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2-Aryl-3-methyloctahydrophenanthrene-2,3,7-triols as Potent Dissociated Glucocorticoid Receptor Agonists†

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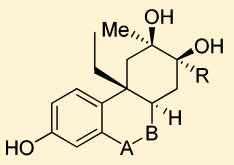
Supporting Information

ABSTRACT: A significant improvement in agonist activity of the previously described 2-aryloctahydrophenanthrene-2,3,7-triol series of dissociated glucocorticoid receptor agonists (DAGRs) was achieved by modifying the substitution at C3 from (*S*)-3-hydroxy to (*R*)-3-hydroxy-3-methyl. The IC_{50} of the prototype **13** in the efficacy assay measuring repression of IL-1 induced MMP-13 expression was 3.5 nM, exhibiting 87% of the maximal effect of dexamethasone (DEX). It displayed a dissociated profile by exhibiting 42% of the maximal effect of DEX in a mouse mammary tumor virus (MMTV) luciferase reporter transactivation assay. Compound **13** and analogues containing heterocyclic replacements for the C2 phenyl and modified B rings showed high repression of TNFα production in human whole blood, with IC_{50} values (43−167 nM) approaching the level of DEX (21 nM). On the basis of X-ray structures and force field calculations, the overall potency of this series was attributed to a favorable conformation of the C2α phenyl, induced by the neighboring C3α methyl.



	R	A-B
13	Ph	CH_2CH_2
22	2-pyridyl	CH_2CH_2
39	2-thiazolyl	CH_2CH_2
40	Ph	$COCH_2$
42	Ph	C(Me)=CH
51	Ph	CH=CH

INTRODUCTION

Glucocorticoid receptor (GR) agonists,[1] such as prednisone (PRED) and dexamethasone (DEX), are among the most effective oral treatments for inflammatory and autoimmune diseases, including rheumatoid arthritis (RA), Crohn's disease, colitis, and multiple sclerosis. They have expanded use in the treatment of asthma and atopic dermatitis as inhalants and topical ointments, respectively, and are also used for treating transplant rejection. Despite their extraordinary efficacy in treating inflammatory diseases, chronic administration of GR agonists results in debilitating side effects.[2] These include diabetes, bone loss, skin thinning, muscle atrophy, and cataracts. Moreover, GR agonists can elicit off-target pharmacology depending upon cross-reactivity with other nuclear hormone receptors, particularly the mineralocorticoid (MR), progesterone (PR), androgen (AR), and estrogen (ERα, ERβ) receptors.

During the past decade new structural classes of GR agonists have emerged with the potential to have improved side effect profiles, as compared with classical steroid agonists.[3a−d,4a,b] The underlying hypothesis for designing these compounds, which we have called "dissociated agonists of the GR" (DAGRs),[5] is evidence that the anti-inflammatory and side-effect activities of ligand-activated GRs occur via dissociated mechanisms—transrepression (TR) and transactivation (TA).[6a,b] According to this hypothesis, the TR pathway is associated with the anti-inflammatory activity of GR agonists and leads to inhibition of transcription factors for genes, including NFκB and AP1, that drive the expression of genes that encode pro-inflammatory proteins, such as IL-1, IL-8, TNFα, and MMP13. On the other hand, the TA pathway activates genes involved in metabolism and endocrine function. In this pathway, the dimeric form of the ligand-activated GR functions as an endogenous transcription factor by binding to specific DNA sequences in the promoter region, called glucocorticoid response elements (GREs). Examples of GRE-driven target genes that may contribute to adverse metabolic and bone effects include tyrosine amino transferase (TAT) and DKK1.[7a,b]

The TR/TA hypothesis, although providing a rationale for discovering safer glucocorticoids, has been criticized as an oversimplification of the complex and unraveling process of gene expression regulation by GR.[8] This is based on recent evidence that is not consistent and even refutes the hypothesis, and that the reporter genes used to measure dissociation are too simplified and unable to effectively target therapeutically relevant genes. Furthermore, targeting functional antagonism rather than TA has been described as an alternative approach of predicting the side effects of GR agonists.[9] Despite this controversy, the screening strategy used herein relies on a measure of intrinsic dissociation, TR/TA in the same SW1353 chondrosarcoma cell background, and having only native GR and associated transcription machinery. This has led to the discovery of a novel pharmacophore that demonstrated a spectrum of TR/TA activities that differed from those of classical GR agonists.[5] Arguably, compounds displaying such dissociated activities represent potential candidates to test for improved safety/efficacy profiles in human. In fact, a recent phase 2 clinical study with DAGR PF-04171327 has shown

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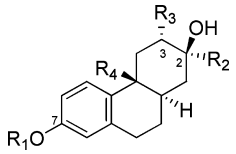
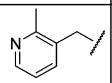
Table 1. Representative Octahydrophenanthrene-2,7-diol Analogues[5]



	R_1	R_2	R_3	R_4	GR Binding IC_{50} (nM)[a]	Transrepression[a,b] MMP-13 IC_{50} (nM) (% max dex)	Transactivation MMTV[a] % max dex
1	H	1-propynyl	H	Allyl	6.2	14 (79)	9
2	H	1-propynyl	H	Et	5.6	14 (86)[c]	6
3	H	1-propynyl	OH	Et	35	63 (79)	13
4	H	Ph	OH	Et	2.5	140 (67)[c,d]	6
5		Ph	OH	Et	1.5	38 (78)[c,e]	6
6	H	Ph	H	Et	21	>5000	1

[a] $n = 1$ (dose response curves run in triplicate), except where otherwise noted. [b] IC_{50}, concentration of test compound that inhibits IL-1 induced MMP-13 production by 50%. [c] Inhibition reversed by co-incubation with RU-486. [d] $n = 7$. [e] $n = 2$ (IC_{50} values = 25 and 50 nM).

similar efficacy to 10 mg of PRED but with effects on bone formation and glucose markers at 5 mg of PRED.[10]

In our first paper, we described the discovery of a novel series of DAGRs containing the octahydrophenanthrene-2,7-diol pharmacophore (**1**−**5**, Table 1).[5] These analogues functioned as agonists in in vitro assays of TR (inhibition of IL-1 induced IL-8 and MMP-13 expression) but possessed reduced capacity for TA in an assay measuring activation of a mouse mammary tumor virus (MMTV) reporter construct, as compared to DEX. In addition, we outlined our early optimization efforts on this series focusing on replacing the allyl (R_4) and particularly the propynyl (R_2) substituents with those more likely to be chemically stable and less likely to produce toxic metabolites. The culmination of this effort was the identification of compounds **4** and **5**, in which the C4a allyl and C2 1-propynyl groups of **1** were replaced with ethyl and phenyl, respectively.

In the discovery of phenyl as a replacement for propynyl at the C2α position, a key structural modification was the introduction of the C3α hydroxyl group. This change was beneficial in three ways: the lipophilicity (clogP) of the octahydrophenanthrene template was reduced; receptor selectivity was increased by reducing binding to ER; and, most importantly, agonist activity of C2α phenyl analogues, which was otherwise lacking, (e.g., **6**), was restored. We speculated that the ability of the C3α hydroxyl to impart agonist activity to analogues with a C2α phenyl is due to a neighboring steric interaction, which stabilizes a particular range of rotamers of the C2α phenyl that is favorable for TR. This conjecture was based, in part, on the fact that introduction of a C3α hydroxyl in the C2α 1-propynyl series (i.e., **3** vs **2**) did not improve agonist potency. In this case, no steric effect is possible because the propynyl is cylindrically symmetric, and hence all rotation is degenerate.

On the basis of our working hypothesis regarding the role of the C3α hydroxyl group in determining the functional agonist potency of C2α phenyl analogues, we sought to explore further structural modifications at C3α with the immediate goal of increasing potency against TR, while also minimizing GR signaling via TA. To accomplish this, we first needed to develop a more efficient and versatile synthesis to allow wider variation of substitutions at C3.[11] We now report a new and efficient synthesis of C2α phenyl analogues containing *alkyl* substitution at C3α, leading to new DAGRs with significantly improved functional potency in both cell-free and whole blood assays, which approaches the level of DEX. We also provide further support for our hypothesis regarding the relationship between

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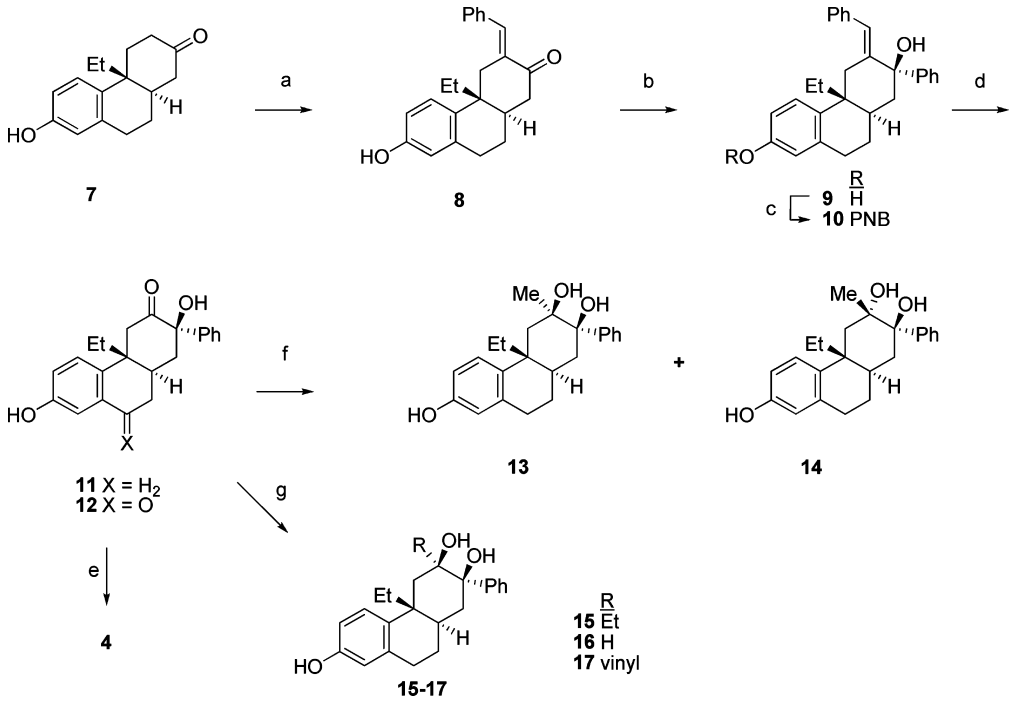
Scheme 1[a]



[a](a) PhCHO, NaOEt, EtOH (81%); (b) PhMgBr, $CeCl_3$, THF, −40 °C (96%); (c) p-$NO_2C_6H_4COCl$, 1 N NaOH, 0 °C (100%); (d) (i) O_3, CH_2Cl_2, MeOH, −78 °C, (ii) DMS, −78 °C to rt, (iii) NaOH, THF, 0 °C (62%, **11**; 11%, **12**); (e) $NaBH_4$, EtOH, THF (95%); (f) MeLi·LiI, THF, −78 °C (72%, **1**; 12%, **14**); (g) EtMgBr or vinylLi, THF, −78 °C (19%, **15**; 18%, **16**; 49%, **17**).

the torsion angle of the C2α phenyl and agonist potency, based on X-ray crystallography and force field calculations.

RESULTS AND DISCUSSION

Chemistry. Our revised synthetic strategy was based on converting the previously described ketone **7**[5] to keto alcohol **11**, a key penultimate intermediate from which new C3α substituents would be introduced directly by organometallic addition to the ketone (Scheme 1). Vital to this approach was the placement of a group at C3 of **7** that would preserve stereoelectronically favored, axial addition of the C2α phenyl to C2 and also serve as a synthon for the C3 ketone of **11**. A planar benzylidine group (i.e., **8**) seemed well-suited for this dual purpose, because it does not contain an α-substituent to interfere with axial attack at C2 and can be easily cleaved by ozonolysis to produce a ketone.

Ketone **7** was condensed with benzaldehyde under thermodynamic conditions to give the desired benzylidine derivative **8**. Addition of excess phenylmagnesium bromide to **8** under Luche conditions occurred stereospecifically from the axial direction to afford the allylic alcohol **9**. The corresponding C2 epimer, the product of equatorial attack, was not detected. Initial attempts to obtain **11** directly from **9** by ozonolysis were accompanied by substantial oxidative degradation of the electron rich phenol (A ring). However, performing the ozonolysis on the *p*-nitrobenzoyl (PNB) derivative **10**, followed by in situ saponification of the PNB group, produced the key intermediate **11** in 62% overall yield from **9**. Diketone **12**, the product of benzylic oxidation, was also isolated in 11% yield. This material proved valuable for the synthesis of B ring analogues, as will be described. The structure of **11** was confirmed by conversion to known analogue **4** by a highly stereoselective directed reduction[12] of the ketone with $NaBH_4$. A small amount (5%) of the corresponding C3 epimer (**16**) was detected by NMR. The structure of **4** was confirmed by X-ray crystallography.

The first analogues were prepared by reacting **11** with MeLi to afford a mixture of C-ring diols **13** and **14** in a ratio of 6:1. These compounds were easily separated by flash chromatography, and their structures were confirmed by X-ray crystallography. In contrast to the $NaBH_4$ reduction of **11**, nucleophilic addition of MeLi occurred predominantly in equatorial fashion, anti to the C2β hydroxyl and syn to the bulkier C2α phenyl. This result is consistent with the model of Rosenberg et al.,[13] in which nucleophilic addition to a cyclohexanone containing an adjacent equatorial dipole group is favored by a trajectory opposite to the negative end of the dipole. In the case of **11**, if deprotonation of the C2 β-hydroxyl had occurred prior to the addition of MeLi, this effect would be amplified because the negative end of the resulting $C-O^-$ dipole would be fully charged. Another factor favoring equatorial addition of MeLi is the avoidance of the 1,3-diaxial interaction with the angular ethyl.

The encouraging activity of analogue **13** led us to increase the size of the C3α substituent by reacting **11** with ethylmagnesium bromide and vinyllithium, respectively, to afford analogues **15** and **17**. In these cases, none of the trans diols were detected, presumably due to the enhanced 1,3-diaxial interaction between the larger incoming nucleophile and the angular ethyl group at C4a. In the reaction with ethylmagnesium bromide, the reduced secondary alcohol **16** (corresponding to the C3 epimer of **4**) was also isolated as the product of β-hydride elimination of the Grignard reagent.[14a,b] The stereospecificity of the reaction is rationalized by a cyclic transition state involving equatorial addition of

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hydride, to minimize the 1,3-diaxial interaction with the angular ethyl (Figure 1).

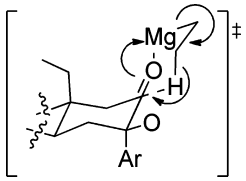


Figure 1. Formation of **16** from **11** by reaction with EtMgBr.

Replacement of the C2α phenyl of analogue **13** by 2-pyridyl was accomplished analogously, although it was first necessary to protect the phenol as the *tert*-butyldimethylsilyl (TBDMS) ether derivative **18** (Scheme 2). Addition of 2-pyridyllithium, generated by transmetalation of 2-bromopyridine, to **18** afforded carbinol **19**, which was converted to PNB ester **20**. Ozonolysis followed by in situ deprotection gave keto alcohol **21**, and subsequent addition of MeLi furnished the desired 2-pyridyl analogue **22**. The corresponding 3- and 4-pyridyl analogues **23** and **24**, respectively, were prepared analogously.

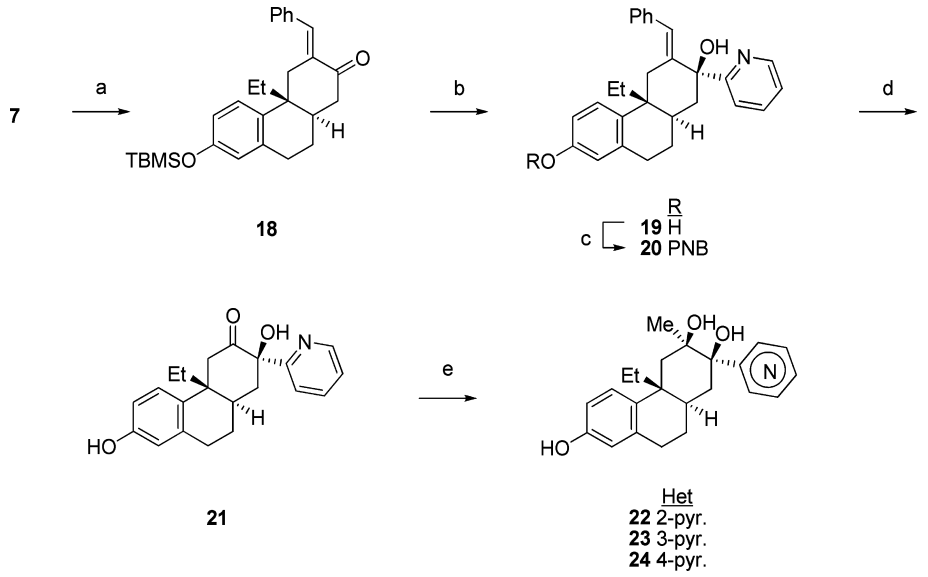
To more efficiently vary the C2α phenyl of analogue **13**, our basic route was modified by reversing the order of installation of the C2α and C3α substituents, respectively, to obtain **29** as an alternative key penultimate intermediate (Scheme 3). Thus, enone **8** was doubly protected by a two-step process involving ketalization to give **25** and subsequent formation of the PNB ester **26**. Ozonolysis of **26** followed by in situ saponification of the PNB group gave the monoprotected α-diketone **27**. The desired penultimate intermediate **29** was then obtained by addition of methyllithium to give carbinol **28**, followed by acidic hydrolysis of the ketal. The stereospecificity of the methyllithium addition from the α-face was explained by the avoidance of the 1,3-diaxial interaction with the angular ethyl.

Reaction of **29** with eight organometallic reagents produced analogues **30**–**37**, respectively. Although the reactions were not generally stereospecific, in most cases the desired 2α-alkyl epimer could be separated by flash chromatography. When separation was not possible, the analogues were tested as mixtures with the ratios determined by NMR. The unexpected erosion in axial selectivity to give mixtures of 2α and 2β alkyl products (vis-à-vis **8**) was explained by initial complexation of the organometallic reagent with the axial 3β hydroxyl leading to equatorial attack. This route provided the theoretically interesting 2α-propynyl analogue **36**, which otherwise would be difficult to obtain by the initial route due to potential oxidation of the triple bond in the ozonolysis step (Scheme 1). Finally, the corresponding 2-thiazolyl analogue **39** was prepared by protection of **29** as the TBDMS protected phenol derivative **38**, followed by addition of 2-lithiothiazole.

Two analogues of **13** containing modifications in the B-ring were initially obtained by treating diketone **12** with excess methyllithium (Scheme 4). Addition occurred completely at the C3 ketone and partially at the C9 ketone (with concomitant dehydration) to provide two pairs of diastereomers epimeric at C3: C9 ketones **40** and **41** and 9-methyl-Δ^9 olefins **42** and **43**, respectively. The four products were easily separable by reverse phase HPLC, and we were able to obtain pure samples of the desired 9-keto and 9-methyl-Δ^9 analogues, **40** and **42**, respectively, corresponding to the C3 stereochemistry of **13**.

The corresponding 9β-hydroxy and Δ^9 analogues, **47** and **51**, respectively, were synthesized from **40** (Scheme 5). First, a more efficient method was developed to prepare **40**, which involved direct benzylic oxidation of **44**, the PNB derivative of **13**, by ozonolysis. Formation of the benzoate derivative **45** and subsequent reduction with $NaBH_4$ gave the 9β-hydroxy derivative **46**,[15] which was saponified to afford analogue **47**. For the synthesis of **51**, the diol of **45** was first protected as the *p*-methoxyphenyl (PMP) acetal to give **48**. The trans relationship between the PMP group in the acetal ring and the angular Me and Ph groups was tentatively assigned by assuming thermodynamic control. Reduction of **48** with $NaBH_4$ furnished alcohol **49**,[15] which underwent dehydration with

Scheme 2[a]



[a](a) TBDMSCl, imidazole, CH_2Cl_2; (b) (i) bromopyridine, *n*-BuLi, THF, −78 °C, (ii) enone **18** addition, warm to 0 °C, (iii) TBAF; (c) p-$NO_2C_6H_4COCl$, 1 N NaOH, 0 °C; (d) (i) O_3, CH_2Cl_2, MeOH, −78 °C, (ii) DMS, −78 °C to rt, (iii) NaOH, THF, 0 °C; (e) MeLi, dimethoxyethane, THF, −78 °C.

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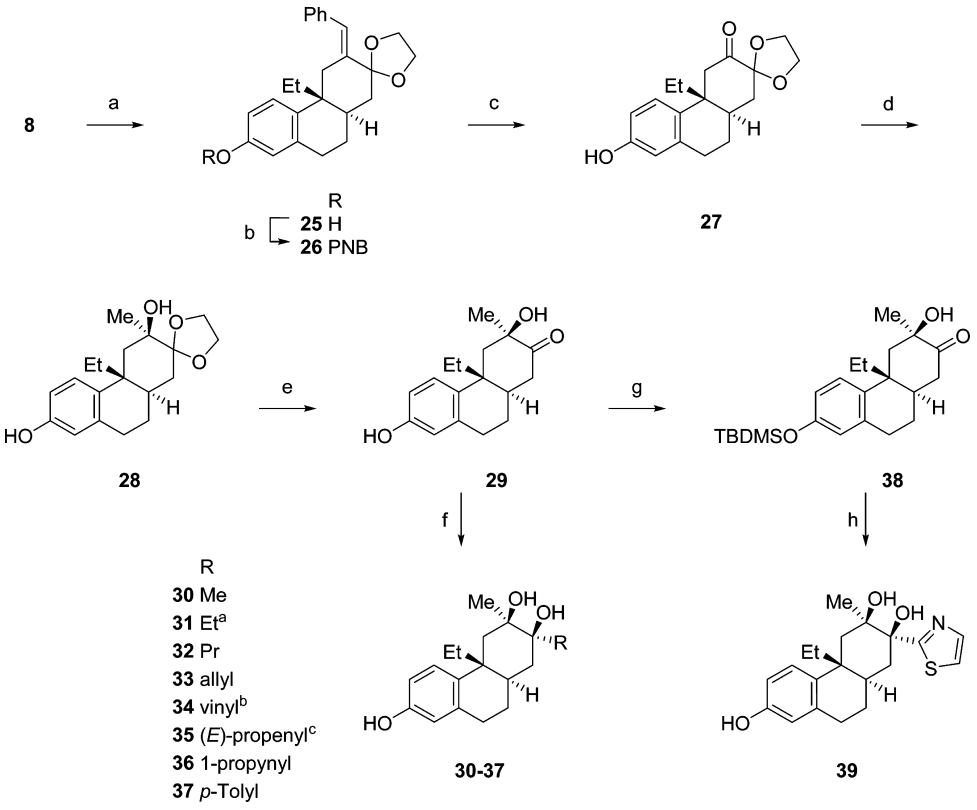
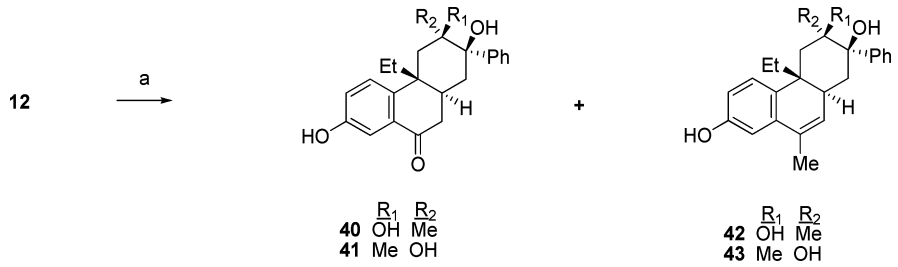
Scheme 3[d]



[a]Contains 25% of β-isomer; [b]Contains 50% of β-isomer; [c]Contains 20% of β-isomer. [d](a) $HOCH_2CH_2OH$, TsOH, toluene, Δ (74%); (b) PNBCl, 1 N NaOH, acetone, 0 °C (74%); (c) (i) O_3, MeOH, CH_2Cl_2, −78 °C, (ii) DMS, (iii) NaOH, THF (98%); (d) MeLi·LiBr, THF, −78 °C (97%); (e) 6 N HCl (87%); (f) RLi or RMgBr, −78 to −30 °C (43%, **30**; 18%, **31**; 6%, **32**; 44%, **33**; 24%, **34**; 28%, **35**; 13%, **36**; 15%, **37**); (g) TBDMSCl, imidazole, CH_2Cl_2 (68%); (h) (i) 2-bromothiazole, *n*-BuLi, THF, −78 °C, (ii) TBAF, THF (43%).

Scheme 4[a]



[a](a) MeLi·LiBr, THF, toluene, 0 °C (28%, **40**; 3%, **41**; 25%, **42**; 5%, **43**).

Burgess reagent to give **50**. Oxidative cleavage of the PMP acetal and subsequent saponification of the benzoate then afforded the desired Δ^9 analogue **51**.

Discussion. Our first objective was to improve the agonist, or TR, activity of analogue **4**, as measured by repression of IL-1 induced MMP-13 expression while maintaining good dissociation with respect to TA in the MMTV assay. According to our hypothesis, this was to be accomplished by replacing the C3α hydroxyl of **4** with bulkier groups capable of enhancing the steric interaction with the adjacent C2α phenyl, thus stabilizing a new set of C2α phenyl rotamers that are more beneficial toward TR. Our new synthesis readily provided analogues with alkyl substitution at C3α and a hydroxyl at C3β.

The first analogue containing a methyl at C3α (**13**) showed vastly improved agonist activity over the earlier lead **4** (Table 2). The IC_{50} of **13** in the MMP-13 assay improved from 140 nM for **4** to 3.5 nM, exhibiting 87% of the maximal effect of DEX. The corresponding analogue **16** lacking a substituent at C3α was inactive as an agonist, despite excellent GR binding (IC_{50} = 3.8 nM), lending support to our hypothesis regarding the steric effect of the C3α substituent on attenuating agonist potency. Analogue **13** retained the dissociative profile of the octahydrophenanthrene-2,7-diol series, exhibiting half the maximal effect of DEX (42%) in the MMTV assay as compared to the MMP-13 assay. This level of dissociation, however, was not as great as that of **4**.

Replacement of the C3α methyl group of **13** with ethyl (i.e., **15**) resulted in ∼2-fold weaker activity in the binding and MMP-13 assays (IC_{50} = 6 nM). No other alkyl substituents at the C3α position were explored, as it was assumed that methyl was optimal and that further increases in size would not be productive. The vinyl analogue **17** was devoid of MMP-13

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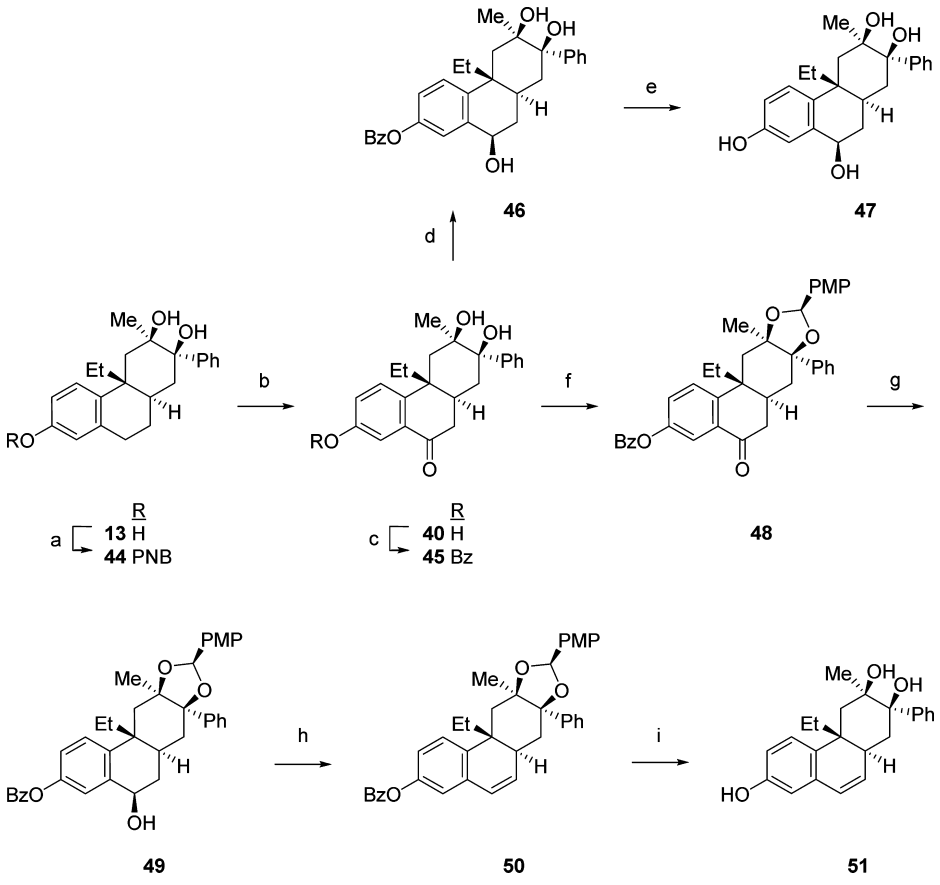
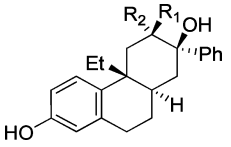
Scheme 5[a]



[a](a) PNBCl, acetone, NaOH (aq); (b) (i) O_3, CH_2Cl_2, MeOH, −30 °C, (ii) DMS, rt; (iii) NaOH; (c) benzoyl chloride, acetone, NaOH; (d) $NaBH_4$, MeOH, THF; (e) NaOH, H_2O, THF; (f) 4-(MeO)C_6H_4CHO, TsOH, benzene, Δ; (g) $NaBH_4$, MeOH, −78 to 0 °C; (h) Burgess salt, benzene, Δ; (i) (i) DDQ, CH_2Cl_2, H_2O, (ii) KOH, EtOH, H_2O.

Table 2. Analogues with Variation at C3



	R_1	R_2	GR binding IC_{50} (nM)[a]	transrepression[a] MMP-13 IC_{50} (nM) (% max DEX)	transactivation[a] MMTV (% max DEX)
13	OH	Me	0.6	3.5 (87)[b]	42
16	OH	H	3.8	>5000[b]	
15	OH	Et	1.2	6 (78)	60
17	OH	vinyl	5.2	>5000	
14	Me	OH	38	>5000	

[a]n = 1 (dose response curves run in triplicate), except where otherwise noted. [b]n = 2.

activity despite good binding (IC_{50} = 5.2 nM). Although similar in length to ethyl, the planar vinyl group is effectively smaller because the α-carbon has one fewer hydrogen to hinder the rotation of the C2α phenyl (−C**H**=CH_2 vs −C**H**$_2$$CH_3$). We presume this induces a different rotamer population that is less beneficial toward agonist activity, as compared to **13**. Analogue **14** (C3 epimer of **13**) containing a C3α hydroxyl was also inactive as an agonist. Comparison of the activity profile of **14** versus **4**, also containing a C3α hydroxyl, suggests that decreased binding resulting from the introduction of the methyl at the C3β-position (IC_{50} = 38 vs 2.5 nM) was a major factor in the abolishment of MMP-13 activity.

With the discovery of the potent analogue **13**, we next explored varying the C2α phenyl to see if further potency increases could be achieved (Table 3). A secondary objective was to lower lipophilicity. Preserving the optimal C2 substitution present in **13**, several analogues containing *p*-tolyl, heteroaryl, alkyl, vinyl, and alkynyl substituents at C2α, respectively, were examined. The *p*-tolyl analogue **37** was 2−3-fold weaker in the binding and MMP-13 assays than **13**, demonstrating that extending the length of the C2α substituent beyond phenyl is not beneficial. In the pyridyl series (**22**−**24**), only the 2-pyridyl analogue **22** showed high potency in the MMP-13 assay (IC_{50} = 1.5 nM), which was 2-fold more potent

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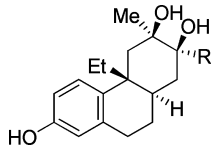
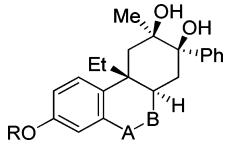
Table 3. Analogues with Variation at C2α



	R	GR binding IC_{50} (nM)	transrepression[a] MMP-13 IC_{50} (nM) (% max DEX)	transactivation[a] MMTV % max DEX	clogP
37	*p*-tolyl	1.3	12 (74)	13	
22	2-Pyr	1.5	1.5 (95)	49	3.0
23	3-Pyr	22	80 (92)	31	
24	4-Pyr	9.1	100 (69)	11	
39	2-Thiaz	0.79	1.5 (93)	53	2.9
30	Me	211	NT		
31	Et[b]	47	>5000		
32	Pr	20.1	>5000		
33	allyl	35.4	>5000		
34	vinyl[c]	12.1	650 (60)	7	
35	(*E*)-1-propenyl[d]	2.1	6 (95)	37	
36	propynyl	1.5	1.9 (89)	43	

[a] $n = 1$ (dose response curves run in triplicate). [b] 3:1 mixture of diastereomers. [c] 1:1 mixture of diastereomers. [d] 4:1 mixture of diastereomers.

Table 4. Analogues with B-Ring Variation



	A–B	R	GR binding IC_{50}[a] (nM)	transrepression[a] MMP-13 IC_{50} (nM) (% max DEX)	transactivation[a] MMTV (% max DEX)	clogP
40	$COCH_2$	H	2.5	5 (87)[b]	41	2.1
47	$CH(OH)CH_2$	H	79	>5000		
51	CH=CH	H	0.3	4.0 (89)	32	4.2
42	C(Me)=CH	H	0.67	0.6 (94)[b]	52	1.8
45	$COCH_2$	Bz	1.7	28 (91)	39	

[a] $n = 1$ (dose response curves run in triplicate), except where otherwise noted. [b] $n = 2$.

than **13**. It was also less lipophilic by 1.5 clogP units. The 2-thiazolyl analogue **39** displayed a similar profile with an excellent combination of agonist potency and relatively low clogP. Both analogues were dissociated, exhibiting about half the maximal effect of DEX in the MMTV assay, as compared to the MMP-13 assay.

None of the analogues with alkyl replacements for the C2α phenyl, **30**–**33**, were active in the MMP-13 assay. The vinyl analogue **34** was weakly active; however, adding a terminal carbon to give the (*E*)-propenyl analogue **35** restored MMP-13 activity to the single-digit nanomolar level (IC_{50} = 6 nM). These results demonstrate that, for optimal activity, the C2α substituent must be attached to C2 by an sp^2 (or sp) carbon and have the length of phenyl (or propynyl).

The C2α propynyl analogue **36** was synthesized to confirm our hypothesis that the main effect of the C2α substituent on agonist potency is to induce a favorable conformation of the adjacent C2α substituent. As predicted, no significant improvement in agonist activity was observed between **36** and the corresponding propynyl analogue **2** lacking substitution at C2, because rotation about the bond connecting C2 and the propynyl substituent is degenerate. The minor 2-fold improvement in MMP-13 activity (IC_{50} = 6 vs 14 nM) was attributed to increased GR binding due to the C3β hydroxyl (IC_{50} = 1.5 vs 5.6 nM). A similar effect was observed between **6** (IC_{50} = 21 nM) and the corresponding C3β hydroxyl analogue **16** (IC_{50} = 3.8 nM).

Modification of the B-ring was explored with the similar goals of improving agonist potency and lowering clogP (Table 4). Compared to **13**, the 9-keto analogue **40** was only slightly weaker in the MMP-13 assay (IC_{50} = 5 nM) but had a considerably lower clogP of 2.1. The corresponding 9-hydroxy analogue **47** was inactive. Placement of an olefinic linkage between C9 and C10 was well tolerated. The corresponding Δ^9 analogue **51** had activity similar to that of **13**, and the change was associated with a modest improvement in dissociation in the MMTV assay (32% max DEX). Moreover, the 9-methyl-Δ^9 analogue **42** was the most potent analogue in the octahydrophenanthrene-2,7-diol series yet discovered with an IC_{50} of 0.6 nM in the MMP-13 assay. However, this compound was slightly less dissociated than **13** (52% max DEX). Finally, the benzoate derivative **45**, tested as an initial probe of the SAR of the C7 phenolic position, was less active in the MMP-13 assay than the corresponding free phenol **40**. Nevertheless, the IC_{50} of 28 nM was respectable and dissociation was retained. This result, together with that of the alkylated phenol derivative **5** from the previous series (Table 1), indicates that further attenuation of activity is possible by modification of the phenol.

An additional in vitro assay was established to measure the efficacy of key analogues in human whole blood (HWB), a more clinically relevant milieu. In this assay, the end point was

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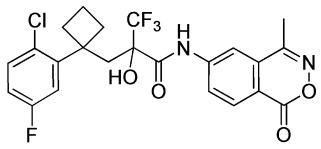
the inhibition of TNFα release induced by lipopolysaccharide (LPS) with DEX used as the positive control. We also tested DAGR analogue **52** (Figure 2) from the trifluoromethylcarbinol series discovered at Schering.[16a,b]



52

Figure 2. Structure of DAGR **52** from the trifluoromethylcarbinol series.[16a,b]

Among the octahydrophenanthrene-2,7-diols, there was a general decline in TR activity in going from the cell-free to the HWB assay, presumably due to protein binding (Table 5). This varied from 14-fold in the case of **40** to 110-fold in the case of **22**. The decline, also seen for DEX (12-fold), did not generally correlate with clogP, although the difference in clogP values was at most not more than 1.8 log units. The most potent analogue was **42** with an IC_{50} of 43 nM, which was only 2-fold weaker than the "gold standard" DEX. The trifluoromethylcarbinol DAGR **52** was weaker than any of the octahydrophenanthrene-2,7-diols in the HWB assay with an IC_{50} of 1000 nM. The large 130-fold decline in activity compared to the cell-free assay was perhaps due to high protein binding, as reflected by the high clogP of 6.7. This compound was also less dissociated than the octahydrophenanthrene-2,7-diols.

Binding assays were performed to determine the affinities of analogue **13**, the prototype of the octahydrophenanthrene-2,7-diol series, and the trifluoromethylcarbinol DAGR **52** for the AR, PR, ERα, and ERβ receptors (Table 6). Analogue **13** was very specific for GR, exhibiting >500-fold selectivity versus AR and PR and even greater selectivity versus ERα and ERβ. On the other hand, **52** was nonselective versus PR but was 100-fold selective versus AR and $>10^5$-selective versus ERα and ERβ.

Finally, we obtained structural information to support our hypothesis regarding the conformation of the C2α phenyl and its effect on agonist efficacy. First, on the basis of the corresponding X-ray structures, the solid state torsion angles of the C2α phenyl (or 2-pyridyl)[17] were determined for key analogues (Table 7). Among those with the same B ring (**13**, **22**, **4**, **14**, and **16**), high agonist potency was associated with narrow torsion angles, −2° and 8°, in which the C2α phenyl and C2α hydroxyl groups were nearly eclipsed (Figure 3). Potency declined as the torsion widened to 44° and was abolished beyond 60°. In the case of the B-ring analogues **42** and **40**, high potency was associated with somewhat larger torsion angles, 13° and 37°, than that of **13**.

Table 6. Affinity of 13 and 52 for Five Steroid Receptors

	binding IC_{50}[a] (nM)				
	GR	AR	PR	ERα	ERβ
13	0.6	300	321	>3200	>3200
52[b]	0.4	40	0.2	$>10^5$	$>10^5$

[a]n = 1 (dose response curves run in triplicate). [b]Tested as the racemate.

Second, Merck Molecular Force Field 94 (MMFF94)[18] calculations were performed on two analogues, **4** and **13**, to obtain a dynamic perspective of the relationship between the torsion angle and agonist activity (Figure 4). These analogues differed only in substitution at C3. The energy profile of analogue **4** containing a C3α hydroxyl showed a steep energy well corresponding to a population of preferred (lowest energy) torsion angles ranging from 40° to 60°. This was consistent with the 44° torsion angle observed in the X-ray structure of **4**. On the other hand, the range of preferred torsion angles for **13** containing a C3α methyl (and C3β hydroxyl), ±5−50°, was broader and shifted toward the eclipsed conformation of 0°. This range of preferred torsion angles was consistent with that observed in the X-ray structures of the more potent analogues (**13**, **22**, **42**, and **40**) containing a C3α methyl.

In summary, both the X-ray data and force field calculations showed a correlation between low torsion angles and high agonist potency, consistent with our original hypothesis. On the basis of the X-ray structures, the optimal torsion angle was between −2° and 37°, although a larger maximum angle of 50° was allowed on the basis of the force field calculations. When the solid state torsion angle was 44°, a transition toward weaker activity occurred, and activity was abolished when the angle reached 60°. These results will be useful for designing more structurally novel analogues.

CONCLUSION

We have shown that simple inversion of the C3α hydroxyl of DAGR **4** and placement of a C3α methyl group result in a significant increase in TR efficacy in vitro. The prototypical analogue **13**, as well as those containing heterocyclic replacements for the C2α phenyl (**22**, **39**) and modifications in the B ring (**40**, **42**, **51**), had low single-digit nanomolar IC_{50} values in the cell-free MMP-13 inhibition efficacy assay. Efficacy in HWB, as measured by inhibition TNFα release, was high with three analogues displaying IC_{50} values <100 nM, or within 2−4-

Table 5. Cell-free and Human Whole Blood TR Activity of Key Analogues Compared to Dexamethasone

	GR binding IC_{50} (nM)	transrepression MMP-13 IC_{50} (nM) (% max DEX)	transactivation MMTV (% max DEX)	transrepression HWB-TNFα IC_{50}[a] (nM) (% max DEX)	clogP
13	0.6	3.5 (87)	42	109 (85)	4.5
22	1.5	1.5 (95)	49	167 (79)	3.0
39	0.79	1.5 (93)	53	83 (88)	2.9
40	2.5	5 (87)	41	72.5 (74)	3.5
42	0.67	0.6 (94)	52	43 (82)	4.7
51	0.3	4.0 (89)	32	400 (60)	4.2
52	3.2	7.5 (98)	66	1000 (52)	6.7
DEX	3.5	1.8	100	21 (100)	

[a]n = 1 (dose response curves run in triplicate).

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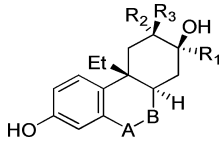
Table 7. Correlation between the Solid State Torsion Angle of the C2α Phenyl and TR Efficacy



	R^1	R^2	R^3	A–B	torsion angle (deg)	transrepression MMP-13 IC_{50} (nM)
13	Ph	Me	OH	CH_2CH_2	−2	3.5
22	2-Pyr	Me	OH	CH_2CH_2	8	1.5
4	Ph	OH	H	CH_2CH_2	44	140
14	Ph	OH	Me	CH_2CH_2	60	>5000
16	Ph	H	OH	CH_2CH_2	77	>5000
42	Ph	Me	OH	C(Me)=CH_2	13	0.6
40	Ph	Me	OH	$COCH_2$	37	5

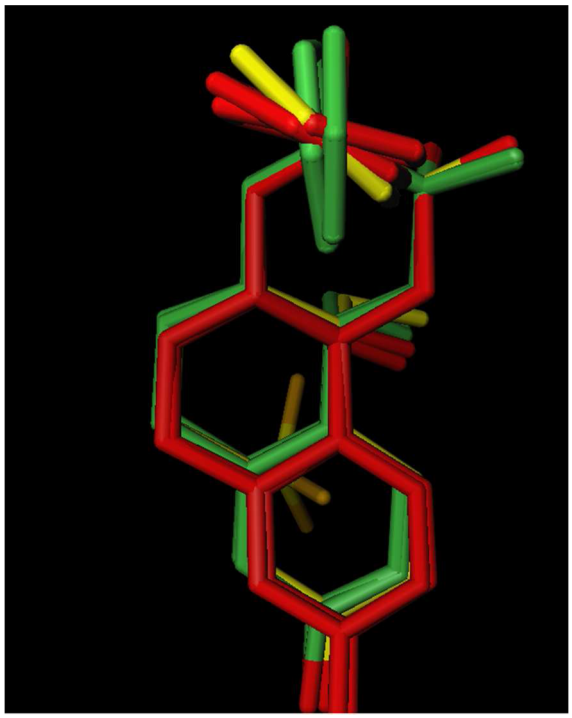


Figure 3. Overlay of the X-ray structures of **13**, **22**, **4**, **14**, and **16**, in counterclockwise order of the orientation of the C2α phenyl, respectively. The color code represents analogues with high (green), moderate (yellow), and minimal (red) agonist potency.

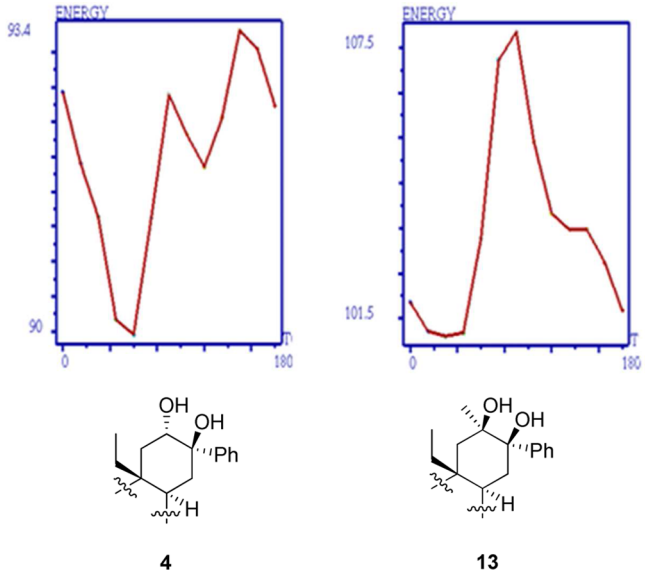


Figure 4. Energy profiles (MMFF94) of **4** and **13** with respect to the torsion angle of the C2α phenyl, defined as 0° when the phenyl is eclipsed to the C2α hydroxyl.

fold of the level of DEX (IC_{50} = 21 nM). These compounds maintained the dissociated profile of a DAGR, exhibiting 1.8–2.8-fold less activity in the MMTV assay relative to DEX (max). Analogue **13** showed minimal affinity for the AR, PR, ERα, and ERβ receptors. Compared to a potent DAGR analogue **52** from the structurally distinct trifluoromethylcarbinol series, the series based on 2-phenyl-3-methyloctahydrophenanthrene-2,3,7-triol **13** has clear advantages with respect to in vitro dissociation, activity in HWB, and affinity for the AR and PR receptors. On the basis of the X-ray structures of key analogues and MMFF94 force field calculations, high agonist potency was correlated with a low torsion angle of the C2α phenyl. Preliminary SAR indicated that potency and physical–chemical parameters can be further attenuated by modification of the C3 phenol, and this has guided subsequent efforts.

Due to its high agonist potency and spectrum of activity, this series is a promising starting point for the identification of developmental candidates through optimization of side-effect dissociation. In fact, potent analogues containing substitutions at the angular ethyl (C4b) and C7 phenolic positions with >99% antagonist activity have been reported and display dissociative activity of target genes in human cell lines.[19]

EXPERIMENTAL SECTION

Chemistry. Reagents were purchased commercially and used without further purification unless otherwise indicated. Reactions were performed under a nitrogen atmosphere, magnetically stirred, and run at room temperature except when otherwise indicated. Solutions of sodium bicarbonate, ammonium chloride, and sodium chloride (brine) were aqueous. Analytical thin-layer chromatography (TLC) was carried out using Merck silica gel 60 F_{254} plates on which compounds were visualized using ultraviolet (UV) light and ceric ammonium molybdate stain. Flash chromatography under medium pressure was performed with Baker silica gel (40 μm) packed manually in glass columns or using a Biotage Flash 40 with 12M, 40S, or 40M columns. In addition, automated flash chromatography was performed using an Isco CombiFlash. Preparative reverse phase chromatography was performed using a Waters Symmetry C8 column

^{1}H NMR spectra were recorded in $CDCl_3$, CD_3OD, or DMSO-d_6 solutions on a Varian Unity 400 or 500 MHz spectrometer. Significant chemical shifts are reported in parts per million (δ) relative to residual $CHCl_3$ (7.24 ppm), DMSO (2.49 ppm), or CD_3OD (3.31 ppm) as

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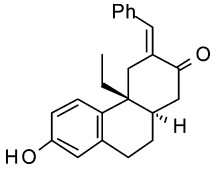
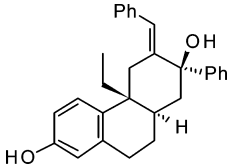
internal reference, respectively. Coupling constants (J) are reported in hertz (Hz). The peak shapes are denoted as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Multiplets can consist of overlapping peaks. Low-resolution liquid chromatography mass spectra (LC-MS) were recorded by positive and negative electrospray ionization using a Waters/Micromass ESI/MS model ZMD/LCZ mass spectrometer equipped with a Gilson 215 liquid handling system and a Hewlett-Packard 1100 diode array detector. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory (Flushing, NY, USA) and Quantitative Technologies, Inc. (Whitehouse, NJ, USA) and are within ±0.4% of the calculated values unless stated otherwise. X-ray crystal structures were collected on a Bruker APEX or Siemens P4 diffractometer.

(3*E*,4a*R*,10a*R*)-3-Benzylidene-4a-ethyl-7-hydroxy-3,4,4a,9,10,10a-hexahydro-1*H*-phenanthren-2-one (8).



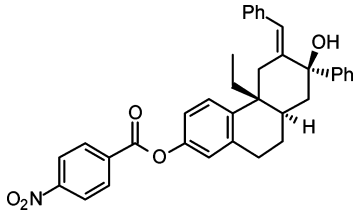
A dry 1 L round-bottom flask was charged with ethanol (500 mL) and cooled to 0 °C. Sodium chunks (3.30 g, 144 mmol) were added in portions, and the mixture was stirred for 1 h. Ketone **7** (14.1 g, 57.5 mmol) was added and the solution stirred for 1 h. A solution of benzaldehyde (6.41 g, 60.4 mmol) in ethanol (50 mL) was added, and the resulting dark red mixture was stirred for 5 h. Additional benzaldehyde (0.91 g, 8.6 mmol) was added, and stirring was continued overnight. The pH was adjusted to 1−2 by the addition of aqueous 1 M hydrochloric acid, and the mixture was diluted with water (1.25 L) and stirred for 1 h. The yellow precipitate was collected by filtration, and the solid (21 g) was stirred in diethyl ether (400 mL) as a suspension for 1 h. The solid was filtered to afford 15.4 g (81%) of title compound **8** as a yellow solid, mp 264−266 °C. An analytical sample was prepared in a previous run by trituration with methanol/THF (1:1), mp 265−267 °C. ^{1}H NMR ($CDCl_3$): δ 0.53 (3H, t, J = 7.5), 1.42−1.55 (2H, m), 1.64−1.72 (1H, m), 1.78−1.90 (1H, m), 2.26−2.34 (1H, m), 2.43−2.51 (2H, m), 2.67 (1H, dd, J = 5.6 and 18.9), 2.82−2.96 (2H, m), 3.68 (1H, d, J = 15.8), 4.70 (1H, br s), 6.59 (1H, d, J = 2.5), 6.64 (1H, dd, J = 2.9 and 8.3), 7.11 (1H, d, J = 8.3), 7.33−7.39 (1H, m), 7.43−7.44 (4H, m), 7.67 (1H, d, J = 2.1). LC-MS: m/z 333.4 $[M + H]^+$. Anal. Calcd for $C_{23}H_{24}O_2$: C, 83.10; H, 7.28. Found: C, 82.83; H, 7.52.

(2*S*,3*E*,4a*R*,10a*R*)-3-Benzylidene-4a-ethyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,7-diol (9).



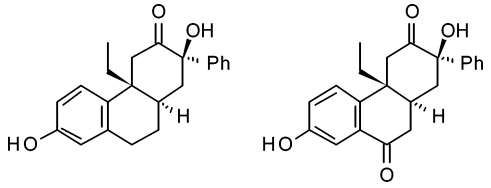
A dry, three-neck, 2 L round-bottom flask was charged with $CeCl_3$ (31.5 g, 128 mmol) and THF (800 mL). The suspension was stirred for 45 min and then cooled to −78 °C. A solution of 1.0 M phenylmagnesium bromide in THF (128 mL, 128 mmol) was added, and the mixture was stirred for 1.5 h. A suspension of ketone **8** (8.50 g, 25.6 mmol) in THF (250 mL) was added via a wide-bore cannula (−70 °C to −55 °C) and then stirred at −40 °C for 2.5 h. A solution of 10% aqueous acetic acid (0.15 L) was added followed by water (400 mL), and the mixture was allowed to warm to room temperature. The aqueous layer was separated and extracted with ethyl acetate (1 × 500 mL, 1 × 250 mL). The combined organic layers were washed with saturated sodium bicarbonate (250 mL) and brine (300 mL), dried ($MgSO_4$), and concentrated to give a greenish brown paste (12.8 g). This material was combined with 1.9 g (4.6 mmol) of crude material prepared similarly in a previous run, and the contents were purified by flash chromatography (400 g silica gel) using ether/hexanes (30, 50, 70%; 2 L each) as eluent to afford 11.95 g (96%, adjusted) of title compound **9** as a beige foam. ^{1}H NMR ($CDCl_3$): δ 0.11 (3H, t, J = 7.6), 1.13−1.26 (1H, m), 1.57−1.90 (6H, m), 2.05 (1H, t, J = 12.7), 2.57 (1H, d, J = 13.3), 2.74−2.82 (2H, m), 3.63 (1H, d, J = 13.7), 6.43−6.48 (2H, m), 6.75 (1H, d, J = 8.7), 7.21−7.45 (10 H, m), 7.58 (2H, J = 7.9). LC-MS: m/z 393.3 $[M + H - H_2O]^+$, 409.4 $[M - 1]^-$, 455.4 $[M - 1 + H_2CO_2]^-$.

(4b*R*,7*S*,8a*R*,*E*)-6-Benzylidene-4b-ethyl-7-hydroxy-7-phenyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl 4-Nitrobenzoate (10).



In a 1 L round-bottom flask, a stirred solution of phenol **9** (11.96 g, 29.1 3 mmol) in acetone (400 mL) was chilled to 0 °C and treated with 1 N aqueous sodium hydroxide (30.5 mL, 30.5 mmol). After 20 min, a solution of 4-nitrobenzoyl chloride (6.48 g, 34.9 mmol) in acetone (50 mL) was added, and the mixture was stirred for 1 h. Additional 1 N aqueous sodium hydroxide (18.3 mL, 18.3 mmol) and 4-nitrobenzoyl chloride (3.60 g, 19.4 mmol) were added in portions as the course of the reaction was monitored by pH (final pH 7−8). The resulting suspension was filtered, and the filtrate was concentrated under vacuum to a volume of approximately 100 mL. Saturated aqueous $NaHCO_3$ solution (250 mL) was added, and the organic phase was extracted with ethyl acetate (1 × 400 mL and 2 × 200 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to afford 16.5 g (theory = 16.3) of title compound **10** as a yellow foam. ^{1}H NMR ($CDCl_3$): δ 0.14 (3H, t, J = 7.6), 1.20−1.32 (1H, m), 1.63−1.95 (6H, m), 2.03−2.13 (1H, m), 2.61 (1H, d, J = 12.9), 2.85−2.90 (2H, m), 3.68 (1H, d, J = 13.3), 6.81 (1H, dd, J = 2.3 and 8.5), 6.85 (1H, s), 6.96 (1H, d, J = 8.3), 7.22−7.48 (9H, m), 7.59 (2H, d, J = 7.9), 8.30 (4H, s). LC-MS: m/z 542.3 $[M + H - H_2O]^+$.

(2*R*,4a*R*,10a*R*)-4a-Ethyl-2,7-dihydroxy-2-phenyl-1,2,4,4a,10,10a-hexahydrophenanthren-3(9*H*)-one (11) and (2*R*,4a*R*,10a*S*)-4a-Ethyl-2,7-dihydroxy-2-phenyl-1,2,4,4a,10,10a-hexahydrophenanthrene-3,9-dione (12).



A 1 L round-bottom flask was charged with olefin **10** (10.4 g, 18.6 mmol), methanol (300 mL), and dichloromethane (300 mL). After the mixture had cooled to −78 °C, ozone was bubbled into it until a dark blue color persisted. The flask was capped and the solution stirred for 3 h, occasionally purging with ozone to maintain a blue color. The mixture was purged with nitrogen for 5 min, and excess dimethyl sulfide (27.5 mL, 23.1 g, 372 mmol) was added. After warming to room temperature over the course of 1 h, the mixture was concentrated to give an oil (10.9 g), which was dissolved in methanol (500 mL) and cooled in an ice bath. A solution of 1 N aqueous sodium hydroxide (37.2 mL, 37.2 mmol) was added, and the mixture was stirred for 2 h at 0 °C. The mixture was poured onto saturated sodium bicarbonate solution (500 mL), and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (1 × 500 mL, 1 × 300 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (1 × 100 mL), brine (1 × 300 mL), dried ($MgSO_4$), and concentrated to give an oil (6.7 g). This material was purified by flash chromatography (350 g silica gel) loading using a minimum amount of ethyl ether (25 mL) and eluting with ethyl ether/hexanes (40, 50, 60, 100%) followed by 100% ethyl acetate. The less polar fractions were evaporated to give 3.9 g (62%) of title ketone **11** as a white solid, mp 97−98 °C. ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, t, J =

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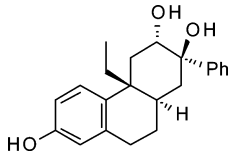
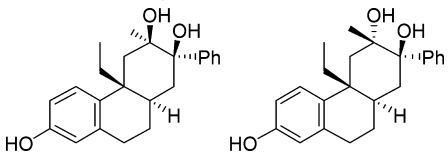
7.5), 1.39−1.50 (2H, m), 1.58 (2H, br s), 1.82−1.92 (2H, m), 2.06 (1H, t, J = 13.9), 2.18−2.30 (1H, m), 2.35 (1H, d, J = 12.8), 2.84−2.92 (3H, m), 3.21 (1H, J = 12.8), 6.50−6.57 (2H, m), 6.81 (1H, d, J = 8.3), 7.24−7.36 (5H, m). LC-MS: m/z 319.4 $[M + 1 - H_2O]^+$, 381.4 $[M - 1 + H_2CO_2]^-$. Anal. Calcd for $C_{22}H_{24}O_3$: C, 78.54; H, 7.16. Found: C, 78.50; H 7.46. The more polar fractions were evaporated to give 0.7 g (11%) of title diketone **12** as a white solid. ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, t, J = 7.3), 1.39−1.50 (1H, m), 1.46−1.82 (3H, overlapping m and br s), 2.17 (1H, t, J = 13.5), 2.46 (1H, d, J = 12.8), 2.69−2.83 (3H, m), 2.89 (1H, d, J = 13.3), 3.28 (1H, d, J = 12.9), 6.93−7.02 (2H, m), 7.21−7.40 (5H, m), 7.49 (1H, d, J = 2.5). LC-MS: m/z 349.3 $[M - 1]^-$, 395.4 $[M - H + H_2CO_2]^-$.

(2*R*,3*S*,4a*R*,10a*R*)-4a-Ethyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (4).



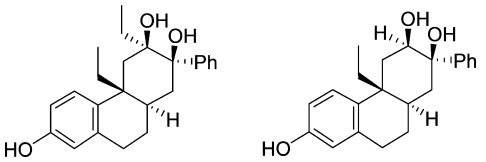
A 30 mL round-bottom flask was charged with ketone **11** (75 mg, 0.22 mmol), THF (5 mL), and ethanol (10 mL). Solid $NaBH_4$ (17 mg, 0.50 mmol) was added, and the mixture was stirred for 2 h. Additional $NaBH_4$ (15 mg, 0.40 mmol) was added, and stirring was continued for 2 h. A solution of 1 N hydrochloric acid (10 mL) and water (40 mL) were added, and the mixture was extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to afford a white solid (78 mg, 100%). The ^{1}H NMR ($CDCl_3$) spectrum showed two isomers (95:5) with the major isomer corresponding to title compound **4**. The minor isomer corresponded to compound **16** (vide infra). The structure of **4** was confirmed by single-crystal X-ray analysis of another sample which had been purified by chromatography and crystallized out of $CDCl_3$ as star needles, mp 191−192 °C. ^{1}H NMR (DMSO-d_6): δ 0.68 (3H, t, J = 7.3), 1.09−1.12 (1H, m), 1.25−1.49 (2H, m), 1.56−1.70 (1H, m), 1.72−1.80 (2H, m), 1.96 (1H, dd, J = 2.9 and 13.7), 2.39 (1H, dd, J = 3.7 and 13.3), 2.54−2.74 (2H, m), 3.83−3.89 (1H, m), 4.82 (1H, s), 4.86 (1H, d, J = 3.3), 4.91 (1H, s), 6.36 (1H, d, J = 2.5), 6.42 (1H, dd, J = 2.7 and 8.4), 6.84 (1H, d, J = 8.3), 7.08−7.12 (1H, m), 7.15−7.21 (2H, m), 7.70 (2H, d, J = 7.5), 8.95 (1H, s). LC-MS: m/z 321.3 $[M + H - H_2O]^+$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (13) and (2*R*,3*S*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (14).



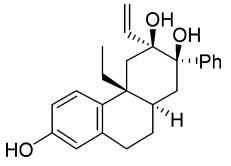
A 1- L round-bottom flask was charged with ketone **11** (6.95 g, 20.7 mmol) and THF (700 mL). The solution was cooled to 0 °C, and a solution of 1 M methyllithium−lithium iodide complex in diethyl ether (100 mL, 100 mmol) was added dropwise. The mixture was allowed to warm to room temperature and then stirred overnight. To the resulting light green mixture was added a solution of 10% aqueous acetic acid (100 mL) followed by water (200 mL). The organic layer was separated and the aqueous layer extracted with ethyl acetate (2 × 300 mL). The combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$), and concentrated. The oily residue (8.2 g) was purified by flash chromatography (dry loaded onto 400 g of silica gel) eluting with ethyl ether/hexanes (30−100% gradient in 10% increments). Evaporation of the less polar fractions afforded 5.2 g (72%) title compound **13** as a white solid. ^{1}H NMR (CD_3OD): δ 0.78 (3H, t, J = 7.1,7.5), 1.12 (3H, s), 1.15−1.31 (1H, m), 1.33−1.40 (1H, m), 1.42 (1H, d, J = 12.8), 1.75−1.92 (3H, m), 2.23−2.37 (2H, m), 2.68−2.80 (3H, m), 6.50 (1H, s), 6.53 (1H, d, J = 8.7), 6.99 (1H, d, J = 8.3), 7.12−7.18 (1H, m), 7.19−7.24 (2H, m), 7.59 (2H, d, J = 7.9). LC-MS: m/z 351.4 $[M - H]^-$, 381.4 $[M - OH + H_2CO_2]^-$. The analytical sample was prepared from a previous sample by crystallization from ethyl ether/hexanes, mp 197−199 °C. Anal. Calcd for $C_{23}H_{28}O_3$: C, 78.38; H, 8.01. Found: C, 78.03; H, 7.91. The structure was confirmed by single-crystal X-ray analysis of a sample that had been crystallized first from chloroform and then recrystallized from ethyl ether/hexanes. Evaporation of the more polar fractions gave title compound **14** as a white solid, 0.90 g (12%). ^{1}H NMR (DMSO-d_6): δ 0.65 (3H, t, J = 7.6), 1.10−1.22 (2H, m), 1.22 (3H, s), 1.28−1.35 (1H, m), 1.55−1.72 (3H, m), 1.78−1.91 (3H, m), 2.52−2.69 (2H, m), 4.12 (1H, s), 4.73 (1H, s), 6.31 (1H, d, J = 2.5), 6.42 (1H, dd, J = 2.5 and 8.7), 6.82 (1H, d, J = 8.3), 7.05 (1H, t, J = 7.3), 7.14 (2H, t, J = 7.4), 7.68 (2H, d, J = 7.5), 8.89 (1H, s). LC-MS: m/z 351.4 $[M - H]^-$, 397.4 $[M - H + H_2CO_2]^-$. The analytical sample was prepared from a previous sample by recrystallization from ethyl ether/hexanes, mp 228−229 °C. Anal. Calcd for $C_{23}H_{28}O_3$: C, 78.38; H, 8.01. Found: C, 78.17; H, 8.29. The structure was confirmed by single-crystal X-ray analysis.

(2*R*,3R,4a*R*,10a*R*)-3,4a-Diethyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (15) and (2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (16).



A dry 20 mL round-bottom flask was charged with ketone **11** (100 mg, 0.30 mmol), lithium chloride (94 mg, 2.2 mmol), and toluene (5 mL). A solution of 1 M ethylmagnesium bromide in THF (2.2 mL, 2.2 mmol) was added, and the mixture was stirred overnight at room temperature. The suspension was treated with 10% aqueous acetic acid and then combined with additional material from a previous reaction run identically (0.45 mmol of **11**). Water (30 mL) was added, and the mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with saturated sodium bicarbonate and brine, dried ($MgSO_4$), and concentrated to give an oil (0.29 g). Purification was performed by flash chromatography (40 g silica gel) eluting with ethyl acetate/hexanes (10, 25, and 35%, 400 mL each). The less polar fractions were pooled and concentrated to afford 51 mg (19%) of title analogue **15** as a foam. ^{1}H NMR (DMSO-d_6): δ 0.67 (3H, t, J = 7.1), 0.79 (3H, t, J = 7.6), 1.09−1.36 (5H, m), 1.49 (1H, d, J = 15.0), 1.62−1.77 (2H, m), 2.10−2.23 (2H, m), 2.58−2.74 (3H, m), 3.67 (1H, s), 5.22 (1H, s), 6.36 (1H, d, J = 2.5), 6.45 (1H, dd, J = 2.5 and 8.3), 6.91 (1H, d, J = 8.7), 7.09 (1H, t, J = 7.3), 7.18 (2H, t, J = 7.5), 7.49 (2H, d, J = 7.5), 8.93 (1H, s). LC-MS: m/z 365.4 $[M - H]^-$, 411.4 $[M - H + H_2CO_2]^-$. The more polar fractions were pooled and concentrated to afford 45 mg (18%) of title alcohol **16** as a foam, which was crystallized from ethyl ether/hexanes, mp 207−209 °C. ^{1}H NMR ($CDCl_3$ + drop of CD_3OD): δ 0.83 (3H, t, J = 7.3), 1.19−1.23 (3H, m), 1.22−1.30 (1H,m), 1.42 (1H, d, J = 13.5), 1.61−1.72 (2H, m), 1.88−2.00 (1H, m), 2.07 (1H, d, J = 13.5), 2.20−2.33 (2H, m), 2.75−2.88 (3H, m), 4.56 (1H, br s), 6.50−6.54 (2H, m), 6.94 (1H, d, J = 8.3), 7.25−7.35 (3H, m), 7.52 (2H, d, J = 7.3). LC-MS: m/z 321.4 $[M + H - H_2O]^+$, 383.4 $[M - 1 + H_2CO_2]^-$. The structure of **16** was confirmed by single-crystal X-ray analysis.

(2*R*,3*S*,4a*R*,10a*R*)-4a-Ethyl-2-phenyl-3-vinyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (17).



To a stirred solution of 1 M vinyl bromide in THF (9 mL, 9 mmol) at −78 °C was added a solution of 1.7 M *tert*-butyllithium in hexane (10 mL, 17 mmol). After stirring for 1 h, the mixture was warmed to 0 °C, and a solution of ketone **11** (0.30 g, 0.89 mmol) in THF (10 mL) was added. The mixture was allowed to warm to room temperature and

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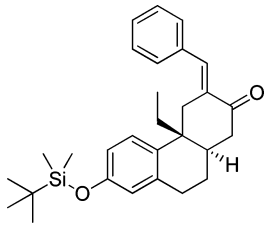
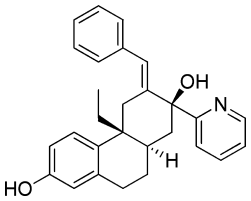
then stirred overnight. The pH was adjusted to 5 by the addition of 10% aqueous acetic acid, and water (30 mL) was added. The mixture was extracted with ethyl acetate (3 × 30 mL), and the combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The oily residue (0.4 g) was purified by flash chromatography (40 g of silica gel) eluting with ethyl ether/hexanes (20−100%) to afford 160 mg (49%) of title compound **17** as an oil. ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, t, J = 7.3), 1.15−1.27 (1H, m), 1.29−1.35 (1H, m), 1.48 (1H, dd, J = 1.6 and 13.0), 1.74−1.84 (2H, m), 1.97 (1H, d, J = 15.0), 2.22−2.32 (2H, m), 2.65−2.74 (3H, m), 3.73 (3H, br s), 5.02 (1H, dd, J = 1.3 and 10.6), 5.37 (1H, dd, J = 1.6 and 17.1), 5.94 (1H, dd, J = 10.9 and 17.1), 6.46 (1H, d, J = 2.6), 6.53 (1H, dd, J = 2.6 and 8.3), 6.97 (1H, d, J = 8.8), 7.12 (1H, t, J = 7.3), 7.17 (2H, t, J = 7.5), 7.51 (2H, d, J = 7.8). LC-MS: m/z 347.1 $[M + H - H_2O]^+$, 363.1 $[M - H]^-$ and 409.0 $[M - H + H_2CO_2]^-$.

(4a*R*,10a*R*,*E*)-3-Benzylidene-7-((*tert*-butyldimethylsilyl)oxy)-4a-ethyl-1,4,4a,9,10,10a-hexahydrophenanthren-2(3*H*)-one (18).



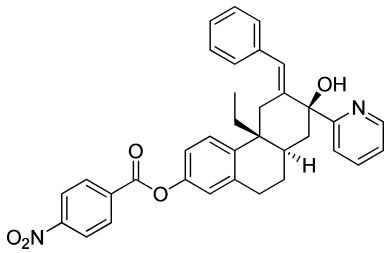
To a solution of enone **7** (10.0 g, 30.0 mmol) in dichloromethane (300 mL) was added imidazole (3.47 g, 51.0 mmol) and *tert*-butyldimethylsilyl chloride (7.69 g, 51.2 mmol). The mixture was stirred overnight at room temperature. An additional 0.33 equiv each of imidazole and *tert*-butyldimethylsilyl chloride was added, and stirring was continued for 8 h. A catalytic amount of 4-dimethylaminopyridine was added, and the mixture was stirred overnight. Aqueous 0.5 N citric acid solution was added, and the organic layer was separated. The aqueous layer was extracted with dichloromethane, and the combined organic layers were washed with water and brine, dried ($MgSO_4$), and concentrated. The residue was triturated with ether to give 1.53 g of recovered **7**. The mother liquor was concentrated, and the residue was purified by flash chromatography using an Isco CombiFlash eluting with ethyl acetate/hexanes (0−30%). The desired fractions were pooled and concentrated to give 6.59 g (49%) of title compound **18** as a waxy solid. ^{1}H NMR ($CDCl_3$) δ 0.18 (6H, s), 0.52 (3H, t, J = 7.5), 0.96 (9H, s), 1.43−1.51 (2H, m), 1.62−1.71 (1H, m), 1.78−1.91 (1H, m), 2.28−2.35 (1H, m), 2.43−2.51 (2H, m), 2.66 (1H, dd, J = 5.8 and 18.7), 2.79−2.96 (2H, m), 3.68 (1H, d, J = 15.8), 6.58 (1H, d, J = 2.5), 6.62 (1H, dd, J = 2.7 and 8.5), 7.06 (1H, d, J = 8.7), 7.35−7.42 (1H, m), 7.42−7.45 (4H, m), 7.65 (1H, d, J = 2.9). LC-MS: m/z 447.1 $[M + H]^+$.

(2*S*,4a*R*,10a*R*,*E*)-3-Benzylidene-4a-ethyl-2-(pyridin-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,7-diol (19).



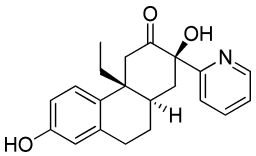
A solution of 2-bromopyridine (23.3 g, 148 mmol) in THF (500 mL) was chilled to −78 °C and treated dropwise with a solution of 2.5 M butyllithium (58.0 mL, 145 mmol) in hexanes. When the addition was complete, the mixture was stirred for an additional 30 min, and a solution of intermediate **18** (6.59 g, 14.8 mmol) in THF (150 mL) was added. Stirring at −78 °C was continued for 4 h, and the mixture was quenched by the addition of water (200 mL). The aqueous layer was extracted twice with ethyl acetate, and the combined extracts were washed with water and brine, dried ($MgSO_4$), and concentrated to give 13.3 g of a red oil. This material was dissolved in THF (230 mL), cooled to 0 °C, and treated with a solution of 1.0 M tetrabutylammonium fluoride (23 mL, 23 mmol) in THF. After stirring for 4 h, the mixture was filtered through a plug of silica gel and concentrated. The residue was purified by flash chromatography using an Isco CombiFlash eluting with ethyl acetate/hexanes (30−65%) followed by an ethyl acetate flush. The desired fractions were pooled and concentrated to give 3.76 g (62%) of title compound **19** as a yellow solid. ^{1}H NMR (CD_3OD): δ 0.09 (3H, t, J = 7.5), 1.16−1.25 (1H, m), 1.56−1.81 (5H, m), 1.88 (1H, t, J = 12.5), 2.70−2.76 (2H, m), 2.87 (1H, d, J = 10.8), 3.68 (1H, d, J = 13.7), 6.36−6.40 (2H, m), 6.66 (1H, d, J = 8.3), 7.19−7.27 (3H, m), 7.32−7.42 (4H, m), 7.64 (1H, d, J = 7.9), 7.74−7.79 (1H, m), 8.51−8.52 (1H, m). LC-MS: m/z 412.3 $[M + H]^+$.

(4b*R*,7*S*,8a*R*,*E*)-6-Benzylidene-4b-ethyl-7-hydroxy-7-(pyridin-2-yl)-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl 4-Nitrobenzoate (20).



A suspension of intermediate **19** (2.27 g, 5.52 mmol) in acetone (60 mL) was cooled to 0 °C and treated with a solution of 1 M sodium hydroxide (5.50 mL, 5.50 mmol). After the solids dissolved, *p*-nitrobenzoyl chloride (1.19 g, 6.41 mmol) was added. The mixture was stirred for 2.5 h and then diluted with saturated sodium bicarbonate solution and extracted twice with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), and concentrated to give 2.83 g (92%) of title compound **20** as a white foam. ^{1}H NMR ($CDCl_3$): δ 0.24 (3H, t, J = 7.5), 1.27−1.33 (1H, m), 1.65 (1H, br s), 1.70−1.78 (3H, m), 2.83−2.91 (3H, m), 2.84 (1H, dd, J = 2.6 and 12.9), 2.85−2.91 (2H, m), 3.79 (1H, d, J = 13.7), 6.88 (1H, dd, J = 2.6 and 8.3), 6.91 (1H, d, J = 2.4), 7.04 (1H, d, J = 8.3), 7.22−7.45 (7H, m), 7.58 (1H, d, J = 7.9), 7.69−7.71 (1H, m), 8.34 (4H, s), 8.63 (1H, dd, J = 0.7 and 1.7). LC-MS: m/z 560.9 $[M + H]^+$.

(2*R*,4a*R*,10a*R*)-4a-Ethyl-2,7-dihydroxy-2-(pyridin-2-yl)-1,4,4a,9,10,10a-hexahydrophenanthren-3(2*H*)-one (21).



Compound **20** (3.70 g, 6.60 mmol) was dissolved in a mixture of dichloromethane (200 mL) and methanol (100 mL), and the contents were chilled to −78 °C and treated with a solution of 6 N hydrochloric acid (1.25 mL, 7.5 mmol). Ozone was bubbled into the mixture until saturated, as indicated by the persistence of a blue color. Stirring was continued until TLC analysis indicated the consumption of starting material. The mixture was purged with oxygen, and then dimethyl sulfide (10 mL) was added. The mixture was allowed to warm to room temperature and concentrated. The solid residue was dissolved in THF (40 mL), and a solution of 1 N sodium hydroxide (20 mL, 20 mmol) was added. The mixture was stirred overnight, neutralized with 1 N hydrochloric acid solution, and extracted twice with ethyl acetate. The combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$), and concentrated to give 2.4 g of a white solid. This material was triturated with ether/hexanes to give 1.38 g (62%) of title compound **21** as a white solid. Additional product (0.58 g, 26%) was obtained by chromatography of the concentrated mother liquor using an Isco CombiFlash eluting with ethyl acetate/hexanes (10−70%). ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, t, J = 7.5), 1.40−1.50 (2H, m), 1.65 (1H, br s), 1.78−1.90 (2H, m), 2.08 (1H, t, J = 13.7), 2.38−2.51 (1H, m), 2.74 (1H, d, J = 12.8), 2.78−2.87 (3H, m), 3.32 (1H, d, J = 12.8), 4.65 (1H, br s), 6.56 (1H, s), 6.59 (1H, d, J = 2.9), 6.89 (1H, d, J = 8.7), 7.18−7.21 (1H, m), 7.51 (1H, d,

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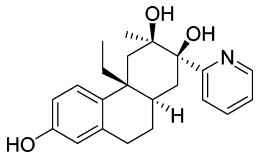
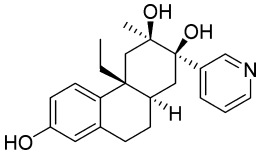
J = 7.9), 7.69−7.74 (1H, m), 8.48 (1H, dd, J = 0.8 and 5.0). LC-MS: m/z 338.3 $[M + H]^+$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-(pyridin-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (22).



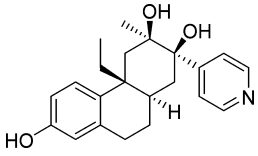
To a solution of intermediate **21** (579 mg, 1.72 mmol) in dimethoxyethane (100 mL) chilled to −15 °C was added dropwise a solution of 1.6 M methyllithium (13 mL, 21 mmol) in ether. The cooling bath was removed, and the mixture was allowed to warm to room temperature. The progress of the reaction was monitored by LC-MS. After stirring overnight, the mixture was quenched with saturated sodium bicarbonate solution, diluted with water, and extracted with ethyl acetate. The combined extracts were filtered to remove undissolved solids, washed with brine, dried ($MgSO_4$), and concentrated to give a tan foam. This material was purified by flash chromatography using an Isco CombiFlash eluting with ethyl acetate/hexanes (10−50%). The desired fractions were pooled and concentrated to give 321 mg (53%) of title compound **22** as white foam. This material was crystallized from ethyl acetate/hexanes to give 133 mg of white crystals suitable for X-ray analysis. The structure was confirmed by single-crystal X-ray analysis. ^{1}H NMR ($CDCl_3$): δ 0.83 (3H, s), 0.85 (3H, t, J = 7.5), 1.24−1.33 (1H, m), 1.50 (1H, dd, J = 3.1 and 13.5), 1.52−1.57 (1H, m), 1.83 (1H, d, J = 14.5), 1.87−1.96 (1H, m), 2.21−2.28 (1H, m), 2.35−2.45 (2H, m), 2.79 (1H, d, J = 15.0). 2.87−2.93 (2H, m), 3.32 (1H, d, J = 1.6), 5.42 (1H, br s), 6.62 (1H, d, J = 2.6), 6.65 (1H, dd, J = 2.9 and 8.5), 7.12 (1H, d, J = 8.8), 7.22−7.25 (1H, m), 7.56 (1H, d, J = 8.3), 7.61−7.64 (1H, m), 8.51−8.52 (1H, m). LC-MS: m/z 354.4 $[M + H]^+$. The more polar fractions were pooled and concentrated to give 100 mg (17%) of recovered **21**.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-(pyridin-3-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (23).



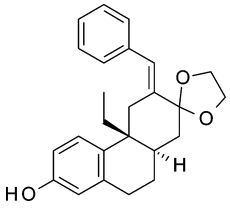
Analogue **23** was synthesized analogously to analogue **22** starting with 3-bromopyridine and intermediate **18**. LC-MS: m/z 354.3 $[M + H]^+$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-(pyridin-4-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (24).



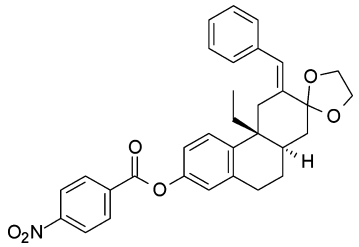
Analogue **24** was synthesized analogously to analogue **22** starting with 4-bromopyridine and intermediate **18**. ^{1}H NMR (CD_3OD): δ 0.79 (3H, t, J = 7.1.7.5), 1.06 (3H, s), 1.20−1.30 (1H, m), 1.38−1.43 (1H, m), 1.41 (1H, d, J = 10.8), 1.83−1.91 (3H, m), 2.26−2.37 (2H, m), 2.76−2.82 (3H, m), 6.47 (1H, d, J = 2.9), 6.53 (1H, dd, J = 2.5 and 8.3), 7.00 (1H, d, J = 8.3), 7.66 (2H, dd, J = 1.7 and 5.0), 8.34 (2H, dd, J = 1.5 and 4.8). LC-MS: m/z 354.4 $[M + H]^+$.

(3′*E*,4a′*R*,10a′*R*)-3′-Benzylidene-4a′-ethyl-3′,4′,4a′,9′,10′,10a′-hexahydro-1′*H*-spiro[[1,3]dioxolane-2,2′-phenanthren]-7′-ol (25).



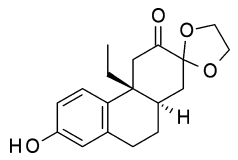
A mixture of enone **8** (19.0 g, 57.2 mmol), ethylene glycol (16.0 mL, 17.8 g, 286 mmol), and *p*-toluenesulfonic acid monohydrate (0.5 g, 2.6 mmol) in toluene (1.4 L) was heated to reflux with water separation using a Dean−Stark trap apparatus. After 12 h, the cooled mixture was washed with saturated sodium bicarbonate solution (1 × 500 mL) and brine (2 × 500 mL), dried (K_2CO_3), and concentrated. The residue was triturated with dichloromethane (40 mL) to afford 13.8 g (64%) of title compound **25** as beige solid. The mother liquor was concentrated and purified by flash chromatography (200g of silica gel) eluting with ethyl acetate/hexanes (10−50%) to afford an additional 3.0 g (14%) of **25**. ^{1}H NMR (CD_3OD): δ 0.07 (3H, t, J = 7.5), 1.09−1.16 (1H, m), 1.35−1.49 (1H, m), 1.52−1.65 (1H, m), 1.69−1.80 (2H, m), 1.85 (1H, t, J = 13.1), 2.02 (1H, d, J = 13.3), 2.06−2.20 (1H, m), 2.72−2.85 (2H, m), 3.55 (1H, d, J = 13.7), 3.81−4.08 (4H, m), 6.44 (1H, d, J = 2.5), 6.46 (2H, s), 6.77 (1H, d, J = 9.1), 6.84 (1H, s), 7.19−7.40 (5H, m). LC-MS: m/z 377.3 $[M + H]^+$.

(3′*E*,4a′*R*,10a′*R*)-3′-Benzylidene-4a′-ethyl-3′,4′,4a′,9′,10′,10a′-hexahydro-1′*H*-spiro[[1,3]dioxolane-2,2′-phenanthrene]-7′-yl 4-Nitrobenzoate (26).



To a stirred solution of ketal **25** (8.70 g, 23.1 mmol) in THF (250 mL) chilled to −78 °C was added dropwise a solution of 2.5 M *n*-butyllithium in hexanes (10.1 mL, 25.2 mmol) over 15 min. After an additional 15 min of stirring, *p*-nitrobenzoyl chloride (5.14 g, 27.7 mmol) was added in one portion. The red mixture was allowed to warm to room temperature for 1 h and then quenched with saturated ammonium chloride solution. The mixture was extracted with ethyl acetate (1 × 200 mL, 1 × 100 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The resulting foam was crystallized from acetone (50 mL) and filtered to afford 8.9 g (74%) of title compound **26** as a white solid. ^{1}H NMR ($CDCl_3$): δ 0.15 (3H, t, J = 7.3), 1.12−1.26 (1H, m), 1.45−1.59 (1H, m), 1.62−1.70 (1H, m), 1.72−1.86 (2H, m), 1.95 (1H, t, J = 13.3), 2.16−2.29 (2H, m), 2.84−3.01 (2H, m), 3.57 (1H, d, J = 13.3), 3,85−3.92 (1H, m), 3.99−4.12 (3H, m), 6.86−6.92 (3H, m), 7.07 (1H, d, J = 8.3), 7.24−7.41 (5H, m), 8.33 (4H, s).

(4a′*R*,10a′*R*)-4a′-Ethyl-7′-hydroxy-4′,4a′,10′,10a′-tetrahydro-1′*H*-spiro[[1,3]dioxolane-2,2′-phenanthren]-3′(9′*H*)-one (27).



A solution of olefin **26** (22.2 g, 42.2 mmol) in a mixture of dichloromethane (440 mL) and methanol (220 mL) was chilled to −78 °C. A stream of ozone was bubbled into the mixture for 15 min, at which time it became blue. Ozone treatment was continued for an additional 15 min, and the excess ozone was purged by bubbling nitrogen into the mixture. Excess dimethyl sulfide (40 mL) was added, and the mixture was slowly allowed to warm to room temperature. The solution was concentrated overnight under a stream of nitrogen. The residual solid was triturated with hexanes (500 mL) and filtered to give beige crystals, which were dissolved in THF (780 mL). The mixture was cooled to 0 °C, treated with a solution of 1 N sodium hydroxide (100 mL, 100 mmol), and then stirred for 5 h. The aqueous layer was separated and extracted with ethyl acetate (1 × 300 mL, 1 × 200 mL). The combined organic layers were washed with brine (2 × 200 mL), dried ($MgSO_4$), and concentrated to afford 12.5 g (98%) of title compound **27** as a foam. ^{1}H NMR ($CDCl_3$): δ 0.72 (3H, t, J =

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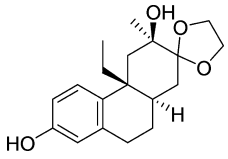
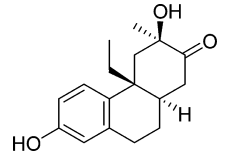
7.5), 1.35−1.43 (2H, m), 1.68−1.88 (2H, m), 1.97 (1H, dd, J = 4.2 and 14.1), 2.09 (1H, apparent t, J = 13.9), 2.33−2.44 (1H, m), 2.67 (1H, dd, J = 0.8, 13.3), 2.81−2.98 (2H, m), 3.10 (1H, d, J = 13.3), 3.73−3.82 (1H, m), 3.92−3.98 (1H, m), 4.05−4.15 (2H, m), 4.99 (1H, br s), 6.59−6.61 (2H, m), 6.92 (1H, d, J = 8.7). LC-MS: m/z 303.4 $[M + H]^+$, 301.3 $[M - H]^-$, 347.3 $[M - H + H_2CO_2]^-$.

(3′*R*,4a′*R*,10a′*R*)-4a′-Ethyl-3′-methyl-3′,4′,4a′,9′,10′,10a′-hexahydro-1′*H*-spiro[[1,3]dioxolane-2,2′-phenanthrene]-3′,7′-diol (28).



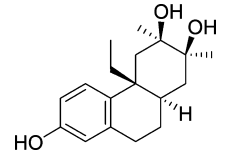
To a solution of ketone **27** (12.5 g, 41.3 mmol) in THF (1 L) chilled to −78 °C was added a solution of 1.5 M methyllithium−lithium bromide complex in diethyl ether (138 mL, 207 mmol) slowly via canula over 15 min. After 1 h, the suspension was allowed to warm to room temperature, and stirring was continued for 12 h. Saturated ammonium chloride solution (200 mL) was added, and the organic layer was separated and saved. The aqueous layer was extracted with ethyl acetate (1 × 500 mL, 1 × 200 mL). The combined organic layers were washed with brine (3 × 500 mL), dried (Na_2SO_4), and concentrated to afford 12.3 g (97%) of title compound **28** as a beige solid. ^{1}H NMR ($CDCl_3$): δ 0.68 (3H, t, J = 6.6), 1.09 (3H, s), 1.08−1.19 (1H, m), 1.30−1.35 (1H, m), 1.39 (1H, d, J = 14.5), 1.48−1.52 (1H, m), 1.72−1.93 (2H, m), 1.97−2.12 (2H, m), 2.42 (1H, d, J = 14.1), 2.70−2.80 (2H, m), 3.85−3.96 (4H, m), 6.43−6.51 (2H, m), 6.89 (1H, d, J = 8.3). LC-MS: m/z 301.4 $[M + H - H_2O]^+$, 317.4 $[M - H]^-$.

(3*R*,4a*R*,10a*R*)-4a-Ethyl-3,7-dihydroxy-3-methyl-3,4,4a,9,10,10a-hexahydrophenanthren-2(1*H*)-one (29).



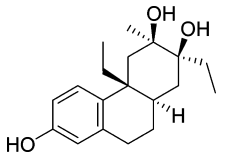
A solution of ketal **28** (12.7 g, 40 mmol) in THF (500 mL) was diluted with a solution of 2 N hydrochloric acid (350 mL), and the mixture was heated to reflux for 2 h. After cooling, the organic layer was separated and saved, and the aqueous layer was extracted with ethyl acetate (1 × 300 mL, 1 × 150 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (1 × 300 mL) and brine (2 × 300 mL), dried (Na_2SO_4), and concentrated. The brown residue was crystallized from ether to give 2.4 g of title compound **29** as a beige solid. The mother liquor was redissolved in ether and stored in the refrigerator to give 2.3 g of a second crop and 1.3 g of a third crop of **29**. The mother liquor from the third crop was purified by flash chromatography (300 g of silica gel) eluting with ethyl ether/hexanes (50−100%). During the charging process, 0.3 g of **29** crystallized out of solution and was recovered. The desired fractions were pooled and concentrated to afford 3.3 g of **29**; total yield = 9.6 g (87%). ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, t, J = 7.3), 1.36 (3H, s), 1.38−1.55 (2H, m), 1.64−1.72 (1H, m), 1.82−1.91 (1H, m), 1.92 (1H, dd, J = 1.2 and 14.5), 2.30−2.40 (1H, m), 2.48−2.66 (2H, m), 2.79 (1H, d, J = 14.5), 2.82−2.90 (2H, m), 6.55 (1H, d, J = 2.5), 6.62 (1H, dd, J = 2.7 and 8.5), 7.00 (1H, d, J = 8.3). LC-MS: m/z 275.3 $[M + H]^+$, 273.3 $[M - H]^-$, 319.4 $[M - H + H_2CO_2]^-$.

Representative Procedure A: (2*S*,3*R*,4a*R*,10a*R*)-4a-Ethyl-2,3-dimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (30).



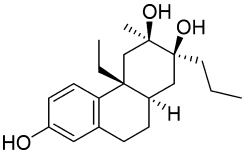
In a 20 mL parallel synthesis reaction vessel, lithium chloride (46 mg, 1.1 mmol) was dried at 150 °C under high vacuum for 30 min. The flask was flushed with nitrogen and chilled to −30 °C. A solution of ketone **29** (50 mg, 0.18 mmol) in THF (10 mL) was added followed by a solution of 1 M methyllithium−lithium iodide complex in ethyl ether (1.1 mL, 1.1 mmol). After stirring for 1 h, the mixture was allowed to warm to room temperature and then stirred for an additional 12 h. The reaction was quenched by the addition of 1 N hydrochloric acid solution (5 mL), diluted with water (15 mL), and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography using a Biotage 12 M and eluting with ethyl acetate/hexanes (20−40%). The desired fractions were pooled and concentrated to afford 21 mg (43%) of title compound **30** as a solid. ^{1}H NMR ($CDCl_3$): δ 0.79 (3H, t, J = 7.5), 1.25 (3H, s), 1.26 (3H, s), 1.26−1.30 (1H, m), 1.36−1.42 (2H, m), 1.58−1.68 (1H, m), 1.73−1.82 (1H, m), 1.84−1.93 (1H, m), 2.04−2.20 (2H, m), 2.55 (1H, d, J = 14.5), 2.85−2.91 (2H, m), 5.30 (1H, br s), 6.55−6.61 (2H, m), 7.00 (1H, d, J = 8.3). LC-MS: m/z 273.7 $[M + H - H_2O]^+$.

Representative Procedure B: (2*S*,3*R*,4a*R*,10a*R*)-2,4a-Diethyl-3-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (31).



In a 20 mL parallel synthesis reaction vessel, lithium chloride (46 mg, 1.1 mmol) was dried at 150 °C under high vacuum for 30 min. The flask was flushed with nitrogen and chilled to −30 °C. A solution of ketone **29** (50 mg, 0.18 mmol) in THF (10 mL) was added followed by a solution of 1 M ethylmagnesium bromide in THF (1.1 mL, 1.1 mmol). After stirring for 1 h, the mixture was allowed to warm to room temperature and then stirred for an additional 12 h. The reaction mixture was quenched by the addition of a solution 1 N hydrochloric acid (5 mL), diluted with water (15 mL), and extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by preparative HPLC using a Waters Symmetry C-8 30 mm × 50 mm column and eluting with acetonitrile/water (30−90% containing 0.1% formic acid) over 15 min (flow rate = 25 mL/min). The desired fractions were pooled and concentrated to afford 10 mg (18%) of title compound **31** (containing 25% of the 2β-ethyl epimer). ^{1}H NMR ($CDCl_3$): δ 0.69−0.74 (3H, two overlapping triplets), 0.84 and 0.91 (3H total, two triplets, J = 7.7, ratio 1:3), 1.13 and 1.22 (3H total, two singlets, ratio 1:3), 1.14−1.26 (1H, m), 1.30−2.18 (10.25H, m), 2.24 (0.75H, d, J = 14.5), 2.78−2.88 (2H, m), 6.49−6.55 (2H, m), 6.93 and 6.94 (1H, two doublets, J = 8.1, ratio 1:3). LC-MS: m/z 303.3 $[M - H]^-$, 349.3 $[M - H + H_2CO_2]^-$.

(2*S*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-propyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (32).



Analogue **32** was obtained following procedure B using a solution of 2.5 M propylmagnesium bromide in hexane; yield = 6%. The 2β-propyl epimer was also isolated in 10% yield. ^{1}H NMR ($CDCl_3$): δ 0.73 (3H, t, J = 7.3), 0.85 (3H, t, J = 6.7), 1.15 (3H, s), 1.11−1.19 (1H, m), 1.20−1.31 (2H, m), 1.32−1.47 (3H, m), 1.50−1.61 (3H, m), 1.74−1.87 (2H, m), 2.08−2.17 (1H, m), 2.47 (1H, d, J = 14.5), 2.80−3.00 (2H, m overlapping with water peak), 6.48−6.56 (2H, m), 6.93 (1H, d, J = 8.3). LC-MS: m/z 301.1 $[M + H - H_2O]^+$, 317.1 $[M - H]^-$, 363.0 $[M - H + H_2CO_2]^-$.

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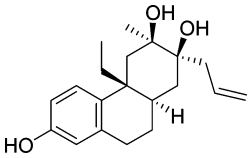
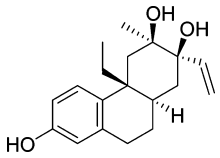
(2*S*,3*R*,4a*R*,10a*R*)-2-Allyl-4a-ethyl-3-methyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (33).



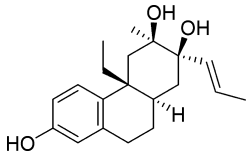
Analogue **33** was obtained following procedure B using a solution of 1 M allylmagnesium bromide in ethyl ether; yield = 44%. The 2β-allyl epimer was also isolated in 9% yield. ^{1}H NMR ($CDCl_3$): δ 0.62 (3H, t, J = 7.5), 1.06−1.12 (1H, m), 1.14 (3H, s), 1.27 (1H, dd, J = 2.9 and 13.7), 1.35−1.47 (1H, m), 1.53 (1H, d, J = 14.1), 1.63−1.79 (2H, m), 1.82−1.95 (2H, m), 2.13 (1H, d, J = 14.2), 2.23−2.39 (2H, m), 2.68−2.74 (2H, m), 4.96−5.05 (2H, m), 5.78−5.88 (1H, m), 6.38−6.45 (2H, m), 6.83 (1H, d, J = 8.3). LC-MS: m/z 299.1 $[M + H - H_2O]^+$, 315.1 $[M - H]^-$, 361.0 $[M - H + H_2CO_2]^-$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-vinyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (34).



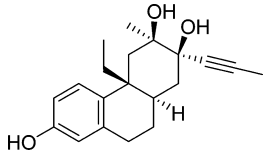
Analogue **34** was obtained following procedure B using a solution of 1 M vinylmagnesium bromide in THF; yield = 24% as a 1:1 mixture of 2α and β epimers. ^{1}H NMR ($CDCl_3$): δ 0.70−0.75 (3H, two overlapping triplets), 1.06 and 1.15 (3H total, two singlets), 1.12−1.24 (1H, m), 1.29−1.37 (1H, m), 1.48−1.55 (1H, m), 1.61 (0.5H, d, J = 14.1), 1.75−1.84 (1.5H, m), 2.00−2.18 (3H, m), 2.28 (0.5H, d, J = 14.5), 2.52 (0.5H, d, J = 14.9), 2.77−2.86 (2H, m), 5.04 (0.5H, d, J = 11.2), 5.18 (0.5H, d, J = 10.8), 5.29 (0.5H, d, J = 17.4), 5.36 (0.5H, d, J = 17.0), 6.09−6.20 (1H, m), 6.48−6.52 (2H, m), 6.93 (1H, d, J = 8.3). LC-MS: m/z 301 $[M - H]^-$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-((*E*)-prop-1-enyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (35).



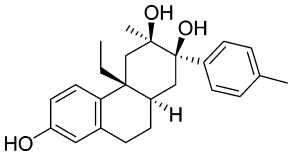
Analogue **35** was obtained following procedure B using a solution of 0.5 M (*E*)-propenylmagnesium bromide in THF; yield = 28% as a 4:1 mixture of 2α- and 2β-(*E*)-prop-1-enyl epimers. ^{1}H NMR ($CDCl_3$): δ 0.78 (3H, t, J = 7.3), 1.12 and 1.20 (3H total, two s), 1.17−1.25 (1H, m), 1.36−1.44 (3H, m), 1.67 (3H, d, J = 5.4), 1.75−1.97 (3H, m), 2.04−2.23 (2H, m), 2.35 (0.2H, d, J = 14.5), 2.56 (0.8H, d, J = 14.9), 2.82−2.88 (2H, m), 5.53−5.55 (0.2H, m), 5.75−5.87 (1.8H, m), 6.55−6.62 (2H, m), 6.98−7.03 (2H, overlapping doublets). LC-MS: m/z 299.3 $[M + H - H_2O]^+$, 315.2 $[M - H]^-$, 361.3 $[M - H + H_2CO_2]^-$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-(prop-1-ynyl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (36).



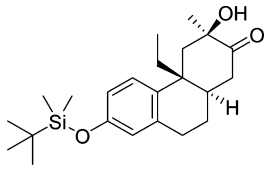
Analogue **36** was obtained following procedure B using a solution of 0.5 M propynylmagnesium bromide in THF; yield = 13%. The 3β-propargyl epimer was also isolated in 36% yield. ^{1}H NMR ($CDCl_3$): δ 0.0.77 (3H, t, J = 7.6), 1.11−1.23 (1H, m), 1.38 (3H, s), 1.52−1.71 (4H, m), 1.77 (3H, m), 2.04−2.15 (3H, m), 2.52 (1H, d, J = 14.5), 2.85−2.94 (2H, m), 6.52−6.61 (2H, m), 7.08 (1H, d, J = 7.9). LC-MS: m/z 297.3 $[M + H - H_2O]^+$, 313.3 $[M - H]^-$, 359.3 $[M - H + H_2CO_2]^-$.

(2*R*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-*p*-tolyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (37).



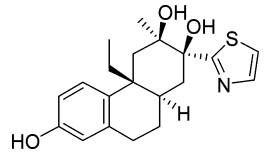
Analogue **37** was obtained following procedure A using a solution of 1 M *p*-tolylmagnesium bromide in THF; yield = 15% after trituration with ether, mp 213−214 °C. ^{1}H NMR ($CDCl_3$): δ 0.78 (3H, t, J = 7,6), 1.11 (3H, s), 1.15−1.30 (1H, m), 1.31−1.43 (1H, m), 1.48 (1H, dd, J = 2.5 and 12.9), 1.75−1.93 (4H, m), 2.15−2.25 (1H, m), 2.25 (3H, s), 2.69−2.78 (3H, m), 6.51 (1H, d, J = 2.5), 6.58 (1H, dd, J = 2.5 and 8.3), 6.98−7.05 (3H, m), 7.45 (2H, J = 8.3). LC-MS: m/z 349.3 $[M + H - H_2O]^+$, 365.3 $[M - H]^-$, 411.2 $[M - H + H_2CO_2]^-$.

(3*R*,4a*R*,10a*R*)-7-(*tert*-Butyldimethylsilyloxy)-4a-ethyl-3-hydroxy-3-methyl-3,4,4a,9,10,10a-hexahydrophenanthren-2(1*H*)-one (38).



A solution of 2.02 g (7.35 mmol) of ketone **29** in dichloromethane (20 mL) was chilled to 0 °C and treated sequentially with imidazole (550 mg, 8.08 mmol) and a solution of 1.0 M TBDMSCl in dichloromethane (8.0 mL, 8.0 mmol). A precipitate formed almost immediately, and the ice bath was removed. After 1.25 h of stirring, the reaction was diluted with water and the layers were separated. The organic layer was dried ($MgSO_4$) and evaporated. The residual oil was purified by flash chromatography using a Biotage 40 Samplet (40S column) eluting with ethyl acetate/heptanes (0−50%). The desired fractions were pooled and concentrated to give 1.94 g (68%) of title compound **38** as a semisolid. ^{1}H NMR (DMSO-d^6): δ 0.12 (6H, s), 0.73 (3H, t, J = 8), 0.90 (9H, s), 1.14 (3H, s), 1.18−1.29 (1H, m), 1.32 (1H, d, J = 12), 1.52−1.60 (1H, m), 1.75−1.94 (2H, m), 2.06 (1H, dd, J = 1 and 12), 2.19−2.27 (1H, m), 2.71 (1H, d, J = 12), 2.78 (2H, br t), 2.93 (1H, t, J = 12), 5.20 (1H, s), 6.49 (1H, s), 6.53 (1H, d, J = 8), 6.98 (1H, d, J = 8). LC-MS: m/z 371.2 $[M + H - H_2O]^+$.

(2*S*,3*R*,4a*R*,10a*R*)-4a-Ethyl-3-methyl-2-(thiazol-2-yl)-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7-triol (39).



A solution of 1.7 M *tert*-butyllithium in pentane (31 mL, 53 mmol) was added dropwise to a prechilled (−78 °C) solution of 2-bromothiazole (8.5 g, 52 mmol) in ethyl ether (100 mL). When the addition was complete, the mixture was stirred for 5 min and a solution of ketone **38** (4.00 g, 10.3 mmol) in ethyl ether (100 mL) was added dropwise over 50 min. The mixture was stirred for an additional 2.5 h and then quenched with ammonium chloride solution. After warming to room temperature, the organic layer was separated, and the aqueous layer extracted with ethyl ether. The combined organic layers were dried (Na_2SO_4) and evaporated. The residual oil was purified by flash chromatography using a Biotage 40S column eluting with ethyl acetate/heptanes (0−10%). The desired fractions were pooled and concentrated to afford 2.36 g (48%) of the desired carbinol as a foam. The impure fractions were evaporated and repurified by flash chromatography eluting with ethyl acetate/heptanes (0−25%) to give an additional 1.84 g (38%) of carbinol. Total yield = 4.2 g (86%). LC-MS: m/z 474.1 $[M + H]^+$.

A solution of 1.88 g (3.97 mmol) of the above material in THF (40 mL) was treated with a solution of 1 M TBAF in THF (4.80 mL, 4.80 mmol). After stirring for 5 min, analysis by LC-MS indicated that the

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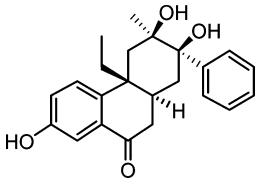
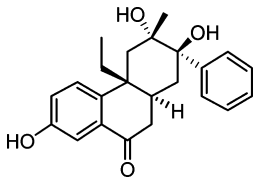
reaction was complete. This mixture was concentrated and purified by flash chromatography using a Biotage 40S column eluting with ethyl acetate/heptanes (0−100%) followed by flushing with dichloromethane/methanol. The desired fractions were pooled and concentrated, and the residue was triturated with ethyl ether/heptanes to afford 1.37 g of a solid, which was a mixture of 2α and 2β isomers by LC-MS. The material was recrystallized from acetonitrile−water at room temperature to afford 537 mg (38%) of title compound **39**. An additional 172 mg (12%) of **39** was obtained similarly by recrystallization of the concentrated mother liquor. Total yield = 705 mg (50%). ^{1}H NMR (DMSO-d^6): δ 0.71 (3H, t, J = 7.1), 0.80 (3H, s), 0.98−1.08 (1H, m), 1.29−1.43 (2H, m), 1.58−1.77 (1H, m), 2.06−2.26 (3H, m), 2.34 (1H, d, J = 14.1), 2.48−2.56 (1H, m), 2.62−2.72 (2H, m), 4.19 (1H, s), 5.70 (1H, s), 6.40 (1H, d, J = 2.5), 6.44 (1H, dd, J = 2.5 and 8.4), 6.89 (1H, d, J = 8.6), 7.47 (1H, d, J = 3.3), 7.56 (1H, d, J = 3.3), 8.89 (1H, s). LC-MS: m/z 360.1 $[M + H]^+$.

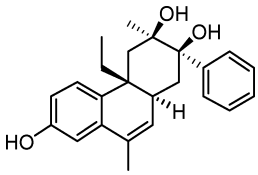
(2*R*,3*R*,4a*R*,10a*S*)-4a-Ethyl-2,3,7-trihydroxy-3-methyl-2-phenyl-2,3,4,4a,10,10a-hexahydrophenanthren-9(1*H*)-one (40).



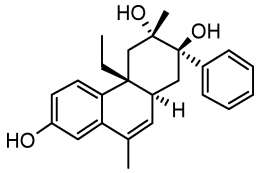
(2*R*,3*S*,4a*R*,10a*S*)-4a-Ethyl-2,3,7-trihydroxy-3-methyl-2-phenyl-2,3,4,4a,10,10a-hexahydrophenanthren-9(1*H*)-one (41).



(2*R*,3*R*,4a*R*,10a*S*)-4a-Ethyl-3,9-dimethyl-2-phenyl-1,2,3,4,4a,10a-hexahydrophenanthrene-2,3,7-triol (42).



(2*R*,3*S*,4a*R*,10a*S*)-4a-Ethyl-3,9-dimethyl-2-phenyl-1,2,3,4,4a,10a-hexahydrophenanthrene-2,3,7-triol (43).



Intermediate **12** (100 mg, 0.285 mmol) was dissolved in a 1:1 mixture of THF and toluene (10 mL) and treated with a solution of 1.5 M methyllithium−lithium bromide complex (1.90 mL, 0.285 mmol) in diethyl ether. The mixture was stirred for 2 h at room temperature, and the excess methyllithium was quenched by the addition of 1 N hydrochloric acid solution. The mixture was extracted with ethyl acetate (3 × 30 mL), and the combined extracts were washed with brine, dried (Na_2SO_4), and concentrated to give 129 mg of an oil. This material was purified by preparative reverse phase HPLC: Waters Symmetry C-8 column (30 mm × 50 mm) eluting with 30−70% acetonitrile in water (each containing 0.1% formic acid) over 15 min at a flow rate of 25 mL/min.

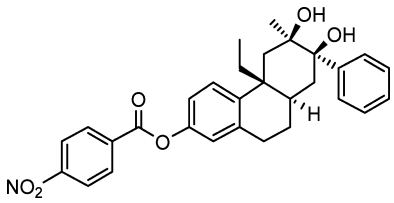
Fraction 2 was concentrated to give 3.5 mg (3%) of analogue **41** as a white solid. ^{1}H NMR (CD_3OD): δ 0.77 (3H, t, J = 7.3), 1.27−1.33 (1H, m), 1.43 (3H, s), 1.97 (1H, dd, J = 3.7 and 14.1), 2.08−2.15 (1H, m), 2.18−2.29 (3H, m), 2.40−2.53 (1H, m), 2.57−2.68 (2H, m), 6.98 (1H, dd, J = 2.6 and 8.5), 7.12−7.17 (1H, m), 7.20−7.29 (4H, m), 7.77−7.79 (2H, m). LC-MS: m/z 367.3 $[M + H]^+$, 365.3 $[M - H]^-$, 411.3 $[M - H + H_2CO_2]^-$.

Fraction 4 was concentrated to give 5.0 mg (5%) of analogue **43** as a white solid. ^{1}H NMR (CD_3OD): δ 0.59 (3H, t, J = 7.5), 1.47 (3H, s), 1.51−1.61 (1H, m), 1.69−1.77 (1H, m), 1.89−1.93 (4H, m), 2.22 (1H, t, J = 13.7), 2.34 (1H, d, J = 14.1), 2.43−2.51 (1H, m), 2.50 (1H, d, J = 14.1), 5.31 (1H, s), 6.60 (1H, dd, J = 2.5 and 8.3), 6.66 (1H, d, J = 2.5), 6.95 (1H, d, J = 8.3), 7.10−7.14 (1H, m), 7.20−7.24 (2H, m), 7.81 (2H, d, J = 8.1). LC-MS: m/z 347.3 $[M + H - H_2O]^+$, 363.3 $[M - H]^-$, 409.3 $[M - H + H_2CO_2]^-$.

Fraction 5 was concentrated to give 26 mg (25%) of analogue **42** as a white solid. ^{1}H NMR (CD_3OD): δ 0.56 (3H, t, J = 7.5), 1.18 (3H, s), 1.45−1.51 (2H, m), 1.92 (3H, s), 2.10 (1H, d, J = 15.0), 2.15−2.27 (1H, m), 2.49−2.60 (1H, m), 2.50 (1H, d, J = 11.6), 2.62 (1H, d, J = 14.5), 5.25 (1H, s), 6.63 (1H, dd, J = 2.5 and 8.3), 6.68 (1H, d, J = 2.5), 6.98 (1H, d, J = 8.3), 7.13−7.17 (1H, m), 7.23 (2H, t, J = 7.7), 7.63 (2H, d, J = 8.1). LC-MS: m/z 347.3 $[M + H - H_2O]^+$, 363.3 $[M - H]^-$, 409.3 $[M - H + H_2CO_2]^-$. The structure was confirmed by single-crystal X-ray analysis on crystals prepared by recrystallization from ethyl acetate−hexanes, mp 257−260 °C.

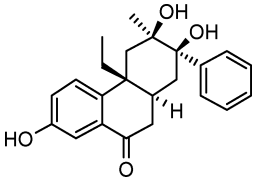
Fraction 8 was concentrated to give 26 mg (28%) of analogue **40** as a white solid. ^{1}H NMR (CD_3OD): δ 0.75 (3H, t, J = 7.3), 1.15−1.20 (1H, m), 1.17 (3H, s), 1.46 (1H, d, J = 10.4), 2.05 (1H, d, J = 15.0), 2.15 (1H, dd, J = 3.9 and 18.9), 2.38−2.45 (2H, m), 2.56−2.65 (2H, m), 2.80 (1H, d, J = 15.0), 7.00 (1H, dd, J = 2.9 and 8.7), 7.11−7.14 (1H, m), 7.15−7.24 (3H, m), 7.31 (1H, d, J = 2.9), 7.61 (2H, d, J = 8.1). LC-MS: m/z 367.3 $[M + H]^+$, 365.3 $[M - H]^-$, 411.3 $[M - H + H_2CO_2]^-$. The structure was confirmed by single-crystal X-ray analysis on crystals prepared by recrystallization from ethanol−water, mp 148−150 °C.

(4b*R*,6*R*,7*R*,8a*R*)-4b-Ethyl-6,7-dihydroxy-6-methyl-7-phenyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl 4-Nitrobenzoate (44).



To a solution of **13** (300 mg, 0.851 mmol) in acetone (50 mL) was added a solution of 1 N sodium hydroxide (0.890 mL, 0.890 mmol) followed by *p*-nitrobenzoyl chloride (189 mg, 1.02 mmol). The mixture was stirred for 1 h, and additional *p*-nitrobenzoyl chloride (90 mg, 0.48 mmol) was added. Additional 1 N sodium hydroxide solution was added periodically to maintain a neutral pH. The mixture was concentrated, diluted with water (75 mL), and extracted with ethyl acetate (2 × 75 mL). The combined extracts were washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$), and concentrated to give 447 mg (>100%) of title compound **44** as a foam. This material was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 0.84 (3H, t, J = 7.6), 1.18 (3H, s), 1.26−1.32 (1H, m), 1.42−1.52 (1H, m), 1.59 (1H, dd, J = 2.7 and 13.1), 1.84−2.04 (3H, m), 2.27−2.38 (2H, m), 2.83 (1H, d, J = 15.0), 2.87−2.92 (2H, m), 6.93 (1H, d, J = 2.5), 6.98 (1H, dd, J = 2.7 and 8.5), 7.19−7.31 (4H, m), 7.61 (2H, d, J = 7.5), 8.35 (4H, s). LC-MS: m/z 546.4 $[M - H + H_2CO_2]^-$.

(2*R*,3*R*,4a*R*,10a*S*)-4a-Ethyl-2,3,7-trihydroxy-3-methyl-2-phenyl-2,3,4,4a,10,10a-hexahydrophenanthren-9(1*H*)-one (40).



A solution of PNP ester **44** (0.851 mmol) dissolved in a mixture of 1:1 dichloromethane and methanol (125 mL) was chilled to −30 °C. Ozone was bubbled into the mixture until a blue color persisted for 1

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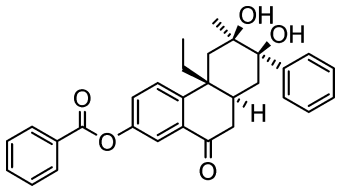
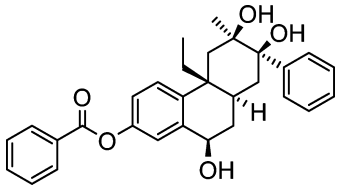
h. The mixture was purged with nitrogen, and excess dimethyl sulfide (ca. 2 mL) was added using a Pasteur pipet. After warming to room temperature and stirring overnight, the mixture was concentrated and the residual oil was dissolved in THF (50 mL). A solution of 1 N sodium hydroxide (2.1 mL, 2.1 mmol) was added and, after 1 h of stirring, additional 1 N sodium hydroxide solution (3.0 mL, 3.0 mmol) was added and stirring was continued for 1 h. Saturated sodium bicarbonate solution (100 mL) was added, and the organic layer was separated and saved. The aqueous layer was extracted with ethyl acetate (100 mL), and the combined organic layers were washed with saturated sodium bicarbonate solution and brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography using a Biotage Flash 40 S eluting with ethyl acetate/hexanes (30−40%). The appropriate fractions were pooled and concentrated to afford 190 mg (61%) of title compound **40**. ^{1}H NMR ($CDCl_3$): δ 0.76 (3H, t, J = 7.6), 1.22 (3H, s), 1.15−1.26 (1H, m), 1.55 (1H, d, J = 10.3), 2.03 (1H, d, J = 15.3), 2.25 (1H, dd, J = 3.9 and 18.9), 2.41−2.48 (2H, m), 2.50−2.57 (1H, m), 2.61−2.73 (1H, m), 2.81 (1H, d, J = 15.0), 2.95 (1H, br s), 3.13 (1H, br s), 7.07 (1H, dd, J = 2.9 and 8.3), 7.15−7.28 (4H, m), 7.55−7.59 (3H, m).

(4b*R*,6*R*,7*R*,8a*S*)-4b-Ethyl-6,7-dihydroxy-6-methyl-10-oxo-7-phenyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl Benzoate (45).



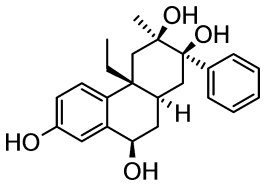
To a solution of phenol **40** (1.374 g, 3.749 mmol) in acetone (50 mL) was added a solution of 1 N sodium hydroxide (3.94 mL, 3.94 mmol). After stirring for 5 min, benzoyl chloride (0.457 mL, 408 mg, 3.94 mmol) was added, and the mixture was stirred for 2.5 h at room temperature. Saturated ammonium chloride solution (5 mL) was added, and the mixture was partially evaporated to remove acetone. The residue was partitioned between saturated ammonium chloride solution (50 mL) and ether (50 mL). The ether layer was separated and the aqueous layer further extracted with ether (50 mL). The combined ether layers were washed with saturated sodium bicarbonate solution (50 mL) and brine (50 mL), dried (Na_2SO_4), and concentrated to give 1.62 g (92%) of title compound **45** as a yellowish white solid. ^{1}H NMR ($CDCl_3$): δ 0.85 (3H, t, J = 7.3), 1.27 (3H, s), 1.23−1.36 (2H, m), 1.40−1.90 (1H, br s), 1.64 (1H, d, J = 11.9), 2.13 (1H, d, J = 14.5), 2.35 (1H, dd, J = 4.1 and 18.7), 2.46−2.77 (4H, m), 2.90 (1H, d, J = 14.5), 7.26−7.70 (10H, m), 7.86 (1H, d, J = 2.1), 8.22 (2H, d, J = 8.3). LC-MS: m/z 471.4 $[M + H]^+$, 469.4 $[M - H]^-$, 515.4 $[M - H + H_2CO_2]^-$.

(4b*R*,6*R*,7*R*,8a*R*,10*R*)-4b-Ethyl-6,7,10-trihydroxy-6-methyl-7-phenyl-4b,5,6,7,8,8a,9,10-octahydrophenanthren-2-yl Benzoate (46).



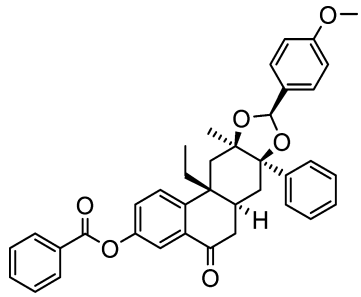
A solution of ketone **45** (249 mg, 0.529 mmol) in methanol (10 mL) was cooled to 0 °C and treated with sodium borohydride (30 mg, 0.80 mmol). The mixture was stirred for 2 h, and additional sodium borohydride (15 mg, 0.40 mmol) was added. The ice bath was removed, and the mixture was stirred for 2 h. Saturated ammonium chloride solution was added, and the mixture was extracted with ethyl acetate (3 × 25 mL). The combined extracts were washed with saturated sodium bicarbonate solution (1 × 25 mL) and brine (1 × 25 mL), dried (Na_2SO_4), and evaporated to give 235 mg of a light brown foam. This material was purified by flash chromatography using a Biotage Flash 40 eluting with 2.75:1 hexanes/ethyl acetate. The appropriate fractions were pooled and concentrated to give 89 mg (36%) of title compound **46** as a white foam. ^{1}H NMR ($CDCl_3$): δ 0.92 (3H, t, J = 6.7,7.3), 1.21 (3H, s), 1.43−1.52 (1H, m), 1.56 (1H, d, J = 13.0), 1.72 (2−3H, br s), 1.87−1.92 (1H, m), 1.97−2.11 (2H, m), 2.39−2.48 (2H, m), 2.86 (1H, d, J = 15.0), 4.83 (1H, t, J = 8.3), 7.11−7.13 (1H, m), 7.28−7.38 (5H, m), 7.52−7.55 (2H, m), 7.62−7.68 (3H, m), 8.21 (2H, d, J = 8.3). LC-MS: m/z 471.3 $[M - H]^-$, 517.3 $[M - H + H_2CO_2]^-$.

(2*R*,3*R*,4a*R*,9*R*,10a*R*)-4a-Ethyl-3-methyl-2-phenyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene-2,3,7,9-tetraol (47).



A solution of benzoate **46** (29 mg, 0.061 mmol) in THF (2 mL) was treated with 1 N sodium hydroxide solution (2.0 mL, 2.0 mL), and the mixture was stirred vigorously overnight. The mixture was poured onto saturated ammonium chloride solution (20 mL) and extracted with ethyl acetate (2 × 20 mL). The combined extracts were washed with saturated sodium bicarbonate solution and brine, dried ($NaSO_4$), and concentrated to give 23 mg (100%) of title compound **47** as an off-white solid. ^{1}H NMR ($CDCl_3$): δ 0.85 (3H, t, J = 7.6), 1.17 (3H, s), 1.32−1.42 (1H, m), 1.48−1.80 (4H br m), 1.52 (1H, dd, J = 2.1 and 12.9), 1.79−1.86 (1H, m), 1.88−2.00 (2H, m), 2.31−2.41 (2H, m), 2.78 (1H, d, J = 15.0), 4.73 (1H, t, J = 8.3), 6.72 (1H, dd, J = 2.7 and 8.5), 6.97 (1H, d, J = 2.9), 7.07 (1H, d, J = 8.3), 7.20−7.31 (3H, m), 7.58 (2H, d, J = 7.1). LC-MS: m/z 333.5 $[M + H - 2H_2O]^+$, 367.5 $[M - H]^-$, 413.5 $[M - H + H_2CO_2]^-$.

(6a*S*,7a*R*,9*R*,10a*R*,11a*R*)-11a-Ethyl-9-(4-methoxyphenyl)-10a-methyl-5-oxo-7a-phenyl-5,6,6a,7,7a,10a,11,11a-octahydrophenanthro[2,3-*d*][1,3]dioxol-3-yl Benzoate (48).



To a solution of intermediate **45** (1.78 g, 3.78 mmol) in benzene (50 mL) was added *p*-anisaldehyde dimethylacetal (6.45 mL, 6.89 g, 37.8 mmol) followed by *p*-toluenesulfonic acid (10 mg). After 5 min of stirring, the mixture became blue and was stirred for 7 h at room temperature. The mixture was poured onto saturated sodium bicarbonate solution (75 mL), and the organic layer was separated and saved. The aqueous layer was extracted with ether (2 × 50 mL), and the combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residual oil was purified by flash chromatography using a Biotage Flash 40 eluting with 10:1 to 5:1 hexanes in ether to give 1.39 g (62%) of title compound **48** as a yellow-white solid. ^{1}H NMR ($CDCl_3$): δ 0.87 (3H, t, J = 7.1), 1.04 (3H, s), 1.38−1.51 (1H, m), 1.79 (1H, dd, J = 3.1 and 14.3), 1.87 (1H, d, J = 15.0), 2.39−2.53 (3H, m), 2.69−2.81 (2H, m), 3.17 (1H, d, J = 15.0), 3.82 (3H, s), 6.22 (1H, s), 6.91−6.94 (2H, m), 7.24−7.54 (11H, m), 7.63−7.67 (1H, m), 7.90 (1H, d, J = 2.5), 8.20 (2H, dd, J = 1.2 and 8.3).

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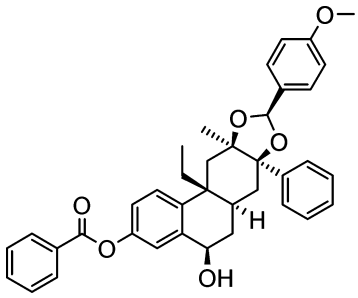
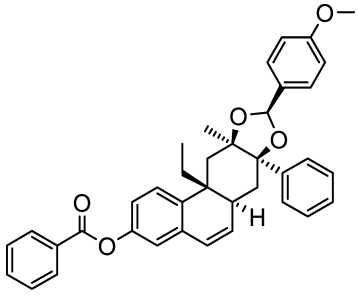
(5*R*,6a*R*,7a*R*,9*R*,10a*R*,11a*R*)-11a-Ethyl-5-hydroxy-9-(4-methoxyphenyl)-10a-methyl-7a-phenyl-5,6,6a,7,7a,10a,11,11a-octahydrophenanthro[2,3-*d*][1,3]dioxol-3-yl Benzoate (49).



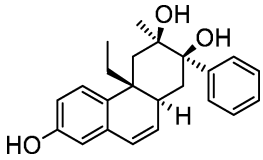
Ketone **48** (994 mg, 1.69 mmol) was dissolved in a mixture of methanol (20 mL) and THF (10 mL), chilled to −78 °C, and treated with sodium borohydride (76.6 mg, 2.02 mmol). The mixture was slowly allowed to warm to 0 °C and then stirred for 1.5 h. The mixture was poured onto saturated ammonium chloride solution (5 mL), and the contents were partially evaporated to remove methanol and THF. The aqueous residue was partitioned between ether (40 mL) and saturated ammonium chloride solution (40 mL). The organic layer was separated and saved and the aqueous layer extracted with ether (40 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give 1.03 g (theory = 997 mg) of title compound **49** as a yellow solid. This material was used directly in the next step without further purification. ^{1}H NMR ($CDCl_3$): δ 0.93 (3H, t, J = 7.1), 1.01 (3H, s), 1.14−1.28 (2H, m), 1.68−1.72 (1H, m), 1.76 (1H, d, J = 10.4), 1.94−2.05 (2H, m), 2.23−2.40 (2H, m), 2.46 (1H, t, J = 13.9), 3.12 (1H, d, J = 15.4), 3.81 (3H, s), 4.92 (1H, t, J = 8.7), 6.18 (1H, s), 6.92 (2H, d, J = 8.7), 7.11 (1H, dd, J = 2.5 and 8.3), 7.25−7.37 (4H, m), 7.42 (1H, d, J = 2.5), 7.49−7.53 (6H, m), 7.62−7.66 (1H, m), 8.20 (2H, d, J = 7.1).

(6a*S*,7a*R*,9*R*,10a*R*,11a*R*)-11a-Ethyl-9-(4-methoxyphenyl)-10a-methyl-7a-phenyl-6a,7,7a,10a,11,11a-hexahydrophenanthro[2,3-*d*][1,3]dioxol-3-yl Benzoate (50).



A mixture of alcohol **49** (1.69 mmol), dry benzene (15 mL), and Burgess reagent (483 mg, 2.03 mmol) was heated to 60 °C for 3 h. Additional Burgess reagent (75 mg, 0.31 mmol) was added, and heating was continued for 3 h. The mixture was cooled and diluted with brine. The organic layer was separated and saved, and the aqueous layer was extracted with ether (2 × 35 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give a yellow solid. This material was purified by flash chromatography using a Biotage Flash 40 eluting with 15:1 hexane/ethyl acetate to give 610 mg (63%) of title compound **50** as a sticky yellow foam. ^{1}H NMR ($CDCl_3$): δ 0.68 (3H, t, J = 7.6), 1.07 (3H, s), 1.68−1.79 (1H, m), 1.87 (1H, dd, J = 3.5 and 13.9), 1.96 (1H, d, J = 15.0), 2.00−2.11 (1H, m), 2.65 (1H, t, J = 14.5), 3.01−3.15 (2H, m), 3.81 (3H, s), 5.62 (1H, d, J = 9.1), 6.19 (1H, s), 6.46 (1H, dd, J = 2.9 and 9.1), 6.91−6.96 (3H, m), 7.07 (1H, dd, J = 2.5 and 8.3), 7.25−7.35 (4H, m), 7.49−7.58 (6H, m), 7.64 (1H, t, J = 7.5), 8.20 (2H, d, J = 7.1).

(2*R*,3*R*,4a*R*,10a*S*)-4a-Ethyl-3-methyl-2-phenyl-1,2,3,4,4a,10a-hexahydrophenanthrene-2,3,7-triol (51).



To a solution of acetal **50** (708 mg, 1.24 mmol) in dichloromethane (40 mL) was added water (3 mL) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (589 mg, 2.58 mmol). The mixture was stirred for 3 h at room temperature and then diluted with saturated sodium bicarbonate solution (50 mL). The organic layer was separated and the aqueous layer extracted with dichloromethane (50 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated to give 727 mg (99%) of a light brown solid. This material was dissolved in a solution of 10% KOH in 1:1 ethanol/water (30 mL), and the mixture was heated to 60 °C for 5 h. After cooling to room temperature, the mixture was diluted with saturated ammonium chloride solution (100 mL) and extracted with ethyl acetate (3 × 75 mL). The combined extracts were washed with saturated sodium bicarbonate solution (100 mL) and brine, dried (Na_2SO_4), and concentrated to give 450 mg (theory = 435 mg) of analogue **51** as a yellow solid, which contained a small amount of residual solvent. ^{1}H NMR (CD_3OD): δ 0.60 (3H, t, J = 7.3), 1.22 (3H, s), 1.55 (1H, dd, J = 2.6 and 12.4), 1.58−1.64 (1H, m), 2.15 (1H, d, J = 15.0), 2.22−2.30 (1H, m), 2.53−2.58 (1H, m), 2.63−2.70 (2H, m), 5.48 (1H, dd, J = 2.3 and 9.6), 6.30 (1H, dd, J = 3.1 and 9.3), 6.49 (1H, d, J = 3.1), 6.64 (1H, dd, J = 2.6 and 8.3), 7.01 (1H, d, J = 8.3), 7.19 (1H, t, J = 7.3), 7.25−7.29 (2H, m), 7.67 (2H, d, J = 7.8). LC-MS: m/z 349.4 $[M - H]^-$, 395.4 $[M - H + H_2CO_2]^-$.

Receptor Binding Assays. Compound affinities for human GR and AR were assessed as previously described.[20] Human PR, ERα, and ERβ were obtained from PanVera (Invitrogen). The ERα and ERβ binding assays were run in a similar manner to that described for AR.[20] The PR assay was run according to the protocol supplied by PanVera (Invitrogen).

Whole Cell Functional Assay: IL-1 Stimulated MMP-13 Production. SW1353 human chondrosarcoma cells were plated at confluence into 96-well plates in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum. After 24 h, medium was removed and replaced with 200 μL/well serum-free DMEM containing 1 mg/L insulin, 2g/L lactalbumin hydrosylate, and 0.5 mg/L ascorbate. The medium was removed 16 h later and replaced with 150 μL/well fresh serum-free medium containing ±20 ng/mL IL-1β, ± 5 nM dexamethasone, ± test compound. After 24 h of incubation at 37 °C under 5% CO_2, 125 μL sample from each well was removed under asceptic conditions for MMP-13 production analysis (Amersham Bio-Trak MMP-13 ELISA) using the manufacturer's protocol.[21]

Whole Cell Functional Assay: MMTV. Clonal SW1353 human chondrosarcoma cells stably transfected with pMAM-neo-luciferase mouse mammary tumor virus (MMTV) reporter construct were plated near confluence into 96-well plates in DMEM with 10% fetal bovine serum. After 24 h, medium was removed and replaced with phenol red-free, serum-free DMEM supplemented with 2 mM L-glutamine, 1 mg/L insulin, 0.5 mg/L ascorbate, and 2 g/L lactalbumin hydrosylate. Treatments were ± dexamethasone or compound. After 16 h of incubation at 37 °C under 5% CO_2, 100 μL of LucLite reagent (Packard) was added to each well. After 5 min of incubation in the dark, luminescence was detected in a TopCount microplate scintillation counter.[21]

Whole Blood Functional Assay: IL-1 Stimulated TNFα Production. Heparinized blood was collected from medication-free human volunteers. The following 10× mixture was added to 96-well U-bottom plates: RPMI/200 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES; Invitrogen) containing drug or DMSO vehicle (1.0%) and LPS (1000 ng/mL, Sigma L-2637, *Escherichia coli* 0111:B4). Human whole blood was then added and gently mixed with the final concentration of DMSO being 0.1% and LPS 100 ng/mL.

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The samples were incubated for 4 h at 37 °C in a humidified 5% CO_2 tissue culture incubator. Plasma was collected following centrifugation at 4 °C. Plasma was immediately diluted 1:5 in TNFα ELISA RD6 diluent (R&D Systems). TNFα levels were determined by ELISA (1:20 final dilution) after calibration to a standard curve as indicated by the manufacturer.

■ ASSOCIATED CONTENT

Supporting Information

X-ray crystallographic parameters and ^{1}H NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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■ DEDICATION

†Dedicated to Dr. M. Ross Johnson for his valued contributions to medicinal chemistry and mentorship to many colleagues.

■ ABBREVIATIONS

AP-1, activator protein-1; DAGR, dissociated agonist of the glucocorticoid receptor; DEX, dexamethasone; DKK1, dickkopf-related protein 1; ER, estrogen receptor; GR, glucocorticoid receptor; RA, rheumatoid arthritis; GRE, glucocorticoid response element; HWB, human whole blood; IL-1, interleukin-1; MMFF94, Merck Molecular Force Field 94; MMP-13, matrix metalloproteinase 13; MMTV, mouse mammary tumor virus; MR, mineralocorticoid receptor; NFκB, nuclear factor kappa B; PR, progesterone receptor; AR, androgen receptor; PRED, prednisone; TA, transactivation; TAT, tyrosine amino transferase; TNFα, tumor necrosis factor alpha; TR, transrepression

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