# DNA Polymerase $\beta$ Inhibitors from *Sandoricum koetjape*

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Bioassay-guided fractionation of *Sandoricum koetjape* using an assay sensitive to DNA polymerase $\beta$ inhibition led to the isolation of three active compounds (**1**−**3**) having IC$_{50}$ values from 20 to 36 $\mu$M. Derivatives **5**−**14** were prepared from compounds **1** and **2**; derivatives **11**, **12**, and **13** showed activity against DNA polymerase $\beta$ with IC$_{50}$ values ranging from 16 to 36 $\mu$M.

DNA repair pathways and their respective repair enzymes have been linked to involvement in resistance to chemotherapeutic agents.[1] Specifically, the repair of DNA damage by DNA polymerase $\beta$ after exposure to DNA-damaging agents such as bleomycin,[2−4] neocarzinostatin,[4] *cis*-platin,[5] and monofunctional DNA-alkylating agents[6] may well result in diminished efficacy of anticancer drug therapies.[7] Inhibition of this repair by simultaneously inhibiting DNA polymerase $\beta$ might well potentiate chemotherapeutic treatment and possibly allow for lower doses of antitumor agent to be administered. In this regard, as well as for an understanding of the role of the enzyme in vivo, the identification of additional DNA polymerase $\beta$ inhibitors is imperative. Although a number of polymerase $\beta$ inhibitors have been reported in recent years, including dideoxythymidine triphosphate (ddTTP),[8] taurospongin A,[9] nigranoic acid,[10] flavonoids,[11] fatty acids,[12] fomitellic acids,[13] bile acids,[14] 3,4,5-tri-*O*-galloylquinic acid,[15] and sulfated glycoglycerolipids,[16] more potent and selective inhibitors of DNA polymerase $\beta$ are needed.

During our survey of plant secondary metabolites for DNA polymerase $\beta$ inhibitors, we found that several soaks of dried plant materials prepared with different solvents were active. In particular, a crude methyl ethyl ketone extract prepared from stem bark and wood of *Sandoricum koetjape* (Burm.f.) Merr. (Meliaceae) showed strong inhibition of DNA polymerase $\beta$ (91% and 83% inhibition at 100 and 50 $\mu$g/mL, respectively). Previously, fatty acids,[17] triterpenes,[18−20] and limonoids[21] have been isolated from this plant. Bioassay-guided fractionation of the extract using an in vitro assay sensitive to DNA polymerase $\beta$ inhibition led to the discovery of three compounds (**1**−**3**) that have been identified previously as natural products.[18,20] Compounds **1** and **2** have been reported to exhibit significant cytotoxicity toward cultured P-388 cells but human tumors represented in the test panel were not sensitive to these compounds.[20] In the hope of identifying more active inhibitors of DNA polymerase $\beta$, compounds **5**−**14** were prepared by semisynthesis from **1** and **2**. Presently, we describe the isolation of compounds **1**−**3**, the preparation of several derivatives of **1** and **2**, and the biochemical activities of the parent compounds and derivatives.

## Results and Discussion

Dried plant material (stem bark and wood) from *Sandoricum koetjape* was soaked successively with hexanes, methyl ethyl ketone, methanol, and water. Using an assay sensitive to DNA polymerase $\beta$ inhibition for bioassay-guided fractionation, the methyl ethyl ketone extract was first fractionated using a polyamide 6S column to permit dereplication of polyphenols. The active 4:1 MeOH−CH$_2$Cl$_2$ fraction was separated further using a C$_{18}$ column, and the active fractions from the C$_{18}$ column were combined and separated further using a C$_8$ column. Compound **1** was obtained as fine, colorless needles from the 90% MeOH fraction from the C$_8$ column. Compounds **2** and **3** were crystallized from active fractions from the C$_{18}$ column, yielding the compounds as colorless needles and colorless prisms, respectively. These three compounds were identified as 3-oxo-olean-12-en-29-oic acid (**1**), katonic acid (**2**), and koetjapic acid (**3**), by comparison with spectral data reported previously.[20]



**1** R = ═ O
**2** R = ⋯ OH        **3**
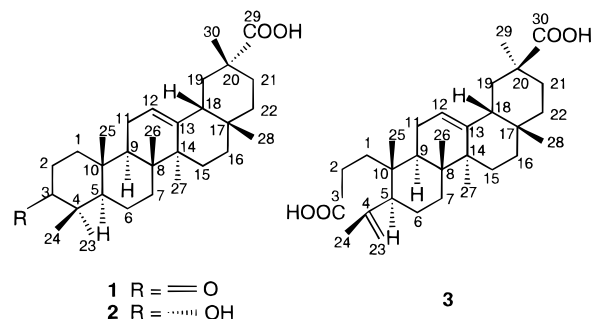
In the hope of finding more active derivatives, compound **1** was modified at positions 29 and 3, containing carboxylic acid and ketone moieties, respectively (Scheme 1). Derivatives **5**−**10** were prepared from **1** by modification of the carboxylate moiety. Compounds **5**−**9** were obtained via the acid chloride **4**, prepared from **1** by treatment with excess (COCl)$_2$ in CH$_2$Cl$_2$. After 30 min, unreacted (COCl)$_2$ was removed under diminished pressure, and intermediate **4** was obtained. CH$_3$(CH$_2$)$_3$NH$_2$, CH$_3$CH$_2$OH, (CH$_3$)$_3$CNH$_2$, (CH$_3$CH$_2$)$_2$NH, and NH$_3$ were added to solutions of crude **4**, affording compounds **5**−**9**, respectively. Compound **10** was obtained from **1** by condensation with MeOH in the presence of DCC and 4-(dimethylamino)pyridine (DMAP). Compounds **11**−**13** were prepared from **1** through modification of the ketone moiety. MeOH or EtOH solutions of C$_6$H$_5$CH$_2$ONH$_2$, CH$_3$ONH$_2$·HCl, or NH$_2$OH were added to **1**; the reactions were stirred at room temperature for 24 h and **11**−**13**, respectively, were obtained after purification. Katonic acid (**2**) was converted to its methyl ester by treatment with MeOH in the presence of TMSCHN$_2$.

The DNA polymerase $\beta$ inhibitory effects of these derivatives were characterized initially by determination of the IC$_{50}$ values. For six derivatives the mode of inhibition and the kinetic constants were also determined (Table 1). Compounds **1**−**3** and **11**−**13** retained a carboxyl group at

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**Scheme 1.** Structure Modification of 3-Oxo-olean-12-en-29-oic Acid (**1**)
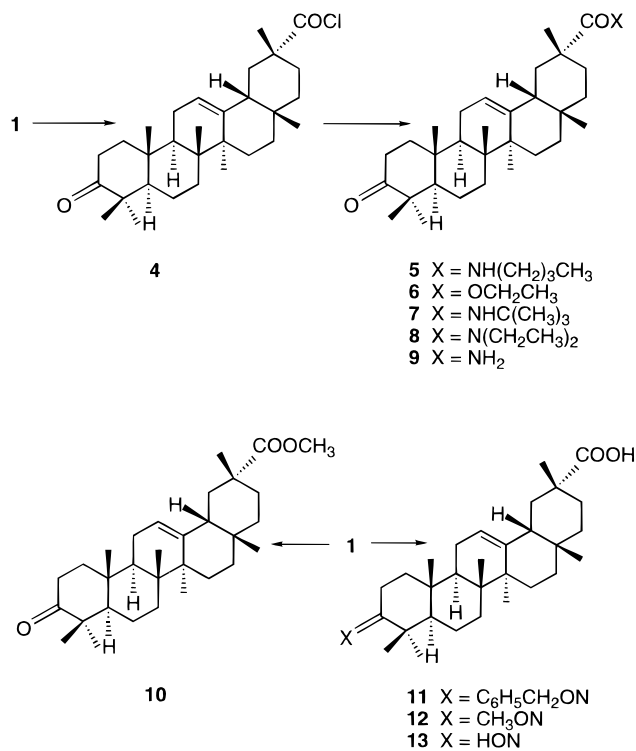


**Table 1.** IC$_{50}$ Values and Kinetic Constants for DNA Polymerase β Inhibitors

| compound | IC$_{50}$ ($\mu$M) | [³H]TTP | | DNA template-primer | |
| --- | --- | --- | --- | --- | --- |
| | | $^aK_{is}$ ($\mu$M) | $^bK_{ii}$ ($\mu$M) | $^aK_{is}$ ($\mu$M) | $^bK_{ii}$ ($\mu$M) |
| **1** | 22 | 60 | 9.1 | 5.5 | 50 |
| **2** | 36 | 60 | 24 | 4.2 | 11 |
| **3** | 20 | 24 | 11 | 5.6 | 18 |
| **5** | NA$^c$ | | | | |
| **6** | NA$^c$ | | | | |
| **7** | NA$^c$ | | | | |
| **8** | NA$^c$ | | | | |
| **9** | NA$^c$ | | | | |
| **10** | NA$^c$ | | | | |
| **11** | 35 | 69 | 22 | 37 | 24 |
| **12** | 36 | 73 | 35 | 9.1 | 35 |
| **13** | 16 | 16 | 10 | $^d$ | $^d$ |
| katonic acid methyl ester | NA$^c$ | | | | |

$^a$ Enzyme–inhibitor dissociation constant. $^b$ Enzyme–substrate-inhibitor dissociation constant. $^c$ Not active. $^d$ Not determined.

position 29. All of these had polymerase β inhibitory activity. In contrast, compounds **5**–**10** and the methyl ester of **2**, which were obtained through modification of the carboxylate moiety, failed to exhibit any inhibitory activity. Because other DNA polymerase β inhibitors such as nigranoic acid,[10] (modified) fatty acids,[12] fomitellic acids,[13] and bile acids[14] possess carboxylic acid moieties, this common structural element may play an important role in DNA polymerase β inhibition, for example, metal ion coordination.

Of those compounds isolated directly from *Sandoricum koetjape*, compound **3** (IC$_{50}$ 20 $\mu$M), having a carboxyl group at position 3, was the most potent inhibitor of DNA polymerase β. Compounds **1** and **2** (IC$_{50}$ values 22 and 36 $\mu$M, respectively) showed slightly less potent inhibition of DNA polymerase β.

Compound **13** (IC$_{50}$ 16 $\mu$M), obtained by derivatization of **1** at position 3 to afford the oxime from the ketone, was the most potent natural product derivative. Derivatization

of **1** at position 3 to afford **11** and **12** (IC$_{50}$ values 35 and 36 $\mu$M, respectively) slightly weakened its inhibitory potency toward DNA polymerase β.

For those compounds and derivatives tested, the type of inhibition determined from Lineweaver–Burk plots was mainly mixed-type inhibition obtained by varying substrate ([³H]TTP) as well as the DNA template-primer concentrations (data not shown). When varying [³H]TTP, the inhibition pattern was intermediate between competitive and noncompetitive inhibition; for the DNA template-primer the pattern was intermediate between competitive and noncompetitive inhibition. From replots of the primary data from the Lineweaver–Burk plots, the $K_{is}$ (enzyme:inhibitor dissociation constant) and the $K_{ii}$ (enzyme:substrate:inhibitor dissociation constant) values were obtained; the slopes from the primary plot versus inhibitor concentrations afforded the $K_{is}$ values, and the $1/v$-axis intercepts versus inhibitor concentrations afforded the $K_{ii}$ values (Table 1).

In mixed-type inhibition, the dissociation constants $K_{is}$ and $K_{ii}$ are not equal, which indicates differential interactions between the inhibitor and the enzyme versus the inhibitor and enzyme:substrate complex. For the substrate [³H]TTP, all the compounds exhibited higher $K_{is}$ values than $K_{ii}$ values; this indicates that there was greater affinity between the inhibitors and the enzyme:[³H]TTP complex than with the enzyme alone. However, this was not the case with the DNA template-primer; the $K_{ii}$ values were greater than the $K_{is}$ values, suggesting that the compounds showed greater affinity for the enzyme than for the enzyme:DNA template-primer complex. For compound **11**, with respect to the DNA template-primer, the $K_{is}$ and $K_{ii}$ values were more nearly equal, which likely demonstrates that the substrate did not affect inhibitor binding; a simple linear noncompetitive inhibition pattern was observed instead of a mixed-type pattern.

Although the three natural products (**1**–**3**) identified as polymerase β inhibitors have been isolated previously, including their identification from the same plant species as cytotoxic agents,[20] the present work is important in a number of regards. These include the identification of **1**–**3** as species capable of inhibiting DNA polymerase β and chemical transformation of **1** and **2** to 10 new compounds, three of which also exhibited significant inhibition of polymerase β. A detailed kinetic analysis of the active compounds has also provided insights into the binding of the individual inhibitors to the enzyme alone and enzyme:substrate complexes, for the [³H]TTP substrate and DNA template-primer. As is clear from Table 1, alteration of the carboxylate moiety at position 29 in **1** or **2** eliminated activity, while alterations at position 3 (**11**–**13**) afforded analogues that retained inhibitory activity toward polymerase β. Interestingly, all six active analogues displayed more potent inhibition of the polymerase β-TTP complex than of the enzyme itself, but four mediated greater inhibition of polymerase β alone than polymerase β bound to the DNA template-primer.

In the aggregate, these data provide important insights into the structural elements required for triterpene inhibition of DNA polymerase β and the nature of the inhibitory processes. The results should facilitate the design of inhibitors with improved properties by delineating those facets of enzyme inhibition that can vary with inhibitor structure.

**Experimental Section**

**General Experimental Procedures.** Melting points were recorded on a Thomas–Hoover capillary melting point ap-

paratus and are uncorrected. The ${}^1$H NMR and ${}^{13}$C NMR spectra were recorded using tetramethylsilane (TMS) as an internal standard, employing a General Electric GE−300 instrument operating at 300 and 75 MHz, respectively. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. LREIMS were recorded on a Finnigan MAT4600 mass spectrometer. HRMS were obtained on a VG ZAB−SE mass spectrometer. Thin-layer chromatography (TLC) was carried out on Merck F$_{254}$ precoated Si gel plates. Spots were visualized by dipping the plates in 10% H$_2$SO$_4$ in EtOH. Column chromatography employed Fluka Si gel 60, mesh size 230−400. All fractionations were carried out using open columns. All reagents were of highest available commercial quality and were used without further purification. Polyamide 6S for column chromatography (a product of Riedel−de Haen, Germany, 50−160 $\mu$m) was purchased from Crescent Chemical Co., Inc. Lipophilic Sephadex LH-20 (a product of Pharmacia Inc., Uppsala, Sweden, bead size 25−100 $\mu$m) was obtained from Sigma Chemicals. Reversed-phase C$_{18}$ (32−63 $\mu$m) and C$_8$ (32−63 $\mu$m) Si gel were obtained from ICN Pharmaceuticals. Unlabeled dNTPs and calf thymus DNA were purchased from Sigma Chemicals. [${}^3$H]-TTP was from ICN Pharmaceuticals. DEAE-cellulose paper (DE−81) was purchased from Whatman. DNA polymerase $\beta$ was a gift from Dr. Li-Kai Wang.

**DNA Polymerase $\beta$ Inhibition Assay.** Crude extract samples and fractions were dissolved in 1:1 DMSO−MeOH. Then, 6 $\mu$L of the sample and 4 $\mu$L of rat DNA polymerase $\beta$[22] (6.9 units, 48,000 units/mg) were added to 50 $\mu$L of 62.5 mM 2-amino-2-methyl-1,3-propanediol buffer, pH 8.6, containing 10 mM MgCl$_2$, 1 mM DTT, 0.1 mg/mL bovine serum albumin, 6.25 $\mu$M dNTPs, 0.04 Ci/mmol [${}^3$H]TTP, and 0.25 mg/mL of activated calf thymus DNA. After incubation at 37 °C for 60 min, the radioactive DNA product was collected on DEAE-cellulose paper, dried, and rinsed successively with 0.4 M K$_2$-HPO$_4$ and 95% EtOH. Studies analyzing kinetics of inhibition were performed using the same assay, except incubation was for 20 min. In one set of experiments the dNTP concentrations were 25, 12.5, 6.25, 3.12, and 1.56 $\mu$M ([${}^3$H]TTP concentrations varied in proportion). Inhibition studies carried out with variable DNA concentrations employed 200, 100, 50, 25, and 12.5 $\mu$M (nucleotide) concentrations of DNA template-primers.

**Extraction and Isolation.** *Sandoricum koetjape* was collected on Oahu, HI, in November 1980. A voucher specimen is stored at the Bishop Museum, Herbarium (BISH), Honolulu, HI. Dried plant materials (stem bark and wood) were soaked successively with hexanes, methyl ethyl ketone, MeOH, and H$_2$O. The methyl ethyl ketone extract was fractionated first using a polyamide 6S column. In a typical experiment, 300 mg of crude extract was applied to a column containing 14.5 g of polyamide 6S; washing was carried out successively with 200-mL portions of H$_2$O, 1:1 MeOH−H$_2$O, 4:1 MeOH−CH$_2$-Cl$_2$, 1:1 MeOH−CH$_2$Cl$_2$, and then 9:1 MeOH−NH$_4$OH. The 4:1 MeOH−CH$_2$Cl$_2$ wash contained 210 mg of material and constituted the major polymerase $\beta$ inhibitory fraction. A portion of this material (170 mg) was fractionated on an 8.5-g C$_{18}$ column, which was washed successively with 200-mL portions of 20% MeOH, 40% MeOH, 60% MeOH, 70% MeOH, 80% MeOH, 90% MeOH, MeOH, and then Me$_2$CO. The 90% MeOH fraction afforded polymerase $\beta$ inhibitory material that deposited compound **2** from MeOH as colorless needles. The MeOH fraction from the C$_{18}$ column afforded compound **3** as colorless prisms. The 70% and 80% MeOH fractions from the C$_{18}$ column were purified further on a C$_8$ column. In a typical experiment, a 2.5-g C$_8$ column was used to fractionate 50 mg of the partially purified material. Successive elution with 50-mL portions of 50% MeOH, 70% MeOH, 90% MeOH, and MeOH resulted in elution of the active principle(s) in the 90% MeOH fraction (17 mg total). This material deposited compound **1** as fine, colorless needles from Me$_2$CO. From approximately 950 mg of crude extract, the foregoing procedures afforded 22 mg of **1**, 160 mg of **2**, and 8 mg of **3**.

**Katonic Acid Methyl Ester.** To 5.0 mg (11 $\mu$mol) of **2** dissolved in 0.9 mL of 7:2 toluene−MeOH was added 16.5 $\mu$L (33 $\mu$mol, 2 M solution in hexanes) of trimethylsilyldiazomethane. The reaction mixture was stirred at room temperature for 2 h. Concentration under diminished pressure afforded a residue that was purified by flash chromatography on a 1-g Si gel column; elution with 7:3 hexanes−EtOAc afforded katonic acid methyl ester as a colorless powder: yield 4.8 mg (92%); Si gel TLC $R_f$ 0.73 (1:1 hexanes−EtOAc); ${}^1$H NMR (CDCl$_3$) $\delta$ 0.85 (6H, s), 0.94 (3H, s), 0.97 (6H, s), 1.15 (3H, s), 1.20 (3H, s), 1.30−2.20 (22H, m), 3.38−3.44 (1H, br s), 3.66 (3H, s), and 5.19−5.25 (1H, br s); FABMS $m/z$ 469.3705 [M − H]$^-$ (calcd for C$_{31}$H$_{49}$O$_3$, 4.69.3684).

**3-Oxo-olean-12-en-29-oic acid (1):[20]** fine, colorless needles from Me$_2$CO; mp 247−250 °C; ${}^1$H NMR and ${}^{13}$C NMR;[20] EIMS $m/z$ 454 (8) [M$^+$], 410 (8), 319 (2), 248 (100), and 187 (45); FABMS $m/z$ 455.3514 [M + H]$^+$ (calcd for C$_{30}$H$_{47}$O$_3$, 455.3525).

**Katonic acid (2):[20]** colorless needles from MeOH; mp 274−275 °C; ${}^1$H NMR and ${}^{13}$C NMR;[20] EIMS $m/z$ 456 (2.7) [M$^+$], 438 (0.7), 423(0.8), 248 (100), 187 (25.7), and 173(22.7); FABMS $m/z$ 456.3616 [M$^+$] (calcd for C$_{30}$H$_{48}$O$_3$, 456.3603).

**Koetjapic acid (3):[20]** colorless prisms from MeOH; mp 307−310 °C; ${}^1$H NMR and ${}^{13}$C NMR; EIMS $m/z$ 470 (20) [M$^+$], 453 (5), 436 (3), 375 (35), and 219 (100); FABMS $m/z$ 471.3461 [M + H]$^+$ (calcd for C$_{30}$H$_{47}$O$_4$, 471.3474).

**Structure Modification of 3-Oxo-olean-12-en-29-oic Acid (1) 3-Oxo-olean-12-en-29-oic Acid *N*-Butylamide (5).** To a solution of 1.7 mg (3.7 $\mu$mol) of **1** in 1 mL of CH$_2$Cl$_2$ was added 0.15 mL of oxalyl chloride. The reaction mixture was stirred at room temperature for 30 min, then concentrated under diminished pressure affording acid chloride **4**. Intermediate **4** was dissolved in 1 mL of CH$_2$Cl$_2$ and treated with 0.2 mL (2.02 mmol) of *n*-butylamine. The reaction mixture was stirred at room temperature for 30 min. Flash chromatography on a 1-g Si gel column, elution with 6:1 hexanes−Me$_2$CO, afforded **5** as a colorless powder: yield 1.2 mg (63%); Si gel TLC $R_f$ 0.45 (5:1 hexanes−Me$_2$CO); ${}^1$H NMR (CDCl$_3$) $\delta$ 0.85 (3H, s), 0.88−0.98 (6H, m), 1.02 (3H, s), 1.05 (3H, s) 1.07 (3H, s), 1.09 (3H, s), 1.15 (3H, s), 1.18 (3H, s), 1.20−2.20 (18H, m), 2.30−2.60 (4H, m), 3.20−3.40 (4H, m), and 5.22−5.27 (1H, br s); FABMS $m/z$ 510.4299 [M + H]$^+$ (calcd for C$_{34}$H$_{56}$NO$_2$, 510.4313).

**3-Oxo-olean-12-en-29-oic Acid Ethyl Ester (6).** To a solution of 1.6 mg (3.52 $\mu$mol) of **1** in 1 mL of CH$_2$Cl$_2$ was added 0.15 mL of oxalyl chloride. The reaction mixture was stirred at room temperature for 30 min and then concentrated under diminished pressure. To the residue was added 0.2 mL of anhydrous EtOH. The reaction mixture was stirred at room temperature for 30 min. Flash chromatography on a 1-g Si gel column, elution with 6:1 hexanes−Me$_2$CO, afforded **6** as a colorless powder: yield 1.2 mg (71%); Si gel TLC $R_f$ 0.45 (5:1 hexanes−Me$_2$CO); ${}^1$H NMR (CDCl$_3$) $\delta$ 0.86 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.08 (3H, s), 1.10 (3H, s), 1.15 (3H, s), 1.19 (3H, s), 1.23 (3H, t, $J$ = 7 Hz), 1.30−2.20 (19H, m), 2.30−2.70 (2H, m), 3.97−4.28 (2H, m), and 5.20−5.30 (1H, br s); FABMS $m/z$ 483.3822 [M + H]$^+$ (calcd for C$_{32}$H$_{51}$O$_3$, 483.3840).

**3-Oxo-olean-12-en-29-oic Acid *N-tert*-Butylamide (7).** To a solution of 1.8 mg (3.96 $\mu$mol) of **1** in 1 mL of CH$_2$Cl$_2$ was added 0.15 mL of oxalyl chloride. The reaction mixture was stirred at room temperature for 30 min and then concentrated under diminished pressure. The residue was then dissolved in 1 mL of CH$_2$Cl$_2$ and treated with 0.2 mL (1.9 mmol) of *tert*-butylamine. The reaction mixture was stirred at room temperature for 30 min. Flash chromatography on a 1-g Si gel column, elution with 6:1 hexanes−Me$_2$CO, afforded **7** as a colorless powder: yield 1.3 mg (64%); Si gel TLC $R_f$ 0.5 (4:1 hexanes−Me$_2$CO); ${}^1$H NMR (CDCl$_3$) $\delta$ 0.85 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.08 (3H, s), 1.10 (3H, s), 1.14 (3H, s), 1.18 (3H, s), 1.20−1.30 (4H, m), 1.32 (9H, s), 1.35−2.20 (15H, m), 2.30−2.60 (4H, s), and 5.21−5.27 (1H, br s); FABMS $m/z$ 510.4295 [M + H]$^+$ (calcd for C$_{34}$H$_{56}$NO$_2$, 510.4313).

**3-Oxo-olean-12-en-29-oic Acid *N,N*-Diethylamide (8).** To a solution of 1.5 mg (3.3 $\mu$mol) of **1** in 1 mL of CH$_2$Cl$_2$ was added 0.15 mL of oxalyl chloride. The reaction mixture was stirred at room temperature for 30 min and then concentrated

under diminished pressure. The residue was dissolved in 1 mL of $CH_2Cl_2$ and treated with 0.2 mL (1.94 mmol) of diethylamine. The reaction mixture was stirred for 30 min at room temperature. Flash chromatography on a 1-g Si gel column, elution with 6:1 hexanes−$Me_2CO$, afforded **8** as a colorless powder: yield 1.2 mg (71%); Si gel TLC $R_f$ 0.5 (4:1 hexanes−$Me_2CO$); $^1$H NMR ($CDCl_3$) δ 0.86 (3H, s), 1.01 (3H, s), 1.06 (6H, t, $J = 6$ Hz), 1.09 (3H, s), 1.11 (3H, s), 1.12 (3H, s), 1.15 (3H, s), 1.25 (3H, s), 1.30−2.30 (19H, m), 2.30−2.60 (4H, m), 3.30−3.43 (4H, q, $J = 7$ Hz), and 5.20−5.27 (1H, br s); FABMS $m/z$ 510.4305 [M + H]$^+$ (calcd for $C_{34}H_{56}NO_2$, 510.4313).

**3-Oxo-olean-12-en-29-oic Acid Amide (9).** To a solution of 1.5 mg (3.3 μmol) of **1** in 1 mL of $CH_2Cl_2$ was added 0.15 mL of oxalyl chloride. The reaction mixture was stirred at room temperature for 30 min, then concentrated under diminished pressure. The residue was dissolved in 1 mL of $CH_2Cl_2$, then treated with a stream of $NH_3$ and stirred at room temperature for 2 h. Flash chromatography on a 1-g Si gel column, elution with 6:1 hexanes−$Me_2CO$, afforded **9** as a colorless powder: yield 0.5 mg (33%); Si gel TLC $R_f$ 0.25 (3:1 hexanes−$Me_2CO$); $^1$H NMR ($CDCl_3$) δ 0.87 (3H, s), 1.02 (3H, s), 1.04 (3H, s), 1.08 (3H, s), 1.10 (3H, s), 1.16 (3H, s), 1.23 (3H, s), 1.30−2.20 (19H, m), 2.30−2.60 (4H, m), and 5.23−5.28 (1H, br s); FABMS $m/z$ 454.3682 [M + H]$^+$ (calcd for $C_{30}H_{48}NO_2$, 454.3687).

**3-Oxo-olean-12-en-29-oic Acid Methyl Ester (10).** A solution containing 2.3 mg (5 μmol) of **1**, 1.2 mg (5.8 μmol) of *N,N*-dicyclohexylcarbodiimide (DCC) and 0.1 mg of 4-(dimethylamino)pyridine in 2 mL of $CH_2Cl_2$ was treated with 0.15 mL of MeOH. The reaction mixture was stirred at room temperature for 30 h. After concentration under diminished pressure, 10 mL of $CH_2Cl_2$ was added, and the solution was washed successively with three 15-mL portions of $H_2O$, three 15-mL portions of 3% HOAc, and another three 15-mL portions of $H_2O$. The organic layer was concentrated, and the residue was purified using flash chromatography on a 1-g Si gel column; elution with 30:1 hexanes−$Me_2CO$ afforded **10** as a colorless powder: yield 0.4 mg (17%); Si gel TLC $R_f$ 0.7 (3:1 hexanes−$Me_2CO$); $^1$H NMR ($CDCl_3$) δ 0.82 (3H, s), 1.02 (3H, s), 1.06 (3H, s), 1.10 (3H, s), 1.15 (3H, s), 1.20 (3H, s), 1.30−2.20 (19H, m), 2.30−2.60 (4H, m), 3.65 (3H, s), and 5.22−5.27 (1H, br s); FABMS $m/z$ 469.3677 [M + H]$^+$ (calcd for $C_{31}H_{49}O_3$, 469.3683).

**3-Benzyloximo-olean-12-en-29-oic Acid (11).** To a solution of 1.5 mg (3.3 μmol) of **1** in 1 mL of MeOH was added a solution of 10 mg (81 μmol) of *o*-benzylhydroxylamine hydrochloride in 1 mL of MeOH. The reaction mixture was stirred at room temperature for 24 h. Concentration under diminished pressure afforded a residue that was partitioned between 10 mL of $CH_2Cl_2$ and 10 mL of $H_2O$. The $CH_2Cl_2$ layer was concentrated under diminished pressure, and the residue was purified by flash chromatography on a 1-g Si gel column; elution with 8:1 hexanes−$Me_2CO$ afforded **11** as a colorless powder: yield 1.4 mg (76%); Si gel TLC $R_f$ 0.33 (3:1 hexanes−$Me_2CO$); $^1$H NMR ($CDCl_3$) δ 0.86 (3H, s), 0.98 (3H, s), 1.01 (3H, s), 1.05 (3H, s), 1.12 (3H, s), 1.17 (3H, s), 1.24 (3H, s), 1.25−2.30 (21H, m), 2.90−3.10 (2H, dt), 5.06 (2H, s), 5.20−5.27 (1H, br s), and 7.28−7.40 (5H, m); FABMS $m/z$ 560.4094 [M + H]$^+$ (calcd for $C_{37}H_{54}NO_3$, 560.4105).

**3-Methyloximo-olean-12-en-29-oic Acid (12).** To a solution of 1.5 mg (3.3 μmol) of **1** in 1 mL of MeOH was added a solution of 10 mg (120 μmol) of methoxylamine hydrochloride in 1 mL of MeOH. The reaction mixture was stirred at room temperature for 24 h. Concentration under diminished pressure afforded a residue that was partitioned between 10 mL of $CH_2Cl_2$ and 10 mL of $H_2O$. The $CH_2Cl_2$ layer was concentrated, and the residue was purified by flash chromatography on a 1-g Si gel column; elution with 8:1 hexanes−$Me_2CO$ afforded **12** as a colorless powder: yield 1.2 mg (75%); Si gel TLC $R_f$ 0.33 (3:1 hexanes−$Me_2CO$); $^1$H NMR ($CDCl_3$) δ 0.86 (3H, s), 0.98 (3H, s), 1.04 (3H, s), 1.07 (3H, s), 1.12 (3H, s), 1.17 (3H, s), 1.24 (3H, s), 1.30−2.20 (21H, m), 2.95 (2H, m), 3.80 (3H, s), and 5.20−5.27 (1H, br s); FABMS $m/z$ 506.3591 [M + Na]$^+$ (calcd for $C_{31}H_{49}NNaO_3$, 506.3610).

**3-Oximo-olean-12-en-29-oic Acid (13).** To a solution of 2.5 mg (5.5 μmol) of **1** in 1 mL of EtOH was added a solution of 10 mg (145 μmol) of hydroxylamine hydrochloride in 3 mL of EtOH. The reaction mixture was stirred at room temperature for 24 h. Concentration afforded a residue that was washed with $CH_2Cl_2$. The filtrate was purified by flash chromatography on a 1-g Si gel column; elution with 40:1 $CH_2Cl_2$−MeOH afforded **13** as a colorless powder: yield 1.4 mg (54%); Si gel TLC $R_f$ 0.25 (30:1 $CH_2Cl_2$−MeOH); $^1$H NMR ($CDCl_3$) δ 0.86 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.10 (3H, s), 1.18 (3H, s), 1.21 (3H, s), 1.27 (3H, s), 1.28−2.20 (21H, m), 3.10−3.20 (2H, m), and 5.22−5.26 (1H, br s); FABMS $m/z$ 492.3452 [M + Na]$^+$ (calcd for $C_{30}H_{47}NNaO_3$, 492.3454).

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