Neurosteroid Analogues. 11. Alternative Ring System Scaffolds: γ-Aminobutyric Acid Receptor Modulation and Anesthetic Actions of Benz[*f*]indenes

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Benz[*f*]indenes are tricyclic compounds with a linear 6-6-5 fused carbocyclic ring system. When properly substituted, benz[*f*]indenes can satisfy the pharmacophore requirements of the critical hydrogen-bond donor and acceptor groups found in neuroactive steroids that modulate γ-aminobutyric acid$_A$ ($GABA_A$) receptor function. Thus, the benz[*f*]indene ring system provides an opportunity to extend the previously well-studied $GABA_A$ receptor structure−activity relationships (SAR) of neuroactive steroids to a different ring system. Depending on whether the stereochemistry of the 6-6-5 ring fusions are trans−trans or cis−trans, either planar or nonplanar benz[*f*]indenes are obtained. We found that the planar trans−trans benz[*f*]indenes are active, but less active than the steroids they were designed to mimic, whereas the nonplanar cis−trans compounds have little, if any, activity. The results provide new insight into the importance of the steroid framework for the actions of neuroactive steroids at $GABA_A$ receptors.

Introduction

In 1941 Hans Selye observed that progesterone induced general anesthesia in rats.[1] Subsequently, it was discovered that allopregnanolone, pregnanolone, and other structurally related 3α-hydroxysteroids are potent anesthetics.[2−6] It is now widely accepted that anesthetic steroids mediate their effects by enhancing the actions of γ-aminobutyric acid (GABA) at $GABA_A$ receptors. Anesthetic steroids modulate a variety of receptor subtypes composed of α, β, γ, and δ subunit isoforms.[7,8] Determination of the location and number of binding sites for steroids on the receptor has been difficult. However, a preliminary report of the structural determinants of a steroid binding site has been recently published.[9]

Numerous structure−activity relationship (SAR) studies of anesthetic steroids at $GABA_A$ receptors have established a pharmacophore for positive modulation of the receptor by steroids. The pharmacophore consists of a hydrogen-bond-accepting group such as acetyl or carbonitrile in a pseudoequatorial configuration at the 17β position and a hydrogen-bond-donating hydroxyl group in the axial 3α configuration (Figure 1).[10−15] It appears that the orientation between these groups is more important than the distance between these groups.[16] It is unclear whether the steroid ring system serves any role other than that of a hydrophobic spacer.

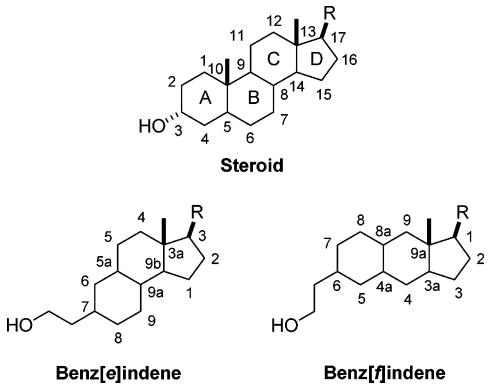

Figure 1. Structure and numbering of the steroid, benz[*e*]indene, and benz[*f*]indene ring systems.

To probe the importance of the steric constraint imposed on the 3-hydroxyl group by the steroid A ring for GABAergic activity, SAR studies of a series of benz[*e*]indene (Figure 1), analogues of pregnanolone (5β-H) and allopregnanolone (5α-H), were performed. Those studies demonstrated that GABAergic activity does not require the steroid 3α-hydroxyl group to be maintained in this position by the rigid steroid A ring.[17−21] These benz[*e*]indenes are nearly as potent as their steroidal counterparts in their enhancement of GABA-mediated currents. However, because the hydrophobic framework of the steroids and benz[*e*]indenes is so similar, little additional information regarding the SAR of the hydrophobic framework is provided by the benz[*e*]indene analogues.

To address further the SAR of the hydrophobic framework of anesthetic steroids, we have synthesized a series of benz[*f*]-indenes (Figure 1 and Chart 1). Depending on which of the two 6*H*-benz[*f*]inden-6-ones (**4a** or **4b**, Scheme 1) was used as the starting material,[22] two types of benz[*f*]indene analogues, trans−trans and cis−trans, with very different shapes were prepared (Figure 2). The trans−trans compounds are planar like 5α-reduced steroids, and even though the ring systems con-

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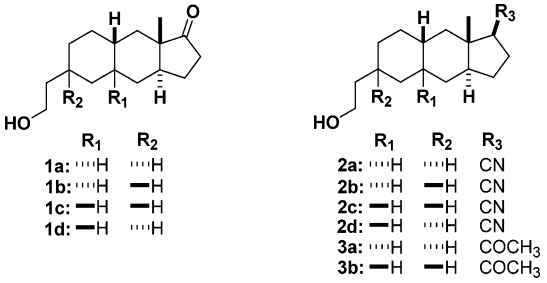
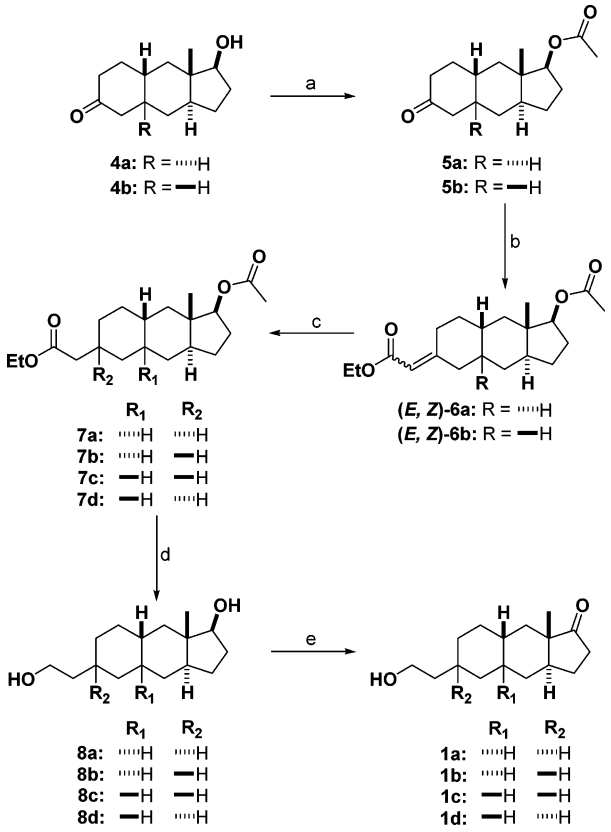
Chart 1



Scheme 1[a]



[a] (a) Ac_2O, pyridine; (b) $Ph_3P{=}CHCOOEt$, 160 °C; (c) Pd−$BaSO_4$, H_2; (d) $LiAlH_4$, THF, reflux; (e) NaOCl, AcOH.

necting the hydrogen-bonding groups within each molecule are different, these functional groups can be similarly located in three-dimensional space as demonstrated in Figure 3A. However, the hydrophobic contacts that large portions of the steroid A and B rings could have with the receptor are missing in the benz[*f*]indene and are replaced by a hydrophobic contact in the region that would be occupied by a two-carbon bridge between steroid carbons 1 and 11.

The cis−trans benz[*f*]indenes are compared to 5β-reduced steroids in Figure 3B. Once again, the geometrical relationships between the hydrogen-bonding groups within each molecule can be very similar. Hydrophobic contacts with the receptor that could be made by the entire steroid A and B rings are absent for the cis−trans benz[*f*]indene and are replaced by new hydrophobic contacts located below the plane of the first ring of the corresponding trans−trans benz[*f*]indene shown in Figure 3A.

In this paper we describe the preparation of the optically pure benz[*f*]indenes from the previously described enantiomerically pure 6*H*-benz[*f*]inden-6-ones (**4a** and **4b**). The activity of the benz[*f*]indenes at $GABA_A$ receptors was evaluated electrophysiologically in *Xenopus laevis* oocytes expressing rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors. The ability of the compounds to bind to $GABA_A$ receptors was measured by the noncompetitive displacement of [^{35}S]-TBPS from the picrotoxin binding site on $GABA_A$ receptors in rat brain membranes. Finally, the anesthetic effects of the compounds were determined by measuring the *X. laevis* tadpole loss of righting reflex (LRR) and loss of swimming reflex (LSR).

We have found that analogues having the trans−trans benz[*f*]indene system are positive $GABA_A$ receptor modulators that have somewhat diminished pharmacological activity in the

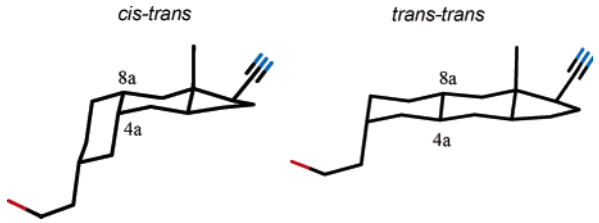


Figure 2. Illustration of how the stereochemistry of the 4a,8a ring fusion affects the shape of the benz[*f*]indene analogues. A cis fusion gives a compound (**2d**) in which there is a bend between the first and second ring, while a trans fusion yields a planar molecule (**2b**). The hydroxyethyl side chain is in the equatorial configuration in the cis−trans ring system and in the axial configuration in the trans−trans ring system. Multiple conformations of the hydroxyethyl side chain are possible. The conformations displayed position the oxygen and nitrogen atoms 11.3 Å from each other in compound **2d** and 10.8 Å apart in compound **2b**. The corresponding distances between these atoms in steroids **14b** and **14a** (see Chart 2 for structures) are 10.6 and 11.1 Å, respectively.

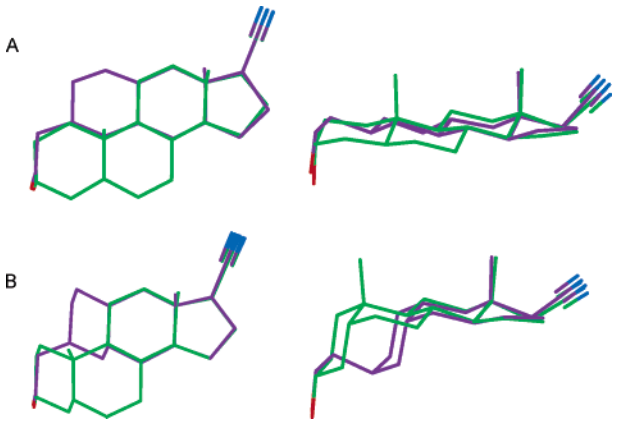


Figure 3. (A) Overlay (top view, left; edge view, right) of trans−trans benz[*f*]indene **2a** (purple) and 5α-reduced steroid **14a** (green). When the structures are compared in this manner, the first six-membered ring of the benz[*f*]indene occupies space that would be occupied by a two-carbon bridge between steroid carbons 1 and 11. Additionally, interactions that the steroid A,B rings (carbons C-4 through C-7) would have with the receptor are not present in the benz[*f*]indene. (B) Overlay (top view, left; edge view, right) of cis−trans benz[*f*]indene **2c** (purple) and 5β-reduced steroid **14b** (green). As aligned, the first ring of these benz[*f*]indenes occupies a different region of space below the plane of the steroid B,C,D rings than the A ring of a 5β-reduced steroid. With the exception of one carbon−carbon bond, there is no overlap between the first benz[*f*]indene ring and the steroid A,B rings. Note that for either the trans−trans or cis−trans benz[*f*]indene the distance and orientation between the hydrogen-bond-donating (OH) and -accepting (CN) groups can be closely mimicked by the flexible hydroxyethyl side chain. Also note that the configuration of the side chain shown in the trans−trans compound is equatorial, whereas it is axial in the cis−trans compound. The distance constraints of the pharmacophore hydrogen-bonding groups can also be maintained when the hydroxyethyl side chain has the opposite configuration in the trans−trans and cis−trans compounds (see Figure 2).

electrophysiological, binding, and tadpole anesthesia assays. In contrast, analogues having the cis−trans benz[*f*]indene system are essentially inactive. In comparative studies with steroids and benz[*e*]indenes, the rank order for positive modulation of $GABA_A$ receptors by the active benz[*f*]indenes was steroids > benz[*e*]indenes > benz[*f*]indenes. The results indicate that the geometrical features of the steroid ring system are an important part of the pharmacophore for neurosteroid action at $GABA_A$ receptors.

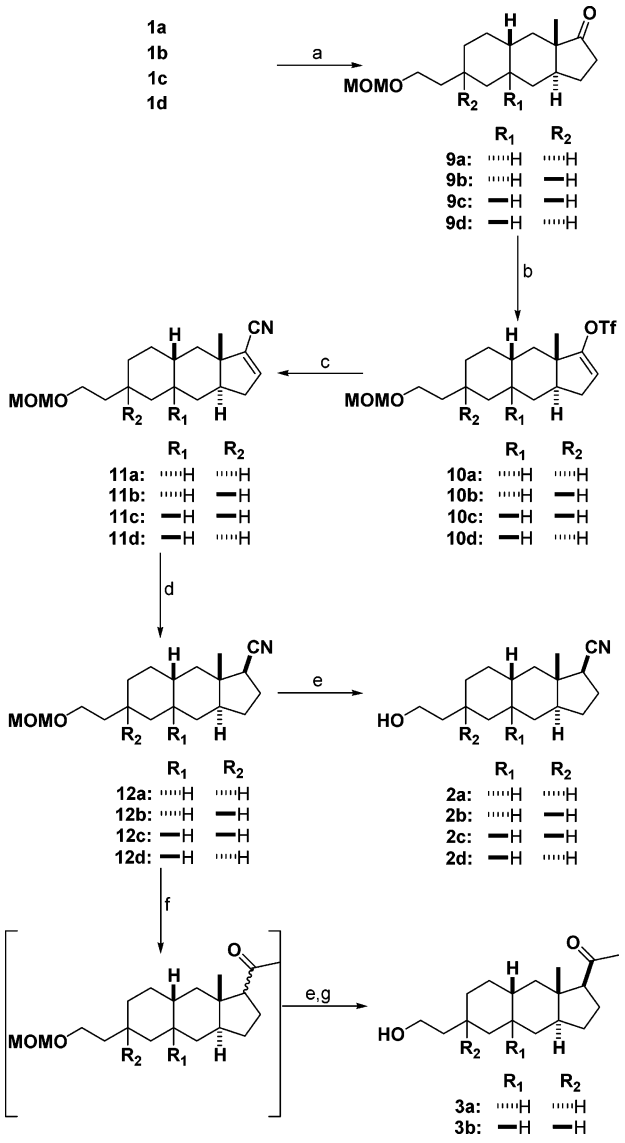
Chemistry. Synthesis of the benz[*f*]indene analogues began by protection of the hydroxyl group of the previously reported 6*H*-benz[*f*]inden-6-ones **4a** (trans−trans) and **4b** (cis−trans) as the acetates **5a** and **5b** in 93% and 78% yield, respectively (Scheme 1). Carbethoxyolefination of ketone **5a** provided a 1:1 isomeric mixture of products (*E*)-**6a** and (*Z*)-**6a** in 86% yield. The same reaction performed with ketone **5b** gave a 1:1 isomeric mixture of products (*E*)-**6b** and (*Z*)-**6b** in 91% yield. For characterization purposes, a portion of both isomeric mixtures was separated by HPLC on a silica gel column to obtain pure compounds (*E*)-**6a**, (*Z*)-**6a**, (*E*)-**6b**, and (*Z*)-**6b**.

The *E* and *Z* configurations of **6a** and **6b** were assigned by a combination of proton two-dimensional (2D) total correlation spectroscopy (TOCSY), 2D nuclear Overhauser effect spectroscopy (NOESY), and natural-abundance carbon ^{13}C heteronuclear single-quantum coherence (HSQC) NMR experiments. All protons in the compounds were assigned by 2D-TOCSY and natural-abundance carbon ^{13}C-HSQC. NOESY spectra were used to determine the NOE build-up between the vinyl proton resonance at δ 5.6 and other protons in the attached ring. The *Z* isomers gave NOE peaks between the vinyl proton and ring protons at positions C-7 and C-8. The *E* isomers gave NOE peaks between the vinyl proton and ring protons at positions C-5 and C-4a.

For the trans−trans system, catalytic hydrogenation of unseparated compounds (*E*/*Z*)-**6a** produced a ∼2.5:1 mixture of products **7a** and **7b** (93%). A portion of this mixture was separated by HPLC on a silica gel column for characterization purposes. Reduction of a mixture of stereoisomers **7a** and **7b** with lithium aluminum hydride (LAH) afforded a mixture of diols **8a** and **8b** (79%). This mixture of diols was selectively oxidized with a 5.25% aqueous solution of NaOCl in AcOH to yield the hydroxy ketones **1a** and **1b** (79%). A portion of this mixture was separated via HPLC for biological evaluation and to be carried forward for characterization of the remaining compounds in the synthetic scheme. Additionally, a portion of the isomerically pure compounds **1a** or **1b** was reduced back to diols **8a** and **8b** with $NaBH_4$ for characterization of the diols. A crystal structure of diol **8a** confirmed that the major isomer in the trans−trans series had an equatorial hydroxyethyl side chain. The majority of the **1a**, **1b** product was carried forward as a mixture and later separated by HPLC as the carbonitriles **2a** and **2b** (cf. Scheme 2.)

For the cis−trans system, catalytic hydrogenation of the remaining (*E*/*Z*)-**6b** compounds produced a ∼3.5:1 mixture of products **7c** and **7d** (91%). Separation of these diastereomers was unsuccessful by silica gel chromatography (column or HPLC). Reduction of a mixture of compounds **7c** and **7d** with LAH gave a mixture of diols **8c** and **8d** (95%). As with the trans−trans system, this mixture of diols was selectively oxidized with a 5.25% aqueous solution of NaOCl in AcOH to yield the hydroxy ketones **1c** and **1d** (85%). All of the isomeric mixture was separated via preparative HPLC (a small amount of the mixture was carried forward in the synthesis and the hydroxy ketones were found to be the easiest compounds to separate). A crystal structure of compound **1c** confirmed that the major isomer in the cis−trans series had an axial hydroxyethyl side chain. A portion of the isomerically pure **1c** product was reduced back to diol **8c** with $NaBH_4$ for characterization of the diol.

Scheme 2[a]



[a] (a) MOMCl, DIPEA, CH_2Cl_2; (b) $PhN(SO_2CF_3)_2$, KHMDS, THF; (c) NaCN, CuI, $Pd(PPh_3)_4$, ACN; (d) 10% Pd−C, H_2; (e) HCl, $MeOH/CH_2Cl_2$; (f) CH_3MgCl, THF; (g) HPLC.

The isomerically pure hydroxy ketones **1a**, **1b**, **1c**, and **1d** were protected as their methoxymethyl ethers **9a** (89%), **9b** (83%), **9c** (82%), and **9d** (82%) (Scheme 2). By use of *N*-phenyltrifluorosulfonimide and potassium bis(trimethylsilyl)-amide, the ketones were converted to enol triflates **10a** (85%), **10b** (73%), **10c** (98%), and **10d** (95%). The triflates were converted in very high yield to the carbonitriles **11a** (96%), **11b** (94%), **11c** (93%), and **11d** (95%) by a palladium-mediated cyanation procedure with NaCN, CuI, and tetrakis(triphenylphosphine)palladium(0).[23] Catalytic hydrogenation of the unsaturated carbonitriles stereoselectively gave products **12a** (96%), **12b** (86%), **12c** (90%), and **12d** (87%). At this point, a portion of each carbonitrile was deprotected to give the target compounds **2a** (86%), **2b** (84%), **2c** (86%), and **2d** (83%). Finally, a small amount of the major isomer in each series (compounds **12a** and **12c**) was converted by reaction with

Table 1. Displacement of [^{35}S]TBPS by Benz[*f*]indenes, Steroids, and Benz[*e*]indenes

compd	IC_{50}[a] (μM)	n_{Hill}
trans−trans Benz[*f*]indenes		
1a [eq, oxo][b]	5.01 ± 0.80	1.18 ± 0.14
1b [ax, oxo]	3.92 ± 0.27	0.89 ± 0.27
2a [eq, CN]	1.22 ± 0.24	0.83 ± 0.10
2b [ax, CN]	0.52 ± 0.12	0.83 ± 0.14
3a [eq, Ac]	2.05 ± 0.40	1.04 ± 0.16
cis−trans Benz[*f*]indenes		
1c [ax, oxo]	>30	
1d [eq, oxo]	>10	
2c [ax, CN]	1.98 ± 0.42	1.08 ± 0.19
2d [eq, CN]	1.90 ± 1.05	0.81 ± 0.25
3b [ax, Ac]	3.51 ± 1.43	0.94 ± 0.22
5α-Reduced Steroids		
13a [oxo]	0.41± 0.13	0.89 ± 0.20
14a[c] [CN]	0.050 ± 0.0046	0.91 ± 0.56
15a[d] [Ac]	0.074 ± 0.0074	0.89 ± 0.06
5*β*-Reduced Steroids		
13b [oxo]	4.14 ± 1.00	1.03 ± 0.16
14b[c] [CN]	0.072 ± 0.0076	0.83 ± 0.05
15b[d] [Ac]	0.071 ± 0.018	0.57 ± 0.06
trans−trans Benz[*e*]indenes		
16a [eq, oxo]	4.17 ± 1.07	1.38 ± 0.39
16b [ax, oxo]	5.42 ± 4.48	0.78 ± 0.26
17a [eq, CN]	0.58 ± 0.10	0.80 ± 0.10
17b [ax, CN]	0.13 ± 0.02	0.72 ± 0.06
18a [eq, Ac]	1.17 ± 0.14	1.01 ± 0.11
18b [ax, Ac]	0.13 ± 0.03	0.83 ± 0.16

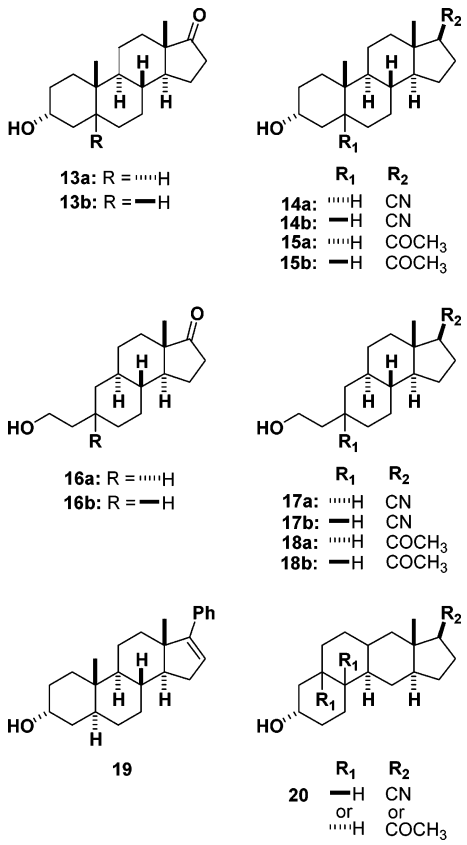
[a] Results presented are from duplicate experiments performed in triplicate. Error limits are calculated as standard error of the mean. [b] eq or ax refers to an equatorial or axial hydroxyethyl side chain; oxo (or CN, Ac) indicates the substituent on the five-membered ring. [c] Values obtained from ref 32. [d] Values obtained from ref 29.

CH_3MgCl to the corresponding methyl ketone and deprotected. In each case, this Grignard reaction afforded a 4:1 mixture of methyl ketone isomers, the major isomer being that in which the methyl ketone is on the same side of the ring system as the 9a-methyl group. The isomers were separated via HPLC to yield products **3a** (35%) and **3b** (31%).

[^{35}S]-TBPS Displacement. The results of the binding experiments are summarized in Table 1. With the exception of ketones **1a** and **1b,** which displaced [^{35}S]-TBPS equally as well as benz[*e*]indenes **16a** and **16b** (Chart 2), the benz[*f*]indenes were less potent displacers of [^{35}S]-TBPS than their corresponding benz[*e*]indenes. The most potent benz[*f*]indene displacer of [^{35}S]-TBPS was **2b**, which was less potent than the analogous benz[*e*]indene **17b** and steroid **14b** by 4- and 7-fold, respectively. Similarly, the other benz[*e*]indenes were all weaker than their analogous steroids, making the general order of binding steroids > benz[*e*]indenes > benz[*f*]indenes. For other compounds in the benz[*f*]indene family with carbonitrile (**2a**, **2c**) or acetyl (**3a**,**3b**) substituents at C-1, binding ability of both trans−trans and cis−trans analogues is approximately the same. For those benz[*f*]indenes with a ketone at C-1 (**1a**−**d**), the cis−trans compounds were much less potent than the trans−trans compounds. The Hill slopes for all the compounds were very similar, indicating they all bind a comparable number of sites.

Electrophysiology. Functional actions of the compounds at $GABA_A$ receptors were determined electrophysiologically in *X. laevis* oocytes expressing rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors (Table 2). For screening purposes, the compounds were evaluated at three different concentrations for their ability to enhance currents mediated by 2 μM GABA. This method is useful for determining whether compounds are active or inactive, but quantitative comparisons are not possible because screening is done on different oocyte preparations (GABA receptor expression varies within and between preparations).

Chart 2



The results of the screening show that none of the benz[*f*]-indenes exhibits any activity at 0.1 μM. At 1 μM, only the trans−trans carbonitrile **2b** had any significant activity over that of GABA alone. With the exception of the cis−trans ketones (**1c** and **1d**) and methyl ketone (**3b**), the remaining benz[*f*]-indenes gave an appreciable response at 10 μM. Direct gating effects of 10 μM compound in the absence of GABA were also assessed. None of the benz[*f*]indenes had any direct gating effects.

To determine a rank order of potency, the carbonitrile steroids, benz[*e*]indenes, and benz[*f*]indenes were applied at 5 μM to the same oocyte, eliminating any differences in receptor expression. The rank order for enhancing GABA-mediated chloride currents at $GABA_A$ receptors was the same as that for TBPS binding: steroids > benz[*e*]indenes > benz[*f*]indenes (Figure 4). However, while the [^{35}S]-TBPS binding data indicated that the trans−trans benz[*f*]indene carbonitriles bound marginally better than the corresponding cis−trans analogues, functionally the trans−trans compounds (**2a** and **2b**) are more potent than cis−trans compounds (**2c** and **2d**).

We have previously reported that (3α,5α)-17-phenylandrost-16-en-3-ol (**19**, Chart 2) selectively antagonizes GABAergic enhancement of 5α- but not 5*β*-reduced steroids.[24] We envisioned the benz[*e*]indenes and trans−trans benz[*f*]indenes that have an equatorial hydroxyethyl side chain (**17a** and **2a**, respectively) to be analogous to 5α-reduced steroids[17,20] and those with an axial side chain (**17b** and **2b**) to be analogous to 5*β*-reduced steroids.[19] Consequently, we were interested in what effect steroid **19** would have on $GABA_A$ currents potentiated by **17a, 17b, 2a,** and **2b**. We found that steroid **19** antagonized both benz[*e*]indenes **17a** (equatorial hydroxyethyl side chain) and **17b** (axial hydroxyethyl side chain) (Figure 5), suggesting

Table 2. Modulation of Rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ Receptor Function by Benz[*f*]indenes, Steroids, and Benz[*e*]indenes

	oocyte electrophysiology[a]			
compd	0.1 μM	1 μM	10 μM	(gating) 10 μM
	trans−trans Benz[*f*]indenes			
1a [eq, oxo][b]	1.00 ± 0.04	1.00 ± 0.06	2.42 ± 0.11	−0.02 ± 0.02
1b [ax, oxo]	0.90 ± 0.04	1.08 ± 0.05	3.54 ± 0.39	−0.02 ± 0.02
2a [eq, CN]	1.02 ± 0.03	1.29 ± 0.05	3.90 ± 0.50	0.00 ± 0.03
2b [ax, CN]	1.02 ± 0.03	2.11 ± 0.03	7.21 ± 0.49	0.01 ± 0.01
3a [eq, Ac]	1.00 ± 0.05	1.15 ± 0.05	3.26 ± 0.22	0.05 ± 0.03
	cis−trans Benz[*f*]indenes			
1c [ax, oxo]	0.93 ± 0.07	0.90 ± 0.04	1.02 ± 0.09	−0.01 ± 0.03
1d [eq, oxo]	1.02 ± 0.03	1.04 ± 0.03	1.21 ± 0.01	−0.05 ± 0.05
2c [ax, CN]	1.01 ± 0.02	1.14 ± 0.03	3.23 ± 0.15	−0.01 ± 0.01
2d [eq, CN]	0.98 ± 0.04	1.03 ± 0.05	2.17 ± 0.19	0.01 ± 0.00
3b [ax, Ac]	1.01 ± 0.06	1.04 ± 0.08	1.35 ± 0.09	−0.01 ± 0.03
	5α-Reduced Steroids			
13a [oxo]	0.97 ± 0.02	1.41 ± 0.01	5.44 ± 0.19	0.02 ± 0.01
14a [CN]	1.44 ± 0.26	5.65 ± 1.73	8.28 ± 2.68	0.66 ± 0.11
15a[c] [Ac]	1.26 ± 0.14	3.89 ± 1.34	9.65 ± 3.87	0.37 ± 0.07
	5β-Reduced Steroids			
13b [oxo]	0.89 ± 0.02	0.87 ± 0.02	1.39 ± 0.05	0.00 ± 0.02
14b [CN]	2.02 ± 0.18	10.45 ± 3.03	20.63 ± 6.62	0.09 ± 0.02
15b[c] [Ac]	1.20 ± 0.10	2.82 ± 0.51	9.77 ± 2.15	0.06 ± 0.03
	trans−trans Benz[*e*]indenes			
16a [eq, oxo]	0.99 ± 0.11	1.03 ± 0.17	1.05 ± 0.11	0.03 ± 0.07
16b [ax, oxo]	0.95 ± 0.10	1.22 ± 0.10	2.11 ± 0.12	−0.16 ± 0.07
17a [eq, CN]	1.29 ± 0.23	2.33 ± 0.48	12.18 ± 2.21	0.54 ± 0.16
17b [ax, CN]	1.45 ± 0.11	3.65 ± 0.14	7.42 ± 1.37	0.01 ± 0.01
18a [eq, Ac]	0.91 ± 0.09	0.96 ± 0.18	3.63 ± 0.76	0.14 ± 0.05
18b [ax, Ac]	1.27 ± 0.12	3.79 ± 0.65	9.35 ± 0.75	0.13 ± 0.09

[a] The GABA concentration used for the control response was 2 μM. Each compound was evaluated on at least four different oocytes at the concentrations indicated, and the results reported are the ratio of the currents measured in the presence/absence of added compound. Gating represents direct current gated by 10 μM compound in the absence of GABA, and this current is reported as the ratio of compound-only current/2 μM GABA current. Error values are calculated as standard error of the mean ($n \geq 4$). [b] eq or ax refers to an equatorial or axial hydroxyethyl side chain; oxo (or CN, Ac) indicates the substituent on the five-membered ring. [c] Values obtained from ref 29.

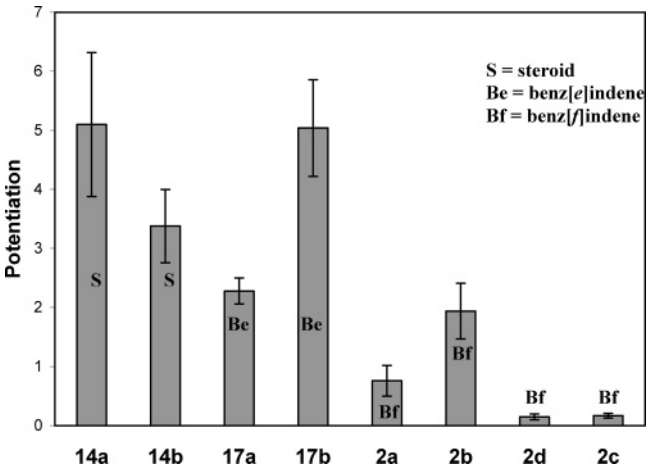


Figure 4. Direct comparison of the ability of carbonitrile steroids, benz[*e*]indenes, and benz[*f*]indenes to modulate $GABA_A$ receptor-mediated chloride currents at 5 μM. The GABA concentration used for the control response was 2 μM. The compounds were evaluated on the same oocytes expressing recombinant rat $\alpha_1\beta_2\gamma_{2L}$ receptors. The steady-state current mediated by GABA alone is set to zero as the control value. Potentiation is calculated as (R2 − R1)/R1, where R2 is the response in the presence of modulators and R1 is the response to GABA alone. Error limits are calculated as standard error of the mean for $n \geq 4$.

that both compounds behave like 5α-reduced steroids at the receptor. In contrast, steroid **19** had no significant effect on

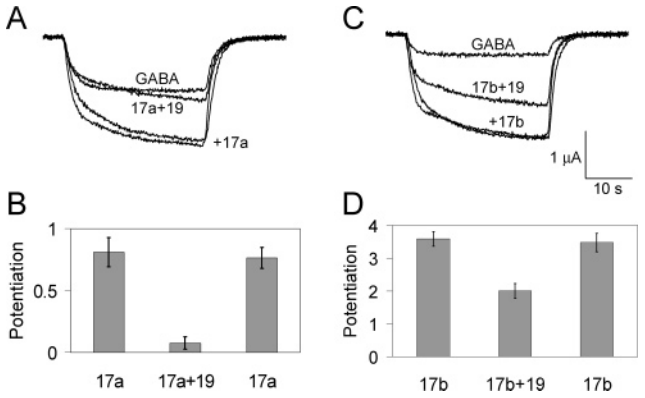


Figure 5. Effect of the steroid antagonist **19** on potentiation by trans−trans benz[*e*]indenes **17a** and **17b**. (A) Raw traces show an oocyte response to 2 μM GABA, 2 μM GABA plus 1 μM benz[*e*]indene **17a**, 2 μM GABA plus 1 μM benz[*e*]indene **17a** plus 10 μM antagonist **19** and 2 μM GABA plus 1 μM benz[*e*]indene after washout of antagonist **19**. (B) Summary of responses from 4 oocytes. The steady-state current mediated by GABA alone is set to zero as the control value. Potentiation is calculated as (R2−R1)/R1, where R2 is the response in the presence of modulators and R1 is the response to GABA alone. (C, D) Same concentrations as in panels A and B but for benz[*e*]indene **17b**. Although the antagonism of compound **17a** is more complete under identical conditions, both trans−trans benz[*e*]indenes, regardless of the stereochemistry of the hydroxyethyl side chain, are antagonized by compound **19**.

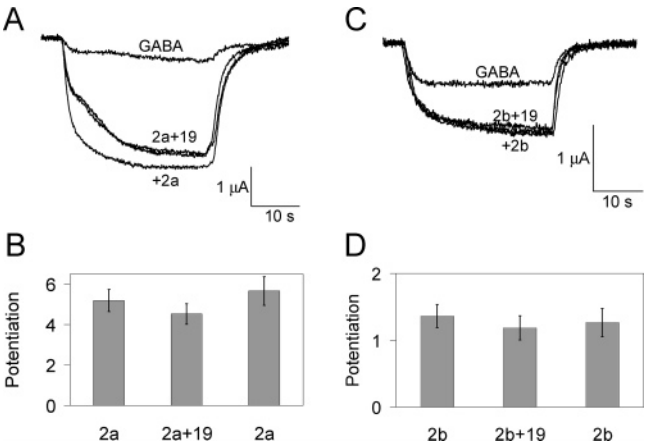


Figure 6. Effect of the steroid antagonist **19** on potentiation by trans−trans benz[*f*]indenes **2a** and **2b**. (A) Raw traces show an oocyte response to 2 μM GABA, 2 μM GABA plus 5 μM benz[*f*]indene **2a**, 2 μM GABA plus 5 μM benz[*f*]indene **2a** plus 10 μM antagonist **19**, and 2 μM GABA plus 1 μM benz[*f*]indene after washout of antagonist **19**. Benz[*f*]indene **2a** does not give an appreciable response at 1 μM. (B) Summary of responses from four oocytes. The steady-state current mediated by GABA alone is set to zero as the control value. Potentiation is calculated as (R2 − R1)/R1, where R2 is the response in the presence of modulators and R1 is the response to GABA alone. (C, D) Similar to panels A and B but for cis−trans benz[*f*]indene **2b**. The concentration of **2b** was 1 μM. Note that compound **19** has no significant effect on either trans−trans benz[*f*]indene regardless of the stereochemistry of the hydroxyethyl side chain.

potentiation caused by either of the benz[*f*]indenes **2a** (equatorial hydroxyethyl side chain) or **2b** (axial hydroxyethyl side chain) (Figure 6).

Tadpole Behavior. The benz[*f*]indenes were less potent at inducing tadpole LRR than their corresponding benz[*e*]indenes or steroids, with the exception of **1a**, which was equally as effective as steroid **13a** and 4-fold better than benz[*e*]indene **16a** (Table 3). The general rank order for inducing LRR was the same as that for [^{35}S]-TBPS displacement and electrophysiology potency, steroids > benz[*e*]indenes > benz[*f*]indenes. Of

Table 3. Effects of Benz[*f*]indenes, Steroids, and Benz[*e*]indenes on Tadpole Righting and Swimming Responses

	tadpole LRR[a]		tadpole LSR[b]	
compd	ED_{50} (μM)	n_{Hill}	ED_{50} (μM)	n_{Hill}
trans−trans Benz[*f*]indenes				
1a [eq, oxo][c]	4.01 ± 0.1 4	−7.37 ± 0.90	none	
1b [ax, oxo]	none[d]		none	
2a [eq, CN]	4.78 ± 0.13	−2.99 ± 0.14	17.3 ± 0.2	−36.2 ± 0.2
2b [ax, CN]	2.88 ± 0.00	−21.0 ± 0.8	5.48 ± 0.17	−33.3 ± 0.1
3a [eq, Ac]	6.63 ± 0.41	−2.42 ± 0.24	17.3 ± 0.2	−36.2 ± 0.2
cis−trans Benz[*f*]indenes				
1c [ax, oxo]	none		none	
1d [eq, oxo]	10.0 ± 0.0	−16.7 ± 0.1	none	
2c [ax, CN]	3.00 ± 0.00	−18.5 ± 0.1	none	
2d [eq, CN]	5.12 ± 0.23	−26.2 ± 0.2	17.3 ± 0.2	−36.2 ± 0.2
3b [ax, Ac]	>10		none	
5α-Reduced Steroids				
13a [oxo]	3.38 ± 0.90	−2.83 ± 2.66	none	
14a[e] [CN]	0.07 ± 0.01	−1.06 ± 0.13	0.71 ± 0.01	−9.8 ± 1.3
15a[f] [Ac]	0.42 ± 0.04	−1.83 ± 0.32	5.5 ± 0.5	−7.5 ± 1.1
5β-Reduced Steroids				
13b [oxo]	>10		none	
14b[g] [CN]	0.08 ± 0.01	−1.75 ± 0.23	0.36 ± 0.01	−4.3 ± 0.6
15b[f] [Ac]	0.06 ± 0.01	−1.54 ± 0.12	0.30 ± 0.01	−6.9 ± 0.5
Benz[*e*]indenes				
16a [eq, oxo]	16.4 ± 0.5	−4.79 ± 0.44	none	
16b [ax, oxo]	8.10 ± 11.6	−0.95 ± 0.63	none	
17a [eq, CN]	0.79 ± 0.10	−1.4 ± 0.24	5.5 ± 0.37	−6.8 ± 0.76
17b [ax, CN]	0.14 ± 0.04	−0.81 ± 0.15	1.73 ± 0.02	−36.5 ± 0.1
18a [eq, Ac]	1.09 ± 0.07	−3.04 ± 0.52	3.15 ± 0.21	−9.95 ± 1.47
18b [ax, Ac]	1.04 ± 0.01	−15.6 ± 0.9	2.84 ± 0.01	−20.3 ± 0.6

[a] LRR = loss of righting response. Error limits are calculated as standard error of the mean (N = 10 animals at each of five or more different concentrations). [b] LSR = loss of swimming response. Error limits are calculated as standard error of the mean (N = 10 animals at each of five or more different concentrations). [c] eq or ax refers to an equatorial or axial hydroxyethyl side chain; oxo (or CN, Ac) indicates the substituent on the five-membered ring. [d] None is defined as no response at 10 μM. [e] Values obtained from ref 33. [f] Values obtained from ref 29. [g] Values obtained from ref 32.

the 10 benz[*f*]indenes, only four of them caused LSR. Not surprisingly, the only one that did so at a concentration below 17 μM was the trans−trans carbonitrile **2b**. This compound was also the most potent in the [^{35}S]-TBPS displacement and electrophysiology experiments.

Discussion

On the basis of the [^{35}S]-TBPS displacement, electrophysiology, and tadpole LRR and LSR experiments, the trans−trans and cis−trans benz[*f*]indenes are weaker than their corresponding benz[*e*]indenes or steroids. Benz[*f*]indenes **2a** and **2c** were evaluated electrophysiologically at 30 and 100 μM, and even at these concentrations there was little increase from the potentiation caused by 10 μM compound (not shown). Therefore, this reduced activity appears to be both a potency and efficacy issue. Several plausible explanations for the reduced overall activity are discussed below. Most likely a combination of factors contributes to the observed outcomes. The trans−trans compounds will be addressed first, then the cis−trans compounds.

One issue to consider with the trans−trans benz[*f*]indenes is the flexibility of the hydroxyethyl side chain. Depending on how the benz[*f*]indenes (and benz[*e*]indenes) are overlaid with their analogous steroids, the flexible side chain could mimic either a 3α- or 3β-hydroxy steroid. 3β-Hydroxypregnane steroids are known blockers of $GABA_A$ receptor function.[25] Some recent work involving single-channel kinetic analysis and whole-cell voltage clamp in human embryonic kidney (HEK) cells transfected with rat $\alpha_1\beta_2\gamma_{2L}$ $GABA_A$ receptors has shown that benz[*e*]indene **17b** has potentiating actions at low concentrations and inhibitory actions at higher concentrations on $GABA_A$ channel function. At low concentrations (2 μM), **17b** prolonged channel openings, resulting in potentiation. But, at higher concentrations (50 μM), **17b** inhibited channel activity by enhancing desensitization (Li et al., submitted for publication). Presumably, these dual actions of **17b** are due to the flexibility of this side chain and the ability of the compound to mimic either a 3α- or 3β-hydroxysteroid. Thus, it may be that potentiating actions of compound **2b** are being countered by inhibitory actions resulting from a different conformation of the compound interacting with the receptor.

A second possibility for the reduced activity of the trans−trans benz[*f*]indenes is that the receptor cannot accommodate their C-7 and C-8 carbons. Those carbons would occupy the space filled by steroid 11α- and 1β-CH_3 groups when the C and D rings of a steroid are superimposed on the corresponding 6-5 fused rings of a benz[*f*]indene. We have prepared allopregnanolone and pregnanolone analogues containing an 11α-CH_3 substituent together with an 11β-OBn substituent and found those compounds to be highly active (Shu et al., submitted for publication), so it seems likely that the C-8 of the benz[*f*]indene ring systems would be sterically tolerated at a steroid binding site. There are no data available for the effect of a steroid 1β-CH_3 group on steroid action at $GABA_A$ receptors, so it is possible that C-7 of a benz[*f*]indene is not sterically tolerated by the receptor. However, steroids with large 2β-substituents are also known to be highly active.[11,26] Therefore, we consider it likely that the receptor can accommodate the benz[*f*]indene C-7 and C-8 methylene groups.

Third, as mentioned in Figure 3a, the trans−trans benz[*f*]indenes are the first analogues where the space that a portion of the steroid A,B rings (steroid carbon atoms C-4 through C-7) is unoccupied. On the basis of previous studies, the C-6/C-7 edge of the steroid B ring appears to provide a critical hydrophobic interaction either with the receptor or the lipid surrounding the receptor.[27−29] Therefore, the total lack of this region would be expected to result in reduced activity.

Finally, because it is known that steroids bind to multiple sites on $GABA_A$ receptors,[30,31] it is possible that the lower activity of the trans−trans benz[*f*]indenes is explained by their binding to only a fraction of the neuroactive steroid binding sites. Partial occupancy of the total steroid sites available could diminish the efficiency of channel opening and might explain the lowered efficacy of the trans−trans benz[*f*]indenes. The methods used here are not adequate to address this possibility, and a single-channel analysis of how the trans−trans benz[*f*]indenes affect channel properties, while having the potential to address this hypothesis, is beyond the scope of this study.

In contrast to the trans−trans compounds, the cis−trans benz[*f*]indenes cause little potentiation over GABA alone. As is the case for the trans−trans benz[*f*]indenes, the flexibility of the hydroxyethyl side chain, the presence of new hydrophobic contacts in disallowed regions of space, and the absence of the required hydrophobic contacts normally provided by the steroid A,B rings could all contribute to the negligible activity of the cis−trans benz[*f*]indenes. It is known that an axial 7α-CH_3 group decreases the $GABA_A$ receptor actions of both allopregnanolone and pregnanolone,[29] so it would not be surprising if the placement of the terminal six-membered ring in the cis−trans benz[*f*]indenes below the plane of the other two rings in the compounds had a similar effect.

To address the possible reasons just discussed for the low or negligible activity of the benz[*f*]indenes, we are preparing a new set of analogues in which the hydroxyethyl side chain will be incorporated into a fourth ring. These analogues will have the 7(8→11)*abeo* steroid ring system (**20**, Chart 2). These new analogues will address the role of conformational flexibility for the hydroxyethyl side chain and restore the hydrophobic contacts that the A rings of either allopregnanolone or pregnanolone are postulated to have with their binding sites on $GABA_A$ receptors. The additional hydrophobic contacts that C-7 and C-8 of the benz[*f*]indenes may make with the receptor will be retained in these analogues, and thus new information regarding the effects of those potential contacts should be forthcoming.

While the majority of the cis−trans compounds caused slight potentiation over GABA alone, the two cis−trans ketones **1c** and **1d** were inactive at all of the initial screening concentrations. Therefore, we screened ketones **1c** and **1d** at 10 μM as antagonists. We are interested in obtaining antagonists as pharmacological tools to understand the endogenous role of neurosteroids in nervous system excitability and to probe the mechanism of neurosteroid actions at $GABA_A$ receptors. To date we have found only one antagonist, **19**, that has limited solubility. Ketones **1c** and **1d** appeared to have antagonistic properties at 10 μM (not shown). However, at concentrations of 30 and 100 μM, **1c** and **1d** exhibited weak potentiation of 2 μM GABA, making these compounds very weak agonists (not shown). Thus, these two compounds are not pure antagonists.

On the basis of the electrophysiology screening and [^{35}S]-TBPS displacement data, it was not surprising that when evaluated on the same oocytes the rank order of activity found was steroids > benz[*e*]indenes > benz[*f*]indenes. In general, 5α-reduced steroids are slightly more active than 5β-reduced steroids (Figure 4, compare **14a** and **14b**). We have considered the benz[*e*]indenes and trans−trans benz[*f*]indenes that have an axial hydroxyethyl side chain (**17b** and **2b**, respectively) to be analogous to 5β-reduced steroids.[19] We were therefore surprised to find that these 5β-like compounds were more potent than their stereoisomers with an equatorial side chain (**17a** and **2a**), which we had previously considered to be 5α-steroid mimics. We also see this trend in the [^{35}S]-TBPS binding data.

To further investigate, we examined the effects of the previously described antagonist **19**, which is selective for 5α-reduced steroids over 5β-reduced steroids, on **17a**,**b** and **2a**,**b**. Compound **19** had a significant effect on both benz[*e*]indenes **17a** and **17b**, suggesting that both compounds, regardless of the stereochemistry of the side chain, behave like 5α-reduced steroids at the receptor. Conversely, steroid **19** had no effect on either benz[*f*]indene **2a** or **2b**. This suggests that either these compounds behave like 5β-reduced steroids at the receptor or they act at a different site. Additional studies are necessary to investigate the hypothesis that the benz[*e*]indene ring system mimics 5α-reduced steroids while the trans−trans benz[*f*]indene system mimics 5β-reduced steroids.

Conclusion

In this study, the SAR of a series of benz[*f*]indene analogues of neuroactive steroids was examined. We have found that the trans−trans benz[*f*]indene analogues have diminished activity compared to their analogous benz[*e*]indenes and neuroactive steroids, while the cis−trans benz[*f*]indenes have little activity over that of GABA alone. In each case, potential explanations for the reduction in activity include the flexibility of the hydroxyethyl side chain, the presence of new hydrophobic contacts in disallowed regions of space, and the absence of required hydrophobic contacts normally provided by the steroid A,B rings. Studies are in progress to sort out the effects of these different factors with a new series of analogues based on the steroid 7(8→11)*abeo* ring system. This is the first study that addresses the role of the steroid backbone in the actions of neuroactive steroids at $GABA_A$ receptors.

Experimental Section

General Methods. Solvents were either used as purchased or dried and purified by standard methodology. Flash chromatography was performed on silica gel (32−63 μm) purchased from Scientific Adsorbents (Atlanta, GA). Melting points were determined on a Kofler micro hot stage and are uncorrected. IR spectra were recorded as films on an NaCl plate (unless stated otherwise) with a Perkin-Elmer FT-IR Spectrum One spectrophotometer. NMR spectra were recorded in $CDCl_3$ (unless stated otherwise) at ambient temperature operating at 300 MHz (^{1}H) or 75 MHz (^{13}C). Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

(1*S*,3a*S*,4a*S*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-6*H*-benz[*f*]inden-6-one (5a) and (1*S*,3a*S*,4a*R*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-6*H*-benz[*f*]inden-6-one (5b). Compound **4a** (2.06 g, 0.009 mol) was prepared as described previously[22] and dissolved in pyridine (50 mL), and AcOAc was added (27 mL). The reaction was stirred overnight (14 h), at which time the reaction was complete by TLC. HCl (10%) was added until the pH was ∼1−2, and the reaction was stirred for an additional 30 min. EtOAc was added and the organic phase was washed with water (3 × 25 mL), saturated $NaHCO_3$ (3 × 25 mL), and brine (3 × 25 mL). The product was dried and filtered, and the solvent was removed in vacuo. The product was purified by silica chromatography (20% EtOAc in hexanes) to yield **5a** as a colorless oil (2.27 g, 93%). The same procedure was followed for **4b** (1.08 g, 0.005 mol, prepared as previously described[22]) to give the white solid **5b** (1.0 g, 78%).

5a: $[\alpha]_D^{25} = +38.1$ ($c = 1.6$, $CHCl_3$); ^{1}H NMR δ 4.69−4.63 (1H, dd, $J = 7.5$, 1.5 Hz), 2.43−2.06 (4H, m), 2.05 (3H, s), 2.04−1.77 (2H, m), 1.66−1.46 (4H, m), 1.42−1.15 (6H, m), 1.00−0.92 (1H, m), 0.87 (3H, s); ^{13}C NMR δ 211.2, 171.1, 82.2, 48.4, 44.2, 43.9, 43.4, 43.3, 41.5, 37.1, 33.1, 32.7, 27.5, 25.1, 21.1, 12.2; IR ν_{max} 2916, 1732, 1373, 1248, 1034 cm^{-1}. Anal. ($C_{16}H_{24}O_3$) C, H.

5b: $[\alpha]_D^{25} = +49.5$ ($c = 1$, $CHCl_3$); mp 104−106 °C; ^{1}H NMR δ 4.78−4.72 (1H, dd, $J = 7.5$, 1.5 Hz), 2.60−2.51 (1H, m), 2.37−2.30 (1H, m), 2.27−2.06 (4H, m), 2.05 (3H, s), 2.04−1.70 (3H, m), 1.69−1.50 (6H, m), 1.47−1.28 (2H, m), 0.87 (3H, s); ^{13}C NMR δ 212.8, 171.2, 82.4, 43.7, 42.8, 37.8, 37.7, 37.1, 37.0, 31.5, 30.2, 30.0, 27.3, 25.1, 21.1, 11.7; IR ν_{max} 2940, 1731, 1705, 1252, 1041 cm^{-1}. Anal. ($C_{16}H_{24}O_3$) C, H.

(2*Z*)-[(1*S*,3a*S*,4a*S*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]inden-6-ylidene]acetic Acid Ethyl Ester [(*Z*)-6a], (2*E*)-[(1*S*,3a*S*,4a*S*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]inden-6-ylidene]acetic Acid Ethyl Ester [(*E*)-6a], (2*Z*)-[(1*S*,3a*S*,4a*R*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]inden-6-ylidene]acetic Acid Ethyl Ester [(*Z*)-6b], and (2*E*)-[(1*S*,3a*S*,4a*R*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]inden-6-ylidene]acetic Acid Ethyl Ester [(*E*)-6b]. Compound **5a** (0.43 g, 1.6 mmol) and (carbethoxymethylene)-triphenylphosphorane (1.2 g, 3.4 mmol) were placed in a flask equipped with a stir bar and condenser. The flask was evacuated and filled with N_2 three times and then heated to 160 °C for 18 h. The reaction was then cooled to room temperature, and EtOAc and water were added. The organic phase was washed with water (3 × 25 mL) and brine (3 × 25 mL). The product was dried and filtered, and the solvent was removed in vacuo. It was then passed down a short column of silica (17% EtOAc in hexanes) to remove the excess (carbethoxymethylene)triphenylphosphorane to yield **6a**, a colorless oil that was a 1:1 mixture of *E* and *Z* isomers (0.462 g, 86%). The same procedure was repeated on **5b** (0.820 g, 3.10 mmol) to yield **6b**, a colorless oil that was a 1:1 mixture of *E* and *Z* isomers (0.950 g, 91%). Portions of the *E*/*Z* isomer mixtures of both **6a** and **6b**

were separated by HPLC on a silica gel column eluted with 5% EtOAc in hexanes for characterization.

(*Z*)-6a: $[\alpha]_D^{25} = +68.3$ ($c = 0.79$, $CHCl_3$); ^{1}H NMR δ 5.60 (1H, s), 4.66−4.60 (1H, dd, $J = 7.2$, 1.8 Hz), 4.17−4.10 (2H, q, $J = 7.2$ Hz), 3.83−3.79 (1H, m), 2.30−2.15 (3H, m), 2.04 (3H, s), 1.77−1.37 (10H, m), 1.30−1.25 (3H, t, $J = 7.2$ Hz), 1.19−0.89 (3H, m), 0.84 (3H, s); ^{13}C NMR δ 171.2, 166.8, 162.5, 113.2, 82.5, 59.5, 45.0, 44.7, 44.0, 43.2, 38.3, 37.8, 36.3, 35.1, 32.7, 27.5, 25.3, 21.2, 14.3, 12.3; IR ν_{max} 2914, 1737, 1714, 1244, 1150 cm^{-1}. Anal. ($C_{20}H_{30}O_4$) C, H.

(*E*)-6a: $[\alpha]_D^{25} = -43.6$ ($c = 0.70$, $CHCl_3$); ^{1}H NMR δ 5.59 (1H, s), 4.65−4.59 (1H, dd, $J = 7.2$, 1.8 Hz), 4.17−4.10 (2H, q, $J = 7.2$ Hz), 3.90−3.85 (1H, m), 2.21−2.14 (3H, m), 2.04 (3H, s), 2.03−1.36 (10H, m), 1.29−1.24 (3H, t, $J = 7.2$ Hz), 1.21−0.88 (3H, m), 0.83 (3H, s); ^{13}C NMR δ 171.2, 166.8, 162.3, 113.4, 82.5, 59.5, 45.7, 44.6, 44.5, 43.8, 43.2, 38.3, 34.4, 32.6, 29.5, 27.4, 25.3, 21.2, 14.3, 12.3; IR ν_{max} 2918, 1737, 1715, 1247, 1142 cm^{-1}. Anal. ($C_{20}H_{30}O_4$) C, H.

(*Z*)-6b: $[\alpha]_D^{25} = +72.8$ ($c = 1.13$, $CHCl_3$); ^{1}H NMR δ 5.59 (1H, s), 4.73−4.68 (1H, dd, $J = 7.8$, 1.2 Hz), 4.15−4.08 (2H, q, $J = 7.2$ Hz), 3.46−3.41 (1H, m), 2.26−2.06 (3H, m), 2.04 (3H, s), 1.98−1.90 (2H, m), 1.77−1.52 (8H, m), 1.49−1.30 (3H, m), 1.28−1.23 (3H, t, $J = 7.2$ Hz), 0.82 (3H, s); ^{13}C NMR δ 171.2, 166.9, 164.4, 112.8, 82.7, 59.4, 43.7, 38.0, 37.8, 37.4, 33.3, 32.6, 30.9, 30.6, 30.3, 27.4, 25.3, 21.1, 14.3, 11.6; IR ν_{max} 2919, 1733, 1713, 1249, 1161 cm^{-1}. Anal. ($C_{20}H_{30}O_4$) C, H.

(*E*)-6b: $[\alpha]_D^{25} = +135.1$ ($c = 1.44$, $CHCl_3$); ^{1}H NMR δ 5.57 (1H, s), 4.72−4.66 (1H, dd, $J = 7.8$, 1.2 Hz), 4.14−4.07 (2H, q, $J = 7.2$ Hz), 3.64−3.60 (1H, m), 2.46−2.38 (1H, m), 2.19−2.09 (1H, m), 2.03 (3H, s), 1.96−1.72 (4H, m), 1.68−1.43 (7H, m), 1.40−1.28 (3H, m), 1.27−1.22 (3H, t, $J = 7.2$ Hz), 0.81 (3H, s); ^{13}C NMR δ 171.2, 166.7, 164.3, 112.8, 82.6, 59.4, 43.7, 38.7, 38.4, 38.0, 37.3, 32.7, 30.8, 30.6, 27.3, 25.3, 24.5, 21.1, 14.3, 11.6; IR ν_{max} 2919, 1734, 1715, 1248, 1157 cm^{-1}. Anal. ($C_{20}H_{30}O_4$) C, H.

(1*S*,3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]indene-6-acetic Acid Ethyl Ester (7a), (1*S*,3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]indene-6-acetic Acid Ethyl Ester (7b), (1*S*,3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]indene-6-acetic Acid Ethyl Ester (7c), and (1*S*,3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-1-Acetyloxydodecahydro-9a-methyl-1*H*-benz[*f*]indene-6-acetic Acid Ethyl Ester (7d). Mixture (*E*,*Z*)-**6a** (2.07 g, 6.19 mmol) was dissolved in EtOAc (150 mL), and 5% Pd on $BaSO_4$ (0.414 g) was added. The reaction was hydrogenated (60 psi, H_2) overnight then filtered through a short column of Celite to remove the catalyst, and washed with EtOAc. The solvent was removed in vacuo to yield a 2.5:1 mixture (1.93 g, 93%) of **7a** (a white solid) to **7b** (yellow oil). The same procedure was repeated on **6b** (1.22 g, 3.65 mmol) to yield a 3.5:1 mixture (1.09 g, 90%) of **7c** to **7d** as an oil. A portion of the mixture of **7a** and **7b** was separated by HPLC on a silica gel column eluted with 5% EtOAc in hexanes for product characterization. The mixture of **7c** and **7d** was inseparable by silica gel chromatography (column or HPLC).

7a: $[\alpha]_D^{25} = -7.7$ ($c = 1.39$, $CHCl_3$); mp 66−67 °C; ^{1}H NMR δ 4.61−4.55 (1H, dd, $J = 7.5$, 1.5 Hz), 4.11−4.04 (2H, q, $J = 7.2$ Hz), 2.14−2.04 (2H, m), 1.99 (3H, s), 1.97−1.27 (11H, m), 1.23−1.19 (3H, t, $J = 7.2$ Hz), 1.10−0.78 (7H, m), 0.75 (3H, s); ^{13}C NMR δ 172.9, 171.1, 82.6, 60.0, 44.7, 44.2, 43.6, 43.1, 42.0, 40.0, 38.0, 35.0, 33.5, 33.0, 32.5, 27.3, 25.3, 21.1, 14.2, 12.2; IR ν_{max} 2911, 1735, 1245, 1187, 1034 cm^{-1}. Anal. ($C_{20}H_{32}O_4$) C, H.

7b: $[\alpha]_D^{25} = +11.6$ ($c = 1.04$, $CHCl_3$); ^{1}H NMR δ 4.61−4.56 (1H, dd, $J = 7.5$, 1.5 Hz), 4.13−4.06 (2H, q, $J = 7.2$ Hz), 2.33 (3H, m), 2.17−2.07 (1H, m), 2.01 (3H, s), 2.00−1.34 (11H, m), 1.25−1.20 (3H, t, $J = 7.2$ Hz), 1.14−0.96 (3H, m), 0.91−0.84 (2H, m), 0.77 (3H, s); ^{13}C NMR δ 173.5, 171.2, 82.7, 60.0, 44.8, 44.5, 43.1, 38.8, 38.1, 37.4, 37.2, 32.6, 30.5, 30.2, 28.7, 27.3, 25.4, 21.1, 14.2, 12.2; IR ν_{max} 2913, 1733, 1259, 1177, 1032 cm^{-1}. Anal. ($C_{20}H_{32}O_4$) C, H.

7c and 7d: IR ν_{max} 2916, 1733, 1248, 1156, 1036 cm^{-1}; resonances for major isomer **7c**, ^{1}H NMR δ 4.69−4.63 (1H, dd, $J = 7.2$, 2.1 Hz), 4.13−4.06 (2H, q, $J = 7.2$ Hz), 2.01 (3H, s), 1.25−1.21 (3H, t, $J = 7.2$ Hz), 0.79 (3H, s); ^{13}C NMR δ 173.0, 171.2, 82.8, 60.0, 43.8, 42.2, 38.4, 37.5, 35.8, 35.5, 33.3, 31.3, 31.2, 30.2, 27.4, 27.3, 25.5, 21.1, 14.2, 11.5.

(1*S*,3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-Dodecahydro-1-hydroxy-9a-methyl-1*H*-benz[*f*]indene-6-ethanol (8a), (1*S*,3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-Dodecahydro-1-hydroxy-9a-methyl-1*H*-benz[*f*]indene-6-ethanol (8b), (1*S*,3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-Dodecahydro-1-hydroxy-9a-methyl-1*H*-benz[*f*]indene-6-ethanol (8c), and (1*S*,3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-Dodecahydro-1-hydroxy-9a-methyl-1*H*-benz[*f*]indene-6-ethanol (8d). LAH (1.6 g, 0.043 mol) was suspended in dry tetrahydrofuran (THF) (30 mL) with stirring under nitrogen. The suspension was cooled to 0 °C in an ice bath, and a mixture of **7a** and **7b** (1.8 g, 0.053 mol) dissolved in THF (10 mL) was added via addition funnel. After addition, the reaction was heated to reflux. After 2 h, the reaction was cooled to 0 °C and the excess LAH was quenched (1.6 mL of H_2O + 1.6 mL of 10% NaOH, 30 min, then 3.2 mL of H_2O, 15 min). The reaction was filtered through Celite and washed with THF. The solvent was removed in vacuo, and chromatography (50% EtOAc in hexanes) yielded the inseparable mixture of diols (1.1 g, 79%) **8a** (white solid) and **8b** (white solid). A portion of purified **1a** and **1b** (vide infra) was reduced back to **8a** and **8b** with $NaBH_4$ for product characterization. The same procedure was repeated on a mixture of **7c** and **7d** (0.59 g, 0.018 mol) to yield the inseparable mixture of diols **8c** and **8d** (0.42 g, 95%). A portion of **1c** (vide infra) was reduced back to **8c** with $NaBH_4$ for product characterization.

8a: $[\alpha]_D^{25} = +4.3$ ($c = 1.02$, THF); mp 146−148 °C; ^{1}H NMR (pyridine-d_5) δ 3.97−3.91 (3H, m), 2.16−2.07 (1H, m), 1.93−1.88 (1H, m), 1.82−1.67 (6H, m), 1.60−1.41 (2H, m), 1.39−1.28 (3H, m), 1.26−1.06 (3H, m), 1.02−0.90 (6H, m), 0.87−0.76 (3H, m); ^{13}C NMR (pyridine-d_5) δ 81.6, 60.3, 45.9, 45.7, 44.9, 44.7, 41.7, 41.4, 39.3, 35.4, 35.0, 34.3, 34.0, 31.3, 26.3, 12.4; IR ν_{max} (KBr) 3332, 2908, 2848, 1385, 1043 cm^{-1}. Anal. ($C_{16}H_{28}O_2$) C, H.

8b: $[\alpha]_D^{25} = +22.2$ ($c = 0.93$, THF); mp 107−109 °C; ^{1}H NMR (pyridine-d_5) δ 3.97−3.92 (3H, m), 2.15−2.08 (3H, m), 1.90−1.75 (5H, m), 1.69−1.45 (5H, m), 1.41−1.11 (7H, m), 1.07−1.02 (4H, m), 0.91−0.83 (1H, m); ^{13}C NMR (pyridine-d_5) δ 81.7, 61.2, 45.9, 45.8, 44.6, 40.0, 39.2, 38.6, 36.2, 34.1, 31.2, 31.1, 30.8, 29.9, 26.4, 12.5; IR ν_{max} (KBr) 3277, 2913, 2857, 1382, 1051 cm^{-1}. Anal. ($C_{16}H_{28}O_2$) C, H.

8c: $[\alpha]_D^{25} = +76.9$ ($c = 0.91$, THF); mp 105−106 °C; ^{1}H NMR (pyridine-d_5) δ 3.99−3.94 (3H, m), 2.18−2.14 (1H, m), 1.97−1.40 (14H, m), 1.36−1.24 (5H, m), 1.13−1.08 (2H, m), 1.03 (3H, s); ^{13}C NMR (pyridine-d_5) δ 81.7, 60.2, 45.1, 41.9, 39.3, 38.6, 37.0, 36.0, 34.6, 32.7, 32.6, 31.5, 31.4, 28.7, 26.5, 11.7; IR ν_{max} (KBr) 3291, 2915, 2853, 1354, 1053 cm^{-1}. Anal. ($C_{16}H_{28}O_2$) C, H.

(3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (1a), (3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (1b), (3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (1c), and (3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (1d). A mixture of **8a** and **8b** (0.21 g, 0.83 mmol) was dissolved in HOAc (4 mL). To this, a 5.25% aqueous solution of NaOCl (2.9 mL, 2.1 mmol) was added dropwise. After 30 min, 2-propanol (2 mL) was added to quench any excess oxidant and water was added (50 mL). The mixture was extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with water (75 mL), saturated $NaHCO_3$ (100 mL), water (100 mL), and brine (100 mL). The mixture was dried, and the solvent was removed in vacuo to yield a mixture of white solids **1a** and **1b** (0.17 g, 79%). A portion of this mixture was separated via HPLC (Beckman Ultrasphere, 5 μm silica column, 250 mm × 10 mm, 45% EtOAc in hexanes, 3 mL/min). The same procedure was repeated on a mixture of **8c** and **8d** (0.17 g, 0.67 mmol) to give the white solids **1c** and **1d**. All of the **1c** and **1d** mixture was separated at this stage via HPLC [Beckman Ultrasphere, 5 μm silica column, 250 mm × 10 mm, EtOAc/hexane/$ClCH_2CH_2Cl$ (4:6:10), 3 mL/min].

1a: $[\alpha]_D^{25} = +64.6$ ($c = 1.07$, $CHCl_3$); mp 94−95 °C; ^{1}H NMR δ 3.71−3.67 (2H, t, $J = 6.0$ Hz), 2.48−2.39 (1H, m), 2.14−2.02 (1H, m), 1.90−1.83 (1H, m), 1.78−1.60 (8H, m), 1.57−1.47 (3H,

m), 1.29−1.09 (3H, m), 1.05−0.93 (4H, m), 0.88 (3H, s); ^{13}C NMR δ 221.1, 60.7, 48.4, 45.7, 44.0, 40.3, 40.0, 38.8, 38.2, 35.6, 34.3, 33.6, 33.2, 32.6, 23.9, 13.6; IR ν_{max} 3396, 2911, 2847, 1733, 1045 cm^{-1}. Anal. ($C_{16}H_{26}O_2$) C, H.

1b: $[\alpha]_D^{25} = +74.3$ ($c = 0.99$, $CHCl_3$); mp 112−114 °C; ^{1}H NMR δ 3.66−3.62 (2H, t, $J = 6.9$ Hz), 2.45−2.36 (1H, m), 2.12−2.00 (1H, m), 1.91−1.82 (2H, m), 1.67−1.38 (11H, m), 1.35−1.11 (5H, m), 1.01−0.93 (1H, m), 0.86 (3H, s); ^{13}C NMR δ 220.8, 61.7, 48.3, 45.9, 39.1, 38.9, 38.5, 37.6, 35.6, 34.8, 32.8, 30.2, 29.7, 28.7, 24.0, 13.7; IR ν_{max} 3470, 2915, 2851, 1738, 1047 cm^{-1}. Anal. ($C_{16}H_{26}O_2$) C, H.

1c: $[\alpha]_D^{25} = +116.3$ ($c = 1.21$, $CHCl_3$); mp 88−90 °C; ^{1}H NMR δ 3.69−3.64 (2H, t, $J = 6.3$ Hz), 2.48−2.38 (1H, m), 2.14−1.94 (2H, m), 1.86−1.68 (4H, m), 1.67−1.40 (10H, m), 1.34−1.19 (4H, m), 0.88 (3H, s); ^{13}C NMR δ 221.4, 60.6, 48.8, 40.3, 39.4, 36.0, 35.7, 34.8, 33.7, 32.1, 31.4 (2× C), 30.5, 27.6, 23.9, 12.8; IR ν_{max} 3429, 2918, 2856, 1738, 1048 cm^{-1}. Anal. ($C_{16}H_{26}O_2$) C, H.

1d: $[\alpha]_D^{25} = +98.0$ ($c = 1.08$, $CHCl_3$); mp 74−76 °C; ^{1}H NMR δ 3.69−3.64 (2H, t, $J = 6.9$ Hz), 2.48−2.39 (1H, m), 2.15−1.91 (4H, m), 1.85−1.49 (9H, m), 1.35−1.25 (6H, m), 1.10−1.06 (1H, m), 0.88 (3H, s); ^{13}C NMR δ 221.3, 61.8, 48.7, 39.2, 35.7, 34.0, 32.0, 31.3, 30.7, 30.3, 29.8, 29.7, 25.8, 24.1, 24.0, 12.8; IR ν_{max} 3436, 2921, 2858, 1738, 1041 cm^{-1}. Anal. ($C_{16}H_{26}O_2$) C, H.

(3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (9a), (3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (9b), (3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (9c), and (3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-one (9d). Compound **1a** (0.17 g, 0.68 mmol) was dissolved in CH_2Cl_2 (5 mL). To this was added *N*,*N*-diisopropylethylamine (0.6 mL, 3 mmol) followed by chloromethyl methyl ether (0.2 mL, 3 mmol). The reaction was stirred for 24 h, at which time the solvent was removed by rotary evaporator to give a yellow oil. After chromatography (10% EtOAc in hexanes), **9a** was obtained as a colorless oil (0.18 g, 89%). The same procedure was repeated on **1b** (82 mg, 0.33 mmol), **1c** (0.193 g, 0.771 mmol), and **1d** (49 mg, 0.20 mmol) to yield colorless oils **9b** (80 mg, 83%), **9c** (0.185 g, 82%), and **9d** (47 mg, 82%).

9a: $[\alpha]_D^{25} = +58.7$ ($c = 0.91$, $CHCl_3$); ^{1}H NMR δ 4.60 (2H, s), 3.57−3.53 (2H, t, $J = 6.0$ Hz), 3.34 (3H, s), 2.46−2.37 (1H, m), 2.13−2.03 (1H, m), 1.88−1.43 (12H, m), 1.24−0.88 (6H, m), 0.86 (3H, s); ^{13}C NMR δ 220.7, 96.4, 65.7, 55.1, 48.4, 45.8, 44.1, 40.4, 39.0, 38.3, 37.0, 35.7, 34.8, 33.7, 33.3, 32.7, 23.9, 13.7; IR ν_{max} 2914, 2850, 1741, 1111, 1044 cm^{-1}.

9b: $[\alpha]_D^{25} = +68.6$ ($c = 1.02$, $CHCl_3$); ^{1}H NMR δ 4.62 (2H, s), 3.56−3.51 (2H, t, $J = 6.0$ Hz), 3.36 (3H, s), 2.47−2.38 (1H, m), 2.14−2.02 (1H, m), 1.93−1.77 (2H, m), 1.69−1.56 (10H, m), 1.52−1.27 (2H, m), 1.23−1.09 (3H, m), 1.04−0.93 (1H, m), 0.88 (3H, s); ^{13}C NMR δ 220.7, 96.4, 66.6, 55.1, 48.3, 45.9, 39.1, 38.9, 38.5, 37.6, 35.6, 32.8, 31.7, 30.1 (2× C), 28.7, 24.0, 13.7; IR ν_{max} 2916, 2852, 1741, 1110, 1041 cm^{-1}.

9c: $[\alpha]_D^{25} = +111.7$ ($c = 1.08$, $CHCl_3$); ^{1}H NMR δ 4.54 (2H, s), 3.51−3.47 (2H, t, $J = 6.0$ Hz), 3.29 (3H, s), 2.42−2.33 (1H, m), 2.09−1.89 (2H, m), 1.81−1.04 (16H, m), 0.98−0.89 (1H, m), 0.83 (3H, s); ^{13}C NMR δ 220.9, 96.2, 65.3, 54.9, 48.6, 39.3, 37.0, 35.9, 35.6, 35.1, 33.6, 32.0, 31.3 (2× C), 30.4, 27.5, 23.8, 12.7; IR ν_{max} 2920, 2858, 1737, 1110, 1043 cm^{-1}. Anal. ($C_{18}H_{30}O_3$) C, H.

9d: $[\alpha]_D^{25} = +74.0$ ($c = 0.91$, $CHCl_3$); ^{1}H NMR δ 4.59 (2H, s), 3.53−3.49 (2H, t, $J = 6.0$ Hz), 3.33 (3H, s), 2.46−2.37 (1H, m), 2.12−1.82 (3H, m), 1.81−1.60 (9H, m), 1.58−1.46 (2H, m), 1.32−1.21 (4H, m), 1.10−1.05 (1H, m), 0.86 (3H, s); ^{13}C NMR δ 221.1, 96.4, 66.5, 55.1, 48.6, 39.2, 35.6, 31.9, 31.3, 30.8, 30.7, 30.2, 30.1, 29.7, 25.8, 24.0 (2× C), 12.7; IR ν_{max} 2921, 2860, 1740, 1110, 1039 cm^{-1}.

(3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-[3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]inden-1-yl]trifluoromethanesulfonate (10a), (3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-[3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]inden-1-yl]trifluoromethanesulfonate (10b), (3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-[3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]inden-1-yl]trifluoromethanesulfonate (10c), and (3a*S*,4a*R*,6*S*,8a*R*,9a*S*)- [3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]-inden-1-yl]trifluoromethanesulfonate (10d). Compound **9a** (0.21 g, 0.71 mmol) was placed in a round-bottom flask equipped with a stir bar. The flask was evacuated and filled with N_2 three times. To this was added *N*-phenyltrifluoromethanesulfonimide (0.77 g, 2.2 mmol) dissolved in THF (10 mL). The reaction was cooled to −78 °C and potassium bis(trimethylsilyl)amide (3 mL) was added. The reaction was stirred for 2 h, at which time 10 mL of saturated NH_4Cl was added. The mixture was extracted with hexanes (3 × 50 mL). The combined organic layers were washed with water (75 mL) and brine (100 mL) and dried, and the solvent was removed in vacuo to give a yellow oil. Chromatography (gradient of 1% EtOAc in hexanes, then 2% EtOAc in hexanes, then 3% EtOAc in hexanes) yielded **10a** (0.26 g, 85%) as a colorless oil. The same procedure was repeated with **9b** (78 mg, 0.26 mmol), **9c** (0.185 g, 0.628 mmol), and **9d** (45 mg, 0.15 mmol) to yield the colorless oils **10b** (83 mg, 73%), **10c** (0.262 g, 98%), and **10d** (62 mg, 95%).

10a: ^{1}H NMR δ 5.53 (1H, m), 4.60 (2H, s), 3.57−3.53 (2H, t, $J = 6.0$ Hz), 3.34 (3H, s), 2.22−2.13 (1H, m), 2.07−1.98 (1H, m), 1.92−1.58 (6H, m), 1.50−1.45 (4H, m), 1.25−1.01 (5H, m), 0.98 (3H, s), 0.85−0.73 (1H, m); ^{13}C NMR δ 159.7, 125.0−112.1 (q, $J_{CF} = 318.8$ Hz), 114.4, 96.4, 65.6, 55.1, 49.3, 45.5, 44.7, 40.4, 39.9, 38.6, 37.0, 34.9, 33.8, 33.4, 31.1, 30.5, 15.7; IR ν_{max} 2919, 1422, 1208, 1143, 1052 cm^{-1}.

10b: ^{1}H NMR δ 5.55 (1H, m), 4.61 (2H, s), 3.55−3.51 (2H, t, $J = 6.0$ Hz), 3.36 (3H, s), 2.22−2.14 (1H, m), 2.08−1.92 (1H, m), 1.91−1.85 (2H, m), 1.69−1.47 (6H, m), 1.42−1.10 (8H, m), 0.99 (3H, s); ^{13}C NMR δ 159.6, 124.9−112.2 (q, $J_{CF} = 318.8$ Hz), 114.4, 96.4, 66.5, 55.1, 49.3, 45.5, 40.0, 39.2, 39.1, 37.6, 31.7, 31.1, 30.6, 30.2, 30.0, 28.8, 15.8; IR ν_{max} 2920, 1422, 1211, 1143, 1047 cm^{-1}.

10c: ^{1}H NMR δ 5.55 (1H, m), 4.60 (2H, s), 3.57−3.53 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.15−1.95 (4H, m), 1.88−1.55 (4H, m), 1.53−1.42 (5H, m), 1.31−1.22 (5H, m), 1.01 (3H, s); ^{13}C NMR δ 159.8, 124.9−112.2 (q, $J_{CF} = 318.8$ Hz), 114.4, 96.4, 65.4, 55.1, 46.0, 42.9, 37.1, 35.9, 35.2, 34.3, 33.7, 31.4, 30.6, 30.5, 30.0, 27.8, 14.8; IR ν_{max} 2922, 1422, 1211, 1143, 1056 cm^{-1}.

10d: ^{1}H NMR δ 5.55 (1H, m), 4.61 (2H, s), 3.55−3.51 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.15−1.86 (4H, m), 1.84−1.67 (4H, m), 1.59−1.48 (1H, m), 1.34−1.03 (9H, m), 1.00 (3H, s); ^{13}C NMR δ 159.8, 124.9−112.2 (q, $J_{CF} = 318.8$ Hz), 114.4, 96.4, 66.5, 55.1, 45.8, 42.7, 33.6, 30.9, 30.8, 30.6, 30.1, 29.8, 29.7, 29.6, 25.7, 24.3, 14.9; IR ν_{max} 2925, 1422, 1212, 1144, 1049 cm^{-1}.

(3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]indene-1-carbonitrile (11a), (3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]indene-1-carbonitrile (11b), (3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz[*f*]indene-1-carbonitrile (11c), and (3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-3a,4,4a,5,6,7,8,8a,9,9a-Decahydro-9a-methyl-6-(methoxymethoxyethyl)-3*H*-benz-[*f*]indene-1-carbonitrile (11d). In a round-bottom flask, compound **10a** (0.25 g, 0.60 mmol) was combined with copper iodide (13 mg, 0.067 mmol), tetrakis(triphenylphosphine)palladium (33 mg, 0.028 mmol), and sodium cyanide (62 mg, 1.3 mmol, dried overnight at 100−105 °C in a vacuum oven). The flask was fitted with a stir bar and condenser, then evacuated and filled with N_2 three times. To this was added acetonitrile (10 mL), and the reaction was heated in an oil bath at 90 °C. After 2 h the reaction was cooled to room temperature, EtOAc was added (20 mL), and the mixture was filtered through Celite. The filtrate was washed with H_2O (2 × 20 mL) and brine (2 × 20 mL) and dried, and the solvent was removed. Chromatography (10% EtOAc in hexanes) gave **11a** as a colorless oil (0.17 mg, 96%). The same procedure was repeated on **10b** (83 mg, 0.19 mmol), **10c** (0.125 g, 0.293 mmol), and **10d** (62 mg, 0.15 mmol) to yield colorless oils **11b** (55 mg, 94%), **11c** (83 mg, 93%), and **11d** (42 mg, 95%).

11a: $[\alpha]_D^{25} = +0.88$ ($c = 1.25$, $CHCl_3$); ^{1}H NMR δ 6.62−6.60 (1H, m), 4.60 (2H, s), 3.57−3.53 (2H, t, $J = 6.0$ Hz), 3.34 (3H, s),

2.35−2.26 (1H, m), 2.18−2.08 (1H, m), 1.82−1.62 (5H, m), 1.56−1.47 (4H, m), 1.33−0.95 (6H, m), 0.92 (3H, s), 0.86−0.75 (1H, m); ^{13}C NMR δ 147.4, 127.7, 115.9, 96.4, 65.6, 55.1, 50.6, 48.8, 44.7, 41.3, 40.4, 38.9, 37.0, 34.9, 34.7, 33.7, 33.3, 31.1, 16.7; IR ν_{max} 2916, 2215, 1448, 1110, 1045 cm^{-1}. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

11b: $[\alpha]_D^{25} = +18.4$ ($c = 1.28$, $CHCl_3$); ^{1}H NMR δ 6.62−6.60 (1H, m), 4.61 (2H, s), 3.55−3.50 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.35−2.26 (1H, m), 2.18−2.08 (1H, m), 1.93−1.85 (1H, m), 1.80−1.71 (2H, m), 1.68−1.11 (13H, m), 0.93 (3H, s); ^{13}C NMR δ 147.5, 127.5, 116.0, 96.4, 66.5, 55.1, 50.7, 48.7, 41.4, 39.5, 39.0, 37.6, 34.7, 31.7, 31.1, 30.1, 30.0, 28.8, 16.7; IR ν_{max} 2918, 2214, 1450, 1110, 1042 cm^{-1}. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

11c: $[\alpha]_D^{25} = +66.9$ ($c = 0.98$, $CHCl_3$); ^{1}H NMR δ 6.64−6.62 (1H, m), 4.60 (2H, s), 3.57−3.53 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.30−2.21(1H, m), 2.14−2.04 (2H, m), 1.98−1.84 (2H, m), 1.79−1.23 (13H, m), 0.95 (3H, s); ^{13}C NMR δ 147.6, 127.9, 116.0, 96.4, 65.4, 55.1, 49.4, 44.3, 37.2, 35.9, 35.1, 35.0, 34.7, 34.2, 31.3, 30.9, 30.0, 27.8, 15.8; IR ν_{max} 2921, 2214, 1449, 1111, 1040 cm^{-1}. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

11d: $[\alpha]_D^{25} = +28.8$ ($c = 1.12$, $CHCl_3$); ^{1}H NMR δ 6.63−6.62 (1H, m), 4.61 (2H, s), 3.55−3.51 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.30−2.21 (1H, m), 2.14−1.64 (10H, m), 1.60−1.44 (2H, m), 1.35−1.15 (4H, m), 1.12−1.08 (1H, m), 0.94 (3H, s); ^{13}C NMR δ 147.5, 127.9, 116.0, 96.4, 66.5, 55.1, 49.2, 44.1, 35.0, 34.8, 31.0, 30.9, 30.7, 30.1, 29.8, 29.6, 25.7, 24.3, 15.9; IR ν_{max} 2922, 2214, 1450, 1110, 1039 cm^{-1}. Anal. ($C_{19}H_{29}NO_2$) C, H, N.

(1*S*,3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (12a), (1*S*,3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (12b), (1*S*,3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (12c), and (1*S*,3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-methoxymethoxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (12d). Compound **11a** (0.17 g, 0.56 mmol) was dissolved in EtOAc (50 mL). To this, 10% Pd on carbon (58 mg) was added. The reaction was hydrogenated (60 psi, H_2) for 3 h, then filtered through a short Celite pad to remove the catalyst, and washed with CH_2Cl_2. The solvent was removed in vacuo to yield the white solid **12a** (0.16 g, 96%). The same procedure was repeated on **11b** (38 mg, 0.13 mmol), **11c** (83 mg, 0.27 mmol), and **11d** (32 mg, 0.11 mmol) to yield colorless oils **12b** (33 mg, 86%) and **12c** (75 mg, 90%) and the white solid **12d** (28 mg, 87%).

12a: $[\alpha]_D^{25} = +54.4$ ($c = 1.09$, $CHCl_3$); ^{1}H NMR δ 4.59 (2H, s), 3.56−3.52 (2H, t, $J = 6.0$ Hz), 3.33 (3H, s), 2.28−2.22 (1H, m), 2.14−2.03 (1H, m), 1.95−1.60 (6H, m), 1.59−1.14 (7H, m), 1.04−0.93 (4H, m), 0.89 (3H, s), 0.84−0.73 (2H, m); ^{13}C NMR δ 121.4, 96.3, 65.5, 55.1, 48.4, 44.8, 44.5, 43.7, 40.2, 39.8, 38.4, 36.9, 34.7, 33.6, 33.1, 32.7, 26.4, 26.2, 14.4; IR ν_{max} 2908, 2235, 1449, 1109, 1042 cm^{-1}. Anal. ($C_{19}H_{31}NO_2$) C, H, N.

12b: $[\alpha]_D^{25} = +68.9$ ($c = 1.08$, $CHCl_3$); ^{1}H NMR δ 4.61 (2H, s), 3.54−3.50 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.29−2.22 (1H, m), 2.14−2.06 (1H, m), 1.95−1.21 (15H, m), 1.17−0.94 (4H, m), 0.91 (3H, s); ^{13}C NMR δ 121.5, 96.4, 66.5, 55.1, 48.5, 44.8, 44.7, 39.9, 39.1, 38.1, 37.4, 32.9, 31.6, 30.0, 29.9, 28.6, 26.3, 26.2, 14.5; IR ν_{max} 2914, 2234, 1450, 1110, 1038 cm^{-1}. Anal. ($C_{19}H_{31}NO_2$) C, H, N.

12c: $[\alpha]_D^{25} = +124.6$ ($c = 0.95$, $CHCl_3$); ^{1}H NMR δ 4.59 (2H, s), 3.55−3.51 (2H, t, $J = 6.0$ Hz), 3.33 (3H, s), 2.33−2.27 (1H, m), 2.14−2.03 (1H, m), 1.95−1.66 (4H, m), 1.64−1.57 (2H, m), 1.54−0.96 (13H, m), 0.91 (3H, s); ^{13}C NMR δ 121.4, 96.3, 65.4, 55.1, 45.5, 42.0, 40.1, 37.7, 37.1, 35.6, 35.2, 33.7, 31.5, 31.3, 30.8, 27.6, 26.4, 26.3, 13.7; IR ν_{max} 2918, 2235, 1450, 1110, 1047 cm^{-1}. Anal. ($C_{19}H_{31}NO_2$) C, H, N.

12d: $[\alpha]_D^{25} = +88.1$ ($c = 1.23$, $CHCl_3$); ^{1}H NMR δ 4.61 (2H, s), 3.55−3.51 (2H, t, $J = 6.0$ Hz), 3.35 (3H, s), 2.34−2.28 (1H, m), 2.15−1.85 (3H, m), 1.79−1.60 (4H, m), 1.59−1.41 (4H, m), 1.34−1.25 (7H, m), 1.09−1.05 (2H, m), 0.92 (3H, s); ^{13}C NMR δ 121.3, 96.5, 66.6, 55.1, 45.4, 41.9, 40.3, 37.7, 31.5, 31.0, 30.9, 30.3, 29.7, 29.4, 26.5, 26.4, 25.8, 24.1, 13.7; IR ν_{max} 2922, 2235, 1451, 1110, 1040 cm^{-1}. Anal. ($C_{19}H_{31}NO_2$) C, H, N.

(1*S*,3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (2a), (1*S*,3a*S*,4a*S*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (2b), (1*S*,3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (2c), and (1*S*,3a*S*,4a*R*,6*S*,8a*R*,9a*S*)-Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]indene-1-carbonitrile (2d). Compound **12a** (73 mg, 0.24 mmol) was dissolved in a 7:2 mixture of MeOH/CH_2Cl_2 (5 mL). To this concentrated HCl (1 mL) was added. The reaction was stirred at room temperature for 2.5 h, at which time the methanol was removed in vacuo. The yellow oil was then redissolved in EtOAc (10 mL), washed with saturated $NaHCO_3$ (3 × 10 mL), H_2O (3 × 5 mL), and brine (3 × 5 mL), and dried, and the solvent was removed. Chromatography (30% EtOAc in hexanes) gave **2a** as a white solid (54 mg, 86%). The same procedure was repeated with **12b** (33 mg, 0.11 mmol), **12c** (60 mg, 0.20 mmol), and **12d** (28 mg, 0.09 mmol) to yield the white solids **2b** (24 mg, 84%), **2c** (44 mg, 86%), and **2d** (20 mg, 83%).

2a: $[\alpha]_D^{25} = +66.3$ ($c = 1.05$, $CHCl_3$); mp 90−92 °C;^{1}H NMR δ 3.69−3.65 (2H, t, $J = 6.0$ Hz), 2.29−2.22 (1H, m), 2.13−2.03 (1H, m), 1.95−1.60 (6H, m), 1.53−1.15 (9H, m), 1.05−0.94 (3H, m), 0.90 (3H, s), 0.84−0.69 (2H, m); ^{13}C NMR δ 121.5, 60.7, 48.4, 44.9, 44.5, 43.7, 40.2, 40.0, 39.8, 38.4, 34.4, 33.7, 33.2, 32.7, 26.4, 26.2, 14.4; IR ν_{max} 3369, 2912, 2235, 1449, 1043 cm^{-1}. Anal. ($C_{17}H_{27}NO$) C, H, N.

2b: $[\alpha]_D^{25} = +74.8$ ($c = 0.95$, $CHCl_3$); mp 94−96 °C; ^{1}H NMR δ 3.67−3.63 (2H, t, $J = 6.0$ Hz), 2.29−2.23 (1H, m), 2.14−2.04 (1H, m), 1.96−1.71 (3H, m), 1.68−1.22 (13H, m), 1.18−0.95 (4H, m), 0.91 (3H, s); ^{13}C NMR δ 121.5, 61.7, 48.5, 44.8, 44.7, 39.9, 39.0, 38.2, 37.5, 34.8, 32.9, 30.1, 29.6, 28.7, 26.3, 26.2, 14.5; IR ν_{max} 3434, 2914, 2235, 1450, 1063 cm^{-1}. Anal. ($C_{17}H_{27}NO$) C, H, N.

2c: $[\alpha]_D^{25} = +149.1$ ($c = 1.01$, $CHCl_3$); mp 97−99 °C; ^{1}H NMR δ 3.68−3.64 (2H, t, $J = 6.0$ Hz), 2.34−2.28 (1H, m), 2.17−1.78 (4H, m), 1.67−1.56 (2H, m), 1.54−1.37 (11H, m), 1.33−1.16 (4H, m), 0.92 (3H, s); ^{13}C NMR δ 121.4, 60.6, 45.5, 42.0, 40.3, 40.1, 37.7, 35.6, 34.8, 33.8, 31.5, 31.3, 30.7, 27.7, 26.4, 26.3, 13.7; IR ν_{max} 3434, 2913, 2235, 1450, 1057 cm^{-1}. Anal. ($C_{17}H_{27}NO$) C, H, N.

2d: $[\alpha]_D^{25} = +88.2$ ($c = 0.42$, $CHCl_3$); mp 122−124 °C; ^{1}H NMR δ 3.68−3.64 (2H, t, $J = 6.0$ Hz), 2.35−2.29 (1H, m), 2.15−2.08 (1H, m), 1.98−1.81 (3H, m), 1.80−1.41 (11H, m), 1.38−1.26 (5H, m), 1.09−1.04 (1H, m), 0.92 (3H, s); ^{13}C NMR δ 121.3, 61.8, 45.4, 42.0, 40.3, 37.7, 34.1, 31.5, 31.0, 30.4, 29.9, 29.5, 26.5, 26.4, 25.8, 24.2, 13.7; IR ν_{max} 3463, 2914, 2239, 1450, 1050 cm^{-1}. Anal. ($C_{17}H_{27}NO$) C, H, N.

(1*S*,3a*S*,4a*S*,6*S*,8a*R*,9a*S*)-1-[Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-yl]ethanone (3a) and (1*S*,3a*S*,4a*R*,6*R*,8a*R*,9a*S*)-1-[Dodecahydro-6-(2-hydroxyethyl)-9a-methyl-1*H*-benz[*f*]inden-1-yl]ethanone (3b). A round-bottom flask equipped with a stir bar and condenser was evacuated and filled with N_2 three times. CH_3MgCl (0.5 mL, 3.0 M in THF) was added, followed by **12a** (92 mg, 0.30 mmol) dissolved in dry THF (5 mL). The reaction was refluxed for 24 h, at which time it was cooled to 0 °C and saturated NH_4Cl (10 mL) was added. The mixture was extracted with EtOAc (3 × 10 mL). The combined organic fractions were washed with brine (2 × 10 mL) and dried, and the solvent was removed. The crude product was dissolved in 7:2 MeOH/CH_2Cl_2 (4 mL), and concentrated HCl (0.8 mL) was added. After the reaction was stirred for 2.5 h, the solvent was removed. The yellow oil was then redissolved in EtOAc (5 mL), washed with saturated $NaHCO_3$ (3 × 5 mL), H_2O (3 × 5 mL), and brine (3 × 5 mL), and dried, and the solvent was removed. The product mixture (1*R*,*S* diastereomers) was separated via HPLC [Beckman Ultrasphere, 5 μm silica column, 250 mm × 10 mm, EtOAc/hexane/$ClCH_2CH_2Cl$ (4:6:10), 3 mL/min] to give the white solid **3a** (29 mg, 35%). The same procedure was repeated on **12c** (40 mg, 0.13 mmol) to yield the colorless oil **3b** (11 mg, 31%).

3a: $[\alpha]_D^{25} = +108.0$ ($c = 1.0$, $CHCl_3$); mp 91−93 °C; ^1H NMR δ 3.70−3.66 (2H, t, $J = 6.0$ Hz), 2.54−2.49 (1H, m), 2.11 (3H, s), 1.87−1.54 (7H, m), 1.46−1.28 (6H, m), 1.27−0.73 (8H, m), 0.59 (3H, s); ^{13}C NMR δ 209.8, 63.5, 60.8, 50.7, 46.6, 44.7, 43.8, 40.4, 40.2, 38.6, 34.4, 34.0, 33.4, 32.6, 31.5, 26.3, 22.7, 13.4; IR ν_{max} 3392, 2911, 1705, 1448, 1053 cm^{-1}. Anal. ($C_{18}H_{30}O_2$) C, H.

3b: $[\alpha]_D^{25} = +152.7$ ($c = 0.37$, $CHCl_3$); ^1H NMR δ 3.72−3.68 (2H, t, $J = 6.0$ Hz), 2.60−2.54 (1H, m), 2.12 (3H, s), 1.99−1.93 (2H, m), 1.78−1.35 (15H, m), 1.32−1.16 (4H, m), 0.62 (3H, s); ^{13}C NMR δ 209.7, 63.8, 60.7, 45.3, 44.1, 40.5, 39.8, 35.7, 34.9, 33.9, 31.7, 31.6, 31.4, 31.0, 27.9, 26.4, 22.7, 12.7; IR ν_{max} 3393, 2916, 1703, 1445, 1054 cm^{-1}. Anal. ($C_{18}H_{30}O_2$) C, H.

[^{35}S]TBPS Binding Methods. The methods used were as described previously.[16]

Xenopus Oocyte Electrophysiological Methods. The methods used were as described previously.[16]

Tadpole Behavioral Methods. The methods used were as described previously.[16]

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Supporting Information Available: Elemental analyses results for target compounds **1a**−**d**, **2a**−**d**, and **3a**,**b** and X-ray crystallographic data and projection views of compounds **8a** and **1c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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