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Synthesis of carbocyclic analogs of dehydroaltenusin: identification of a stable inhibitor of calf DNA polymerase α

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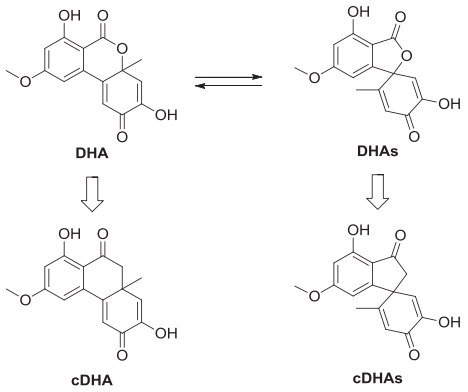
ABSTRACT

Syntheses of carbocyclic analogs of dehydroaltenusin tautomers are reported. Both target compounds, **cDHA** (2,8-dihydroxy-6-methoxy-10a-methyl-10,10a-dihydrophenanthrene-3,9-dione) and **cDHAs** (4′,5-dihydroxy-6′-methoxy-2-methylspiro[cyclohexa[2,5]diene-1,1′-indene]-3′,4(2′H)-dione), were prepared from 3,5-dimethoxybenzaldehyde in 11 and 13 steps, respectively. Unlike dehydroaltenusin, both **cDHA** and **cDHAs** are stable and their structures were confirmed by X-ray crystallography. Compound **cDHA** was found to be active against calf DNA polymerase α but not related isozymes, while the spirocyclic analog **cDHAs** was inactive.

1. Introduction

Proteins and pathways involved in DNA damage/repair continue to be intensely studied, especially in the context of development of new cancer therapeutic agents.[1] The inhibition of DNA polymerases (pols) seems to be a proper component of synthetic lethal combination therapy.[2] Specifically, deletion of pol α was found to be synthetic lethal with elimination or inhibition of CHK1 kinase, but elimination of additional pols (delta and epsilon) resulted in significantly weaker synthetic lethal response.[3] Selective inhibitors of pol α could therefore be of considerable therapeutic potential. Of the compounds that are known to inhibit this enzyme,[1] only two seem to be sufficiently selective: nucleoside analog BuPdGTP and its derivatives,[4] and dehydroaltenusin.[5] Both substances possess suboptimal pharmacological properties: BuPdGTP exhibits only weak activity in the cell,[6] while chemical stability of dehydroaltenusin is limited, as it undergoes a rearrangement in aqueous solutions to give a mixture of the spirocyclic and non-spirocyclic forms (structures **DHAs** and **DHA** in Scheme 1; absolute configurations are not known).[7] It is not clear which of these forms is active and it has been suggested that the rearrangement *o*-quinone intermediate might be also responsible for the inhibitory activity.[8] In order to help elucidate this issue, we decided to carry out bioisosteric replacement of the lactone ring oxygen by methylene group and prepare the carbocyclic analogs of the spirocyclic and non-spirocyclic forms of dehydroaltenusin – **cDHAs** and **cDHA**, respectively (Scheme 1). These compounds were envisioned to be significantly more stable and resistant to the rearrangement.



Scheme 1. Spirocyclic and non-spirocyclic isomers of dehydroaltenusin and their carbocyclic analogs. Of note, the central scaffolds of both c**DHA** and c**DHAs** are only sporadically documented in the literature.

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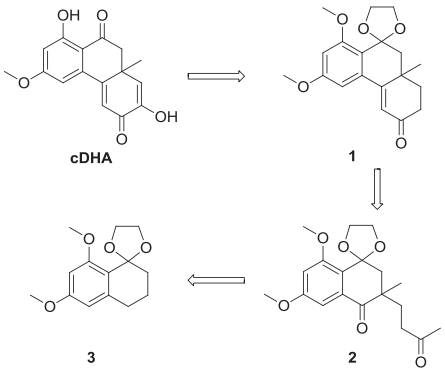
0040-4020/

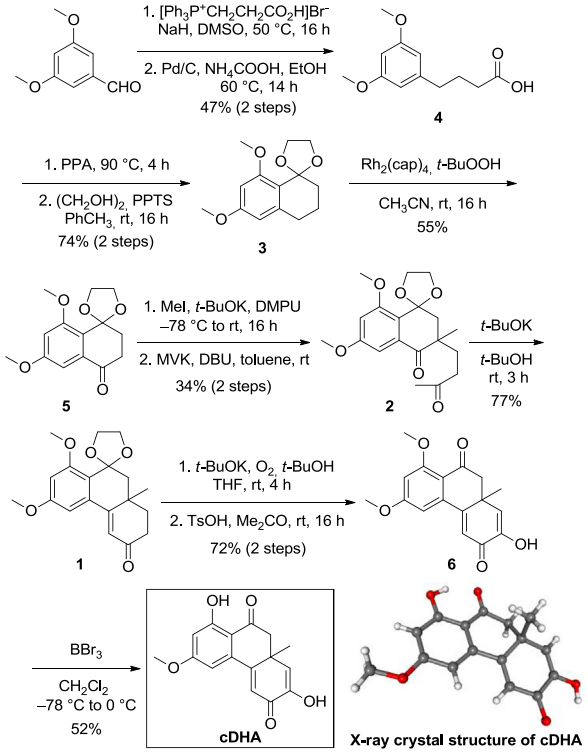
2. Results and discussion

The retrosynthetic analysis of **cDHA**, depicted in Scheme 2, relies on benzylic oxidation and alkylation of protected tetralone **3** to provide intermediate **2** possessing the required quaternary center, aldol cyclization forming the tricyclic scaffold **1**, and final oxidation and deprotection to yield the target compound (**cDHA**).



Scheme 2. Retrosynthetic analysis of **cDHA**. Construction of the quaternary center and the tricyclic scaffold.

Starting from 3,5-dimethoxybenzaldehyde, Wittig reaction followed by hydrogenation afforded acid **4**, whose intramolecular Friedel–Crafts cyclization gave the required tetralone (not shown).[9] Attempted protections of the tetralone using ethanediol, 2-methoxy-1,3-dioxolane, ethanediol plus $CH(OCH_3)_3$, or 2-methoxy-1,3-dioxolane in the presence of TsOH did not provide the desired product **3** in acceptable yields, likely due to the sensitivity of the substance to strong acids. However, reaction with ethanediol in the presence of PPTS in refluxing benzene afforded **3** in satisfactory yield (Scheme 3).



Scheme 3. Synthesis and structural assignment of **cDHA**.

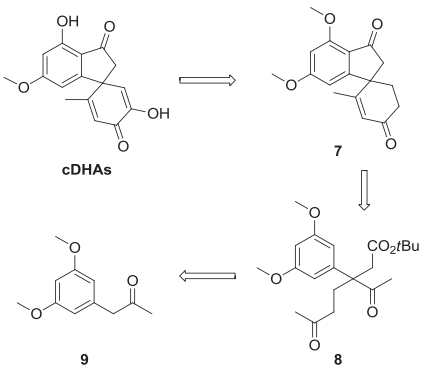
Benzylic oxidation of **3** using $Cr(CO)_6$ or PDC with *tert*-butylhydroperoxide[10] afforded the desired ketone **5** in modest yields (10% and 35%, respectively). Best result (55%) was obtained with dirhodium caprolactamate plus *tert*-butylhydroperoxide in acetonitrile, buffered with $NaHCO_3$.[11]

Methylation (*t*-BuOK, MeI) of **5** led to a mixture of mono- and dimethylated ketones, along with the unreacted starting material. Reasonable yield (55%) of the desired mono-methylated product was obtained when the alkylation was carried out in the presence of DMPU. Subsequent reaction with methyl vinyl ketone (MVK) proved difficult and out of many conditions tried, only the method by von Doering[12] afforded the desired diketone **2** in acceptable (61%) yield.

Intramolecular aldol condensation of **2** using *t*-BuOH/*t*-BuOK provided tricyclic ketone **1**. Subsequent oxidation with oxygen under basic conditions, followed by acidic cleavage of the ketal provided compound **6** in good overall yield (Scheme 3).

Finally, selective demethylation of **6** using BBr_3 gave the desired target compound **cDHA**, whose structure was confirmed by X-ray crystallography (Scheme 3).

The retrosynthetic analysis of **cDHAs** (depicted in Scheme 4) relies on double alkylation of ketone **9**, regioselective ring closure of **8** leading to the spirocyclic intermediate **7**, and final oxidation and selective demethylation to afford **cDHAs**.

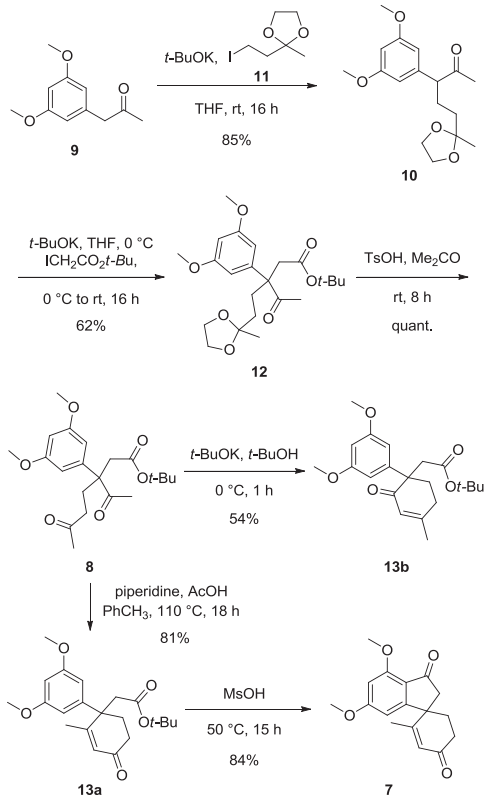


Scheme 4. Retrosynthetic analysis of **cDHAs**. Construction of the spirocyclic core.

Ketone **9** was prepared in two steps by condensation of 3,5-dimethoxybenzaldehyde with nitroethane, followed by reduction with iron in AcOH.[13] Because our subsequent attempts to add MVK to **9** failed under a variety of conditions and in most cases lead only to the recovery of the starting material, we attempted alkylations with MVK equivalents. Best results were obtained with iodide **11**, which we prepared in 80% yield by modification of the procedure published for the analogous bromide.[14] This way we obtained intermediate **10** that underwent second alkylation with *t*-butyl-2-iodoacetate.[15] The resulting ester **12** was then deprotected to give diketone **8**, which was used for the subsequent intramolecular aldol closure (Scheme 5).

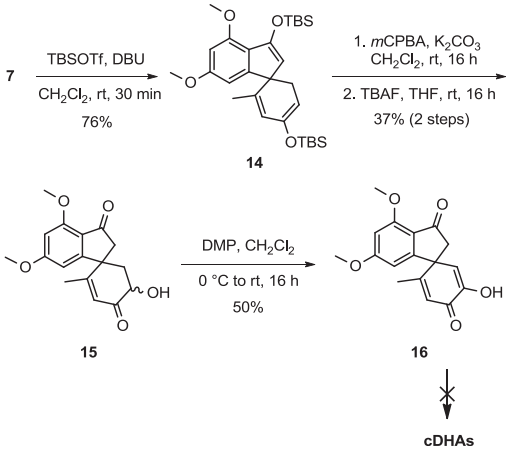
The regioselectivity of the aldol closure proved very dependent on the reaction conditions. Condensation using piperidine/AcOH in toluene,[16] which may proceed via enamine formation at the less sterically hindered carbonyl, afforded the desired enone **13a** (81%). In contrast, when carried out under basic conditions (*t*-BuOK/THF), the condensation yielded exclusively the isomer **13b** (Scheme 5). Construction of the spirocyclic scaffold was completed by intramolecular Friedel–Crafts reaction of **13a** in the presence of MsOH, which provided the spirocyclic enone **7** in good yield (Scheme 5).

In contrast to the non-spirocyclic series, we were not able to oxidize enone **7** directly. We thus attempted to convert it into a silyl enol ether that could be used for subsequent Rubottom oxidation. In accordance with the literature,[17] regioselective formation of the desired silyl enol ether proved non-trivial: numerous attempts to carry out mono-silylation of **7** yielded only mixtures of the desired product, often along with its exocyclic isomer, bis-silyl enol ether **14**



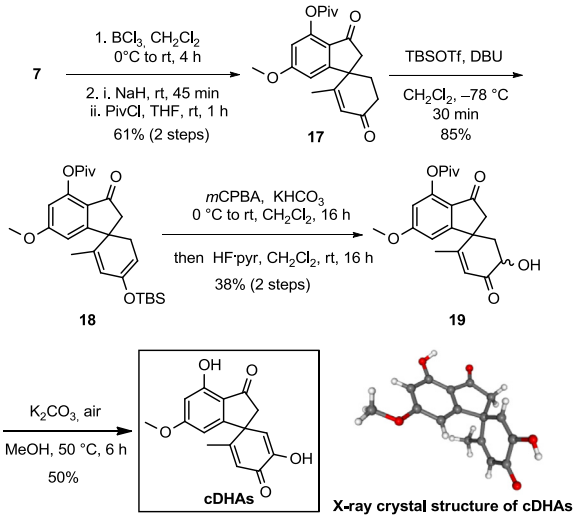
Scheme 5. Construction of the spirocyclic scaffold.

and unreacted **7**. However, with excess of TBSOTf the bis-silylation of **7** proceeded cleanly and the subsequent epoxidation occurred predominantly at the six membered ring (Scheme 6). The resulting hydroxyketone **15** (as ca. 5:1 mixture of diastereomers) was oxidized to **16**. However, all our attempts to cleave the phenolic methyl ether in the vicinity of indanone carbonyl (e.g., using BBr_3 or BCl_3) failed and yielded complex mixtures that contained only traces of the desired product **cDHAs**.



Scheme 6. Attempted conversion of enone **7** into **cDHAs**.

Fortunately, selective demethylation was possible at the stage of spirocyclic enone **7**, which enabled us to introduce an alternative protecting group that could be cleaved more easily at the end of the synthesis (Scheme 7). Along this line we decided to prepare pivaloyl ester **17**. Interestingly (and in contrast to **7**), ester **17** underwent clean *mono*-silylation to afford enol ether **18**, which was then oxidized to hydroxyketone **19**. Exposure of methanolic solution of **19** to air and K_2CO_3, which effected the desired oxidation and cleavage of the pivalate, afforded the target compound **cDHAs**, whose structure was again unambiguously confirmed by X-ray crystallography (Scheme 7).



Scheme 7. Completion of synthesis of **cDHAs**.

In the biochemical assay we developed previously,[18] we tested both **cDHA** and **cDHAs** against the following mammalian pols: calf pol α and human pols γ, κ, and λ, which belong to the B-, A-, Y-, and X-family of pols, respectively. As shown in Table 1, **cDHA** specifically inhibits pol α. In contrast, **cDHAs** inhibits none of the tested pols.

Table 1
IC_{50} values of **cDHA** and **cDHAs** for mammalian DNA polymerases[a]

Polymerase	IC_{50} value (μM)	
	cDHA	cDHAs
Calf pol α	5.5±0.29	>100
Human pol γ	>100	>100
Human pol κ	>100	>100
Human pol λ	>100	>100

[a] Data are shown as the means ± SE of three independent experiments. Description of the assay is given in Experimental section.

3. Conclusions

In conclusion, we prepared racemic novel carbocyclic analogs of dehydroaltenusin **cDHA** and **cDHAs** in 11 and 13 steps, respectively, starting from 3,5-dimethoxybenzyldehyde. While **cDHA** is approximately eight times weaker inhibitor than dehydroaltenusin itself (previously reported IC_{50} value for dehydroaltenusin against calf pol α is 0.68 μM),[5] it is stable and can be stored in the solid state as well as in aqueous DMSO solution for at least four weeks without noticeable decomposition. In addition, the enantiomers of **cDHA** can be efficiently separated by HPLC on chiral stationary phase (details given in Supplementary data). Bioisosteric replacement of oxygen atom by the methylene group is commonly used in medicinal chemistry;[19] however, in the context of this project, it enabled not only identification of more stable analogs, but also provided significant (albeit indirect) information on which of the dehydroaltenusin isomers is responsible for its biological activity. Our observation of the dramatic difference in activities of **cDHA** and **cDHAs** strongly suggests that dehydroaltenusin's inhibitory activity

toward pols is due to its non-spirocyclic form **DHA** and while the presence reactive *o*-quinone intermediate may contribute to dehydroaltenusin's inhibitory activity, it may not be absolutely required.

We believe the methodology presented above will enable preparation of additional analogs possessing the rare and non-trivial tricyclic central motifs. In addition, our results could be used to guide further development of potent and selective inhibitors of mammalian polymerases, namely pol α, exploiting the recently published crystal structure.[20]

4. Experimental section

4.1. General

All reagents and solvents were of reagent grade and used without further purification. Anhydrous solvents (THF, dichloromethane, CH_3CN) were purchased from commercial suppliers (Aldrich, Acros) and stored over 4 Å molecular sieves. All reactions were carried out in oven-dried glassware and under N_2 atmosphere unless stated otherwise. Column chromatography was carried on silica gel (230–400 mesh). TLC plates were visualized under UV and stained with phosphomolybdic acid or $KMnO_4$ solution or Vaughn's reagent ($(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$Ce(SO_4)_2\cdot 4H_2O$/conc. H_2SO_4/H_2O).

NMR spectra were recorded on Bruker Avance 300 and 500 MHz spectrometers, with operating frequencies 300.13, 500.13 MHz for ^1H and 75.48, 125.77 MHz for ^{13}C. The ^1H and ^{13}C NMR chemical shifts (δ in ppm) were referenced to the residual signals of solvents: $CDCl_3$ [7.24 (^1H) and 77.23 (^{13}C)]. Structural assignments of resonances have been performed with the help of 2D NMR gradients experiments (HSQC, HMBC, NOESY).

High resolution mass spectra were measured on Agilent 6224 Accurate-Mass TOF LC-MS with dual electrospray/chemical ionization mode with mass accuracy greater than 2 ppm, applied mass range was from 25 to 20,000 Da.

IR spectra (4000–400 cm^{-1}) were collected on an EQUINOX 55/S/NIR FTIR spectrometer. Samples were prepared as KBr pellets.

The diffraction data for sample **cDHA** (CCDC number 1012667) were collected with a KUMA KM-4 κ-axis diffractometer equipped with a Sapphire2 CCD detector and a Cryostream Cooler (Oxford Cryosystems, UK). Mo Kα radiation (λ=0.71073 Å, fine-focus sealed tube, graphite monochromator) was used.

The diffraction data for sample **cDHAs** (CCDC number 1012668) were collected with a Rigaku partial χ geometry diffractometer equipped with a Saturn 944+ HG CCD detector and a Cryostream Cooler (Oxford Cryosystems, UK). Cu Kα radiation (λ=1.54184 Å, MicroMax-007HF rotating anode source, multilayer optic VariMax) was used. Data reduction and final cell refinement were carried out using the CrysAlisPro software ([CrysAlisPro] CrysAlisPRO, Agilent Technologies UK Ltd).

4.2. 4-(3,5-Dimethoxyphenyl)but-3-enoic acid

To NaH (60% suspension in mineral oil, 1.90 g, 49.7 mmol) was added dry DMSO (36 mL) and the mixture was stirred and heated at 70 °C. After the evolution of bubbles ceased, the mixture was cooled to 10 °C. To this mixture, a solution of (2-carboethoxy)triphenylphosphonium bromide (11.00 g, 26.5 mmol) in DMSO (26 mL) was added dropwise. To the resulting dark red reaction mixture, a solution of 3,5-dimethoxybenzaldehyde (2.0 g, 12.0 mmol) in DMSO (5 mL) was added. The reaction mixture was stirred at 50 °C for 16 h, then cooled, poured into ice–water (200 mL), acidified with conc. HCl to pH<4, and extracted with EtOAc (4×50 mL). The combined organic layers were washed with H_2O (2×50 mL), dried over Na_2SO_4 and concentrated. The product was purified by column chromatography (silica gel, EtOAc/hexane (1:1)) to obtain the title 4-(3,5-dimethoxyphenyl)but-3-enoic acid as a yellow solid (1.80 g, 66%). Mp 58–60 °C; ^1H NMR (300 MHz, $CDCl_3$) δ=6.55 (d, J=2.3 Hz, 2H), 6.47 (d, J=15.9 Hz, 1H), 6.39 (t, J=2.3 Hz, 1H), 6.28 (dt, J=15.9 Hz, J=7.2 Hz, 1H), 3.80 (s, 6H), 3.30 (d, J=7.2 Hz, 2H).^1H NMR spectrum was identical with the published literature.[21]

4.3. 4-(3,5-Dimethoxyphenyl)butanoic acid (4)

A solution of 4-(3,5-dimethoxyphenyl)but-3-enoic acid (2.00 g, 9.0 mmol) in EtOH (24 mL) was added to Pd/C (10% Pd, 478 mg) and ammonium formate (1.70 g, 27.0 mmol). The reaction mixture was stirred at 60 °C for 14 h, then cooled, filtered through a pad of Celite, and concentrated under reduced pressure. The residue was diluted with H_2O (100 mL), acidified with conc. HCl to pH=4, and extracted with EtOAc (3×50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/hexane (1:1)) to afford **4** as a white solid (1.44 g, 71%). Mp 63–65 °C; ^1H NMR (300 MHz, $CDCl_3$) δ=6.35 (d, J=2.0 Hz, 2H), 6.32 (dd, J=2.0 Hz, 1H), 3.78 (s, 6H), 2.62 (t, J=7.6 Hz, 2H), 2.38 (t, J=7.4 Hz, 2H), 2.02–1.90 (m, 2H).

4.4. 6,8-Dimethoxy-3,4-dihydronaphthalen-1(2*H*)-one

A mixture of **4** (1.44 g, 6.42 mmol) and polyphosphoric acid (14 mL) was stirred at 90 °C for 4 h, then it was poured into ice–-water (250 mL) and extracted with EtOAc (3×100 mL). The combined organic extracts were washed with saturated aqueous solution of $NaHCO_3$ (2×50 mL) and dried over Na_2SO_4. Removal of the solvent under reduced pressure afforded the product as a yellow solid (1.24 g, 93%). Mp 62–63 °C; ^1H NMR (300 MHz, $CDCl_3$) δ=6.35–6.30 (m, 3H), 3.87 (s, 2H), 3.84 (s, 3H), 2.87 (t, J=6.0 Hz, 2H), 2.57 (t, J=6.5 Hz, 2H), 2.07–1.96 (m, 2H). ^1H NMR spectrum was identical with the published literature.[22]

4.5. 6′,8′-Dimethoxy-3′,4′-dihydro-2′*H*-spiro[[1,3]dioxolane-2,1′-naphthalene] (3)

A mixture of 6,8-dimethoxy-3,4-dihydronaphthalen-1(2*H*)-one (206 mg, 1.00 mmol), pyridinium *p*-toluenesulfonate (25 mg, 0.01 mmol), and ethylene glycol (170 μL, 3.00 mmol) in benzene (25 mL) was heated at reflux in a flask equipped with a Dean-Stark apparatus for 14 h. The mixture was cooled to 25 °C, K_2CO_3 (100 mg) was added, and the solvent was evaporated. The residue was purified by column chromatography (silica gel, EtOAc/hexane/Et_3N (100:50:1)) to afford **3** as a white solid (200 mg, 80%), which was used immediately in the next step. Mp 85 °C (dec); ^1H NMR (500 MHz, $CDCl_3$) δ=6.33 (d, J=2.4 Hz, 1H), 6.21 (d, J=2.4 Hz, 1H), 4.25–4.18 (m, 2H), 4.08–4.01 (m, 2H), 3.80 (s, 3H), 3.76 (s, 3H), 2.75–2.70 (m, 2H), 1.96–1.91 (m, 2H) 1.86–1.79 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=160.2, 160.0, 142.3, 117.8, 108.1, 104.3, 98.0, 65.3 (2C), 55.8, 55.1, 36.8, 31.1, 21.1; HRMS (APCI): calcd for $C_{14}H_{19}O_4$ $[M+H]^+$: 251.1283, found: 251.1348.

4.6. 6′,8′-Dimethoxy-2′*H*-spiro[[1,3]dioxolane-2,1′-naphthalen]-4′(3′*H*)-one (5)

$Rh_2(cap)_4$ (9 mg, 0.01 mol) and *t*-BuOOH (5.5 M in decane, 900 μL, 4.95 mmol) were added to a mixture of **3** (250 mg, 1.00 mmol) and $NaHCO_3$ (42 mg, 0.50 mmol) in CH_3CN (2 mL). The reaction mixture was stirred at 25 °C for 16 h, then concentrated, and purified by column chromatography (silica gel, EtOAc/hexane (1:2)) to yield **5** as a white solid (145 mg, 55%). Mp 81 °C (dec); ^1H NMR (300 MHz, $CDCl_3$) δ=7.11 (d, J=2.5 Hz, 1H), 6.71 (d, J=2.5 Hz, 1H), 4.31–4.23 (m, 2H), 4.13–4.05 (m, 2H), 3.86 (s, 3H), 3.84 (s, 3H), 2.79 (m, 2H), 2.28 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=197.3, 161.0, 159.1, 135.2, 123.4, 106.8, 106.0, 100.9, 65.8 (2C), 56.3, 55.6, 36.2,

34.4; IR (KBr): $\tilde{\nu}$=2962 cm^{-1} (m), 2939 (w), 2887 (w), 1685 (s), 1603 (s), 1466 (m), 1430 (w), 1354 (s), 1321 (s), 1300 (s), 1217 (s), 1163 (s), 1114 (s), 1040 (s), 980 (w), 916 (m), 842 (m); HRMS (APCI): calcd for $C_{14}H_{17}O_5$ $[M+H]^+$: 265.1071, found: 265.1068.

4.7. 6′,8′-Dimethoxy-3′-methyl-2′*H*-spiro[[1,3]dioxolane-2,1′-naphthalen]-4′(3′*H*)-one

A solution of compound **5** (100 mg, 0.38 mmol) and 1,3-dimethyltetrahydropyrimidin-2(1*H*)-one (330 μL) in THF (1 mL) was cooled to −78 °C, *t*-BuOK (46.8 mg, 0.42 mmol) was added, and the mixture was stirred at −78 °C for 45 min. Iodomethane (30 μL, 0.48 mmol) was added, the mixture was allowed to warm to 25 °C and stirred for 16 h, then diluted with H_2O (50 mL), and extracted with CH_2Cl_2 (3×20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/hexane (1:2)) to yield 6′,8′-dimethoxy-3′-methyl-2′*H*-spiro[[1,3]dioxolane-2,1′-naphthalen]-4′(3′*H*)-one as a white solid (58 mg, 55%). Mp 99 °C (dec); ^{1}H NMR (500 MHz, $CDCl_3$) δ=7.11 (d, *J*=2.6 Hz, 1H), 6.70 (d, *J*=2.6 Hz, 1H), 4.33–4.24 (m, 2H), 4.17–4.13 (m, 1H), 4.11–4.06 (m, 1H), 3.86 (s, 3H), 3.85 (s, 3H), 3.00–2.92 (m, 1H) 2.25 (m, 1H), 2.12 (m, 1H), 1.22 (d, *J*=6.7 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=199.6, 161.0, 159.1, 135.4, 123.0, 106.7, 105.5, 100.9, 65.9, 65.8, 56.1, 55.5, 42.7, 39.9, 14.6; IR (KBr): $\tilde{\nu}$=2987 cm^{-1} (w), 2968 (m), 2893 (m), 2860 (m), 2839 (w), 1680 (s), 1603 (s), 1470 (m), 1456 (s), 1365 (m), 1323 (s), 1300 (s), 1229 (s), 1207 (s), 1157 (m), 1115 (s), 1082 (m), 1041 (m), 964 (m), 949 (w), 941 (w), 839 (s); HRMS (APCI): calcd for $C_{15}H_{19}O_5$ $[M+H]^+$: 279.1227, found: 279.1227.

4.8. 6′,8′-Dimethoxy-3′-methyl-3′-(3-oxobutyl)-2′*H*-spiro[[1,3]dioxolane-2,1′-naphthalen]-4′(3′*H*)-one (2)

A stream of nitrogen saturated by MVK was bubbled through a solution of 6′,8′-dimethoxy-3′-methyl-2′*H*-spiro[[1,3]dioxolane-2,1′-naphthalen]-4′(3′*H*)-one (40 mg, 0.14 mmol) and DBU (22 μL, 0.14 mmol) in toluene (1 mL). Upon completion, indicated by TLC (silica gel, EtOAc/hexane (1:2)), the solvent was evaporated. The crude residue was purified by column chromatography (silica gel, EtOAc/hexane (1:2)) to afford **2** as a colorless wax (29.7 mg, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.10 (d, *J*=2.5 Hz, 1H), 6.71 (d, *J*=2.5 Hz, 1H), 4.33–4.20 (m, 2H), 4.16–4.05 (m, 2H), 3.84 (s, 6H), 2.57–2.44 (m, 1H), 2.39–2.27 (m, 1H), 2.22 (d_{AB}, *J*=14.4 Hz, 1H), 2.12 (s, 3H), 2.10 (d_{AB} *J*=14.4 Hz, 1H) 2.06–1.85 (m, 2H), 1.20 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=208.1, 201.5, 161.2, 159.1, 133.9, 122.4, 105.8, 105.6, 101.3, 65.64, 65.56, 56.1, 55.5, 44.9, 44.5, 38.6, 31.3, 29.8, 23.1; IR (KBr): $\tilde{\nu}$=2964 cm^{-1} (w), 2937 (w), 2899 (w), 1713 (s), 1687 (s), 1601 (s), 1464 (m), 1356 (m), 1323 (s), 1296 (s), 1221 (m), 1159 (s), 1115 (m), 1090 (m), 1045 (m), 1011 (w), 948 (w), 848 (w); HRMS (APCI): calcd for $C_{19}H_{25}O_6$ $[M+H]^+$: 349.1646, found: 349.1647.

4.9. 6′,8′-Dimethoxy-10a′-methyl-10′,10a′-dihydro-1′*H*-spiro[[1,3]dioxolane-2,9′-phenanthren]-3′(2′*H*)-one (1)

t-BuOK (57.9 mg, 0.52 mmol) was added to a solution of **2** (150 mg, 0.43 mmol) in *t*-BuOH (2 mL), the reaction mixture was stirred at 25 °C for 3 h H_2O (5 mL) was added and the mixture was extracted with EtOAc (3×10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated in a vacuum to afford **1** as a white solid (110 mg, 77%), which was used in the next step without further purification. Mp 126 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.70 (d, *J*=2.3 Hz, 1H), 6.60 (d, *J*=2.3 Hz, 1H), 6.47 (br s, 1H), 4.37–4.27 (m, 1H), 4.23–4.04 (m, 3H), 3.86 (s, 3H), 3.82 (s, 3H), 2.70–2.54 (m, 1H), 2.48–1.39 (m, 1H), 2.14–1.85 (m, 4H), 1.30 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=199.6, 162.2, 160.9, 160.0, 135.6, 121.9, 118.7, 106.3, 102.9, 101.3, 65.8, 65.2, 56.0, 55.4, 49.3, 36.8, 35.6, 33.2, 21.7; IR (KBr): $\tilde{\nu}$=2950 cm^{-1} (m), 2922 (w), 2890 (w), 1660 (s), 1603 (s), 1579 (s), 1466 (s), 1425 (m), 1356 (s), 1327 (s), 1296 (s), 1275 (s), 1207 (s), 1157 (s), 1134 (s), 1101 (s), 1147 (s), 1020 (m), 989 (m), 950 (m), 849 (w), 837 (m), 821 (w); HRMS (APCI): calcd for $C_{19}H_{23}O_5$ $[M+H]^+$: 331.1540, found: 331.1543.

4.10. 2-Hydroxy-6,8-dimethoxy-10a-methyl-10,10a-dihydrophenanthrene-3,9-dione (6)

t-BuOK (110 mg, 0.30 mmol) was added to a solution of **1** (186 mg, 0.33 mmol) in *t*-BuOH (2 mL) and THF (2 mL) and the mixture was stirred under O_2 atmosphere at 25 °C for 4 h. The reaction mixture was diluted with saturated aqueous solution of NH_4Cl (30 mL) and extracted with CH_2Cl_2 (3×20 mL). The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was evaporated. The residue was dissolved in acetone (5 mL), TsOH (10 mg, 0.05 mmol) was added, and the mixture was stirred at 25 °C for 16 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, EtOAc/CH_2Cl_2 (1:1)) to afford **6** (71 mg, 72%) as a white solid. Mp 218–220 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.84 (s, 1H), 6.73 (d, *J*=2.3 Hz, 1H), 6.61 (d, *J*=2.3 Hz, 1H), 6.37 (br s, 1H), 6.13 (s, 1H), 3.95 (s, 3H), 3.94 (s, 3H), 2.83 (d_{AB}, *J*=16.1 Hz, 1H), 2.57 (d_{AB}, *J*=16.1 Hz, 1H), 1.34 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=192.0, 181.7, 164.8, 162.6, 162.3, 146.5, 141.8, 123.8, 122.3, 114.0, 102.5, 101.1, 56.3, 55.7, 51.0, 42.0, 25.8; IR (KBr): $\tilde{\nu}$=3234 cm^{-1} (w), 3018 (w), 2972 (w), 2931 (w), 1657 (s), 1606 (s), 1589 (s), 1558 (s), 1477 (m), 1454 (m), 1407 (m), 1377 (w), 1342 (s), 1306 (w), 1265 (s), 1245 (s), 1215 (s), 1207 (s), 1165 (s), 1115 (s), 1026 (w), 999 (m), 957 (w), 895 (w), 850 (w), 839 (w); HRMS (APCI): calcd for $C_{17}H_{17}O_5$ $[M+H]^+$: 301.1071, found: 301.1067.

4.11. 2,8-Dihydroxy-6-methoxy-10a-methyl-10,10a-dihydrophenanthrene-3,9-dione (cDHA)

BBr_3 (1 M in CH_2Cl_2, 0.2 mL, 0.2 mmol) was added dropwise to a solution of **6** (30 mg, 0.1 mmol) in anhydrous CH_2Cl_2 (3 mL) at −78 °C. The reaction mixture was stirred at −78 °C for 1 h, then allowed to warm to 0 °C, and quenched with aqueous 10% NaOH (1 mL). The mixture was acidified with 1 M HCl to pH=6 and extracted with CH_2Cl_2 (2×10 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by preparative TLC (CH_2Cl_2/EtOAc (1:1)) to afford **cDHA** (15 mg, 52%) as a pale yellow solid. Mp 199 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ=12.61 (s, 1H), 6.84 (s, 1H), 6.72 (d, *J*=2.3 Hz, 1H), 6.54 (d, *J*=2.3 Hz, 1H), 6.38 (s, 1H), 6.13 (s, 1H), 3.90 (s, 3H), 2.85 (d_{AB}, *J*=17.0 Hz, 1H), 2.67 (d_{AB}, *J*=17.0 Hz, 1H), 1.35 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=199.1, 181.5, 166.5, 165.5, 160.4, 146.6, 139.6, 123.0, 122.3, 109.1, 105.5, 102.8, 55.9, 48.8, 41.6, 26.3; IR (KBr): $\tilde{\nu}$=3375 cm^{-1} (m), 2964 (w), 2926 (w), 1632 (s), 1564 (w), 1479 (w), 1454 (w), 1429 (m), 1389 (m), 1365 (m), 1343 (w), 1292 (s), 1238 (m), 1203 (s), 1159 (s), 1126 (w), 1101 (w), 1067 (w), 978 (w), 903 (w), 879 (w); HRMS (APCI): calcd for $C_{16}H_{15}O_5$ $[M+H]^+$: 287.0914, found: 287.0912.

Crystal data for **cDHA**: CCDC ref. No. 1012667. Crystallized from $CHCl_3$.

4.12. 1,3-Dimethoxy-5-(2-nitroprop-1-en-1-yl)benzene

A mixture of 3,5-dimethoxybenzaldehyde (10.0 g, 60.18 mmol), nitroethane (45 mL, 630.68 mmol) and NH_4OAc (3.36 g, 43.57 mmol) was stirred and heated at reflux for 4 h (oil bath 130 °C). The reaction mixture was cooled to 25 °C, diluted with Et_2O (200 mL), and washed with brine (4×150 mL). The organic layer was dried over Na_2SO_4, filtered, and the solvent was evaporated. 1,3-Dimethoxy-5-(2-nitroprop-1-en-1-yl)benzene (13.04 g, 97%)

was obtained as a yellow solid. Mp 86–87 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ=7.99 (br s, 1H), 6.54 (d, J=2.2 Hz, 2H), 6.51 (d, J=2.2 Hz, 1H), 3.81 (s, 6H), 2.43 (d, J=0.9 Hz, 3H).

4.13. 1-(3,5-Dimethoxyphenyl)propan-2-one (9)

Fe powder (1.60 g, 28.7 mmol) was added to a solution of 1,3-dimethoxy-5-(2-nitroprop-1-en-1-yl)benzene (493 mg, 2.21 mmol) in AcOH (13.9 mL, 221.0 mmol) and the mixture was heated at reflux for 3 h. The mixture was cooled to 25 °C, filtered, diluted with water (70 mL), and extracted with CH_2Cl_2 (3×30 mL). Combined organic extracts were washed with aqueous 5% NaOH (2×30 mL), brine (2×30 mL), dried over Na_2SO_4, and concentrated. The crude residue was purified by column chromatography (silica gel, EtOAc/hexane (2:1)) to provide **9** (303 mg, 71%) as a colorless oil. ^{1}H NMR (300 MHz, CDCl3) δ=6.37–6.31 (m, 3H), 3.74 (s, 6H), 3.57 (s, 2H), 2.11 (s, 3H).

4.14. 2-(2-Iodoethyl)-2-methyl-1,3-dioxolane (11)

Iodotrimethylsilane (1.7 mL, 12 mmol) was added dropwise at 0 °C to a mixture of methyl vinyl ketone (90%, 833 μL, 10 mmol) and ethylene glycol (1.24 mL, 22 mmol). The reaction mixture was stirred for 2 h while allowed to warm to 25 °C, then it was diluted with pentane (20 mL) and washed successively with 5% aqueous Na_2CO_3 (10 mL) and 5% aqueous $Na_2S_2O_3$ (10 mL). The organic layer was dried over K_2CO_3 and concentrated under reduced pressure to provide **11** (1.93 g, 80%) as a pale yellow oil, which was used directly in the next step without further purification. ^{1}H NMR (300 MHz, $CDCl_3$) δ=4.01–3.87 (m, 4H), 3.21–3.12 (m, 2H), 2.35–2.24 (m, 2H), 1.30 (s, 3H).

4.15. 3-(3,5-Dimethoxyphenyl)-5-(2-methyl-1,3-dioxolan-2-yl)pentan-2-one (10)

t-BuOK (677 mg, 6.04 mmol) was added to a solution of **9** (1.02 g, 5.24 mmol) in anhydrous THF (12 mL) and the reaction mixture was stirred at 25 °C for 1 h. **11** (1.7 g, 7.02 mmol) was added dropwise over the period of 5 min and the reaction mixture was stirred at 25 °C for 16 h. Saturated aqueous solution of $NaHCO_3$ (1 mL) was added, the solvents were evaporated and the residue was directly loaded onto a column and purified by column chromatography (silica gel, EtOAc/hexane (1:2)). **10** (1.37 g, 85%) was obtained as a pale yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.35 (s, 3H), 3.94–3.84 (m, 4H), 3.77 (s, 6H), 3.54 (t, J=7.4 Hz, 1H), 2.16–2.02 (m, 1H), 2.05 (s, 3H), 1.86–1.71 (m, 1H), 1.64–1.43 (m, 2H), 1.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ=207.9, 161.2 (2C), 141.2, 109.9, 106.4 (2C), 99.2, 64.61, 64.58, 59.7, 55.3 (2C), 36.6, 28.8, 26.0, 23.8; IR (KBr): $\tilde{\nu}$=2958 cm^{-1} (w), 2941 (w), 1712 (s), 1670 (w), 1605 (s), 1595 (s), 1460 (m), 1431 (m), 1352 (w), 1205 (s), 1157 (s), 1065 (s), 858 (w), 837 (w); HRMS (ESI): calcd for $C_{17}H_{24}O_5Na$ [M+Na]$^+$: 331.1516, found: 331.1517.

4.16. *tert*-Butyl 3-acetyl-3-(3,5-dimethoxyphenyl)-5-(2-methyl-1,3-dioxolan-2-yl)pentanoate (12)

t-BuOK (580 mg, 5.17 mmol) was added to a solution of **10** (1.39 g, 4.50 mmol) in THF (16 mL) at 0 °C and the resulting yellow mixture was stirred at 0 °C for 1 h. Then, *t*-butyl-2-iodoacetate (2.0 g, 5.85 mmol) was added, the mixture was allowed to warm to 25 °C and stirred for 20 h. Reaction was quenched by saturated solution of $NaHCO_3$ (1 mL). The solvents were evaporated and the residue was purified by column chromatography (silica gel, EtOAc/hexane (1:4 to 1:1)) to yield **12** (924 mg, 62%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.36–6.33 (m, 3H), 3.93–3.80 (m, 2H), 3.76 (s, 6H), 2.96 (d_{AB}, J=14.7 Hz, 1H), 2.81 (d_{AB}, J=14.7 Hz, 1H), 2.40–2.27 (m, 1H), 2.07–1.96 (m, 3H), 1.98 (s, 3H), 1.45–1.37 (m, 2H), 1.35 (s, 9H), 1.30 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=207.8, 170.2, 161.0 (2C), 142.6, 109.8, 105.2 (2C), 98.9, 81.0, 64.6, 64.5, 57.4, 55.3 (2C), 39.5, 33.2, 27.8 (3C), 27.5, 25.4, 23.7; IR (KBr): $\tilde{\nu}$=2978 cm^{-1} (w), 2937 (w), 2885 (w), 1726 (s), 1713 (s), 1598 (s), 1458 (m), 1425 (m), 1369 (m), 1352 (m), 1296 (w), 1205 (s), 1157 (s), 1064 (m), 858 (w), 845 (w); HRMS (ESI): calcd for $C_{23}H_{34}O_7Na$ [M+Na]$^+$: 445.2197, found: 445.2198.

4.17. *tert*-Butyl 3-acetyl-3-(3,5-dimethoxyphenyl)-6-oxoheptanoate (8)

TsOH (75 mg, 0.17 mmol) was added to a solution of **12** (721 mg, 1.71 mmol) in acetone (10 mL) and the mixture was stirred at 25 °C for 8 h. The solvent was evaporated, the residue was dissolved in Et_2O (20 mL) and washed with saturated aqueous solution of $NaHCO_3$ (10 mL). The aqueous phase was re-extracted with Et_2O (2×10 mL). The combined organic extracts were washed with H_2O (10 mL), dried over Na_2SO_4 and concentrated in a vacuum to yield **8** (641 mg, 100%) as a white solid. Mp 94–97 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ=6.36 (t, J=2.2 Hz, 1H), 6.30 (d, J=2.2 Hz, 2H), 3.76 (s, 6H), 2.98 (d, J=14.3 Hz, 1H), 2.79 (d, J=14.3 Hz, 1H), 2.48–2.42 (m, 1H), 2.29–2.15 (m, 3H), 2.07 (s, 3H), 1.97 (s, 3H), 1.35 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=207.6, 207.4, 170.0, 161.2 (2C), 142.2, 105.0 (2C), 99.0, 81.4, 57.1, 55.4 (2C), 39.8, 38.5, 29.8, 27.8 (3C), 27.5, 25.6; IR (KBr): $\tilde{\nu}$=2975 cm^{-1} (w), 2939 (w), 1720 (s), 1707 (s), 1593 (s), 1466 (m), 1427 (m), 1365 (m), 1352 (s), 1311 (s), 1292 (m), 1211 (s), 1146 (s), 1068 (m), 1051 (m), 924 (w), 840 (w); HRMS (ESI): calcd for $C_{21}H_{30}O_6Na$ [M+Na]$^+$: 401.1935, found: 401.1935.

4.18. *tert*-Butyl 2-(3′,5′-dimethoxy-6-methyl-4-oxo-1,2,3,4-tetrahydro-[1,1′-biphenyl]-1-yl)acetate (13a)

AcOH (0.12 mL, 2.13 mmol) and piperidine (0.19 mL, 1.93 mmol) were added to a solution of **8** (732 mg, 1.93 mmol) in toluene (10 mL) and the mixture was heated at reflux (oil bath 110 °C) for 18 h. The solvent was evaporated in a vacuum, the residue was dissolved in EtOAc (150 mL) and washed with H_2O (100 mL) and then with brine (100 mL). The organic layer was dried over Na_2SO_4, concentrated, and the residue was purified by column chromatography (silica gel, EtOAc/hexane (1:2)) to afford **13a** as a white semi-solid (563 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.40 (d, J=2.1 Hz, 2H), 6.36 (t, J=2.1 Hz, 1H), 6.16 (br s, 1H), 3.76 (s, 6H), 2.91 (s, 2H), 2.72–2.60 (m, 1H), 2.32–2.01 (m, 3H), 1.98 (d, J=1.2 Hz, 3H), 1.43 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=198.7, 169.8, 163.5, 161.0 (2C), 144.7, 130.4, 105.7 (2C), 96.1, 81.4, 55.3 (2C), 46.8, 43.8, 35.7, 34.2, 28.0 (3C), 21.7; IR (KBr): $\tilde{\nu}$=2976 cm^{-1} (w), 2937 (w), 1724 (s), 1672 (s), 1601 (s), 1456 (s), 1425 (m), 1369 (m), 1348 (m), 1310 (m), 1207 (s), 1159 (s), 1068 (w), 1043 (w), 841 (w); HRMS (ESI): calcd for $C_{21}H_{28}O_5Na$ [M+Na]$^+$: 383.1829, found: 383.1829.

4.19. *tert*-Butyl 2-(3′,5′-dimethoxy-4-methyl-6-oxo-1,2,3,6-tetrahydro-[1,1′-biphenyl]-1-yl)acetate (13b)

t-BuOK (14.6 mg, 0.13 mmol) was added to a solution of **8** (50 mg, 0.13 mmol) in THF (1 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, then saturated aqueous solution of $NaHCO_3$ (1 mL) was added. The solvent was evaporated and the residue was loaded directly onto a column and purified by column chromatography (silica gel, EtOAc/hexane (1:2)) to give **13b** as a colorless oil (25.2 mg, 54%). ^{1}H NMR (500 MHz, $CDCl_3$) δ=6.40 (d, J=2.2 Hz, 2H), 6.34 (t, J=2.1 Hz, 1H), 5.95 (br s, 1H), 3.75 (s, 6H), 2.93 (d_{AB}, J=16.2, 1H), 2.73–2.66 (m, 1H), 2.56 (d_{AB}, J=16.2, 1H), 2.51–2.45 (m, 1H), 2.34–2.24 (m, 1H), 2.17–2.10 (m, 1H), 1.84 (s, 3H), 1.36 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=199.2, 170.6, 161.4, 160.8 (2C), 141.8, 126.4, 105.3 (2C), 96.7, 80.4, 55.3 (2C), 50.9, 45.1, 31.3, 28.7 (3C), 28.2, 24.1; IR (KBr): $\tilde{\nu}$=2974 cm^{-1} (w), 2931 (w), 1730 (s), 1668 (s), 1637 (w), 1595 (s), 1458 (s), 1425 (m), 1367 (m), 1348 (m), 1308 (m), 1294 (w), 1205 (s),

1159 (s), 1080 (w), 1063 (w), 1049 (w), 843 (w); HRMS (APCI): calcd for $C_{21}H_{29}O_5$ $[M+H]^+$: 361.2010, found: 361.2004.

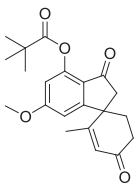
4.20. 4′,6′-Dimethoxy-2-methylspiro[cyclohex[2]ene-1,1′-indene]-3′,4(2′*H*)-dione (7)

A solution of **13a** (415 mg, 1.15 mmol) in MsOH (4.2 mL) was heated at 50 °C for 15 h. The mixture was cooled to 25 °C, poured into cold saturated solution of $NaHCO_3$ (30 mL), and extracted with EtOAc (5×30 mL). The combined organic extracts were dried over Na_2SO_4, evaporated, and the residue was purified by gradient column chromatography (silica gel, EtOAc/hexane (1:2 to pure EtOAc)) to afford **7** (275 mg, 84%) as a pale brown solid. Mp 159–164 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.37 (d, J=1.9 Hz, 2H), 6.32 (d, J=1.9 Hz, 1H), 6.00 (br s, 1H), 3.93 (s, 3H), 2.86 (s, 3H), 2.82 (d_{AB}, J=18.5, 1H), 2.65 (d_{AB}, J=18.5, 1H), 2.56–2.48 (m, 2H), 2.46–2.29 (m, 1H), 2.06–1.97 (m, 1H), 1.64 (d, J=1.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=199.2, 197.9, 167.5, 163.8, 162.5, 159.5, 128.4, 118.8, 100.4, 98.3, 55.91, 55.89, 47.4, 46.7, 37.2, 34.9, 20.4; IR (KBr): $\tilde{\nu}$=2953 cm^{-1} (w), 2927 (w), 1697 (s), 1664 (s), 1576 (s), 1460 (m), 1439 (w), 1427 (w), 1350 (w), 1315 (s), 1234 (s), 1203 (s), 1159 (s), 1107 (w), 1053 (m), 1026 (w), 860 (m); HRMS (APCI): calcd for $C_{17}H_{19}O_4$ $[M+H]^+$: 287.1278, found: 287.1279.

4.21. 4′-Hydroxy-6′-methoxy-2-methylspiro[cyclohex[2]ene-1,1′-indene]-3′,4(2′*H*)-dione

BCl_3 (1 M solution in CH_2Cl_2, 0.96 mL, 0.96 mmol) was added dropwise to a solution of **7** (275 mg, 0.96 mmol) in CH_2Cl_2 (10 mL) at 0 °C and the mixture was stirred at 0 °C. Additional five 0.96 mL portions of 1 M BCl_3 in CH_2Cl_2 (total volume of 4.8 mL, 4.8 mmol) were added in one hour intervals and then the mixture was stirred for 16 h while allowed to warm to 25 °C. Then the reaction mixture was cooled to 0 °C, additional four 2.88 mL portions of 1 M BCl_3 in CH_2Cl_2 (total volume of 11.5 mL, 11.5 mmol) were added in three hour intervals and the mixture was stirred for additional 16 h while allowed to warm to 25 °C. Then it was quenched with cold saturated aqueous solution of $NaHCO_3$ (50 mL), and extracted with CH_2Cl_2 (3×50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/hexane (2:1)) to afford 4′-hydroxy-6′-methoxy-2-methylspiro[cyclohex[2]ene-1,1′-indene]-3′,4(2′*H*)-dione (170 mg, 65%) as a white solid. Mp 128–129 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ=9.07 (s, 1H), 6.34 (d, J=1.9 Hz 1H), 6.31 (d, J=1.9 Hz, 1H), 5.98 (br s, 1H), 3.82 (s, 3H), 2.85 (d_{AB}, J=18.9 Hz, 1H), 2.69 (d_{AB}, J=18.9 Hz, 1H), 2.61–2.46 (m, 2H), 2.44–2.28 (m, 1H), 2.10–2.00 (m, 1H), 1.64 (d, J=1.2 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=204.0, 197.7, 168.4, 162.0, 160.5, 159.1, 128.3, 116.2, 102.8, 100.1, 55.9, 47.6, 46.9, 36.7, 34.8, 20.3; IR (KBr): $\tilde{\nu}$=3433 cm^{-1} (w), 2976 (w), 2928 (w), 2918 (w), 1672 (s), 1659 (s), 1624 (s), 1597 (s), 1491 (m), 1443 (m), 1367 (s), 1325 (m), 1254 (m), 1205 (m), 1151 (s), 1022 (w), 989 (w), 962 (w), 864 (w), 849 (w); HRMS (APCI): calcd for $C_{16}H_{17}O_4$ $[M+H]^+$: 273.1121, found: 273.1125.

4.22. 6′-Methoxy-2-methyl-3′,4-dioxo-2′,3′-dihydrospiro[cyclohex[2]ene-1,1′-inden]-4′-yl pivalate (17)



A solution of 4′-hydroxy-6′-methoxy-2-methylspiro[cyclohex[2]ene-1,1′-indene]-3′,4(2′*H*)-dione (295 mg, 1.08 mmol) in dry THF (5 mL) was added to NaH (60% dispersion in mineral oil, 65 mg, 1.63 mmol) and the mixture was stirred at 25 °C for 45 min. Pivaloyl chloride (189 μL, 1.52 mmol) was added and the mixture was stirred for additional 1 h. Water (50 mL) was added and the mixture was extracted with EtOAc (3×50 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (silica gel, EtOAc/hexane (1:1)) to afford **17** (352 mg, 92%) as a white solid. Mp 136–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ=6.63 (br s, 1H), 6.59 (br s, 1H), 6.00 (br s, 1H), 3.86 (s, 3H), 2.81 (d_{AB}, J=18.7 Hz, 1H), 2.64 (d_{AB}, J=18.9 Hz, 1H), 2.57–2.30 (m, 3H), 2.11–2.01 (m, 1H), 1.64 (br s, 3H); 1.40 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ=198.2, 197.6, 176.0, 166.5, 162.14, 162.09, 149.5, 128.5, 121.9, 109.1, 106.6, 55.1, 47.4, 46.8, 39.1, 37.1, 34.9, 27.1 (3C), 20.4; IR (KBr): $\tilde{\nu}$=2978 cm^{-1} (w), 2956 (w), 2931 (w), 1759 (s), 1711 (s), 1670 (s), 1591 (m), 1481 (m), 1438 (m), 1340 (w), 1332 (w), 1310 (s), 1261 (m), 1244 (m), 1142 (s), 1109 (s), 1084 (s), 1036 (m), 1009 (m), 897 (w), 856 (w); HRMS (APCI): calcd for $C_{21}H_{25}O_5$ $[M+H]^+$: 357.1697, found: 357.1695.

4.23. 4-((*tert*-Butyldimethylsilyl)oxy)-6′-methoxy-2-methyl-3′-oxo-2′,3′-dihydrospiro-[cyclohexa[2,4]diene-1,1′-inden]-4′-yl pivalate (18)

TBSOTf (143 μL, 0.61 mmol) was added to a solution of **17** (135 mg, 0.38 mmol) in CH_2Cl_2 (1 mL) at −78 °C, then DBU (92 μL, 0.61 mmol) was added dropwise and the reaction mixture was stirred at −78 °C for 30 min, filtered through a pad of silica gel (1 cm height, 2 cm diameter), and the pad was quickly washed with EtOAc (100 mL). After evaporation of the solvents from the filtrate, **18** (152 mg, 85%) was obtained as a colorless semi-solid, which was used in the next step without any further purification. Analytically pure sample was obtained by preparative TLC (silica gel, EtOAc/hexane (1:2)); ^{1}H NMR (300 MHz, $CDCl_3$)=6.84 (d, J=2.0 Hz, 1H), 6.54 (d, J=2.0 Hz, 1H), 5.67 (br s, 1H), 4.85–4.79 (m, 1H), 3.88 (s, 3H), 2.81 (d_{AB}, J=18.3 Hz, 1H), 2.74 (dd, J=16.9 Hz, J=2.6 Hz, 1H), 2.45 (d_{AB}, J=18.3 Hz, 1H), 2.26 (dd, J=16.9 Hz, J=5.8 Hz, 1H), 1.53 (s, 3H), 1.41 (s, 9H), 0.95 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=200.3, 176.2, 166.1, 163.9, 149.1, 148.9, 141.0, 124.2, 122.0, 108.6, 107.3, 99.0, 56.0 (2C), 48.4, 46.4, 39.1, 38.6, 27.2 (3C), 25.7 (3C), 19.5, 18.1, −4.4; IR (KBr): $\tilde{\nu}$=2958 cm^{-1} (m), 2931 (m), 2856 (w), 1761 (s), 1711 (s), 1674 (s), 1610 (s), 1589 (s), 1479 (m), 1462 (m), 1461 (m), 1441 (m), 1396 (w), 1340 (m), 1310 (s), 1273 (m), 1244 (m), 1194 (w), 1144 (s), 1109 (s), 1186 (s), 1036 (m), 862 (w), 839 (w); HRMS (APCI): calcd for $C_{27}H_{39}O_5Si$ $[M+H]^-$: 471.2561, found: 471.2560.

4.24. 5-Hydroxy-6′-methoxy-2-methyl-3′,4-dioxo-2′,3′-dihydrospiro[cyclohex[2]ene-1,1′-inden]-4′-yl pivalate (19)

*m*CPBA (77%, 110 mg, 0.49 mmol) was added to a mixture of **18** (155 mg, 0.33 mmol) and $KHCO_3$ (77 mg, 0.77 mmol) in CH_2Cl_2, (5 mL) at 0 °C and the reaction mixture was stirred at 25 °C for 16 h. EtOAc (50 mL) was added and the mixture was washed with saturated aqueous solution of $NaHCO_3$ (20 mL), then with H_2O (30 mL), dried over Na_2SO_4, filtered, and concentrated in a vacuum. The residue was dissolved in CH_2Cl_2 (10 mL), pyr HF (1 mL) was added, and the mixture was stirred at 25 °C for 16 h. The mixture was diluted with H_2O (10 mL) and extracted with EtOAc (3×30 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by preparative TLC (silica gel, EtOAc/hexane (2:1) to afford **19** (45 mg, 37%) as a colorless semi solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ=6.71 (d, J=2.0 Hz, 1H), 6.63 (d, J=2.0 Hz, 1H), 6.16–6.15 (m, 1H), 4.57 (dd, J=12.9 Hz, J=5.8 Hz, 1H), 3.87 (s, 3H), 3.08 (d_{AB}, J=18.7 Hz, 1H), 2.56 (d_{AB}, J=18.7 Hz, 1H), 2.50 (dd, J=13.2 Hz, J=5.8 Hz, 1H), 2.27 (dd, J=13.2 Hz, J=12.9 Hz, 1H), 1.78 (d, J=1.2 Hz, 3H), 1.43 (s, 9H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=198.4, 198.1, 176.1, 166.3, 163.5, 159.0,

150.1, 125.9, 121.3, 109.0, 107.6, 69.0, 56.2, 51.1, 48.2, 44.1, 39.2, 27.2 (3C), 20.1; IR (KBr): $\tilde{\nu}$=2976 cm^{-1} (w), 2933 (w), 1759 (s), 1711 (s), 1684 (s), 1608 (s), 1587 (s), 1479 (w), 1440 (w), 1340 (m), 1308 (m), 1244 (m), 1142 (s), 1109 (s), 1053 (w), 1032 (w), 899 (w), 867 (w); HRMS (APCI): calcd for $C_{21}H_{25}O_6$ $[M+H]^+$: 373.1646, found: 373.1641.

4.25. 4′,5-Dihydroxy-6′-methoxy-2-methylspiro[cyclohexa[2,5]diene-1,1′-indene]-3′,4(2′*H*)-dione (cDHAs)

K_2CO_3 (44.5 mg, 0.322 mmol) was added to a solution of **19** (38 mg, 0.107 mmol) in MeOH (4 mL), and the mixture was stirred in an open flask at 50 °C for 6 h. MeOH was then evaporated and the residue was dissolved in H_2O (10 mL) and the pH was adjusted to 7 with 1 M HCl. The mixture was extracted with EtOAc (4×10 mL), the combined organic extracts were dried over Na_2SO_4, filtered, and the solvent was evaporated. The residue was purified by preparative TLC (silica gel, CH_2Cl_2/EtOAc (2:1)) to afford **cDHAs** (14.5 mg, 50%) as a white solid. Mp 177 °C (dec); ^{1}H NMR (300 MHz, $CDCl_3$) δ=9.02 (br s, 1H), 6.42–6.33 (m, 3H), 6.14 (d, J=1.7 Hz, 1H), 6.03, (s, 1H), 3.83 (s, 3H), 2.92 (s, 2H), 1.75 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ=203.4, 181.6, 168.8, 162.8, 159.3, 156.8, 146.0, 125.5, 120.0, 116.0, 102.6, 101.0, 56.1, 50.8, 47.3, 19.5; IR (KBr): $\tilde{\nu}$=3387 cm^{-1} (w), 2955 (w), 2922 (m), 2850 (w), 1680 (s), 1639 (s), 1628 (s), 1433 (w), 1420 (w), 1377 (m), 1311 (m), 1205 (s), 1176 (m), 1148 (s), 1105 (w), 1022 (w), 887 (w); HRMS (APCI): calcd for $C_{16}H_{15}O_5$ $[M+H]^+$: 287.0914, found: 287.0912.

Crystal data for **cDHAs**: CCDC ref. No. 1012668. Crystallized from $CHCl_3$.

4.26. Measurement of inhibitory activities of mammalian pols

The four mammalian pols α, γ, κ, and λ were prepared, and the reaction mixtures for these pols, as described previously.[23] Briefly, poly(dA)/oligo(dT)$_{18}$ (A/T, 2:1) and [^{3}H]-dTTP (100 cpm/pmol) were used as the DNA template-primer substrate and nucleotide (dNTP; 2′-deoxynucleotide-5′-triphosphate) substrate, respectively. The tested compounds were dissolved in distilled dimethyl sulfoxide (DMSO) at concentrations 0–200 μM. Subsequently, 4-μL aliquots were mixed with 16 μL of each enzyme (0.05 units) in 50 mM Tris–HCl (at pH 7.5) containing 1 mM dithiothreitol, 50% glycerol (v/v), and 0.1 mM ethylenediaminetetraacetic acid, and were held at 0 °C for 10 min. Subsequently, 8 μL of these inhibitor-enzyme mixtures were added to 16-μL aliquots of enzyme standard reaction mixture containing 50 mM Tris–HCl (at pH 7.5), 1 mM dithiothreitol, 1 mM $MgCl_2$, 15% glycerol, 5 μM poly(dA)/oligo(dT)$_{18}$ (A/T, 2:1) and 10 μM [^{3}H]-dTTP, and were incubated at 37 °C for 60 min. The enzyme activity in the absence of inhibitor was taken as 100% (the activity without the enzyme was considered 0%), and the inhibitory activity was determined for each inhibitor concentration. One unit of pol activity was defined as the amount of each enzyme that catalyzed incorporation of 1 nmol dTTP into synthetic DNA template-primers in 60 min at 37 °C, under standard reaction conditions.[24] The 50% inhibitory concentration (IC_{50} value) of the enzyme inhibitor was determined by constructing a dose–response curve and examining the effect of different concentrations of inhibitor on reversing enzyme activity (functional antagonist assay).

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Supplementary data

Supplementary data (^{1}H, ^{13}C NMR, IR and HRMS spectra plus conditions for separation of the enantiomers of **cDHA** are available.) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2015.08.005.

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