A New 7,8-Euphadien-Type Triterpenoid from *Brackenridgea nitida* and *Bleasdalea bleasdalei* That Inhibits DNA Polymerase β

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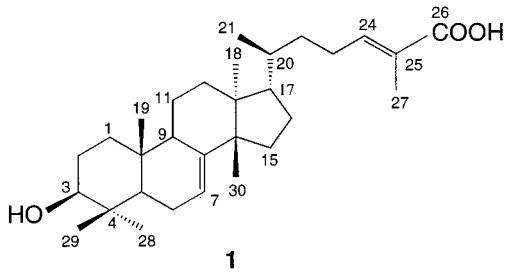
Bioassay-guided fractionation of extracts prepared from *Brackenridgea nitida* and *Bleasdalea bleasdalei*, using an assay to detect DNA polymerase β inhibition, resulted in the isolation of the inhibitory principle, (24*E*)-3β-hydroxy-7,24-euphadien-26-oic acid (**1**), a new euphane triterpenoid. The structure of **1** was established on the basis of HRMS and 1D and 2D NMR spectroscopic methods and was confirmed further by X-ray crystallographic analysis. Compound **1** inhibited rat DNA polymerase β with an IC_{50} value of 23 μM in the presence of bovine serum albumin (BSA) and 9.7 μM in the absence of BSA, consistent with the possibility that **1** may be of utility in vivo. This possibility was further supported by the finding that **1** potentiated the inhibitory action of the anticancer drug bleomycin in cultured P-388D_1 cells, reducing the number of viable cells by 48% when employed at a concentration of 25 μM in the presence of an otherwise nontoxic (75 nM) concentration of bleomycin. Compound **1** is the first euphane-type triterpenoid found to inhibit DNA polymerase β.

DNA repair pathways and their respective repair enzymes have been linked to involvement in resistance to chemotherapeutic agents.[1,2] Specifically, the role of eukaryotic DNA polymerase β in repairing DNA damage after exposure to DNA-damaging agents such as bleomycin (BLM), monofunctional DNA alkylation agents, cisplatin, and neocarzinostatin results in diminished efficacy of such anticancer drug therapies.[3−6] Selective inhibition of DNA polymerase β concomitant with adminstration of DNA-damaging agents could potentiate chemotherapeutic treatment and possibly allow for the use of lower doses of DNA-damaging antitumor agents. Indeed, recent studies in our laboratory using isolated DNA polymerase β inhibitors revealed that inhibition of this enzyme function in cultured cells resulted in potentiation of BLM and cisplatin cytotoxicity.[7−9] To provide access to more inhibitors having potential as antitumor agents and to facilitate an understanding of the role of polymerase β, we have attempted to identify additional naturally occurring DNA polymerase β inhibitors.

During a survey of crude plant extracts for DNA polymerase β inhibitory principles,[7−13] we found that two methyl ethyl ketone extracts prepared, respectively, from *Brackenridgea nitida* (F. Muell.) Kanis (Ochraceae) and *Bleasdalea bleasdalei* (F. Muell.) A. C. SM. et J. Haas (Proteaceae) exhibited potent DNA polymerase β inhibitory activity. Subsequent bioassay-guided fractionation of the two extracts, using an assay to monitor DNA polymerase β inhibition, led to the isolation of the polymerase β inhibitor (24*E*)-3β-hydroxy-7,24-euphadien-26-oic acid (**1**), a new euphane triterpenoid. Herein, we report the isolation of **1** from the two extracts through bioassay-guided fractionation, its structure elucidation, and its potency as a DNA polymerase β inhibitor, as well as the potentiation of BLM cytotoxicity by **1** in cultured cells.



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Results and Discussion

During a survey of plant extracts for naturally occurring DNA polymerase β inhibitors, methyl ethyl ketone extracts prepared from dried leaves of *B. nitida* and stem bark of *B. bleasdalei* were shown to exhibit strong inhibitory activity toward DNA polymerase β. The two crude extracts were still strongly active after passage through polyamide 6S columns to permit removal of polyphenols, which tend to be strong but nonspecific inhibitors of the enzyme. Accordingly, the two extracts were subjected to bioassay-guided fractionation, using a DNA polymerase β inhibition assay, to permit isolation and characterization of the principle(s) responsible for inhibition of DNA polymerase β.

Isolation and Structure Elucidation. Each of the two methyl ethyl ketone extracts, *B. nitida* and *B. bleasdalei*, was fractionated initially on a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4-OH. The 4:1 MeOH−CH_2Cl_2 fractions, which had significant DNA polymerase β inhibitory activity, were subjected to bioassay-guided fractionation. A combination of Sephadex LH-20 and reversed-phase C_{18} column chromatography, followed by crystallization from MeOH, afforded inhibitory principle **1**.

Compound **1** was obtained as colorless plates having $[\alpha]^{22}_D$ −38° (*c* 1.1, $CHCl_3$). The molecular formula $C_{30}H_{48}O_3$ was established from the high-resolution electron impact mass spectrum (HREIMS). The MS showed fragment ions

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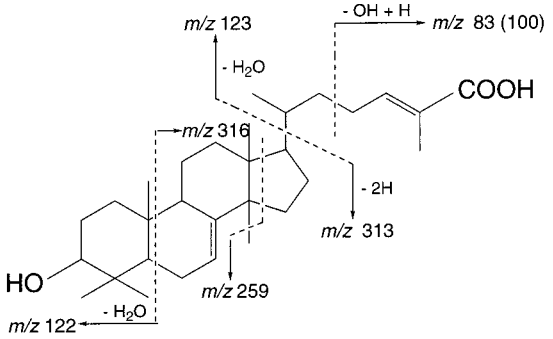

Figure 1. Important MS fragment ions observed for **1**.

at *m*/*z* 441 $[M - CH_3]^+$, 423 $[M - CH_3 - H_2O]^+$, and 405 $[M - CH_3 - 2H_2O]^+$. Also observed were fragment ions at *m*/*z* 313 and 259, corresponding to $[M -$ side chain $- 2H]^+$ and $[M -$ side chain $-$ part of ring D $- CH_3]^+$, which are characteristic of lanostane triterpenes having a mono-unsaturated side chain and a mono-unsaturated skeleton (Figure 1).[14] The intensity of the ion at *m*/*z* 83 (100%), which arose from allylic cleavage of the double bond and loss of elements of H_2O, strongly supported the presence of a methyl-substituted conjugated carboxyl group located at the terminus of the side chain.[14,15]

The ^{1}H NMR spectrum of **1** displayed signals for five methyls on quaternary carbons, a methyl doublet signal [δ_H 0.87 (3 H, d, J = 6.8 Hz)], an olefinic methyl group [δ_H 1.84 (3 H, br s)], two olefinic protons [δ_H 5.26 (1 H, m) and 6.89 (1 H, t, J = 5.8 Hz)], and an oxygen-substituted methine proton [δ_H 3.22 (1 H, dd, J = 11.5, 3.5 Hz)]. The ^{13}C NMR and DEPT spectra indicated that **1** was a tetracyclic triterpenoid composed of seven methyl, nine methylene, four methine, one oxygen-substituted methine (δ_C 78.9), two vinylic (δ_C 117.5 and 145.3), four quaternary, two fully substituted olefinic (δ_C 126.5 and 144.9), and one carboxyl (δ_C 172.6) carbons. The five singlet proton signals at high field due to the methyl group attached to quaternary carbons (δ_H 0.74, 0.80, 0.88, 0.96, and 0.97) and the high-field methylene carbon signal at δ_C ~18.0 for C-11 indicated that **1** was a $\Delta^{7,8}$ euphane triterpenoid.[16] The $\Delta^{7,8}$ assignment was supported further by the olefinic proton signal at δ_H 5.26 (m) and the MS fragmentation of ring B through a reverse Diels–Alder cleavage (Figure 1); this was confirmed from the key correlations of H-7 with C-5, C-9, and C-4 in the HMBC experiment of **1**. The proton signal at δ_H 3.22 (1H, dd, J = 11.5, 3.5 Hz) in the ^{1}H NMR spectrum of **1** was assigned to H-3; an α-orientation for H-3 was assignable on the basis of the coupling constant.

The geometry of the terminal methyl and carboxyl-substituted olefin (C-24 and C-25) for **1** was determined from the chemical shift value of H-24. With the strong deshielding of the carboxyl group, the proton signal due to H-24 in **1** appeared at lower field [δ_H 6.89 (1H, t, J = 5.8 Hz)], indicating that conjugated double bond for **1** had the *E*-configuration, compared with the corresponding chemical shift of δ_H ~5.90 that would be expected for the corresponding *Z* configuration.[17] The configuration at C-20 in **1** was deduced to be *S* (20β-CH_3) from the chemical shift value of C-18[14,16] at δ_C 21.8, in contrast to the corresponding carbon at the higher field (δ_C ~14.0[14]) because of the strong shielding of the 20α-CH_3. Therefore, the structure of **1** was established as (24*E*)-3β-hydroxy-7,24-euphadien-26-oic acid. Protons and carbons of **1** were assigned from the ^{1}H and ^{13}C NMR, ^{13}C–^{1}H COSY, and DEPT spectra and by comparison with structurally related compounds.[14–17] The structure of **1** was further confirmed by X-ray crystal analysis (Figure 2 and Tables 1 and 2). Compound **1** is the first euphane triterpenoid reported to have the 24*E* configuration. It may be noted that some phytochemical studies on *Brackenridgea* have been reported previously.[18–20]

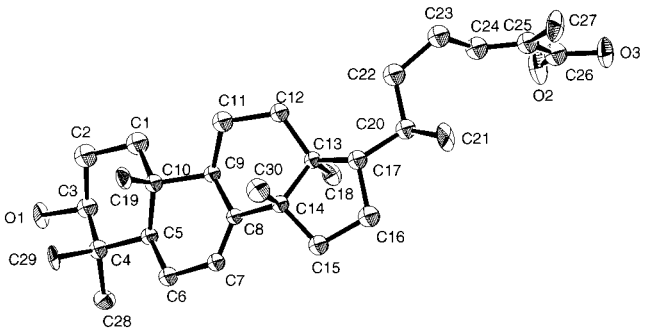

Figure 2. X-ray crystal structure for **1**.

Table 1. Crystal and Structure Refinement Data for **1**

empirical formula	$C_{30}H_{48}O_3 \cdot CH_3OH$
M	488.75
crystal system	orthorhombic
a/Å	10.551(3)
b/Å	35.67(2)
c/Å	7.624(2)
Z	4
D_c/Mg m^{-3}	1.13
μ(Mo Kα)/mm^{-1}	0.072
R_{int}	0.038
F(000)	1080
reflections collected	2688
unique reflections	2352
no. reflections [$I > 3\sigma(I)$]	1118
data/restraints/parameters	1118/0/192
residuals *R*, *R′*	0.075, 0.092

Table 2. Positional Parameters

atom	*x*	*y*	*z*
O(1)	0.8204(8)	0.0776(2)	0.444(1)
O(2)	0.5004(8)	0.4502(2)	0.324(1)
O(3)	0.5419(7)	0.5087(2)	0.266(1)
O(4)	1.3062(8)	0.5186(2)	0.190(1)
C(1)	0.915(1)	0.1782(3)	0.517(2)
C(2)	0.905(1)	0.1356(3)	0.551(2)
C(3)	0.842(1)	0.1165(3)	0.398(2)
C(4)	0.9145(9)	0.1216(3)	0.226(1)
C(5)	0.9250(8)	0.1640(3)	0.194(1)
C(6)	0.9854(10)	0.1741(3)	0.021(1)
C(7)	1.0104(9)	0.2159(3)	−0.007(1)
C(8)	0.9969(9)	0.2401(3)	0.120(1)
C(9)	0.9566(8)	0.2302(3)	0.302(1)
C(10)	0.9849(9)	0.1875(3)	0.345(1)
C(11)	1.006(1)	0.2569(3)	0.443(1)
C(12)	0.9841(10)	0.2990(3)	0.400(1)
C(13)	0.9548(8)	0.3082(3)	0.208(1)
C(14)	1.0323(9)	0.2810(3)	0.088(1)
C(15)	1.0073(10)	0.2961(3)	−0.093(1)
C(16)	0.993(1)	0.3395(3)	0.066(2)
C(17)	0.999(1)	0.3462(3)	0.133(1)
C(18)	0.8132(9)	0.3033(3)	0.174(2)
C(19)	1.127(1)	0.1818(3)	0.363(2)
C(20)	0.9248(10)	0.3821(3)	0.185(2)
C(21)	0.981(1)	0.4155(3)	0.088(2)
C(22)	0.929(1)	0.3882(3)	0.379(2)
C(23)	0.887(1)	0.4267(3)	0.453(2)
C(24)	0.759(1)	0.4378(3)	0.398(2)
C(25)	0.711(1)	0.4718(3)	0.358(2)
C(26)	0.582(1)	0.4755(4)	0.319(2)
C(27)	0.796(1)	0.5054(3)	0.349(2)
C(28)	0.831(1)	0.1041(3)	0.077(2)
C(29)	1.037(1)	0.0995(3)	0.226(2)
C(30)	1.179(1)	0.2840(3)	0.121(2)
C(31)	1.205(1)	0.4893(4)	0.200(2)

Table 3. IC_{50} Values[a] and Kinetic Constants[b] for **1**

	DNA		[^{3}H]dTTP	
IC_{50}	K_{is}	K_{ii}	K_{is}	K_{ii}
23 (BSA)[c]	15	2.8	82	28
9.7 (no BSA)				

[a] Obtained from the DNA polymerase β inhibition assay; reported in μM. [b] Reported in μM. [c] BSA, bovine serum albumin at 0.1 mg/mL.

Interestingly, one of the species isolated was a compound having physical constants similar to **1**, but was assigned a slightly different structure.[18]

Although masticadienolic acid [(24*Z*)-3α-hydroxy-7,24-euphadien-27-oic acid] has been reported to be a specific competitive inhibitor of secreted phospholipase A_2,[21] compound **1** is the first example of a euphane triterpenoid that inhibits DNA polymerase β.

Potency and Kinetic Analysis of DNA Polymerase *β*. In the DNA polymerase β assay, compound **1** had an IC_{50} value of 23 μM in the presence of bovine serum albumin (BSA) and 9.7 μM in the absence of BSA (Table 2). Kinetic analysis of enzyme inhibition revealed that **1** was a mixed inhibitor with respect to both activated DNA and [^{3}H]dTTP. A noncompetitive-uncompetitive pattern of inhibition is evident from the observed K_{is} and K_{ii} values; for activated DNA, $K_{is} = 15$ μM and $K_{ii} = 2.8$ μM, and for [^{3}H]dTTP, $K_{is} = 82$ μM and $K_{ii} = 28$ μM (Table 3). These values demonstrate that there was a stronger interaction between **1** and DNA polymerase β when DNA was varied than when dTTP was varied. Greater affinity was also observed for the ternary complexes when either DNA or dTTP was varied. Overall, the most efficient binding was observed when only activated DNA was bound by the enzyme, as evidenced by the lowest kinetic dissociation constant ($K_{ii} = 2.8$ μM).

Potentiation of BLM Cytotoxicity in Cultured P-388D_1 Cells. BLM and other DNA-damaging antitumor agents induce, among other lesions, single-strand DNA breaks,[22,23] which are repaired readily by DNA polymerase β.[3−6] The constitutive cellular expression of DNA polymerase β in vivo, as well as its overexpression in cells after exposure to various DNA-damaging agents,[2] makes this enzyme a good target for potentiation of the action of DNA-damaging agents. As shown in Figure 3, when used at a 25-μM concentration, compound **1** did not decrease the number of viable cells significantly. The same was also true of BLM when this agent was employed at a 75-nM concentration. However, when 75 nM BLM was added simultaneously with 25 μM **1**, the number of viable cells decreased by 48%. These findings suggest that DNA polymerase β inhibition by **1** could be of utility in cancer chemotherapeutic treatment; potentiation of the action of DNA-damaging agents such as BLM could plausibly be enhanced by inhibition of the DNA polymerase β-dependent DNA repair pathway.

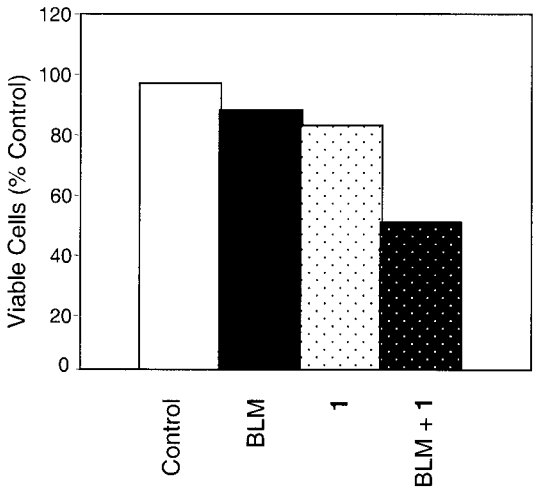

Figure 3. Effect of compound **1** on bleomycin-induced inhibition of the growth of P-388D_1 cells. Cells were treated as described for 6 h. Viability was assessed by trypan blue exclusion. White bars, control, no treatment; black bars, 0.075 μM blenoxane; black dots, 25 μM compound **1**; white dots, 25 μM compound **1** + 0.075 μM BLM. Results are presented as percent of control (untreated) cells.

Experimental Section

General Experimental Procedures. Polyamide 6S was obtained from Crescent Chemical Co. Sephadex LH-20 (Pharmacia; 40 μm) was purchased from Sigma Chemicals. Silica reversed-phase C_8 and C_{18} resins (32−60 μm) were obtained from ICN Pharmaceuticals. The melting point was recorded on a Thomas-Hoover capillary point apparatus and is uncorrected. Optical rotations were measured on a Perkin-Elmer 243 B polarimeter. ^{1}H and ^{13}C NMR were recorded on General Electric GN-300 or QE-300 NMR spectrometers. Low-resolution chemical ionization (CI) and electron impact (EI) mass spectra were recorded on a Finnigan MAT 4600 mass spectrometer. HRMS were obtained on a VG ZAB-SE mass spectrometer. Compound **1** was dissolved in 1:1 DMSO−methanol for the DNA polymerase β inhibition assay and for the kinetic studies; and in 100% DMSO for the cell culture studies, with a final DMSO concentration of 0.25% in each culture medium. Recombinant rat liver DNA polymerase β was a gift from Dr. Xiangyang Wang and Hongge Wang, prepared as described previously.[24−28] Antibiotic antimycotic solution, Hank's balanced salt solution, Dulbecco's modified Eagle's medium containing 4500 mg glucose/L, unlabeled dNTPs, and calf thymus DNA were purchased from Sigma Chemicals; calf thymus DNA was activated by the method described previously.[26] [^{3}H]dTTP (15 Ci/mmol) was purchased from ICN Pharmaceuticals. DEAE-cellulose paper (DE-81) was from Whatman. The P-388D_1 cell line (mouse, lymphoid neoplasm) was purchased from American Type Culture Collection. Trypan blue dye and donor horse serum were from Gibco BRL. Blenoxane, the clinically used mixture of bleomycins consisting predominantly of BLM A_2 and BLM B_2, was a gift from Bristol Myers Squibb Pharmaceuticals.

Plant Material. Leaves of *B. nitida* were collected in Australia in October 1979. Voucher specimen VKM-2681 is deposited at the U.S. National Arboretum, Herbarium (NA). Stem bark of *B. bleasdalei* was collected in Australia in October 1979. Voucher specimen VKM-2683 is deposited at the NA.

Extraction and Isolation. The dried leaves of *B. nitida* were soaked successively with hexanes, methyl ethyl ketone, methanol, and water. The methyl ethyl ketone extract exhibited inhibition of DNA polymerase β (97% inhibition at 100 μg/mL). The crude extract continued to have significant activity after passage through a polyamide 6S column to remove polyphenols. A total of 874 mg of methyl ethyl ketone crude extract was used for the bioassay-guided fractionation; a typical set of experiments is described below. The crude extract (258 mg) was applied to a 15-g polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4-OH. The 4:1 MeOH−CH_2Cl_2 fraction (115 mg) strongly inhibited DNA polymerase β (97% inhibition at 100 μg/mL); this material was fractionated further on a 10-g Sephadex LH-20 column, which was washed successively with hexanes, 1:1 hexanes−CH_2Cl_2, CH_2Cl_2, 1:1 CH_2Cl_2−acetone, acetone, and MeOH. The hexanes (37 mg) and 1:1 hexanes−CH_2Cl_2 (21 mg) fractions, which showed the strongest activity (93% and 83% inhibition at 50 μg/mL, respectively), were combined (58 mg) and fractionated further on a 12-g C_{18} reversed-phase open

column, which was washed successively with 2:8, 4:6, 8:2, 9:1, and 10:0 MeOH−H_2O. The 8:2 MeOH−H_2O fraction (15 mg) displayed the strongest inhibition of DNA polymerase β. Crystallization of the active principle from MeOH afforded compound **1** (9 mg) as colorless plates.

Extraction and Fractionation of *B. bleasdalei*. The dried stem bark of *B. bleasdalei* was soaked successively with hexanes, methyl ethyl ketone, methanol, and water. The methyl ethyl ketone extract exhibited inhibition for DNA polymerase β (91% inhibition at 100 μg/mL). The crude extract continued to exhibit activity after passage through a polyamide 6S column. The crude extract (1.13 g) was fractionated initially on a 35-g polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH−H_2O, 4:1 MeOH−CH_2Cl_2, 1:1 MeOH−CH_2Cl_2, and 9:1 MeOH−NH_4OH. The 4:1 MeOH−CH_2Cl_2 fraction (850 mg) strongly inhibited DNA polymerase β (88% inhibition at 100 μg/mL) and was fractionated further on a 30-g Sephadex LH-20 column. The column was washed successively with hexanes, 1:1 hexanes−CH_2Cl_2, CH_2Cl_2, 1:1 CH_2Cl_2−acetone, acetone, and MeOH. The 1:1 hexanes−CH_2Cl_2 fraction (255 mg), which showed the strongest activity (91% inhibition at 50 μg/mL), was purified further using a 20-g C_{18} reversed-phase open column, which was washed successively with 70%, 80%, 90% MeOH, and MeOH. The most active (90% MeOH) fraction (138 mg) provided 60 mg of crystalline **1** from MeOH.

(24*E*)-3β-Hydroxy-7,24-euphadien-26-oic acid (1): colorless plates; mp 145−146 °C; $[\alpha]^{22}_D$ −38° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) 0.74 (3H, s, 19-H_3), 0.80 (3H, s, 18-H_3), 0.87 (3H, d, J = 6.8 Hz, 21-H_3), 0.88 (3H, s, 29-H_3), 0.96 (3H, s, 30-H_3), 0.97 (3H, s, 28-H_3), 1.84 (3H, br s, 27-H_3), 3.22 (1H, dd, J = 11.5, 3.5 Hz, 3-H), 5.26 (1H, m, 7-H), and 6.89 (1H, t, J = 5.8 Hz, 24-H); ^{13}C NMR ($CDCl_3$, 75 MHz) 11.7 (C-19), 12.7 (C-29), 14.4 (C27), 17.7 (C-21), 18.1 (C-11), 21.8 (C-18), 23.6 (C-6), 25.9 (C-28), 26.9 (C-23), 27.1 (C-22), 27.2 (C-30), 28.1 (C-16), 33.5 (C-12), 34.5 (C-15), 35.5 (C-2), 36.8 (C-10), 38.5 (C-1), 43.1 (C-13), 48.4 (C-4), 50.2 (C-17), 50.9 (C-9), 51.0 (C-14), 52.8 (C-5 and C-20), 78.9 (C-3), 117.5 (C-7), 126.5 (C-25), 144.9 (C-8), 145.3 (C-24), and 172.6 (C-26); CI *m*/*z* 457 ([M + H]$^+$, 8.5), 439 ([M − H_2O + H]$^+$, 7.5), 423 ([M − CH_3 − H_2O]$^+$, 1.5), 259 (2.5), 123 (2.8), 83 (100); EI *m*/*z* 456 (M$^+$, 3.2), 441 ([M − CH_3]$^+$, 10.5), 423 ([M − CH_3 − H_2O]$^+$, 18.5), 316 (0.8), 313 (1.1), 259 (1.8), 123 (1.2), 122 (0.8), 83 (100); HREI *m*/*z* 456.3614 [M$^+$] (calcd for $C_{30}H_{48}O_3$ 456.3603).

X-ray Crystallography of 1. Compound **1** was crystallized from methanol solution by the vapor diffusion method. A crystal of dimensions 0.39 × 0.19 × 0.46 mm was selected for the data collection. All measurements were performed on a Rigaku AFC6S diffractometer at 273 K using Mo Kα radiation (λ = 0.71069 Å). Calculations were carried out on a Silicon Graphics Indigo 2 Extreme computer with the teXsan 1.7 package.[29] Relevant crystallographic data are listed in Tables 1 and 2. Unit cell dimensions were determined by applying the setting angles of 25 high-angle reflections. Three standard reflections were monitored during the data collection, showing no significant variance. The structure was solved by direct methods (SIR92).[30] Full-matrix least-squares refinement with anisotropic thermal displacement parameters for the O and terminal C atoms of the compound yielded the final R value of 0.075. The H atoms were found in the difference Fourier maps and were included in the calculations without further refinement. The goodness-of-fit was 2.13. The final electron density map was featureless, with the highest peak of 0.23 eÅ^{-3}.

DNA Polymerase β Inhibition Assay. To 50 μL of 62.5 mM 2-amino-2-methyl-1,3-propanediol buffer, pH 8.6, containing 10 mM $MgCl_2$, 1 mM DTT, 100 μg/mL BSA, 6.25 μM dNTPs including 0.04 Ci/mmol [^{3}H]dTTP, and 0.25 mg/mL activated calf thymus DNA was added 6 μL of a solution containing each test sample and 4 μL of recombinant rat DNA polymerase β preparation (6.9 units, 4.8 × 10^4 units/mg). After incubation at 37 °C for 60 min, the radiolabeled DNA product was collected on DEAE-cellulose paper (DE-81), dried, and rinsed successively with 0.4 M K_2HPO_4, pH 9.4, and 95% ethanol for radioactivity determination. For the kinetic studies, inhibitor constants (K_{is} and K_{ii}) were obtained using the same assay, except incubation was for 20 min and the samples were immediately placed on ice before application to DE-81 paper. Additionally, dNTP concentrations were varied {25, 12.5, 6.25, 3.12, and 1.56 μM ([^{3}H]dTTP was added in proportion, such that its specific activity remained constant)} or the activated DNA concentration was varied (200, 100, 50, 25, and 12.5 μM nucleotide concentration).

BLM Cytotoxicity Assay in Cell Culture. P-388D_1 cells were maintained as suspension cultures in 90% (v/v) Dulbecco's modified Eagle's medium containing 4500 mg glucose/L, 10% (v/v) donor horse serum, and 100 IU penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B/mL at 37 °C in a 5% CO_2 in air atmosphere. To 12.5-mL tissue culture flasks were added 5 mL of cell suspension containing ca. 5 × 10^5 cells/mL; these were incubated for 1 h to stabilize the cells. Assays were carried out in 6-mL reaction cultures containing the desired amounts of compound **1** and blenoxane dissolved in media. Cultures were incubated at 37 °C in a 5% CO_2 in air atmosphere for 6 h followed by cell viability determination using trypan blue dye.

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Supporting Information Available: Details of the X-ray structure analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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