Chemistry and Structure−Activity Relationship of HIV-1 Integrase Inhibitor Integracide B and Related Natural Products

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Integracides, 4,4-dimethylergostane triterpenoids, are inhibitors of HIV-1 integrase, a critical enzyme in replication of HIV-1. The chemistry and structure−activity relationship of integracide B and related natural products are described. A charged group, e.g., a sulfate, carboxyl, or amino, is required for the HIV-1 integrase activity. These compounds showed HIV-1 integrase activity with IC_{50} values in the range 4.8−15 μM and exhibited antiviral activity in a viral spread assay, but with only a small or no therapeutic window.

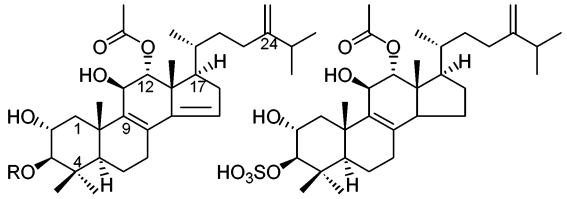
Integration is an essential step catalyzed by HIV-1 integrase, an enzyme responsible for three distinct steps including assembly of proviral DNA onto integrase, endonucleolytic cleavage of the proviral DNA, and strand transfer of the proviral DNA into the host cell DNA.[1] This distinctive retroviral process is absent in its mammalian host cells and, therefore, presents a safe target for development of anti-HIV therapy. This concept appears to have recently been validated with the discovery of some diketo acid (DKA)-based inhibitors,[2a,b] and at least two inhibitors are in clinical development.[2c]

Over the past few years we have reported a number of natural product integrase inhibitors from the screening of extracts derived from microbial, terrestrial, and marine organisms. These include equisetin,[3] integric acid,[4] complestatin,[5] integracins,[6] integrastatins,[7] integramycin,[8] chaetochromin and derivatives,[9] and integracides.[10]

Integracides are 4,4-dimethylergostane derivatives that are moderately potent and selective inhibitors of HIV-1 integrase. Integracide A (**1**), a 3-sulfate ester of integracide B (**2**), is the most active member of the five natural products reported.[10] To explore the structure−activity relationship of this class of compounds, we undertook additional studies including evaluation of related compounds from our natural products collection and selective derivatization of **2**. We describe herein the syntheses and HIV-1 integrase structure−activity relationship of these derivatives and related natural products.

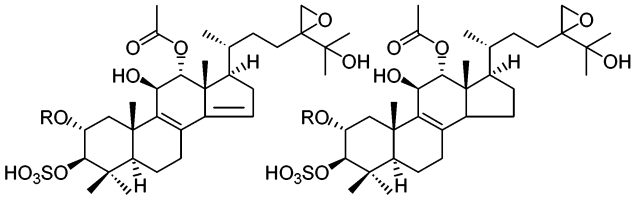
Results and Discussion

Sources of Inhibitors. (a) Natural Products and Related Compounds from Sample Collection. Compounds **3**−**6** were obtained from our natural products collection. Compounds **3**−**5** were originally isolated from a *Fusarium* sp. as inhibitors of elastase.[11] Compound **6** was discovered as an antifungal agent. Clavaric acid (**7**), its methyl ester **8**, and clavarinone (**9**) were discovered as Ras FPTase inhibitors.[12] Compounds **10**−**13** were obtained as side products of the sulfate hydrolysis of **1**, and their preparation and characterization were reported earlier.[13] The structure and purity of these compounds were verified by LCMS analysis.

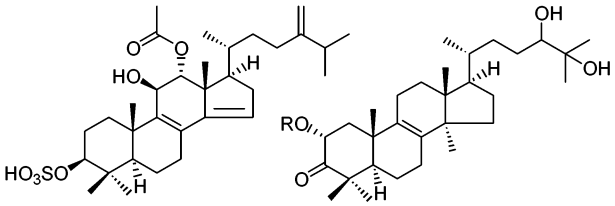


1: R = SO_3H
2: R = H

3



4: R = $CO(CH_2)_{14}CH_2OH$ **5**: R = $CO(CH_2)_{14}CH_2OH$



6

7: R = $COCH_2C(CH_3,OH)CH_2CO_2H$
8: R = $COCH_2C(CH_3,OH)CH_2CO_2Me$
9: R = H

(b) Chemical Derivatization of 2. Acetylation of **2** with acetic anhydride exclusively produced tetraacetate **14** (Scheme 1). Base hydrolysis of **2** yielded the 12-hydroxy derivative **15** in >70% yield. Reaction of **2** with 2,2-dimethoxypropane under pyridinium para-toluenesulfonate (PPTS) catalysis afforded acetonide **16** (80% yield). Reaction of **2** with methane sulfonyl chloride selectively produced the 2-mesylate, which was heated with DBU in toluene at 50 °C to give the 2,3-epoxide **17** in 70% yield. Benzoylation of **2** with benzoic anhydride at room temperature afforded a 2:2:3 mixture of 2,3-di, 3-, and 2-mono benzoates, **18**, **19**, and **20**, respectively, in 80% combined yield after silica gel chromatography. A similar reaction of **2** with MOM chloride produced three MOM ethers, **21**−**23**, in analogous ratios. Heating of **2** with succinic anhy-

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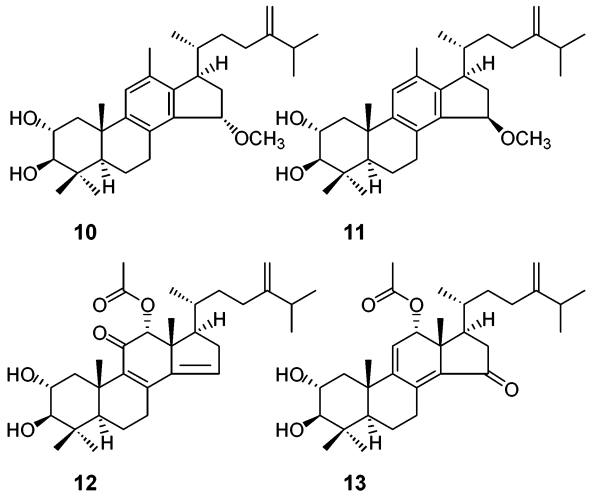
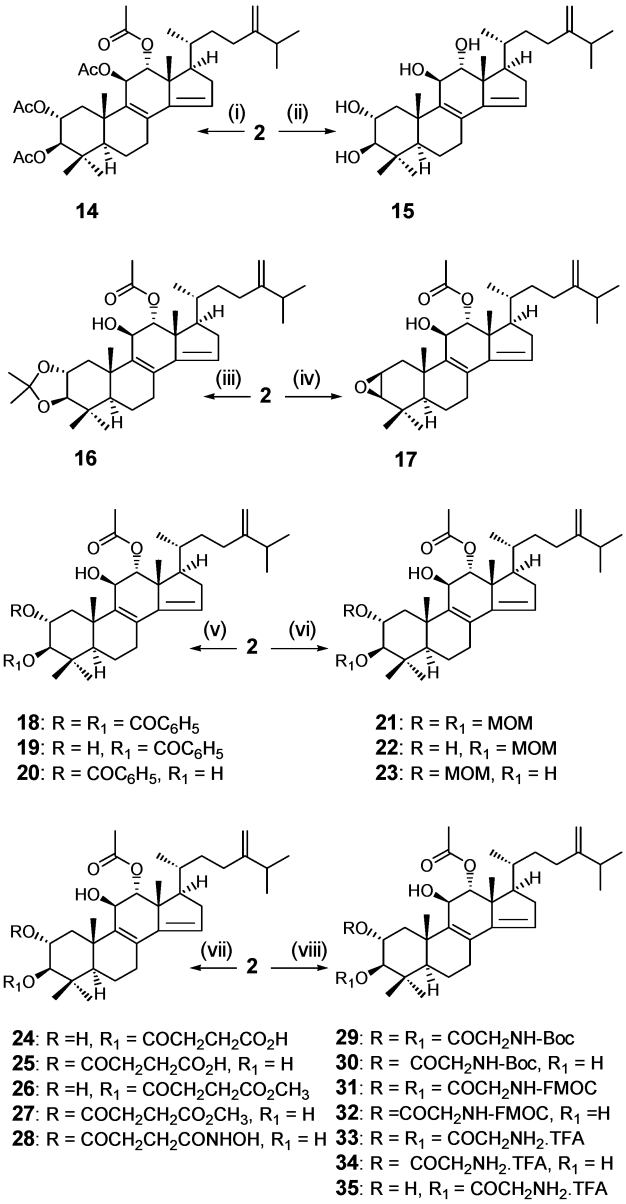


dride at 50 °C afforded a mixture of the two monohemisuccinates **24** and **25** in a ratio of 1:3. These compounds were purified by reversed-phase HPLC under acetic conditions. Methylation of these hemisuccinates with diazomethane furnished their respective methyl esters **26** and **27**. The standard mixed anhydride activation of the carboxyl group of **25** followed by reaction with hydroxylamine and reversed-phase HPLC yielded the hydroxymate **28** in a 60% yield. Acylation of **2** with N-*t*-Boc-Gly succinimide ester surprisingly afforded 2,3-diester and only the 2-monoester **29** and **30** in a 2:1 ratio. A similar product distribution [2,3-di (**31**) and 2-mono (**32**)] was also observed upon the reaction of **2** with FMOC-Gly-OPFP ester. The product distribution in the last two acylation reactions was different from the benzoylation reaction. Standard deprotection of the Boc group with TFA caused decomposition of the desired products **33** and **34**. However, deprotection of the FMOC protected esters **31** and **32** with piperidine in DMF followed by purification by reversed-phase HPLC afforded **33** and **34**, respectively. During the deprotection of the monoester **32**, about 20% of the 3-monoester **35** was also recovered as a result of acyl transfer from C-2 to C-3. A similar C-2 to C-3 acyl transfer was also observed in the hemisuccinate series wherein **24** was produced from **25** after several months of storage.

HIV-1 Integrase Structure−Activity Relationship. All compounds described in this paper were first evaluated for their ability to inhibit the coupled and strand transfer reactions of recombinant HIV-1 integrase by an assay protocol described earlier,[2a] and the data are presented in Table 1. Like integracide A (**1**), all natural products with a sulfate ester at the C-3 position (cf. **3**−**5**) were essentially equipotent and exhibited IC_{50} values of 5−6 μM in the coupled assay. The 2-deoxy compound **6** showed slightly better activity in this assay and displayed an IC_{50} value of 3.2 μM. A bulky ester group (cf. **4** and **5**) at C-2 did not have any impact on the coupled reaction inhibitory activity. While these compounds were essentially equipotent in their ability to inhibit the coupled reaction, they did show significant differences in their ability to inhibit the strand transfer reaction. The 2-deoxy compound **6** was the best inhibitor (IC_{50} = 4.8 μM) of the strand transfer reaction in this series with a 2-fold better potency than integracide A (**1**). Compound **4** was slightly less active (IC_{50} = 14 μM) than **1** in the strand transfer assay, but the dihydro analogue **5** was ~3-fold more active than **4** and was equipotent in both coupled and strand transfer assays. The dihydro analogue **3** (IC_{50} = 15 μM) was slightly less active than **1** (IC_{50} = 9 μM) in the strand transfer assay. The

Scheme 1[a]



[a] Reagents: (i) Ac_2O, C_5H_5N; (ii) LiOH, dioxane−H_2O; (iii) 2,2-dimethoxypropane, PPTS, CH_2Cl_2; (iv) (a) MsCl, DIPEA, DMAP, CH_2Cl_2; (b) DBU, toluene, 50 °C; (v) $(C_6H_5CO)_2O$, TEA, DMAP, THF; (vi) MOMCl, DIPEA, CH_2Cl_2; (vii) succinic anhydride, TEA, DMAP, CH_2Cl_2, 50 °C; (viii) N-*t*-Boc-Gly-Osu, or FMOC-Gly-OPFP, DIPEA, DMAP, THF−CH_2Cl_2, (**24** → **26** and **25** → **27**) CH_2N_2, CH_2Cl_2, (**25** → **28**) isobutyl chloroformate, CH_2Cl_2, NH_2OH.

structure−activity relationship of these compounds indicated that oxygenation (e.g., C-25 hydroxy and 23,24-epoxide in **4** and **5**) of the C-17 side chain plays no role in the activity of these compounds. Clavaric acid (**7**) was significantly less active than the sulfated compounds and showed IC_{50} values of 47 and 85 μM in the coupled and the strand transfer assays, respectively. The methyl ester **8** and clavarinone **9** were inactive in both assays. The neutral compounds **10**−**23** (i.e., the compounds without sulfate or any other charged group) were either significantly less active or inactive in both assays. The only exception was the 15-keto analogue **13**, which showed IC_{50} values of 25 and 40 μM in the coupled and the strand transfer assays, respectively.

Like the sulfated esters, compounds with either a free carboxyl group, for example, hemisuccinates **24** and **25**, or a free amino group, for example, the glycine esters **33**−**35**, showed variable levels of activities in the coupled and

Table 1. HIV-1 Integrase Activity of Integracides and Analogues[a]

compd	coupled[b] IC_{50} (μM)	ST[b,c] IC_{50} (μM)	3′-end processing[b] IC_{50} (μM)	antiviral activity[b] CIC_{95} (μM)	MTT toxicity[b,d] Hela cells (μM)	assembly[b] (IC_{50}, μM)	PIC[b] (IC_{50}, μM)
1	4 (±1)	9 (±3)	5	25	25		50
2	82	>100					
3	5 (±2)	15 (±3)	5	50	50	10	30
4	5 (±2)	14 (±3)	10	6	12		
5	5.6 (±2)	5.6 (±2)	2	>50	50	12	50
6	3.1 (±1)	4.8 (±1)					
7	47	85					
8	70	>100					
9	68	>100					
10	50	>100					
11	50	>100					
12	75	>100					
13	25	40					
14	>50	>50					
15	48	>50					
16	>50	>50					
17	>50	>50					
18	>69	>69					
19	>80	>80					
20	>80	>80					
21	>80	>80					
22	>80	>80					
23	>80	>80					
24	3 (±1)	12 (±3)	5	6	12.5		12.5
25	3 (±1)	12 (±3)		6	6		6
26	>50	>50					
27	>50	>50					
28	8	>50					
29	>50	>50					
30	>50	>50					
31	>50	>50					
32	>50	>50					
33	1.7	11.5					
34	23	>50					
35	18	>50					

[a] Blank spaces indicate that the compounds were not tested in that assay. The IC_{50} values without any error limits were obtained from the average of a duplicate or triplicate data set. [b] See ref 2a and references therein. [c] ST = strand transfer. [d] MTT.

the strand transfer assays. Both 2- and 3-hemisuccinates exhibited indistinguishable activities and each displayed IC_{50} values of 3 and 12 μM, respectively, in the coupled and the strand transfer assays. The monoamino esters **34** and **35** were both weakly active in the coupled assay and were essentially inactive in the strand transfer assay at 50 μM. In contrast, the bis-amino derivative **33** was found to be one of the most potent compounds of this series in the coupled assay and showed an IC_{50} value of 1.7 μM. This compound also showed good activity in the strand transfer assay (IC_{50} = 11.5 μM). Compounds with a protected acid group (**26** and **27**) or protected amino groups, **29**−**32**, were completely inactive in both assays. The hydroxymate **28** showed weak activity against the coupled reaction with an IC_{50} = 8 μM but was inactive in the strand transfer assay. The activity observed by the charged compounds would indicate that these compounds may potentially interact with bivalent metal (e.g., Mg^{2+}) at the active site. However, the lack of the strand transfer activity of the hydroxymate **28** contradicts this hypothesis.

All of these compounds were tested in generic DNA cleavage assays such as DNAse or ECOR1.[3b] All compounds were inactive in these assays with measured IC_{50} > 50 μM, except for the bis-amino compound **33**, which inhibited the DNAse assay with an IC_{50} value of 47 μM, and indicated that HIV-1 integrase inhibition by most of these compounds is selective.

Many of the potent compounds from this series were further evaluated for their ability to inhibit the 3′-end processing reaction of HIV-1 integrase.[2a,3b] Integracide A (**1**) and compounds **3** and **24** each inhibited this reaction with an IC_{50} value of 5 μM. The potencies of the compounds with a 2-ester and a 3-sulfate group (**4** and **5**) were 5-fold apart from each other. The dihydro compound **5** exhibited an IC_{50} of 2 μM, whereas the IC_{50} of compound **4** was 10 μM.

Compounds **3** and **5** inhibited the assembly formation[2a,3b] with IC_{50} values of 10 and 12 μM, respectively. A few of these compounds, such as **1**, **3**, and **5**, were tested for their effect against preintegration complex. These compounds exhibited IC_{50} values of 50, 30, and 50 μM, respectively, in the PIC assay.[2a,3b]

Selected compounds of the series were evaluated in a multiple cycle antiviral assay[2a] using HIV-1-infected H9 T-lymphoid cells, and their CIC_{95} values (concentration of the inhibitor required for >95% protection from viral infection) are reported in Table 1. In addition, these compounds were also tested for their cytotoxicity in cell viability MTT staining assay using H9 cells, and these data are also reported in Table 1. Many of these compounds exhibited CIC_{95} values of 6−50 μM in this antiviral viral spread assay, but unfortunately, they also showed toxicity at about the same levels and therefore provided only a small or no therapeutic window.

In summary, we have described in this paper a series of natural products and derivatives of integracide B with a range of HIV-1 integrase activities including activity against preintegration complex formation.

Experimental Section

For general experimental procedures see ref 14.

HIV-1 Integrase Coupled, Assembly, and Strand Transfer Assays. In the coupled assay, an unprocessed donor DNA and inhibitor are incubated with the HIV-1 integrase at the same time for 30 min followed by addition of target DNA. In the assembly assay, integrase and inhibitor were incubated

with preprocessed donor DNA followed by washing of unbound integrase and addition of target DNA. In the strand transfer assay, the reaction is repeated like the assembly assay with a preprocessed donor DNA except that the target DNA and inhibitor are added after washing of excess integrase. In both assays, the final product that is measured is the result of the strand transfer reaction. For coupled and strand transfer HIV-1 integrase assays see ref 3b. All reactions were performed in a 96-well plate in a final concentration of 10% DMSO. IC_{50} values were determined using a series of 2-fold dilutions in duplicate or triplicate.

3′-End Processing Assay. The assay for specific 3′-end processing of LTR donor sequence oligonucleotide substrates was performed as detailed in ref 15, except the reactions were performed in the presence of 25 nM $MnCl_2$ and 250 μM integrase and are summarized in ref 3b.

Preintegration Complex Assay. PICs were isolated from HIV-1 infected cells and partially purified, and reaction was performed as described in ref 16. The integration reaction was analyzed by Southern blotting, and reaction products were quantified by PhosphorImager analysis.

Integracide B (2). A solution of **1** (160 mg) in dioxane (16 mL) was heated at 66 °C for 5 min. After addition of sodium bicarbonate (300 mg) the reaction mixture was filtered through a bed of sodium sulfate and washed with ethyl acetate (150 mL). The combined filtrate was washed once each with 50 mL of 10% aqueous sodium bicarbonate and 50 mL of water, dried over sodium sulfate, concentrated under reduced pressure, and chromatographed over a silica gel column. Elution with 50% ethyl acetate in hexane gave 13 mg of fraction A, 27 mg of fraction B, and 72 mg (33%) of **2** as a gum. Lyophilization of **2** from acetonitrile−water gave a colorless powder: ^{1}H NMR ($CDCl_3$) δ 5.61 (1H, t, J = 2.5 Hz, H-15), 4.97 (1H, d, J = 2.0 Hz, H-12), 4.73 (1H, brs, H-28), 4.66 (1H, d, J = 1.0 Hz, H-28), 4.24 (1H, brs, H-11), 3.81 (1H, ddd, J = 11.5, 10, 4 Hz, H-2), 3.20 (1H, brs, OH), 3.05 (1H, d, J = 9.5 Hz, H-3), 2.45 (2H, m, H-16, H-7β), 2.37 (1H, dd, J = 12, 5 Hz, H-1β), 2.32 (1H, dd, J = 17.5, 7 Hz, H-7α), 2.23 (1H, heptet, J = 7 Hz, H-25), 2.10 (1H, m, H-23), 2.06 (1H, m, H-16), 2.05 (3H, s, H_3-32), 1.97 (H, dt, J = 10.5, 7.5 Hz, H-17), 1.89 (1H, m, H-23), 1.77 (1H, brdd, J = 13.5, 7.5 Hz, H-6β), 1.69 (1H, m, H-6α), 1.65 (1H, m, H-20), 1.57 (1H, m, H-22), 1.31 (3H, s, H_3-19), 1.27 (1H, t, J = 12 Hz, H-1α), 1.27 (1H, dd, J = 12.5, 3.0 Hz, H-5), 1.15 (1H, m, H-22), 1.08 (3H, s, H_3-18), 1.06 (3H, s, H_3-30), 1.03, 1.01 (6H, d, J = 7 Hz, H_3-26, H_3-27), 0.89 (3H, s, H_3-29), 0.88 (3H, d, J = 7 Hz, H_3-21); ^{13}C NMR ($CDCl_3$) δ 171.19 (C-31), 156.58 (C-24), 146.73 (C-14), 138.03 (C-9), 125.77 (C-8), 121.45 (C-15), 106.09 (C-28), 83.40 (C-3), 79.23 (C-12), 69.13 (C-2), 68.88 (C-11), 50.11 (C-5), 49.10 (C-17), 46.58 (C-13), 42.76 (C-1), 39.31 (C-4), 38.37 (C-10), 35.30 (C-16), 34.45 (C-22), 33.79 (C-25), 33.25 (C-20), 30.90 (C-23), 28.66 (C-30), 26.77 (C-7), 23.24 (C-19), 21.98, 21.85 (C-26, C-27), 21.28 (C-32), 18.19 (C-21), 18.00 (C-6), 16.74 (C-18), 16.69 (C-29); ESIMS (m/z) 1046 $[2M + NH_4]^+$, 532 $[M + NH_4]^+$, 497 $[M + H]^+$, 437 $[M + H - H_2O - AcOH]^+$, 1141 $[2M + CF_3CO_2]^-$, 627 $[M + CF_3CO_2]^-$, HREIMS m/z 454.3448 ($[M - AcOH]^+$, calcd for $C_{30}H_{46}O_3$, 454.3447), 439.3219 ($[M - AcOH - CH_3]^+$, calcd for $C_{29}H_{43}O_3$, 439.3212), 311.1990 ($[M - AcOH - H_2O$ − C-17 side chain$]^+$, calcd for $C_{21}H_{27}O_2$, 311.2010).

Tetraacetate 14. To a solution of **2** (10 mg) in pyridine (0.5 mL) was added acetic anhydride (0.3 mL), and the solution was stirred at room temperature overnight under an inert atmosphere followed by heating at 50 °C for 3 h. Methanol was added to consume excess acetic anhydride. The solvent and volatile material was removed under a stream of N_2. The product was purified by preparative TLC (SiO_2, hexane−EtOAc, 7:3). The band was eluted with EtOAc. Evaporation of EtOAc under reduced pressure afforded the tetraacetate **14** as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 5.73 (1H, brs, H-15), 5.33 (1H, brs, H-11), 5.18 (1H, brdt, J = 11.6, 4.4 Hz, H-2), 5.13 (1H, d, J = 2.0 Hz, H-12), 4.77 1H, d, J = 10 Hz, H-3), 4.74 (1H, brs, H-28), 4.68 (1H, brs, H-28), 2.50 (1H, m, H-16), 2.48 (1H, m, H-7β), 2.40 (1H, brdd, J = 18.4, 6.8 Hz, H-7α), 2.24 (1H, doublet of heptet, J = 6.4, 0.8 Hz, H-25), 2.13 (2H, m, H-23, H-16), 2.10 (3H, s, $COCH_3$), 2.09 (3H, s, $COCH_3$), 2.08 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), 1.95 (1H, dd, J = 12, 4 Hz, H-1), 1.86 (1H, m, H-23), 1.85 (1H, m, H-23), 1.82 (1H, m, H-5), 1.81 (1H, m, H-6), 1.70 (1H, m, H-6), 1.61 (1H, m, H-20), 1.56 (1H, m, H-22), 1.41 (1H, t, J = 12 Hz, H-1α), 1.38 (1H, m, H-5), 1.27 (3H, s, H_3-19), 1.16 (2H, m, H-22), 1.05 (3H, s, CH_3), 1.04, 1.03 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.99 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.89 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 658 $(M + NH_4)^+$; HREIMS (m/z) 598.3837 ($M - COCH_2$, calcd for $C_{36}H_{54}O_7$, 598.3869).

12-Desacetyl Compound (15). To a solution of **2** (10 mg) in dioxane−water (2:1, 1.5 mL) was added LiOH (13.4 mg), and the yellowish solution was stirred at room temperature overnight. EtOAc (50 mL) was added, and the solution was washed with 2 × 20 mL of water. The EtOAc layer was dried (Na_2SO_4), evaporated under reduced pressure, and chromatographed on preparative TLC (SiO_2, hexane−EtOAc, 3:7). Elution of the major band gave 2.3 mg of desacetyl compound **15** as a colorless amorphous powder: ^{1}H NMR ($CDCl_3$ + CD_3-OD, 10:1) δ 5.59 (1H, brs, H-15), 4.66 (1H, brs, H-28), 4.60 (1H, brs, H-28), 4.23 (1H, brs, H-11), 3.69 (1H, dt, J = 11.2, 4 Hz, H-2), 3.69 (1H, d, J = 1.6 Hz, H-12), 2.90 (1H, d, J = 9.2 Hz, H-3), 2.36 (2H, m, H-16, H-7β), 2.27 (1H, dd, J = 11, 6 Hz, H-1β), 2.20 (1H, m, H-7α), 2.20 (1H, m, H-17), 2.19 (1H, m, H-25), 2.07 (1H, m, H-23), 1.97 (1H, m, H-16), 1.86 (1H, m, H-23), 1.69 (1H, m, H-6β), 1.61 (1H, m, H-6α), 1.61 (1H, m, H-20), 1.51 (1H, m, H-22), 1.27 (1H, t, J = 12 Hz, H-1α), 1.23 (3H, s, H_3-19), 1.20 (2H, m, H-5, H-22), 0.97 (3H, s, H_3-18), 0.97 (3H, d, J = 6.4 Hz, H_3-21), 0.96 (3H, s, H_3-30), 0.96, 0.95 (6H, d, J = 5.6 Hz, H_3-26, H_3-27), 0.81 (3H, s, H_3-29); HREIMS (m/z) 472.3543 (M^+, calcd for $C_{30}H_{48}O_4$, 472.3552).

2,3-Acetonide (16). To a solution of **2** (10 mg) in CH_2Cl_2 (1 mL) was added 2,2-dimethoxypropane (0.1 mL) and pyridinium *p*-toluenesulfonic acid (5 mg), and the solution was stirred at room temperature for 30 min. Water (20 mL) and EtOAc (50 mL) were added and the layers were separated. The organic layer was sequentially washed with 20 mL each of 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and water. The EtOAc extract was dried (Na_2SO_4), concentrated under reduced pressure, and chromatographed over preparative TLC (SiO_2, hexane−EtOAc, 7:3). Elution of the band with EtOAc and evaporation of the solvent gave acetonide **16** (4.2 mg) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 5.65 (1H, brs, H-15), 5.01 (1H, d, J = 2.0 Hz, H-12), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.6 Hz, H-28),4.28 (1H, brd, $J_{H,OH}$ = 4.8 HZ, H-11), 3.85 (1H, ddd, J = 12.8, 9.6, 3.6 Hz, H-2), 3.11 (1H, d, J = 9.6 Hz, H-3), 2.54 (1H, dd, J = 10.8, 3.2 Hz, H-1), 2.49 (1H, m, H-7), 2.47 (1H, m, H-16), 2.37 (1H, brdd, J = 18, 7.2 Hz, H-7α), 2.25 (1H, heptet, J = 6.8 Hz, H-25), 2.09 (1H, m, H-16), 2.08 (3H, s, $COCH_3$), 2.06 (1H, m, H-23), 1.95 (1H, m, H-17), 1.92 (1H,m, H-23), 1.91 (1H, d, J = 5.2 Hz, OH), 1.83 (1H, m, H-6), 1.71 (1H, m, H-6), 1.65 (1H, m, H-20), 1.57 (1H, m, H-22), 1.47, 1.45 (3H each, s, 2 × CH_3), 1.45 (1H, t, J = 13.2 Hz, H-1α), 1.36 (3H, s, CH_3), 1.29 (1H, dd, J = 12.8, 2.4 Hz, H-5), 1.20 (1H, m, H-22), 1.10 (6H, s, 2 × CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.98 (3H, s, CH_3), 0.91 (3H, d, J = 6.4 Hz, H_3-21); HREIMS (m/z) 554.3947 (M^+, calcd for $C_{35}H_{54}O_5$, 554.3971).

2,3-Epoxide (17). To a cooled (−40 °C) solution of **2** (42 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) was added diisopropylethylamine (33 μL), (dimethylamino)pyridine (5 mg), and methane sulfonyl chloride (14 μL). The reaction mixture was stirred for 20 min and was allowed to warm to room temperature and quenched by addition of ice. EtOAc (50 mL) was added, and the layers were separated. The organic layer was sequentially washed with 20 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and finally water, dried (Na_2SO_4), and evaporated under reduced pressure to give clean 2-mesylate (40 mg) as a foam: ^{1}H NMR ($CDCl_3$) δ only distinct signals are listed, 5.64 (1H, brs, H-15), 5.00 (1H, d, J = 0.8 Hz, H-12), 4.81 (1H, ddd, J = 11.6, 9.6, 4.0 Hz, H-2), 4.75 (1H, brs, H-28), 4.68 (1H, d, J = 0.2 Hz, H-28), 4.19 (1H, brs, H-11), 3.27 (1H, d, J = 9.6 Hz, H-3), 3.16 (3H, brs, $SOCH_3$), 2.11 (3H, s, $COCH_3$), 1.30 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.96 (3H, s, CH_3), 0.90 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 610 (M +

$NH_4)^+$. The mesylate (12 mg) in 1 mL of toluene and 50 μL of DBU was heated at 50 °C for 30 min. Ice followed by EtOAc (50 mL) was added to the reaction after it was cooled to room temperature. The EtOAc layer was sequentially washed with 20 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and finally water, dried (Na_2SO_4), evaporated under reduced pressure, and chromatographed over preparative TLC (SiO_2, hexane−EtOAc, 7:3). The band was eluted with EtOAc to give 2,3-epoxide **17** (4 mg) as an amorphous powder: ^{1}H NMR ($CDCl_3$) δ 5.62 (1H, t, J = 2 Hz, H-15), 5.01 (1H, d, J = 2.4 Hz, H-12), 4.73 (1H, brs, H-28), 4.67 (1H, d, J = 2 Hz, H-28), 4.31 (1H,brd, $J_{H,OH}$ = 6.4 HZ, H-11), 3.34 (1H, dt, J = 4, 2 Hz, H-2), 2.86 (1H, d, J = 4.4 Hz, H-3), 2.51 (1H, dd, J = 14.4, 2 Hz, H-1), 2.45 (1H, ddd, J = 16, 6.8, 3.2 Hz, H-16), 2.32 (2H, m, H-7), 2.23 (1H, heptet, J = 7.2 Hz, H-25), 2.10 (1H, m, H-16), 2.07 (1H, m, H-23), 2.06 (3H, s, $COCH_3$), 1.99 (1H, m, H-17), 1.89 (1H, m, H-23), 1.79 (1H, d, J = 5.6 Hz, OH), 1.65 (1H, m, H-20), 1.61 (2H, m, H-6), 1.60, 1.54 (2H, m, H-22), 1.48 (1H, d, J = 14 Hz, H-1α), 1.39 (3H, s, CH_3), 1.16 (1H, m, H-22), 1.084 (6H, s, 2 × CH_3), 1.078 (3H, s, CH_3), 1.03, 1.02 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.89 (3H, d, J = 6.4 Hz, H_3-21); ESIMS m/z 514 $(M + NH_4)^+$; HREIMS (m/z) 436.3344 ([M − AcOH]$^+$, calcd for $C_{30}H_{44}O_2$, 436.3341).

Benzoates (18−20). Triethylamine (20 μL), DMAP (5 mg), and benzoic anhydride (17.2 mg, 0.076 mmol) were added to a stirred solution of **2** (10 mg, 0.019 mmol) in anhydrous THF (1 mL). The reaction mixture was stirred overnight under nitrogen. Ice (5 g) was added to quench the reaction, and the mixture was diluted with EtOAc (50 mL). The organic layer was separated and sequentially washed with 20 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and water and dried (Na_2SO_4), and EtOAc was evaporated under reduced pressure. The mixture was chromatographed by preparative TLC (SiO_2, hexane−EtOAc, 7:3) to give **18** (3.4 mg), **19** (3.4 mg), and **20** (4.6 mg), all as colorless amorphous powders. **18:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 7.99 (2H, dd, J = 7.6, 1.6 Hz, ArH), 7.92 (2H, dd, J = 8.4, 1.2 Hz, ArH), 7.47 (2H, m, ArH), 7.37 (2H, t, J = 8 Hz, ArH), 7.35 (2H, t, J = 7.6 Hz, ArH), 5.68 (1H, brs, H-15), 5.62 (1H, dt, J = 11.6, 4.4 Hz, H-2), 5.26 (1H, d, J = 10.4 Hz, H-3), 4.99 (1H, d, J = 2.0 Hz, H-12), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.0 Hz, H-28), 4.23 (1H, bd, $J_{H,OH}$ = 5.0 Hz, H-11), 2.10 (3H, s, H_3-32), 1.83 (1H, d, $J_{H,OH}$ = 5.6 Hz, 11-OH), 1.52 (3H, s, CH_3), 1.20 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.06, 1.05 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.92 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 1462 (85%, $2M + NH_4)^+$, 740 (100%, $M + NH_4)^+$, 705 (35%, $M - H_2O + H)^+$. **19:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 8.12 (2H, d, J = 7.2, ArH), 7.61 (1H, t, J = 7.6 Hz, ArH), 7.49 (2H, t, J = 8 Hz, ArH), 7.35 (2H, t, J = 7.6 Hz, ArH), 5.67 (1H, brs, H-15), 5.02 (1H, brs, H-12), 4.84 (1H, d, J = 10 Hz, H-3), 4.76 (1H, brs, H-28), 4.70 (1H, d, J = 1.0 Hz, H-28), 4.32 (1H, brs, H-11), 4.11 (1H, dt, J = 10, 3.6 Hz, H-2), 2.10 (3H, s, H_3-32), 1.39 (3H, s, CH_3), 1.12 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.06, 1.05 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 1.01 (3H, s, CH_3), 0.92 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 1254 (10%, $2M + NH_4)^+$, 636 (100%, $M + NH_4)^+$, 601 (30%, $M - H_2O + H)^+$. **20:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 8.10 (2H, dd, J = 7.6, 1.6 Hz, ArH), 7.60 (1H, t, J = 7.6 Hz, ArH), 7.48 (2H, t, J = 8 Hz, ArH), 7.35 (2H, t, J = 7.6 Hz, ArH), 5.67 (1H, brs, H-15), 5.36 (1H, dt, J = 11.2, 4.4 Hz, H-2), 4.99 (1H, brs, H-12), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.0 Hz, H-28), 4.23 (1H, brs, H-11), 3.41 (1H, d, J = 10 Hz, H-3), 2.09 (3H, s, H_3-32), 1.43 (3H, s, CH_3), 1.14 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 1.02 (3H, s, CH_3), 0.91 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 1254 (10%, $2M + NH_4)^+$, 636 (100%, $M + NH_4)^+$, 601 (30%, $M - H_2O + H)^+$.

Methoxymethyl Ethers (21−23). To a cold (0 °C) solution of **2** (10 mg, 0.019 mmol) in 1 mL of CH_2Cl_2 were added diisopropylethylamine (15.5 μL, 0.114 mmol) and methoxymethyl chloride (7.4 μL, 0.095 mmol). The solution was stirred at 0 °C for 2 h followed by stirring at room temperature overnight. The reaction mixture was quenched with ice, and 50 mL of EtOAc was added. The organic layer was washed sequentially with 50 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and then water and dried (Na_2SO_4). The solvent was removed under reduced pressure, and products were purified by preparative TLC (SiO_2) using hexane−EtOAc (1:1). The three bands were eluted with EtOAc to give bis-MOM ether **21** (1.5 mg), 3-MOM ether **22** (3.4 mg), and 2-MOM ether **23** (2.9 mg) as amorphous powders. **21:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.65 (1H, brs, H-15), 5.01 (1H, d, J = 2.0 Hz, H-12), 4.95 (1H, d, J = 6.4 Hz, OCH_2O), 4.77 (2H, brs, OCH_2O), 4.76 (1H, d, J = 6.4 Hz, OCH_2O), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.0 Hz, H-28), 4.26 (1H, bd, $J_{H,OH}$ = 5.6 Hz, H-11), 3.86 (1H, ddd, J = 12, 10, 4 Hz, H-2), 3.46 (3H, s, OCH_3), 3.43 (3H, s, OCH_3), 3.06 (1H, d, J = 10 Hz, H-3), 2.07 (3H, s, H_3-32), 1.77 (1H, d, $J_{H,OH}$ = 5.6 Hz, 11-OH), 1.32 (3H, s, H_3-19), 1.10 (3H, s, H_3-18), 1.08 (3H, s, H_3-30), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.94 (3H, s, H_3-29), 0.92 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 620 (100%, $M + NH_4)^+$, 603 (5%, $M + H)^+$, 585 (30%, $M - H_2O + H)^+$. **22:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.64 (1H, t, J = 2.0 Hz, H-15), 5.02 (1H, d, J = 1.2 Hz, H-12), 4.87 (1H, d, J = 6.4 Hz, OCH_2O), 4.75 (1H, brs, H-28), 4.69 (1H, brs, H-28), 4.66 (1H, d, J = 6.4 Hz, OCH_2O), 4.31 (1H, bd, $J_{H,OH}$ = 4.4 Hz, H-11), 3.84 (1H, ddd, J = 12.8, 9.6, 4 Hz, H-2), 3.49 (3H, s, OCH_3), 2.85 (1H, dd, $J_{2,3}$ = 9.6 Hz, $J_{H,OH}$ = 2 Hz, H-3), 2.05 (3H, s, H_3-32), 1.95 (1H, d, $J_{H,OH}$ = 5.2 Hz, 11-OH), 1.33 (3H, s, H_3-19), 1.10 (3H, s, H_3-18), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 1.02 (3H, s, H_3-30), 0.93 (3H, s, H_3-29), 0.92 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z): 576 (60%, $M + NH_4)^+$, 541 (90%, $M - H_2O + H)^+$, 481 (100%, $M + H - H_2O - AcOH)^+$. **23:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.65 (1H, t, J = 2.0 Hz, H-15), 5.01 (1H, d, J = 1.2 Hz, H-12), 4.83 (1H, d, J = 6.8 Hz, OCH_2O), 4.76 (1H, d, J = 6.8 Hz, OCH_2O), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.2 Hz, H-28), 4.27 (1H, bd, $J_{H,OH}$ = 5.2 Hz, H-11), 3.68 (1H, ddd, J = 13.6, 9.6, 4 Hz, H-2), 3.47 (3H, s, OCH_3), 3.31 (1H, d, $J_{H,OH}$ = 2 Hz, 3-OH), 3.12 (1H, dd, $J_{2,3}$ = 10 Hz, $J_{H,OH}$ = 2 Hz, H-3), 2.07 (3H, s, H_3-32), 1.80 (1H, d, $J_{H,OH}$ = 6.0 Hz, 11-OH), 1.31 (3H, s, H_3-19), 1.12 (3H, s, H_3-18), 1.10 (3H, s, H_3-30), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.93 (3H, s, H_3-29), 0.91 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 576 (100%, $M + NH_4)^+$, 541 (40%, $M - H_2O + H)^+$.

Hemisuccinates (24, 25). To a THF (2 mL) solution of **2** (10 mg, 0.019 mmol) was added triethylamine (60 μL), DMAP (5 mg), and succinic anhydride (22 mg). The mixture was stirred at room temperature overnight and heated at 50 °C for 2 h. The reaction mixture was allowed to cool and then ice followed by EtOAc (50 mL) was added. The layers were separated, and the organic layer was sequentially washed with 2 × 20 mL each of water, 10% aqueous citric acid, and water and then dried (Na_2SO_4). EtOAc was removed under reduced pressure. Chromatography of the mixture on reversed-phase HPLC (Zorbax RX C-8, 22 × 250 mm, gradient of 60% to 75% CH_3CN in H_2O, both containing 0.05% TFA, flow rate 8 mL/min) followed by lyophilization gave **24** (2 mg) and **25** (6 mg) as colorless amorphous powders. **24:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.64 (1H, brs, H-15), 5.04 (1H, brs, H-12), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.0 Hz, H-28), 4.65 (1H, d, J = 9.6 Hz, H-3), 4.23 (1H, brs, H-11), 4.00 (1H, m, H-2), 2.73 (4H, m, 2 x CH_2CO), 2.07 (3H, s, H_3-32), 1.34 (3H, s, CH_3), 1.11 (3H, s, CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.97 (3H, s, CH_3), 0.93 (3H, s, CH_3), 0.91 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 1246 (10%, $2M + NH_4)^+$, 632 (100%, $M + NH_4)^+$, 597 (35%, $M - H_2O + H)^+$. **25:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.65 (1H, brs, H-15), 5.15 (1H, dt, J = 11.6, 4.4 Hz, H-2), 4.98 (1H, brs, H-12), 4.76 (1H, brs, H-28), 4.69 (1H, d, J = 1.0 Hz, H-28), 4.22 (1H, brs, H-11), 3.29 (1H, d, J = 10 Hz, H-3), 2.76 (2H, m, 2 × CH_2CO), 2.68 (2H, m, 2 × CH_2CO), 2.10 (3H, s, H_3-32), 1.36 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.95 (3H, s, CH_3), 0.91 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 1246 (10%, $2M + NH_4)^+$, 632 (100%, $M + NH_4)^+$, 597 (35%, $M - H_2O + H)^+$.

Hemisuccinate Methyl Esters (26, 27). A mixture of 16 mg of hemisuccinates, described in the previous experiment,

was dissolved in 0.1 mL of CH_2Cl_2 and cooled to 0 °C. An ethereal solution of freshly prepared diazomethane was added, and the solution was kept at 0 °C overnight. Volatile material was evaporated under a stream of nitrogen, and the methyl esters were purified by preparative TLC (SiO_2, hexane−EtOAc, 3:1). Elution of the bands with EtOAc gave monomethyl esters **26** (3 mg) and **27** (12 mg) as amorphous powders. **26:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.63 (1H, t, J = 2.8 Hz, H-15), 4.98 (1H, d, J = 1.6 Hz, H-12), 4.73 (1H, brs, H-28), 4.67 (1H, d, J = 1.6 Hz, H-28), 4.62 (1H, d, J = 9.6 Hz, H-3), 4.27 (1H, brs, H-11), 3.95 (1H, ddd, J = 11.6, 10, 4.4 Hz, H-2), 3.70 (3H, s, OCH_3), 2.68 (4H, m, 2 × CH_2CO), 2.05 (3H, s, H_3-32), 1.32 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.03, 1.02 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.94 (3H, s, CH_3), 0.92 (3H, s, CH_3), 0.89 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 629 (50%, M + H)$^+$; HREIMS (m/z) 628.4009 (calcd for $C_{37}H_{56}O_8$, 428.3975). **27:** ^{1}H NMR ($CDCl_3$) (only distinct signals are presented) δ 5.63 (1H, t, J = 2.8 Hz, H-15), 5.15 (1H, ddd, 11.6, 10, 4.4 Hz, H-2), 4.97 (1H, d, J = 1.6 Hz, H-12), 4.73 (1H, brs, H-28), 4.67 (1H, d, J = 1.0 Hz, H-28), 4.18 (1H, brs, H-11), 3.70 (3H, s, OCH_3), 3.23 (1H, d, 10 Hz, H-3), 2.68 (4H, m, 2 × CH_2CO), 2.07 (3H, s, H_3-32), 1.34 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.07 (3H, s, CH_3), 1.03, 1.02 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.94 (3H, s, CH_3), 0.89 (3H, d, J = 6.4 Hz, H_3-21); ESIMS (m/z) 629 (100%, M + H)$^+$; HREIMS (m/z) 628.3978 (calcd for $C_{37}H_{56}O_8$, 428.3975).

Succinate Hydroximate (28). To a cooled (−40 °C) solution of **25** (10 mg, 0.016 mmol) in THF (0.5 mL) was added *N*-methylmorpholine (15 μL) followed by allyl chloroformate (10 μL). The reaction mixture was allowed to warm to room temperature. After stirring for 30 min under nitrogen it was recooled at −23 °C and an aqueous solution of hydroxylamine was added via a syringe. The mixture was stirred at 0 °C for 30 min and then quenched with ice and diluted with EtOAc (50 mL). The ethyl acetate layer was sequentially washed with 20 mL each of 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and water. The EtOAc extract was dried (Na_2SO_4), concentrated under reduced pressure, and chomatographed by reversed-phase HPLC (Zorbax RX C-8, 22 × 250 mm, 20 to 80% CH_3CN in H_2O (+0.1% TFA) gradient in 40 min, at 8 mL/min). The fractions containing the product were lyophilized to give hydroxymate **28** (4 mg) as an amorphous powder. **28:** ^{1}H NMR ($CDCl_3$) δ (only distinct signals are listed, spectrum was very broad) 5.64 (1H, brs, H-15), 5.14 (1H, m, H-2), 4.98 (1H, brs, H-12), 4.75 (1H, brs, H-28), 4.69 (1H, brs, H-28), 4.19 (1H, brs, H-11), 3.30 (4H, m, 2 x CH_2), 2.75 (1H, H-3), 2.23 (1H, heptet, J = 7.2 Hz, H-25), 2.09 (3H, s, $COCH_3$), 1.35 (3H, s, CH_3), 1.09 (6H, s, 2 × CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.94 (3H, s, CH_3), 0.90 (3H, d, J = 6 Hz, H_3-21); ESIMS m/z 630 (M + H)$^+$.

***t*-Boc-Glycine Esters (29, 30).** To an anhydrous solution of **2** (40 mg, 0.078 mmol) in a 2:1 mixture of CH_2Cl_2−THF (1.5 mL) was added N-t-Boc-glycine-succinimide ester (103 mg, 0.39 mmol) followed by diisopropylethylamine (64 μL) and (dimethylamino)pyridine (5 mg). The homogeneous mixture was stirred overninght under nitrogen followed by heating at 50 °C for 2 h. The reaction mixture was quenched by addition of ice and was diluted with EtOAc (50 mL). The organic layer was sequentially washed with 20 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and finally water, dried (Na_2SO_4), evaporated under reduced pressure, and chromatographed over a preparative TLC column (SiO_2, hexane−EtOAc, 7:3). The two bands were eluted with EtOAc to give diester **29** (15.9 mg) and monoester **30** (7.6 mg) both as amorphous powders. **29:** ^{1}H NMR ($CDCl_3$) δ (only distinct signals are listed) 5.66 (1H, brs, H-15), 5.48 (1H, brt, J = 6 Hz, NH), 5.37 (1H, dt, J = 11.6, 3.6 Hz, H-2), 5.30 (1H, t, J = 6 Hz, NH), 4.99 (1H, d, J = 0.8 Hz, H-12), 4.81 (1H, d, J = 10 Hz, H-3), 4.75 (1H, brs, H-28), 4.69 (1H, d, J = 1.2 Hz, H-28), 4.20 (1H, brd, $J_{H,OH}$ = 4.8 Hz, H-11), 3.93 (2H, m, gly-CH_2), 3.83 (2H, m, gly-CH_2), 2.26 (1H, heptet, J = 7.2 Hz, H-25), 2.10 (3H, s, $COCH_3$), 1.94 (1H, d, J = 5.2 Hz, OH), 1.48, 1.46 (9H each, s, $C(CH_3)_3$), 1.38 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.05, 1.04 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 1.00 (3H, s, CH_3), 0.95 (3H, s, CH_3), 0.92 (3H, d, J = 6.8 Hz, H_3-21); ESIMS m/z 846 (M + NH_4)$^+$. **30:** ^{1}H NMR ($CDCl_3$) δ (only distinct signals are listed) 5.66 (1H, brs, H-15), 5.18 (1H, dt, J = 11.6, 4 Hz, H-2), 5.10 (1H, t, J = 6 Hz, NH), 4.99 (1H, d, J = 0.8 Hz, H-12), 4.76 (1H, brs, H-28), 4.69 (1H, d, J = 1.6 Hz, H-28), 4.19 (1H, brd, $J_{H,OH}$ = 5.2 Hz, H-11), 3.97 (1H, dd, J = 17.6, 5.0 Hz, gly-CH), 3.92 (1H, dd, J = 17.6, 5.6 Hz, gly-CH), 3.22 (1H, d, J = 7.6 Hz, H-3), 2.26 (1H, heptet, J = 7.2 Hz, H-25), 2.10 (3H, s, $COCH_3$), 1.93 (1H, d, J = 5.2 Hz, OH), 1.48 (9H, s, $C(CH_3)_3$), 1.37 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.09 (3H, s, CH_3), 1.06, 1.05 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.96 (3H, s, CH_3), 0.91 (3H, d, J = 6.8 Hz, H_3-21); ESIMS m/z 689 (M + NH_4)$^+$.

Fmoc-Glycine Esters (31, 32). Diisopropylethylamine (128 μL, 0.7 mmol) and (dimethylamino)pyridine (10 mg) followed by Fmoc-glycine-pentafluorophenyl ester were added to a solution of **2** (80 mg, 0.14 mmol) in a 2:3 mixture of CH_2Cl_2−THF (5 mL). The solution was stirred overnight under nitrogen. Water followed by EtOAc (50 mL) was added after completion of the reaction, and the layers were separated. The organic layer was sequentially washed with 2 × 20 mL each of water, 10% aqueous citric acid, water, 10% aqueous $NaHCO_3$, and finally water, dried (Na_2SO_4), evaporated under reduced pressure, and chromatographed over preparative TLC (SiO_2, hexane−EtOAc, 7:3). The two bands were eluted with EtOAc to give diester **31** (23.9 mg) and monoester **32** (19.9 mg) both as amorphous pale powders. **31:** ^{1}H NMR ($CDCl_3$) δ (only distinct signals are listed) 7.76 (4H, d, J = 7.5 Hz, ArH), 7.60 (4H, t, J = 8 Hz, ArH), 7.38 (4H, t, J = 7.5 Hz, ArH), 7.26 (4H, m, ArH), 5.85 (1H, t, J = 6 Hz, NH), 5.72 (1H, t, J = 6.5 Hz, NH), 5.67 (1H, t, J = 2.5 Hz, H-15), 5.37 (1H, ddd, J = 12, 10.5, 4.5 Hz, H-2), 4.99 (1H, d, J = 1.5 Hz, H-12), 4.82 (1H, d, J = 10.5 Hz, H-3), 4.74 (1H, brs, H-28), 4.69 (1H, d, J = 1.5 Hz, H-28), 4.40−4.30 (4H,m, 2 × CH_2O−), 4.24−4.16 (3H, m, Fmoc-CH, H-11), 3.90 (4H, m, 2 × gly-CH_2), 2.25 (1H, doublet of heptet, J = 7, 1 Hz, H-25), 2.04 (3H, s, $COCH_3$), 1.37 (3H, s, CH_3), 1.08 (3H, s, CH_3), 1.04, 1.03 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.98 (3H, s, CH_3), 0.94 (3H, s, CH_3), 0.91 (3H, d, J = 6.8 Hz, H_3-21); HRFABMS m/z 1095.5364 (calcd for $C_{66}H_{76}N_2O_{11}Na$, 1095.5347). **32:** ^{1}H NMR ($CDCl_3$) δ (only distinct signals are listed) 7.81 (2H, d, J = 7.5 Hz, ArH), 7.65 (2H,m, ArH), 7.43 (2H, brt, J = 8 Hz, ArH), 7.36 (2H, m, ArH), 5.67 (1H, t, J = 2.5 Hz, H-15), 5.51(1H, t, J = 6.0 Hz, NH), 5.14 (1H, ddd, J = 14, 11.5, 4.0 Hz, H-2), 5.00 (1H, d, J = 1.0 Hz, H-12), 4.76 (1H, d, J = 1.5 Hz, H-28), 4.70 (1H, q, J = 1.5 Hz, H-28), 4.45 (2H, d, J = 7.5 Hz, Fmoc-CH_2), 4.28 (1H, t, J = 7.5 Hz, Fmoc-CH), 4.00 (2H, m, gly-CH_2), 3.19 (1H, d, J = 10 Hz, H-3), 2.27 (1H, doublet of heptet, J = 7, 1 Hz, H-25), 2.05 (3H, s, $COCH_3$), 1.37 (3H, s, CH_3), 1.10 (3H, s, CH_3), 1.06 (3H, s, CH_3), 1.06, 1.05 (6H, d, J = 6.8 Hz, H_3-26, H_3-27), 0.94 (3H, s, CH_3), 0.92 (3H, d, J = 6.5 Hz, H_3-21); HRFABMS m/z 816.4488 (calcd for $C_{49}H_{63}NO_8Na$, 816.4452).

Glycine Ester (33). Piperidine (20 μL) was added to a solution of **31** (12 mg) in dimethylformamide (200 μL), and the solution was stirred at room temperature for 5 min. Volatile material was removed under a stream of nitrogen and chromatographed over a reversed-phase HPLC column (Zorbax RX C-8, 22 × 250 mm, gradient of 20 to 70% aqueous CH_3CN containing 0.1% TFA, flow rate 8 mL/min). The product eluted from 50 to 58 min. The combined fractions were directly lyophilized to yield a colorless powder of the trifluoroacetate salt of **33** (4 mg): ^{1}H NMR (CD_3CN−$CDCl_3$, 1:1) δ (only distinct signals are listed) 5.37 (1H, t, J = 2 Hz, H-15), 5.05 (1H, dt, J = 12, 4.5 Hz, H-2), 4.77 (1H, d, J = 0.8 Hz, H-12), 4.63 (1H, d, J = 10 Hz, H-3), 4.48 (1H, brs, H-28), 4.43(1H, d, J = 1.2 Hz, H-28), 3.86 (1H, brs, H-11), 3.65 (4H, m, 2 × gly-CH_2), 2.01 (1H, heptet, J = 7.2 Hz, H-25), 1.81 (3H, s, $COCH_3$), 1.10 (3H, s, CH_3), 0.81 (3H, s, CH_3), 0.79, 0.78 (6H, d, J = 7 Hz, H_3-26, H_3-27), 0.75 (3H, s, CH_3), 0.71 (3H, s, CH_3), 0.65 (3H, d, J = 6.5 Hz, H_3-21); ESIMS m/z 629 (M + H)$^+$; HREIMS (m/z) 628.4012 (calcd for $C_{36}H_{56}N_2O_7$: 628.4087).

Glycine Esters (34, 35). **32** (8 mg) was reacted in DMF (100 μL) with piperidine (20 μL). The products were chromatographed and lyophilized in a manner similar to the procedure described above to give 0.8 mg of the trifluoroacetate salt of **35** as an amorphous powder: ^{1}H NMR (CD_3CN−$CDCl_3$, 1:1) δ (only distinct signals are listed) 5.35 (1H, brs, H-15), 4.76

(1H, brs, H-12), 4.48 (1H, brs, H-28), 4.43 (1H, brs, H-28), 4.40 (1H, d, J = 9.5 Hz, H-3), 3.97 (1H, brs, H-11), 3.66 (3H, m, H-2, gly-CH_2), 2.01 (1H, heptet, J = 7.2 Hz, H-25), 1.76 (3H, s, $COCH_3$), 1.06 (3H, s, CH_3), 1.03 (3H, s, CH_3), 0.82 (3H, s, CH_3), 0.79, 0.78 (6H, d, J = 7 Hz, H_3-26, H_3-27), 0.70 (3H, s, CH_3), 0.66 (3H, d, J = 6.5 Hz, H_3-21); ESIMS m/z 572 $(M + H)^+$.

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