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Synthesis of plakevulin A and structure–activity relationships of its related compounds against DNA polymerases

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Abstract—Synthesis of plakevulin A and structure–activity relationships of its related compounds against DNA polymerases is described. We have achieved a total synthesis and revised the structure of plakevulin A. Several analogues including untenone A, manzamenone A, and optically active plakevulin A, were prepared and tested with an enzyme inhibition assay for mammalian DNA polymerases. The effect of the methyl ester moiety, and the substituents at the 1- and 4-positions of plakevulin A on DNA polymerase activities are discussed.

1. Introduction

The cytotoxic oxylipin, plakevulin A (**1**), was isolated from an Okinawan sponge *Plakortis* sp by Kobayashi et al.[1] Compound **1** exhibited cytotoxicity against murine leukemia L1210 and epidermoid carcinoma KB cells (Fig. 1). It was also reported that **1** inhibited the activities of DNA polymerases (pol) α and γ. Although the proposed structure was the levulinyl ester as depicted for **1a**, our synthetic studies and enzyme-inhibitory assays revealed that the structure of plakevulin A is actually as shown for **1**.[2] Recently a synthesis of optically active (+)-**1** has been reported by Honda et al.[3]

Untenone A (**2**), which inhibited the cell proliferation of L1210 cells, was isolated from the genus *Plakortis*.[4,5] The structurally related manzamenone A (**3**), a unique dimeric fatty acid derivative, was also isolated from the *Plakortis*.[6,7] Untenone A has been considered to be a plausible intermediate in the biosynthetic pathways of manzamenone A and plakevulin A.[1,7] Both **2** and **3** were found to inhibit mammalian pol α,β, and human terminal deoxynucleotidyl transferase (TdT).[8,9]

In this article, we fully describe our synthetic studies and biological evaluation of a series of plakevulin A analogues.

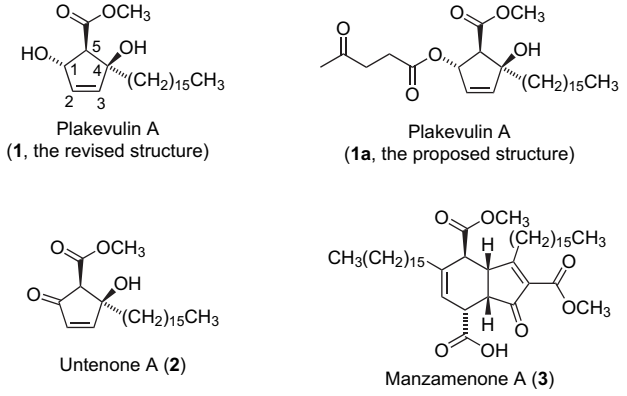

Figure 1. The revised structure of plakevulin A (**1**), the proposed structure of plakevulin A (**1a**), untenone A (**2**), and manzamenone (**3**).

2. Results and discussions

2.1. Synthesis and structural revision of plakevulin A

Our synthetic approach towards the proposed structure of plakevulin A (**1a**) was based on the assumed biosynthetic pathway.[1,2,7] The reduction of untenone A (**2**), followed by esterification of the resulting alcohol would provide **1a**. Our route to synthesize untenone A was based on the modified protocol reported by Yamada et al.[5b]

Protection of the alcohol (±)-**4** with TMSOTf and *i*-Pr_2NEt gave a TMS ether (±)-**5** (Scheme 1). Methoxycarboxylation

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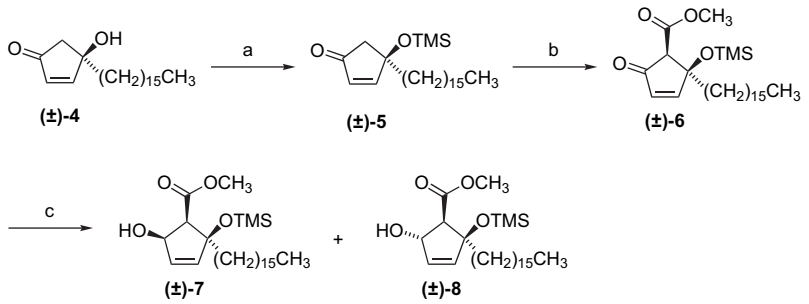


Scheme 1. Synthesis of the key intermediate (±)-**7**. (a) TMSOTf, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 90%; (b) LDA, THF/HMPA, then $NCCO_2CH_3$, −42 °C, 63% (dr=4.2:1) and (c) DIBAL, CH_2Cl_2, −78 °C, 41% for (±)-**7** and 6% for (±)-**8**.

of (±)-**5** with LDA and $NCCO_2CH_3$ afforded (±)-**6** as a 4.2:1 mixture of inseparable diastereomers in 63% yield.[5c] Reduction of (±)-**6** with DIBAL (2 equiv) in CH_2Cl_2 gave (±)-**7** and (±)-**8** in 41% and 6% yields, respectively, as the identified products. In this reaction, β-hydroxyaldehyde was obtained as the major byproduct, as a mixture that proved difficult to separate. When 1 equiv of DIBAL was used, no reaction occurred. Although the use of other reducing agents such as $NaBH_4$–$CeCl_3$, $LiBH_4$, $ZnBH_4$, and (*i*-PrO)$_3$Al/*i*-PrOH was examined, none or only a trace amount of (±)-**7** was obtained. The stereochemistry of the major isomer (±)-**7** was determined by its NOESY spectrum (Fig. 2a). The NOESY correlation between H-1 and H-5 in (±)-**7** indicated the *syn* relation for H-1/H-5. The *anti* relation for 4-OTMS and H-5 in (±)-**7** was determined by the NOESY correlations between H-5 and H-6, and between 4-OTMS and H-23.

The esterification of (±)-**7** was attempted with both inversion and retention of the stereochemistry at C-1 (Scheme 2). The Mitsunobu esterification of (±)-**7** with levulinic acid afforded (±)-**9** in 52% yield.[10] The observed NOEs between H-1 and 4-OTMS and between H-1 and H-23 of (±)-**9** indicated that the configuration of **9** was 1*S**,4*S**,5*R** (Fig. 2b). Deprotection of TMS ether (±)-**9** with TBAF provided the proposed structure of plakevulin A (**1a**). On the other hand, the esterification of (±)-**7** with levulinic acid by EDCI, followed by deprotection of the TMS ether afforded (±)-**11**, the 1-*epi*-isomer of **1a**.

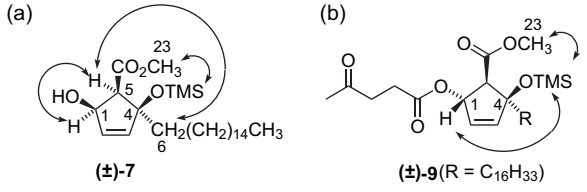


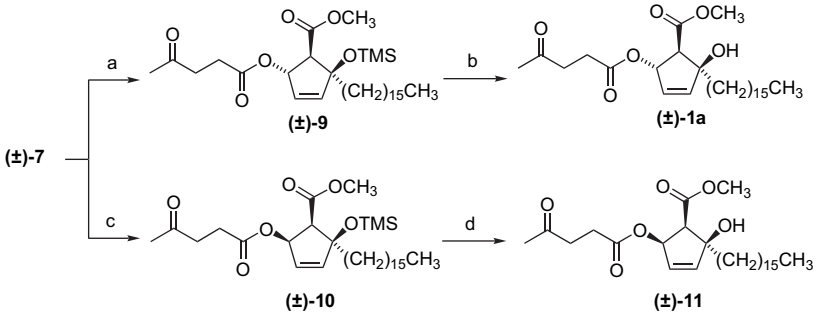
Figure 2. The NOESY correlations for (±)-**7** (left) and (±)-**9** (right).

Selected ^1H and ^{13}C NMR spectral data of (±)-**1a** and (±)-**11** are summarized in Table 1. As shown in Table 1, the ^1H and ^{13}C NMR spectral data of (±)-**1a** and (±)-**11** were different from those of the natural plakevulin A. In particular, both the proton and carbon signals at C-1 in (±)-**1a** appeared further downfield from those in the natural plakevulin A. These observations suggested that the natural plakevulin A is not the levulinyl ester, but the delevulinyl form.

Based on these considerations, the removal of the levulinyl moiety of (±)-**1a** was attempted (Scheme 3). Treatment of (±)-**1a** with hydrazine in pyridine and acetic acid gave the alcohol (±)-**1** in 92% yield.[11] The ^1H NMR and ^{13}C NMR spectral data of synthetic (±)-**1** were in good agreement with those of the natural plakevulin A except for the peaks derived from levulinic acid. Therefore the sample of the natural plakevulin A could be estimated to be an 1:1 mixture of (+)-**1** and levulinic acid. On the other hand, deprotection of TMS ether (±)-**7** with TBAF afforded (±)-**12** in 64% yield. The ^1H NMR and ^{13}C NMR spectral data of (±)-**12** were actually different from those of **1**.

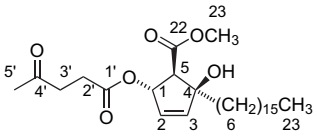
2.2. Synthesis of (+)- and (−)-plakevulin A, and (+)- and (−)-untenone A

Since natural plakevulin A is optically active ($[\alpha]_D^{25}$ +19 (*c* 2.0, $CHCl_3$)),[1] (+)-plakevulin A was synthesized from **13**.[5b] Compound **13** was prepared from (*S*)-(*tert*-butyldimethylsilyloxy)-2-cyclopentenone (99% ee by a chiral HPLC), which was derived from *cis*-3,5-diacetoxycyclopent-1-ene

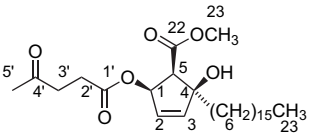


Scheme 2. Synthesis of the proposed structure of (±)-plakevulin A (**1a**) and (±)-**11**. (a) levulinic acid, DIAD, PPh_3, toluene, 52%; (b) TBAF, THF, 0 °C, 94%; (c) levulinic acid, EDCI, DMAP, 1,4-dioxane, 83% and (d) TBAF, THF, 0 °C, 49%.

Table 1. The selected ^{1}H and ^{13}C NMR spectral data of the natural plakevulin A, (±)-**1a**, and (±)-**11**

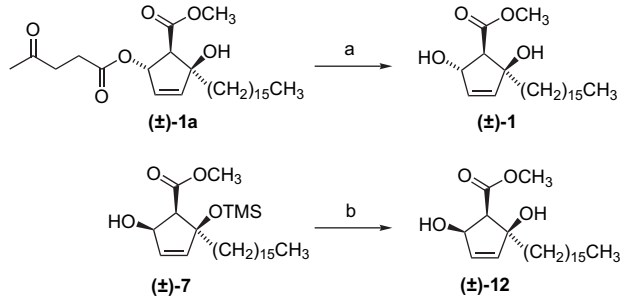


(±)-1a (the proposed structure)



(±)-11

^{1}H NMR	Natural plakevulin A	1a	11
	δ (m, Hz)	δ (m, Hz)	δ (m, Hz)
1	5.34 ddd, 5.2, 1.7, 1.5	6.04 ddd, 4.4, 1.5, 0.8	5.82 br d, 6.9
2	5.92 dd, 5.6, 1.8	5.91 dd, 5.4, 1.5	5.90 dd, 5.6, 2.1
3	5.85 dd, 5.6, 1.5	5.94 dd, 5.4, 0.8	6.08 d, 5.6
5	2.82 d, 5.2	2.96 d, 4.4	3.22 dd, 6.9, 1.0
6	1.81 m	1.80 m	1.65 m
23	3.78 s	3.76 s	3.75 s
2′	2.63 t, 6.4	2.56 m	2.55 t, 6.5
3′	2.75 t, 6.4	2.75 m	2.72 m
5′	2.20 s	2.18 s	2.19 m
^{13}C NMR			
1	78.2	80.9	76.8
2	135.7	131.5	129.4
3	136.9	139.8	142.2
4	84.9	85.3	83.4
5	60.6	57.7	53.9
6	40.6	40.8	40.4
22	172.7	171.8	171.5
23	52.1	52.2	52.1
1′	177.6	172.4	172.0
2′	27.6	27.9	28.0
3′	37.7	37.8	37.7
4′	206.5	206.3	206.3
5′	29.7	29.8	29.8



Scheme 3. Synthesis of the alcohol (±)-**1** and (±)-**12**. (a) $NH_2NH_2 \cdot H_2O$, pyridine/AcOH, 92% and (b) TBAF, THF, 64%.

(Scheme 4).[12] Desilylation of **13** with TBAF afforded (−)-**14** in 82% yield. Oxidation of (−)-**14** with Jones reagent, followed by protection of the *tert*-alcohol as a TMS ether gave (−)-**5** in 90% yield (99% ee by a chiral HPLC). Treatment of (−)-**5** with LDA followed by $NCCO_2CH_3$ in THF/HMPA gave **6** in a 4.2:1 diastereomeric mixture.

Reduction of **6** with DIBAL afforded (−)-**7** and (+)-**8** in 36% and 5%, respectively (Scheme 5). Esterification of (−)-**7** with *p*-nitrobenzoic acid under Mitsunobu conditions gave (+)-**15** in 61% yield. Methanolysis of the *p*-nitrobenzoate **15** and spontaneous migration of TMS group provided 1-*O*-TMS ether **16**. Finally desilylation of (+)-**16** with TBAF afforded (+)-plakevulin A (**1**) in 73% yield. The optical rotation of our synthetic (+)-**1** ($[\alpha]_D^{21}$ +27.1 (*c* 0.55, $CHCl_3$)) was slightly higher than that of natural **1** ($[\alpha]_D^{25}$ +19 (*c* 2.0, $CHCl_3$)),[1] and almost the same value as that of the synthetic (+)-**1** reported by Honda et al. ($[\alpha]_D^{22}$ +24.1 (*c* 0.6, $CHCl_3$)).[3] Compound (+)-**1** was also obtained by desilylation of (+)-**8** with TBAF.

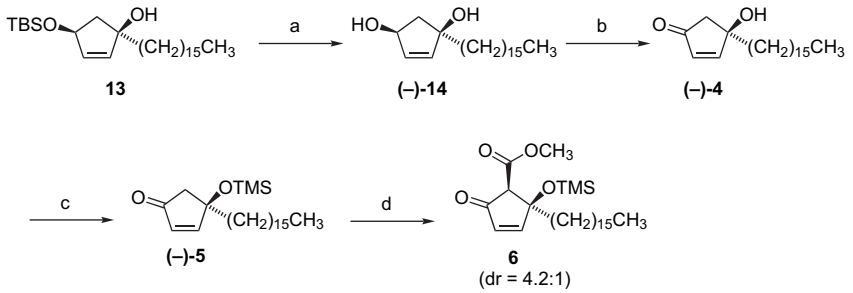
Since deprotection of TMS ether **6** could give optically active (−)-untenone A (**2**), deprotection of **6** was attempted (Scheme 6). First, treatment of **6** with TBAF in THF gave **2** as a racemic form ($[\alpha]_D^{23}$ ~0 (*c* 0.25, $CHCl_3$)). Although the formation of the acylic β-ketoester could not be observed in this reaction, the basic conditions would induce the retro-aldol reaction of the β′-hydroxy-β-ketoester and promote the racemization of **2**. Thus deprotection of **6** was performed under acidic conditions. Treatment of **6** with a catalytic amount of concd HCl in methanol gave optically active (−)-untenone A (**2**) in 94% yield. The optical rotation of our synthetic (−)-**2** ($[\alpha]_D^{23}$ −71.3 (*c* 0.94, $CHCl_3$)) was identical with those reported in the literature ($[\alpha]_D^{27}$ −73.3 (*c* 1.2, $CHCl_3$))[5c] by Asami et al., and $[\alpha]_D^{26}$ −79.7 (*c* 1.0, $CHCl_3$) by Honda et al.[3]

(−)-Plakevulin A (**1**) ($[\alpha]_D^{21}$ −25.7 (*c* 0.10, $CHCl_3$)) and (+)-untenone A (**2**) ($[\alpha]_D^{23}$ +72.2 (*c* 0.50, $CHCl_3$)) were prepared starting from **17**[5b] according to the same procedures (Scheme 7).

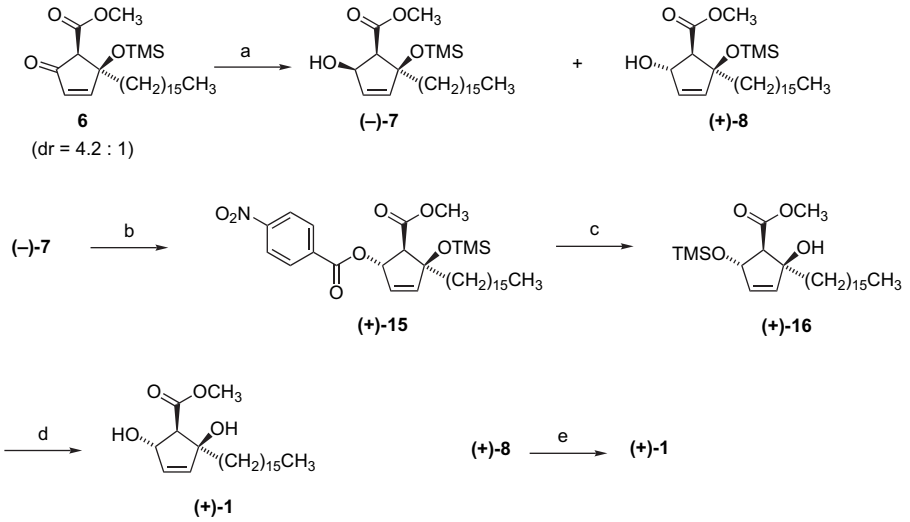
2.3. Preparation of (±)-untenone A, (±)-manzamenone A, and untenone A derivatives

In order to examine the structure–activity relationships, we prepared a number of untenone A derivatives.[9]

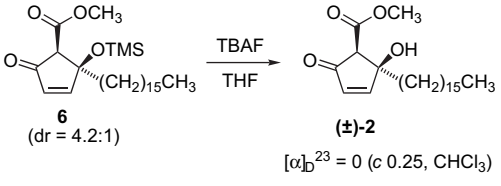
(±)-Untenone A (**2**) was prepared from (±)-**6**, from which in turn (±)-manzamenone A (**3**) was prepared by heating via a unique biogenetic pathway reported by Whitehead et al. (Scheme 8).[7]

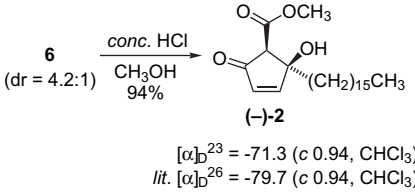


Scheme 4. Synthesis of the key intermediate **6**. (a) TBAF, THF, 84%; (b) Jones reagent, acetone, 77%; (c) TMSOTf, *i*-Pr_2NEt, CH_2Cl_2, 0 °C, 92% and (d) LDA, THF/HMPA, then $NCCO_2CH_3$, −42 °C, 69% (dr=4.2:1).

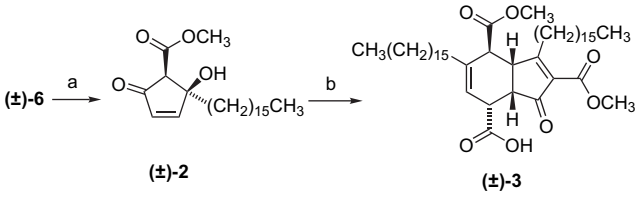


Scheme 5. Synthesis of (+)-plakevulin A (**1**). (a) DIBAL, CH_2Cl_2, −78 °C, 36% for (−)-**7** and 5% for (+)-**8**; (b) DIAD, PPh_3, *p*-nitrobenzoic acid, THF, 61%; (c) $NaOCH_3$, CH_3OH, 81%; (d) TBAF, THF, 73% and (e) TBAF, THF, 52%.

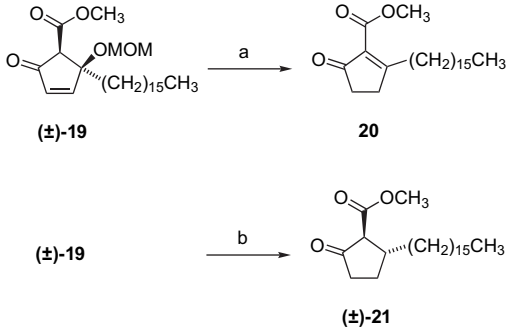




Scheme 6. Deprotection of TMS ether of **6**. (a) TBAF, THF and (b) concd HCl (cat), CH_3OH, 94%.

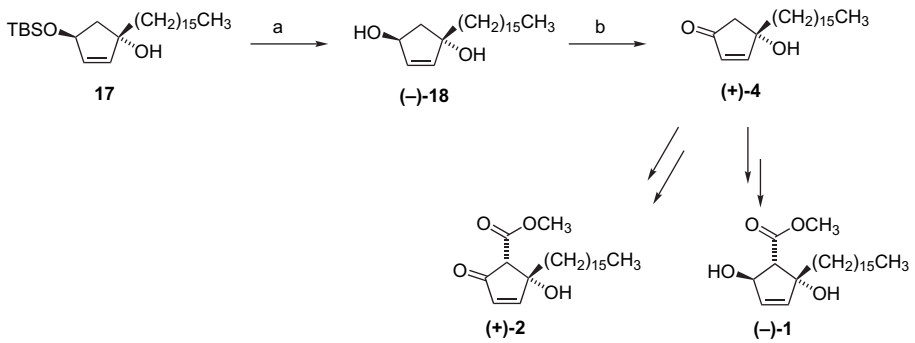


Scheme 8. Synthesis of (±)-**2** and (±)-**3**. (a) concd HCl, CH_3OH and (b) △.



Scheme 9. Synthesis of (±)-**20** and (±)-**21**. (a) H_2, Pd/C, EtOAc, quant. and (b) H_2, $Pd(OH)_2$/C, EtOAc, quant.

Hydrogenation of the double bond and elimination of the methoxymethyloxy group occurred by treatment of (±)-**19**[5b] with 10% Pd on carbon under an H_2 atmosphere to yield **20** (Scheme 9). On the other hand, treatment of (±)-**19** with $Pd(OH)_2$ under an H_2 atmosphere afforded (±)-**21**. Compound (±)-**21** was produced by further hydrogenation and the isomerization of the β-ketoester. The stereochemistry of (±)-**21** was established by the NOESY experiment (Fig. 3). The NOESY correlations between H-3α and H-5, H-3α and H-6, and H-5 and H-6, and the NOESY correlation between H-3β and H-4 indicated the



Scheme 7. Synthesis of (−)-**1** and (+)-**2**. (a) TBAF, THF, 77% and (b) Jones reagent, acetone, 0 °C, 66%.

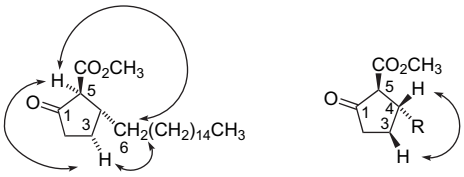


Figure 3. The NOESY correlations for (±)-**21**.

anti-relation for H-4/H-5. Reduction of the double bond of (±)-**19** was unsuccessful by hydrogenation or 1,4-reduction. Hydrogenation of (±)-**19** with Rh–Al_2O_3 and PtO_2 gave (±)-**21**. The use of other conditions ($NaBH_4$/MeOH, Mg/MeOH, and CuCl, $PhMe_2SiH$/DMF, etc.) gave complex mixture.

2.4. Structure–activity relationships of synthetic derivatives for inhibition of DNA polymerases

Synthetic (±)-**1**, (±)-**1a**, (±)-**11**, and (±)-**12** were tested with an enzyme inhibition assay for mammalian DNA polymerases α (pol α) and β (pol β). Table 2 shows the value of 50% inhibitory concentrations of these compounds. Compound (±)-**1** inhibited pol α (IC_{50}=61 μM) and weakly inhibited pol β (IC_{50}=179 μM), whereas the levulinyl ester (±)-**1a** did not influence pol α and pol β at concentrations lower than 200 μM. Interestingly, although (±)-**12** had no influence on pol α and pol β at concentrations lower than 200 μM, the levulinyl ester (±)-**11** inhibited the activity of pol α (IC_{50}=66 μM) and pol β (IC_{50}=132 μM). These results indicate that the stereochemistry and its functionality at C-1 greatly influenced the inhibitory activities against pol α and pol β.

Table 2. The IC_{50} values for enzymatic inhibition of DNA polymerase α (pol α) and β (pol β) by (±)-**1**, (±)-**1a**, (±)-**11**, and (±)-**12**

Compounds	IC_{50}(μM)	
	Pol α	Pol β
(±)-**1**	66	179
(±)-**1a**	>200	>200
(±)-**11**	61	132
(±)-**12**	>200	>200

The inhibitory activities of (±)-**2**, (±)-**3**, (±)-**4**, (±)-**19**, **20**, and (±)-**21** against pol α, pol β, and TdT are summarized in Table 3. We found that synthetic (±)-untenone A (**2**) possessed selective inhibitory activity against the enzymes (IC_{50}=4.3 μM for pol α, IC_{50}=57 μM for pol β, and IC_{50}=16 μM for TdT). (±)-Manzamenone A (**3**) was found to have strong inhibitory activity against all of these enzymes in the micromolar range. The β-hydroxyketone (**4**) showed no inhibitory activity against pol α, pol β, and TdT. Methoxymethyl-protected untenone A (**19**) showed inhibitory activity against the enzymes in the submicromolar and micromolar range, but exhibited nonselective inhibitory activity against the enzymes when compared to (±)-**2**. Both the α,β-unsaturated β-ketoester (**20**) and the saturated deoxygenated derivative (**21**) showed weaker inhibitory activities against polymerases than untenone A (**2**). These results indicate that the methyl ester moiety and the substituents at C-4 affected the inhibitory activities against pol α, pol β, and TdT.

Table 3. The IC_{50} values for enzymatic inhibition of DNA polymerase α (pol α) and β (pol β), and human terminal deoxynucleotidyl transferase (TdT) by (±)-**2**, (±)-**3**, (±)-**4**, (±)-**19**, **20**, and (±)-**21**

Compounds	IC_{50} (μM)		
	Pol α	Pol β	TdT
(±)-**2**	4.3	57	16
(±)-**3**	1.9	3.2	2.5
(±)-**4**	>200	>200	>200
(±)-**19**	5.9	9.3	18
20	17	107	129
(±)-**21**	20	90	84

We newly carried out DNA polymerase assays using pol α and pol β in order to test the influence of the chirality of plakevulin A (**1**) and untenone A (**2**) on the inhibitory activities. Table 4 shows the inhibitory effects of (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** against pol α and pol β. The inhibitory activities of (−)-**1** against these enzymes (IC_{50}=49 μM for pol α, IC_{50}=72 μM for pol β) were slightly stronger than those of (+)-**1** (IC_{50}=137 μM for pol α, IC_{50}=189 μM for pol β). In contrast, there are no significant differences in the inhibitory activities between (+)-**2** and (−)-**2**.

Table 4. The IC_{50} values for enzymatic inhibition of DNA polymerase α (pol α) and β (pol β) by (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2**

Compounds	IC_{50}(μM)	
	Pol α	Pol β
(+)-**1**	137	189
(−)-**1**	49	72
(+)-**2**	13	91
(−)-**2**	19	54

3. Conclusion

We have achieved a total synthesis of the proposed structure of plakevulin A (**1a**). However, the ^1H and ^{13}C NMR spectral data of **1a** were different from those of natural plakevulin A. The chemical shifts of the proton and the carbon at C-1 of **1a** especially deviated downfield from those of natural plakevulin A. Thus **1a** was converted into the corresponding alcohol (**1**) by removal of the levulinyl ester. The NMR data of **1** was identical with that of the natural product.

We have prepared optically active plakevulin A (**1**) and untenone A (**2**) according to the modified protocol reported by Yamada et al. Compounds (+)-**1**, (−)-**1**, (+)-**2**, and (−)-**2** were prepared and tested with an enzyme inhibition assay for mammalian DNA polymerases α (pol α) and β (pol β). Several analogues were also prepared in order to examine the structure–activity relationships of **1** in the inhibition of DNA polymerases. We found that the methyl ester of **1** was important for the inhibitory activity and that the substituents at C-1 and C-4 greatly influenced the activity. Although the inhibitory activity of (−)-**1** against pol α and pol β was slightly more potent than that of (+)-**1**, there were no significant differences in the inhibitory activity between (+)-**2** and (−)-**2**. Among the synthetic analogues, manzamenone (**3**) showed the most potent activity against pol α, pol β, and TdT.

4. Experimental

4.1. General

^{1}H and ^{13}C NMR were recorded on a JEOL JNM-LD400, or on a BRUKER DXR400 or DRX600. Chemical shifts were reported in δ, parts per million (ppm), relative to TMS as an internal standard or calibrated using residual undeuterated solvent as an internal reference. IR spectra were recorded on a JASCO FT/IR-410 spectrometer. Mass spectra were obtained on API QSTAR Pulsar i spectrometer. Optical rotations were measured on a JASCO P-1030 digital polarimeter. Melting points were determined with Yanaco MP-3S melting point apparatus. Column chromatography was carried out on Fuji Silisia PSQ100B. Analytical thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F_{254} plates, and compounds were visualized by UV illumination (254 nm) or heating at 150 °C after spraying phosphomolybdic acid in ethanol. THF was distilled from sodium/benzophenone. CH_2Cl_2 was distilled from P_2O_5. HMPA and diisopropylamine were distilled from CaH_2. All other solvent and reagents were obtained from commercial sources and used without further purification. Organic extracts were dried over Na_2SO_4, filtered, and concentrated using a rotary evaporator. Involatile oils and solids were vacuum dried.

4.1.1. 4-Hexadecyl-4-trimethylsiloxy-2-cyclopenten-1-one, (±)-5. To a solution of (±)-**5** (637 mg, 1.98 mmol) and *i*-Pr_2NEt (690 μL, 3.96 mmol) in CH_2Cl_2 (20 mL) was added TMSOTf (390 μL, 2.18 mmol) at 0 °C and the mixture was stirred at 0 °C for 15 min. Then the mixture was quenched by the addition of satd aq $NaHCO_3$ and extracted with ether. The organic layer was washed with H_2O and brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (10:1 hexane/EtOAc) to give TMS ether (703 mg, 90%) as a white solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44 (1H, d, *J*=5.7 Hz), 6.11 (1H, d, *J*=5.7 Hz), 2.50 (2H, m), 1.68 (2H, m), 1.31–1.25 (28H, br m), 0.88 (3H, t, *J*=6.9 Hz), 0.11 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.9, 166.9, 132.8, 81.3, 49.6, 41.9, 31.9, 29.9, 29.68 (×4), 29.65 (×2), 29.61, 29.55, 29.5, 29.4, 24.3, 22.7, 14.1, 2.1 (×3); IR (KBr) 2925, 2853, 1726, 1591, 1465, 1407, 1341, 1253, 1200, 1077, 938, 841, 756 cm^{-1}; HRMS calcd for $C_{24}H_{46}O_2NaSi$ ([M+Na]$^+$) 417.3159, found 417.3171.

4.1.2. 4-Hexadecyl-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopenten-1-one, (±)-6. To a solution of diisopropylamine (560 μL, 3.92 mmol) in THF (13 mL) was added *n*-BuLi (2.5 mL of a 1.58 M solution in hexane, 3.92 mmol) at 0 °C and the mixture was stirred at 0 °C for 10 min. The mixture was cooled to −78 °C. A solution of (±)-**5** (703 mg, 1.78 mmol) in THF/HMPA (10:1, 5 mL) was added to the mixture at −78 °C and the mixture was stirred at −78 °C for 80 min. Then methyl cyanoformate (340 μL, 4.27 mmol) was added and the mixture was stirred at −45 °C for 55 min. The mixture was quenched by the addition of satd NH_4Cl and extracted with ether. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (4:1 hexane/EtOAc) to give methyl ester (505 mg, 63%) as a 4.2:1 diasteromeric mixture as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) major isomer: δ 7.46 (1H, d, *J*=5.8 Hz), 6.27 (1H, d, *J*=5.8 Hz), 3.69 (3H, s), 3.38 (1H, s), 1.87 (1H, m), 1.69 (1H, m), 1.35–1.25 (28H, br m), 0.88 (3H, t, *J*=6.8 Hz), 0.11 (9H, s), minor isomer: δ 7.55 (1H, d, *J*=5.8 Hz), 6.19 (1H, d, *J*=5.8 Hz), 3.76 (3H, s), 3.51 (1H, s), 1.78 (1H, m), 1.66 (1H, m), 1.35–1.25 (28H, br m), 0.88 (3H, t, *J*=6.8 Hz), 0.14 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) major isomer: δ 201.4, 167.6, 164.6, 133.5, 81.3, 62.0, 51.9, 41.6, 31.9, 29.7, 29.63 (×4), 29.60, 29.58, 29.54, 29.48, 29.4, 29.3, 24.1, 22.6, 14.1, 2.2 (×3), minor isomer: δ 200.6, 168.6, 165.9, 131.7, 83.6, 64.8, 52.1, 38.3, 31.9, 29.9, 29.7, 29.6 (×6), 29.5 (×3), 23.8, 22.6, 14.1, 1.9 (×3); IR (neat) 2925, 2853, 1750, 1718, 1464, 1436, 1340, 1315, 1252, 1151, 1103, 1051, 1009, 941, 757 cm^{-1}; HRMS calcd for $C_{26}H_{48}O_2NaSi$ ([M+Na]$^+$) 475.3214, found 475.3189.

4.1.3. (1*RS*,4*SR*,5*RS*)-4-Hexadecyl-1-hydroxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (±)-7. To a solution of (±)-**6** (200 mg, 0.44 mmol) in CH_2Cl_2 (5 mL) was added DIBAL (940 μL of a 0.95 M solution in CH_2Cl_2, 0.89 mmol) at −78 °C. After the mixture was stirred at −78 °C for 2.5 h, the mixture was diluted with Et_2O. Then 1.0 mL of MeOH, followed by Celite was added, and the mixture was stirred at rt for 1 h. The mixture was filtrated through Celite and the filtrate was concentrated. The residue was purified by silica gel column chromatography (9:1→4:1 hexane/EtOAc) to give both (±)-**7** (82 mg, 41%) and (±)-**8** (12 mg, 6%) as colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.94 (1H, dd, *J*=5.6 Hz, 2.0 Hz), 5.84 (1H, d, *J*=5.6 Hz), 4.72 (1H, m), 4.08 (1H, d, *J*=9.5 Hz), 3.70 (3H, s), 3.30 (1H, d, *J*=7.1 Hz), 1.72 (1H, m), 1.58 (1H, m), 1.32–1.22 (28H, br m), 0.88 (3H, t, *J*=7.2 Hz), 0.09 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 172.3, 136.9, 135.2, 87.9, 75.0, 56.7, 51.5, 42.5, 31.9, 29.8, 29.68 (×4), 29.65 (×2), 29.61, 29.57, 29.5, 29.3, 24.3, 22.7, 14.1, 2.2 (×3); IR (neat) 3511, 3018, 2926, 2854, 1717, 1466, 1438, 1415, 1360, 1253, 1176, 1099, 1067, 991, 843, 668 cm^{-1}; HRMS calcd for $C_{26}H_{50}O_4NaSi$ ([M+Na]$^+$) 477.3370, found 447.3380.

4.1.4. (1*SR*,4*SR*,5*RS*)-4-Hexadecyl-1-hydroxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (±)-8. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (1H, d, *J*=6.0 Hz), 5.97 (1H, dd, *J*=6.0 Hz, 2.4 Hz), 4.96 (1H, m), 4.03 (1H, d, *J*=4.0 Hz), 3.77 (3H, s), 3.07 (1H, d, *J*=5.6 Hz), 1.64 (2H, m), 1.32–1.25 (28H, br m), 0.88 (3H, t, *J*=7.2 Hz), 0.11 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) 173.5, 140.7, 132.7, 88.2, 74.4, 58.8, 51.7, 40.7, 31.9, 30.0, 29.7 (×6), 29.65, 29.6 (×2), 29.4, 23.9, 22.7, 14.1, 2.1 (×3); IR (neat) 3471, 3018, 2926, 2854, 1712, 1464, 1439, 1355, 1253, 1215, 1095, 843 cm^{-1}; HRMS calcd for $C_{26}H_{50}O_4NaSi$ ([M+Na]$^+$) 477.3370, found 447.3392.

4.1.5. (1*SR*,4*SR*,5*RS*)-4-Hexadecyl-1-levuloyloxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (±)-9. To a solution of (±)-**7** (3.3 mg, 7.3 μmol), levulinic acid (7.0 μL, 68.4 μmol) and PPh_3 (19.2 mg, 73.2 μmol) was added DIAD (40% in toluene, 3.7 μL, 7.32 μmol). The reaction mixture was stirred for 2 h. The resulting mixture was concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/AcOEt 4:1) to afford (±)-**9** (2.0 mg, 52%) as a white solid. Mp=32–33 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.12 (1H, d, *J*=4.6 Hz), 5.93 (1H, d,

J=5.7 Hz), 5.91 (1H, d, J=5.7 Hz), 3.70 (3H, s), 2.95 (1H, d, J=4.6 Hz), 2.74 (2H, m), 2.55 (2H, m), 2.18 (3H, s), 1.79 (2H, br m), 1.34–1.26 (28H, br m), 0.88 (3H, t, J=6.0 Hz), 0.04 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.4, 172.3, 170.8, 138.8, 132.1, 87.9, 80.8, 59.0, 51.7, 42.1, 37.9, 31.9, 29.9, 29.9, 29.7 (×5), 29.7 (×2), 29.6, 29.6, 29.4, 27.9, 24.6, 22.7, 14.1, 2.0 (×3); IR (film) 3020, 2927, 2854, 1737, 1437, 1361, 1160, 1084, 846, 669 cm^{-1}; HRMS calcd for $C_{31}H_{56}O_6Na$ [(M+Na)$^+$] 575.3738, found 575.3766.

4.1.6. (1*SR*,4*SR*,5*RS*)-4-Hexadecyl-4-hydroxy-1-levuloyloxy-5-methoxycarbonyl-2-cyclopentene (the proposed structure of plakevulin A), (±)-1a. To a solution of (±)-**9** (5.5 mg, 9.9 μmol) in dry THF (0.5 mL) was added dropwise a solution of TBAF in THF (20 μL of 1.0 M solution in THF, 20 μmol) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. The resulting mixture was quenched with water, and extracted with Et_2O. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/AcOEt=5:1 to 2:1) to afford (±)-**1a** (4.5 mg, 94%) as a white solid. Mp=45–47 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.04 (1H, ddd, J=4.4 Hz, 1.5 Hz, 0.8 Hz), 5.94 (1H, dd, J=5.4 Hz, 0.8 Hz), 5.91 (1H, dd, J=5.4 Hz, 1.5 Hz), 3.76 (3H, s), 2.96 (1H, d, J=4.4 Hz), 2.75 (2H, m), 2.56 (2H, m), 2.30 (1H, s), 2.18 (3H, s), 1.80 (2H, m), 1.34–1.26 (28H, br m), 0.88 (3H, t, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3, 172.4, 171.8, 139.8, 131.5, 85.3, 80.9, 57.7, 52.2, 40.8, 37.8, 31.9, 29.9, 29.8, 29.7 (×5), 29.7 (×2), 29.6, 29.6, 29.4, 27.9, 24.2, 22.7, 14.1; IR (film) 3482, 3018, 2925, 2854, 1739, 1462, 1438, 1363, 1265, 1201, 1160, 1020, 759, 667 cm^{-1}; HRMS calcd for $C_{28}H_{48}O_6Na$ ([M+Na]$^+$) 503.3343, found 503.3307.

4.1.7. (1*RS*,4*SR*,5*RS*)-4-Hexadecyl-1-levuloyloxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (±)-10. To a solution of (±)-**7** (30 mg, 66 μmol) and levulinic acid (13.5 μL, 132 μmol) in 1,4-dioxane (0.7 mL) were added EDCI (25.3 mg, 132 μmol) and DMAP (0.8 mg, 6.6 μmol) at rt. The mixture was stirred at rt for 2 h. The resulting mixture was quenched with water, and extracted with Et_2O. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/AcOEt=20:1 to 2:1) to afford (±)-**10** (30.3 mg, 83%) as white wax and the recovered (±)-**7** (3.8 mg, 13%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.01 (1H, d, J=5.8 Hz), 5.87 (1H, dd, J=5.8 Hz, 2.0 Hz), 5.61 (1H, br d, J=7.2 Hz), 3.64 (3H, s), 3.39 (1H, d, J=7.2 Hz), 2.73 (2H, m), 2.58 (2H, m), 2.18 (3H, s), 1.69 (1H, m), 1.58 (1H, m), 1.34–1.26 (28H, br m), 0.88 (3H, t, J=6.7 Hz), 0.10 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.5, 172.5, 168.7, 139.8, 129.9, 87.2, 75.9, 56.7, 51.2, 42.3, 37.8, 31.9, 29.8, 29.7 (×4), 29.6 (×3), 29.6, 29.6, 29.5, 29.3, 28.1, 24.3, 22.7, 14.1, 2.0 (×3); IR (film) 3022, 2926, 2854, 1744, 1464, 1436, 1359, 1252, 1158, 1098, 919, 843, 667 cm^{-1}; HRMS calcd for $C_{31}H_{56}O_6SiNa$ ([M+Na]$^+$) 575.3738, found 575.3777.

4.1.8. (1*RS*,4*SR*,5*RS*)-4-Hexadecyl-4-hydroxy-1-levuloyloxy-5-methoxycarbonyl-2-cyclopentene, (±)-11. To a solution of (±)-**10** (8.2 mg, 15 μmol) in dry THF (0.5 mL) was added TBAF (40 μL of 1.0 M solution in THF, 40 μmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. The resulting mixture was quenched with water, and extracted with Et_2O. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (5:1→2:1 hexane/EtOAc) to afford (±)-**11** (3.5 mg, 49%) as a white solid. Mp=79–81 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.08 (1H, d, J=5.6 Hz), 5.90 (1H, dd, J=5.6 Hz, 2.1 Hz), 5.82 (1H, dd, J=6.9 Hz, 1.5 Hz), 4.06 (1H, s), 3.75 (3H, s), 3.22 (1H, dd, J=6.9 Hz, 1.0 Hz), 2.72 (2H, m), 2.55 (2H, t, J=6.5 Hz), 2.19 (3H, s), 1.65 (2H, m), 1.33–1.24 (28H, br m), 0.88 (3H, t, J=6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.3, 172.0, 171.5, 142.2, 129.4, 83.4, 76.8, 53.9, 52.1, 40.4, 37.7, 31.9, 29.9, 29.8, 29.7 (×4), 29.6 (×2), 29.6, 29.6, 29.5, 29.4, 28.0, 24.1, 22.7, 14.1; IR (film) 3505, 3020, 2926, 2854, 1722, 1519, 1465, 1439, 1408, 1359, 1216, 1158, 1076, 1030, 929, 757, 669 cm^{-1}; HRMS calcd for $C_{28}H_{48}O_6Na$ ([M+Na]$^+$) 503.3343, found 503.3311.

4.1.9. (±)-Plakevulin A (±)-1. To a solution of (±)-**1a** (1.1 mg, 2.6 μmol) in dry pyridine (1.0 mL) was added a solution hydrazine monohydrate (2.0 μL, 41.2 μmol) in pyridine/AcOH (3:2, 1.0 mL). The reaction mixture was stirred for 20 min. The resulting mixture was quenched with water, and extracted with EtOAc. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (1:1 hexane/EtOAc) to afford (±)-**1** (0.9 mg, 92%) as a white solid. Mp=80–82 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.94 (1H, dd, J=5.7 Hz, 1.8 Hz), 5.84 (1H, dd, J=5.7 Hz, 1.5 Hz), 5.34 (1H, m), 3.79 (3H, s), 2.83 (1H, d, J=5.3 Hz), 2.45 (1H, s), 2.00 (1H, br s), 1.81 (2H, m), 1.37–1.22 (28H, br m), 0.88 (3H, t, J=6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 172.6, 137.0, 135.7, 84.8, 78.2, 60.5, 52.1, 40.6, 31.9, 29.9, 29.7 (×5), 29.7 (×2), 29.6, 29.5, 29.4, 24.5, 22.7, 14.1; IR (film) 3445, 3019, 2927, 2855, 1724, 1520, 1465, 1439, 1374, 1041, 928, 759, 669 cm^{-1}; HRMS calcd for $C_{23}H_{42}O_4Na$ ([M+Na]$^+$) 405.2975, found 405.2972.

4.1.10. (1*RS*,4*SR*,5*RS*)-4-Hexadecyl-1,4-dihydroxy-5-methoxycarbonyl-2-cyclopentene, (±)-12. To a solution of (±)-**7** (8.9 mg, 19.5 μmol) in THF (2 mL) was added TBAF (30 μL of 1.0 M solution in THF, 30 μmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min. The resulting mixture was quenched with water, and extracted with Et_2O. The combined extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel chromatography (hexane/AcOEt 5:1 to 2:1) to afford (±)-**12** (4.8 mg, 64%) as white wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.09 (1H, dd, J=5.6 Hz, 2.4 Hz), 6.03 (1H, d, J=5.6 Hz), 4.82 (1H, m), 3.80 (3H, s), 3.50 (1H, s), 3.04 (1H, br d, J=8.0 Hz), 2.99 (1H, d, J=6.1 Hz), 1.73 (2H, m), 1.30–1.25 (28H, br m), 0.88 (3H, t, J=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.0, 140.0, 134.7, 83.9, 75.8, 55.0, 52.0, 39.3, 31.9, 29.9, 29.7 (×4), 29.6 (×2), 29.6, 29.6, 29.5, 29.3, 24.4, 22.7, 14.1; IR (film) 3440, 3014, 2925, 2854, 1718, 1464, 1440, 1357, 1214, 1176, 1064, 931, 759, 667 cm^{-1}; HRMS calcd for $C_{23}H_{42}O_4Na$ ([M+Na]$^+$) 405.2975, found 405.2989.

4.1.11. (1*S*,4*R*)-(−)-4-Hexadecyl-1,4-dihydroxy-2-cyclopentene, (−)-14. To a solution of **13** (2.6 g, 4.7 mmol) in THF (25 mL) was added TBAF (4.7 mL of a 1.0 M solution in THF, 4.7 mmol) at 0 °C and the mixture was stirred at rt for 30 min. Then the mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (4:1 hexane/EtOAc) to give diol (1.3 g, 84%) as a white solid. Mp=79–80 °C; $[\alpha]_D^{23}$ −45.9 (*c* 0.80, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 5.91 (1H, dd, *J*=5.6 Hz, 2.0 Hz), 5.88 (1H, d, *J*=5.6 Hz), 4.67 (1H, m), 2.55 (2H, br s), 2.41 (1H, dd, *J*=14.3 Hz, 7.0 Hz), 1.73 (1H, d, *J*=14.3 Hz, 3.1 Hz), 1.58 (2H, m), 1.30–1.25 (28H, br m), 0.88 (3H, t, *J*=6.7 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 140.0, 134.9, 84.1, 75.3, 48.0, 40.5, 31.9, 30.0, 29.68 (×4), 29.65 (×3), 25.59, 29.57, 29.4, 24.3, 22.7, 14.1; IR (KBr) 3317, 2918, 2849, 1469, 1360, 1302, 1213, 1159, 1086, 1054, 995, 971, 935, 873, 825, 783, 722, 603 cm^{-1}; HRMS calcd for $C_{21}H_{40}O_2Na$ ([M+Na]$^+$) 347.2920, found 347.2916.

4.1.12. (4*R*)-(−)-4-Hexadecyl-4-hydroxy-2-cyclopenten-1-one, (−)-4. To a solution of (−)-**14** (503 mg, 1.55 mmol) in acetone (15 mL) was added the Jones reagent (0.5 mL) in portions. Then the mixture was quenched by the addition of 2-propanol (0.5 mL) and diluted with EtOAc and H_2O. The organic layer was separated and washed with H_2O, brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (3:1 hexane/EtOAc) to give (−)-**4** (383 mg, 77%) as a white solid. The product was recrystallized from Et_2O to obtain colorless crystals. White solid, mp=52–53 °C; colorless crystal, mp=45–46 °C; $[\alpha]_D^{23}$ −54.5 (*c* 1.10, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 (1H, d, *J*=5.7 Hz), 6.09 (1H, d, *J*=5.7 Hz), 2.53 (1H, d, *J*=18.6 Hz), 2.41 (1H, d, *J*=18.6 Hz), 2.07 (1H, br s), 1.71 (2H, m), 1.35–1.23 (28H, br m), 0.85 (3H, t, *J*=6.6 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.9, 165.8, 133.3, 79.2, 48.8, 40.3, 31.9, 29.8, 29.7 (×3), 29.63 (×2), 29.61, 29.59, 29.5, 29.4, 29.3, 24.2, 22.7, 14.1; IR (KBr) 3418, 3017, 2925, 2854, 1715, 1466, 1404, 1339, 1215, 1067 cm^{-1}; HRMS calcd for $C_{21}H_{38}O_2Na$ ([M+Na]$^+$) 345.2764, found 345.2765.

4.1.13. (4*R*)-(−)-4-Hexadecyl-4-trimethylsiloxy-2-cyclopenten-1-one, (−)-5. To a solution of (−)-**4** (311 mg, 0.96 mmol) and *i*-Pr_2NEt (480 μL, 2.75 mmol) in CH_2Cl_2 (5 mL) was added TMSOTf (250 μL, 1.38 mmol) at 0 °C and the mixture was stirred at 0 °C for 15 min. Then the mixture was quenched by the addition of H_2O and extracted with CH_2Cl_2. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (10:1 hexane/EtOAc) to give (−)-**5** (351 mg, 92%) as a white solid. Mp=31–32 °C; $[\alpha]_D^{23}$ −16.0 (*c* 1.17, $CHCl_3$).

4.1.14. 4-Hexadecyl-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopenten-1-one (6). To a solution of diisopropylamine (250 μL, 1.76 mmol) in THF (5 mL) was added *n*-BuLi (1.1 mL of a 1.57 M solution in hexane, 1.73 mmol) at 0 °C and the mixture was stirred at 0 °C for 10 min. The mixture was cooled to −78 °C. A solution of (−)-**5** (309 mg, 0.78 mmol) in THF/HMPA (10:1, 4.4 mL) was added to the mixture at −78 °C and the mixture was stirred at −78 °C for 15 min. Then methyl cyanoformate (170 μL, 2.14 mmol) was added and the mixture was stirred at −45 °C for 2 h. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (4:1 hexane/EtOAc) to give **6** (245 mg, 69%) as a 4.2:1 diasteromeric mixture as colorless oil. The ^1H NMR, ^{13}C NMR, and IR data of **6** were identical with those of (±)-**6**.

4.1.15. (1*R*,4*S*,5*R*)-(−)-4-Hexadecyl-1-hydroxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (−)-7. To a solution of **6** (125 mg, 0.28 mmol) in CH_2Cl_2 (5 mL) was added DIBAL (620 μL of a 0.94 M solution in CH_2Cl_2, 0.58 mmol) at −78 °C. After the mixture was stirred at −78 °C for 1 h, the mixture was diluted with Et_2O. Then 1.0 mL of MeOH, followed by Celite was added, and the mixture was stirred at rt for 1 h. The mixture was filtrated through Celite and the filtrate was concentrated. The residue was purified by silica gel column chromatography (9:1→4:1 hexane/EtOAc) to give both (−)-**7** (45.5 mg, 36%) and (+)-**8** (6.8 mg, 5%) as colorless oil. $[\alpha]_D^{23}$ −12.7 (*c* 0.45, $CHCl_3$).

4.1.16. (1*S*,4*S*,5*R*)-4-Hexadecyl-1-hydroxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (+)-8. Colorless oil. $[\alpha]_D^{21}$ +54.0 (*c* 0.33, $CHCl_3$).

4.1.17. (1*S*,4*S*,5*R*)-(+)-4-Hexadecyl-5-methoxycarbonyl-1-(4-nitrobenzoyl)-4-trimethylsiloxy-2-cyclopentene, (+)-15. To a solution of (−)-**7** (30.1 mg, 66.2 μmol), *p*-nitrobenzoic acid (58.0 mg, 347 μmol) and PPh_3 (93.2 mg, 355 μmol) in THF (1.5 mL) was added DIAD (180 μL of a 40% solution in toluene, 356 μmol) at rt and the mixture was stirred at rt for 10 min. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (30:1 hexane/EtOAc) to give (+)-**15** (24.2 mg, 61%) as a white solid. Mp=85–86 °C; $[\alpha]_D^{21}$ +117.3 (*c* 1.47, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (2H, d, *J*=6.8 Hz), 8.17 (2H, d, *J*=6.8 Hz), 6.42 (1H, m), 6.07 (1H, dd, *J*=5.8 Hz, 1.9 Hz), 6.03 (1H, dd, *J*=5.8 Hz, 1.4 Hz), 3.75 (3H, s), 3.15 (1H, d, *J*=4.8 Hz), 1.84 (2H, m), 1.33–1.24 (28H, br m), 0.88 (3H, t, *J*=6.8 Hz), 0.09 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 164.3, 150.6, 139.7, 135.4, 131.5, 130.8 (×2), 123.5 (×2), 87.9, 82.1, 59.1, 51.8, 42.1, 31.9, 29.9, 29.7 (×6), 29.64 (×2), 29.61, 29.3, 24.7, 22.7, 14.1, 2.0 (×3); IR (KBr) 2919, 2850, 1744, 1720, 1606, 1525, 1333, 1274, 1116, 968, 844, 721 cm^{-1}; HRMS calcd for $C_{33}H_{53}NO_7NaSi$ ([M+Na]$^+$) 626.3483, found 626.3510.

4.1.18. (1*S*,4*S*,5*R*)-(±)-4-Hexadecyl-4-hydroxy-5-methoxycalbonyl-1-trimethylsiloxy-2-cyclopentene, (+)-16. To a solution of (+)-**15** (26.5 mg, 43.9 μmol) in THF–MeOH (1/1, 3 mL) was added NaOMe (52.0 μL of a 1 M solution in MeOH, 52.0 μmol) at rt and the mixture was stirred at rt for 20 min. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography

(5:1 hexane/EtOAc) to TMS ether (16.2 mg, 81%) as colorless oil. $[\alpha]_D^{23}$ +73.2 (*c* 1.0, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 5.92 (1H, dd, *J*=5.8 Hz, 1.7 Hz), 5.80 (1H, dd, *J*=5.8 Hz, 1.7 Hz), 5.45 (1H, m), 3.71 (3H, s), 2.81 (1H, d, *J*=5.5 Hz), 2.21 (1H, br s), 1.81 (2H, m), 1.30–1.25 (28H, br m), 0.88 (3H, t, *J*=6.8 Hz), 0.02 (9H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 136.3, 136.2, 87.8, 77.7, 62.5, 51.6, 41.6, 31.9, 29.9, 29.69 (×5), 29.65 (×2), 29.6 (×2), 29.4, 24.9, 22.7, 14.1, 2.0 (×3); IR (film) 3444, 2925, 2854, 1734, 1463, 1438, 1359, 1250, 1216, 1082, 937, 844 cm^{-1}; HRMS calcd for $C_{26}H_{50}O_4NaSi$ ($[M+Na]^+$) 477.3370, found 477.3356.

4.1.19. (+)-Plakevulin A (1). To a solution of (+)-**18** (14.4 mg, 31.7 μmol) in THF (1.0 mL) was added TBAF (40.0 μL of 1.0 M solution in THF, 40.0 μmol) and the mixture was stirred at rt for 1 h. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (2:1 hexane/EtOAc) to give (+)-plakevulin A (8.9 mg, 73%) as colorless wax. The product was recrystallized from hexane/EtOAc to obtain white solid. Colorless wax, mp=61–62 °C; White solid, mp=73–74 °C; $[\alpha]_D^{21}$ +27.1 (*c* 0.55, $CHCl_3$). The ^1H NMR, ^{13}C NMR, and IR data of (+)-**1** were identical with those of (±)-**1**.

4.1.20. (−)-Untenone A (2). To a solution of **6** (18.7 mg, 0.041 mmol) in MeOH (1 mL) was added one drop of concd HCl (ca. 10 μL) at rt and the mixture was stirred at rt for 15 min. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (9:1 hexane/EtOAc with 1% AcOH) to give (−)-untenone A (**2**) (14.7 mg, 94%) as colorless wax. The product was recrystallized from hexane to obtain white solid. Colorless wax, mp=~30 °C; White solid, mp=60–62 °C; $[\alpha]_D^{23}$ −71.3 (*c* 0.94, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ 7.53 (1H, d, *J*=5.7 Hz), 6.19 (1H, d, *J*=5.7 Hz), 3.80 (3H, s), 3.65 (1H, br s), 3.47 (1H, s), 1.81 (1H, m), 1.70 (1H, m), 1.32–1.25 (28H, br m), 0.88 (3H, t, *J*=6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.0, 169.1, 167.1, 132.3, 79.9, 60.8, 52.9, 40.4, 31.9, 29.74, 29.67 (×4), 29.64, 29.62, 29.58, 29.5, 29.4, 29.3, 23.8, 22.7, 14.1; IR (neat) 3451, 2919, 2850, 1740, 1712, 1467, 1437, 1321, 1256, 1154, 1035, 817, 763, 721 cm^{-1}; HRMS calcd for $C_{23}H_{41}O_4Na$ ($[M+Na]^+$) 381.3004, found 381.3012.

4.1.21. (1*S*,4*S*)-(−)-4-Hexadecyl-1,4-dihydroxy-2-cyclopentene, (−)-18. To a solution of **17** (326 mg, 0.74 mmol) in THF (6 mL) was added TBAF (0.92 mL of a 1.0 M solution in THF, 0.92 mmol) at 0 °C and the mixture was stirred at rt for 7 h. Then the mixture was quenched by the addition of H_2O and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (4:1 hexane/EtOAc) to give (−)-**18** (185 mg, 77%) as a white solid. Mp=83 °C; $[\alpha]_D^{23}$ −28.7 (*c* 0.68, $CHCl_3$). ^1H NMR (600 MHz, $CDCl_3$) δ 5.91 (1H, dd, *J*=5.6 Hz, 2.0 Hz), 5.87 (1H, dd, *J*=5.6 Hz, 1.2 Hz), 5.04 (1H, m), 2.30 (1H, dd, *J*=14.0 Hz, 6.8 Hz), 1.81 (1H, dd, *J*=14.0 Hz, 4.0 Hz), 1.73–1.63 (4H, m), 1.30–1.26 (28H, br m), 0.88 (3H, t, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 139.6, 135.9, 85.1, 76.1, 48.1, 41.6, 31.9, 30.0, 29.7 (×6), 29.64 (×2), 29.58, 29.3, 24.4, 22.7, 14.1; IR (KBr) 3330, 2919, 2850, 1465, 1406, 1346, 1268, 1190, 1160, 1126, 1103, 1045, 915, 867, 783, 722 cm^{-1}; HRMS calcd for $C_{21}H_{40}O_2Na$ ($[M+Na]^+$) 347.2920, found 347.2913.

4.1.22. (4*S*)-(+)-4-Hexadecyl-4-hydroxy-2-cyclopenten-1-one, (+)-4. To a solution of (−)-**18** (505 mg, 1.55 mmol) in acetone (30 mL) was added the Jones reagent (0.6 mL) in portions. The mixture was stirred at rt for 30 min. Then the mixture was quenched by the addition of 2-propanol (1.0 mL) and diluted with EtOAc and H_2O. The organic layer was separated and washed with H_2O, brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel column chromatography (3:1 hexane/EtOAc) to give (+)-**4** (332 mg, 66%) as a white solid. The product was recrystallized from Et_2O to obtain colorless crystals. Mp=45–46 °C; $[\alpha]_D^{20}$ +51.9 (*c* 1.10, $CHCl_3$).

4.1.23. (4*S*)-(+)-4-Hexadecyl-4-trimethylsiloxy-2-cyclopenten-1-one, (+)-5. White solid, mp=30–31 °C; $[\alpha]_D^{21}$ +16.1 (*c* 1.27, $CHCl_3$).

4.1.24. (1*S*,4*R*,5*S*)-(+)-4-Hexadecyl-1-hydroxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (+)-7. Colorless oil; $[\alpha]_D^{22}$ +12.7 (*c* 0.67, $CHCl_3$).

4.1.25. (1*R*,4*R*,5*S*)-4-Hexadecyl-1-hydroxy-5-methoxycarbonyl-4-trimethylsiloxy-2-cyclopentene, (−)-8. Colorless oil; $[\alpha]_D^{21}$ −52.4 (*c* 0.10, $CHCl_3$).

4.1.26. (1*R*,4*R*,5*S*)-(−)-4-Hexadecyl-5-methoxycarbonyl-1-(4-nitrobenzoyl)-4-trimethylsiloxy-2-cyclopentene, (−)-15. White solid, mp=82–83 °C; $[\alpha]_D^{19}$ −111.7 (*c* 0.55, $CHCl_3$).

4.1.27. (1*R*,4*R*,5*S*)-(−)-4-Hexadecyl-4-hydroxy-5-methoxycalbonyl-1-trimethylsiloxy-2-cyclopentene, (−)-16. Colorless oil; $[\alpha]_D^{21}$ −73.3 (*c* 0.25, $CHCl_3$).

4.1.28. (−)-Plakevulin A (1). Colorless wax, mp=60–62 °C; white soild, mp=70–71 °C; $[\alpha]_D^{21}$ −25.7 (*c* 0.10, $CHCl_3$).

4.1.29. (+)-Untenone A (2). White solid, mp=60–62 °C; $[\alpha]_D^{23}$ +72.2 (*c* 0.50, $CHCl_3$).

4.1.30. (±)-Manzamenone A (3). Compound (±)-**2** (20 mg, 47 μmol) was heated at ~70 °C for 24 h. The residue was purified by PTLC (hexane/EtOAc/AcOH 150:60:1) to give (±)-**3** (3.7 mg, 20%). ^1H NMR (600 MHz, $CDCl_3$) δ 6.18 (1H, br s), 3.88 (3H, s), 3.62 (1H, m), 3.55 (3H, s), 3.51 (1H, d, *J*=5.9 Hz), 3.20 (1H, dd, *J*=7.7 Hz, *J*=6.2 Hz), 3.13 (1H, m), 2.93 (1H, t, *J*=8.3 Hz), 2.45 (1H, m), 2.19 (2H, m), 1.60–1.26 (56H, br m), 0.88 (6H, t, *J*=6.6 Hz).

4.1.31. 2-Methoxycarbonyl-3-hexadecyl-2-cyclopenten-1-one (20). A solution of (±)-**19** (2.5 mg, 5.9 μmol) and 10% Pd on carbon (0.7 mg) in EtOAc (1 mL) was stirred at rt under an H_2 atmosphere for 14.5 h. The mixture was filtrated through Celite and washed with EtOAc. The solvent was removed under a reduced pressure. The residue was

purified by silica gel column chromatography (4:1 hexane/EtOAc) to give **20** (1.8 mg, 100%) as colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.84 (3H, s), 2.76 (2H, t, *J*=7.9 Hz), 2.68 (2H, m), 2.49 (2H, m), 1.57 (4H, m), 1.36 (1H, m), 1.26–1.32 (23H, br m), 0.88 (3H, t, *J*=7.0 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.8, 189.0, 163.8, 51.8, 34.9, 32.7, 31.9, 30.4, 29.7 (×7), 29.6, 29.6, 29.5, 29.4, 29.3, 27.7, 22.7, 14.1; IR (film) 3020, 2926, 2854, 1739, 1714, 1620, 1465, 1437, 1361, 1295, 1259, 1216, 1155, 1026, 758, 667 cm^{-1}; HRMS calcd for $C_{23}H_{40}O_3Na$ ([M+Na]$^+$) 387.2869, found 387.2868.

4.1.32. (2*RS*,3*SR*)-2-Methoxycarbonyl-3-hexadecylcyclopentanone, (±)-21. A solution of (±)-**19** (8.4 mg, 20 μmol) and 20% $Pd(OH)_2$ on carbon (2.3 mg) in EtOAc (1 mL) was stirred at rt under an H_2 atmosphere for 26 h. The mixture was filtrated through Celite and washed with EtOAc. The solvent was removed under a reduced pressure. The residue was purified by silica gel column chromatography (4:1 hexane/EtOAc) to give (±)-**21** (7.1 mg, 100%) as a white solid. Mp=38–41 °C. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.76 (3H, s), 2.83 (1H, d, *J*=11.2 Hz), 2.57 (1H, m), 2.42 (1H, dd, *J*=8.3 Hz, 18.7 Hz), 2.32 (1H, m), 2.23 (1H, m), 1.54 (1H, m), 1.47 (1H, m), 1.43 (1H, m), 1.36 (1H, m), 1.31–1.27 (27H, br m), 0.88 (3H, t, *J*=6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.1, 170.1, 61.9, 52.4, 41.5, 38.5, 35.0, 31.9, 29.7 (×7), 29.6, 29.6, 29.5, 29.4, 27.4, 27.2, 22.7, 14.1; IR (film) 3021, 2927, 2855, 1754, 1726, 1463, 1439, 1216, 1129, 927, 759, 669 cm^{-1}; HRMS calcd for $C_{23}H_{42}O_3Na$ ([M+Na$^+$]) 389.3026, found 389.3039.

4.2. DNA polymerase assay[13]

Calf pol α was purified by immuno-affinity column chromatography as described previously.[14] Recombinant rat pol β was purified based on the method described by Date et al.[15] Recombinant human TdT was purified as described by Ibe et al.[16] The synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 1 min. Five micro liters of these compounds in 40% DMSO were mixed with 10 μl of 2× reaction mixture (100 mM Tris–HCl (pH 7.5), 10 mM $MgCl_2$, 2 mM dithiothreitol, 15% glycerol, 250 μg/ml activated DNA, 100 μM each of dATP, dGTP, and dCTP, and 0.2 μM [^{3}H]dTTP) and 5 μl of each enzyme (0.01 units). These mixtures were incubated on ice for 10 min, then at 37 °C for 60 min. The amount of incorporated [^{3}H]dTMP into activated DNA without inhibitors was considered 100%. One unit of the activity was defined as the amount of enzyme that catalyzes the incorporation of 1 nmol of deoxyribonucleotide triphosphates into template DNA in 60 min at 37 °C.

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