Pharmacophoric Requirements for the Cannabinoid Side Chain. Probing the Cannabinoid Receptor Subsite at C1′

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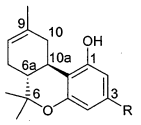
Earlier work from our laboratories has provided evidence for the existence of a subsite within the CB1 and CB2 cannabinoid receptor binding domain corresponding to substituents at the benzylic side chain position of classical cannabinoids. The existence and stereochemical features of this subsite have now been probed through the synthesis of a novel series of (−)-Δ^8-tetrahydrocannabinol analogues bearing C1′-ring substituents. Of the compounds described here, those with C1′-dithiolane (**1c**), C1′-dioxolane (**2d**), and cyclopentyl (**2a**) substituents exhibited the highest affinities for CB1 and CB2. We used molecular modeling approaches to better define the stereochemical limits of the putative subsite. In vitro pharmacological testing found **1c** to be a potent CB1 agonist.

Introduction

The discovery[1,2] and cloning[2−5] of CB1 and CB2, the two known $G_{i/o}$-protein-coupled cannabinoid receptors,[6−8] as well as the isolation and characterization of two families of endogenous cannabinergic ligands represented by arachidonylethanolamide[9] (anandamide) and 2-arachidonylglycerol[10,11] (2AG), have opened new horizons in this newly discovered field of biology. Furthermore, a considerable number[12−15] of cannabinoid analogues were synthesized and tested, thus providing substantial information on the stereoelectronic requirements for receptor recognition and activation. Structure−activity relationship (SAR) studies recognize four pharmacophores within the cannabinoid prototype: a phenolic hydroxyl, a lipophilic alkyl side chain, a northern aliphatic hydroxyl, and a southern aliphatic hydroxyl.[12] The first two are encompassed in the plant-derived cannabinoids while all four pharmacophores are represented in some of the synthetic nonclassical cannabinoids developed by Pfizer and exemplified by CP-55,940.[16] Our continued efforts in cannabinoid medicinal chemistry have sought to carefully characterize and optimize all four pharmacophores.[12,15] It is now well established that among these, the side chain plays a crucial role in determining cannabinergic potency. Previous SAR studies[17−19] seeking to probe chain length and substitution pattern requirements, have suggested that the 1′,1′-dimethylheptyl group **1b** (Table 1) is optimal for activity. Additionally, the design and synthesis of novel analogues in which the side chain is conformationally constrained[19−22] has provided information on its optimal stereochemical features.

Table 1. Affinities (K_i) of Δ^8-THC Analogues for CB1 and CB2 Cannabinoid Receptors (95% confidence limits)



compd	R	CB1 (Ki, nM)[a]	CB2 (Ki, nM)[a]
1a		22 ± 4[b]	—
1b		0.83[c]	0.49[c]
1c		0.32[d]	0.52[d]
2a		0.45 ± 0.07	1.92 ± 0.4
2b		1.8 ± 0.7	3.6 ± 1.3
2c		168 ± 18	103 ± 16
2d		0.52 ± 0.11	0.22 ± 0.06
2e		32.3 ± 4.0	19.7 ± 2.7
2f		56.9 ± 6.8	257 ± 41

[a] Affinities for CB1 and CB2 were determined using rat brain (CB1) or mouse spleen (CB2) membranes and [^{3}H]CP-55,940 as the radioligand following previously described procedures.[35] K_i values were obtained from three independent experiments run in duplicate and are expressed as the mean of the three values. [b] Reported previously.[18] [c] Reported previously.[15] [d] Reported previously.[22]

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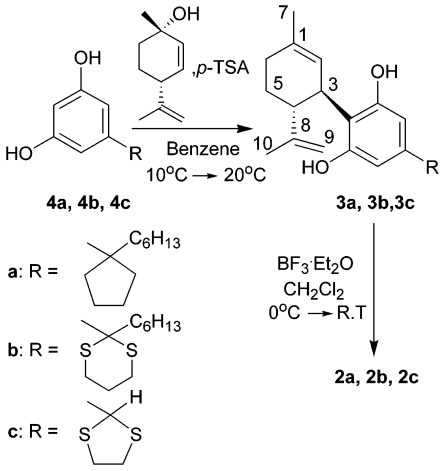
Scheme 1



Earlier efforts in our laboratories, aimed at exploring the pharmacophoric requirements of the side chain within the classical tetrahydrocannabinol (THC) template, led to the development of cannabinergic ligands possessing high affinities for both cannabinoid receptors.[22,23] One of the most successful compounds to result from this work was the C1′-dithiolane analog[22,24] **1c** (Table 1) with K_i values of 0.32 and 0.52 nM for CB1 and CB2, respectively. This increase in affinity was attributed to a hydrophobic subsite in both CB1 and CB2 at the level of the benzylic side chain carbon. To explore in more detail the stereoelectronic requirements of this subsite, we have now designed and synthesized a series of classical cannabinoid analogues depicted in Table 1. The current study focuses on the C1′-position of the side chain and explores the effect of C1′-ring substituents on the abilities of these analogues to recognize the two known cannabinoid receptors. As with earlier work, we used (−)-Δ^8-THC (Table 1, R = *n*-C_5H_{11}) as our prototype, favoring it over the less stable and almost equipotent isomer (−)-Δ^9-THC, while the length of the side chain was optimized to seven carbon atoms. All analogues were tested for their respective affinities for CB1 and CB2. The results were used to explore the binding domain for the cannabinoid benzylic subsite within each receptor using computational molecular graphics. Two of the analogues synthesized, namely (6a*R*-*trans*)-3-(1-hexylcyclopentyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol **2a** (Table 1) and (6a*R*-*trans*)-3-[2-hexyl-(1,3)-dioxolan-2-yl]-6a,7,-10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol **2d** (Table 1), were found to possess remarkably high affinities for the CB1 and CB2. The observed CB1 or CB2 selectivities of the analogues reported here are relatively modest. However, they do provide a good starting point for the development of more subtype-selective second-generation ligands. In vitro pharmacological testing of **1c** indicated that this compound behaves as a very potent CB1 agonist.

Chemistry

Entries into the synthesis of Δ^8-THC analogues frequently involve condensation of an appropriately 5-substituted resorcinol with a chiral monoterpenoid alcohol. Following a well-established protocol,[22,25,26] Friedel−Crafts allylation (Scheme 1) of resorcinol derivatives **4a**, **4b**, and **4c** with (+)-*cis*/*trans*-*p*-mentha-2,8-dien-1-ol[27] catalyzed by *p*-toluenesulfonic acid afforded cannabidiols **3a**, **3b**, and **3c** in 85%, 43%, and 30% yield, respectively. These intermediates were readily cyclized in the presence of boron trifluoride etherate to give the corresponding tetrahydrocannabinol analogues **2a**, **2b**, and **2c**[28] (Table 1) in 79%, 69%, and 50% yield.

Recently we developed[29] a general and efficient method for substituting activated aryl methylenes with cycloalkyl groups of varying ring size. We now report the synthesis of the key intermediate resorcinol **4a** using the above-mentioned approach (Scheme 2). The starting point for this synthesis is (3,5-dimethoxyphenyl)acetonitrile (**5**) which was, in turn, obtained from commercially available 3,5-dimethoxybenzaldehyde in three steps. This involved reduction with sodium borohydride[30] to afford 3,5-dimethoxybenzyl alcohol which was converted to the respective chloride by treatment[31] with triphenylphosphine and carbon tetrachloride in an 80% overall yield, followed by cyanide displacement in dimethyl sulfoxide. The yield of this last reaction (93%) is greater than when a mixture of ethanol and water is used[32] as solvent. Sequential deprotonation of **5** with potassium bis(trimethylsilyl)amide and cyclobisalkylation using 1,4-dibromobutane afforded (3,5-dimethoxyphenyl)cyclopentane carbonitrile **6** in a very good yield (88%). The cyano group of **6** was then reduced (87% yield)[33] to aldehyde **7**, which upon Wittig olefination using (butylmethylene)triphenylphosphorane gave intermediate **8** in excellent yield (96%) after purification. Catalytic hydrogenation of **8** led to the resorcinol dimethyl ether **9** which was demethylated using boron tribromide[34] to give **4a** in an overall 85% yield.

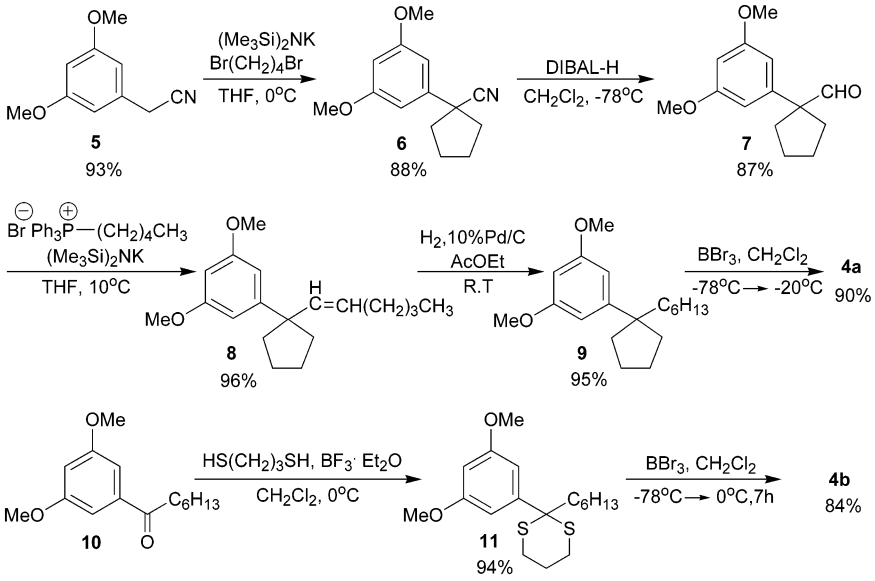
The dithioresorcinols **4b** and **4c** were prepared from the respective keto precursors as shown in Schemes 2 and 3. Thus, boron trifluoride etherate-catalyzed reaction of phenone **10**[22] with 1,3-propanedithiol provided 2-(3,5-dimethoxyphenyl)-2-hexyl-(1,3)-dithiane **11** in 94% yield. This was followed by demethylation under boron tribromide conditions affording resorcinol **4b** in 84% yield. Similarly, 3,5-dimethoxybenzaldehyde **12** upon treatment with 1,2-ethanedithiol and boron trifluoride etherate gave thioketal **13** (87% yield) which was converted to the corresponding resorcinol **4c**[28] in 87% yield, by demethylation using boron tribromide at 0 °C for 48 h.

(6a*R*-*trans*)-3-(1-Oxoheptyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol **14** whose synthesis was previously described[22] served as a starting point for the side chain analogues **2d**, **2e**, and **2f** (Table 1) using acetalization or thioacetalization reaction conditions depicted in Scheme 3. Treatment of **14** with 1,2-dimethyl-1,2-ethanedithiol afforded **2e** as a 2.6:1 mixture of two diastereomeric pairs (**2e$_1$** and **2e$_2$**, respectively) as confirmed by ^{1}H NMR data.

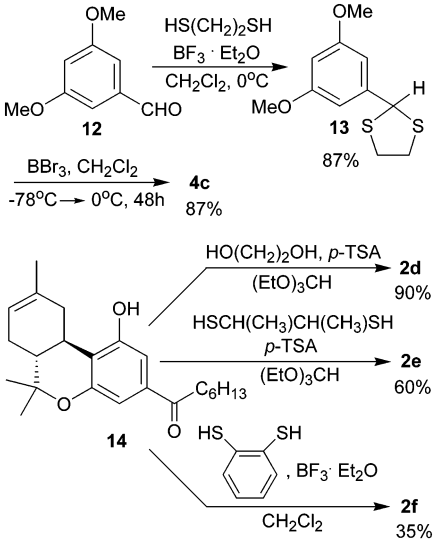
Receptor Binding Studies

The abilities of **2a**−**f** to displace radiolabeled CP-55,-940 from purified rat forbrain synaptosomes and mouse spleen synaptosomes were determined as described in the Experimental Section. K_i values calculated from the respective displacement curves are listed in Table 1 and serve as indicators for the affinities of these Δ^8-THC analogues for the CB1 and CB2 receptors.

Scheme 2



Scheme 3



Results and Discussion

The binding data depicted in Table 1 indicate that the introduction of different ring substituents at the C1′-position of the side chain can lead to analogues exhibiting an over 1170-fold range of affinities for CB1 and CB2. The causes for these large differences maybe 2-fold. First, the different C1′-substituents can lead to variations in the conformational properties of individual side chains, thus affecting the interaction of this pharmacophoric group with the binding site. The second cause for this wide range of CB1/CB2 affinities maybe related to differences in the abilities of the C1′-substituents to engage in optimal interactions at the putative receptor-binding subsite. Thus, substituents with a "favorable fit" for the subsite may produce an enhancement of the ligand's affinity while substituents that undergo unfavorable interactions with the receptor subsite may reduce the ligand's affinity.

A first step in the evaluation of our binding data was a comparison of the stereochemical features of the individual side chain C1′-substituents. For this purpose we used molecular mechanics/molecular dynamics calculations to identify the low energy conformations of all the analogues. The starting point for our calculations was the low energy conformation of CP-47,497,[36] a nonclassical cannabinoid carrying a 1′,1′-dimethylheptyl side chain. Dynamics and minimization runs were then performed to establish our analogues' low energy conformations. The results show that in their respective low energy conformations all analogues have their seven-carbon chains in a similar conformation with the plane of the chain approximately orthogonal to that of the tricyclic ring system. In the preferred conformers, the bulky tricyclic ring system adopts an equatorial conformation within the respective five- or six-membered C1′-rings while the seven-carbon chain is axial. The low energy conformation of the high affinity analogue **2a** is shown in Figure 1.

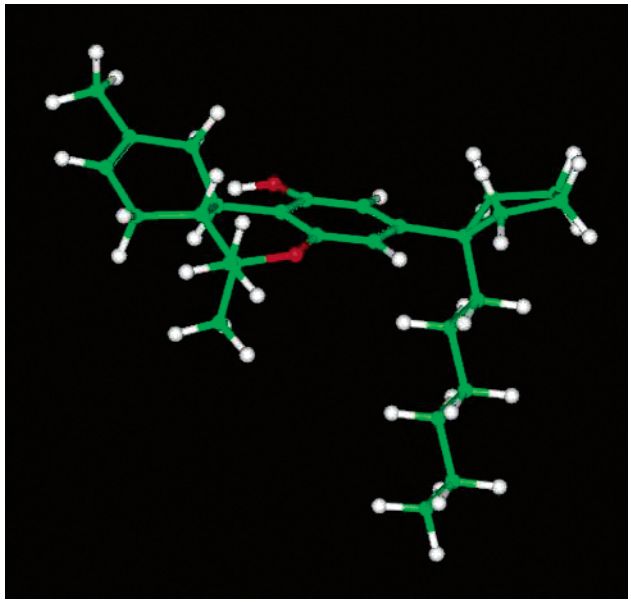


Figure 1. The low energy conformation of **2a** as determined using molecular mechanics/molecular dynamics calculations.

Our molecular modeling also reveals significant stereochemical differences between the different C1′-ring substituents.

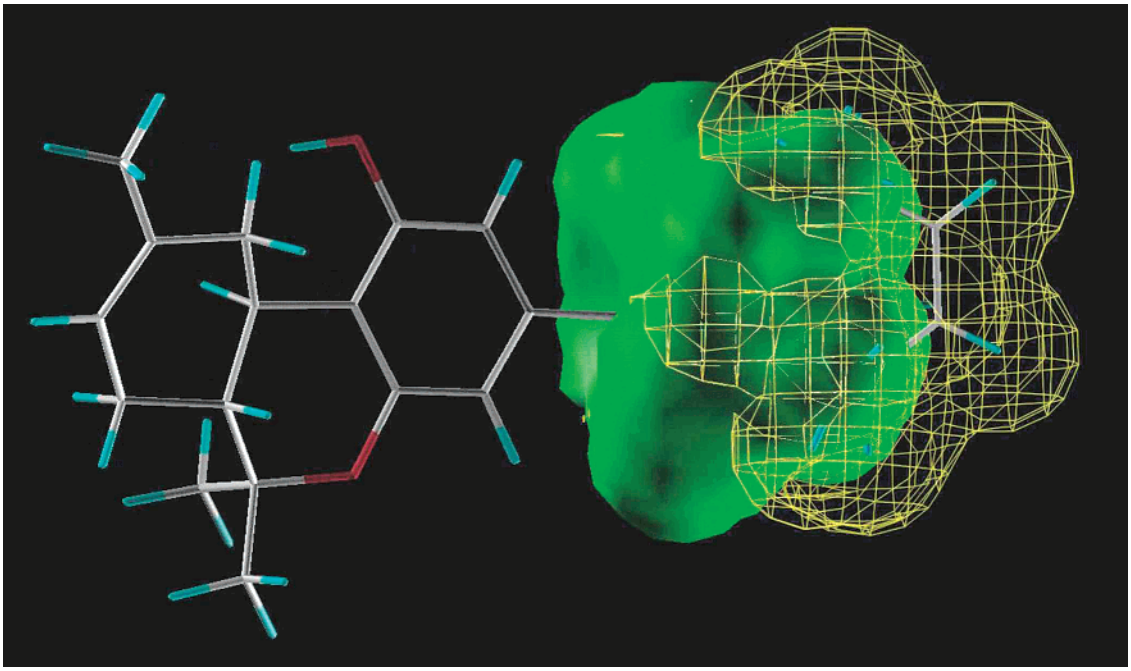


Figure 2. CB1/CB2 receptor essential volume map (yellow grid) and excluded-volume map (green contours) for the C1′-subsite. In this top-view, only the C1′-position of the side chain is shown. The remaining carbons (not visible) in the chain are approximately orthogonal with the plane of the tricyclic ring and below the plane of the paper. The receptor essential volume map is calculated by subtracting the sum of the van der Waals volumes of the superimposed "active" analogues (**1b**, **1c**, **2a**, **2d**) from that of the "subactive" analogues (**2b**, **2e**, **2f**). C1′-substituents falling within the yellow grid experience unfavorable or less favorable interactions at the binding site. The receptor excluded volume map is calculated by addition of the van der Waals volumes of the superimposed "active" analogues; thus, the green area represents the free space within the receptor region that accommodates high affinity C1′-substituted ligands.

The above data thus suggest that the observed variations in the affinity of the series of C1′-substituted analogues are not due to conformational differences in the backbone of their seven-membered side chain, but are probably the result of differences in the stereochemical features of the C1′-substituents. Therefore, it can be argued that these stereoelectronic differences determine to what extent the individual analogues can engage in an optimal pharmacophoric fit with the receptor. Furthermore, a comparison of the dithiolane analogue **1c** with its congener **2c** in which the last six carbons of the side chain are truncated reveals a drop in affinity of over 2 orders of magnitude for both receptors, thus confirming the key role of the side chain pharmacophore.

A comparison of the binding data of *n*-heptyl Δ^8-THC **1a** and its 1′,1′-dimethylheptyl analogue **1b** suggests that the presence of the two C1′-methyl groups enhances the ligand's affinity for the CB1 receptor. This interaction is optimized when the geminal dimethyl substitution is modified into a five-membered ring as seen in the dithiolane analogue **1c** and the dioxolane analogue **2d**. The above two analogues appear to exhibit no significant preference for either of the two receptors. The corresponding C1′-carbocyclic congener **2a** also maintains a high affinity for CB1 and CB2 with a 3-fold preference for CB1. Thus, within CB1, the putative subsite appears to be indifferent to the presence of the oxygen, sulfur atoms, or methylene groups attached to the C1′-position. Conversely, the CB2 receptor appears to show preference for the smaller dioxolane five-membered ring compared to the slightly larger dithiolane or the more hydrophobic cyclopentyl analogue **2a**. Enlargement of the size of the C1′-substituent to a six-membered dithiane ring **2b** is accompanied with a relatively small but distinct reduction in affinity which is more accentuated in CB2. The steric limits of C1′-substitution become progressively more pronounced with increases in size. This is clearly seen in analogue **2e** in which two vicinal methyl groups were added to the southern end of the dithiolane ring, and more so in **2f** where the C1′-substituent is a bulky benzodithiolane group. Again the CB2 receptor appears to exhibit greater sensitivity to steric variations.

We used standard molecular graphics procedures to obtain a more detailed representation of the putative C1′-side chain subsite. Following molecular mechanics and molecular dynamics-based minimization procedures,[37] the low energy conformers of individual analogues were superimposed and then used as a basis for generating receptor essential volume maps and receptor-excluded volume maps.[37,38] These volume maps were computed based on the assumption that a family of high affinity ("active") ligands adopt similar shapes and occupy similar volumes at the receptor site. Conversely, lower affinity ("subactive") ligands adopt shapes and occupy binding spaces which differ from the "active" ligands, resulting in reduced binding affinities. More specifically, analogues **1b**, **1c**, **2a**, and **2d** were designated as "active" and analogues **2b**, **2e**, and **2f** were designated as "subactive". These analogues were superimposed using the carbon atoms of the cannabinoid aromatic ring as superimposition points. The sum of the van der Waals volumes of the "subactive" analogues minus the sum of the "active" ones is designated as the "receptor essential volume map" and is depicted by the yellow area in Figure 2. This yellow grid can be interpreted to represent those regions within the bind-

ing site for which the ligand experiences unfavorable or less favorable interactions. The sum of the van der Waals volumes of the "active" ligands is designated as "receptor-excluded volume" and is depicted by the green contours in Figure 2. The green area represents the free space within the receptor region that accommodates the "active" ligand moieties. It includes C1′-substituents from analogues **1b**, **1c**, **2a**, **2d** and, within the class of analogues discussed here, represents the area that the pharmacophore must occupy in order to engage in a maximum affinity interaction. On the basis of these volume maps, the C1′-substituents of analogues **1b**, **1c**, **2a**, and **2d** optimally fit in a putative binding pocket within each of the two cannabinoid receptors. Conversely, analogues **2e** and **2f**, in which the C1′-substituents are relatively bulky, experience a less favorable interaction with the two receptors as is reflected in their larger K_i values. In the case of analogue **2b**, its six-membered C1′-ring substituent enhances its affinity for both CB1 and CB2. However, this enhancement is less effective than its five-membered counterpart **1c**. For this reason, we designate the C1′-dithiane substituent in **2b** as a "threshold pharmacophoric group", indicating that it may reside, in part, at the buffer zone between the model's yellow and green zones.

The above data support the presence of respective subsites within the cannabinoid binding domains of CB1 and CB2 and offer opportunities for the development of novel improved classical cannabinoid ligands. The results also point to possible subsite differences between the two receptors. These are currently being elaborated with the design of next generation more selective CB1/CB2 analogues.

The analogue in this series with the highest affinities for the two cannabinoid receptors (**1c**) was subjected to in vitro evaluation using mouse isolated vasa deferentia. Previous experiments had shown that cannabinoid receptor agonists very potently inhibit the electrically evoked contractions[39,40] of this tissue (twitch response), and that the potency of cannabinoids as inhibitors of this effect correlates well with their affinities for the CB1 receptors.[41] Our experiment showed that **1c** produced a concentration-related inhibition of the twitch response acting as a potent CB1 agonist with an IC_{50} value of 0.27 nM, closely resembling its K_i value from the receptor binding experiments. Our functional results suggest that there is a high likelihood that all of the "active" analogues identified in this communication are potent CB1 agonists. A more thorough pharmacological evaluation of the key compound included here is underway.

Experimental Section

Materials. All reagents and solvents were purchased from Aldrich Chemical Co. unless specified otherwise and used without further purification. All anhydrous reactions were performed under a static argon or nitrogen atmosphere in flame-dried glassware using scrupulously dry solvents. Organic phases were dried over $MgSO_4$ and evaporated under reduced pressure. Flash column chromatography employed silica gel 60 (230−400 mesh). All compounds were demonstrated to be homogeneous by analytical TLC on precoated silica gel TLC plates (Merck, 60 F_{245} on glass, layer thickness 250 μm), and chromatograms were visualized by phosphomolybdic acid straining. Melting points were determined on a micro-melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker DMX-500 or on a Bruker AC 300 spectrometer operating at 500 and 300 MHz, respectively. All NMR spectra were recorded in $CDCl_3$ unless otherwise stated, and chemical shifts are reported in units of δ relative to internal TMS. Multiplicities are indicated as br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet), and coupling constants (J) are reported in hertz (Hz). Low and high-resolution mass spectra were performed in School of Chemical Sciences, University of Illinois at Urbana−Champaign or were recorded on a HP6890 GC/MS instrument. Elemental analyses were obtained in Baron Consulting Co, Milford, CT, or carried out by the microanalytical section of the Institute of Organic and Pharmaceutical Chemistry, National Hellenic Research Foundation.

1-(3,5-Dimethoxyphenyl)cyclopentanecarbonitrile (6). To a solution of **5** (2.0 g, 11.3 mmol) in dry THF (99 mL) at 0 °C, under an argon atmosphere, was added potassium bis-(trimethylsilyl)amide (6.77 g, 34.0 mmol). The mixture was stirred at the same temperature for 3 min, and then a solution of 1,4-dibromobutane (2.7 g, 12.5 mmol) in dry THF (14 mL) was added over a period of 10 min. Following the addition, the reaction was stirred for 5 min at 0 °C and then quenched by the addition of saturated aqueous NH_4Cl. The mixture was diluted with EtOAc, the organic layer separated, and the aqueous phase extracted with EtOAc. The combined organic layer was washed with brine and dried over $MgSO_4$ and the solvent evaporated under reduced pressure to give an oily residue. Purification by flash column chromatography (30% diethyl ether−petroleum ether) afforded 2.3 g (88% yield) of the compound **6** as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.60 (d, J = 1.6 Hz, 2H), 6.39 (t, J = 1.6 Hz, 1H), 3.81 (s, 6H), 2.47−2.43 (m, 2H), 2.10−1.98 (m, 4H), 1.96−1.91 (m, 2H); mass spectrum m/z (relative intensity) 231 (M^+, 33), 203 (5), 190 (100), 165 (9), 138 (6). Exact mass calculated for $C_{14}H_{17}NO_2$, 231.1259; found, 231.1257.

1-(3,5-Dimethoxyphenyl)cyclopentanecarboxaldehyde (7). To a solution of **6** (2.0 g, 8.66 mmol), in dry CH_2Cl_2 (87 mL) at −78 °C under an argon atmosphere, was added diisobutylaluminum hydride (21.7 mL, 1 M solution in hexanes) over a period of 15 min. The reaction mixture was stirred at the same temperature for 1 h and then quenched by dropwise addition of potassium sodium tartrate (10% solution in water). The resulting mixture was warmed to room temperature, stirred vigorously for 40 min, and then diluted with EtOAc. The organic phase was separated and the aqueous phase extracted with EtOAc. The combined organic layer was washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The crude product was purified by flash column chromatography on silica gel using 15% diethyl ether−petroleum ether as eluent to give compound **7** as a white solid in 87% yield (1.77 g). mp 47−48 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.38 (s, 1H), 6.39 (d, J = 1.7 Hz, 2H), 6.36 (t, J = 1.7 Hz, 1H), 3.78 (s, 6H), 2.47−2.43 (m, 2H), 1.90−1.84 (m, 2H), 1.78−1.71 (m, 2H), 1.67−1.61 (m, 2H); mass spectrum m/z (relative intensity) 234 (M^+, 23), 205 (100), 177 (13), 165 (17), 151 (58), 77 (8), 67 (53). Exact mass calculated for $C_{14}H_{18}O_3$, 234.1256; found, 234.1256.

1,3-Dimethoxy-5-[1-(hex-1-enyl)cyclopentyl]benzene (8). To a suspension of pentyltriphenylphosphonium bromide (15.0 g, 36.3 mmol) in dry THF (200 mL) at 0 °C, under an argon atmosphere, was added potassium bis(trimethylsilyl)amide (7.01 g, 35.6 mmol). The mixture was warmed to 10 °C and stirred for an additional 30 min to ensure complete formation of the orange (butylmethylene)triphenylphosphorane. To the resulting slurry, at the same temperature, was added dropwise a solution of **7** (1.7 g, 7.26 mmol) in dry THF (17 mL). The reaction was stirred for 45 min and upon completion was quenched by the addition of saturated aqueous NH_4Cl. The organic layer was separated, and the aqueous phase was extracted with diethyl ether. The combined organic layer was washed with brine and dried over $MgSO_4$, and the solvent was evaporated under reduced pressure. The residue was purified through a short column of silica gel using 5% diethyl ether−petroleum ether as eluent to afford the compound **8** as a

colorless liquid in 96% yield (2.0 g). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.54 (d, J = 2.0 Hz, 2H), 6.28 (t, J = 2.0 Hz, 1H), 5.69 (d, J = 11.0 Hz, 1H), 5.29 (dt, J = 11.0 Hz, J = 7.4 Hz, 1H), 3.78 (s, 6H), 2.02−1.98 (m, 2H), 1.95−1.90 (m, 2H), 1.76−1.67 (m, 6H), 1.14−1.08 (m, 4H), 0.76 (t, J = 7.0 Hz, 3H); mass spectrum m/z (relative intensity) 288 (M^+, 40), 257 (12), 245 (40), 231 (46), 219 (27), 205 (100), 194 (40), 151 (59), 77 (16), 67 (31). Exact mass calculated for $C_{19}H_{28}O_2$, 288.2089; found, 288.2091. Anal. ($C_{19}H_{28}O_2$) C, H.

1,3-Dimethoxy-5-(1-hexylcyclopentyl)benzene (9). To a solution of **8** (1.5 g, 5.21 mmol) in EtOAc (47 mL) was added 10% Pd/C (255 mg), and the resulting suspension was stirred vigorously under hydrogen atmosphere, overnight at room temperature. The catalyst was removed by filtration through Celite, and the filtrate was evaporated under reduced pressure to afford the crude product. Purification through a short column of silica gel using 5% diethyl ether−petroleum ether yielded compound **9** as a colorless liquid (1.43 g, 95% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.45 (d, J = 1.9 Hz, 2H), 6.31 (t, J = 1.9 Hz, 1H), 3.81 (s, 6H), 1.92−1.86 (m, 2H), 1.80−1.76 (m, 2H), 1.74−1.63 (m, 4H), 1.57−1.54 (m, 2H), 1.23−1.15 (m, 6H), 1.03−0.97 (m, 2H), 0.84 (t, J = 7.0 Hz, 3H); mass spectrum m/z (relative intensity) 290 (M^+, 20), 206 (100), 194 (16), 177 (7), 165 (24), 151 (31), 67 (24). Exact mass calculated for $C_{19}H_{30}O_2$, 290.2246; found, 290.2241. Anal. ($C_{19}H_{30}O_2$) C, H.

5-(1-Hexylcyclopentyl)resorcinol (4a). To a solution of **9** (1.2 g, 4.14 mmol) in dry CH_2Cl_2 (130 mL) at −78 °C under an argon atmosphere was added boron tribromide (10.0 mL, 1 M solution in CH_2Cl_2). Following the addition, the reaction temperature was gradually raised over a period of 3h to −20 °C. Stirring was continued at that temperature until completion of the reaction (5 days). Unreacted boron tribromide was destroyed by addition of methanol and ice at 0 °C. The resulting mixture was warmed at room temperature and stirred for 40 min, and the volatiles were removed in vacuo. The residual was diluted with EtOAc and washed with saturated $NaHCO_3$ solution, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (40% diethyl ether−petroleum ether) afforded 975 mg (90% yield) of the compound **4a** as a slightly brown solid. mp 82−83 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.36 (d, J = 1.6 Hz, 2H), 6.19 (t, J = 1.6 Hz, 1H), 5.78 (br s, 2H, OH), 1.83−1.77 (m, 2H), 1.73−1.58 (m, 6H), 1.51−1.48 (m, 2H), 1.22−1.12 (m, 6H), 1.02−0.94 (m, 2H), 0.83 (t, J = 7.1 Hz, 3H); mass spectrum m/z (relative intensity) 262 (M^+, 22), 178 (100), 166 (16), 137 (19), 123 (51), 77 (7), 67 (38). Exact mass calculated for $C_{17}H_{26}O_2$, 262.1933; found, 262.1923. Anal. ($C_{17}H_{26}O_2$) C, H.

(−)-2-[3−3,4-*trans*-*p*-Menthadien-(1,8)-yl]-5-(1-hexylcyclopentyl)resorcinol (3a). To a solution of **4a** (571 mg, 2.18 mmol) in dry benzene (22 mL) at 10 °C under an argon atmosphere was added *p*-toluenesulfonic acid (79 mg, 0.42 mmol) followed by the addition of a solution of (+)-*cis*/*trans*-*p*-mentha-2,8-dien-1-ol (464 mg, 3.05 mmol) in dry benzene (6 mL). The reaction mixture was stirred at 10 °C to 20 °C for 1 h, at which time TLC indicated the complete consumption of starting material. The reaction mixture was diluted with diethyl ether, and the ethereal solution was washed with saturated $NaHCO_3$ solution, water, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by flash column chromatography (7% diethyl ether−petroleum ether) afforded 736 mg (85% yield) of the title compound **3a** as colorless viscous oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.35 (br s, 1H, ArH), 6.25 (br s, 1H, ArH), 5.97 (br s, 1H, OH), 5.60 (br s, 1H, 2-H), 4.66 (s, 1H, >C=CH_2), 4.63 (br s, 1H, OH), 4.54 (s, 1H, >C=CH_2), 3.82 (m as br d, J = 11.0 Hz, 1H, 3-H), 2.37 (td, J = 10.7 Hz, J = 3.4 Hz, 1H, 4-H), 2.28−2.19 (m, 1H), 2.14−2.07 (m, 1H), 1.87−1.74 (m, 7H, especially 1.80, s, 3H, 7-CH_3), 1.73−1.57 (m, 9H, especially 1.63, s, 3H, 10-CH_3), 1.48 (m, 2H, 2′-CH_2-), 1.22−1.11 (m, 6H, 4′-CH_2-, 5′-CH_2-, 6′-CH_2-), 0.92 (m, 2H, 3′-CH_2-), 0.82 (t, J = 7.1 Hz, 3H, 7′-CH_3); mass spectrum m/z (relative intensity) 396 (M^+, 11), 381 (4), 328 (44), 313 (100), 289 (9), 275 (21), 243 (40), 121 (13). Exact mass calculated for $C_{27}H_{40}O_2$, 396.3028; found, 396.3019. Anal. ($C_{27}H_{40}O_2$) C, H.

(6a*R*-*trans*)-3-(1-Hexylcyclopentyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (2a). To a solution of **3a** (601 mg, 1.52 mmol) in anhydrous CH_2Cl_2 (43 mL) at 0 °C under an argon atmosphere was added boron trifluoride etherate (1.32 mL 10.6 mmol). Following the addition, the mixture was stirred at 0 °C for 1 h and then at room temperature for 7 h. The reaction was quenched by the addition of saturated $NaHCO_3$ solution, and the volatiles were removed under reduced pressure. The crude residual was diluted with EtOAc, and the organic layer was washed with water and brine and dried over $MgSO_4$. Solvent evaporation and purification by flash column chromatography on silica gel (6% diethyl ether−petroleum ether) afforded 476 mg (79% yield) of the title compound **2a** as white foam. mp 50−52 °C dec; ^{1}H NMR (500 MHz, $CDCL_3$) δ 6.33 (d, J = 1.9 Hz, 1H, 4-H), 6.17 (d, J = 1.9 Hz, 1H, 2-H), 5.43 (d, J = 3.9 Hz, 1H, 8-H), 4.65 (s, 1H, OH), 3.19 (dd, J = 16.9 Hz, J = 4.0 Hz, 1H, 10α-H), 2.69 (ddd as td, J = 10.6 Hz, J = 4.6 Hz, 1H, 10a−H), 2.16−2.12 (m, 1H, 7α-H), 1.93−1.77 (m, 5H, 1″-CH, 4″-CH, 10β-H, 7β-H, 6a−H), 1.72−1.58 (m, 9H, especially 1.70, s, 9-CH_3), 1.48 (m, 2H, 2′-CH_2-), 1.38 (s, 3H, 6β-CH_3), 1.22−1.12 (m, 6H, 4′-CH_2-, 5′-CH_2-, 6′-CH_2-), 1.11 (s, 3H, 6α-CH_3), 1.04−0.95 (m, 2H, 3′-CH_2-), 0.82 (t, J = 7.1 Hz, 3H, 7′-CH_3); mass spectrum m/z (relative intensity) 396 (M^+, 39), 381 (4), 357 (7), 312 (100), 300 (17), 271 (9), 243 (8), 190 (9). Exact mass calculated for $C_{27}H_{40}O_2$, 396.3028; found, 396.3026. Anal. ($C_{27}H_{40}O_2$) C, H.

2-(3,5-Dimethoxyphenyl)-2-hexyl-(1,3)-dithiane (11). To a solution of **10** (0.615 g, 2.46 mmol) in CH_2Cl_2 (10 mL) and 1,3-propanedithiol (0.37 mL, 3.69 mmol) at 0 °C was added boron trifluoride etherate (0.058 mL, 0.49 mmol). The reaction mixture was stirred at that temperature for 2 h, and at completion a saturated solution of $NaHCO_3$ (10 mL) was added. The mixture was diluted with diethyl ether, the organic layer washed with water and brine, dried (Na_2SO_4), and evaporated to afford 0.786 g (94% yield) of **11** as viscous oil, sufficiently pure for the following step. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 2.4 Hz, 2H), 6.37 (d, J = 2.4 Hz, 1H), 3.80 (s, 6H), 2.86−2.60 (m, 4H), 1.96−1.88 (m, 4H), 1.32−1.10 (m, 8H), 0.82 (t, J = 6.7 Hz, 3H, 7′-CH_3); ^{13}C NMR ($CDCl_3$) δ 160.9, 144.9, 107.3, 98.6, 59.4, 55.4, 45.3, 31.5, 29.3, 27.8, 25.3, 23.8, 22.5, 14.0; Anal. ($C_{18}H_{28}O_2S_2$) C, H.

5-[2-Hexyl-(1,3)-dithian-2-yl]resorcinol (4b). The title compound was prepared from **11** (0.83 g, 2.44 mmol), in anhydrous CH_2Cl_2 (61 mL) and boron tribromide (0.52 mL, 5.36 mmol), following the procedure described for compound **4a**. Purification by flash column chromatography (50% diethyl ether−petroleum ether as eluent) gave 640 mg (84%) of compound **4b** as viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$−CD_3-$COCD_3$) δ 7.21 (br s, 2H, OH), 6.89 (d, J = 1.8 Hz, 2H), 6.24 (d, J = 1.8 Hz, 1H), 2.70−2.49 (m, 4H), 1.84−1.78 (m, 4H), 1.16−1.08 (br s, 8H), 0.72 (t, J = 6.7 Hz, 3H). Anal. ($C_{16}H_{24}O_2S_2$) C, H.

(−)-2-[3−3,4-*trans*-*p*-Menthadien-(1,8)-yl]-5-[2-hexyl-(1,3)-dithian-2-yl]resorcinol (3b). The synthesis was carried out analogous to the preparation of **3a** using **4b** (0.3 g, 0.96 mmol), (+)-*cis*/*trans*-*p*-mentha-2,8-dien-1-ol (0.182 g, 1.2 mmol), and *p*-toluenesulfonic acid (0.034 g, 0.178 mmol) in anhydrous benzene (10 mL); yield: 43% (0.22 g); yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.97 (br s, 2H, ArH), 6.37 (br s, 1H, OH), 5.53 (br s, 1H, 2-H), 4.97 (s, 1H, >C=CH_2), 4.91 (s, 1H, >C=CH_2), 3.75−3.60 (m, 1H, 3-H), 2.79−2.63 (m, 5H), 2.50−2.17 (m, 8H), 1.71 (s, 3H, 7-CH_3), 1.4 (s, 3H, 10-CH_3), 1.26 (br s, 8H), 0.87 (t, J = 7.2 Hz, 3H).

(6a*R*-*trans*)-3-[2-Hexyl-(1,3)-dithian-2-yl]-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (2b). The synthesis was carried out analogous to the preparation of **2a** using **3b** (0.22 g, 0.493 mmol) and boron trifluoride etherate (0.22 mL, 1.763 mmol) in anhydrous CH_2Cl_2 (14 mL); yield: 69% (152 mg); yellow gum. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.98 (d, J = 2.0 Hz, 1H, 4-H), 6.85 (d, J = 2.0 Hz, 1H, 2-H),

5.44 (br s, 1H, 8-H), 4.74 (s, 1H, OH), 3.26−3.20 (m, 1H, 10α-H), 2.8−2.6 (m, 5H), 2.18 (m, 1H, 10a−H), 1.93−1.80 (m, 7H, 2′-CH_2, 6a−H, 7-CH, 10*β*-H, SCH_2CH_2), 1.70 (s, 3H, 9-CH_3), 1.40 (s, 3H, 6-CH_3), 1.26−1.18 (br s, 8H,-CH_2-), 1.19 (s, 3H, 6-CH_3), 0.82 (t, 3H, J = 8.5 Hz, 7′-CH_3); ^{13}C NMR ($CDCl_3$) *δ* 155.2, 154.9, 142.1, 134.7, 119.3, 111.8, 111.1, 107.7, 76.8, 58.8, 55.5, 45.1, 44.8, 35.9, 31.7, 31.5, 29.3, 27.8, 27.6, 25.3, 23.8, 23.5, 22.5, 18.5, 14.0; Anal. ($C_{26}H_{38}O_2S_2$) C, H.

2-(3,5-Dimethoxyphenyl)-(1,3)-dithiolane (13). The synthesis was carried out analogous to the preparation of **11** using **12** (1.0 g, 6.0 mmol), 1,2-ethanedithiol (0.755 mL, 9.0 mmol), and boron trifluoride etherate (0.14 mL, 1.2 mmol), in anhydrous CH_2Cl_2 (24 mL); yield: 87% (1.27 g); light yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 6.70 (d, J = 2.2 Hz, 2H), 6.36 (t, J = 2.2 Hz, 1H), 5.58 (s, 1H, 1′-H), 3.80 (s, 6H, OCH_3), 3.50−3.30 (m, 4H, $-S(CH_2)_2S$-); Anal. ($C_{11}H_{14}O_2S_2$) C, H.

5-(1,3-Dithiolan-2-yl)resorcinol (4c). The synthesis was carried out analogous to the preparation of **4a** using **13** (0.64 g, 2.64 mmol) and boron tribromide (0.56 mL, 5.81 mmol) in anhydrous CH_2Cl_2 (66 mL). The reaction was completed in 48 h at −78 °C to 0 °C; yield: 87% (0.49 g); yellow gum; (lit.[28] gum, no data reported). ^{1}H NMR (300 MHz, $CDCl_3$−CD_3COCD_3) *δ* 6.81 (br s, 2H, OH) 6.53 (d, J = 2.4 Hz, 2H), 6.25 (t, J = 2.4 Hz, 1H), 5.45 (s, 1H, 1′-H), 3.43−3.21 (m, 4H, $-S(CH_2)_2S$-); ^{13}C NMR ($CDCl_3$−CD_3COCD_3) *δ* 157.5, 142.8, 106.8, 102.5, 55.9, 93.8, 10.0; Anal. ($C_9H_{10}O_2S_2$) C, H.

(−)-2-[3−3,4-*trans*-*p*-Menthadien-(1,8)-yl]-5-(1,3-dithiolan-2-yl)resorcinol (3c). The synthesis was carried out analogous to the preparation of **3a** using **4c** (0.440 g, 2.52 mmol), (+)-*cis*/*trans*-*p*-mentha-2,8-dien-1-ol (0.390 g, 2.56 mmol), and *p*-toluenesulfonic acid (0.073 g, 0.38 mmol) in anhydrous benzene (19 mL); yield: 30% (0.21 g); yellow viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 6.55 (br s, 2H), 5.53 (br s, 1H, 2-H), 5.48 (s, 1H, 1′-H), 4.64 (s, 1H, >C=CH_2), 4.54 (s, 1H, >C=CH_2), 3.85 (m, 1H, 3-H), 3.50−3.27 (m, 4H, $-S(CH_2)_2S$-), 2.4 (m, 1H), 2.30−2.11 (m, 2H), 1.8 (br s, 5H), 1.65 (s, 3H, 10-CH_3); Anal. ($C_{19}H_{24}O_2S_2$) C, H.

(6a*R*-*trans*)-3-(1,3-Dithiolan-2-yl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (2c). The synthesis was carried out analogous to the preparation of **2a** using **3c** (0.20 g, 0.574 mmol) and boron trifluoride etherate (0.26 mL, 2.05 mmol) in anhydrous CH_2Cl_2 (16.4 mL); yield: 50% (0.10 g); yellow solid. mp 56−59 °C (lit.[28] no data reported); ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 6.59 (s, 1H, ArH), 6.46 (s, 1H, ArH), 5.47 (s, 1H, 8-H), 5.41 (s, 1H, 1′-H), 4.96 (br s, 1H, OH), 3.46−3.28 (m, 4H, -$S(CH_2)_2S$-), 3.25−3.13 (m, 1H, 10α-H), 2.72−2.60 (m, 1H, 10a−H), 2.14−1.72 (m, 4H, 6α-H, 7-CH_2-, 10*β*-H), 1.70 (s, 3H, CH_3) 1.35 (s, 3H, CH_3), 1.07 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) *δ* 155.1, 154.9, 140.1, 134.7, 119.3, 113.1, 110.0, 106.5, 76.6, 55.7, 44.8, 40.1, 35.8, 31.7, 30.0, 27.8, 27.5, 23.5, 18.5; mass spectrum *m*/*z* (relative intensity) 348 (M^+, 100), 333 (10), 305 (24), 289 (60), 265 (76), 105 (29). Exact mass calculated for $C_{19}H_{24}O_2S_2$; 348.1218; found, 348.1221. Anal. ($C_{19}H_{24}O_2S_2$) C, H.

(6a*R*-*trans*)-3-[2-Hexyl-(1,3)-dioxolan-2-yl]-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (2d). To a solution of **14** (0.031 g, 0.087 mmol) in ethylene glycol (0.051 mL, 0.932 mmol) and triethyl orthoformate (0.014 mL, 0.087 mmol) at 0 °C was added *p*-toluenesulfonic acid (0.31 mg, 0.002 mmol). The reaction mixture was stirred at that temperature for 24 h, and at completion a saturated solution of $NaHCO_3$ was added. The mixture was extracted with diethyl ether and the organic layer washed with water and brine and dried over Na_2SO_4. Solvent evaporation and purification by flash column chromatography on silica gel (30% diethyl ether−petroleum ether) afforded 31.4 mg (90% yield) of the title compound **2d** as a yellow gum. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 6.50 (d, J = 1.2 Hz, 1H, 4-H), 6.42 (d, J = 1.2 Hz, 1H, 2-H), 5.49 (br s, 1H, OH), 5.41 (br s, 1H, 8-H), 3.97 (m, 2H, $-O(CH_2)$-), 3.80 (m, 2H, $-O(CH_2)$-), 3.29−3.18 (m, 1H, 10α-H), 2.75−2.65 (m, 1H, 10a−H), 2.15−2.10 (m, 1H, 7α-H), 1.91−1.80 (m, 5H, 6α-H, 7*β*-H, 10*β*-H, 2′-CH_2-), 1.70 (s, 3H, 9-CH_3), 1.40 (s, 3H, 6-CH_3), 1.20 (br s, 8H,-CH_2-), 1.10 (s, 3H, 6-CH_3), 0.82 (t, 3H, J = 6.1 Hz, 7′-CH_3); ^{13}C NMR ($CDCl_3$) *δ* 155.0, 154.8, 142.2, 134.7, 119.3, 112.6, 110.5, 107.5, 104.8, 104.7, 76.8, 64.4, 44.8, 40.2, 35.8, 31.7, 29.4, 27.8, 27.5, 23.5, 22.6, 18.5, 14.0; mass spectrum *m*/*z* (relative intensity) 400 (M^+, 4), 352 (3), 315 (100), 77 (27). Exact mass calculated for $C_{25}H_{36}O_4$; 400.2614; found, 400.2618. Anal. ($C_{25}H_{36}O_4$) C, H.

(6a*R*-*trans*)-3-[2-Hexyl-4,5-dimethyl-(1,3)-dithiolan-2-yl]-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (2e). To a solution of **14** (0.050 g, 0.14 mmol) in 1,2-dimethyl-1,2-ethanedithiol (0.086 mL, 0.70 mmol) and triethyl orthoformate (0.024 mL, 0.14 mmol) at 0 °C was added *p*-toluenesulfonic acid (0.51 mg, 0.003 mmol). Following the addition of the acid, the reaction mixture was stirred at room temperature for 10 h. The workup was carried out analogous to the preparation of **2d**. Yield: 60% (38.7 mg); yellow gum. ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 6.72 (d, J = 1.9 Hz, 1H, ArH, **2e$_2$**), 6.65 (d, J = 1.9 Hz, 1H, ArH, **2e$_1$**), 6.63 (d, J = 1.9 Hz, 1H, ArH, **2e$_2$**), 6.57 (d, J = 1.9 Hz, 1H, ArH, **2e$_1$**), 5.43 (br s, 2H, 8-H, **2e$_1$**, **2e$_2$**), 4.75 (br s, 2H, OH, **2e$_1$**, **2e$_2$**), 3.86−3.84 (m, 2H, -SCHCHS-, **2e$_1$**), 3.55−3.45 (m, 1H, -SCHS-, **2e$_2$**), 3.35−3.30 (m, 1H, -SCHS-, **2e$_2$**), 3.29−3.16 (m, 2H, 10α-H, **2e$_1$**, **2e$_2$**), 2.78−2.67 (m, 2H, 10a−H, **2e$_1$**, **2e$_2$**), 2.31−2.10 (m, 6H, 7-CH, 2′-CH_2-, **2e$_1$**, **2e$_2$**), 1.92−1.75 (m, 6H, 6a−H, 7-CH, 10*β*-H, **2e$_1$**, **2e$_2$**), 1.70 (s, 6H, 9-CH_3, **2e$_1$**, **2e$_2$**), 1.40 (s, 6H, 6-CH_3, **2e$_1$**, **2e$_2$**), 1.38−1.25 (m, 12H, $-SC(CH_3)$-, **2e$_1$**, **2e$_2$**), 1.21 (br s, 16H,-CH_2-, **2e$_1$**, **2e$_2$**), 1.10 (s, 6H, 6-CH_3, **2e$_1$**, **2e$_2$**), 0.83 (t, 6H, J = 6.9 Hz, 7′-CH_3, **2e$_1$**, **2e$_2$**); ^{13}C NMR ($CDCl_3$) *δ* 157.7, 154.3, 144.3, 134.7, 119.3, 112.3, 108.8, 106.2, 76.6, 72.8, 56.7, 55.7, 53.7, 48.8, 47.5, 44.7, 35.8, 31.6, 29.7, 27.8, 27.5, 27.2, 26.8, 23.5, 22.6, 18.5, 14.1; mass spectrum *m*/*z* (relative intensity) 460 (M^+, 5), 446 (10), 404 (6), 375 (100). Exact mass calculated for $C_{27}H_{40}O_2S_2$; 460.2470; found, 460.2464. Anal. ($C_{27}H_{40}O_2S_2$) C, H.

(6aR-*trans*)-3-[2-Hexylbenzo(1,3)dithiol-2-yl]-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (2f). The title compound was prepared from **14** (0.10 g, 0.28 mmol), in anhydrous CH_2Cl_2 (1.12 mL), 1,2-benzenedithiol (0.06 mL, 0.5 mmol) and boron trifluoride etherate (0.01 mL, 0.084 mmol), following the procedure described for compound **11**. Purification by flash column chromatography (20% diethyl ether−petroleum ether as eluent) gave 47.2 mg (35%) of **2f** as white foam. mp 57−60 °C dec; ^{1}H NMR (300 MHz, $CDCl_3$) *δ* 7.18−7.14 (m, 2H), 7.00−6.97 (m, 2H), 6.66 (d, J = 1.9 Hz, 1H, 4-H), 6.60 (d, J = 1.9 Hz, 1H, 2-H), 5.43 (d, J = 3.7 Hz, 1H, 8-H), 4.90 (s, 1H, OH), 3.20−3.14 (dd, J = 20 Hz, J = 4 Hz, 1H, 10α-H), 2.70 (m, 1H, 10a−H), 2.41−2.39 (m, 2H, 2′-CH_2-), 2.36−2.12 (m, 1H, 7-H), 1.92−1.77 (m, 3H), 1.69 (s, 3H, 9-CH_3), 1.40 (s, 3H, 6-CH_3), 1.30−1.10 (m, 8H, -CH_2-), 1.09 (s, 3H, 6-CH_3), 0.80 (t, J = 6.5 Hz, 3H, 7′-CH_3); ^{13}C NMR ($CDCl_3$) *δ* 154.9, 154.6, 141.6, 138.1, 134.6, 125.4, 122.3, 119.3, 112.7, 108.7, 106.5, 76.6, 74.8 (1′-C), 44.7, 44.5, 35.7, 31.6, 29.1, 27.8, 27.5, 26.2, 23.4, 22.5, 18.5, 14.0; mass spectrum *m*/*z* (relative intensity) 480 (M^+, 4), 446 (8), 395 (100). Exact mass calculated for $C_{29}H_{36}O_2S_2$; 480.2157; found, 480.2154. Anal. ($C_{29}H_{36}O_2S_2$) C, H.

Radioligand Binding Assay. Forebrain synaptosomal membranes were prepared from frozen rat brains by the method of Dodd et. Al.[42] and were used to assess the affinities of the novel analogues for the CB1 binding sites, while affinities for the CB2 sites were measured using a membrane preparation from frozen mouse spleen using a similar procedure.[35] The displacement of specifically tritiated CP-55,940 from these membranes was used to determine the IC_{50} values for the test compounds. The assay was conducted in a 96-well microfilter plate. The samples were filtered using a Packard Filtermate Harvester and Whatman GF/B unifilter-96 plates, and 0.5% BSA was incorporated into the wash buffer. Radioactivity was detected using MicroScint 20 scintillation cocktail added to the dried filter plates and was counted using a Packard Instruments Top Count. Data were collected from three independent experiments between 100% and 0% specific binding for [^{3}H]CP-55,940, determined using 0 and 100 nM CP-55,940. The normalized data from three independent experiments were combined and analyzed using a four-

papameter logistic equation to yield IC_{50} values which were converted to K_i values using the assumptions of Cheng and Prussoff.[43]

Molecular Modeling. Computational studies were performed on the cannabinoid analogues using the InsightII/Discover software program[44] on a SGI Indigo2 workstation. Each molecule was first constructed with bond angles and bond distances supplied by the molecule builder feature and was then given the CVFF force field. The following angles, corresponding to cannabinoid low energy conformations, were then set for all analogues: C2−C1−O−H at 167°, C2−C3−C1′-C2′ at 240°, and C3−C1′-C2′-C3′ at 60°.[36] In ligands with rings at the C1′-position the lower energy ring conformation was used in which the bulkier tricyclic group is equatorial while the seven-carbon chain is axial. Molecular dynamics[37] were performed at 300 K for 1000 iterations (1 fs per iteration, with morse terms, with cross terms) to allow for equilibration. Next, the analogues were minimized with the steepest descent method for 100 iterations followed by the conjugate gradient method until the maximum derivative was less than 0.001 kcal/mol. The resulting structures were then superimposed by minimum rmsd alignment of the analogues. The six carbons of each analog's cannabinoid aromatic ring served as the superimposition points. Based on these calculations, receptor volume maps[37,38] were generated with the TRIPOS SYBYL[45] multiple-contour module to compare the topographical differences among the analogues.

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Supporting Information Available: In vitro pharmacological data for analogue **1c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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