2.06–2.19 (m, 1H), 2.50 (t, J = 7.9 Hz, 2H), 2.44–2.76 (m, 2H), 5.42 (br s, 1H), 6.63 (s, 1H), 6.67 (d, J = 7.7 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.1, 22.5, 23.4, 27.5, 27.6, 30.9, 31.6, 32.1, 35.5, 36.6, 42.8, 76.5, 116.8, 119.9, 120.1, 122.7, 126.3, 133.3, 142.1, 152.8; MS (EI) m/z 298 (33), 255 (21), 243 (11), 230 (21), 215 (100), 185 (25), 177 (26), 173 (11), 159 (17); HRMS: calcd for $C_{21}H_{30}O$ 298.2297, found 298.2295.

1-Deoxy-3-butyl-Δ^8-tetrahydrocannabinol (7). 3-Butyl-Δ^8-tetrahydrocannabinol[18] was converted to 1-deoxy-3-butyl-Δ^8-tetrahydrocannabinol by the procedure used in the preparation of deoxy-Δ^8-THC. From 0.42 g (1.40 mmol) of 3-butyl-Δ^8-THC there was obtained 0.47 g (77%) of pure phosphate ester. From 0.301 g (0.669 mmol) of phosphate ester there was obtained 0.22 g (84%) of deoxy-cannabinoid **7** as a colorless oil after chromatography (petroleum ether:ethyl acetate 200:1). R_f 0.37 (petroleum ether:ethyl acetate 100:1), 0.77 (petroleum ether:ethyl acetate 25:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (t, J = 7.3 Hz, 3H), 1.18 (s, 3H), 1.28–1.46 (m, 2H), 1.42 (s, 3H), 1.56–1.98 (m, 5H), 1.76 (s, 3H), 2.16–2.22 (m, 1H), 2.56 (t, J = 7.8 Hz, 2H), 2.58–2.76 (m, 2H), 5.48 (br s, 1H), 6.66 (d, J = 1.2 Hz, 1H), 6.73 (dd, J = 1.2, 7.8 Hz, 1H), 7.14 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 13.9, 19.1, 22.4, 23.4, 27.5, 27.7, 32.1, 33.3, 35.2, 36.6, 42.8, 76.7, 116.9, 119.9, 120.2, 122.8, 126.4, 133.4, 142.2, 152.8; MS (EI) m/z 285 (25), 284 (90), 201 (100); $[\alpha]_D^{20}$ −122° (c 0.88, $CHCl_3$); HRMS: calcd for $C_{20}H_{28}O$ 284.2140, found 284.2135.

1-Deoxy-3-hexyl-Δ^8-tetrahydrocannabinol (8). 3-Hexyl-Δ^8-tetrahydrocannabinol[19] was converted to the 1-deoxy analogue by the procedure described above. From 0.56 g (1.70 mmol) of 3-hexyl-Δ^8-THC there was obtained 0.285 g (54% for two steps) of pure deoxy-cannabinoid **8** after flash chromatography (petroleum ether:ethyl acetate 20:1): R_f 0.25 (petroleum ether:ether 50:1), 0.69 (petroleum ether:ether 20:1): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.4 Hz, 3H), 1.24 (s, 3H), 1.36 (s, 3H), 1.71 (s, 3H), 1.19–1.47 (m, 6H), 1.51–2.02 (m, 5H), 2.06–2.19 (m, 1H), 2.50 (t, J = 7.9 Hz, 2H), 2.44–2.76 (m, 2H), 5.38–5.46 (m, 1H), 6.63 (s, 1H), 6.67 (d, J = 7.7 Hz, 1H), 7.07 (d, J = 7.8 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.1, 22.5, 23.4, 27.4, 27.6, 29.1, 30.9, 31.6, 32.1, 35.5, 36.6, 42.8, 76.5, 116.8, 119.9, 120.1, 122.7, 126.3, 133.3, 142.1, 152.8; $[\alpha]_D^{20}$ −160°; HRMS: calcd for $C_{22}H_{32}O$ 312.2453, found 312.2452.

2 Methyl-2-(3,5-dimethoxyphenyl)propane. A solution of 1.7 g (23.0 mmol) of *tert*-butyl alcohol, 3.5 g (22.7 mmol) of 2,6-dimethoxyphenol and 4.0 mL of methanesulfonic acid was stirred at 50°C for 3 h and at ambient temperature for an additional 14 h. The reaction mixture was poured onto ice, extracted with CH_2Cl_2, and the extracts were washed with water, saturated aqueous $NaHCO_3$ and dried ($MgSO_4$). The solvent was removed in vacuo to give 4.2 g (88%) of a brown oil which was used in the subsequent step without further purification.

The phenol was converted to 2-methyl-2-(3,5-dimethoxyphenyl)propane via the phosphate ester using the procedure described above for the preparation of deoxy-cannabinoid **6**. From 4.40 g (21.0 mmol) of phenol there was obtained 3.5 g (86%) of 2-methyl-2-(3,5-dimethoxyphenyl)propane as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 (s, 9H), 3.80 (s, 6H), 6.32 (t, J = 2.2 Hz, 1H), 6.56 (d, J = 2.2 Hz, 2H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 31.2, 34.9, 55.1, 96.7, 104.0, 153.8, 160.5.

(1′,1′-Dimethylethyl)-Δ^8-tetrahydrocannabinol. To 1.75 g (9.0 mmol) of 2-methyl-2-(3,5-dimethoxyphenyl)propane at 0°C was added 22.0 mL (22.0 mmol) of 1.0 M BBr_3 in CH_2Cl_2. The reaction mixture was warmed to ambient temperature, stirred for 18 h and carefully poured into ice water. After extraction with CH_2Cl_2, the organic extracts were washed with water, and dried ($MgSO_4$). The solvent was removed to give 1.50 g (100%) of crude substituted resorcinol as a brown oil which was used in the next step without further purification: A mixture of 1.50 g (9.0 mmol) of crude resorcinol, 1.40 g (9.2 mmol) of *trans-p*-menthadienol and 0.20 g of toluenesulfonic acid monohydrate in 100 mL of benzene was heated at reflux for 3 h. After cooling, the mixture was diluted with ether, washed with brine and dried ($MgSO_4$). The solvent was removed in vacuo to give a brown gum which was chromatographed using petroleum ether:ethyl acetate (19:1) to afford 2.1 g (77%) of cannabinoid as a viscous pale yellow oil: R_f 0.38 (petroleum ether:ethyl acetate 9:1), 0.58 (petroleum ether:ethyl acetate 4:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.12 (s, 3H), 1.23 (s, 9H), 1.40 (s, 3H), 1.70 (s, 3H), 1.75–1.94 (m, 3H), 2.14 (m, 1H), 2.72 (dt, J = 4.6, 10.8 Hz, 1H), 3.22 (dd, J = 4.6, 16.3 Hz, 1H), 5.04 (s, 1H), 5.43 (d, J = 4.0 Hz, 1H), 6.30 (d, J = 1.6 Hz, 1H), 6.46 (d, J = 1.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 18.5, 23.5, 27.6, 27.8, 31.1, 31.4, 34.3, 35.9, 44.8, 76.7, 104.9, 107.3, 110.3, 119.3, 134.7, 151.1, 154.4, 154.5. MS (EI) m/z (rel intensity) 301 (30), 300 (90), 217 (100).

1-Deoxy-3-(1′,1′-dimethylethyl)-Δ^8-tetrahydrocannabinol (2, n = 0). Dimethyl-ethyl-Δ^8-THC was converted to the corresponding deoxy-cannabinoid by the procedure used for the preparation of deoxy-Δ8-THC. From 1.0 g (3.3 mmol) of 3-(1′,1′-dimethylethyl)-Δ^8-THC there was obtained 1.0 g (69%) of phosphate ester. Reduction of 0.70 g (1.61 mmol) of this phosphate ester gave 0.35 g (77%) of deoxy-cannabinoid as a yellow solid, mp 101–103°C, after chromatography (petroleum ether:ethyl acetate 100:1): R_f 0.39 (petroleum ether:ethyl acetate 100:1), 0.71 (petroleum ether:ethyl acetate 25:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.19 (s, 3H), 1.32 (s, 9H), 1.42 (s, 3H), 1.69–2.03 (m, 3H) 1.72 (s, 3H), 2.15 (m, 1H), 2.61–2.76 (m, 2H), 5.48 (br s, 1H), 6.86 (d, J = 1.9 Hz, 1H), 6.94 (dd, J = 1.9, 8.1 Hz, 1H), 7.17 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 19.2, 23.5, 27.5, 27.7, 31.3,

32.0, 34.4, 36.4, 42.8, 76.8, 114.1, 117.1, 119.9, 122.6, 126.2, 133.4, 150.6, 152.6; MS (EI) *m/z* (rel intensity) 284 (30), 201 (30), 57 (100); $[\alpha]_D^{20}$ −168° (*c* 1.30, $CHCl_3$); anal. calcd for $C_{20}H_{28}O$: C, 84.45; H, 9.92; found: C, 84.39; H, 10.00.

1-Deoxy-3-(1′,1′-dimethylpropyl)-Δ⁸-tetrahydrocannabinol (2, *n* = 1). Deoxy-cannabinoid **2**, *n* = 1, was obtained as a colorless oil after chromatography (petroleum ether:ethyl acetate 100:1): R_f 0.36 (petroleum ether:ethyl acetate 100:1), 0.72 (petroleum ether:ethyl acetate 25:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.69 (t, *J* = 7.3, 3H), 1.16 (s, 3H), 1.25 (s, 6H), 1.40 (s, 3H), 1.60 (q, *J* = 7.3 Hz, 2H), 1.67–1.97 (m, 6H), 2.13 (m, 1H), 2.58–2.73 (m, 2H), 5.45 (br s, 1H), 6.77 (d, *J* = 2.0 Hz, 1H), 6.85 (dd, *J* = 2.0, 8.1 Hz, 1H), 7.14 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 9.2, 19.2, 23.5, 27.5, 27.7, 28.3, 32.0, 36.5, 36.8, 37.6, 42.8, 76.7, 114.8, 117.8, 119.9, 122.5, 126.0, 133.4, 149.1, 152.5; MS (EI) *m/z* (relative intensity) 298 (10), 167 (100); $[\alpha]_D^{20}$ −143° (*c* 1.25, $CHCl_3$); HRMS: calcd for $C_{21}H_{30}O$ 298.2297, found 298.2297.

1-Deoxy-3-(1′,1′-dimethylbutyl)-Δ⁸-tetrahydrocannabinol (2, *n* = 2). Deoxy-cannabinoid **2**, *n* = 2, was obtained as a colorless oil after chromatography (petroleum ether:ethyl acetate 100:1): R_f 0.42 (petroleum ether:ethyl acetate 100:1), 0.81 (petroleum ether:ethyl acetate 25:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, *J* = 7.3, 3H), 1.08–1.20 (m, 2H), 1.23 (s, 3H), 1.28 (s, 6H), 1.42 (s, 3H), 1.53–1.59 (m, 2H), 1.66–2.21 (m, 3H) 1.75 (s, 3H), 2.15 (m, 1H), 2.60–2.75 (m, 2H), 5.48 (br s, 1H), 6.78 (d, *J* = 1.8 Hz, 1H), 6.78 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.15 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.8, 17.9, 19.2, 23.4, 27.5, 27.7, 28.8, 28.9, 32.0, 36.5, 37.4, 42.8, 47.0, 76.7, 114.6, 117.6, 119.9, 122.4, 126.0, 133.3, 149.3, 152.5; MS (EI) *m/z* (rel intensity) 312 (30), 269 (45), 57 (100); $[\alpha]_D^{20}$ −185° (*c* 1.65, $CHCl_3$); HRMS: calcd for $C_{22}H_{32}O$ 312.2453, found 312.2451.

1-Deoxy-3-(1′,1′-dimethylpentyl)-Δ⁸-tetrahydrocannabinol (2, *n* = 3). Deoxy-cannabinoid **2**, *n* = 3, was obtained from 3-(1′,1′-dimethylpentyl-Δ^8-tetrahydrocannabinol[20] as a colorless oil after flash chromatography (petroleum ether:ethyl acetate 20:1): R_f 0.50 (hexanes:ethyl acetate 50:1), 0.61 (hexanes:ethyl acetate 20:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, *J* = 7.1 Hz, 3H), 1.15 (s, 3H), 1.25 (s, 6H), 1.38 (s, 3H), 1.71 (s, 3H), 1.00–2.05 (m, 9H), 2.07–2.22 (m, 1H), 2.52–2.75 (m, 2H), 5.43 (s, 1H), 6.76 (d, *J* = 1.7 Hz, 1H), 6.83 (dd, *J* = 8.1, 1.7 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 19.1, 23.4, 23.5, 26.9, 27.5, 27.7, 28.8, 32.0, 36.5, 37.2, 42.8, 44.3, 76.6, 114.6, 117.6, 119.9, 122.3, 126.0, 133.3, 149.3, 152.5; MS (EI) *m/z* (rel intensity) 326 (23), 269 (100), 243 (18); $[\alpha]_D^{20}$ −152° (*c* 1.24, $CHCl_3$); HRMS: calcd for $C_{23}H_{34}O$ 326.2610, found 326.2609.

1-Deoxy-3-(1′,1′-dimethylhexyl)-Δ⁸-tetrahydrocannabinol (2, *n* = 4). Pure deoxy-cannabinoid **2**, *n* = 4, was obtained as a yellow oil: R_f 0.39 (hexanes:ethyl acetate 100:1), 0.58 (hexanes:ethyl acetate 25:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.82 (t, *J* = 7.1 Hz, 3H), 1.18 (s, 6H), 1.01–2.04 (m, 14H), 1.39 (s, 3H), 1.68 (s, 3H), 2.07–2.23 (m, 1H), 2.51–2.77 (m, 2H), 5.44 (s, 1H), 6.76 (d, *J* = 2.0 Hz, 1H), 6.83 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 19.2, 22.5, 23.5, 24.3, 27.5, 27.7, 28.8, 28.9, 32.0, 32.6, 36.5, 37.3, 42.9, 44.5, 76.7, 114.6, 117.6, 119.9, 122.4, 126.0, 133.4, 149.4, 152.5; MS (EI) *m/z* (rel intensity) 340 (43), 270 (75), 269 (100), 257 (49); $[\alpha]_D^{20}$ −134° (*c* 1.33, $CHCl_3$); HRMS: calcd for $C_{24}H_{36}O$ 340.2766, found 340.2765.

1-Deoxy-3-(1′,1′-dimethyloctyl)-Δ⁸-tetrahydrocannabinol (2, *n* = 6). Pure deoxy-cannabinoid **2**, *n* = 6, was obtained as an oil after flash chromatography (petroleum ether:ethyl acetate 20:1): R_f 0.40 (hexanes:ethyl acetate 100:1), 0.60 (hexanes:ethyl acetate 25:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, *J* = 7.1 Hz, 3H), 1.15 (s, 3H), 1.25 (s, 6H), 1.39 (s, 3H), 1.72 (s, 3H), 0.97–2.06 (m, 15H), 2.06–2.24 (m, 1H), 2.51–2.74 (m, 2H), 5.44 (s, 1H), 6.76 (d, *J* = 1.7 Hz, 1H), 6.83 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 19.2, 22.6, 23.5, 24.7, 27.5, 27.7, 28.8, 29.2, 30.3, 31.9, 32.0, 36.5, 37.3, 42.8, 44.5, 76.7, 114.6, 117.6, 119.9, 122.4, 126.0, 133.4, 149.4, 152.5; MS (EI) *m/z* (relative intensity) 369 (26), 285 (14), 269 (100), 185 (13); $[\alpha]_D^{20}$ −142° (*c* 1.33, $CHCl_3$); HRMS: calcd for $C_{26}H_{40}O$ 368.3079, found 368.3080.

1-Deoxy-3-(1′,1′-dimethylnonyl)-Δ⁸-tetrahydrocannabinol (2, *n* = 7). Pure deoxy-cannabinoid **2**, *n* = 7, was obtained as an oil after flash chromatography (petroleum ether:ethyl acetate 20:1): R_f 0.62 (hexanes:ethyl acetate 25:1), 0.43 (hexanes:ethyl acetate 100:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.9 Hz, 3H), 1.16 (s, 3H), 1.25 (s, 6H), 0.98–1.35 (m, 15H), 1.39 (s, 3H), 1.48–1.61 (m, 2H), 1.61–2.06 (m, 3H), 2.06–2.24 (m, 1H), 2.52–2.76 (m, 2H), 5.43 (s, 1H), 6.76 (d, *J* = 1.8 Hz, 1H), 6.83 (dd, *J* = 8.1, 2.1 Hz, 1H), 7.11 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 4.1, 19.2, 22.7, 23.5, 24.7, 27.5, 27.7, 28.8, 29.3, 30.4, 31.9, 32.0, 36.5, 37.3, 42.8, 44.5, 76.7, 114.6, 117.6, 119.9, 122.4, 126.0, 133.4, 149.5, 152.5; MS (EI) *m/z* (rel intensity) 383 (33), 299 (11), 269 (100), 201 (20); $[\alpha]_D^{20}$ −150° (*c* 0.48, $CHCl_3$); HRMS: calcd for $C_{27}H_{42}O$ 382.3236, found 382.3237.

1-Deoxy-3-(1,1-dimethylheptyl)-Δ⁸⁽¹¹⁾-THC (17). To a stirred solution of 1.84 g (5.15 mmol) of methyltriphenylphosphonium bromide in 20 mL of dry THF, at ambient temperature, was added dropwise 1.90 mL (4.75 mmol) of 2.5 M *n*-butyllithium in hexanes. After stirring for 5 min, a solution of 0.166 g (0.466 mmol) of deoxynabilone (**16**) in 3 mL of dry THF was added dropwise. The reaction mixture was stirred at ambient temperature for 2 h, and heated at reflux with stirring for 18 h. After cooling, the reaction was quenched with water, and extracted with three portions of ether. The combined extracts were washed with brine and dried ($MgSO_4$). The solvent was removed in vacuo to give an oil which was chromatographed (petroleum ether:ether 30:1) to provide 0.137 g (82%) of **17**: R_f 0.25 (hexanes), 0.55 (hexanes:ethyl acetate 40:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.80 (t, *J* = 6.9 Hz, 3H), 1.09 (s, 3H), 1.21 (s, 6H), 0.92–1.29 (m, 9H), 1.38 (s, 3H), 1.45–1.62 (m, 3H), 1.81–2.16 (m, 3H), 2.32–2.49 (m, 2H), 2.89–3.06 (m, 1H), 4.72 (s, 1H), 4.77 (s, 1H), 6.71 (d, *J* = 1.8 Hz, 1H), 6.79 (dd, *J* = 1.8, 8.0 Hz, 1H), 7.09 (d, *J* = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 20.1, 22.6, 24.6, 28.1, 28.6,

28.8, 30.0, 31.7, 34.4, 36.7, 37.3, 39.3, 44.5, 46.7, 77.0, 108.7, 114.5, 117.4, 121.5, 125.2, 147.5, 149.6, 152.6; MS (EI) m/z (relative intensity) 354 (26), 335 (29), 269 (100), 185 (9); $[\alpha]_D^{25}$ 14.0° (c 3.0, $CHCl_3$); HRMS: calcd for $C_{25}H_{38}O$ 354.2923, found 354.2921.

(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-6,6-dimethyl-9*H*-dibenzo[b,d]pyran-9-β-ol (18). To a stirred solution of 0.238 g (0.668 mmol) of deoxynabilone (**16**) in 2 mL of dry THF at −78°C was added 0.051 g (1.3 mmol) of $LiAlH_4$. The reaction mixture was stirred at −78°C for 2 h, warmed to ambient temperature and stirred for 18 h. After quenching with water, and acidification with 10% HCl, the reaction mixture was extracted with ether. The ether extracts were washed with brine, dried ($MgSO_4$), and the solvent removed in vacuo to give an off white gum. Chromatography (petroleum ether:ether 1:2) gave 0.177 g (72%) of **18** as an off white solid: mp 110–111°C; R_f 0.13 (hexanes:ethyl acetate 5:1), 0.49 (hexanes:ethyl acetate 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 6.9 Hz, 3H), 0.97–1.48 (m, 12H), 1.14 (s, 3H), 1.24 (s, 6H), 1.41 (s, 3H), 1.48–1.59 (m, 2H), 1.64 (br s, 1H), 1.84–1.96 (m, 1H), 2.07–2.22 (m, 1H), 2.39–2.55 (m, 1H), 2.65–2.80 (m, 1H), 3.72–3.89 (m, 1H), 6.74 (d, J = 1.8 Hz, 1H), 6.83 (dd, J = 1.8, 8.1 Hz, 1H), 7.10 (d, J = 8.1 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 20.2, 22.7, 24.6, 25.8, 28.2, 30.0, 31.8, 33.7, 35.4, 37.3, 39.8, 44.5, 46.2, 70.6, 77.1, 114.6, 117.5, 121.0, 125.1, 149.8, 152.7; MS (EI) m/z (relative intensity) 340 (21), 321 (48), 255 (100), 235 (12), 213 (11); $[\alpha]_D^{25}$ −29.9° (c 1.10, $CHCl_3$); HRMS: calcd for $C_{24}H_{38}O_2$ 358.2872, found 358.2871.

(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-6,6a,7,8,10,10a-hexahydro-6,6-dimethyl-9*H*-dibenzo[b,d]pyran-9-α-ol (19). To a solution of 0.252 g (0.707 mmol) of deoxynabilone in 2 mL of dry THF at −78°C was added 1.50 mL (1.50 mmol) of 1.0 M K-selectride in THF. The reaction mixture was stirred at −78°C for 2 h and at ambient temperature for 1 h. A solution of 1 mL of water in 5 mL of EtOH was added, followed by 2 mL of 15% aqueous NaOH and 2 mL of 30% H_2O_2. The mixture was extracted with ether, the ether extracts were washed with brine, dried ($MgSO_4$) and the solvent removed at reduced pressure to afford an oil. Chromatography (petroleum ether: ether 1:1) gave 0.199 g (79%) of **19** as an oil: R_f 0.26 (hexanes:ethyl acetate 5:1), 0.52 (hexanes:ethyl acetate 1:2); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.84 (t, J = 7.0 Hz, 3H), 0.99–1.31 (m, 8H), 1.17 (s, 3H), 1.24 (s, 6H), 1.40 (s, 3H), 1.35–1.68 (m, 7H), 1.84–2.00 (m, 1H), 2.05 (s, 1H), 2.45–2.60 (m, 1H), 2.81–2.96 (m, 1H), 4.26 (s, 1H), 6.74 (d, J = 1.7 Hz, 1H), 6.81 (dd, J = 1.9, 8.1 Hz, 1H), 7.07 (d, J = 8.0 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.0, 20.2, 21.5, 22.6, 24.6, 27.8, 28.8, 29.0, 30.0, 31.7, 32.6, 37.2, 37.6, 44.5, 47.0, 66.0, 77.0, 114.5, 117.3, 121.8, 125.1, 149.4, 152.9; MS (EI) m/z (relative intensity) 340 (32), 321 (76), 255 (100), 251 (19); $[\alpha]_D^{25}$ −19.4° (c 4.90, $CHCl_3$); HRMS: calcd for $C_{24}H_{38}O_2$ 358.2872, found 358.2871.

3-(1′,1′-Dimethylheptyl)-*Δ*^{8}-tetrahydrocannabinol methyl ether (3). To a suspension of 2.0 g (14.5 mmol) of anhydrous potassium carbonate and 0.25 g (0.68 mmol) of 3-(1′,1′-dimethylheptyl)-Δ^8-THC in 10 mL of freshly distilled acetone was added 1.3 mL (13.8 mmol) of dimethyl sulfate. The resulting mixture was refluxed overnight, cooled to ambient temperature and diluted with distilled water. The acetone was removed under reduced pressure and the aqueous residue extracted three times with ether. The combined organic extracts were washed with water, brine and dried ($MgSO_4$). Concentration and chromatography (petroleum ether: ethyl acetate 100:1) gave the methyl ether as a colorless oil: R_f 0.34 (petroleum ether), 0.74 (petroleum ether: ethyl acetate 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, J = 6.9 Hz, 3H), 1.10 (s, 3H), 1.05–1.35 (m, 8H), 1.25 (s, 6H), 1.39 (s, 3H), 1.62 (m, 2H) 1.74 (s, 3H), 1.75–1.90 (m, 3H), 2.14 (m, 1H), 2.67 (dt, J = 4.8, 10.9 Hz, 1H), 3.17 (dd, J = 4.0, 17.6 Hz, 1H), 3.81 (s, 3H), 5.42 (d, J = 4.2 Hz, 1H), 6.38 (d, J = 1.6 Hz, 1H), 6.43 (d, J = 1.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 18.4, 22.7, 23.5, 24.6, 27.6, 28.0, 28.8, 30.0, 31.8, 36.2, 37.7, 44.5, 45.1, 55.0, 76.7, 100.6, 108.3, 111.6, 119.2, 135.0, 149.6, 154.0, 158.7; MS (EI) m/z (relative intensity) 384 (40), 300 (100).

Nabilone methyl ether. Nabilone[16] was converted to the corresponding methyl ether by the procedure described above for the preparation of 3-(1′,1′-dimethylheptyl)-Δ^8-tetrahydrocannabinol methyl ether. From 0.18 g (0.48 mmol) of nabilone there was obtained 0.14 g (75%) of methyl ether after chromatography (petroleum ether:ethyl acetate 9:1). The ^{1}H and ^{13}C NMR spectra were identical to those of a sample prepared by an alternative route.[10]

3-(1′,1′-Dimethylheptyl)-*Δ*$^{9(11)}$-tetrahydrocannabinol methyl ether (4). Reaction of 0.10 g (0.26 mmol) of nabilone methyl ether with the ylide derived from 0.925 g (2.59 mmol) methyl triphenylphosphonium bromide was carried out as described above for the preparation of **17**, to give 0.40 g (40%) of methyl ether **4** as a colorless oil after chromatography (petroleum ether:ethyl acetate 19:1): R_f 0.38 (hexane), 0.64 (petroleum ether:ethyl acetate 9:1); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, J = 6.9 Hz, 3H), 1.04 (s, 3H), 1.08–1.19 (m, 9H), 1.23 (s, 6H), 1.39 (s, 3H), 1.53 (m, 2H), 1.60–1.80 (m, 2H), 1.92 (m, 1H), 2.20 (m, 1H), 2.43 (dt, J = 3.1, 11.5 Hz, 2H), 3.63 (d, J = 11.5 Hz, 1H), 3.81 (s, 3H), 4.72 (s, 1H), 4.77 (s, 1H), 6.36 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 1.6 Hz, 1H); ^{13}C NMR (75.5 MHz, $CDCl_3$) δ 14.1, 19.0, 22.7, 24.6, 27.8, 28.8, 28.9, 30.0, 31.8, 35.0, 36.7, 37.7, 39.1, 44.5, 48.9, 55.1, 76.7, 100.7, 108.2, 108.6, 110.8, 148.8, 149.8, 154.1, 158.6; MS (EI) m/z (relative intensity) 384 (40), 300 (100).

Receptor binding assays

CB$_1$ assay. [^{3}H]CP-55,940 (K_D = 690 nM) binding to P_2 membranes was conducted as described elsewhere,[21] except whole brain (rather than cortex only) was used. Displacement curves were generated by incubating drugs with 1 nM of [^{3}H]CP-55,940. The assays were performed in triplicate, and the results represent the combined data from three individual experiments.

CB$_2$ assay. Human embryonic kidney 293 cells were maintained in Dulbecco′s modified Eagle′s medium

(DMEM) with 10% fetal clone II (HyClone, Logan, UT) and 5% CO_2 at 37°C in a Forma incubator. Cell lines were created by transfection of CB_2pcDNA3 into 293 cells by the Lipofectamine reagent (Life Technologies, Gaithersburg, MD). The human CB_2 cDNA was provided by Dr. Sean Munro (MRC, Cambridge, England). Stable transformants were selected in growth medium containing geneticin (1 mg/mL reagent, Life Technologies, Gaithersburg, MD). Colonies of about 500 cells were picked (about 2 weeks post transfection) and allowed to expand, then tested for expression of receptor mRNA by Northern blot analysis. Cell lines containing moderate to high levels of receptor mRNA were tested for receptor binding properties. Transfected cell lines were maintained in DMEM with 10% fetal clone II plus 0.3–0.5 mg/mL geneticin and 5% CO_2 at 37°C in a Forma incubator.

The current assay is a modification of Compton et al.[13] Cells were harvested in phosphate-buffered saline containing 1 mM EDTA and centrifuged at 500 *g*. The cell pellet was homogenized in 10 mL of solution A (50 mM Tris–HCl, 320 mM sucrose, 2 mM EDTA, 5 mM $MgCl_2$, pH 7.4). The homogenate was centrifuged at 1600 *g* (10 min), the supernatant saved, and the pellet washed three times in solution A with subsequent centrifugation. The combined supernatants were centrifuged at 100,000 *g* (60 min). The (P_2 membrane) pellet was resuspended in 3 mL of buffer B (50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, pH 7.4) to yield a protein concentration of approximately 1 mg/mL. The tissue preparation was divided into equal aliquots, frozen on dry ice, and stored at −70°C. Binding was initiated by the addition of 40–50 μg membrane protein to silanized tubes containing [^{3}H]CP-55,940 (102.9 Ci/mmol) and a sufficient volume of buffer C (50 mM Tris–HCl, 1 mM EDTA, 3 mM $MgCl_2$, and 5 mg/mL fatty acid free BSA, pH 7.4) to bring the total volume to 0.5 mL. The addition of 1 μM unlabeled CP-55,940 was used to assess nonspecific binding. Following incubation (30°C for 1 h), binding was terminated by the addition of 2 mL of ice cold buffer D (50 mM Tris–HCl, pH 7.4, plus 1 mg/mL BSA) and rapid vacuum filtration through Whatman GF/C filters (pretreated with polyethyleneimine (0.1%) for at least 2 h). Tubes were rinsed with 2 mL of ice cold buffer D, which was also filtered, and the filters subsequently rinsed twice with 4 mL of ice cold buffer D. Before radioactivity was quantitated by liquid scintillation spectrometry, filters were shaken for 1 h in 5 mL of scintillation fluid.

CP-55,940 and all cannabinoid analogues were prepared by suspension in assay buffer from a 1 mg/mL ethanolic stock without evaporation of the ethanol (final concentration of no more than 0.4%). When anandamide was used as a displacing ligand, experiments were performed in the presence of phenylmethylsulfonyl fluoride (50 μM). Competition assays were conducted with 1 nM [^{3}H]CP-55,940 or 1 nM [^{3}H]SR141716A and 6 concentrations (0.1 nM to 10 μM displacing ligands). Displacement IC_{50} values were originally determined by unweighted least-squares linear regression of log concentration–percent displacement data and then converted to K_i values using the method of Cheng and Prusoff.[22]

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