Published in final edited form as:

Org Biomol Chem. 2016 October 18; 14(41): 9790–9805. doi:10.1039/c6ob01726f.

Structure–activity studies of non-steroid analogues structurally-related to neuroprotective estrogens†

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Abstract

Estrone and 17β-estradiol are phenolic steroids that are known to be neuroprotective in multiple models of neuronal injury. Previous studies have identified the importance of their phenolic steroid A-ring for neuroprotection and have identified *ortho* substituents at the C-2 and C-4 positions on the phenol ring that enhance this activity. To investigate the importance of the steroid ring system for neuroprotective activity, phenolic compounds having the cyclopent[*b*]anthracene, cyclopenta[*b*]phenanthrene, benz[*f*]indene, benz[*e*]indene, indenes linked to a phenol, and a phenolic spiro ring system were prepared. New synthetic methods were developed to make some of the cyclopent[*b*]anthracene analogues as well as the spiro ring system. Compounds were evaluated for their ability to protect HT-22 hippocampal neurons from glutamate neurotoxicity and their activity relative to a potent neuroprotective analogue of 17β-estradiol was determined. An adamantyl substituent placed *ortho* to the phenolic hydroxyl group gave neuroprotective analogues in all ring systems studied.

Introduction

The estrogens estrone (E_1) and 17β-estradiol (E_2) have been shown to provide neuroprotection against oxidative damage in many different models of neuronal injury (Figure 1).[1–12] These compounds are free radical scavengers.[13,14] Since methylation of the aromatic hydroxyl group of an estrogen analogue eliminates neuroprotective action, it has

†Electronic supplementary information (ESI) available: ^{1}H NMR and ^{13}C NMR data. Crystal data for compounds **7**, **21**, **37** and **57**, CCDC reference numbers: 1451350, 1497517, 1451351 and 1497516, respectively.

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been concluded that the antioxidative properties of phenols are responsible for neuroprotection by this class of compounds.[10] In a previous structure–activity study we reported that electron donating alkyl groups positioned on either or both sides of the phenolic hydroxyl group in either E_1 or E_2 increase neuroprotective potency.[10] ZYC-26 (Figure 1) was the most potent compound identified in that study. In addition to the electron donating properties of the adamantyl group, an NMR study of ZYC-5 supports the hypothesis that another contributing factor for the high potency of *ortho*-substituted adamantyl analogues is the effect that this substituent has on orienting the steroid in the membrane.[15]

In this study we address the importance of the steroid ring system for the neuroprotective actions of phenolic analogues of E_1, E_2 and ZYC-26. We prepared analogues in six different ring systems (Figure 2): 1) tetracyclic cyclopent[*b*]anthracenes (**1**–**8**) and cyclopenta[*b*]phenanthrenes (**9**–**12**); 2) tricyclic benz[*f*]indenes (**13**–**16**) and benz[*e*]indenes (**17**–**20**); 3) indenes connected by a $–(CH_2)_n–$ linker to a phenol (**21**–**24**); and 4) a spiro ring system (**25**, **26**). In all ring systems we prepared analogues with an adamantyl group *ortho* to the phenolic hydroxyl group to evaluate the impact of this substituent on neuroprotective activity. Preparation of cyclopent[*b*]anthracenes **4**–**8** was accomplished using newly developed synthetic routes. Additionally, an efficient ring closure reaction leading to spiro compounds **25** and **26** was implemented. Neuroprotection was evaluated in a neuronal cell culture model of glutamate induced neurotoxicity. In the absence of an adamantyl group, E_2 and its analogues did not have significant neuroprotective activity at the highest concentration tested (1 μM). Several analogues containing an adamantyl group had neuroprotective activity similar to that of ZYC-26 at the highest concentration evaluated (1 μM).

Results and Discussion

Chemistry

Four Fused Rings – cyclopent[b]anthracenes (CP[b]A) and cyclopenta[b]phenanthrenes (CP[b]P)—Throughout all Schemes (S)-OH indicates that the stereochemistry of the hydroxyl group is the same as that of the steroid 17β-OH. The syntheses of compounds prepared in the CP[*b*]A ring system are shown in Schemes 1–4. The synthesis of **1**–**4** from the previously prepared CP[*b*]A **27**[16] is shown in Scheme 1. Jones oxidation of CP[*b*]A **27** gave compound **28.** The ring containing the enone group was then aromatized using $CuBr_2$/LiBr to give compound **1**. $NaBH_4$ reduction converted compound **1** to compound **2**. Reaction of compounds **1** and **2** with 1-adamantanol and BF_3•Et_2O gave compounds **3** and **4**, respectively.

CP[*b*]A compounds **5** and **6** were prepared by a newly developed route (Scheme 2). These two analogues have the same stereochemistry at all ring fusions as the just described CP[*b*]A compounds **1**–**4**. However, the position of the hydroxyl group is different. Indenone **29** was prepared from optically pure Hajos–Parrish ketone as described previously.[17] Attempts to convert indenone **29** directly into benzylated indenone **32** using $Pd(PPh_3)_4$ or $Pd_2(dba)_3$ catalysts with either phosphine ligands 2-biphenyldi-*tert*-butyl phosphine or 2-

biphenyldicyclohexyl phosphine as described in the literature for the benzylation of ketones failed.[18] Accordingly, indenone **29** was first carbomethoxylated with dimethyl carbonate to give enol **30** as described previously.[19,20] Benzylation then was carried out using a modified Yadav's method[21] to give compound **31**. The NMR spectrum of compound **31** indicated that it was 5:1 mixture of diastereomers resulting from the new chiral center formed in the benzylation reaction. Decarbomethoxylation of compound **31** using Krapcho's method[22] gave ketone **32** as a single stereoisomer with the benzyl side chain assigned as having the thermodynamically more stable equatorial configuration. This stereochemical assignment was later confirmed (vide infra). Ketone **32** was next converted into exocyclic olefin **33** by a Wittig reaction. Hydroboration of olefin **33** using BH_3•THF complex afforded primary alcohol **34** as a mixture of C-6 diastereomers (~4.5:1). Swern oxidation of diastereomeric alcohol **34** gave aldehyde **35** as a single product. Aldehyde **35** was then cyclized using aqueous 3 N HCl in MeOH to form CP[*b*]A **36**. The stereochemistry of the groups at the C-6 stereocenter in compounds **34** and **35** was not established as this stereocenter is eliminated upon formation of product **36**.

The double bond of compound **36** was reduced using Na/liq. NH_3 in the presence of added aniline. As reported previously, aniline addition was found to improve the yield of a reduction reaction of this type.[23] The ^{1}H NMR showed that the crude product was a mixture of diastereomers (4:1, 4a*S*:4a*R*) which was not separable by flash column chromatography. Separation was achieved chromatographically after removal of the *tert*-butyl protecting group using 3 N HCl in refluxing methanol to yield CP[*b*]A **37**. The minor stereoisomer having the 4a*R* stereochemistry was not isolated in pure form or further characterized. An X-ray structure determination confirmed the structure of product **37**. If the *tert*-butyl group was removed by HCl treatment before the Na/liq. NH_3 reduction, more than half of the trisubstituted olefin **36** rearranged to the tetrasubstituted olefin. Finally, the methyl group of the methoxy ether was removed with DIBAL–H in refluxing toluene to give CP[*b*]A **5**. If BBr_3 was used as the demethylating reagent, only 64% of compound **5** was obtained. The adamantyl group of CP[*b*]A **6** was then added.

The synthetic routed used to prepare CP[*b*]A **27** as a starting material for the synthesis of CP[*b*]A **1** is not optimal. That synthetic route requires the separation of CP[*b*]A **27** from another product formed in the CP[*b*]P ring system.[16] Hence, realizing that if indenone **29** could be efficiently converted into indenone **41**, it might be possible to obtain CP[*a*]A **27** in improved yield (i.e., products in the CP[*b*]P ring system would not also be formed), we explored this possibility (Schemes 3 and 4). Although indenone **41** has been prepared previously,[24] we developed a new route for its synthesis from enol **30**. $LiAlH_4$ reduction of enol **30** formed allylic alcohol **38**[25] which was subsequently acetylated to form compound **39**. Ozonolysis then gave α-acetoxyketone **40**, and reduction of compound **40** with SmI_2, using a procedure we reported previously,[26,27] afforded the desired indenone **41**.

Indenone **41** was then converted into CP[*b*]A **48** (Scheme 4) using the same sequence of reactions shown in Scheme 2 for the conversion of indenone **29** into CP[*b*]A **36**. Carbomethoxylation of indenone **41** gave enol **42**. Benzylation of enol **42** gave product **43**, and decarbomethyoxylation of compound **43** gave ketone **44** as a single stereoisomer with

the benzyl group assigned the more thermodynamically stable equatorial configuration. This stereochemical assignment was later confirmed (vide infra). Ketone **44** was transformed to exocyclic olefin **45** by a Wittig reaction using THF as the solvent. If the Wittig reaction was run in benzene, no desired olefin was formed and starting material was fully recovered. Olefin **45** was hydroborated using BH_3•THF to afford primary alcohol **46** as essentially a single C-6 stereoisomer rather than a mixture of diastereomers at this newly formed stereocenter. Swern oxidation of alcohol **46** gave aldehyde **47** and cyclization of aldehyde **47** using 3 N HCl in MeOH yielded product **48**. The stereochemistry of the groups at the C-6 stereocenter in compounds **46** and **47** was not established as this stereocenter is eliminated upon formation of product **48**.

The double bond of compound **48** was reduced using Na/liq. NH_3 in the presence of added aniline. The ^{1}H NMR showed that the crude product was a mixture of diastereomers (12:1, 10a*S*:10a*R*) which was not separable by flash column chromatography. CP[*b*]A **49** was obtained by chromatographic purification after removal of the *tert*-butyl protecting group. The minor stereoisomer having the 10a*R* stereochemistry was not isolated in pure form or further characterized. In analogy to what we found for the Na/liq. NH_3 reduction of CP[*b*]A **36**, we expected that the double bond reduction product with the 10a*R* configuration (*trans* 4a*R*,10a*R* ring fusion) would be the major product, not the product with the 10a*S* configuration (*cis* 4a*R*,10a*S* ring fusion). In an attempt to produce the double bond reduction product with the 10a*R* configuration, we carried out a Pd/C catalyzed hydrogenation of compound **48**. No detectable reduction product with the 10a*R* configuration was detected by either ^{1}H NMR or ^{13}C NMR. We hypothesize that a steric effect of the 11a*S* methyl group explains the stereochemical outcome found for both double bond reduction methods. Thus, indenone **41** could not be efficiently converted into a CP[*b*]A having the *trans* 4a*R*,10a*R* ring fusion. However, instead, indenone **41** did provide ready access to CP[*b*]A analogues having the *cis* 4a*R*,10a*S* ring fusion. Consequently, the methoxy group of compound **49** was cleaved with DIBAL–H in refluxing toluene to give CP[*b*]A **7**. Compound **7** was then converted into its adamantyl substituted analogue **8**. An X-ray structure determination for compound **7** confirmed the stereochemistry of all chiral centers.

Compounds **9** and **10** in the CP[*b*]P ring system (Scheme 5) were prepared as described previously.[20] The adamantyl groups were then added to CP[*b*]Ps **9** and **10** to obtain compounds **11** and **12**, respectively.

Three Fused Rings – benz[f]indenes and benz[e]indenes—Compounds **50** (Scheme 6) and **52** (Scheme 7), the starting materials for the preparation of tricyclic compounds **13–16** and **17–20**, respectively, were prepared as previously described.[25,28] Each starting material was oxidized to a diketone (**51** and **53**, Schemes 6 and 7, respectively) before the enone ring was aromatized to yield compounds **13** and **17**. The adamantyl group was then introduced to form compounds **14** and **18**. Reduction of the ketone group of compounds **13** and **17** yielded compounds **15** and **19** and addition of the adamantyl group to **15** and **19** gave compounds **16** and **20**, respectively.

Indenes connected to a phenol and a spiro ring system—A Wittig reaction was used to connect a *p*-methoxybenzene group with the ketone group of indenone **29** to form compounds **54**, **55** and **56** (Scheme 8). For compounds **54** and **55**, the exocyclic double bond was isomerized to the endocyclic double bond using HCl/MeOH to yield compounds **58** and **59**, respectively. BBr_3 cleavage of the *p*-methoxy group yielded compounds **21** and **23**. An X-ray structure determination for compound **21** established the position of the double bond. The position of the double bond in compounds **58**, **59** and **23**, was assigned by inference to be the same as it was in compound **21**. The ^{13}C NMR spectrum of compound **23** revealed that it consisted of two rotamers that were not interconverting at room temperature on the NMR time scale. The adamantyl group was then added to compounds **21** and **23** to obtain compounds **22** and **24**, respectively.

Similar treatment of compound **56** with HCl/MeOH resulted in a ring closure reaction to form spiro compound **57** whose structure was established by an X-ray diffraction analysis. BBr_3 cleavage of the *p*-methoxy group yielded compound **25**, and subsequent addition of the adamantyl group yielded compound **26**.

Biological Evaluation

The compounds were evaluated for neuroprotective activity in a cell culture model that used HT-22 hippocampal neurons (Table 1). Glutamate (3 mM) and each test compound (100 nM or 1μM) were co-incubated with the cells for 24 h and then cell viability was determined by a fluorescence assay (see Experimental for details). The neuroprotective activity of all compounds was compared to that of ZYC-26 at 1 μM.

The purpose of comparing the analogues without the adamantyl group to E_2 was to determine if any of the ring systems among the different analogues was superior to the steroid ring system of E_2. No ring system was superior to that of the steroid ring system. Differences in potency may be found at concentrations above 1 μM, but we did not test higher concentrations because compounds with weaker effects than those of ZYC-26 were not of interest.

To determine if any of the ring systems lacked neuroprotective activity, an adamantyl group was introduced into the analogues. For compounds with four fused rings, the effect of the adamantyl group is apparent when the following compounds are compared: **1** and **3; 2** and **4; 5** and **6; 7** and **8; 9** and **11**; and **10** and **12**. Only the activity of compound **6**, relative to that of compound **5**, failed to gain activity by introduction of the adamantyl group. At a concentration of 1 μM, compounds **3**, **4**, **8**, **11** and **12**, like ZYC-26, fully protected the neurons against death in the assay. The planarity of compounds in the CP[*b*]A ring system appeared less important as a determinant of activity than the position of the hydroxyl group on the aromatic ring (compare compounds **4** and **8**; and compounds **4** and **6**). The importance of the position of the aromatic hydroxyl group (C-3 vs. C-2) and the lack of a strict requirement for compound planarity was also noted in an earlier study of E_2 analogues.[10]

For the compounds with three fused rings, the effect of the adamantyl group can be observed by comparing compounds **13** and **14; 15** and **16; 17** and **18**; and **19** and **20**. The adamantyl

group increased activity more markedly when the functional group on the five-membered ring was a hydroxyl group (**16** and **20**) than when it was a carbonyl group (**14** and **18**). The neuroprotective activity of compound **14** failed to achieve statistical significance because of the high degree of neuronal death found for the no compound control. Overall, these result parallels results in the steroid ring system where the activity of compounds with a 17β-OH group are higher than those of the corresponding steroid analogues with the 17-carbonyl group.[10] At a concentration of 1μM, compounds **16** and **20** were as neuroprotective as ZYC-26. We conclude that these tricyclic ring systems can be neuroprotective when properly substituted.

For the compounds in the linked and spiro ring systems, the effect of the adamantyl group can be observed by comparing compounds **21** and **22; 23** and **24**; and **25** and **26**. Although the adamantyl group increased the activity of compounds **21** and **23**, the corresponding adamantyl analogues **22** and **24** were not as neuroprotective at 1 μM as ZYC-26 was at this concentration. This suggests that the flexibility of these two linked systems is unfavorable for high neuroprotective activity. For the spiro ring system, compounds **25** and **26** can be compared. The adamantyl group greatly increased activity. Unlike the adamantyl compounds **22** and **24** in the linked system, adamantyl spiro compound **26** is rigid and the compound at 1 μM fully protects the neurons. This occurs even though two of the rings are orthogonal to the other two rings.

Conclusions

In summary, we have reported two new synthetic routes to compounds in the CP[*b*]A ring systems and a spiro ring system. These compounds, in conjunction with compounds in the other ring systems evaluated, have provided new information on the role that the steroid ring system has on the neuroprotective actions of A-ring phenolic steroids.

Experimental

General Methods

Solvents were either used as purchased or dried and purified by standard methodology. Extraction solvents were dried with anhydrous Na_2SO_4 and, after filtration, removed on a rotary evaporator. Flash column chromatography was performed using silica gel (32–63 μm) purchased from Scientific Adsorbents (Atlanta, GA). Melting points were determined on a Kofler micro hot stage and are uncorrected. Infrared spectra were recorded as films on a NaCl plate on a Perkin–Elmer Spectrum One FT-IR spectrometer. Optical rotations were measured on a Perkin–Elmer Model 341 Polarimeter in the solvent indicated. NMR spectra were recorded on a Varian NMR spectrometer in $CDCl_3$, acetone-d_6, or DMSO-d_6 at ambient temperature at 300 MHz (^{1}H) or 75 MHz (^{13}C). Chemical shifts are reported as δ values relative to internal chloroform (δ = 7.27) for ^{1}H and chloroform (δ = 77.0) for ^{13}C. Elemental analyses were performed by M-H-W Laboratories (Phoenix, AZ).

(3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-7-hydroxy-11a-methyl-1*H*-cyclopent[*b*]anthracen-1-one (1)—$CuBr_2$ (892 mg, 4 mmol) and LiBr (209 mg, 2.4 mmol) were added to compound **27** (544 mg, 2 mmol) in CH_3CN (20 mL).

The reaction was stirred at room temperature and monitored by TLC. After 3 h at room temperature, the reaction was quenched with 3 N HCl (50 mL) and the products were extracted into EtOAc (50 mL × 3), dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel, eluted with 20% EtOAc in hexanes) to give compound **1** (448 mg, 83%): mp 268–270 °C; $[\alpha]_D^{25}$ +164.8 (*c* = 0.29, DMSO); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.87 (s, br, 1H), 6.71 (d, *J* = 8.3 Hz, 1H), 6.40–6.34 (m, 2H), 2.61–0.82 (m, 15H), 0.74 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 219.5, 155.0, 137.0, 129.1, 126.3, 114.5, 113.0, 47.7, 44.5, 38.9, 38.7, 36.7, 36.5, 35.2, 34.1, 31.8, 23.6, 13.2; IR (film, cm^{-1}) 3431, 1725. Anal. Calcd for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20; found: C, 79.89; H, 7.96.

(1*S*,3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-11a-methyl-1*H*-cyclopent[*b*]anthracene-1,7-diol (2)—$NaBH_4$ (120 mg, 3 mmol) was added to a solution of compound **1** (250 mg, 0.93 mmol) in EtOH (20 mL) at room temperature. After 1 h, 3 N HCl (20 mL) was added and the product was extracted into CH_2Cl_2 (50 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give compound **2** (233 mg, 92%): mp 260–262 °C; $[\alpha]_D^{20}$ +118.5 (*c* = 0.27, DMSO); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.90 (d, *J* = 1.3 Hz, 1H), 6.71 (d, *J* = 8.2 Hz, 1H), 6.69–6.33 (m, 2H), 4.40 (d, *J* = 4.9 Hz, 1H), 3.43–3.36 (m, 1H), 2.57–0.62 (m, 15H), 0.58 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 154.9, 137.2, 129.1, 126.6, 114.5, 112.9, 80.0, 44.3, 44.1, 43.2, 38.7, 37.1, 36.8, 34.5, 32.6, 29.7, 25.2, 11.3; IR (film, cm^{-1}) 3462, 1500, 1258. Anal. Calcd for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88; found: C, 79.19; H, 8.63.

(3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-8-(adamant-1-yl)-7-hydroxy-11a-methyl-1*H*-cyclopent[*b*]anthracen-1-one (3)—BF_3•Et_2O (1 mL) was added to a solution of compound **1** (100 mg, 0.37 mmol) and 1-adamantanol (60 mg, 0.4 mmol) in CH_2Cl_2 (10 mL) at 0 °C. After 30 min, the reaction was warmed to room temperature for 2 h, and then water (20 mL) was added. The product was extracted into CH_2Cl_2 (50 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give compound **3** (122 mg, 81%): mp 256–258 °C; $[\alpha]_D^{20}$ +110.0 (*c* = 0.32, $CHCl_3$); ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ 8.74 (s, 1H), 6.58 (s, 1H), 6.32 (s, 1H), 2.50–0.86 (m, 30H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 219.4, 153.6, 133.8, 133.1, 125.9, 125.8, 115.6, 47.6, 44.5, 40.1 (3 × C), 39.8, 38.7, 36.7 (3 × C), 36.0, 35.7, 35.2, 34.2, 31.9, 28.4 (3 × C), 23.6,13.2; IR (film, cm^{-1}) 3422, 1725, 1417. Anal. Calcd for $C_{28}H_{36}O_2$: C, 83.12; H, 8.97; found: C, 83.21; H, 9.09.

(1*S*,3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-8-(adamant-1-yl)-11a-methyl-1*H*-cyclopent[*b*]anthracene-1,7-diol (4)—Compound **4** (100 mg, 75%) was prepared from compound **2** (90 mg, 0.33 mmol) using the procedure described for the preparation of compound **3**. Compound **4** had: mp 144–146 °C; $[\alpha]_D^{20}$ +78.5 (*c* = 0.26, DMSO); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89 (s, 1H), 6.39 (s, 1H), 5.17 (s, br, 1H), 3.75–3.73 (m, 1H), 2.72–0.88 (m, 31H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.3, 134.9, 134.1, 128.4, 127.0, 116.3, 81.9, 44.6, 44.3, 43.6, 40.7 (3 × C), 39.7, 37.4, 37.1 (3 ×

C), 36.7, 36.3, 34.9, 32.8, 30.3, 29.0 (3 × C), 25.4, 11.0; IR (film, cm^{-1}) 3358, 1698, 1418. Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found: C, 82.59; H, 9.42.

(1*S*,3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-11a-methyl-1*H*-cyclopent[*b*]anthracene-1,8-diol (5)—DIBAL–H (20 mL, 1.0 M in toluene, 20 mmol) was slowly added to a solution of compound **37** (1.65 g, 5.77 mmol) in toluene (30 mL). After gas evolution ceased, the reaction was refluxed for 24 h. The reaction was slowly quenched with aqueous NH_4Cl, followed by aqueous 6 N HCl until both phases became clear. The product was extracted into EtOAc (150 mL × 3). The combined extracts were dried, filtered, and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give compound **5** (1.34 g, 85%): mp 192–194 °C; $[\alpha]_D^{20}$ +76.0 (*c* = 0.35, acetone); ^{1}H NMR (300 MHz, acetone-d_6) δ 7.89 (s, 1H), 6.78 (d, *J* = 8.0 Hz, 1H), 6.49–6.44 (m, 2H), 3.61–3.55 (m, 2H), 2.64–1.06 (m, 15H), 0.72 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 155.2, 137.8, 129.4, 127.5, 114.8, 113.1, 81.1, 44.8 (2 × C), 43.7, 40.5, 38.3, 36.7, 34.8, 33.0, 30.1, 25.4, 10.9; IR (film, cm^{-1}) 3400, 1501. Anal. Calcd for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88; found: C, 79.48; H, 8.75.

(1*S*,3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-7-(adamant-1-yl)-11a-methyl-1*H*-cyclopent[*b*]anthracene-1,8-diol (6)—Compound **6** (131 mg, 80%) was prepared from compound **5** (100 mg, 0.40 mmol) using the procedure described for the preparation of compound **3**. Compound **6** had: mp 294–296 °C; $[\alpha]_D^{20}$ +80.0 (*c* = 0.23, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (s, 1H), 6.58 (s, 1H), 6.31 (s, 1H), 4.39 (d, *J* = 4.7 Hz, 1H), 3.41–3.3.37 (m, 1H), 2.54–0.62 (m, 30H), 0.58 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.1, 134.8, 133.8, 126.6, 126.5, 116.3, 80.6, 44.9, 44.8, 40.7 (3 × C), 40.4, 37.6, 37.3 (3 × C), 37.1, 36.4, 34.9, 33.3, 30.3, 29.1 (4 × C), 25.8, 11.9; IR (film, cm^{-1}) 3347, 1715, 1614, 1264. Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found: C, 82.53; H, 9.50.

(1*S*,3a*S*,4a*S*,10a*S*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-11a-methyl-1*H*-cyclopent[*b*]anthracene-1,7-diol (7)—Compound **7** (1.27 g, 94%) was prepared from compound **49** (1.40 g, 4.97 mmol) using the procedure described for the preparation of compound **5**. Compound **7** had: mp 122–124 °C; $[\alpha]_D^{20}$ −9.5 (*c* = 0.44, DMSO); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.82 (s, 1H), 6.71 (d, *J* = 9.1 Hz, 1H), 6.39–6.30 (m, 2H), 4.38 (d, *J* = 4.7 Hz, 1H), 3.40–3.30 (m, 1H), 2.79 (dd, *J* = 16.2 Hz, 6.6 Hz, 1H), 2.59–2.22 (m, 3H), 1.92–0.88 (m, 11H), 0.58 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.5, 136.6, 127.8, 127.7, 114.3, 112.0, 80.8, 44.6, 42.4, 41.6, 35.4, 34.8, 32.5, 32.1, 29.2, 29.1, 24.8, 13.3; IR (film, cm^{-1}) 3342, 1704, 1610, 1500, 1270. Anal. Calcd for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88; found: C, 79.21; H, 8.82.

(1*S*,3a*S*,4a*S*,10a*S*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-8-(adamant-1-yl)-11a-methyl-1*H*-cyclopent[*b*]anthracene-1,7-diol (8)—Compound **8** (121 mg, 85%) was prepared from compound **7** (95 mg, 0.35 mmol) using the procedure described for the preparation of compound **3**. Compound **8** had: mp 130–132 °C; $[\alpha]_D^{20}$ +9.0 (*c* = 0.20, acetone); ^{1}H NMR (300 MHz, acetone-d_6) δ 7.74 (s, 1H), 6.72 (s, 1H), 6.43 (s, 1H), 3.54–3.52 (m, 2H), 2.86–0.79 (m, 30H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 153.7,

134.1, 133.5, 128.5, 125.9, 116.2, 82.5, 46.1, 43.2, 42.7, 40.7 (3 × C), 37.2 (3 × C), 36.3, 35.4, 33.9, 33.0, 30.0, 29.9, 29.3 (3 × C), 28.3, 25.6, 13.9; IR (film, cm^{-1}) 3347, 1715, 1614, 1264. Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found: C, 82.53; H, 9.30.

(6a*R*, 7a*S*, 10a*S*, 11a*R*) -5, 6, 6a, 7, 7a, 9, 10, 10a, 11, 11a-Decahydro-3-hydroxy-7a-methyl-8*H*-cyclopenta[*b*] phenanthren-8-one (9)—Compound **9** was prepared as described previously.[20]

(6a*R*, 7a*S*, 8*S*, 10a*S*, 11a*R*) -6, 6a, 7, 7a, 8, 9, 10, 10a, 11, 11a-Decahydro-7a-methyl-5*H*-cyclopenta[*b*] phenanthrene-3, 8-diol (10)—Compound **10** was prepared as described previously.[20]

(6a*R*, 7a*S*, 10a*S*, 11a*R*) -5, 6, 6a, 7, 7a, 9, 10, 10a, 11, 11a-Decahydro-2-(adamant-1-yl)- 3-hydroxy-7a-methyl-8*H*-cyclopenta[*b*] phenanthren-8-one (11)—Compound **11** (95 mg, 71%) was prepared from compound **9** (95 mg, 0.35 mmol) using the procedure described for the preparation of compound **3**. Compound **11** had: mp 268–270 °C; $[\alpha]_D^{20}$ −9.5 (*c* = 0.20, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$/acetone-d_6) δ 7.15 (s, 1H), 6.43 (s, 1H), 5.35 (s, 1H), 2.85–1.15 (m, 30H), 0.91 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$/acetone-d_6) δ 220.5, 153.4, 134.8, 133.7, 130.5, 123.2, 116.6, 48.0, 45.9, 44.4, 40.5 (3 × C), 39.1, 37.1 (3 × C), 36.6, 35.7, 35.6, 30.9, 30.4, 29.6, 29.1 (3 × C), 24.0, 13.6; IR (film, cm^{-1}) 1734, 1413. Anal. Calcd for $C_{28}H_{36}O_2$: C, 83.12; H, 8.97; found: C, 82.99; H, 8.77.

(6a*R*, 7a*S*, 8*S*, 10a*S*, 11a*R*) -6, 6a, 7, 7a, 8, 9, 10, 10a, 11, 11a-Decahydro-2-(adamant-1-yl)-7a-methyl-5*H*-cyclopenta[*b*] phenanthrene-3, 8-diol (12)—Compound **12** (95 mg, 71%) was prepared from compound **10** (90 mg, 0.33 mmol) using the procedure described for the preparation of compound **3**. Compound **12** had: mp 248–250 °C; $[\alpha]_D^{20}$ −42.9 (*c* = 0.21, DMSO); ^{1}H NMR (300 MHz, $CDCl_3$/acetone-d_6) δ 7.02 (s, 1H), 6.94 (s, 1H), 6.38 (s, 1H), 3.66–3.61 (m, 1H), 2.72–0.79 (m, 31H), 0.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$/acetone-d_6) δ 153.2, 134.9, 133.6, 131.2, 123.2, 116.5, 81.5, 45.4, 44.7, 44.4, 43.3, 40.5 (3 × C), 37.1 (3 × C), 36.6, 35.9, 31.2, 30.9, 30.4, 29.9, 29.1 (3 × C), 25.5, 11.2; IR (film, cm^{-1}) 3364, 1412. Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found: C, 82.82; H, 9.25.

(3a*S*,9a*S*)-2,3,3a,4,9,9a-Hexahydro-6-hydroxy-9a-methyl-1*H*-benz[*f*]inden-1-one (13)—Compound **13** (751 mg, 74%) was prepared from compound **51** (980 mg, 4.5 mmol) using the procedure described for the preparation of compound **1**. Compound **13** had: mp 268–270 °C; $[\alpha]_D^{25}$ +205.0 (*c* = 1.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.00 (d, *J* = 8.0 Hz, 1H), 6.70–6.66 (m, 2H), 6.02 (s, 1H), 2.95–1.69 (m, 9H), 0.88 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 222.6, 154.0, 136.8, 131.8, 126.8, 115.8, 114.0, 47.4, 41.9, 36.7, 36.4, 31.6, 24.5, 13.5; IR (film, cm^{-1}) 3391, 1723. Anal. Calcd for $C_{14}H_{16}O_2$: C, 77.75; H, 7.46; found: C, 77.75; H, 7.61.

(3a*S*,9a*S*)-2,3,3a,4,9,9a-Hexahydro-7- (adamant-1-yl)-6-hydroxy-9a-methyl-1*H*-benz[*f*]inden-1-one (14)—Compound **14** (175 mg, 71%) was prepared from compound **13** (150 mg, 0.7 mmol) using the procedure described for the preparation of compound **3**.

Compound **14** had: mp 225–227 °C; $[\alpha]_D^{20}$ +115.0 (*c* = 0.1, $CHCl_3$); ^1H NMR (300 MHz, DMSO-*d*$_6$) δ 8.83 (s, 1H), 6.67 (s, 1H), 6.41 (s, 1H), 3.24 (s, 2H), 2.69–1.52 (m, 22H), 0.64 (s, 3H); ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ 220.9, 154.4, 134.4, 133.6, 128.4, 124.9, 117.2, 47.2, 41.7, 40.5 (3 × C), 37.3 (3 × C), 37.1, 36.4, 36.3, 30.9, 29.0 (3 × C), 24.5, 13.7; IR (film, cm^{-1}) 3400, 1651, 1050. Anal. Calcd for $C_{24}H_{30}O_2$: C, 82.24; H, 8.63; found: C, 82.38; H, 8.54.

(1*S*,3a*S*,9a*S*)-2,3,3a,4,9,9a-Hexahydro-9a-methyl-1*H*-benz[*f*]indene-1,6-diol (15)—Compound **15** (264 mg, 81%) was prepared from compound **13** (324 mg, 1.5 mmol) using the procedure described for the preparation and of compound **2**. Compound **15** had: mp 112–113 °C; $[\alpha]_D^{20}$ +86.6 (*c* = 0.58, $CHCl_3$); ^1H NMR (300 MHz, acetone-*d*$_6$) δ 7.94 (s, 1H), 6.80 (d, *J* = 8.0 Hz, 1H), 6.53–6.50 (m, 2H), 3.88 (d, *J* = 8.0 Hz, 1H), 3.77–3.71 (m, 1H), 2.66–1.29 (m, 9H), 0.63 (s, 3H); ^{13}C NMR (75 MHz, acetone-*d*$_6$) δ 155.9, 138.0, 131.6, 128.0, 116.4, 114.2, 81.7, 43.4, 42.9, 42.0, 32.9, 31.2, 26.3, 10.4; IR (film, cm^{-1}) 3402, 1045. Anal. Calcd for $C_{14}H_{18}O_2$: C, 77.03; H, 8.31; found: C, 76.83; H, 8.13.

(1*S*,3a*S*,9a*S*)-2,3,3a,4,9,9a-Hexahydro-7-(adamant-1-yl)-9a-methyl-1*H*-benz[*f*]indene-1,6-diol (16)—Compound **16** (150 mg, 61%) was prepared from compound **15** (150 mg, 0.7 mmol) using the procedure described for the preparation of compound **3**. Compound **16** had: mp 217–219 °C; $[\alpha]_D^{20}$ +55.0 (*c* = 0.32, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.90 (s, 1H), 6.43 (s, 1H), 6.36 (s, 1H), 3.93 (t, *J* = 8.3 Hz, 1H), 2.80–1.26 (m, 25H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.6, 134.6, 134.2, 128.6, 127.7, 117.1, 82.0, 42.8, 42.2, 41.3, 40.9 (3 × C), 37.3 (3 × C), 36.5, 31.4, 30.9, 29.3 (3 × C), 25.7, 10.1; IR (film, cm^{-1}) 3324, 1042; Anal. Calcd for $C_{24}H_{32}O_2$: C, 81.77; H, 9.15; found: C, 81.89; H, 9.15.

(3a*S*, 9b*S*) -1, 2, 3a, 4, 5, 9b-Hexahydro-7-hydroxy-3a-methyl-3*H*-benz[*e*] inden-3-one (17)—Known compound **17**[29,30] (500 mg, 58%) was prepared from compound **53** (877 mg, 3.9 mmol) using the procedure described for the preparation and purification of compound **1**. Compound **17** had: mp 78–80 °C; ^1H NMR ($CDCl_3$) δ 7.00 (d, *J* = 8.8 Hz, 1H), 6.68–6.66 (m, 2H), 4.91 (s, 1H), 2.94–1.74 (m, 9H), 0.73 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 220.8, 154.3, 138.3, 130.3, 126.4, 115.4, 113.0, 48.2, 46.1, 36.7, 29.0, 26.7, 21.6, 14.1.

(3a*S*, 9b*S*) -1, 2, 3a, 4, 5, 9b-Hexahydro-8-(adamant-1-yl)-7-hydroxy-3a-methyl-3*H*-benz[*e*] inden-3-one (18)—Compound **18** (70 mg, 50%) was prepared from compound **17** (86 mg, 0.4 mmol) using the procedure described for the preparation of compound **3**. Compound **18** had: mp 210–212 °C; $[\alpha]_D^{20}$ +45.8 (*c* = 0.45, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.93 (s, 1H), 6.47 (s, 1H), 4.70 (s, 1H), 2.95–1.24 (m, 24H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 221.3, 153.2, 134.6, 134.3, 129.3, 123.6, 116.8, 48.2, 46.4, 40.8 (3 × C), 37.3 (3 × C), 36.7, 36.6, 29.3 (3 × C), 29.1, 25.9, 21.7, 13.8; IR (film, cm^{-1}) 3400, 1725, 1415. Anal. Calcd for $C_{24}H_{30}O_2$: C, 82.24; H, 8.63; found: C, 82.20; H, 8.45.

(3*S*, 3a*S*, 9b*S*)-2, 3, 3a, 4, 5, 9b-Hexahydro-3a-methyl-1*H*-benz[*e*] indene-3, 7-diol (19)—Known compound **19**[31] (90 mg, 83%): was prepared from compound **17** (108 mg, 0.5 mmol) using the procedure described for the preparation of compound **2**. Compound **19** had: mp 74–76 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (d, *J* = 8.3 Hz, 1H), 6.62–6.33 (m, 2H), 5.29 (s, br, 1H), 3.93–3.87 (m, 1H), 2.90–1.23 (m, 10H), 0.64 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 137.8, 131.8, 126.8, 115.2, 122.7, 81.3, 45.8, 43.5, 33.9, 31.4, 27.0, 23.3, 10.7.

(3*S*, 3a*S*, 9b*S*)-2, 3, 3a, 4, 5, 9b-Hexahydro-8-(adamant-1-yl)- 3a-methyl-1*H*-benz[*e*]indene-3, 7-diol (20)—Compound **20** (78 mg, 56%) was prepared from compound **19** (85 mg, 0.4 mmol) using the procedure described for the preparation of compound **3**. Compound **20** had: mp 221–223 °C; $[\alpha]_D^{20}$ −22.0 (*c* = 0.10, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.80 (s, 1H), 6.45 (s, 1H), 4.75 (s, 1H), 3.92 (t, *J* = 7.2 Hz, 1H), 2.85–1.24 (m, 25H), 0.67 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.4, 134.4, 133.9, 131.5, 124.2, 116.7, 81.3, 46.1, 43.5, 41.0 (3 × C), 37.3 (3 × C), 36.6, 34.0, 31.5, 29.3 (3 × C), 26.3, 23.4, 10.7; IR (film, cm^{-1}) 3400, 1598; Anal. Calcd for $C_{24}H_{32}O_2$: C, 81.77; H, 9.15; found: C, 81.68; H, 9.05.

(1*S*,3a*S*,7a*S*)-2,3,3a,4,7,7a-Hexahydro-5-(4-hydroxybenzyl)-7a-methyl-1*H*-inden-1-ol (21)—BBr_3 in CH_2Cl_2 (8 mL) was added to compound **58** (194 mg, 0.72 mmol) dissolved in CH_2Cl_2 (8 mL) at −78 °C. After 30 min, the reaction mixture was warmed to room temperature for 2 h, and then CH_3OH (3 mL) and water (20 mL) were added. The product was extracted into CH_2Cl_2 (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) to give compound **21** (143 mg, 77%): mp 165–166 °C; $[\alpha]_D^{20}$ +66.4 (*c* = 0.28, acetone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (s, 1H), 6.93 (d, *J* = 8.5 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 5.36 (s, 1H), 3.70–3.64 (m, 2H), 2.03–1.10 (m, 13H), 0.61 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.8, 137.2, 131.2, 129.7 (2 × C), 121.7, 115.2 (2 × C), 81.0, 43.3, 41.7, 41.3, 38.5, 31.0, 30.4, 25.5, 9.9; IR (film, cm^{-1}) 3335, 1510, 1236. Anal. Calcd for $C_{18}H_{24}O_2$: C, 79.03; H, 8.58; found: C, 78.98; H, 8.80.

(1*S*,3a*S*,7a*S*)-2,3,3a,4,7,7a-Hexahydro-5-[3-(adamantan-1-yl)-4-hydroxybenzyl]-7a-methyl-1*H*-inden-1-ol (22)—Compound **22** (84 mg, 79%) was prepared from compound **21** (70 mg, 0.27 mmol) using the procedure described for the preparation of compound **3**. The ^{1}H and ^{13}C NMR spectra of compound **22** were consistent with it being a 2.5:1 mixture of rotamers. Crystals of the compound melted at two different temperatures. Compound **22** had: mp 218–220 °C and 227–229 °C; major rotamer ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.89 (s, 1H), 6.75 (s, 1H), 6.65 (d, *J* = 6.0 Hz, 1H), 6.56 (d, *J* = 6.0 Hz, 1H), 5.30 (s, 1H), 4.41 (d, *J* = 4.7 Hz, 1H), 3.49 (t, *J* = 8.5 Hz, 1H), 3.28 (s, 1H), 2.08–0.76 (m, 25H), 0.48 (s, 3H); major rotamer ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.7, 154.5, 137.4, 135.7, 135.6, 131.3, 130.4, 126.8, 126.7, 122.0, 116.7, 80.4, 45.1, 43.9, 43.5, 43.2, 41.8, 41.3, 41.0, 40.6, 38.8, 37.3, 36.7, 32.6, 31.5, 30.7, 29.1, 25.8, 11.1, 10.9; IR (film, cm^{-1}) 3282, 1417, 1276. Anal. Calcd for $C_{27}H_{38}O_2$: C, 82.61; H, 9.24; found: C, 82.77; H, 9.43.

(1*S*,3a*S*,7a*S*)-2,3,3a,4,7,7a-Hexahydro-5-(4-hydroxyphenethyl)-7a-methyl-1*H*-inden-1-ol (23)—Compound **23** (102 mg, 72%) was prepared from compound **59** (150 mg, 0.53 mmol) using the procedure described for the preparation of compound **21**. Compound **23** had: mp 95–98 °C; $[\alpha]_D^{20}$ +78.2 (*c* = 0.50, acetone); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (d, *J* = 8.2 Hz, 2H), 6.80 (d, *J* = 8.2 Hz, 2H), 5.34–5.33 (m, 1H), 3.83 (t, *J* = 8.5 Hz, 1H), 2.73–1.26 (m, 15H), 0.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.1, 136.6, 134.2, 129.4 (2 × C), 120.5, 115.4 (2 × C), 82.1, 41.6, 41.2, 39.8, 38.2, 33.8, 31.6, 30.6, 25.6, 10.2; IR (film, cm^{-1}) 3339, 1514. Anal. Calcd for $C_{18}H_{24}O_2$: C, 79.37; H, 8.88; found: C, 79.15; H, 9.03.

(1*S*,3a*S*,7a*S*)-2,3,3a,4,7,7a-Hexahydro-5-[3-(adamantan-1-yl)-4-hydroxyphenethyl]-7a-methyl-1*H*-inden-1-ol (24)—Compound **24** (65 mg, 73%) was prepared from compound **23** (60 mg, 0.22 mmol) using the procedure described for the preparation of compound **3**. Compound **24** had: mp 210–212°C; $[\alpha]_D^{20}$ +19.1 (*c* = 0.32, acetone); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 (s, 1H), 6.74 (s, 1H), 6.70 (d, *J* = 9.4 Hz, 1H), 6.56 (d, *J* = 9.4 Hz, 1H), 5.16–5.14 (m, 1H), 4.41 (d, *J* = 4.7 Hz, 1H), 3.48–3.30 (m, 1H), 3.28 (s, 1H), 2.48–1.06 (m, 27H), 0.46 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 154.5, 136.8, 135.5, 132.3, 126.8, 126.4, 121.1, 116.8, 80.6, 41.7, 41.0, 40.7 (3 × C), 39.9, 38.8, 37.3 (3 × C), 36.6, 34.2, 31.8, 30.7, 29.1 (3 × C), 25.9, 10.9; IR (film, cm^{-1}) 3368, 1720. Anal. Calcd for $C_{28}H_{38}O_2$: C, 82.71; H, 9.42; found: C, 82.34; H, 9.19.

(1*S*,3a*S*,4a*R*,7a*S*,)-1,2,3,3a,3′,4,4′,6,7,7a-Decahydro-7a-methyl-2′*H*-spiro[indene-5,1′-naphthalene]-1,7′-diol (25)—DIBAL–H (1.0 M in toluene, 3.6 mL, 3.6 mmol) was added to a solution of compound **57** (180 mg, 0.6 mmol) in toluene (15 mL) at room temperature and then the reaction was refluxed for 16 h. After cooling to room temperature, water (2 mL) and 6 N HCl (10 mL) were added and the product was extracted into CH_2Cl_2 (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 25% EtOAc in hexanes) to give compound **25** (160 mg, 94%): mp 161–163°C; $[\alpha]_D^{20}$ +34.9 (*c* = 0.18, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.84 (s, 1H), 6.73 (s, 1H), 6.70 (d, *J* = 7.9 Hz, 1H), 6.39 (d, *J* = 7.9 Hz, 1H), 4.43 (d, *J* = 4.1 Hz, 1H), 3.50–3.48 (m, 1H), 2.45–1.06 (m, 17H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.6, 152.3, 134.7, 132.3, 118.6, 118.2, 85.2, 47.9, 45.2, 45.4, 43.4, 39.8, 38.5, 38.0, 35.2, 35.1, 30.8, 24.8, 15.5; IR (film, cm^{-1}) 3436, 1494, 1228. Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; found: C, 79.48; H, 8.97.

(1*S*,3a*S*,4a*R*,7a*S*)-1,2,3,3a,3′,4,4′,6,7,7a-Decahydro-6′-(adamantan-1-yl)-7a-methyl-2′*H*-spiro[indene-5,1′-naphthalene]-1,7′-diol (26)—Compound 2**6** (86 mg, 83%) was prepared from compound **25** (70 mg, 0.245 mmol) using the procedure described for the preparation of compound **3**. Compound **26** had: mp 228–230°C; $[\alpha]_D^{20}$ +25.8 (*c* = 0.36, acetone); mp 228–230°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (s, 1H), 6.83 (s, 1H), 6.68 (s, 1H), 3.67–3.60 (m, 1H), 2.87–1.13 (m, 33H), 0.84 (s, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ 154.1, 144.3, 133.6, 127.6, 126.7, 114.8, 81.0, 43.1, 40.6 (3 × C), 40.2, 39.8, 38.0, 37.2 (3 × C), 36.2, 34.8, 33.5, 33.2, 30.4, 30.2, 29.3 (3 × C), 25.8, 20.0, 9.78; IR (film,

cm^{-1}) 3306, 1508, 1233. Anal. Calcd for $C_{29}H_{40}O_2$: C, 82.81; H, 9.59; found: C, 82.66; H, 9.18.

(1*S*, 3a*S*, 4a*S*, 9a*R*, 10a*R*, 11a*S*) -1, 2, 3, 3a, 4, 4a, 5, 8, 9, 9a, 10, 10a, 11, 11a-Tetradecahydro-1-hydroxy-11a-methyl-7*H*-Cyclopent[*b*] anthracen-7-one (27)— Compound **27** was prepared as described previously.[13]

(3a*S*,4a*S*,9a*R*,10a*R*,11a*S*)-2,3,3a,4a,5,8,9,9a,10,10a,11,11a-Dodecahydro-11a-methyl-1*H*-cyclopenta[*b*]anthracene-1,7(4H)-dione (28)—Jones reagent was added at 0 °C to a solution of compound **27** (548 mg, 2 mmol) dissolved in acetone (50 mL) until the color of excess reagent persisted. After 15 min, 2-propanol (1 mL) was added to consume excess reagent. Brine (50 mL) was then added and the product was extracted into EtOAc (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **28** (544 mg, ~100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.71 (s, 1H), 2.40–0.86 (m, 20H), 0.83 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 219.7, 199.6, 165.6, 124.3, 47.8, 45.1, 43.9, 41.9, 41.0, 38.0, 37.5, 36.8, 36.4, 35.4, 31.9, 28.8, 23.5, 13.4; HRMS (ESI) calcd for $[C_{18}H_{24}O_2+Na]^+$: 295.1669, found: 295.1672.

(1*S*, 3a*S*, 7a*S*) -1- (1, 1-Dimethylethoxy)octahydro-7a-methyl-5*H*-inden-5-one (29)—Optically pure (>99% ee as determined by optical rotation measurement) compound **29** was prepared as described previously.[17]

(3*S*,3a*S*,7a*S*)-3-(1,1-Dimethylethoxy)-2,3,3a,4,7,7a-hexahydro-6-hydroxy-3a-methyl-1*H*-indene-5-carboxylic acid, methyl ester (30)—Compound 30 (13.1 g, 93%) was prepared from compound **29** (13.5 g, 150 mmol) as described previously.[25] Compound **30** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.30 (s, 1H), 3.75 (s, 3H), 3.58 (t, J = 7.6 Hz, 1H), 2.38–1.24 (m, 9H), 1.17 (s, 9H), 0.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.7, 172.2, 96.8, 80.0, 72.4, 51.3, 41.5, 39.8, 35.0, 32.1, 31.4, 28.8 (3 × C), 25.6, 10.5.

(3*S*,3a*S*,7a*S*)-3-(1,1-Dimethylethoxy)octahydro-5-(3-methoxybenzyl)-3a-methyl-6-oxo-1*H*-indene-5-carboxylic acid, methyl ester (31)—NaH (800 mg, 60% in mineral oil, 20 mmol) was added to a solution of compound **30** (4.48 g, 20 mmol) in DMF/toluene (40 mL/120 mL) at room temperature and the resulting mixture was stirred until gas evolution ceased (ca. 30 min). 3-Methoxybenzyl bromide (3.6 g, 20 mmol) was added and the reaction was refluxed for 2 h. The reaction was cooled to room temperature, aqueous NH_4Cl was added, and the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **31** as an unresolved mixture of two diastereomers due to the formation of the new chiral center at C-5 (6.83 g, 85%). The major diastereomer had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (t, J = 7.1 Hz, 1H), 6.78–6.70 (m, 3H), 3.76 (s, 3H), 3.63 (s, 3H), 3.33 (t, J = 7.1 Hz, 1H), 3.19 (d, J = 13.4 Hz, 1H), 3.01 (d, J = 13.4 Hz, 1H), 2.56–2.35 (m, 3H), 1.70–1.15 (m, 6H), 1.12 (s, 9H), 0.82 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 207.5, 173.8, 159.4, 138.5, 128.9, 123.7, 116.6, 112.7, 80.4, 72.8, 59.4, 55.2, 52.5, 45.6, 45.2, 42.5, 42.4, 41.9, 31.5,

28.7 (3 × C), 25.7, 11.6; IR (film, cm^{-1}) 1711, 1600. Anal. Calcd for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51; found: C, 71.42; H, 8.27.

(1*S*,3a*S*,6*R*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-7a-methyl-5*H*-inden-5-one (32)—LiCl (2.17 g, 51 mmol) was added to a solution of compound **31** (6.80 g, 17.0 mmol) in dry DMF (85 mL) at room temperature. The reaction was refluxed for 14 h. The reaction was quenched with aqueous NH_4Cl and the product extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **32** (5.26 g, 90%): $[\alpha]_D^{20}$ +22.0 (c = 0.20, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (t, *J* = 8.0 Hz, 1H), 6.74–6.70 (m, 3H), 3.78 (s, 3H), 3.43 (t, *J* = 7.4 Hz, 1H), 3.30 (dd, *J* = 13.7 Hz, 5.0 Hz, 1H), 2.64–1.38 (m, 11H), 1.10 (s, 9H), 0.94 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.8, 159.6, 142.2, 129.3, 121.6, 115.0, 111.2, 79.2, 72.2, 55.1, 53.7, 51.1, 47.2, 43.5, 35.5, 32.1, 31.1, 28.6 (3 × C), 24.9, 11.9; IR (film, cm^{-1}) 1708, 1602, 1362. Anal. Calcd for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36; found: C, 76.52; H, 9.15.

(1*S*,3a*S*,6*R*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-7a-methyl-5-methylene-1*H*-indene (33)—NaH (1.5 g, 37.5 mmol) was added to a suspension of methyltriphenylphosphonium bromide (13.39 g, 37.5 mmol) in benzene (100 mL) at room temperature and then refluxed for 30 min. Compound **32** (5.24 g, 15.2 mmol) in benzene (30 mL) was added. After 3 h, the reaction was quenched by the addition of aqueous NH_4Cl and the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **33** (4.89 g, 94%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 (t, *J* = 7.7 Hz, 1H), 6.76–6.71 (m, 3H), 4.77 (d, *J* = 23.1 Hz, 1H), 3.79 (s, 3H), 3.36 (t, *J* = 7.4 Hz, 1H), 3.13 (dd, *J* = 12.7 Hz, 4.1 Hz, 1H), 2.47–1.31 (m, 12H), 1.06 (s, 9H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 153.3, 143.2, 129.2, 121.7, 114.8, 111.4, 106.3, 80.5, 72.4, 55.3, 47.4, 44.5, 43.2, 39.5, 38.9, 37.2, 31.8, 28.8 (3 × C), 25.8, 11.5; IR (film, cm^{-1}) 1639, 1602, 1487; MS (ESI) for $[C_{23}H_{34}O_2+H]^+$: 343.2, found: 343.3.

(1*S*,3a*S*,6*R*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-7a-methyl-1*H*-indene-5-methanol (34)—BH_3•THF complex (15 mL, 1.0 M in THF, 15 mmol) was added to a solution of compound **33** (4.86 g, 14.2 mmol) in THF (100 mL) at 0 °C. After 30 min, the reaction was allowed to warm to room temperature. After an additional 1 h, the reaction was quenched with aqueous 3 N NaOH (40 ml) and 30% H_2O_2 (20 mL). The mixture was stirred at room temperature for 1 h and the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give product **34** (4.22 g, 82%) as a mixture of C-5 diastereomers (ratio ~4.5:1). The major isomer had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (t, *J* = 7.7 Hz, 1H), 6.74–6.70 (m, 3H), 3.80–3.73 (m, 1H), 3.78 (s, 3H), 3.66–3.60 (m, 1H), 3.37 (t, *J* = 8.5 Hz, 1H), 2.62–1.11 (m, 14H), 1.09 (s, 9H), 0.94 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 159.8, 143.0, 129.3, 121.4, 114.8, 111.2, 80.9, 72.3, 60.4, 55.3, 43.3, 40.4, 39.8, 39.0, 36.8, 31.4, 28.9 (3 ×

C), 28.8, 26.6, 25.9, 11.4; IR (film, cm^{-1}) 3349, 1601, 1259; MS (ESI) for $[C_{23}H_{36}O_3+NH_4]^+$: 378.3, found: 378.3.

(1*S*,3a*S*,6*R*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-7a-methyl-1*H*-indene-5-carboxyaldehyde (35)—DMSO (1.13 g, 14.4 mmol) in CH_2Cl_2 (20 mL) was added to a solution of oxalyl chloride (1.69 g, 13.3 mmol) in CH_2Cl_2 (100 mL) at −78 °C. After 10 min, diastereomeric compound **34** (4.20 g, 11.7 mmol) in CH_2Cl_2 (40 mL) was added and the reaction was stirred at −78 °C for 1 h. Et_3N (3.43 g, 34 mmol) was then added in one portion at −78 °C. After 30 min, the reaction was warmed to room temperature for 1 h. The reaction was quenched with water (40 mL) and the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **35** (4.19 g, 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.68 (s, 1H), 7.13 (t, J = 7.7 Hz, 1H), 6.67–6.61 (m, 3H), 3.70 (s, 3H), 3.36 (t, J = 7.4 Hz, 1H), 2.88 (dd, J = 13.5 Hz, 9.3 Hz, 1H), 2.68–1.14 (m, 12H), 1.04 (s, 9H), 0.68 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 205.1, 159.8, 142.7, 129.5, 121.4, 114.7, 111.4, 80.5, 72.4, 55.2, 49.4, 43.2, 41.4, 4.10, 39.7, 36.8, 31.3, 28.8 (3 × C), 25.8, 25.4, 11.3.

(3*S*,3a*S*,4a*R*,11a*S*)-3-(1,1-Dimethylethoxy)-2,3,3a,4,4a,5,11,11a-octahydro-7-methoxy-3a-methyl-1*H*-cyclopent[*b*]anthracene (36)—3 N HCl (50 ml) was added to a solution of compound **35** (4.15 g, 11.5 mmol) in MeOH (100 mL) at 0 °C. After 30 min, the reaction was warmed to room temperature. The reaction was complete after 1 h at room temperature. The product was extracted into EtOAc (100 ml × 3). The combined extracts were washed with aqueous $NaHCO_3$, brine, dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **36** (3.28 g, 84%): $[\alpha]_D^{20}$ −8.3 (c = 0.12, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89–6.86 (m, 1H), 6.66–6.64 (m, 2H), 6.13 (s, 1H), 3.76 (s, 3H), 3.44 (t, J = 7.7 Hz, 1H), 2.76–1.22 (m, 12H), 1.15 (s, 9H), 0.77 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 158.2, 140.5, 136.6, 128.2, 126.0, 122.3, 113.4, 111.1, 80.5, 72.4, 55.4, 45.6, 44.4, 43.2, 37.6, 34.4, 32.8, 31.5, 29.0 (3 × C), 26.0, 10.9; IR (film, cm^{-1}) 1608, 1500, 1253; MS (ESI) for $[C_{23}H_{32}O_2+NH_4]^+$: 358.3, found: 358.2.

(1*S*,3a*S*,4a*S*,10a*R*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-8-methoxy-11a-methyl-1*H*-cyclopent[*b*]anthracen-1-ol (37)—Na metal (1.04 g, 45 mmol) was added to an oven dried flask under N_2. The flask was equipped with a Dry Ice cooled condenser and anhydrous NH_3 (100 mL) was condensed in the flask. Aniline (6 ml) was then added at −78 °C. After 30 min, compound **36** (3.25 g, 9.5 mmol) in THF (40 mL) was added. The reaction was stirred for 2 h, and then quenched with solid NH_4Cl until the blue color disappeared. The flask was allowed to warm up to room temperature for 14 h to allow NH_3 to evaporate. Aqueous NH_4Cl was added and the product was extracted into EtOAc (100 ml × 3). The combined extracts were dried, filtered and the solvents removed. The ^{1}H NMR showed that the crude product was a mixture of diastereomers (4:1, 4a*S*:4a*R*). The unseparated diastereomeric 4a*S*:4a*R* products were dissolved in aqueous 6 N HCl in MeOH (100 mL) and refluxed 14 h. After cooling to room temperature, the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the

solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes). Purification removed the minor diastereomer (not characterized) yielding isolated pure product **37** (1.68 g, 62%, 2 steps): $[\alpha]_D^{20}$ +116.5 (*c* = 0.17, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.92 (d, *J* = 8.3 Hz, 1H), 6.63–6.54 (m, 2H), 3.70 (s, 3H), 3.64 (t, *J* = 8.2 Hz, 1H), 2.72–1.04 (m, 15H), 0.86 (t, *J* = 7.7 Hz, 1H), 0.74 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.6, 138.0, 129.6, 129.0, 113.4, 112.0, 81.9, 55.3, 44.7, 44.5, 43.7, 40.2, 38.5, 36.7, 34.6, 32.9, 30.5, 25.5, 11.2; IR (film, cm^{-1}) 3401, 1614, 1503. Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; found: C, 79.46; H, 9.08.

(1*S*,3a*S*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-7a-methyl-6-methylene-1*H*-inden-5-ol (38)—Compound **38** (4.19 g, 88%) was prepared from compound **30** (5.64 g, 20 mmol) as described previously.[25] Compound **38** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.01 (d, *J* = 1.4 Hz, 1H), 4.72 (d, *J* = 1.4 Hz, 1H), 3.94–3.89 (m, 1H), 3.41 (t, *J* = 7.4 Hz, 1H), 2.84 (s, br, 1H), 2.26–1.08 (m, 9H), 1.07 (s, 9H), 0.59 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6, 107.3, 79.1, 72.5, 72.1, 45.0, 43.9, 43.1, 35.5, 31.6, 28.6 (3 × C), 24.8, 10.7; IR (film, cm^{-1}) 3357, 1651, 1362; MS (ESI) for $[C_{15}H_{26}O_2+Na]^+$: 261.2, found: 261.2.

Acetic acid, (1*S*,3a*S*,7a*S*)-1-(1,1-dimethylethoxy)octahydro-7a-methyl-6-methylene-1*H*-inden-5-yl ester (39)—Ac_2O (3.57 g, 35 mmol), Et_3N (5.35 g, 52.5 mmol), and DMAP (108 mg, 0.88 mmol) were added to a solution of compound **38** (4.15 g, 17.4 mmol) in CH_2Cl_2 (80 mL) at room temperature. After 10 min, water (30 mL) was added and the product was extracted into CH_2Cl_2 (50 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography to afford product **39** (4.87 g, ca. 100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.15–5.10 (m, 1H), 4.83 (d, *J* = 27.2 Hz, 2H), 3.46 (t, *J* = 8.0 Hz, 1H), 2.31 (d, *J* = 12.9 Hz, 1H), 2.08 (s, 3H), 2.03–1.15 (m, 8H), 1.10 (s, 9H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 143.7, 107.9, 79.0, 73.9, 72.2, 45.2, 43.8, 42.8, 32.0, 31.6, 28.5 (3 × C), 24.8, 21.0, 10.8; IR (film, cm^{-1}) 1741, 1652, 1464, 1362; HRMS (ESI) calcd for $[C_{17}H_{28}O_3+Na]^+$: 303.1931, found: 303.1936.

Acetic acid, (1*S*,3a*S*,7a*S*)-1-(1,1-dimethylethoxy)octahydro-7a-methyl-6-oxo-1*H*-inden-5-yl ester (40)—A solution of compound **39** (4.85 g, 17.3 mmol) in MeOH (100 mL) and EtOAc (10 ml) was treated with ozone at −78 °C until a purple color persisted (ca. 30 min). Oxygen was passed through the solution for 20 min until the purple color disappeared, Me_2S (10 mL) was added and the reaction was allowed to warm to room temperature for 14 h. The solvents were removed under reduced pressure and the residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give product **40** (4.88 g, 17.3 mmol, ~100%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.08 (t, *J* = 7.2 Hz, 1H), 3.56 (t, *J* = 8.7 Hz, 1H), 2.38 (d, *J* = 13.2 Hz, 1H), 2.13 (d, *J* = 13.2 Hz, 1H), 2.05 (s, 3H), 2.38–1.31 (m, 7 H), 1.03 (s, 9H), 0.63 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 204.0, 169.9, 78.7, 75.8, 72.6, 51.3, 47.0, 41.7, 32.2, 30.8, 28.5 (3 × C), 2.45, 20.7, 11.6; MS (ESI) for $[C_{16}H_{26}O_4+H]^+$: 283.2, found: 283.2.

(1*S*,3a*S*,7a*S*)-3-(1,1-Dimethylethoxy)octahydro-3a-methyl-5*H*-inden-5-one (41)—Iodine (12.7 g, 50.0 mmol) in dry THF (150 mL) was added to Sm metal filings (7.80 g,

52 mmol) by cannula under N_2. The reaction was stirred at room temperature for 1 h forming a deep blue solution. Compound **40** (4.86 g, 17.2 mmol) in dry THF (75 mL) and MeOH (5 mL) was added to the SmI_2-THF solution and the reaction was stirred for 30 min. The reaction was poured into 20% aqueous Na_2CO_3 (300 mL) and the product was extracted into EtOAc (100 mL × 3). The combined extracts were washed with water (30 mL), brine (50 mL), dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to afford product **41** (3.27 g, 85%): $[\alpha]_D^{20}$ +75.4 (*c* = 0.54, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.49 (t, *J* = 8.5 Hz, 1H), 2.25–1.19 (m, 11H), 0.96 (s, 9H), 0.54 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.0, 79.2, 72.4, 53.7, 46.4, 43.2, 40.6, 32.1, 28.6 (3 × C), 25.1, 24.8, 11.8; IR (film, cm^{-1}) 1654, 1441, 1218. Anal. Calcd for $C_{14}H_{24}O_2$: C, 74.95; H, 10.78; found: C, 75.01; H, 10.87.

(1*S*,3a*S*,7a*S*)-1-(1,1-Dimethylethoxy)-2,3,3a,4,7,7a-hexahydro-6-hydroxy-7a-methyl-1*H*-indene-5-carboxylic acid, methyl ester (42)—Dimethyl carbonate (3.48 g, 43.5 mmol) was added to a suspension of NaH (1.16 g, 29 mmol) in THF (100 mL) at room temperature. The reaction was then refluxed for 30 min. Compound **41** (3.25 g, 14.5 mmol) in THF (50 mL) was added and the reaction was refluxed for 14 h. After cooling to room temperature, the reaction was slowly quenched with acetic acid until pH 4–5 and water was added (50 ml). The product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **42** (3.97 g, 97%): $[\alpha]_D^{20}$ +73.8 (*c* = 0.80, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.17 (s, 1H), 3.63 (s, 3H), 3.44 (t, *J* = 7.6 Hz, 1H), 2.36–1.20 (m, 9H), 1.03 (s, 9H), 0.62 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 172.0, 96.8, 80.3, 72.5, 51.4, 42.8, 41.9, 40.5, 31.6, 28.8 (3 × C), 25.7, 24.8, 11.2; IR (film, cm^{-1}) 1654, 1609, 1361, 1218; MS (ESI) for $[C_{16}H_{26}O_4+Na]^+$: 305.2, found: 305.2.

(1*S*,3a*S*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-5-(3-methoxybenzyl)-7a-methyl-6-oxo-1*H*-indene-5-carboxylic acid, methyl ester (43)—Compound **43** (4.38 g, 81%) was prepared from compound **42** (3.95 g, 14.0 mmol) using the procedure described for the preparation of compound **31** as an unresolved mixture of two diastereomers due to the formation of the new chiral center at C-5. The major diastereomer of compound **43** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11–7.06 (m, 1H), 6.86–6.60 (m, 3H), 3.67 (s, 3H), 3.66 (s, 3H), 3.54–3.46 (m, 1H), 3.27 (d, *J* = 13.7 Hz, 1H), 3.00 (d, *J* = 13.7 Hz, 1H), 2.38–1.01 (m, 9H), 1.04 (s, 9H), 0.71 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 209.2, 172.7, 159.6, 137.7, 129.3, 122.9, 116.3, 112.4, 79.3, 72.6, 62.2, 55.1, 52.3, 51.6, 46.5, 38.7, 38.6, 31.9, 30.6, 28.7 (3 × C), 25.0, 12.1; IR (film, cm^{-1}) 1704, 1601, 1263; MS (ESI) for $[C_{24}H_{34}O_5+H]^+$: 403.3, found: 403.3.

(3*S*,3a*S*,6*S*,7a*S*)-3-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-3a-methyl-5*H*-inden-5-one (44)—Compound **44** (3.58 g, 92%) was prepared from compound **43** (4.35 g, 11.3 mmol) using the procedure described for the preparation and purification of compound **32**. Compound **44** had: $[\alpha]_D^{20}$ +12.8 (*c* = 0.545, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11 (t, *J* = 7.7 Hz, 1H), 6.64–6.20 (m, 3H), 3.68 (s, 3H), 3.53 (t, *J* = 8.0 Hz, 1H), 3.20 (d, *J* = 9.6 Hz, 1H), 2.38–1.10 (m, 11H), 1.03 (s, 9H), 0.57 (s,

3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 211.8, 159.6, 142.2, 129.3, 121.6, 114.9, 111.2, 79.2, 72.5, 55.1, 53.7, 51.1, 47.1, 43.5, 35.5, 32.1, 31.1, 28.6 (3 × C), 25.0, 11.9; IR (film, cm^{-1}) 1705, 1602, 1256. Anal. Calcd for $C_{22}H_{32}O_3$: C, 76.70; H, 9.36; found: C, 76.90; H, 9.18.

(1*S*,3a*S*,5*S*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-5-(3-methoxybenzyl)-7a-methyl-6-methylene-1*H*-indene (45)—Compound **45** (3.45 g, 98%) was prepared from compound **44** (3.55 g, 10.3 mmol) using the procedure described for the preparation of compound **33** except that the reaction solvent was THF instead of benzene. Compound **45** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (t, *J* = 7.7 Hz, 1H), 6.68–6.60 (m, 3H), 4.74 (d, *J* = 13.5 Hz, 1H), 3.69 (s, 3H), 3.39 (t, *J* = 8.2 Hz, 1H), 3.08 (dd, *J* = 13.2 Hz, 4.1 Hz, 1H), 2.37–1.10 (m, 8H), 1.06 (s, 9H), 1.05–0.75 (m, 4H), 0.55 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 150.4, 143.2, 129.3, 121.7, 115.1, 111.0, 108.5, 80.0, 72.4, 55.2, 48.7, 45.1, 44.6, 44.1, 39.1, 32.0, 29.9, 28.9 (3 × C), 25.6, 11.2; MS (ESI) for $[C_{23}H_{34}O_2+H]^+$: 343.3, found: 343.3.

(3*S*,3a*S*,6*S*,7a*S*)-3-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-3a-methyl-1*H*-indene-5-methanol (46)—Compound **46** (3.06 g, 86%) was prepared from compound **45** (3.42 g, 10.0 mmol) using the procedure described for the preparation of compound **34**. Compound **46** had: $[\alpha]_D^{20}$ +16.0 (*c* = 0.43, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21–7.15 (m, 1H), 6.77–6.70 (m, 3H), 3.89–3.83 (m, 1H), 3.78 (s, 3H), 3.68–3.62 (m, 1H), 3.35 (t, *J* = 7.4 Hz, 1H), 2.83 (dd, *J* = 13.7 Hz, 5.0 Hz, 1H), 2.46–2.39 (m, 1H), 2.06–1.14 (m, 12H), 1.11 (s, 9H), 0.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.7, 143.3, 129.2, 121.6, 115.0, 111.0, 81.7, 72.3, 64.1, 55.2, 46.3, 43.5, 42.3, 41.3, 40.1 (2 × C), 31.2, 28.9 (3 × C), 27.8, 25.9, 13.4; IR (film, cm^{-1}) 3543, 1600, 1258; MS (ESI) for $[C_{23}H_{36}O_3+NH_4]^+$: 378.3, found: 378.2.

(3*S*,3a*S*,6*S*,7a*S*)-3-(1,1-Dimethylethoxy)octahydro-6-(3-methoxybenzyl)-3a-methyl-1*H*-indene-5-carboxaldehyde (47)—Compound **47** (3.01 g, ~100%) was prepared from compound **46** (3.04 g, 8.4 mmol) using the procedure described for the preparation of compound **35**. Compound **47** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.81 (s, 1H), 7.12 (t, *J* = 7.4 Hz, 1H), 6.66–6.60 (m, 3H), 3.69 (s, 3H), 3.33 (t, *J* = 6.9 Hz, 1H), 2.98–2.75 (m, 2H), 1.96–1.10 (m, 11H), 1.04 (s, 9H), 0.53 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 205.3, 159.7, 142.8, 129.4, 121.4, 114.8, 111.3, 80.8, 72.4, 55.2, 49.3, 45.8, 42.9, 42.7, 39.6, 39.5, 31.2, 29.1, 28.8 (3 × C), 25.9, 13.1.

(1*S*,3a*S*,4a*S*,11a*S*)-1-(1,1-Dimethylethoxy)-2,3,3a,4,4a,5,11,11a-octahydro-7-methoxy-11a-methyl-1*H*-cyclopent[*b*]anthracene (48)—Compound **48** (2.42 g, 85%) was prepared from compound **47** (3.00 g, 8.4 mmol) using the procedure described for the preparation of compound **36**. Compound **48** had: $[\alpha]_D^{20}$ +33.3 (*c* = 0.70, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.89–6.86 (m, 1H), 6.65–6.64 (m, 2H), 6.13 (s, 1H), 3.77 (s, 3H), 3.50 (t, *J* = 8.0 Hz, 1H), 2.84–2.38 (m, 4H), 1.96–1.25 (m, 8H), 1.16 (s, 9H), 0.72 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.2, 139.6, 136.3, 128.3, 126.0, 123.5, 113.4, 111.1, 80.0, 72.4, 55.4, 46.4, 43.8, 43.6, 37.1, 36.1, 34.5, 31.5, 28.9 (3 × C), 25.8, 11.5; IR (film, cm^{-1}) 1609, 1251. Anal. Calcd for $C_{23}H_{32}O_2$: C, 81.13; H, 9.47; found: C, 81.30; H, 9.34.

(1*S*,3a*S*,4a*S*,10a*S*,11a*S*)-2,3,3a,4,4a,5,10,10a,11,11a-Decahydro-7-methoxy-11a-methyl-1*H*-cyclopent[*b*]anthracen-1-ol (49)—Na metal (805 mg, 35 mmol) was added to an oven dried flask under N_2. The flask was equipped with a Dry Ice cooled condenser and anhydrous NH_3 (100 mL) was condensed into the flask. Aniline (5 ml) was then added at −78 °C. After 30 min, compound **48** (2.41 g, 7.1, mmol) in THF (50 mL) was added. The reaction was stirred for 2 h, and then was quenched with solid NH_4Cl until the blue color disappeared. The mixture was allowed to warm to room temperature for 14 h, aqueous NH_4Cl was added and the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered, and the solvents removed. The ^1H NMR of the crude product showed the diastereomeric 10a*S*:10a*R* products in a 12:1 ratio.

The unseparated 10a*S*:10a*R* diastereomers were dissolved in MeOH (50 mL) and aqueous 6 N HCl (25 mL) was added at room temperature. The reaction was refluxed for 3 h. After cooling to room temperature, the product was extracted into EtOAc (100 mL × 3). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 20% EtOAc in hexanes) to give compound **49** (1.42 g, 70%, 2 steps): mp 88–90 °C; $[\alpha]_D^{20}$ −8.7 (*c* = 0.46, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 6.92 (d, *J* = 8.0 Hz, 1H), 6.64–6.59 (m, 2H), 3.97 (s, 3H), 3.63 (t, *J* = 7.7 Hz, 1H), 2.99 (dd, *J* = 16.2 Hz, 6.6 Hz, 1H), 2.73–2.42 (m, 3H), 2.15–1.10 (m, 12H), 0.76 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.6, 137.7, 130.4, 128.7, 113.7, 111.3, 83.0, 55.2, 45.8, 42.9, 42.5, 36.5, 35.8, 33.2, 32.9, 30.3, 29.9, 25.6, 13.8; IR (film, cm^{-1}) 3390, 1610, 1502, 1257. Anal. Calcd for $C_{19}H_{26}O_2$: C, 79.68; H, 9.15; found: C, 79.79; H, 8.98.

(1*S*, 3a*S*, 8a*R*, 9a*S*) -1, 2, 3, 3a, 4, 7, 8, 8a, 9, 9a-Decahydro-1-hydroxy-9a-methyl-6*H*-benz[*f*] inden-6-one (50)—Compound **50** was prepared as described previously.[25]

(3a*S*,8a*R*,9a*S*)-2,3,3a,4,7,8,8a,9-Octahydro-9a-methyl-3*H*-benz[*f*]indene-1,6(3a*H*)-dione (51)—Compound **51** (980 mg, 97%) was prepared from compound **50** (1.02 g, 4.6 mmol) using the procedure described for the preparation of compound **28**. Compound **51** had: ^1H NMR (300 MHz, $CDCl_3$) δ 5.79 (s, 1H), 2.53–1.04 (m, 14H), 0.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 218.6, 199.4, 164.1, 126.5, 47.7, 44.7, 38.9, 37.3, 35.7, 35.2, 33.9, 30.2, 23.8, 12.9.

(3*S*, 3a*S*, 9a*S*, 9b*S*) -1, 2, 3, 3a, 4, 5, 8, 9, 9a, 9b-Decahydro-3-hydroxy-3a-methyl-7*H*-benz[*e*] inden-7-one (52)—Compound **52** was prepared as described previously.[28]

(3a*S*, 9a*S*, 9b*S*) -1, 2, 4, 5, 8, 9, 9a, 9b-Octahydro-3a-methyl-3*H*-benz[*e*] indene-3, 7(3a*H*) -dione (53)—Compound **53** (877 mg, 97%) was prepared from compound **52** (900 mg, 4 mmol) using the procedure described for the preparation and purification of compound **28**. Compound **53** had: ^1H NMR (300 MHz, $CDCl_3$) δ 5.80 (s, 1H), 2.51–1.13 (m, 14H), 0.95 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 218.9, 199.0, 164.4, 125.9, 50.6, 47.6, 37.6, 36.6, 35.8, 30.8, 30.7, 26.7, 21.9, 13.0.

(1*S*,3a*S*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-5-(4-methoxybenzylidene)-7a-methyl-1*H*-indene (54)—Na metal (92 mg, 4.0 mmol) was dissolved in EtOH (30 mL) and then 1-(1-methylenetriphenylphosphine)-4-methoxybenzene bromide (1.85 g, 4.0 mmol) was added and the reaction was refluxed for 1 h. Compound **29** (448 mg, 2.0 mmoL) in THF (10 mL) was then added. After 16 h at reflux, the reaction mixture was cooled to room temperature, water was added (30 mL) and the product was extracted into EtOAc (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **54** (420 mg, *E* and *Z* mixture 1/1, 64%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19–7.05 (m, 2H), 6.78 (d, *J* = 8.2 Hz, 2H), 6.14 (s, 1H), 3.74 (s, 3H), 3.34 (t, *J* = 7.4 Hz, 1H), 2.72–0.84 (m, 11H), 1.07 (s, 9H), 0.80 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.9, 141.5, 141.4, 131.3, 131.1, 130.3, 122.9, 122.7, 113.7, 80.6, 80.5, 72.4, 55.4, 46.3, 45.6, 42.8, 42.7, 38.3, 37.7, 37.6, 33.0, 31.6, 31.4, 29.9, 29.7, 28.9, 26.1, 24.7, 10.6.

(1*S*,3a*S*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-5-[2-(4-methoxyphenyl)ethylidene]-7a-methyl-1*H*-indene (55)—NaH (80 mg, 60% in mineral oil, 2.0 mmol) suspended in THF was added to a suspension of 1-(2-ethyltriphenylphosphine)-4-methoxybenzene iodide (1.03 g, 2.0 mmol) in THF (20 mL) at room temperature. The mixture was refluxed for 1 h and then compound **29** (224 mg, 1.0 mmoL) in THF (5 mL) was added while reflux was maintained. After 1 h, the reaction was cooled to room temperature, water was added (30 mL) and the product was extracted into EtOAc (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **55** (274 mg, E and Z mixture 1/1, 80%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (dd, *J* = 1.6 Hz, 8.5 Hz, 2H), 6.75 (dd, *J* = 1.6 Hz, 8.5 Hz, 2H), 5.22–5.19 (m, 1H), 3.67 (s, 3H), 3.33 (t, *J* = 6.9 Hz, 1H), 3.27–3.20 (m, 2H), 2.49–0.73 (m, 11H), 1.05 (s, 9H), 0.75 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.95, 139.7, 134.0, 133.8, 129.3, 128.9, 128.7, 121.7, 121.5, 114.0, 80.7, 80.6, 72.3, 55.4, 46.4, 45.8, 43.0, 38.4, 37.8, 37.1, 33.0, 32.8, 32.5, 31.6, 31.5, 29.9, 28.9, 28.6, 26.2, 26.0, 23.9, 10.6, 10.5.

(1*S*,3a*S*,7a*S*)-1-(1,1-Dimethylethoxy)octahydro-5-[3-(4-methoxyphenyl)propylidene]-7a-methyl-1*H*-indene (56)—NaH (80 mg, 60% in mineral oil, 2.0 mmol) suspended in THF was added to a suspension of 1-(3-propyltriphenylphosphine)-4-methoxybenzene iodide (900 mg, 2.0 mmol) in THF (20 mL) at room temperature. The mixture was refluxed for 1 h and then compound **29** (224 mg, 1.0 mmoL) in THF (5 mL) was added while reflux was maintained. After 1 h, the reaction mixture was cooled to room temperature, water was added (30 mL) and the product was extracted into EtOAc (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 10% EtOAc in hexanes) to give product **56** (328 mg, E and Z mixture 1/1, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99 (d, *J* = 8.2 Hz, 2H), 6.72 (d, *J* = 8.2 Hz, 2H), 5.06 (t, *J* = 6.6 Hz, 1H), 3.67 (s, 3H), 3.25 (t, *J* = 7.7 Hz, 1H), 2.56–0.72 (m, 15H), 1.03 (s, 9H), 0.69 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 139.8, 139.6, 134.4, 129.5, 129.4, 129.3, 128.7, 128.6, 128.5, 121.8, 121.5, 113.8, 113.7, 80.5, 80.4, 79.5, 72.5, 72.1, 55.2,

46.3, 45.3, 44.7, 43.0, 42.8, 42.1, 38.4, 37.4, 37.0, 35.6, 35.3, 32.5, 31.9, 31.5, 31.4, 29.8, 29.7, 29.5, 28.8, 28.7, 28.5, 26.0, 25.9, 23.7, 10.4, 10.3.

(1*S*,3a*S*,4a*R*,7a*S*)-1,2,3,3a,3′,4,4′,6,7,7a-Decahydro-7′-methoxy-7a-methyl-2′H-spiro[indene-5,1′-naphthalen]-1-ol (57)—6 N HCl (20 mL) was added to a solution of compound **56** (356 mg, 0.92 mmol) in MeOH (20 mL) at room temperature. The reaction was refluxed for 16 h and then was cooled to room temperature. The product was extracted into CH_2Cl_2 (100 mL × 2). The combined extracts were dried, filtered and the solvents removed. The residue was purified by flash column chromatography (silica gel eluted with 15% EtOAc in hexanes) to give product **57** (200 mg, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (d, *J* = 8.5 Hz, 2H), 6.87 (dd, *J* = 2.7 Hz, 8.5 Hz, 1H), 3.83 (s, 3H), 3.78 (t, *J* = 7.7 Hz, 1H), 2.72–2.69 (m, 1H), 2.20–1.34 (m, 17H), 0.98 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 147.6, 129.8, 113.3, 110.7, 81.8, 55.3, 43.0, 39.9, 39.8, 38.8, 34.7, 33.3, 33.0, 30.5, 30.3, 25.8, 19.8, 10.2; HRMS (ESI) calcd for $[C_{20}H_{28}O_2+Na]^+$: 323.1982, found: 323.1982.

(1*S*,3a*S*,7a*S*)-2,3,3a,4,7,7a-Hexahydro-5-(4-methoxybenzyl)-7a-methyl-1*H*-inden-1-ol (58)—Compound **58** (241 mg, 70%) was prepared from compound **54** (420 mg, 1.28 mmol) using the procedure described for the preparation of compound **57**. Compound **58** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.5 Hz, 2H), 6.87 (d, *J* = 8.5 Hz, 2H), 5.41–5.40 (m, 1H), 3.78 (s, 3H), 3.76 (t, *J* = 7.7 Hz, 1H), 3.21 (s, 1H), 2.16–1.23 (m, 11H), 0.65 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.1, 137.0, 132.6, 129.8 (2 × C), 121.9, 113.8 (2 × C), 81.9, 55.4, 43.5, 41.7, 41.3, 38.4, 31.2, 30.9, 25.6, 10.3; HRMS (ESI) calcd for $[C_{18}H_{24}O_2+Na]^+$: 295.1669, found: 295.1672.

(1*S*,3a*S*,7a*S*)-2,3,3a,4,7,7a-Hexahydro-5-(4-methoxyphenethyl)-7a-methyl-1*H*-inden-1-ol (59)—Compound **59** (150 mg, 89%) was prepared from compound **55** (200 mg, 0.6 mmol) using the procedure described for the preparation of compound **57**. Compound **59** had: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 5.34–5.32 (m, 1H), 3.76 (s, 3H), 3.72 (t, *J* = 7.8 Hz, 1H), 2.68–1.24 (m, 14H), 0.68 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.8, 136.6, 134.6, 129.3 (2 × C), 120.6, 114.0, 113.8 (2 × C), 81.9, 55.3, 41.2, 39.8, 38.3, 33.8, 31.7, 30.8, 25.7, 10.1.

Cell culture conditions

HT-22 cells are a murine hippocampal cell line donated by David Schubert, Salk Institute, San Diego, CA. HT-22 cells were maintained in Dulbecco's modified Eagle's (DMEM) media (GIBCO, Gaithersburg, PA) supplemented with 10% charcoal-stripped fetal bovine serum (HyClone, Logan, UT) and 20 Ag/ml gentamicin (Sigma, St. Louis, MO) under standard cell culture conditions (5% CO2, 95% air, 37 8C). HT-22 cells were seeded into Costar 96-well plates (Corning, NY) at a density of 5,000 cells per well.

Compound neuroprotection evaluation

Compounds were administered simultaneously with the glutamate insults. HT-22 cells were incubated with 3mM glutamate for 24 h and then assessed for viability. Glutamate-induced cell death in HT-22 cells is through inhibition of the glutamate/cysteine antiporter leading to the depletion of glutathione and thus oxidative stress.[32] For each compound tested,

glutamate toxicity was assessed in the absence of compound, or in the presence of 100nM or 1 μM compound. As a positive control, ZYC-26, a compound that is potently neuroprotective,[11] was tested at 1μM in the assay for each compound.

Cell viability determination

Cell viability was determined by calcein acetoxymethyl (AM) assay (Molecular Probes, Eugene, OR). The calcein AM assay measures cellular esterase activity and plasma membrane integrity. For complete cell death, several wells received MeOH for 15 min before calcein AM assay. Wells were rinsed with phosphate-buffered saline (PBS), after which a 2.5 AM solution of calcein AM in PBS was added. After incubation at room temperature for 15 min, fluorescence was determined (excitation 485 nm, emission 530 nm) using a fluorescence FL600 microplate reader (Biotek, Winooski, VT). We have shown a strong linear relationship between calcein fluorescence (relative fluorescence units; RFU) and cell number for HT-22 cells (range 1000 to 10,000 cells per well).

Statistical Analysis

Data from each compound represent the mean ± SEM of at least 8 independent experiments. Data were analyzed with GraphPad Prism 5.0 (La Jolla, CA) using one-way analysis of variance (ANOVA) with $p < 0.05$ as the threshold for statistical significance. Because our interest was in comparing each of the three treatment conditions to the No Cmpd control group, we followed up significant ANOVAs with Dunnett's post hoc test for pairwise comparisons to control for the inflated Type I error rate associated with multiple two-group comparisons. Bartlett's tests were used to identify statistically significant ($p<0.05$) or marginal ($0.05<p<0.1$) violations of homogeneity of variance in the ANOVA tests conducted for each compound of interest. An important consequence to violations of this crucial assumption of ANOVA is an inflation of the Type I error rate, meaning an increased risk of detecting a significant effect in the sample when one does not actually exist in the population.[33] In the event such violations were detected for a given compound, data were log transformed and the ANOVA/post-hoc tests were interpreted using the transformed data, provided the violation of the homogeneity of variance was corrected by the transformation. If the log transformation failed to correct the homogeneity of variance violation, non-parametric testing using the Kruskal-Wallis test, which does not rely as heavily on the assumptions underlying ANOVA, was used. As well, using the Shapiro-Wilk test, all data for each compound, even log-transformed data, were assessed for adherence to the normality assumption of ANOVA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The Pharmacological assessment of the compounds was completed at the University of North Texas Health Science Center. The data analysis and statistical assessment was done at West Virginia University. This work was supported by NIH Grants P20 GM109098, P01 AG022550, P01 AG027956 and U54 GM1049492 to JWS; and The Taylor Family Institute for Innovative Psychiatric Research to DFC. The X-ray structure determinations were made

possible by NSF Shared Instrument Grant No. CHE-042097. Support for the Washington University in St. Louis Mass Spectrometry Resource is provided by NIH grant P41 GM103422.

Notes and references

1. Bishop J, Simpkins JW. Mol Cell Neurosci. 1994; 5:303. [PubMed: 7804599]
2. Behl C, Widmann M, Trapp T, Holsboer F. Biochem Biophys Res Commun. 1995; 216:473. [PubMed: 7488136]
3. Behl C, Skutella T, Lezoualc'h F, Post A, Widmann M, Newton CJ, Holsboer F. Mol Pharmacol. 1997; 51:535. [PubMed: 9106616]
4. Simpkins JW, Rajakumar G, Zhang YQ, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL. J Neurosurg. 1997; 87:724. [PubMed: 9347981]
5. Sawada H, Ibi M, Kihara T, Urushitani M, Akaike A, Shimohama S. J Neurosci Res. 1998; 54:707. [PubMed: 9843162]
6. Goodman Y, Bruce AJ, Cheng B, Mattson MP. J Neurochem. 1996; 66:1836. [PubMed: 8780008]
7. Moosmann B, Behl C. Proc Natl Acad Sci U S A. 1999; 96:8867. [PubMed: 10430862]
8. Chen J, Xu W, Jiang H. Anesth Analg. 2001; 92:1520. [PubMed: 11375837]
9. Culmsee C, Vedder H, Ravati A, Junker V, Otto D, Ahlemeyer B, Krieg JC, Krieglstein J. J Cereb Blood Flow Metab. 1999; 19:1263. [PubMed: 10566973]
10. Perez E, Cai ZY, Covey DF, Simpkins JW. Drug Dev Res. 2005; 66:78.
11. Perez E, Liu R, Yang SH, Cai ZY, Covey DF, Simpkins JW. Brain Res. 2005; 1038:216. [PubMed: 15757637]
12. Gatson JW, Liu MM, Abdelfattah K, Wigginton JG, Smith S, Wolf S, Simpkins JW, Minei JP. J Neurotrauma. 2012; 29:2209. [PubMed: 22435710]
13. Niki E, Nakano M. Methods Enzymol. 1990; 186:330. [PubMed: 2172709]
14. Lacort M, Leal AM, Liza M, Martin C, Martinez R, Ruizlarrea MB. Lipids. 1995; 30:141. [PubMed: 7769970]
15. Cegelski L, Rice CV, O'Connor RD, Caruano AL, Tochtrop GP, Cai ZY, Covey DF, Schaefer J. Drug Dev Res. 2005; 66:93.
16. Jastrzebska I, Scaglione JB, DeKoster GT, Rath NP, Covey DF. J Org Chem. 2007; 72:4837. [PubMed: 17530900]
17. Hajos ZG, Parrish DR. Org Synth. 1984; 64:26.
18. Martin R, Buchwald SL. Acc Chem Res. 2008; 41:1461. [PubMed: 18620434]
19. Collins DJ, Fallon GD, Skene CE. Aust J Chem. 1992; 45:71.
20. Qian M, Covey DF. Adv Synth Catal. 2010; 352:2057. [PubMed: 22022301]
21. Jeyaraj DA, Kapoor KK, Yadav VK, Gauniyal HM, Parvez M. J Org Chem. 1998; 63:287.
22. Krapcho AP. Synthesis. 1982:805.
23. Kutney JP, Winter J, McCrae W, By A. Can J Chem. 1963; 41:470.
24. Tietze LF, Ramachandar T, Voch C. Synlett. 2003:118.
25. Scaglione J, Rath NP, Covey DF. J Org Chem. 2005; 70:1089. [PubMed: 15675880]
26. Qian M, Krishnan K, Kudova E, Li P, Manion BD, Taylor A, Elias G, Akk G, Evers AS, Zorumski CF, Mennerick S, Covey DF. J Med Chem. 2014; 57:171. [PubMed: 24328079]
27. Wang C, Rath NP, Covey DF. Tetrahedron. 2007; 63:7977. [PubMed: 18698337]
28. Hu Y, Zorumski CF, Covey DF. J Org Chem. 1995; 60:3619.
29. Matsuya Y, Masuda S, Itoh T, Murai T, Nemoto H. J Org Chem. 2005; 70:6898. [PubMed: 16095311]
30. Komeno T, Ishihara S, Itani H. Tetrahedron. 1972; 28:4719.
31. Morales-Alanis H, Brienne MJ, Jacques J, Bouton MM, Nedelec L, Torelli V, Tournemine C. J Med Chem. 1985; 28:1796. [PubMed: 4068004]
32. Li Y, Maher P, Schubert D. Proc Natl Acad Sci U S A. 1998; 95:7748. [PubMed: 9636222]

33. Keppel, G., Wickens, TD. Design and Analysis: A Researcher's Handbook. Vol. Chapter 7. Prentis Hall; 2004. p. 143

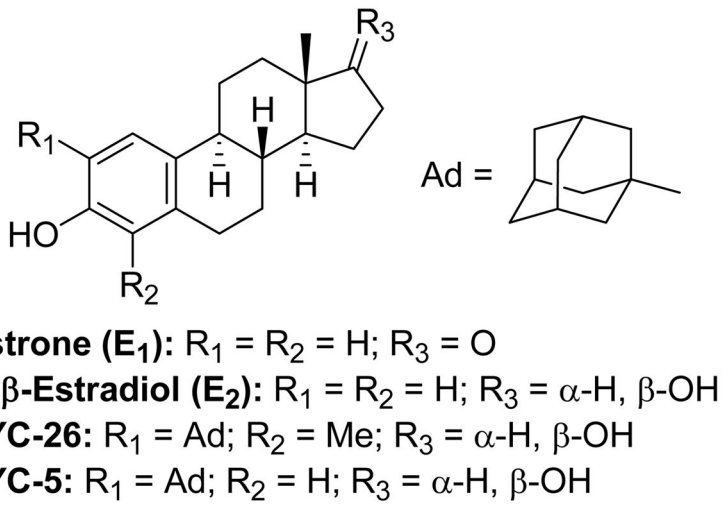


Figure 1.
Structures of neuroprotective steroids: estrone, 17β-estradiol, ZYC-26 and ZYC-5.

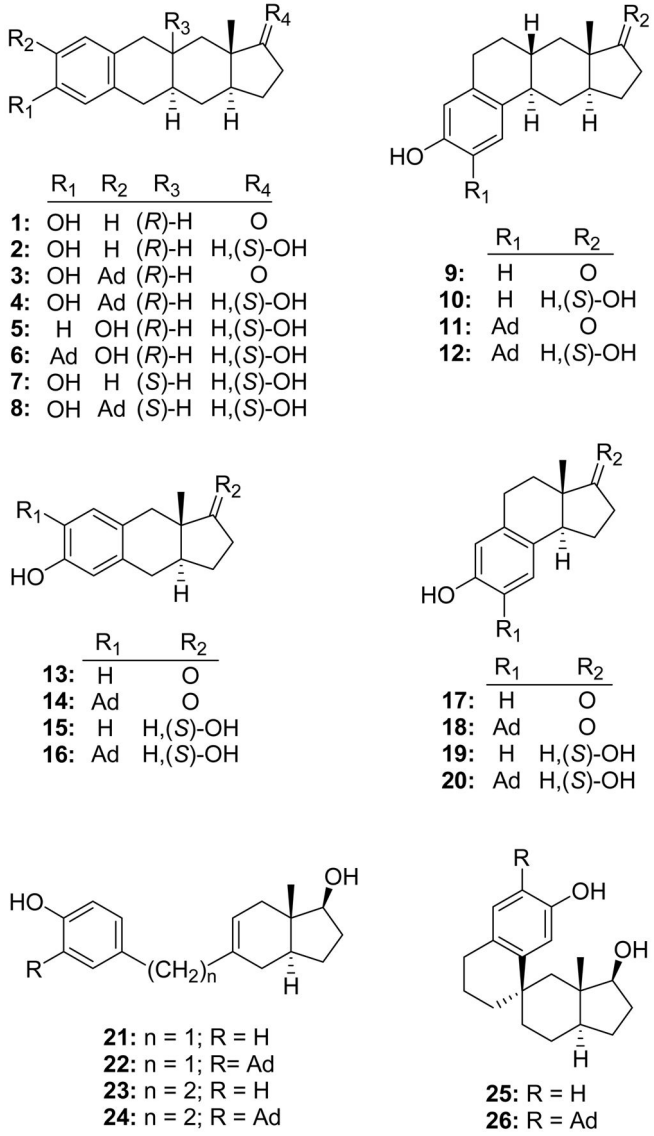
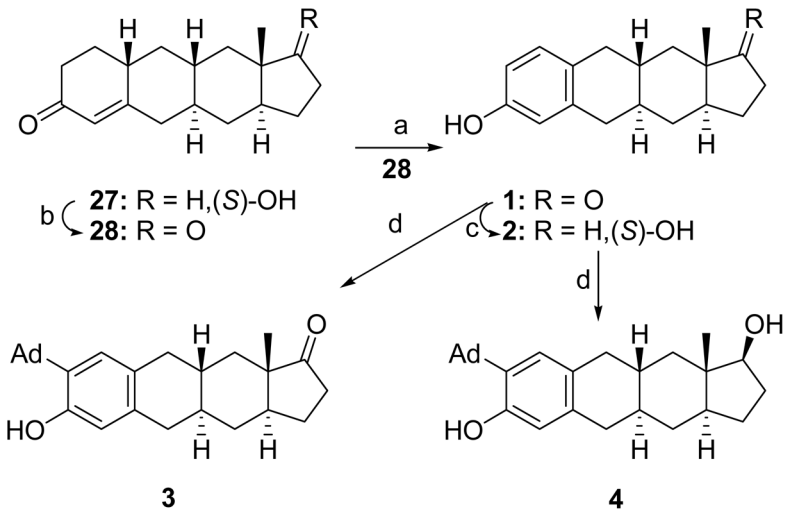


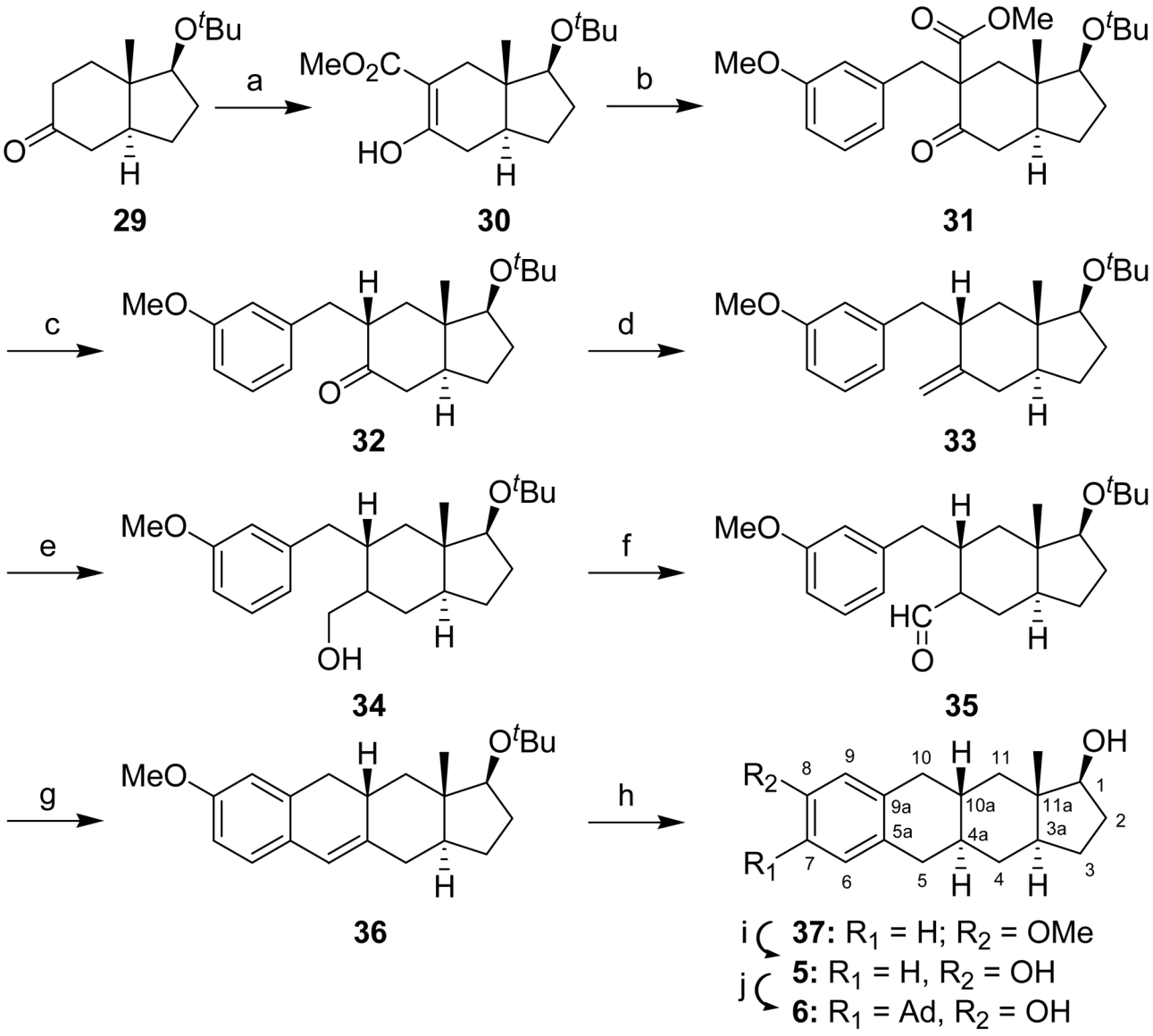
Figure 2.

Structures of the non-steroidal analogues synthesized and evaluated. The designation (*S*)-OH indicates the stereochemistry of the hydroxyl group is the same as that of a steroid 17β-OH group.



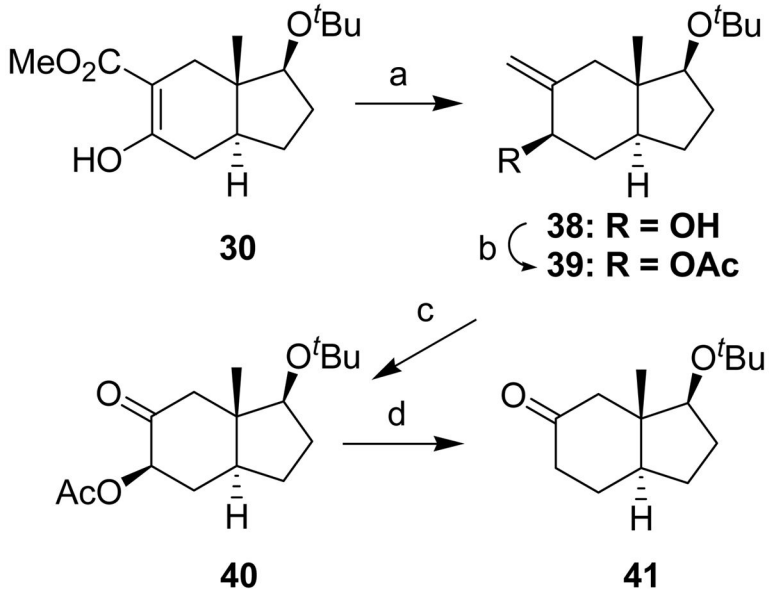
Scheme 1[a]

[a]Reagents: (a) $CuBr_2$, LiBr, CH_3CN, (83%); (b) Jones Reagent, acetone, (ca. 100%); (c) $NaBH_4$, EtOH, (92%); (d) 1-adamantanol, BF_3•Et_2O, CH_2Cl_2, (**3**, 81%), (**4**, 75%).



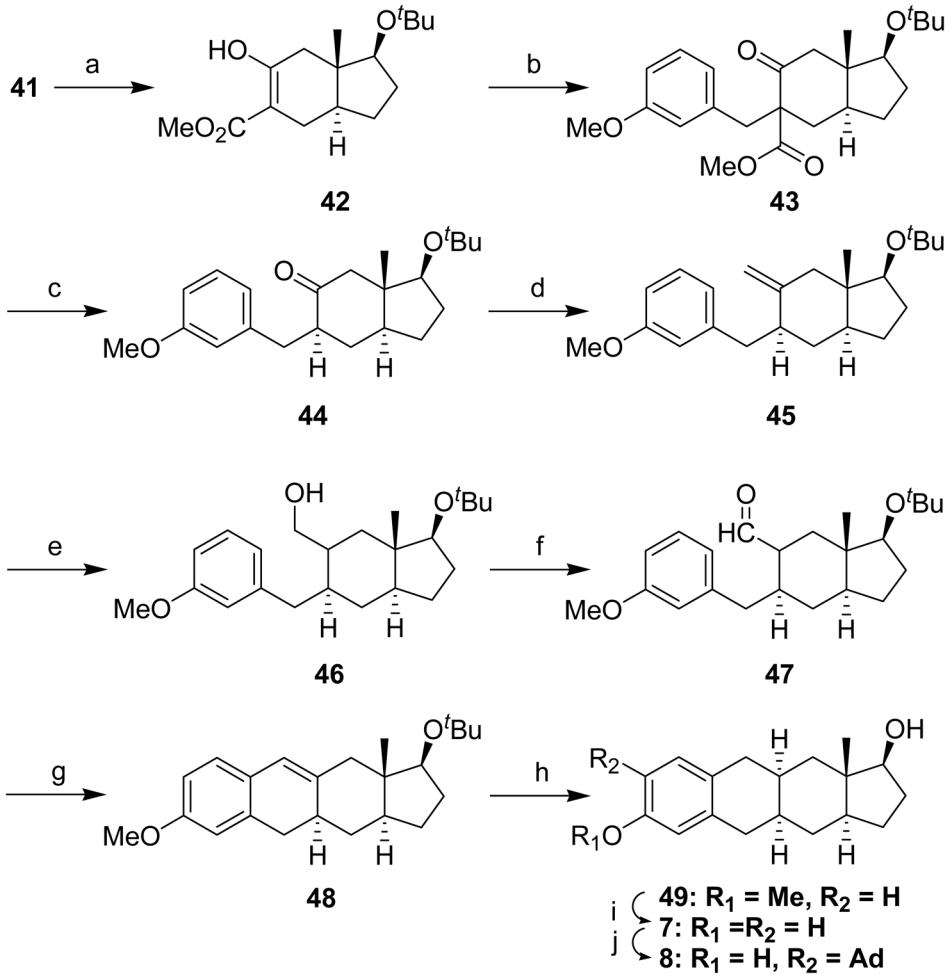
Scheme 2[a]

[a]Reagents: (a) NaH, $(CH_3O)_2CO$, THF, (93%); (b) NaH, 3-Methoxybenzyl bromide, DMF/toluene, (85%); (c) LiCl, DMF, (90%); (d) NaH, methyltriphenylphosphonium bromide, benzene, (94%); (e) i: BH_3•THF, THF; ii: 3 N NaOH, H_2O_2, (82%); (f) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N, (ca. 100%); (g) 3 N HCl, MeOH, (84%); (h) i: Na, liq. NH_3, aniline, THF; ii: 6 N HCl, MeOH, (62%, 2 steps); (i) DIBAL–H, toluene, (85%); (j) 1-adamantanol, BF_3•Et_2O, CH_2Cl_2, (80%).



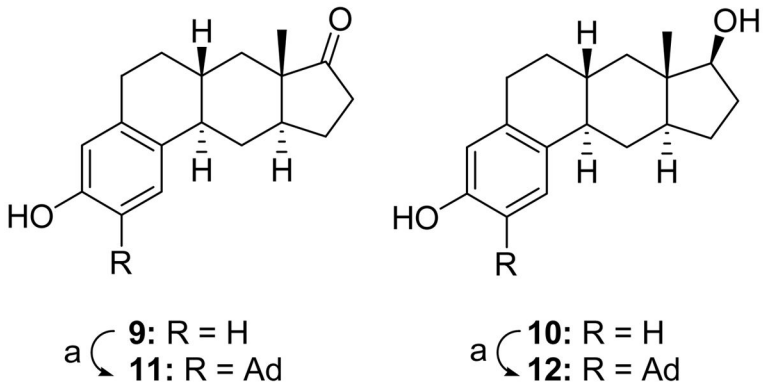
Scheme 3[a]

[a]Reagents: (a) $LiAlH_4$, THF, (88%); (b) $(AcO)_2O$, Et_3N, DMAP, CH_2Cl_2 (ca. 100%); (c) i: O_3, MeOH/EtOAc; ii: Me_2S, (ca. 100%); (d) SmI_2, THF/MeOH, (85%)



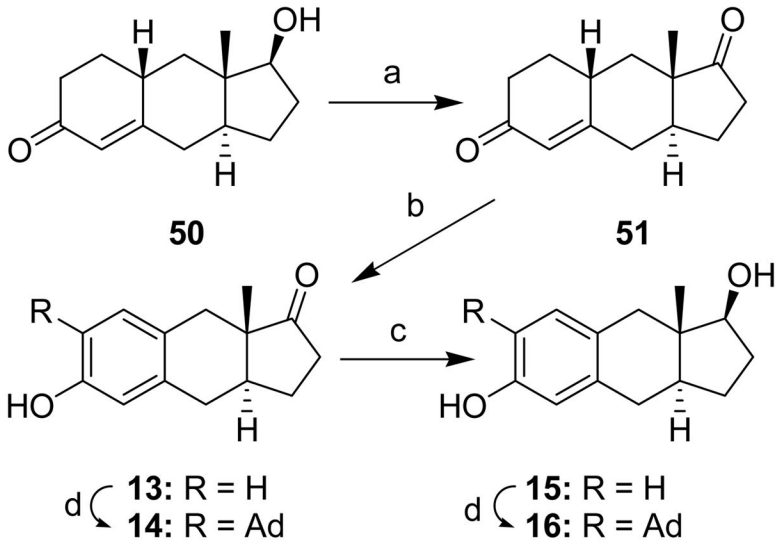
Scheme 4[a]

[a]Reagents: (a) NaH, $(CH_3O)_2CO$, THF, (97%); (b) NaH, 3-Methoxybenzyl bromide, DMF/toluene, (81%); (c) LiCl, DMF, (92%); (d) NaH, methyltriphenylphosphonium bromide, benzene, (98%); (e) i: BH_3•THF, THF; ii: 3 N NaOH, H_2O_2, (86%); (f) $(COCl)_2$, DMSO, CH_2Cl_2, Et_3N (ca. 100%); (g) 3 N HCl, MeOH, (85%); (h) i: Na, liq. NH_3, aniline, THF; ii: 6 N HCl, MeOH (70%, 2 steps); (i) DIBAL–H, toluene, (94%); (j) 1-adamantanol, BF_3•Et_2O, CH_2Cl_2, (85%).



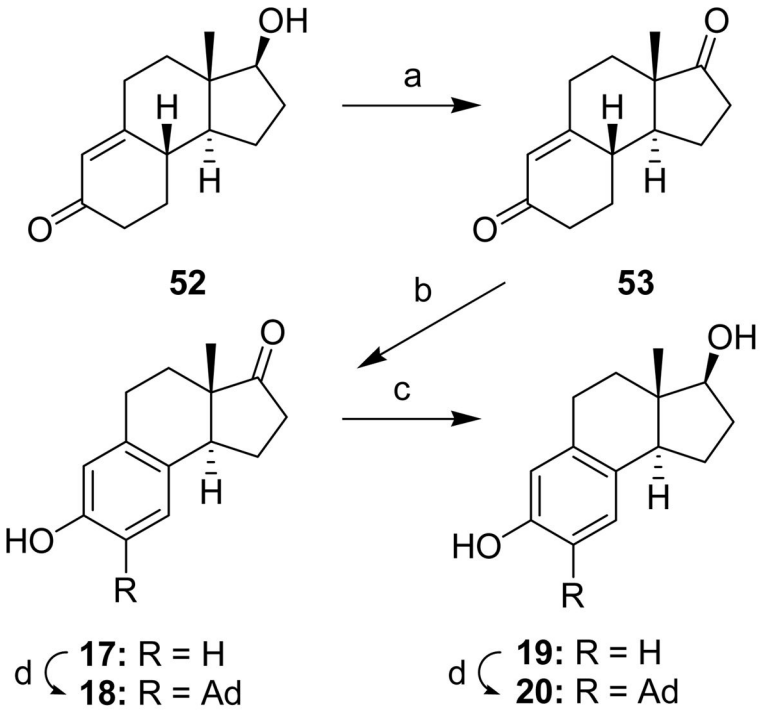
Scheme 5[a]

[a]Reagents:(a) 1-adamantanol, BF_3•Et_2O, CH_2Cl_2, (**11**,71%), (**12**, 71%).



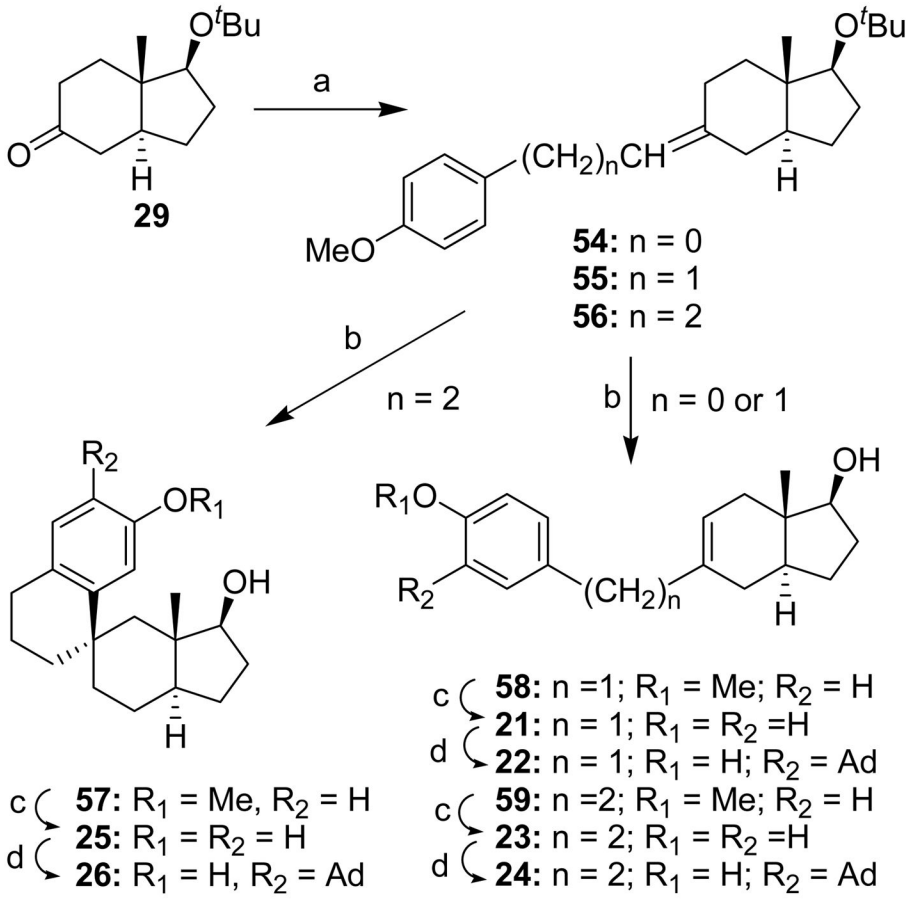
Scheme 6[a]

[a]Reagents: (a) Jones Reagent, acetone, (97%); (b) $CuBr_2$, LiBr, CH_3CN, (74%); (c) $NaBH_4$, EtOH, (81%); (d) 1-adamantanol, BF_3•Et_2O, CH_2Cl_2, (**14**, 71%), (**16**, 61%).



Scheme 7[a]

[a]Reagents: (a) Jones Reagent, acetone, (97%); (b) $CuBr_2$, LiBr, CH_3CN, (58%); (c) $NaBH_4$, EtOH, (83%); (d) 1-adamantanol, BF_3•Et_2O, CH_2Cl_2, (**18**, 50%), (**20**, 56%).



Scheme 8[a]

[a]Reagents: (a) **54**, 4-MeOC$_6$H$_4$CH$_2$PPh$_3$Br, Na, EtOH, (64%); **55**, 4-MeOC$_6$H$_4$CH$_2$CH$_2$PPh$_3$Br, NaH, THF, (80%); **56**, 4-MeOC$_6$H$_4$CH$_2$CH$_2$CH$_2$PPh$_3$Br, NaH, THF (92%); (b) 6 N HCl, MeOH, (**57**, 72%), (**58**, 70%), (**59**, 89%); (c) BBr$_3$, CH$_2$Cl$_2$, (**21**,77%); DIBAL–H, toluene, (**23**, 72%); DIBAL-H, toluene, (**25**, 94%); (d) 1-adamantanol, BF$_3$•Et$_2$O, CH$_2$Cl$_2$ (**22**, 79%) (**24**, 73%), (**26**, 83%).

Org Biomol Chem. Author manuscript; available in PMC 2017 October 18.

Table 1

Neuroprotective activity against glutamate-induced neurotoxicity for E_2 and the non-steroidal analogues in cultures of the HT-22 hippocampal cell line.

Cmpd	Ketone or Hydroxyl[a]	No Cmpd	Cmpd @ 100 nM	Cmpd @ 1 μM	ZYC-26 @ 1 μM
		% Survival from 3mM glutamate toxicity (mean±SEM)[b]			
Compounds Without Adamantyl Group Compared to ZYC-26					
Four Fused Rings					
E_2	OH	37.3 ± 1.0	39.7 ± 2.3	43.6 ± 2.3	99.5 ± 2.6*
1	=O	33.2 ± 3.4	42.5 ± 2.9	40.9 ± 2.5	102 ± 7.7^{*N}
2	OH	33.2 ± 3.4	38.1 ± 3.0	34.9 ± 2.4	102 ± 7.7^{*N}
5	OH	31.8 ± 3.5	38.8 ± 0.5	38.0 ± 1.3	87.9 ± 5.0^{*N}
7	OH	30.4 ± 2.4	39.8 ± 3.9	41.5 ± 4.6	112 ± 10.4^{*L}
9	=O	34.8 ± 5.3	48.6 ± 4.2	42.2 ± 3.5	113 ± 6.4^{*N}
10	OH	34.8 ± 5.3	62.8 ± 5.7^{*N}	43.3 ± 4.8	113 ± 6.4^{*N}
Three Fused Rings					
13	=O	43.6 ± 2.7	41.3 ± 2.2	43.0 ± 1.9	102±6.5^{*N}
15	OH	43.6 ± 2.7	38.7 ± 2.3	40.3 ± 2.4	102±6.5^{*N}
17	=O	39.6 ± 1.6	37.9 ± 1.3	39.6 ± 1.4	96.5 ± 2.2^{*N}
19	OH	41.2 ± 2.1	46.0 ± 3.4	45.1 ± 2.9	99.1 ± 3.0*
Phenolic Rings Connected via Linkers to Indenes; Spiro Rings					
21	OH	38.5 ± 2.1	34.9 ± 1.8	40.9 ± 1.9	103 ± 5.2^{*L}
23	OH	42.5 ± 3.6	43.2 ± 3.0	49.2 ± 5.3	101 ± 5.6*
25	OH	34.2 ± 1.5	35.8 ± 1.4	34.5 ± 1.5	102 ± 2.8*
Compounds with Adamantyl Group Compared to ZYC-26					
Four Fused Rings					
3	=O	35.4 ± 1.5	55.1 ± 3.5^{*L}	117 ± 6.0^{*L}	121 ± 5.2^{*L}
4	OH	35.4 ± 1.5	52.9 ± 3.2^{*L}	108 ± 4.9^{*L}	121 ± 5.2^{*L}
6	OH	31.8 ± 3.5	37.5 ± 0.8	45.9 ± 1.5	87.9 ± 4.7^{*N}
8	OH	30.4 ± 2.4	55.8 ± 6.6	106 ± 9.3^{*N}	112 ± 10.4^{*N}

Cmpd	Ketone or Hydroxyl[a]	No Cmpd	Cmpd @ 100 nM	Cmpd @ 1 μM	ZYC-26 @ 1 μM
		% Survival from 3mM glutamate toxicity (mean±SEM)[b]			
11	=O	29.3 ± 4.9	52.3 ± 2.9	105 ± 6.1^{*N}	127 ± 2.2^{*N}
12	OH	29.3 ± 4.9	54.1 ± 2.3	133 ± 2.8^{*N}	127 ± 2.2^{*N}
Three Fused Rings					
14	=O	51.4 ± 2.1	54.2 ± 4.8	55.5 ± 3.1	101 ± 4.6^{*N}
16	OH	51.4 ± 2.1	56.5 ± 2.8	99.1 ± 5.5^{*L}	101 ± 4.6^{*L}
18	=O	39.6 ± 1.6	39.6 ± 1.4	50.0 ± 1.6^{*N}	96.5 ± 2.2^{*N}
20	OH	41.2 ± 2.1	45.7 ±2.4	93.5±4.5*	99.1 ± 3.0*
Phenolic Rings Connected via Linkers to Indenes; Spiro Rings					
22	OH	38.5 ± 2.1	36.5 ± 2.0	61.5 ± 4.7^{*N}	103 ± 5.2^{*L}
24	OH	42.5 ± 3.6	49.2 ± 5.3	82.6 ± 4.7*	101 ± 5.6*
26	OH	34.2 ± 1.5	54.2 ± 2.1*	105 ± 3.5*	102 ± 2.8*

[a]Ketone or hydroxyl refers to the substituent on the five membered ring in the analogues.

[b]Symbols used to identify significant comparisons indicate the following: * = statistically significant (p<0.05) difference between marked group and the No Cmpd comparison group using ANOVA; ^{*L} = statistically significant (p<0.05) difference between marked group and the No Cmpd comparison group using ANOVA applied to data that had been log-transformed to correct a violation of homogeneity of variance (as indicated by significant Bartlett's tests); ^{*N} = statistically significant (p<0.05) difference between marked group and the NoCmpd comparison group using non-parametric testing (Kruskal-Wallis tests) applied to data that violated the normality assumption (as indicated by significant Shapiro-Wilk tests). See Statistical Analysis in Experimental section for further details.