Δ^9-Tetrahydrocannabinol Immunochemical Studies: Haptens, Monoclonal Antibodies, and a Convenient Synthesis of Radiolabeled Δ^9-Tetrahydrocannabinol

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Immunopharmacotherapy as an approach to combat drugs of abuse has become an active area of investigation. Marijuana is the most commonly used illicit drug in the U.S. The main active chemical in marijuana is Δ^9-tetrahydrocannabinol (Δ^9-THC); hence, monoclonal antibodies with high affinity and specificity for Δ^9-tetrahydrocannabinol could be valuable immunopharmacotherapeutic intervention and diagnostic tools. We have synthesized immunoconjugates that induce an effective immune response to Δ^9-THC and describe a convenient synthesis of radiolabeled Δ^9-THC. We demonstrate the value and use of this probe to select anti-Δ^9-THC antibodies that bind Δ^9-THC with good affinity. The synthetic route to radiolabeled Δ^9-THC has enabled the correct assessment of the affinity of these antibodies to their ligand and may facilitate future binding studies between Δ^9-THC and its analogues and the cannabinoid receptors.

Introduction

Marijuana obtained from *Cannabis sativa* has been used by humans for over 4000 years and continues to be one of the most common recreational drugs among substance abusers. The plant contains a mixture of natural cannabinoids, and the study of these compounds has intensified greatly since the early 1990s when the chief cannabinoid receptor (CB1) and its endogenous ligand, anandamide, were identified.[1] The main psychoactive constituent of marijuana, (−)-Δ^9-tetrahydrocannabinol (Δ^9-THC), binds to the CB1 receptor and is quickly metabolized principally to 11-hydroxy-Δ^9-THC, 9-carboxy-11-nor-Δ^9-THC, and 8β,11-dihydroxy-Δ^9-THC, as well as their glucuronide conjugates.[2,3] The structure of Δ^9-THC was first elucidated about 40 years ago by Gaoni and Mechoulam,[4] and total syntheses for many of the natural cannabinoids have been reported.[5,6] Endocannabinoid receptors have been recognized as mediators of key physiological processes such as pain, motility, memory, and appetite.[2]

Marijuana has been the most abused illicit substance used in the U.S. for several decades.[7,8] Understanding changes in the use of marijuana over time is important for a number of reasons. Marijuana use is associated with impaired educational attainment, reduced workplace productivity, and increased risk of use of other substances. Marijuana use plays a major role in motor vehicle crashes and has adverse effects on the respiratory and cardiovascular systems.[7,8] The increasing spread of marijuana use, especially among adolescents and young adults, and the potential regulated availability of marijuana for medical use have heightened societal awareness of the risks and possible benefits[9] associated with this drug. This has further highlighted the need to fully understand its mechanism of action and the treatment of adverse effects.

Immunopharmacotherapy is based on the elicitation or administration of antibodies that are capable of binding the targeted drug before it can reach the brain. This strategy has emerged as a powerful tool at the interface of chemistry and biology. Immunopharmacotherapeutic studies have been reported to treat addiction to cocaine, nicotine, PCP, and methamphetamine.[10] In this study, we report our efforts to develop an immunotherapeutic strategy to combat marijuana dependence. Several different THC-based haptens were designed and synthesized, and their immunogenic properties were studied in mice. Whereas several previously reported immunoconjugates are based on conjugation to the carrier protein through the phenol moiety of Δ^9-THC, our approach is based on conjugation through the aliphatic side chain. Most of the immunoconjugates showed good immunogenicity, and several monoclonal antibodies (mAbs) with good affinity and specificity for THC were isolated. The binding propertes of mAbs to Δ^9-THC were assessed using radiolabeled Δ^9-THC and analogues. Radiolabeled Δ^9-THC is not commercially available perhaps because of a lack of demand, given that past efforts to use it for detecting endogenous cannabinoids were unsuccessful.[11] Hence, one of our aims was to synthesize the tritium-labeled compound, based on literature procedures but without the use of tritium gas that is subject to safety issues and regulatory complications. The syntheses of THC-based haptens, generation of anti-THC antibodies, the synthesis of radiolabeled Δ^9-THC and analogues for the study of antibody binding properties are presented herein.

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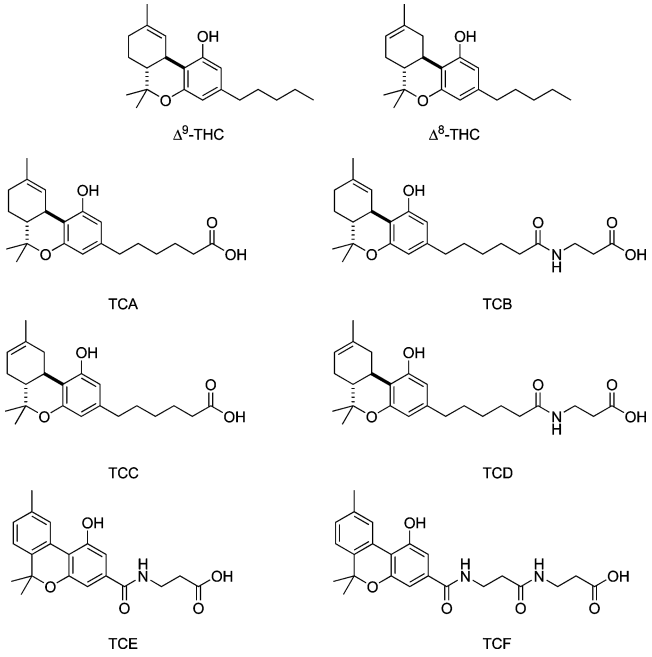


Figure 1. Structures of Δ^9-THC, Δ^8-THC, and various haptens.

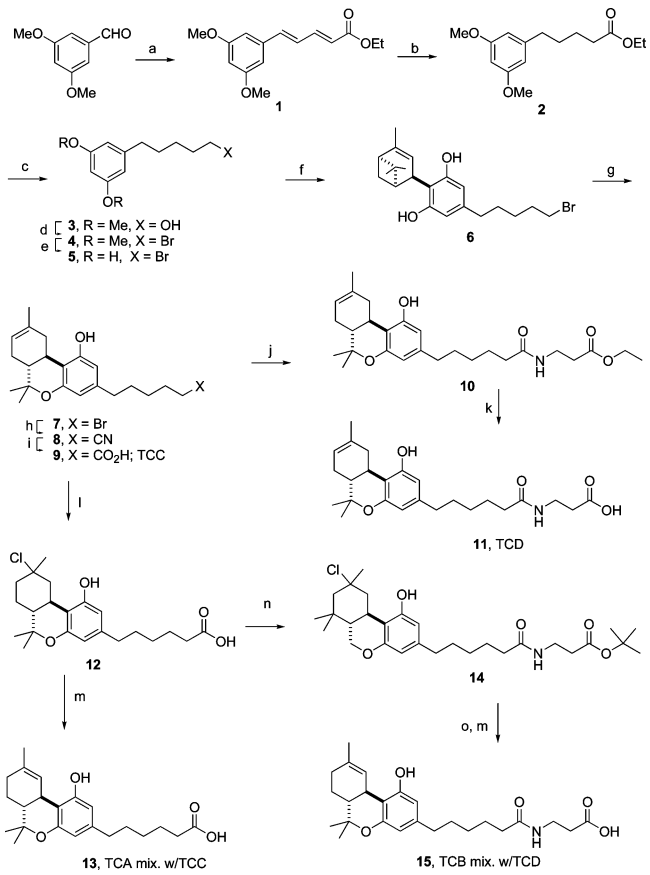
Results and Discussion

1. Chemistry. 1.1. Hapten Design. Although Δ^9-THC is the major component of marijuana (Figure 1), Δ^8-THC shows roughly similar bioactivities as Δ^9-THC and is thermodynamically stable.[12] Therefore, we chose to design haptens based on both THC isomers.

Previously reported THC-related haptens typically contained short linkers attached to the phenol moiety. In our design, the linker is conjugated to the aliphatic side chain that is distant from the tricyclic core structure. A more specific immune response is expected due to the intact THC core structure and the functional groups in these haptens. Haptens TCA, TCB, TCC, TCD, TCE, and TCF are shown in Figure 1. In a previous study, we had reported a beneficial linker effect on antibody generation when β-alanine was incorporated in the hapten design.[13] We continue to study the potential generality of this phenomenon. With the new amide functionality and increased linker length, the haptens are expected to be more effective in generating specific anti-THC antibodies. We also designed TCE and TCF, which were modified from original THC structures by using more "aromatic elements" and amide bonds for increased immunogenicity.

1.2. Hapten Synthesis. The syntheses for TCA, TCB, TCC, and TCD are shown in Scheme 1. Starting from the commercially available 3, 5-dimethoxybenzaldehyde, the aromatic moiety was prepared according to Huffman's method[14] of reacting 3,5-dimethoxybenzaldehyde with triethyl-4-phosphonocrotonate to provide ester **1**. Catalytic hydrogenation followed by $LiAlH_4$ reduction gave alcohol **3** in good yield. Bromination followed by demethylation gave bromide **5**. Coupling of **5** with (*s*)-cis-verbenol under TsOH provided compound **6**, which was cyclized to the brominated Δ^8-THC analogue **7**. The bromide was then converted to give hapten TCC (**9**) by cyanation and hydrolysis. TCC was coupled with β-alanine ethyl ester to give TCD precursor **10**, which was hydrolyzed by NaOH to obtain TCD (**11**). TCA (**13**) was

Scheme 1. Synthesis of Haptens TCA, TCB, TCC, and TCD[a]

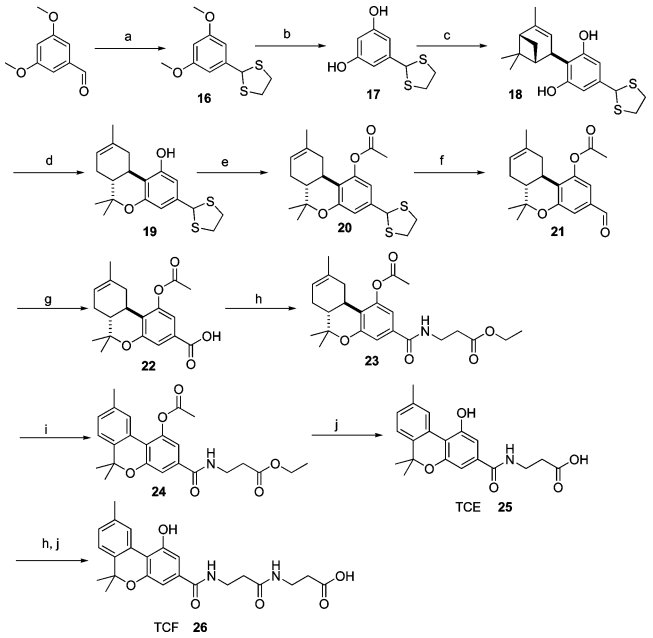


[a] Reagents and conditions: (a) triethyl 4-phosphonocrotonate, NaH, 50%; (b) H_2, Pd/C and $Pd(OH)_2$, 85%; (c) LAH, 99%; (d) PPh_3, CBr_4, 86%; (e) BBr_3, 98%; (f) (*s*)-*cis*-verbenol, TsOH, 47%; (g) BF_3/OEt_2, 71%; (h) $OH(Me_2)CCN$, DBU, 86%; (i) NaOH, 94%; (j) H-β-alanine-OEt, HBTU, 83%; (k) NaOH, 81%; (l) HCl, $ZnCl_2$, 92%; (m) KO*t*Bu; (n) H-β-alanine-O*t*Bu, HBTU, 86%; (o) TFA.

prepared as a mixture with TCC by migration of the double bond in TCC following published procedures.[15,16] TCB (**15**) was prepared as a mixture with TCD from compound **12**, followed by purification. We decided to prepare TCA and TCB based immunoconjugates as mixtures incorporating TCC and TCD, respectively, partly because of difficulties related to pure TCA and TCB hapten preparation and partly because of our experience in past immunization strategies that this strategy may yield antibodies with specificity for Δ^9-THC as well as antibodies that will bind both isomers. The latter could be advantageous because Δ^8-THC, which has roughly 75% of the bioactivity of Δ^9-THC, is present in low amounts in marijuana; furthermore, since this compound is more stable than Δ^9-THC, its bioavailability may be significantly higher.

Haptens TCE and TCF were prepared as shown in Scheme 2. Commercially available 3,5-dimethoxybenzaldehyde was reacted with ethanedithiol in the presence of BF_3 etherate, after which demethylation yielded compound **17**, which was reacted with (*s*)-*cis*-verbenol in the presence of TsOH to give diol **18**. BF_3 etherate mediated ring closure, followed by phenol protection with acetic anhydride, and deprotection of the aldehyde moiety gave **21**, which was converted to carboxylic acid **22** and coupled to β-alanine ethyl ester to provide compound **23**. The aromatic THC analogue was obtained

Scheme 2. Synthesis of Haptens TCE and TCF[a]



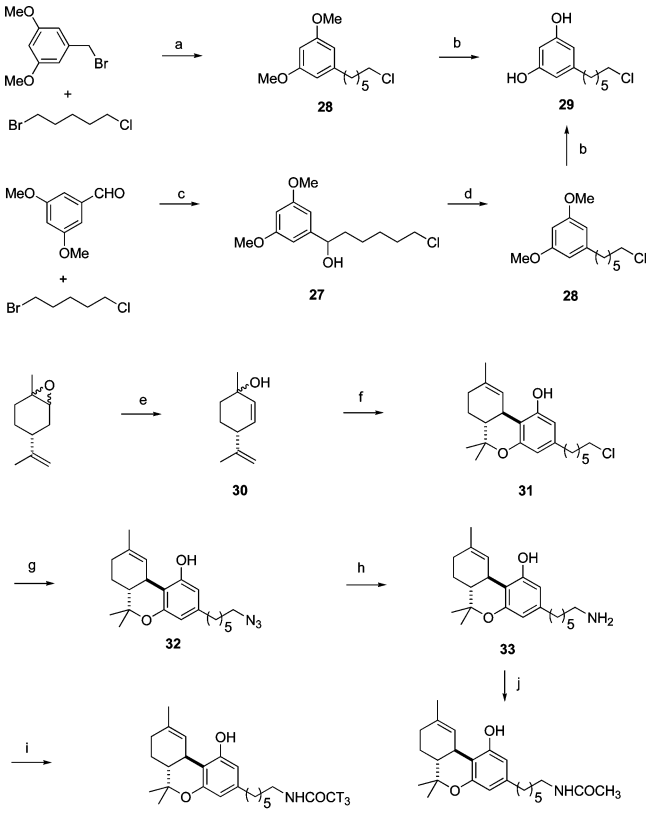
[a] Reagents and conditions: (a) ethanedithiol, BF_3/Et_2O, 99%; (b) BBr_3, 69%; (c) (*s*)-*cis*-verbenol, TsOH, 43%; (d) BF_3/Et_2O, 54%; (e) Ac_2O, Py, 75%; (f) $AgNO_3$; (g) NaH_2PO_3, $NaClO_2$, 2-methyl-2-butene; (h) H-β-Ala-OEt, HBTU, Et_3N, 24% (three steps); (i) sulfur, 200 °C, 49%; (j) LiOH, H_2O, THF, 84%.

by the method of Tuchinda[17] through treatment of **23** with sulfur at high temperature for 45 min. Removal of protecting groups gave hapten TCE (**25**); this compound was then coupled to β-alanine ethyl ester, followed by deprotection to give hapten TCF (**26**).

1.3. Design and Synthesis of Radiolabeled Δ9-THC and Analogues. To assess the binding properties of mAbs to Δ9-THC, we set out to synthesize radiolabeled Δ9-THC and some closely related analogues. The first radiolabeled analogue was designed on the basis of the structure of TCA, and its synthesis is shown in Scheme 3. Chloride **28** was prepared from the commercially available reagents by a Grignard mediated coupling reaction.[18] However, we note that purification of **28** from the starting materials was complicated; hence, an alternative method was sought.[19] 3,5-Dimethoxybenzaldehyde was reacted with the Grignard reagent to give benzylic alcohol **27**, which was dehydroxylated by catalytic hydrogenation to chloride **28**. Demethylation of **28** provided chloride **29** in very pure form. Compound **30** was synthesized from (+)-limonene oxide by a two-step approach.[20] Acid-catalyzed coupling between compounds **30** and **29** gave the desired product with high purity after flash chromatography. The chloride **31** was converted to azide **32**, which was reduced to primary amine **33** by a Staudinger reaction using polymer-supported triphenylphosphine. A selective model acylation of the primary amine gave target amide **34a**, using polymer-supported pyridine as base to facilitate its workup. Reaction of **33** with tritium-labeled acetic anhydride afforded the tritium labeled analogue **34b** in good yield.

With the completion of a route to **34b**, binding analysis using equilibrium dialysis with several mAbs that had shown good binding to Δ9-THC using competition ELISA was undertaken; however, we note that **34b**

Scheme 3. Synthesis of Tritium Labeled Δ9-THC Analogue **34b**[a]



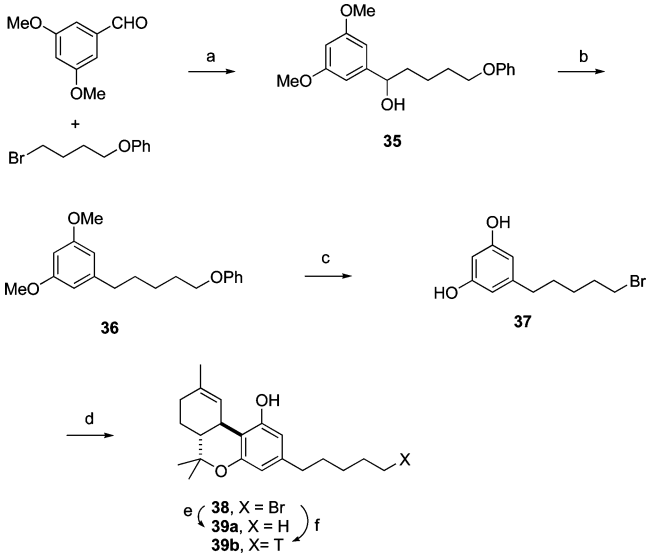
[a] Reagents and conditions: (a) (i) Mg, THF, reflux; (ii) Li_2CuCl_4, THF, −78 °C to room temp, 81%; (b) BBr_3, CH_2Cl_2, −78 °C to room temp, 84%; (c) Mg, THF, reflux −78 °C to room temp, 67%; (d) Pd/C (10%), H_2, AcOH, 54%; (e) (i) $(PhSe)_2$, $NaBH_4$, EtOH; (ii) H_2O_2, THF; (iii) room temp, then reflux, 52% for three steps; (f) BF_3/Et_2O, CH_2Cl_2, $MgSO_4$, 0 °C, **29**, 42%; (g) NaN_3, DMF, NaI (cat.), 60 °C, 92%; (h) THF/H_2O, polymer-supported Ph_3P; (i) polymer-supported pyridine, Ac_2O-(tritium-6), CH_2Cl_2, 80%; (j) polymer-supported pyridine, Ac_2O, CH_2Cl_2, 88% for two steps.

is not a good Δ9-THC mimic because poor binding was observed with the antibodies tested. This was rather surprising because the structure of **34b** is highly congruent to that of the TCB hapten used to elicit these antibodies.

We next decided to concentrate our efforts on the synthesis of [3H]-Δ9-THC. Owing to the lack of availability of tritium as reducing agent, a synthesis with high yield of radiolabel incorporation proved to be difficult. With this second setback we decided to explore a new synthetic approach that would require reduction of a bromo-THC analogue to [3H]-Δ9-THC with $LiAlT_4$ or $NaBT_4$.

3,5-Dimethoxybenzaldehyde was reacted with an in situ generated Grignard reagent to give benzylic alcohol **35** (Scheme 4). Subsequent dehydroxylation by catalytic hydrogenation was ineffective. After extensive experiments, we found that a TMSCl/NaI combination effected the transformation successfully.[21] Product **36** was deprotected by BBr_3 etherate, and the resulting primary alcohol was converted to bromide **37** simultaneously. Coupling of **37** with **30** under acidic conditions provided the brominated Δ9-THC analogue **38**. A model reduction of the bromide with $LiAlH_4$ or $NaBH_4$ selectively debrominated **38** to give Δ9-THC without affecting the internal double bond. This procedure was repeated

Scheme 4. Synthesis of Tritium-Labeled Δ^9-THC Analogue **39b**[a]



[a] Reagents and conditions: (a) Mg, THF, reflux −78 °C to room temp, 93%; (b) TMSCl, MeCN, Et_2O/H_2O, room temp, 24 h, 88%; (c) BBr_3, CH_2Cl_2, −78 °C to room temp, 36 h, 80%; (d) BF_3/Et_2O, CH_2Cl_2, $MgSO_4$, 0 °C, **30**, 38%; (e) LAH, THF, room temp or $NaBH_4$, DMSO 47%; (f) tritium-LAH, THF, room temp, 50% or tritium-$NaBH_4$, DMSO, room temp, 80%.

Table 1. Serum Antibody Titers and Relative Affinities for Δ^9-THC and Δ^8-THC

hapten	average titer	Δ^9-THC affinity, μM	Δ^8-THC affinity, μM
TCA	1870	39	20
TCB	11600	48	48
TCC	2530	72	85
TCD	8800	51	53
TCE	25600	>128	>128
TCF	14400	>128	>128

successfully, using $LiAlT_4$ and $NaBT_4$, to give tritium-labeled Δ^9-THC analogue **39b**. We note that the $NaBT_4$-mediated reduction was more efficient, with an increase in yield (80% versus 50%) and resulting in a 17-fold higher specific activity of the final product (4.0 Ci/mmol). This increase in specific activity of the probe was crucial in our endeavor to correctly assess the binding constants of potential mAbs with high affinity for Δ^9-THC.

2. Immunochemistry. 2.1. Murine Immunization. Immunoconjugates of haptens conjugated to bovine serum albumin (BSA) and keyhole limpet hemocyanin (KLH) were prepared using standard EDC coupling procedures and dialyzed against PBS, pH 7.4. The KLH immunoconjugates were used to immunize mice (strain 129 GIX^+) that were not older than 3 months of age. The first injection (200 μL) contained the immunoconjugate (100 μg based on KLH) and RIBI adjuvant (50 μg) reconstituted in PBS. The injection was administered ip. A second booster injection was given 2 weeks later in a similar fashion. Bleeds of mice 7−10 days later were used to assess antibody titers and specificities using enzyme linked immunosorbent assay (ELISA) and competition ELISA with Δ^9-THC and Δ^8-THC (Table 1). On the basis of the titers and relative affinities of the sera, we used mice that were immunized with TCB-KLH for hybridoma production. A final tail vein iv injection of KLH immunoconjugate (50 μg) in PBS (150 μL) was given 1 month after the final ip boost. Three days later, the spleen was fused with a myeloma cell line (X63-Ag8.653) to produce hybridomas according to standard techniques[22] with some modifications developed in our laboratory. The hybridomas were cloned into 96-well plates and screened against the respective BSA conjugates by ELISA during the cloning process. Each member of a final panel of 60 mAbs then was assessed for binding and specificity to Δ^9-THC by using competition ELISA. This assay yielded several good mAbs, of which three were evaluated in more detail by equilibrium dialysis.

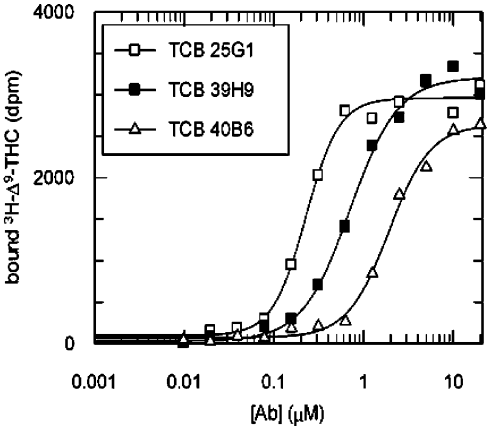


Figure 2. Binding curves for mAbs TCB 25G1, TCB 39H9, and TCB 40B6 with Δ^9-THC analogue **39b** as ligand.

2.2. Monoclonal Antibody Binding Studies. We used equilibrium dialysis, a powerful tool in the determination of physiologically relevant protein−ligand dissociation constants,[23] to study the affinity of mAbs TCB25G1, TCB39H9, and TCB40B6 for [^{3}H]-Δ^9-THC. This technique is more quantitative than competition ELISA. Since the results of this assay are obtained under equilibrium conditions, the physiological interaction can be studied and low affinity interactions that are not detectable with high reliability using other methods can also be accomplished. The results of this assay are shown in Figure 2. The mAbs TCB25G1, TCB39H9, and TCB40B6 showed moderate to good binding to Δ^9-THC with $K_d = 0.23 \pm 0.02$, 0.71 ± 0.06, and 1.41 ± 0.14 μM, respectively, a striking difference to the results obtained from the assay with radiolabeled compound **34b**. This points to a relatively specific interaction between the aliphatic side chain of Δ^9-THC and the selected mAbs. Hence, by employing [^{3}H]-Δ^9-THC, we were able to identify mAbs that have good affinity for Δ^9-THC. On the basis of Δ^9-THC serum concentrations that reach up to 0.8 μM after smoking,[24] mAb TCB25G1 can be considered a good candidate to bind Δ^9-THC in vivo with potential physiological consequences.

Conclusions

In this study, we synthesized several haptens based on the Δ^9-THC core and developed a new and convenient route to radiolabeled Δ^9-THC and related analogues. Six THC-based immunoconjugates were prepared and used for the immunization of mice, which in several cases showed a strong immune response and a reasonable degree of binding specificity for Δ^9-THC. Our convenient synthesis of tritium-labeled Δ^9-THC allowed assessment of binding constants for several mAbs that were elicited

using the immunoconjugate TCB-KLH. The synthetic strategy developed for [^{3}H]-Δ^9-THC provides an efficient route from readily available starting materials while avoiding the use of tritium gas as a reducing agent. The mAb TCB25G1 that we obtained using immunization of mice with TCB-KLH showed good affinity for Δ^9-THC ($K_d = 0.23\ \mu$M) and we are currently studying the effects of mAb TCB25G1 on the behavior of rats that are treated with Δ^9-THC.

Experimental Section

Chemistry. ^{1}H and ^{13}C NMR spectra were measured at 399.7 MHz on a Varian INOVA-400, 300 MHz on a Varian MERCURY-300BB, 400 and 500 MHz on Bruker AMX-400 and DRX-500 spectrometers. Chemical shifts (ppm) are reported relative to internal TMS (^{1}H, 0.00 ppm) and $CDCl_3$ (^{13}C, 77.0 ppm). HRMS and high-accurate spectra were measured using MALDI and ESI-TOF techniques. Solvents were dried by standard methods. Flash chromatography was performed on silica gel 60 (230−400 mesh) and thin-layer chromatography (TLC) on glass plates coated with a 0.25-mm layer of silica gel 60 F-254.

5-(3,5-Dimethoxyphenyl)penta-2,4-dienoic Acid Ethyl Ester (1). A mixture of NaH (50%, 3.5 g, 72.2 mmol) in THF (60 mL) was stirred at 0 °C under Ar. To the mixture, triethyl 4-phosphonocrotonate (18 g, 72.2 mmol) in THF (40 mL) was added dropwise in 20 min. After 15 h, 3,5-dimethoxybenzaldehyde (10 g, 60.2 mmol) in THF (30 mL) was added slowly in 15 min at 0 °C. The mixture was allowed to warm to room temperature. After being stirred for 12 h, the reaction mixture was poured into water and extracted two times with Et_2O. The combined organic extracts were washed with water and then saturated brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:10) to afford the desired compound **1** as yellow crystals (7.93 g, 30.3 mmol, 50%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.30 (t, J = 7 Hz, 3H), 3,79 (s, 6H), 4.21 (q, J = 7 Hz, 2H), 5.97 (d, J = 15.6 Hz, 1H), 6.42 (t, J = 2.2 Hz, 1H), 6.59 (d, J = 2.3 Hz, 2H), 6.75−6.85 (m, 2H), 7.33−7.44 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 55.3, 60.3, 101.2, 105.0, 121.4, 126.6, 137.8, 140.2, 144.2, 160.9, 166.9. HRMS (MALDI-FTMS) calcd for $C_{15}H_{19}O_4$ (MH^+) 263.1278, found 263.1274.

5-(3,5-Dimethoxyphenyl)pentanoic Acid Ethyl Ester (2). A solution of 5-(3,5-dimethoxyphenyl)penta-2,4-dienoic acid ethyl ester (7.93 g, 30.3 mmol) and 10% Pd/C (400 mg) in MeOH (150 mL) was stirred under H_2. After 3.5 h, 20% Pd-$(OH)_2$ (400 mg) was added and the mixture was stirred for 13 h. The mixture was filtered and evaporated, and the residue was purified by chromatography on silica gel, with Et_2O as eluant, to afford the desired compound **2** as a pale-yellow oil (6.86 g, 25.7 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.24 (t, J = 7 Hz, 3H), 1.57−1.75 (m, 4H), 2.31 (t, J = 7 Hz, 2H), 2.57 (t, J = 7.1 Hz, 2H), 3.33 (s, 6H), 4.12 (q, J = 7 Hz, 2H), 6.29−6.31 (m, 1H), 6.32−6.34 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.2, 24.5, 30.6, 34.1, 35.8, 55.1, 60.1, 97.6, 106.3, 144.5, 160.6, 173.6. HRMS (MALDI-FTMS) calcd for $C_{15}H_{22}O_4$-Na (M Na^+) 289.141, found 289.1412.

5-(3,5-Dimethoxyphenyl)pentan-1-ol (3). To a mixture of $LiAlH_4$ (2.0 g, 52.5 mmol) in THF (175 mL), 5-(3,5-dimethoxyphenyl)pentanoic acid ethyl ester **2** (14.0 g, 52.5 mmol) in THF (100 mL) was added by a dropping funnel over 40 min at 0 °C. After 2 h 40 min, H_2O (2 mL), 2 N NaOH (2 mL), and H_2O (6 mL) were added to the reaction mixture successively. After the precipitate (aluminum hydroxide) was removed by filtration, the clear solution was evaporated to afford **3** as a colorless liquid (11.7 g, 52.0 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.35−1.45 (m, 2H), 1.55−1.70 (m, 4H), 1.84−1.89 (m, 1H), 2.57 (t, J = 7.8 Hz, 2H), 3.65 (t, J = 6.6 Hz, 2H), 3.78 (s, 6H), 6.30 (t, J = 2.1 Hz, 1H), 6.34 (d, J = 2.1 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.3, 31.0, 32.6, 36.2, 55.2, 62.8, 97.5, 106.4, 145.0, 160.6. HRMS (MALDI-FTMS) calcd for $C_{13}H_{21}O_3$ (MH^+) 225.1485, found 225.1487.

1-(5-Bromopentyl)-3,5-dimethoxybenzene (4). To a solution of 5-(3,5-dimethoxyphenyl)pentan-1-ol (717 mg, 3.20 mmol) in CH_2Cl_2 (11 mL) at 0 °C, were added PPh_3 (1.43 g, 5.44 mmol) and CBr_4 (1.59 g, 4.80 mmol). The mixture was allowed to warm to room temperature. After being stirred for 14 h at room temperature, the solution was filtered by silica gel and washed by CH_2Cl_2. After evaporation, the residue was purified by chromatography on silica gel (EtOAc/hexane 1:10) to afford the desired compound **4** as a yellow oil (793 mg, 2.76 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.42−1.54 (m, 2H), 1.60−1.70 (m, 2H), 1.83−1.93 (m,2H), 2.57 (t, J = 7.6 Hz, 2H), 3.41 (t, J = 6.8 Hz, 2H), 3.78 (s, 6H), 6.31 (t, J = 2.4 Hz, 1H), 6.34 (d, J = 2.4 Hz, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 27.9, 30.4, 32.8, 33.9, 36.1, 55.3, 97.6, 106.3, 144.5, 160.4. HRMS (MALDI-FTMS) calcd for $C_{13}H_{20}BrO_2$ (MH^+) 287.0641, found 287.0637.

5-(5-Bromopentyl)benzene-1,3-diol (5). A solution of 1-(5-bromopentyl)-3,5-dimethoxybenzene (**4**, 762 mg, 2.65 mmol) and BBr_3 (7.95 mL, 1 M solution in CH_2Cl_2) was stirred for 45 min at −78 °C, after which the mixture was allowed to warm to room temperature. After being stirred for 6 h, the mixture was poured into saturated $NaHCO_3$ and extracted four times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:1) to afford the desired compound **5** as a yellow oil (748 mg, 2.60 mmol, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35−1.45 (m, 2H), 1.46−1.60 (m, 2H), 1.75−1.88 (m, 2H), 1.53 (pent, J = 6.9 Hz, 2H), 2.43 (t, J = 7.8 Hz, 2H), 3.50 (t, J = 6.9 Hz, 2H), 6.15−6.18 (m, 1H), 6.23 (d, J = 2.4 Hz, 2H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 27.8, 30.1, 32.6, 34.0, 35.5, 100.4, 108.2, 145.6, 156.1. HRMS (MALDI-FTMS) calcd for $C_{11}H_{16}BrO_2$ (MH^+) 259.0328, found 259.0331.

5-(5-Bromopentyl)-2-[(1*S*-*cis*)-4,6,6-trimethylbicyclo-[3.1.1]hept-3-en-2-yl]benzene-1,3-diol (6). To a solution of 5-(5-bromopentyl)benzene-1,3-diol (**5**, 4.5 g, 17.4 mmol) and *p*-toluenesulfonic acid (331 mg, 1.74 mmol) in $CHCl_3$ (100 mL) at room temperature under Ar was added (*s*)-*cis*-verbenol (2.6 g, 17.4 mmol, 50% ee) in $CHCl_3$ (50 mL). After being stirred for 2.5 h, the reaction mixture was poured into saturated $NaHCO_3$ and extracted two times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:9) to afford **6** as a yellow oil (3.23 g, 8.21 mmol, 47%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.96 (s, 3H), 1.32 (s, 3H), 1.40−1.52 (m, 3H), 1.59 (pent, J = 7.6 Hz, 2H), 1.84−1.91 (m, 5H), 2.16−2.20 (m, 1H), 2.25−2.33 (m, 2H), 2.45 (t, J = 7.6 Hz, 2H), 3.40 (t, J = 7.0 Hz, 2H), 3.90−3.93 (m, 1H), 5.70 (brs, 1H), 6.19 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 23.7, 25.9, 27.8, 30.0, 32.6, 33.8, 35.2, 37.8, 40.7, 47.0, 47.8, 76.7, 108.2, 112.4, 116.4, 119.3, 134.7, 142.0, 142.4, 152.7, 155.5. HRMS (MALDI-FTMS) calcd for $C_{21}H_{30}BrO_2$ (MH^+) 393.1424, found 393.1428.

3-(5-Bromopentyl)-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (7). To a solution of 5-(5-bromopentyl)-2-(4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl)-benzene-1,3-diol (**6**, 3.23 g, 8.21 mmol) in CH_2Cl_2 (150 mL) at 0 °C under Ar was added BF_3/Et_2O (2.08 mL, 16.4 mmol), and the mixture was allowed to warm to room temperature. After being stirred for 2 h, the mixture was poured into saturated $NaHCO_3$ and extracted two times with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/hexane (1:1), to yield **7** as a yellow oil (2.29 g, 5.83 mmol, 71%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 3H), 1.38 (s, 3H), 1.41−1.52 (m, 2H), 1.52−1.68 (m, 2H), 1.70 (brs, 3H), 1.76−1.96 (m, 5H), 2.06−2.22 (m, 1H), 2.45 (t, J = 7.5 Hz, 1H), 2.64−2.76 (m, 1H), 3.20 (dd, J = 16.5 Hz, 4.2 Hz, 1H), 3.39 (t, J = 6.7 Hz, 2H), 5.02 (s, 1H), 5.42 (brd, J = 3.9 Hz, 1H), 6.09 (d, J = 1.2 Hz, 1H), 6.25 (d, J = 1.2 Hz, 1H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 18.6, 23.5, 27.6, 27.88, 27.9, 30.1, 31.6, 32.7, 33.9, 35.2, 36.0, 44.9, 76.7, 107.5, 109.9, 110.6, 119.2, 134.6, 141.8, 154.6, 154.7. HRMS (MALDI-FTMS) calcd for $C_{21}H_{30}BrO_2$ (MH^+) 393.1424, found 393.1412.

6-(1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanenitrile (8). To a solution of 3-(5-bromopentyl)-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (**7**, 2.16 g, 5.49 mmol) in acetonitrile (60 mL) at room temperature under Ar were added DBU (2.51 g, 16.5 mmol) and acetone cyanohydrin (1.40 g, 16.5 mmol). The reaction mixture was stirred for 17 h. After evaporation of the solvent, the residue was partitioned between water and EtOAc. The organic layer was washed with water and then brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:3), to afford compound **8** as a yellow oil (1.61 g, 4.74 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 3H), 1.38 (s, 3H), 1.42−1.52 (m, 2H), 1.54−1.68 (m, 5H), 1.70 (br s, 3H), 1.74−1.90 (m, 2H), 2.10−2.20 (m, 1H), 2.33 (t, J = 7.0 Hz, 2H), 2.47 (dt, J = 2.8 and 7.6 Hz, 2H), 2.70 (dt, J = 11.2 and 4.8 Hz, 1H), 3.19 (dd, J = 16 and 4.4 Hz, 1H), 4.73 (s, 1H), 5.43 (brd, J = 4.4 Hz, 1H), 6.10 (d, J = 1.6 Hz, 1H), 6.25 (d, J = 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.1, 18.5, 23.5, 25.2, 27.5, 27.8, 28.2, 29.9, 30.3, 31.5, 34.9, 35.9, 44.8, 107.6, 109.9, 110.8, 119.3, 119.8, 125.5, 134.7, 141.6, 154.9. HRMS (MALDI-FTMS) calcd for $C_{22}H_{29}NO_2Na$ (MNa^+) 362.2096, found 362.2091.

6-(1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoic Acid (9, TCC). 6-(1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]-chromen-3-yl)hexanenitrile (**8**, 1.06 g, 2.65 mmol) in a solution of 50% aqueous MeOH (25 mL) containing 15% NaOH was refluxed for 4 h. The reaction mixture was acidified with 1 N HCl and extracted two times with EtOAc. The combined extracts were washed with saturated brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatographyon silica gel, eluting with EtOAc/hexane (1:1), to afford the desired compound **9** (TCC) as a yellow amorphous solid (895 mg, 2.50 mmol, 94%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 3H), 1.32−1.42 (m, 2H), 1.37 (s, 3H), 1.53−1.72 (m, 5H), 1.70 (brs, 3H), 1.74−1.90 (m, 2H), 2.06−2.20 (m, 1H), 2.35 (t, J = 7.3 Hz, 2H), 2.41−2.45 (m, 2H), 2.69 (dt, J = 4.8 and 10.8 Hz, 1H), 3.19 (dd, J = 4.2 and 16.2 Hz, 1H), 4.55−4.95 (br, 1H), 5.43 (d, J = 4.0 Hz, 1H), 6.09 (d, J = 1.6 Hz, 1H), 6.26 (d, J = 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4, 23.5, 24.4, 27.5, 27.8, 28.6, 30.4, 31.5, 33.9, 35.1, 35.9, 44.8, 76.7, 107.7, 109.9, 110.7, 119.3, 134.7, 142.1, 154.7, 154.9, 180.0. HRMS (MALDI-FTMS) calcd for $C_{22}H_{30}O_4Na$ (MNa^+) 381.2036, found 381.2040.

3-[6-(1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoylamino]propionic Acid Ethyl Ester (10). To a mixture of 6-(1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoic acid (**9**, 237 mg, 0.661 mmol), Et_3N (0.1 mL, 0.728 mmol), and HBTU (276 mg, 0.728 mmol) in DMF (12 mL) at room temperature under Ar was added H-β-alanine ethyl ester hydrochloride (112 mg, 0.728 mmol). After being stirred for 2 h, the reaction mixture was poured into water and extracted two times with Et_2O/EtOAc (1:1). The organic layer was washed with saturated brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:1), to afford **10** as a yellow oil (244 mg, 0.550 mmol, 83%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.10 (s, 3H), 1.27 (t, J = 7.2 Hz, 3H), 1.24−1.35 (m, 2H), 1.37 (s, 3H), 1.47−1.68 (m, 5H), 1.69 (s, 3H), 1.72−1.87 (m, 2H), 2.06−2.20 (m, 1H), 2.16 (t, J = 7.2 Hz, 2H), 2.41 (t, J = 7.8 Hz, 2H), 2.54 (t, J = 6 Hz, 2H), 2.70 (dt, J = 4.4 and 10.8 Hz, 1H), 3.28 (dd, J = 4.4 and 16 Hz, 1H), 3.52 (q, J = 6.0 Hz, 2H), 4.16 (q, J = 7.2 Hz, 2H), 5.41 (brd, J = 4.0 Hz, 1H), 6.16 (brs, 1H), 6.19 (brs, 1H), 6.27 (brt, J = 6.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 18.4, 23.5, 25.4, 27.5, 27.9, 28.6, 30.4, 31.6, 33.9, 34.8, 35.1, 35.9, 36.6, 44.9, 60.8, 76.6, 107.7, 109.2, 110.7, 119.1, 134.9, 141.8, 154.6, 155.7, 172.9, 173.7. HRMS (MALDI-FTMS) calcd for $C_{27}H_{40}NO_5$ (MH^+) 458.2902, found 458.2092.

3-[6-(1-Hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoylamino]propionic Acid (11, TCD). To a solution of 3-[6-(1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoylamino]propionic acid ethyl ester (**10**, 80 mg, 0.180 mmol) in THF/H_2O (3 mL/1 mL) at 60 °C under Ar was added LiOH/H_2O (15 mg, 0.360 mmol). After being stirred for 1 h, the reaction mixture was poured into water and extracted two times with EtOAc. The organic layer was washed with saturated brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel, eluting with MeOH/CH_2Cl_2 (1:9), to afford the desired compound **11** (TCD) as a yellow oil (60.9 mg, 0.146 mmol, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H), 1.20−1.36 (m, 2H), 1.37 (s, 3H), 1.45−1.66 (m, 5H), 1.68 (s, 3H), 1.72−1.87 (m, 2H), 2.08−2.20 (m, 1H), 2.16 (t, J = 7.4 Hz, 2H), 2.39 (t, J = 7.4 Hz, 2H), 2.59 (t, J = 5.6 Hz, 2H), 2.64−2.75 (m, 1H), 3.27 (brd, J = 16.8 Hz, 1H), 3.45−3.60 (m, 2H), 5.41 (brd, J = 2.8 Hz, 1H), 6.12 (brs, 1H), 6.20 (brs, 1H), 6.39 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.4, 23.5, 25.4, 27.5, 27.9, 28.5, 29.7, 30.3, 31.6, 35.0, 35.3, 35.9, 36.3, 44.9, 76.8, 107.8, 109.3, 110.9, 119.2, 134.8, 141.8, 154.5, 155.7, 174.9. HRMS (MALDI-FTMS) calcd for $C_{25}H_{35}NO_5Na$ (MNa^+) 452.2407, found 452.2400.

6-(9-Chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoic Acid (12). To a solution of 6-(1-hydroxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoic acid (400 mg, 1.12 mmol) and $ZnCl_2$ (80 mg, 0.587 mmol) in toluene at −10 °C (40 mL) was bubbled gasous HCl for 2 h. The mixture was stirred for 1.5 h after stopping the bubbling of HCl. The reaction mixture was poured into water and extracted two times with EtOAc. The organic layer was washed with saturated brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:1), to afford chloride **12** as a yellow oil (409 mg, 1.03 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H), 1.30−1.50 (m, 3H), 1.39 (s, 3H), 1.54−1.70 (m, 8H), 1.67 (s, 3H), 1.71−1.79 (m, 2H), 2.14−2.22 (m, 1H), 2.35 (t, J = 7.4 Hz, 2H), 2.44 (dd, J = 6.8 Hz, 8.4 Hz, 2H), 3.05 (dt, J = 2.8 Hz, 11.2 Hz, 1H), 3.44 (dt, J = 14 Hz, 2.8 Hz, 1H), 6.08 (d, J = 1.6 Hz, 1H), 6.24 (d, J = 1.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.1, 24.2, 24.4, 27.6, 28.5, 30.3, 31.3, 33.9, 34.2, 35.0, 42.0, 44.7, 48.7, 72.7, 76.9, 107.7, 109.1, 109.9, 142.3, 154.6, 155.0, 179.8. HRMS (MALDI-FTMS) calcd for $C_{22}H_{32}ClO_4$ (MH^+) 395.1984, found 395.1990.

6-(1-Hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6H-benzo[*c*]chromen-3-yl)hexanoic Acid (13, TCA). To a solution of KO^{t}Bu (44 mg, 0.391 mmol) in benzene (4 mL) at 4 °C under Ar was added 6-(9-chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoic acid (**12**, 52 mg, 0.112 mmol) in benzene (2 mL). After being stirred for 30 min, the mixture was warmed to 65 °C for 20 min, cooled, and bubbled with CO_2 for 30 min. The reaction mixture was poured into water and extracted two times with EtOAc. The organic extract was washed with saturated brine, dried over $MgSO_4$, and evaporated. The desired compound **13** (TCA) was obtained as a 1:1 mixture with **9** (TCC), in the form of a brown oil (41 mg, 96%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H), 1.30−1.43 (m, 4H), 1.41 (s, 3H), 1.50−1.70 (m, 5H), 1.67 (brs, 3H), 1.86−1.95 (m, 1H), 2.12−2.18 (m, 1H), 2.34 (t, J = 7.6 Hz, 2H), 2.44 (t, J = 7.6 Hz, 2H), 3.15−3.22 (m, 1H), 6.13 (brs, 1H), 6.24 (brs, 1H), 6.32 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.2, 23.3, 24.5, 25.0, 27.5, 28.5, 29.7, 30.4, 31.1, 33.6, 35.0, 45.7, 77.3, 107.6, 109.2, 109.9, 123.8, 134.2, 142.2, 154.3, 154.7, 180.0. HRMS (MALDI-FTMS) calcd for $C_{22}H_{30}O_4Na$ (MNa^+) 381.2036, found 381.2036.

3-[6-(9-Chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoylamino]propionic Acid *tert*-Butyl Ester (14). To a mixture of 6-(9-chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoic acid (**12**, 200 mg, 0.506 mmol), Et_3N (0.18 mL, 1.27 mmol), and HBTU (231 mg, 0.608 mmol) in DMF (6 mL) at room temperature under Ar was added H-β-alanine *tert*-butyl ester hydrochloride (114 mg, 0.608 mmol). After being stirred for 1 h, the reaction mixture was poured into water and extracted two times with Et_2O/EtOAc (1:1). The organic extract was washed with saturated

brine, dried over $MgSO_4$, and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:1), to afford **14** as a yellow oil (229 mg, 0.438 mmol, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.13 (s, 3H), 1.23−1.35 (m, 4H), 1.38 (s, 3H), 1.40−1.50 (m, 1H), 1.46 (s, 9H), 1.50−1.70 (m, 5H), 1.65 (s, 3H), 1.70−1.84 (m, 1H), 2.10−2.22 (m, 1H), 2.15 (t, J = 7.4 Hz, 2H), 2.40 (t, J = 7.8 Hz, 2H), 2.46 (t, J = 5.8 Hz, 2H), 3.55 (brd, J = 14 Hz, 1H), 6.14 (brs, 1H), 6.18 (brs, 1H), 6.18−6.25 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.1, 24.2, 25.5, 27.7, 28.1, 28.6, 30.4, 31.4, 34.2, 34.98, 35.04, 36.7, 42.1, 44.6, 48.7, 72.7, 76.7, 81.3, 107.7, 109.0, 109.3, 142.1, 154.9, 155.4, 172.3, 173.5.

3-[6-(1-Hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexanoylamino]propionic Acid (15, TCB). To a solution of 3-[6-(9-chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)-hexanoylamino]propionic acid *tert*-butyl ester (**14**, 200 mg, 0.383 mmol) in CH_2Cl_2 (1.7 mL) at 0 °C was added TFA (5 mL) which was precooled at 0 °C, and the mixture was stirred for 3 h at room temperature. The mixture was coevaporated with toluene (three times) to give 3-[6-(9-chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl)-hexanoylamino]propionic acid, which was used for the next reaction without further purification.

To a solution of KO^{t}Bu (71 mg, 0.630 mmol) in benzene (5 mL) at 4 °C under Ar was added 3-[6-(9-chloro-1-hydroxy-6,6,9-trimethyl-6a,7,8,9,10,10a-hexahydro-6*H*-benzo[*c*]chromen-3-yl-)hexanoylamino]propionic (58.7 mg, 0.126 mmol) in benzene (1 mL). After being stirred for 30 min, the mixture was warmed to 65 °C for 20 min, cooled, and bubbled with CO_2 for 30 min. The reaction mixture was poured into water and extracted two times with EtOAc. The organic extract was washed with saturated brine, dried over $MgSO_4$, and evaporated. The desired compound **15** (TCB) was obtained as a 1:1 mixture with **11** (TCD), as a brown oil (41 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H), 1.20−1.36 (m, 2H), 1.37 (s, 3H), 1.45−1.66 (m, 5H), 1.68 (s, 3H), 1.72−1.87 (m, 2H), 2.08−2.20 (m, 1H), 2.16 (t, J = 7.4 Hz, 2H), 2.39 (t, J = 7.4 Hz, 2H), 2.59 (t, J = 5.6 Hz, 2H), 2.64−2.75 (m, 1H), 3.27 (brd, J = 16.8 Hz, 1H), 3.45−3.60 (m, 2H), 5.41 (brd, J = 2.8 Hz, 1H), 6.12 (brs, 1H), 6.20 (brs, 1H), 6.39 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.9, 23.3, 24.6, 25.0, 27.5, 28.3, 29.2, 29.8, 30.6, 31.2, 33.9, 34.7, 35.8, 45.8, 77.4, 107.8, 109.1, 110.7, 123.5, 134.5, 141.9, 154.1, 154.4, 175.2. HRMS (MALDI-FTMS) calcd for $C_{25}H_{35}NO_4Na$ (MNa^+) 452.2407, found 452.2407.

2-(3,5-Dimethoxyphenyl)[1,3]dithiolane (16). To 2.8 g (16.8 mmol) of 3,5-dimethoxybenzaldehyde and 2.1 mL (25.2 mmol) of ethanedithiol in 80 mL of dry CH_2Cl_2 under N_2 was added 0.64 mL (5.05 mmol) of BF_3/Et_2O, and the mixture was stirred for 15 h. Then the reaction was quenched with saturated Na_2CO_3, and the appropriate layer was extracted with CH_2Cl_2 twice. The combined organic extracts were washed with brine and dried over $MgSO_4$. After concentration, the residue was purified by column chromatography on silica gel, eluting with EtOAc/hexane (1:9), to afford **16** as a yellow oil (4.09 g, 16.6 mmol, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.30−3.38 (m, 2H), 3.44−3.52 (m, 2H), 3.79 (s, 6H), 5.58 (s, 1H), 6.36 (t, J = 2.4 Hz, 1H), 6.69 (d, J = 2.4 Hz, 2H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$) δ 40.1, 55.3, 56.3, 100.0, 105.8, 142.7, 160.7 ppm. ESI-MS 259 (MH^+), 281 (MNa^+).

5-[1,3]Dithiolan-2-ylbenzene-1,3-diol (17). A solution of 2-(3,5-dimethoxyphenyl)[1,3]dithiolane (**16**, 6.4 g, 26.4 mmol) and BBr_3 (66 mL, 1 M solution in CH_2Cl_2) was stirred for 2 h at −78 °C, after which the mixture was allowed to warm to room temperature. After being stirred for 2 h, the mixture was cooled to 0 °C and BBr_3 (20 mL, 1 M solution in CH_2Cl_2) was added again. The mixture was allowed to warm to room temperature and stirred overnight. Then the mixture was poured into saturated $NaHCO_3$ and extracted four times with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:1), to give **17** as a brown oil (3.9 g, 18.2 mmol, 69%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.29−3.37 (m, 2H), 3.42−3.50 (m, 2H), 5.02 (brs, 2H, OH × 2), 5.51 (s, 1H, PhCH), 6.26 (t, J = 2.4 Hz, 1H, Ph), 6.59 (d, J = 2.4 Hz, 2H, Ph). GC−MS 214 (M^+).

5-[1,3]Dithiolan-2-yl-2-(4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-yl)benzene-1,3-diol (18). To a mixture of **17** (2.0 g, 9.40 mmol) and CSA (racemic) (281 mg, 0.94 mmol) in $CHCl_3$ (100 mL) at room temperature under Ar was added (*s*)-*cis*-verbenol (1.43 g, 9.40 mmol, 50% ee) in $CHCl_3$ (50 mL). After being stirred for 2 h, the reaction mixture was poured into saturated $NaHCO_3$ and extracted two times with CH_2Cl_2. The CH_2Cl_2 layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (2:8), to afford the desired diol **18** as a yellow oil (1.39 g, 4.00 mmol, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.95 (s, 3H, −Me), 1.35 (s, 3H, −Me), 1.45−1.48 (m, 2H), 1.85 (dd, J = 1.6 and 2.4 Hz, 3H), 2.15−2.20 (m, 1H), 2.22−2.34 (m, 2H), 3.28−3.36 (m, 2H), 3.42−3.50 (m, 2H), 3.89−3.93 (m, 1H), 5.49 (s, 1H), 5.68 (brs, 1H), 6.53 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.4, 23.7, 25.9, 27.8, 37.9, 40.0, 40.7, 46.8, 47.8, 55.6, 107.7, 114.8, 116.0, 140.3, 153.0, 155.1. HRMS (MALDI-FTMS) calcd for $C_{19}H_{25}O_2S_2$ (M^+ + H) 349.1296, found 349.1296.

3-[1,3]Dithiolan-2-yl-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (19). To a solution of diol **18** (1.0 g, 2.87 mmol) in CH_2Cl_2 (70 mL) at 0 °C under Ar was added BF_3/Et_2O (0.73 mL, 5.74 mmol). The mixture was allowed to warm to room temperature and stirred for 1 h, after which the mixture was poured into saturated $NaHCO_3$ and extracted two times with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and evaporated. The residue was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:9), to afford the desired target product **19** as a yellow oil (541 mg, 1.55 mmol, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H), 1.37 (s, 3H), 1.69 (brs, 3H), 1.71−1.88 (m, 4H), 2.07−2.18 (m, 1H), 2.69 (dt, J = 4.8 and 10.8 Hz, 1H), 3.14−3.30 (dd, J = 4.4 and 6.8 Hz, 1H), 3.25−3.35 (m, 2H), 3.40−3.49 (m, 2H), 5.08 (s, 1H), 5.42 (d, J = 4.0 Hz, 1H), 5.49 (s, 1H), 6.47 (d, J = 2.0 Hz, 1H), 6.56 (d, J = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5, 23.5, 27.5, 27.8, 31.6, 35.6, 30.96, 40.0, 44.6, 55.6, 77.3, 106.5, 109.8, 113.1, 119.2, 134.7, 140.0, 154.9, 155.1. HRMS (MALDI-FTMS) calcd for $C_{19}H_{25}O_2S_2$ (M^+ + H) 349.1296, found 349.1295.

Acetic Acid 3-[1,3]Dithiolan-2-yl-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-yl Ester (20). To a solution of **19** (510 mg, 1.55 mmol) in pyridine (4.0 mL) was added acetic anhydride (4.0 mL) at room temperature. After being stirred overnight, the reaction mixture was concentrated under reduced pressure. The residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/hexane (1:3), to afford the desired product **20** (456 mg, 1.17 mmol, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H), 1.37 (s, 3H), 1.68 (brs, 3H), 1.70−1.95 (m, 4H), 2.08−2.18 (m, 1H), 2.28 (s, 3H, Ac), 2.60 (dt, J = 4.8 Hz, 10.8 Hz, 1H), 2.71 (dd, J = 4.4 Hz, 16.8 Hz, 1H), 3.25−3.35 (m, 2H), 3.40−3.48 (m, 2H), 5.43 (brd, J = 3.0 Hz, 1H), 5.52 (s, 1H), 6.75 (d, J = 2.0 Hz, 1H), 6.88 (d, J = 2.0 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.5, 21.2, 23.5, 27.3, 27.6, 31.9, 35.8, 39.9, 40.0, 44.4, 55.3, 77.1, 113.6, 114.8, 118.5, 119.7, 133.7,140.6, 149.8, 154.7, 168.7. HRMS (MALDI-FTMS) calcd for $C_{21}H_{27}O_3S_2$ (M^+ + Na) 413.1215, found 413.1206.

3-[(1-Acetoxy-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromene-3-carbonyl)amino]propionic Acid Ethyl Ester (23). To a stirred solution of dithiolan **20** (794 mg, 2.03 mmol) in ethanol (50 mL) and water (5 mL) at room temperature was added $AgNO_3$ (1.04 g, 6.10 mmol). After 20 h, the reaction mixture was filtered through a pad of Celite. After the filtrate was dried and concentrated, the crude reaction mixture was used in the next reaction without purification. The crude aldehyde was dissolved in ^{t}BuOH (30 mL) and water (8 mL). Sodium dihydrogen phosphate (122 mg, 1.02 mmol), a 2 M THF solution of 2-methyl-2-butene (1.5 mL, 3.0 mmol), and sodium chlorite (188 mg, 2.03 mmol) were subsequently added at room temperature. The mixture was stirred for 5 h, and the reaction was then quenched with 1 N HCl. The aqueous organic phases were washed with brine,

dried over $MgSO_4$, filtered, and evaporated to dryness. The crude carboxylic acid was dissolved in DMF (20 mL). Triethylamine (0.4 mL, 2.84 mmol), β-alanine ethyl ester hydrochloride (217 mg, 1.42 mmol), and HBTU (539 mg, 1.42 mmol) were subsequently added. After the mixture was stirred overnight at room temperature, the reaction was quenched with water. The mixture was then extracted with Et_2O/EtOAc (1:1, two times), and the combined extracts were washed with water and then brine, dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting crude product was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:2 to 1:1), to afford **23** as a white amorphous solid (206 mg, 0.480 mmol, 24% yield in three steps). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.09 (s, 3H, Me), 1.27 (t, J = 7.2 Hz, Et), 1.39 (s, 3H, Me), 1.64 (s, 3H, Me), 1.55−1.95 (m, 3H), 2.09−2.24 (m, 1H), 2.31 (s, 3H, Ac), 2.60 (t, J = 6.0 Hz, 2H, CH_2CO_2), 2.60−2.68 (m, 1H), 2.69−2.77 (m, 1H), 3.68 (q, J = 6.0 Hz, 2H, NCH_2), 4.16 (q, J = 7.2 Hz, 2H, Et), 5.41−5.46 (m, 1H, olefinic), 6.75 (brt, J = 6.0 Hz, 1H, amide), 7.01 (d, J = 1.6 Hz, 1H, Ph), 7.07 (d, J = 1.6 Hz, 1H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.14, 18.43, 21.16, 23.50, 27.26, 27.58, 32.09, 33.90, 35.21, 35.71, 44.36, 60.80, 77.47, 113.18, 113.89, 119.75, 122.54, 133.62, 133.91, 150.11, 154.99, 166.05, 168.73, 172.83. HRMS (MALDI-FTMS) calcd for $C_{24}H_{32}NO_6$ (M^+ + H) 430.2224, found 430.2216.

3-[(1-Acetoxy-6,6,9-trimethyl-6*H*-benzo[*c*]chromene-3-carbonyl)amino]propionic Acid Ethyl Ester (24). The method of Tuchinda was employed. The olefinic compound (**23**, 79 mg, 0.184 mmol) was heated with sulfur (24 mg, 0.736 mmol) at 180−200 °C for 45 min. After the mixture was cooled to room temperature, concentration of the mixture and purification by preparative TLC (silica, eluant EtOAc/hexane, 4:6) gave the title compound **24** as a pale-yellow oil (38.3 mg, 0.09 mmol, 49%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.25 (t, J = 7.2 Hz, 3H, Et), 1.58 (s, 6H, Me × 2), 2.34 (s, 3H), 2.39 (s, 3H), 2.65 (t, J = 6.8 Hz, 2H, CH_2CO_2), 3.63 (t, J = 6.8 Hz, 2H, NH_2), 4.15 (q, J = 7.2 Hz, 2H, Et), 7.12 (brd, J = 8.0 Hz, 1H), 7.21 (d, J = 2.0 Hz, 1H), 7.26 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.87 (brs, 1H). HRMS (MALDI-FTMS) calcd for $C_{24}H_{28}NO_6$ (M^+ + H) 426.1911, found. 426.1910.

3-[(1-Hydroxy-6,6,9-trimethyl-6*H*-benzo[*c*]chromene-3-carbonyl)amino]propionic Acid (25, TCE). To a stirred solution of ethyl ester **24** (38.3 mg, 0.090 mmol) in THF (6 mL) and water (2 mL) was added lithium hydroxide monohydrate (11 mg, 0.270 mmol). After 2 h at 50 °C, the mixture was concentrated in vacuo. Purification of the residue by column chromatography on silica gel, eluting with MeOH/CH_2Cl_2 (3:7), gave the title product as a yellow crystalline solid (27 mg, 0.0753 mmol, 84%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.56 (s, 6H, Me × 2), 2.36 (s, 3H, Me), 2.54 (t, J = 6.8 Hz, 2H, CH_2CO_2), 3.60 (t, J = 6.8 Hz, 2H, NCH_2), 6.84−6.87 (m, 1H), 6.94−6.97 (m, 1H), 7.11 (brd, J = 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 8.43 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.0, 21.5, 27.1, 33.9, 35.1, 35.7, 60.9, 77.5, 107.9, 108.9, 114.2, 122.3, 127.0, 127.6, 128.5, 133.5, 136.8, 137.1, 154.7, 155.3, 167.6, 172.1, 172.5. HRMS (MALDI-FTMS) calcd for $C_{20}H_{22}NO_5$ (M^+ + Na) 378.1312, found 378.1311.

3-{3-[(1-Hydroxy-6,6,9-trimethyl-6*H*-benzo[*c*]chromene-3-carbonyl)amino]propionylamino}propionic Acid Ethyl Ester. The carboxylic acid (TCE, 27 mg, 0.0753 mmol) was dissolved in DMF (3.7 mL). Triethylamine (0.052 mL, 0.377 mmol), β-alanine ethyl ester hydrochloride (23 mg, 0.151 mmol), and HBTU (57 mg, 0.151 mmol) were subsequently added. After 3.5 h at room temperature, the reaction was quenched with water. The mixture was then extracted with Et_2O/EtOAc (1:1, two times), and combined organic phases were washed with water and then brine, dried over $MgSO_4$, filtered, and evaporated to dryness. The resulting crude was purified by chromatography on silica gel, eluting with EtOAc/hexane (1:2 to 1:1) to afford, in order of elution, the desired title ethyl ester as a yellow solid (21.6 mg, 0.0475 mmol, 63%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.19 (t, J = 7.2 Hz, 3H, Et), 1.57 (s, 6H, Me × 2), 2.37 (s, 3H, Me), 2.47−2.60 (m, 2H, CH_2 × 2), 3.50 (q, J = 6.0 Hz, 2H, NCH_2), 3.73 (q, J = 6.0 Hz, 2H, $PhCONCH_2$), 4.07 (q, J = 7.2 Hz, 2H, Et), 6.80 (t, J = 6.0 Hz, amide), 6.89 (d, J = 1.6 Hz, 1H), 7.09 (dd, J = 1.2 and 7.6 Hz, 1H), 7.12 (d, J = 7.6 Hz, 1H), 7.47 (t, J = 6.0 Hz, 1H, amide), 8.43 (brs, 1H), 9.37 (s, 1H, OH). HRMS (MALDI-FTMS) calcd for $C_{25}H_{30}N_2O_6$ (M^+ + H) 455.2177, found 455.2181.

3-{3-[(1-Hydroxy-6,6,9-trimethyl-6*H*-benzo[*c*]chromene-3-carbonyl)amino]propionylamino}propionic Acid (26, TCF). To a stirred solution of the ethyl ester (21.6 mg, 0.0475 mmol) in THF (4 mL) and water (1 mL) was added lithium hydroxide monohydrate (4.0 mg, 0.095 mmol). After being stirred for 2.5 h at 50 °C, the mixture was concentrated under reduced pressure. Purification of the residue by column chromatography on silica gel, eluting with MeOH/CH_2Cl_2 (2:8), gave the title product as a yellow crystalline solid (15.5 mg, 0.037 mmol, 78%). ^{1}H NMR (400 MHz, CD_3OD): δ 1.56 (s, 6H, Me × 2), 2.36 (s, 3H, Me), 2.39−2.51 (m, 2H), 3.45 (t, J = 7.2 Hz, 4H, CH_2CO_2, CH_2CON), 3.61 (t, J = 6.8 Hz, 4H, NCH_2 × 2), 6.83 (d, J = 2.0 Hz, 1H), 6.97 (d, J = 2.0 Hz, 1H), 7.11 (brd, J = 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 8.44 (brs, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 21.73, 27.66, 30.87, 36.91, 37.22, 37.70, 78.67, 109.14, 109.74, 123.63, 128.57, 128.96, 129.59, 135.68, 137.85, 138.59, 156.05, 156.99. HRMS (MALDI-FTMS) calcd for $C_{23}H_{27}N_2O_6$ (M^+ + H) 427.1864, found 427.1860.

6-Chloro-1-(3,5-dimethoxyphenyl)hexan-1-ol (27). To an oven-dried flask equipped with an addition funnel, condenser, and stirring bar were added Mg turnings (406.5 mg, 16.7 mmol) under Ar at room temperature. A solution of 1-bromo-5-chloropentane (2 mL, 15.2 mmol) in dry THF (20 mL) was added dropwise via the addition funnel (after the first several drops of the solution were added, the flask was heated with hot gun to reflux; the rest was added dropwise). The mixture was stirred at room temperature for 30 min, then heated to reflux for an additional 1 h. After the Grignard reagent was cooled to room temperature, it was transferred into another flask containing 3,5-dimethoxybenzaldehyde (1.486 g, 8.94 mmol) in dry THF (30 mL) via cannula at −78 °C under Ar over 30 min. The temperature was gradually raised to room temperature (30 min), and the mixture was stirred for 1 h. The reaction was quenched by dropwise addition of a saturated aqueous NH_4Cl solution (20 mL). The crude suspension was diluted with EtOAc (20 mL) and brine (10 mL) and then separated. The aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phases were dried over $MgSO_4$, and after filtration the solvent was removed in vacuo. The crude product was purified by column chromatography (silica, EtOAc/hexane 1:4 to 3:7) to give the product (**27**, 1.64 g, 67%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.49 (d, J = 2.23 Hz, 2H), 6.37 (t, J = 2.26 Hz, 1H), 4.60 (t, J = 6. 55 Hz, 1 H), 3.79 (s, 6 H), 3.51 (t, J = 6.67 Hz, 2 H), 1.76 (m, 4 H), 1.69 (m, 2 H), 1.45 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ160.89, 147.36, 103.72, 99.34, 74.56, 55.34, 45.02, 38.70, 32.48, 26.73, 25.07. HRMS (ESI-TOF high-acc) calcd $[M + H]^+$ for $C_{14}H_{21}ClO_3$ 273.1252, found 273.1259.

1-(6-Chlorohexyl)-3,5-dimethoxybenzene (28, Method B). To a flask containing 10% Pd/C (66.6 mg) was added glacial acetic acid (16 mL). The mixture was degassed, and the benzylic alcohol **27** (1.309 g, 4.8 mmol) was added. The mixture was stirred overnight under a H_2 atmosphere at room temperature. Upon completion the mixture was diluted with ether (30 mL) and water (10 mL) and the catalyst was removed by filtration through Celite. The organic layer was separated and diluted with water (10 mL) and neutralized by portionwise addition of solid $NaHCO_3$. The aqueous phase was extracted with ether (3 × 20 mL). The organic layers were dried over $MgSO_4$, and after filtration the solvent was removed in vacuo. The residue was purified by column chromatography (silica, EtOAc/hexane 1:100 to 1:4) to give product **28** as a light-yellow oil (666 mg, 54% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.41 (d, J = 2.20 Hz, 2 H), 6.37 (t, J = 2.19 Hz, 1 H), 3.81 (s, 6 H), 3.55 (t, J = 6.78 Hz, 2 H), 2.61 (t, J = 7.69 Hz, 2 H), 1.80 (quint, J = 7.08 Hz, 2 H), 1.68 (quint, J = 7.57 Hz, 2 H), 1.50 (quint, J = 7.41 Hz, 2 H), 1.40 (quint, J = 7.50 Hz, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.48, 144.62, 106.12, 97.27,

54.74, 44.70, 35.83, 32.31, 30.79, 28.25, 26.47. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{14}H_{21}ClO_2$ 257.1303, found 257.1288.

5-(6-Chlorohexyl)benzene-1,3-diol (29). To a solution of the chloride (**28**, 565.2 mg, 2.2 mmol) in dry CH_2Cl_2 (13 mL) at −78 °C was added dropwise BBr_3 (1 M in CH_2Cl_2, 6.6 mL, 6.6 mmol) over 15 min, after which the mixture was warmed to room temperature during 35 min. The mixture was stirred for 3 h at room temperature. The reaction was quenched with a saturated aqueous $NaHCO_3$ solution and extracted with DCM (3 × 20 mL). The organic layers were dried over $MgSO_4$ and filtered. The solvent was removed under reduced pressure. The residue was purified by column chromatography (silica, EtOAc/hexane 1:4 to 2:3) to give diol **29** as a colorless oil (424 mg, 84%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.26 (d, J = 1.81 Hz, 2 H), 6.19 (broad, 1 H), 3.53 (t, J = 6.69 Hz, 2 H), 2.49 (t, J = 7.68 Hz, 2 H), 1.76 (quint, J = 7.12 Hz, 2 H), 1.58 (quint, J = 7.63 Hz, 2 H), 1.45 (quint, J = 7.56 Hz, 2 H), 1.34 (m, 2 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.48, 145.83, 108.10, 100.27, 45.15, 35.59, 32.47, 30.74, 28.37, 26.64. HRMS (ESI-TOF high-acc): calcd $[M - H]^-$ for $C_{12}H_{17}ClO_2$ 227.0844, found 227.0844.

4-Isopropenyl-1-methylcyclohex-2-enol (30). To a solution of PhSeSePh (5.49 g, 17.59 mmol) in dry EtOH (17 mL) was added $NaBH_4$ (1.32 g, 34.86 mmol) under Ar. The mixture was stirred, and after it turned colorless, a solution of (+)-limonene oxide (2.72 mL, 16.60 mmol) in EtOH (6.5 mL) was added dropwise. The mixture was stirred and heated to reflux for 2 h under Ar, after which the reaction was quenched with 1 M HCl and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic extract was washed with a saturated $NaHCO_3$ solution, water, and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was dissolved in THF (200 mL), and 35% H_2O_2 (14.22 mL, 166.0 mmol) was added dropwise at 0 °C. The mixture was stirred at room temperature for 1 h and then heated to reflux for 2 h. The mixture was stirred overnight at room temperature. The reaction was quenched with water, and the aqueous layer was extracted with EtOAc (3 × 200 mL). The organic extract was washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified over silica gel, eluting with EtOAc/hexane (1:10 to 2:3) to provide **30** as a light-yellow oil (1.30 g, 52%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.71 (ddd, J = 10.01, 2.26, 1.28 Hz, 1 H), 5.65 (ddd, J = 10.01, 2.28, 0.97 Hz, 1 H), 4.78 (m, 1 H), 4.75 (m, 1 H), 2.66 (m, 1 H), 1.75−1.85 (m, 2 H), 1.74 (m, 3 H), 1.58−1.65 (m, 2 H), 1.29 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 148.14, 133.99, 132.14, 110.59, 67.43, 43.49, 36.78, 29.44, 24.92, 20.81. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{10}H_{16}O$ 153.1274, found 153.1269.

3-(6-Chlorohexyl)-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (31). A suspension of chloride **29** (248 mg, 1.08 mmol), dienol **30** (181.43 mg, 1.192 mmol), and anhydrous $MgSO_4$ (812.2 mg) in dry CH_2Cl_2 (14 mL) was cooled to −3 °C, and BF_3/Et_2O (68.3 μL, 0.539 mmol) was added dropwise under Ar. The mixture was stirred for 2.5 h at 0 °C, and then 1.8 g of anhydrous $NaHCO_3$ was added. The mixture was warmed to room temperature, stirred vigorously for 30 min, and filtered through Florisil, after which the filtrate was evaporated under reduced pressure. The residue was purified with flash chromatography (silica gel, Et_2O/hexane 0:100 to 1:9) to give product **31** (164 mg, 42%) as a light-yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.30 (m, 1 H), 6.27 (m, 1 H), 6.14 (d, J = 1.45 Hz, 1H), 4.76 (b, 1H), 3.53 (t, J = 6.79 Hz, 2 H), 3.21 (d, J = 11.94 Hz, 1 H), 2.46 (t, J = 7.70 Hz, 2 H), 2.18 (m, 2 H), 1.93 (m, 1 H), 1.77 (m, 2 H), 1.69 (m, 4 H), 1.58 (m, 2 H), 1.42 (m, 6 H), 1.34 (m, 2 H), 1.09 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.82, 154.18, 142.41, 134.48, 123.63, 110.08, 109.13, 107.49, 45.78, 45.12, 35.30, 33.55, 32.53, 31.15, 30.69, 28.44, 27.56, 26.72, 25.00, 23.35, 19.26. HRMS (ESI-TOF high-acc): calcd $[M - H]^-$ for $C_{22}H_{31}ClO_2$ 361.194, found 361.1926.

3-(6-Azidohexyl)-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (32). A suspension of chloride **31** (29.3 mg, 0.08 mmol) in DMF (27 mL) was treated with NaN_3 (7.8 mg, 0.12 mmol) and a catalytic amount of NaI (0.4 mg) under Ar at room temperature. The mixture was then warmed to 66 °C and stirred for 12 h. The mixture was then diluted with CH_2Cl_2 (20 mL), after which the solid was removed by filtration and the solvent was evaporated in vacuo. The residue was filtered through a short pad of silica gel with ether as eluent. The crude product was purified with column chromatography (silica, Et_2O/hexane 1:4) to give product **32** as a colorless oil (27.3 mg, 92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.30 (m, 1 H), 6.27 (d, J = 1.34 Hz, 1 H), 6.14 (d, J = 1.40 Hz, 1 H), 4.78 (s, 1 H, OH), 3.25 (t, J = 6.94 Hz, 2 H), 3.19−3.21 (m, 1 H), 2.45 (t, J = 7.73 Hz, 2 H), 2.15−2.19 (m, 2 H), 1.89−1.95 (m, 1 H), 1.68−1.72 (m, 4 H), 1.53−1.62 (m, 4 H), 1.31−1.42 (m, 8 H), 1.10 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.80, 154.18, 142.38, 134.48, 123.61, 110.06, 109.13, 107.49, 51.43, 45.76, 35.29, 33.53, 31.14, 30.70, 28.72, 28.69, 27.55, 26.55, 24.99, 23.37, 19.25. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{22}H_{31}N_3O_2$ 370.2489, found 370.2478.

3-(6-Aminohexyl)-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (33). The azide (**32**, 25.3 mg, 0.068 mmol) was treated with polymer supported Ph_3P (27.3 mg, 3 mmol/g, 0.082 mmol) in THF/H_2O (10:1.5, 0.1 mL) at room temperature, and the mixture was shaken overnight. The resin was filtered out and washed sequentially by MeOH (2 × 2 mL), CH_2Cl_2 (2 × 2 mL), and THF (2 × 2 mL), and the organic mixture was dried over Na_2SO_4. After filtration the solvent was removed under reduced pressure. The crude product (24.0 mg) was not further purified for the next step. LC−MS analysis (HP-1100, using a flow rate of 0.75 mL/min in a gradient of 25−99% acetonitrile in water (0.5% formic acid) in 8 min) showed only one peak at 3.983 min, $[M + H]^+$: 344.2. ^{1}H NMR (CD_3OD, 500 MHz): δ 6.38 (br s, 1 H), 6.14 (d, J = 1.42 Hz, 1 H), 6.04 (d, J = 1.48 Hz, 1 H), 3.12 (d, J = 10.88 Hz, 1 H), 2.60 (t, J = 7.11 Hz, 2 H), 2.38 (t, J = 7.59 Hz, 2 H), 2.11 (m, 2 H), 1.86−1.94 (m, 1 H), 1.60−1.70 (m, 4 H), 1.50−1.59 (m, 4 H), 1.40−1.46 (m, 2 H), 1.28−1.36 (m, 6 H), 1.01 (s, 3 H).

***N*-[6-(1-Hydroxy-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl)hexyl]acetamide (34a and 34b).** The amine (**33**, 20.3 mg, 0.059 mmol) was treated with polymer supported pyridine (5.48 mmol/g, 29.0 mg, 0.159 mmol) in CH_2Cl_2 (0.1 mL) and shaken for 30 min. Then Ac_2O (8.9 uL, 0.094 mmol) was added and shaking was continued for 4 h. The resin was removed by filtration and washed with CH_2Cl_2 (2 × 2 mL), MeOH (2 × 2 mL), and CH_2Cl_2 (2 × 2 mL) sequentially. The solution was concentrated in vacuo, and the residue was purified using PTLC, eluting with EtOAc/hexane (1:4) to give product **34a** (20.1 mg, 88% for two steps). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.37 (br, 1 H), 6.22 (br, 1 H), 6.19 (d, J = 1.31 Hz, 1 H), 5.53 (br, 1 H), 3.18−3.30 (m, 3 H), 2.45 (dt, J = 7.78, 2.43 Hz, 2 H), 2.13−2.20 (m, 2 H), 2.00 (s, 3 H), 1.86−1.96 (m, 1 H), 1.62−1.73 (m, 6 H), 1.43−1.50 (m, 4 H), 1.41 (s, 3 H), 1.30−1.36 (m, 2 H), 1.09 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.38, 154.79, 154.70, 142.09, 133.97, 123.98, 109.66, 109.16, 107.51, 45.79, 39.46, 34.78, 33.64, 31.18, 30.11, 29.21, 28.07, 27.58, 26.23, 25.03, 23.39, 23.36, 19.27. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{24}H_{35}NO_3$ 386.2690, found 386.2691.

The isotopic analogue **34b** was synthesized the same way with use of tritium-labeled Ac_2O (47.5 mCi in toluene), providing the product (13.4 mg, 80%) with low radiochemical yield (0.347 mCi, 0.73%).

1-(3,5-Dimethoxyphenyl)-5-phenoxypentan-1-ol (35). To a flame-dried flask containing Mg (530.5 mg, 21.82 mmol) was added THF (22 mL), and a solution of 4-phenoxybutyl bromide (5.257 g, 21.82 mmol) was added dropwise while the suspension was kept mildly refluxing for 20 min. Then the mixture was heated to reflux for 1 h. After cooling to room temperature, the solution of Grignard reagent was transferred to a solution of 3,5-dimethoxybenzaldehyde (2.133 g, 12.84 mmol) in THF (43 mL) at −78 °C during 30 min. The mixture was then warmed to room temperature and stirred for 1 h. The reaction was quenched by dropwise addition of saturated aqueous NH_4Cl (20 mL), and the mixture was diluted with EtOAc (40 mL) and brine (20 mL). The organic layer was separated, and the aqueous phase was extracted with EtOAc

(3 × 50 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified (silica gel, EtOAc/hexane 1:4 to 2:3) to give **35** (3.77 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.26−7.31 (m, 2 H), 6.94 (t, J = 7.34 Hz, 1 H), 6.89 (dd, J = 8.66, 0.87 Hz, 2 H), 6.52 (d, J = 2.27 Hz, 2 H), 6.39 (t, J = 2.28 Hz, 1 H), 4.65 (dd, J = 7.43, 5.63 Hz, 1 H), 3.96 (t, J = 6.46 Hz, 2 H), 3.80 (s, 6 H), 1.72−1.91 (m, 5 H), 1.63 (m, 1 H), 1.50 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.85, 158.95, 147.35, 129.38, 120.49, 114.43, 103.71, 99.37, 67.57, 55.31, 38.60, 29.12, 22.40. HRMS (MALDI-FTMS high-acc): calcd $[M + Na]^+$ for $C_{19}H_{24}O_4$ 339.1567, found 339.1566.

1-(3,5-Dimethoxyphenyl)-5-phenoxypentane (36). TMSCl (3.33 mL, 26.22 mmol) and NaI (3.943 g, 26.30 mmol) in MeCN (1.8 mL) were stirred at room temperature for 15 min. To this mixture was added a solution of compound **35** (1.3813 g, 4.37 mmol) in Et_2O/hexane (3 mL /2.3 mL), and the mixture was stirred at room temperature for 24 h under Ar. The reaction was quenched with H_2O, and the appropriate layer was extracted with Et_2O (3 × 10 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_3$ solution and brine. The solution was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the reduced product **36** (1.16 g, 88%) as a light-yellow oil. The product was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.29 (d, J = 8.69 Hz, 1 H), 7.28 (d, J = 8.56 Hz, 1 H), 6.92 (m, 3 H), 6.36 (d, J = 2.23 Hz, 2 H), 6.31 (t, J = 2.25 Hz, 1 H), 3.96 (t, J = 6.52 Hz, 2 H), 3.79 (s, 6 H), 2.60 (t, J = 7.69 Hz, 2 H), 1.83 (quint, J = 6.64 Hz, 2 H), 1.70 (quint, J = 7.65 Hz, 2 H), 1.52 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 160.70, 159.04, 144.96, 129.38, 120.47, 114.47, 106.47, 97.64, 67.70, 55.23, 36.19, 31.01, 29.17, 25.76. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{19}H_{24}O_3$: 301.1798, found 301.1793.

5-(5-Bromopentyl)benzene-1,3-diol (37). To a solution of phenoxyether **36** (835.1 mg, 2.78 mmol) in CH_2Cl_2 (6 mL) at −78 °C was added 13.9 mL of a 1 M BBr_3 solution in CH_2Cl_2. The mixture was slowly warmed to room temperature and stirred for 36 h. The reaction was quenched by careful addition of a saturated aqueous $NaHCO_3$ solution at 0 °C. The mixture was extracted with ether (3 × 30 mL), and the combined organic layers were washed with saturated aqueous $NaHCO_3$ solution (3 × 20 mL), followed by brine (3 × 20 mL), then dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, EtOAc/hexane 1:4 to 2:3) to give product **37** (1.236 g, 80%) as a light-brown oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.27 (d, J = 1.9 Hz, 2 H), 6.21 (s, 1 H), 3.36 (t, J = 6.8 Hz, 2 H), 2.43 (t, J = 7.6 Hz, 2 H), 1.81 (quint, J = 7.13 Hz, 2 H), 1.52 (quint, J = 7.54 Hz, 2 H), 1.39 (quint, J = 7.32,2 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.11, 145.68, 108.20, 100.39, 35.43, 33.98, 32.50, 29.97, 27.67. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{11}H_{15}BrO_2$ 259.0328, found 259.0327.

3-(5-Bromopentyl)-6,6,9-trimethyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (38). A suspension of bromide **37** (120 mg, 0.463 mmol), dienol **30** (77 mg, 0.51 mmol), and anhydrous $MgSO_4$ (347.3 mg) in dry CH_2Cl_2 (6 mL) was cooled to −3 °C, and BF_3/Et_2O (28.5 μL, 0.062 mmol) was added dropwise under Ar. The mixture was stirred for 2.5 h at 0 °C and then 150 mg of anhydrous $NaHCO_3$ was added. The mixture was warmed to room temperature, stirred vigorously for 30 min, and filtered through Florisil. The filtrate was evaporated under reduced pressure, and the residue was purified by flash chromatography (silica gel, Et_2O/hexane 0:100 to 10:100) to give bromide **38** (68.3 mg, 37.5%) as a light-yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.29 (m, 1 H), 6.26 (d, J = 1.51 Hz, 1 H), 6.14 (d, J = 1.53 Hz, 1 H), 4.76 (s, 1 H), 3.40 (t, J = 6.86 Hz, 2 H), 3.20 (m, 1 H), 2.47 (m, 2 H), 2.17 (m, 2 H), 1.85−1.92 (m, 4 H), 1.69 (m, 3 H), 1.57 (s, 3 H), 1.44−1.48 (m, 2 H), 1.42 (s, 3 H), 1.11 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.85, 154.20, 142.12, 134.54, 123.56, 110.05, 109.20, 107.48, 45.76, 35.21, 33.81, 33.53, 32.70, 31.14, 30.06, 27.79, 27.56, 24.99, 23.38, 19.26. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{21}H_{29}BrO_2$ 393.1424, found 393.1417.

6,6,9-Trimethyl-3-pentyl-6a,7,8,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (Δ^9-THC, 39a). To a solution of bromide **38** (4.7 mg, 0.015 mmol) in dry THF (0.5 mL) was added dropwise LAH (1 M in THF, 30 μL) at room temperature under Ar. The mixture was stirred for 1 h. The reaction was monitored by TLC and quenched with EtOAc (1 mL) and aqueous NaOH solution (0.5 mL, 1 M). The mixture was filtered through a short pad of Celite and anhydrous Na_2SO_4, and the filtrate was concentrated. The residue was purified by PTLC (EtOAc/hexane 1:4) to give Δ^9-THC (**39a**, 2.2 mg, 47%). ^{1}H NMR ($CDCl_3$, 600 MHz): δ 6.31 (m, 1 H), 6.28 (m, 1 H), 6.15 (m, 1 H), 4.73 (s, 1 H), 3.21 (d, J = 3.21 Hz, 1 H), 2.46 (m, 2 H), 2.18 (d, J = 5.14 Hz, 2 H), 1.92 (m, 1 H), 1.70−1.77 (m, 2 H), 1.69 (s, 3 H), 1.58 (m, 5 H), 1.40−1.48 (m, 2 H), 1.25−1.35 (m, 2 H), 1.10 (s, 3 H), 0.89 (t, J = 6.53 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.76, 154.11, 152.90, 142.81, 134.44, 123.65, 110.08, 107.49, 45.76, 35.45, 33.53, 31.49, 30.65, 27.56, 24.99, 23.37, 22.53, 19.25, 14.01. HRMS (ESI-TOF high-acc): calcd $[M + H]^+$ for $C_{21}H_{30}O_2$ 315.2318, found 315.2315.

39b. The tritium-labeled analogue **39b** was prepared using the following procedures.

(a) By LAH. Bromide **38** (1.64 mg, 4.16 μmol) in dry THF was treated with regular LAH (1.0 M in THF, 4.2 μL) and stirred for 30 min. Then tritium-labeled LAH (80 Ci/mmol, 100 mCi) was added and stirring was continued at room temperature for 1 h. Regular LAH (1.0 M in THF, 1.0 μL, 1.0 μmol) was added, and the mixture was stirred for 10 min. After workup and preparative thin-layer chromatography (PTLC) (EtOAc/hexane 1:9), product **39b** was obtained (0.65 mg, 2.08 μmol, 50% yield, 0.23 Ci/mmol, 0.48 mCi, radiochemical yield 1.9%).

(b) By $NaBH_4$. Bromo-THC (1.01 mg, 2.57 μmol) in DMSO was treated with tritium-labeled $NaBH_4$ (100 mCi, 80 Ci/mmol) at room temperature, and the mixture was stirred for 2.5 h, after which regular $NaBH_4$ (0.1 mg, 2.64 μmol) was added to complete the reaction. The mixture was extracted with with 1:4 ether/hexane (×3) and filtered through a short pad of silica gel (EtOAc/hexane 1:9). The solvent was removed in vacuo to give product **39b** (one spot on TLC, 0.65 mg, 2.056 μmol, 80% yield, 8.0 mCi, 4 Ci/mmol, radiochemical yield 32%).

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