Anti-AIDS Agents 69. Moronic Acid and Other Triterpene Derivatives as Novel Potent Anti-HIV Agents

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In a continuing structure−activity relationship study of potent anti-HIV agents, seven new triterpene derivatives were designed, synthesized, and evaluated for in vitro antiviral activity. Among them, moronic acid derivatives **19**, **20**, and **21** showed significant activity in HIV-1 infected H9 lymphocytes. Compounds **19** and **20** were also evaluated against HIV-1 NL4-3 and drug resistant strains in the MT-4 cell line. Compounds **19** and **20** showed better antiviral profiles than the betulinic acid analogue **8** (PA-457), which has successfully completed a Phase IIa clinical trial. Compound **20** showed potent anti-HIV activity with EC_{50} values of 0.0085 μM against NL4-3, 0.021 μM against PI-R (a multiple protease inhibitor resistant strain), and 0.13 μM against FHR-2 (an HIV strain resistant to **8**). Promising compound **20** has become a new lead for modification, and further development of **20**-related compounds as clinical trial candidates is warranted.

Introduction[1]

Acquired immunodeficiency syndrome (AIDS) remains an exceptional crisis beccause of both its emergent and long-term development. The epidemic remains extremely dynamic, growing and changing character as the human immunodeficiency virus (HIV) exploits new opportunities for transmission. No region of the world has been spared, and the AIDS pandemic continues to outpace the global response to HIV treatment.[2] Although current anti-AIDS drugs include inhibitors of reverse transcriptase (RT), protease, and fusion,[3] the increasing prevalence of drug resistant HIV strains is one of the major problems for the treatment of HIV infection, driving the demand for the development of novel drugs with new mechanisms of action.

Triterpenes, including betulinic acid (**1**), constitute a promising class of anti-HIV agents. Two types of anti-HIV **1** derivatives have exhibited potent anti-HIV profiles. In our prior studies, we found that 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid (**8**) inhibits HIV-1 maturation by interfering with HIV-1 P24/P25 processing, which results in a noninfectious HIV-1 particle.[4−7] The second type of anti-HIV **1** analogues contain various C-28 amide modifications. IC9564 (**9**), a statine analogue of **1**, represents this compound type and acts at an early stage of viral infection.[8,9]

Compound **8**, designated as **PA-457** by Panacos Pharmaceuticals, Inc.,[10] is a maturation inhibitor directed against a novel viral target. Panacos, Inc. recently concluded a successful Phase IIa clinical trial with **8**.[10] Because **8** has a different target than that of approved HIV drugs, it retains activity against virus isolates resistant to currently available treatments, including RT and protease inhibitors.

In the current study, we synthesized a series of new triterpene derivatives with 3-, 28-, and both substitutions on different triterpene skeletons, including betulinic acid (BA[a]), glycyrrhetinic acid (GLA), moronic acid (MA), oleanolic acid (OA), and ursolic acid (UA). This article reports their design, synthesis, and SAR.

Design. From previous SAR studies of **8**-type analogues, the most potent derivatives contain either 3′,3′-dimethylsuccinyl or 3′,3′-dimethylglutaryl moieties at C-3.[8,9,11] Thus, these side chains were also used in the current study.

Our first aim was to investigate the SAR of the triterpene core. To optimize this core and gain more information about target binding requirements, we designed and synthesized three substituted derivatives of triterpenes other than **1**. Moreover, by replacing the betulin core with other triterpenes, we could potentially lower cytotoxicity, improve pharmacological profiles, and obtain more SAR information. Lupeol (**2**) has the same skeleton as **1** but lacks the C-28 carboxylic acid. OA (**3**) and UA (**4**) have a six-membered E ring rather than the five-membered E ring found in **1**. MA (**5**) and **3** differ at the double bond position. GLA (**7**) has a carboxylic acid at C-30 rather than at C-28. Activity data with these latter compounds will provide information on the contribution and positional requirement of the carboxylic acid on antiviral activity. Previously synthesized compounds **12**−**15** were also included in the current study.[12]

Among many C-28 amide BA derivatives, a relatively long C-28 amide side chain is a common structural feature that is essential for potent anti-HIV entry activity.[8] These results suggest that BA derivatives may interfere at more than one stage of HIV infection, depending on the side chains on C-3 and C-28. Two compounds (**10** and **11**) with C-3 ester and C-28 amide side chains exhibited both anti-fusion and maturation activity.[13] These observations prompted us to hypothesize that the betulin moiety serves as a molecular scaffold or docker, the C-28 amide side chain is the pharmacophore for anti-HIV entry activity, and the C-3 ester group has an important role in target interaction during HIV-1 maturation. On the basis of this hypothesis, a compound with both DMS on C-3 and a proper amide on C-28 will probably exhibit dual mechanisms of action, both anti-fusion

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[a] Abbreviations: BA, betulinic acid; MA, moronic acid: OA, oleanolic acid; UA, ursolic acid; GLA, glycyrrhetinic acid.

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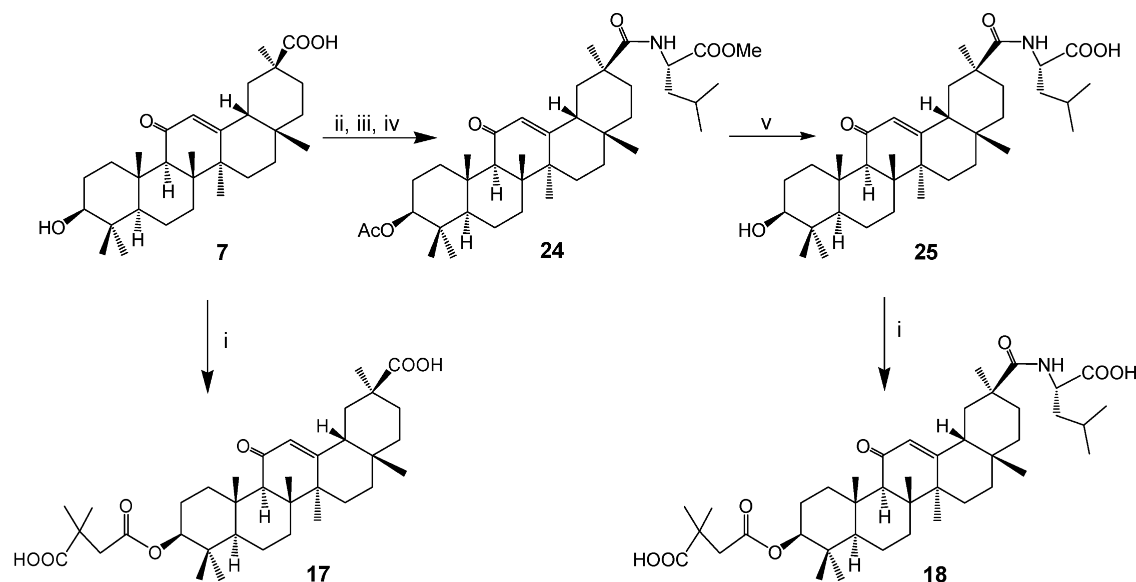
Scheme 1[a]



[a] Conditions: (i) 2,2-dimethylsuccinyl anhydride, DMAP, pyridine, reflux 12 h; (ii) Ac_2O, py, rt; (iii) oxalyl chloride, CH_2Cl_2, rt; (iv) leucine methyl ester, Et_3N, CH_2Cl_2, rt; (v) 2 N KOH, THF-MeOH (1:2), rt.

and anti-maturation, to increase potency and decrease HIV-resistance profiles.

Our previous article reported that **5**, which is isolated from Brazilian propolis, a herbal medicine widely used worldwide today, exhibited significant anti-HIV activity (EC_{50} <0.1 μg/mL; TI >186) in H9 lymphocytes.[14] In addition, the observed toxicity of MA derivatives is generally lower than that of BA derivatives. This finding implies that changing the betulin core to **5** may alter the cytotoxicity profile without impairing anti-HIV potency. In our continuing development of novel triterpene anti-HIV agents, compound **5** was dually functionalized at C-3 and C-28 on the basis of the SAR of BA derivatives.

The β-amyrin series triterpene glycyrrhizin or glycyrrhizic acid (**6**) is used in Japanese clinics by intravenous administration for the treatment of chronic viral hepatitis B. In some clinical studies in Japan, the administration of **6** to AIDS patients resulted in delayed progression of HIV infection symptoms.[15] Therefore, we also modified **7**, the aglycone of **6**, with both ester and amide side chains.

Chemistry. Compounds **2** and **7** were reacted separately with 2,2-dimethylsuccinic anhydride to yield target compounds **16** and **17**, respectively. Compound **23**, the acetate of **7**, was then treated with oxalyl chloride and, without isolation, further reacted with leucine methyl ester hydrochloride to afford **24** in a yield of 95% over two steps. Compound **24** was hydrolyzed under basic conditions to **25** in a 95% yield. The 3β-hydroxy group of **25** was reacted with 2,2-dimethylsuccinic anhydride to yield final target product **18** in a 65% yield (Scheme 1).

The methanol extract (600 g) of propolis, collected by Africanized *Apis mellifera* in southern Brazil, gave **5** in good yield (1.5 g, 0.25%).[14] Scheme 2 shows the synthetic steps to preparing moronic acid derivatives **19**−**22**. Using the same method used for compound **24**, compounds **26** and **27** were synthesized from **5**. To reduce the C-3 keto group, sodium borohydride ($NaBH_4$) was added to a solution of **26** or **27** in MeOH and THF to produce the corresponding reduction products **28** and **29** in yields of 33% and 85%, respectively. At the same time, **26** was also reduced to **32** in a 60% yield. Compounds **28** and **29** were hydrolyzed to **30** and **31** and yielded final target products **20**−**22** in 30−40% yield. Compound **32** reacted with 2,2-dimethylsuccinyl anhydride to yield crude disuccinated compound **33**. Without isolation, **33** was treated with 2 N KOH in MeOH and THF at 0 °C to afford target compound **19** in 70% yield (Scheme 2).

Results and Discussion

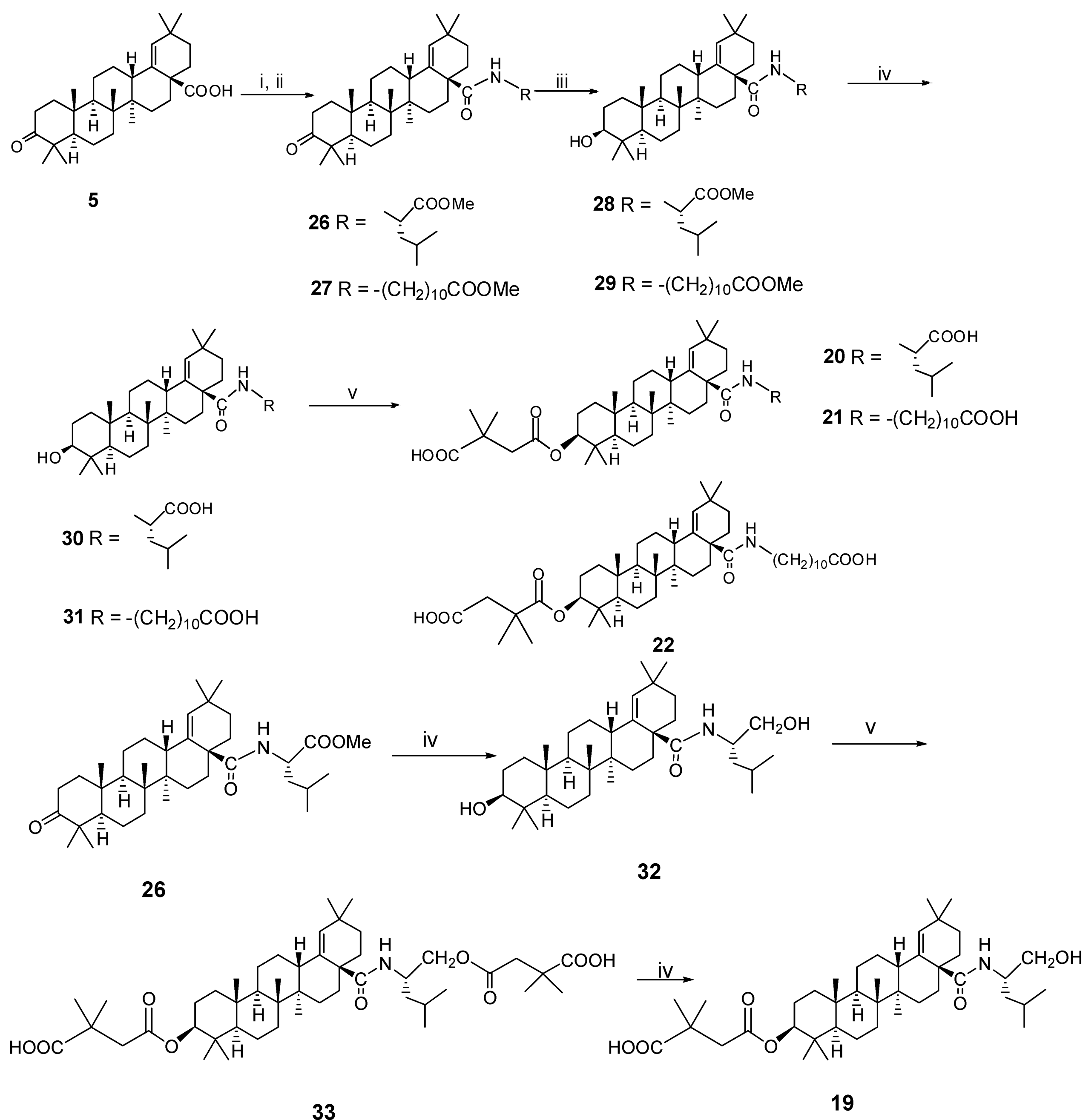
In our current study, newly synthesized triterpene derivatives **16**−**22** were evaluated for anti-HIV-1 replication activity against two different viral strains in two different cell lines in parallel with **8** and other previously synthesized compounds **10**−**15**. Two MA derivatives **19** and **20** were also evaluated for anti-HIV-1 replication activity against three different viral strains in MT-4 cells in parallel with **8** and compound **10**, which has the same C-3 and C-28 substituents as **20** but with a BA core. The results are summarized in Tables 1 and 2, respectively.

First, our new results confirm prior reports that a 3′,3′-dimethylsuccinyl side chain is superior to 3′,3′-dimethylglutaryl at the C-3 position. Accordingly, OA derivative **13** with the latter substituent was inactive, whereas OA analogue **12** with the former substituent had an EC_{50} value of 0.32 μM. Similarly, among comparable UA analogues, **15** was 10-fold weaker than **14**. (Due to changes in the screening system after 2000, our prior publications reported different activities for **12**−**15** in H9 lymphocytes.[12,16])

Compounds **16** and **8** have identical structures, except at C-28, which is a methyl group in the former and a carboxylic acid in the latter compound. Compound **16** showed dramatically decreased activity in both HIV-1 infected H9 lymphocytes and MT-4 cells with EC_{50} values of 2.6 and 21.6 μM, respectively. OA analogue **12** and UA analogue **14**, which also have a carboxylic acid at C-28, were 10−50-fold more potent than **16** in the H9 lymphocyte assay; however, they were still less active than **8**. GLA analogue **17**, which has a C-28 methyl and a C-30 carboxylic acid, was inactive in both assays. These results indicate that the carboxylic acid at C-28 might be required for high anti-HIV activity in this compound type.

From the 3D structures of **1**, 3β-hydroxymoronic acid, and **7** shown in Figure 4, the C-28 carboxylic acid is oriented in the same direction in **1** and 3β-hydroxymoronic acid, even though they have different E rings. However, the C-30 carboxylic acid of **7** is oriented in a different direction. Accordingly, a

Scheme 2[a]



[a] Conditions: (i) oxalyl chloride, CH_2Cl_2, rt; (ii) amino acid methyl esters, Et_3N, CH_2Cl_2, rt; (iii) $NaBH_4$, MeOH/THF, rt; (iv) 2 N KOH, THF-MeOH (1:2), rt; (v) 2,2-dimethylsuccinyl anhydride, DMAP, pyridine, reflux 12 hr.

substituent on this position of **7** will create a totally different molecular shape compared to that of analogous BA and MA derivatives. Therefore, like **17**, disubstituted derivative **18** was inactive in both assays.

Similar to disubstituted BA derivatives (**10** and **11**), disubstituted MA derivatives (**19**−**21**) also exhibited high potency. Compound **21** with 3-(3′,3′-dimethylsuccinyl) and 28-undecanoic amide substituents exhibited the highest potency (EC_{50} = 0.007 μM; TI = 3400, in H9 lymphocytes), with results similar to those of **8** in the same assay. Compounds **19** and **20** also demonstrated high potencies with EC_{50} values of 0.017 and 0.016 μM, respectively. Compound **22**, the 3-(2′,2′-dimethylsuccinyl) isomer of **21**, almost lost anti-HIV activity.

These compounds were also tested in an HIV-1 replication assay using MT-4 cells infected with the NL4-3 strain, which is a T-cell adapted X4 wild-type HIV-1 virus. Most compounds exhibited weaker activity in this system. Interestingly, unlike in H9 lymphocytes, compound **20**, which showed the best activity (EC_{50} = 0.0085 μM) in MT-4 cells, was more potent than **8** in this assay. Compound **19** also exhibited slightly better activity than **8** with an EC_{50} value of 0.045 μM. However, the most active compound in H9 lymphocytes, **21**, showed an EC_{50} value of only 0.11 μM. The discrepancy might result from differences in the assay protocols, especially the viral infecting dose.

Because **19** and **20** were active in both assays, these two compounds, together with **10**, were also screened against other viral strains. The data are shown in Table 2. PI-R is HIV-1 M46I/L63P/V82T/I84V, an HIV-1 strain resistant to multiple protease inhibitors,[17] and FHR-2 is an HIV-1 strain resistant to **8**.[7] Both **19** and **20** showed better activity (EC_{50} = 0.088 and 0.021 μM) than **8** against the multi-PI resistant strain but were

Table 1. Anti-HIV Activities of Triterpene Derivatives Against HIV-1 Strains in H9 Lymphocytes and MT-4 Cells

	HIV-1$_{IIIB}$ in H9 lymphocytes			NL4−3 in MT-4 cells		
compd	IC$_{50}$ (μM)	EC$_{50}$ (μM)	TI	IC$_{50}$ (μM)	EC$_{50}$ (μM)	TI
8	>42.8	0.007	>6250	>5[a]	0.096	>52
10		NT		12.2	0.010	>1220
11		NT		11	0.007	1570
12	>42.7	0.32	>133	20.2	8.3	2.4
13		NS			NS	
14	28.5	0.079	360	21	8.3	2.5
15	28.5	0.68	42		NS	
16	>45.1	2.6	>17.2	>36.1	21.6	>1.7
17		NS			NS	
18		NS			NS	
19	>42.7	0.017	2500	23.5	0.045	522
20	>42.7	0.0156	2735	22.5	0.0085	2650
21	24.0	0.007	3400	19.5	0.11	177.3
22	>32.6	9.3	>3.5		NS	

[a] The highest concentration tested for **8** was 5 μM; there was no observable cytotoxicity at this concentration. NT, not tested in this assay; NS, no suppression.

Table 2. Comparison of Anti-HIV Activities of Selected Derivatives Against Several HIV-1 Strains in MT-4 Cells[a]

	EC$_{50}$ (μM) in different viral strains			
compd	NL4-3	PI-R	FHR-2	IC$_{50}$ (μM)
8	0.096	0.43	NS	>5[b]
10	0.010	0.006	0.05	12.2
19	0.045	0.088	2.78	23.5
20	0.0085	0.021	0.13	21.0
AZT	0.013	0.019	0.019	>37.5

[a] NL4-3 is a T-cell adapted HIV-1 strain X4 wild-type virus; PI-R is an HIV-1 strain HIV-1 M46I/L63P/V82T/I84V, resistant to multiple protease inhibitors;[17] and FHR-2 is an HIV-1 strain resistant to **8**.[7] EC$_{50}$ is the concentration that inhibits HIV-1 replication by 50%, and IC$_{50}$ is the concentration that decreases 50% of the viable cell number. [b] The highest concentration tested for DSB was 5 μM; there was no observable cytotoxicity at this concentration. NS, no suppression at testing concentration, 5 μM.

Table 3. Inhibition of HIV-1 Envelope-Mediated Membrane Fusion[a]

compd	EC$_{50}$ (μM)	IC$_{50}$ (μM)
8	>17.1	>17.1
9	0.0086	>13.2
19	5.85	>14.6
20	3.58	>14.3
21	5.21	>13.0

[a] EC$_{50}$ is the drug concentration that inhibits HIV envelope-mediated membrane fusion by 50%; IC$_{50}$ values (cytotoxicity) for both cell types were determined in a one-day assay because the fusion assay is also a one-day assay. The highest concentration tested: 10 μg/mL.

less active than **10** (EC$_{50}$ = 0.006 μM). As for the FHR-2 HIV-1 strain, which is resistant to **8**, compound **20** retained some potency with an EC$_{50}$ of 0.13 μM but was less active than **10** (EC$_{50}$ = 0.05 μM), whereas **19** showed only weak activity (EC$_{50}$ = 2.78 μM). With a reduced carboxylic acid in the 28-side chain, **19** showed less activity than **20** against all tested viral strains; thus, a carboxylic acid within the leucine side chain is more favorable for target interaction. Our results indicate that MA derivatives **19**, **20**, and **21** exhibited weak anti-HIV fusion activity (Table 3). BA derivatives **10** and **11** also exhibited anti-fusion activity in a previous study.[13] The anti-fusion activity of these compounds are weaker than that of **9** (EC$_{50}$ 0.0086 μM). These data suggest that the disubstituted compounds **10**, **11**, **19**, **20**, and **21** can block HIV-1 entry.

The key structural feature of disubstituted compounds **10**, **11**, **19**, **20**, and **21** is the presence of both side chains at positions 3 and 28. This feature enables our target compounds to be active against viruses that are resistant to **8**, which has a side chain only at C-3. Therefore, this modification study indicated that the BA scaffold can be replaced by an analogous triterpene, MA, without a loss of activity. Disubstituted MA derivatives with side chains on both C-3 and C-28 are likely to have dual functions and lower cytotoxicity as well as better drug resistance profiles than their parent compound **8**. However, the detailed molecular mechanisms of action of these new disubstituted MA derivatives are yet to be determined. Their mechanisms of action might be different from that of **8**. Additional modification and SAR studies are in progress with an aim to continually improve potency, particularly against drug-resistant viral strains. Further development of compounds related **20** as next generation clinical trial candidates is warranted.

Experimental Section

Chemistry. Melting points were measured with a Fisher-Johns melting apparatus without correction. The proton nuclear magnetic resonance (^{1}H NMR) spectra were measured on 300 MHz Varian Gemini 2000 and 500 MHz Varian Inova spectrometers using TMS as internal standard. The solvent used was $CDCl_3$ unless indicated. Mass spectra were obtained on an Agilent 1100 MSD ion trap mass spectrometer or a PE-SCIEX API-3000 with a turbo ion spray source. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. All target compounds were analyzed for C, H, and N and gave values within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was performed on PTLC silica gel 60 F_{254} plates (0.5 mm, Merck). Teledyn-Isco companion systems were used as medium-pressure column chromatography. Compound **7** was provided by Tokiwa Pharmaceuticals, Inc. All other chemicals were obtained from Aldrich, Inc.

3*β*-*O*-(3′,3′-Dimethylsuccinyl)-lupeol (16). Compound **2** (21.3 mg, 0.05 mmol) was heated with 2,2-dimethylsuccinic anhydride (25.6 mg, 0.2 mmol) and 4-dimethylamino pyridine (DMAP, 12.2 mg, 0.1 mmol) in pyridine (2 mL) to reflux overnight. After the addition of EtOAc (50 mL), the mixture was worked up with 2 N HCl and H_2O. The evaporation of EtOAc gave a mixture of the starting material and succinate, which was separated by SiO_2 column chromatography (CH_2Cl_2/MeOH 100:2). Yield 63%; white amorphous powder; mp 193−194 °C; MS (ESI−) *m/z*: 553.5 (M^- − 1) for $C_{36}H_{58}O_4$. ^{1}H NMR (500 MHz) δ 0.79, 0.81, 0.83, 0.84, 0.94, 1.03 (3H each, s, CH_3-23, 24, 25, 26, 27 and 28), 1.29, 1.31 (3H each, s, CH_3-3′×2), 1.68 (3H, s, CH_3-30), 1.91 (2H, m, H-2), 2.37 (1H, m, H-19), 2.57, 2.67 (1H each, d, J = 15.5 Hz, H-2′), 4.49 (1H, dd, J = 11.0, 5.5 Hz, H-3), 5.30 (1H, s, COOH-4′), 4.68, 4.56 (1H each, d, J = 2 Hz, H-29). Anal. ($C_{36}H_{58}O_4$), C 77.77, H 10.50.

3*β*-*O*-(3′,3′-Dimethylsuccinyl)-glycyrrhetinic Acid (17). Following the procedure described for **16**, compound **17** was obtained from **7** as a white amorphous powder (235 mg, 0.5 mmol) in a yield of 60%; mp >300 °C; MS (ESI−) *m/z*: 597.4 (M^- − 1) for $C_{36}H_{54}O_7$. ^{1}H NMR (500 MHz, CD_3OD) δ 0.83, 0.89, 0.90, 0.92, 1.14, 1.16 (3H each, s, CH_3-23, 24, 25, 26, 27 and 28), 1.25, 1.26 (3H each, s, CH_3-3′×2), 1.43 (3H, s, CH_3-29), 2.28 (1H, m, H-18), 2.51 (2H, m, H-7), 4.48 (1H, m, H-3), 5.58 (1H, s, H-12). Anal. ($C_{36}H_{54}NO_7$), C 72.30, H 8.90.

3*β*-*O*-Acetylglycyrrhetinic Acid (23). Compound **7** (935 mg, 2 mmol) was reacted with Ac_2O (0.5 mL) in pyridine (5 mL) and dichloromethane (10 mL) at room temperature for 1 day. The mixture was washed with 20% HCl and H_2O. The evaporation of CH_2Cl_2 followed by silica gel column chromatography (CH_2Cl_2/MeOH, 100:2) gave **23**. Yield 54%; white powder; mp 175−177 °C; MS (ESI−) *m/z*: 511.5 (M^- − 1) for $C_{32}H_{48}O_5$. ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.76, 0.83, 1.04, 1.07, 1.10, 1.15 (3H each, s, CH_3-23, 24, 25, 26, 27 and 28), 1.37 (3H, s, CH_3-29), 2.0 (3H, s,

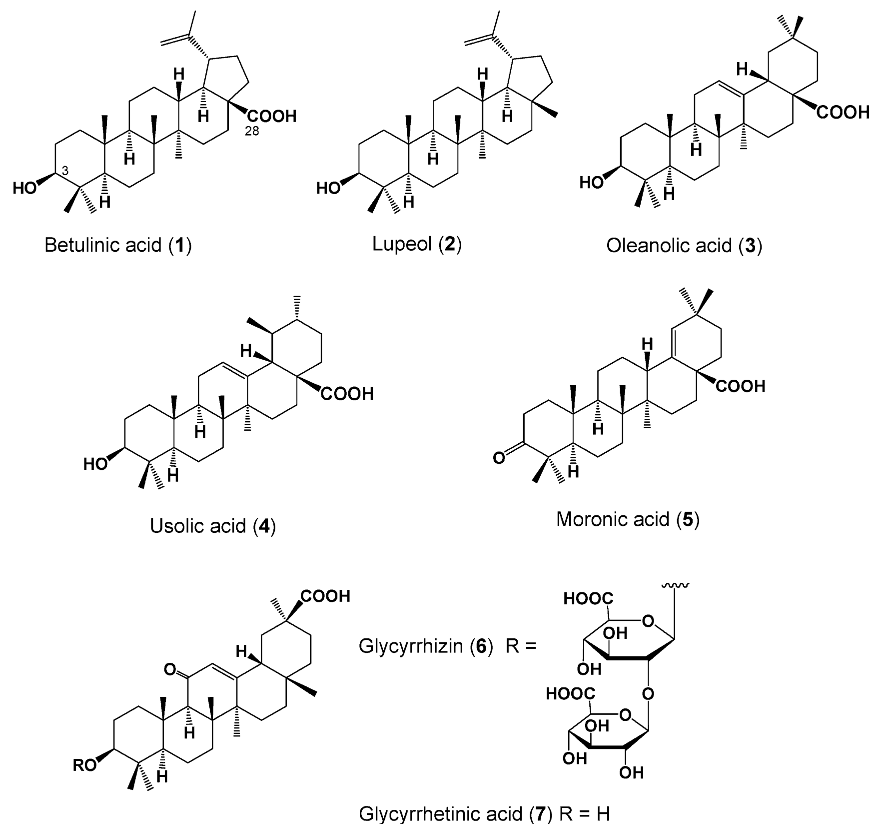


Figure 1. Related triterpene natural products.

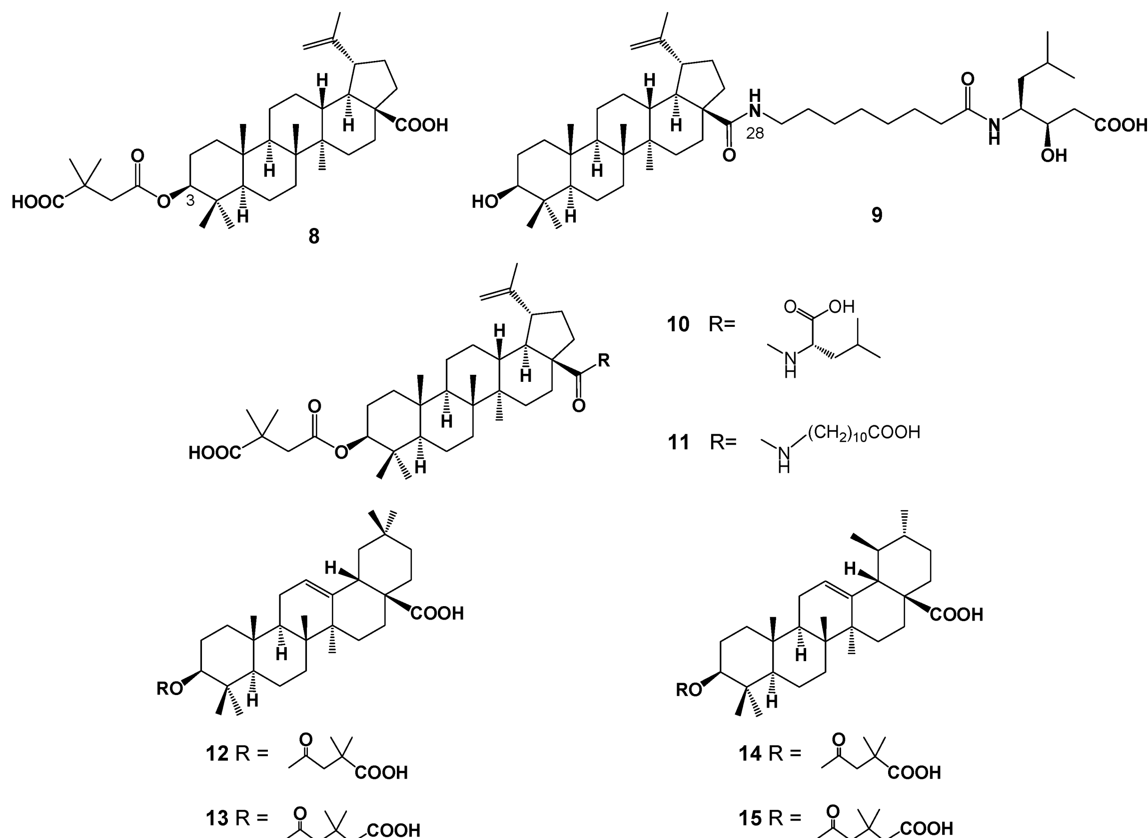


Figure 2. Previously modified anti-HIV triterpene derivatives.

CH_3CO-3), 2.07 (1H, m, H-18), 2.63 (1H, m, H-7), 4.42 (1H, dd, $J = 12.0, 4.5$ Hz, H-3), 5.41 (1H, s, H-12), 12.18 (1H, COOH-30).

***N*-(Olean-3*β*-*O*-acetyl-11-oxo-12-en-30-oyl)-L-leucine Methyl Ester (24).** Following the procedure described for **26**, compound **24** was obtained in 99% yield from compound **23**; white amorphous powder; mp 103−105 °C; MS (ESI+) *m/z*: 662.7 ($M^+ + Na$), 640.7 ($M^+ + 1$) for $C_{39}H_{61}NO_6$. ^1H NMR (500 MHz) δ 0.81, 0.88, 1.14, 1.15, 1.17, 1.38 (3H each, s, CH_3-23, 24, 25, 26, 27 and 28), 0.94, 0.95 (3H each, d, $J = 6.5$ Hz, -CH(CH_3)$_2$-30 side chain), 1.57 (3H, s, CH_3-29), 2.05 (3H, s, CH_3CO-3), 2.28 (1H, m, H-18),

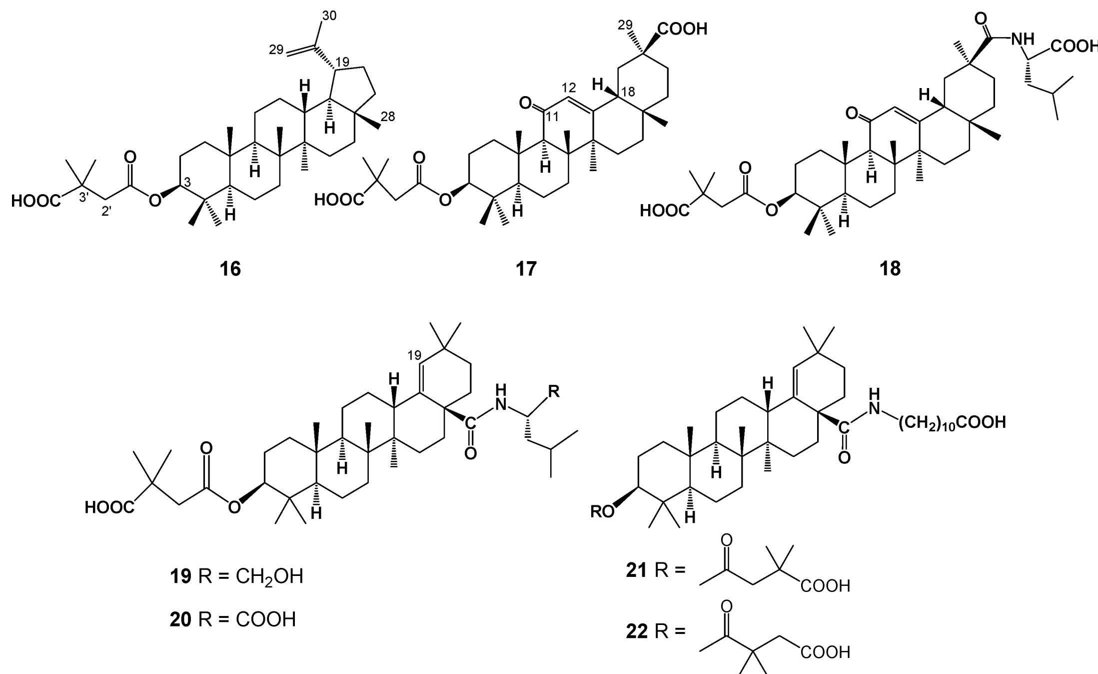


Figure 3. Newly synthesized triterpene derivatives.

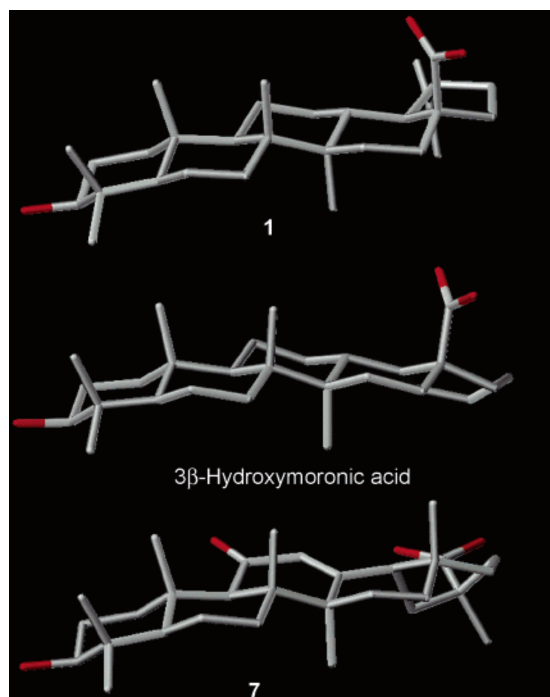


Figure 4. 3D structures of **1**, 3β-hydroxymoronic acid, and **7**.

2.81 (1H, m, H-7), 3.75 (3H, s, COO*CH*$_3$), 4.52 (1H, dd, $J = 12.0$, 5.2 Hz, H-3), 4.67 (1H, m, -NHC*H*-), 5.77 (1H, s, H-12), 5.93 (1H, d, $J = 8.5$ Hz, -CO*NH*-).

***N*-(Olean-3β-hydroxy-11-oxo-12-en-30-oyl)-L-leucine (25).** Following the procedure described for **30**, compound **25** was obtained in 98% yield from **24**; white amorphous powder; mp 184−186 °C; MS (ESI−) *m*/*z*: 582.7 ($M^- - 1$) for $C_{36}H_{57}NO_5$. ^{1}H NMR (500 MHz, CD_3OD) δ 0.79, 0.80, 0.96, 1.13, 1.14, 1.25 (3H each, s, CH$_3$-23, 24, 25, 26, 27 and 28), 0.93, 0.95 (3H each, d, $J = 6.0$ Hz, -CH(*CH*$_3$)$_2$-30 side chain), 1.42 (3H, s, *CH*$_3$-29), 3.16 (1H, dd, $J = 12.0$, 5.0 Hz, H-3), 4.52 (1H, m, -NHC*H*-), 5.74 (1H, s, H-12).

***N*-[3β-*O*-(3′,3′-Dimethylsuccinyl)-olean-11-oxo-12-en-30-oyl]-L-leucine (18).** Yield 65% from **25**; white amorphous powder; mp 145−147 °C; MS (ESI+) *m*/*z*: 734.5 ($M^+ + Na$) for $C_{42}H_{65}NO_8$. ^{1}H NMR (300 MHz) δ 0.78, 0.84, 0.86, 1.08, 1.12, 1.14 (3H each, s, CH$_3$-23, 24, 25, 26, 27, and 28), 0.91, 0.93 (3H each, d, $J = 7.2$ Hz, -CH(*CH*$_3$)$_2$-30 side chain), 1.29 (6H, s, 2 × CH$_3$-3′), 1.30 (3H, s, *CH*$_3$-29), 4.51 (1H, m, H-3), 4.69 (1H, m, -NHC*H*-), 5.68 (1H, s, H-12), 6.49 (1H, br.s, -CO*NH*-). Anal. ($C_{42}H_{65}NO_8$), C 71.14, H 9.18, N 2.22.

***N*-(Olean-3-oxo-18-en-28-oyl)-leucine Methyl Ester (26).** MA (**5**, 454 mg, 1 mmol) was dissolved in CH_2Cl_2 (5 mL) and ice-cooled. To this solution, oxalyl chloride (20 mL of 2.0 M solution in CH_2Cl_2) was added dropwise with stirring. The mixture was stirred at room temperature for 2 h, then CH_2Cl_2 was evaporated. To the residue, CH_2Cl_2 (5 mL, ×3) was added and evaporated to give a yellow solid. This residue was dissolved in CH_2Cl_2 (5 mL), and a CH_2Cl_2 solution (10 mL) of leucine methyl ester hydrochloride (543 mg, 3 mmol) and Et_3N (303 mg, 3 mmol) was added. The reaction mixture was left overnight at room temperature. After washing with water three times, CH_2Cl_2 was removed to give a viscous oil, which was then subjected to silica gel column chromatography (CH_2Cl_2/MeOH, 100:1) to give **26**. 88% yield; mp 86−88 °C; MS (ESI+) *m*/*z*: 582.6 ($M^+ + 1$) for $C_{37}H_{59}NO_4$. ^{1}H NMR (300 MHz) δ 0.78, 0.96, 0.99, 1.01, 1.01, 1.03, 1.08 (3H each, s, CH$_3$-23, 24, 25, 26, 27, 29 and 30), 0.93, 0.94 (3H each, d, $J = 6$ Hz, -CH(*CH*$_3$)$_2$-28 side chain), 3.73 (3H, s, COO*CH*$_3$), 4.61 (1H, m, H-3), 5.37 (1H, s, H-19), 6.13 (1H, d, $J = 7.8$ Hz, -CO*NH*-).

***N*-(Olean-3-oxo-18-en-28-oyl)-aminoundecanoic Acid Methyl Ester (27).** Following the procedure described for **26**, compound **27** was obtained in 51% yield; white amorphous powder; mp 90−92 °C; MS (ESI+) *m*/*z*: 652.9 ($M^+ + 1$) for $C_{42}H_{69}NO_4$. ^{1}H NMR (500 MHz) δ 0.77, 0.95, 0.98, 0.98, 1.01, 1.03, 1.08 (3H each, s, CH$_3$-23, 24, 25, 26, 27, 29 and 30), 3.30 (2H, m, −NHC*H*$_2$-), 3.67 (3H, s, COO*CH*$_3$), 5.32 (1H, s, H-19), 5.82 (1H, t, $J = 5.8$ Hz, -CO*NH*-).

***N*-(Olean-3β-hydroxy-18-en-28-oyl)-leucine Methyl Ester (28).** Compound **26** (920 mg, 1.58 mmol) was dissolved in a mixture of MeOH and THF (50 mL, 3 mL) and stirred in an ice bath. To the solution, solid $NaBH_4$ (597 mg, 15.8 mmol) was slowly added, and the reaction mixture was kept overnight at room temperature. Water and CH_2Cl_2 (50 mL, 100 mL) were added to the warmed reaction mixture, and insoluble solid was filtered off. The aqueous and organic layers were separated, and evaporation of the organic solvent gave an oily residue, which was subjected to silica gel

column chromatography (CH_2Cl_2/MeOH, 200:1). Compounds **32** (300 mg, 60%) and **28** (560 mg, 33%) were isolated and crystallized from a mixture of CH_2Cl_2 and MeOH; mp 145−146 °C; MS (ESI−) *m/z*: 582.7 ($M^- - 1$) for $C_{37}H_{61}NO_4$; ^{1}H NMR (300 MHz) δ 0.76, 0.76, 0.87, 0.97, 0.98, 0.99, 1.00 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 0.93, 0.94 (3H each, d, J = 6.3 Hz, -CH(CH_3)$_2$-28 side chain), 3.20 (1H, dd, J = 10.6, 5.6 Hz, H-3), 3.72 (3H, s, -COOCH_3-28 side chain), 4.61 (1H, td, J = 8.4, 4.5 Hz, -*CH*(CH_3)$_2$-28 side chain), 5.36 (1H, s, H-19), 6.12 (1H, d, J = 8.4 Hz, -CO*NH*-).

***N*-(Olean-3-hydroxy-18-en-28-oyl)-aminoundecanoic Acid Methyl Ester (29).** Following the procedure described for **28**, compound **29** was obtained in yield 85% from **27**; white amorphous powder; mp 152−154 °C; MS (ESI+) *m/z*: 676.8 ($M^+ + Na$), 654.7 ($M^+ + 1$) for $C_{42}H_{71}NO_4$. ^{1}H NMR (300 MHz) δ 0.74, 0.75, 0.84, 0.94, 0.95, 0.96, 0.99 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 3.18 (2H, m, −NHCH_2-), 3.29 (1H, m, H-3), 3.65 (3H, s, COOCH_3), 5.30 (1H, s, H-19), 5.80 (1H, t, J = 6.0 Hz, -CO*NH*-).

***N*-(Olean-3β-hydroxy-18-en-28-oyl)-leucine (30).** Compound **28** (25 mg) was dissolved in a mixture of MeOH (2 mL) and THF (1 mL), and 2 N KOH (0.5 mL) was added to this mixture at 0 °C with stirring. The reaction mixture was acidified with 2 N HCl and extracted with CH_2Cl_2 to yield compound **30** in a 96% yield after the evaporation of solvent; mp 245−247 °C; MS (ESI−) *m/z*: 568.6 ($M^- - 1$) for $C_{36}H_{59}NO_4$. ^{1}H NMR (300 MHz, in CD_3-OD and $CDCl_3$ mixture) δ 0.70, 0.76, 0.84, 0.90 (6H, br. s), 0.95 (br. s), 0.98 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 0.90, 0.95 (3H each, br.s, -CH(CH_3)$_2$-28 side chain), 3.09 (1H, dd, J = 10.8, 5.2 Hz, H-3), 4.46 (1H, m, -*CH*(CH_3)$_2$-28 side chain), 5.34 (1H, s, H-19), 6.54 (1H, d, J = 8.7 Hz, -CO*NH*-).

***N*-(Olean-3β-hydroxy-18-en-28-oyl)-aminoundecanoic Acid (31).** Following the procedure described for **30**, compound **31** was obtained in 85% yield from **29**; white amorphous powder; mp 168−170 °C; MS (ESI+) *m/z*: 640.7 ($M^+ + 1$) for $C_{41}H_{69}NO_4$. ^{1}H NMR (300 MHz) δ 0.74, 0.75, 0.85, 0.94, 0.95, 0.96, 0.97 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 3.17 (2H, m, −NHCH_2-), 3.30 (1H, m, H-3), 5.30 (1H, s, H-19), 5.83 (1H, t, J = 5.7 Hz, -CO*NH*-).

***N*-(Olean-3β-hydroxy-18-en-28-oyl)-(1-hydroxymethyl-3-methyl)-butylamide (32).** Compound **32** was obtained in 60% yield from compound **26**; mp 253−254 °C; MS (ESI−) *m/z*: 554.7 ($M^- - 1$) for $C_{36}H_{61}NO_3$; ^{1}H NMR (300 MHz) δ 0.76, 0.77, 0.86, 0.97, 0.98, 0.99, 1.00 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 0.91, 0.93 (3H each, d, J = 6.6 Hz, -CH(CH_3)$_2$-28 side chain), 3.20 (1H, dd, J = 10.8, 5.1 Hz, H-3), 3.51 (1H, dd, J = 10.5, 6.3 Hz, -CH_2OH-28 side chain), 3.66 (1H, dd, J = 10.5, 3.5 Hz, -CH_2-OH-28 side chain), 4.02 (1H, m, -*CH*(CH_3)$_2$-28 side chain), 5.33 (1H, s, H-19), 5.87 (1H, d, J = 8.1 Hz, -CO*NH*-).

***N*-[3β-*O*-(3′,3′-Dimethylsuccinyl)-olean-18-en-28-oyl]-(1-hydroxymethyl-3-methyl)-butylamide (19).** Compound **32** (130 mg, 0.23 mmol) was heated with 2,2-dimethylsuccinic anhydride (180 mg, 1.38 mmol) and DMAP (56 mg, 0.46 mmol) in pyridine (6 mL) to reflux overnight. After the addition of EtOAc (50 mL), the mixture was washed twice with 2 N HCl (5 mL) and H_2O. The evaporation of EtOAc gave crude disuccinates **33** as an oil. The oil was treated with 2 N KOH (7 mL) in a mixture of MeOH (15 mL) and THF (2 mL) under ice-cooling and then kept at room temperature overnight. The reaction mixture was acidified with 2 N HCl, and the organic solvent was evaporated. The residue was extracted by CH_2Cl_2 to give an oil, which was subjected to silica gel column chromatography. Using a mixture of CH_2Cl_2 and MeOH (100:1), compound **19** was obtained as an oil and was crystallized from EtOAc to give 105 mg of **19** as a white amorphous powder in a yield of 70.0%; mp 237−238 °C; MS (ESI−) *m/z*: 682.8 ($M^- - 1$) for $C_{42}H_{69}NO_6$. ^{1}H NMR (300 MHz) δ 0.76, 0.80, 0.83, 0.87, 0.97, 0.99 (6H) (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 0.90, 0.92 (3H each, d, J = 4.4 Hz, -CH(CH_3)$_2$-28 side chain), 1.27, 1.29 (3H each, s, 2 × CH_3-3′), 3.49 (1H, dd, J = 10.8, 3.9 Hz, -CH_2OH-28 side chain), 3.64 (1H, dd, J = 10.8, 6.3 Hz, -CH_2-OH-28 side chain), 4.03 (1H, m, −NHC*H*-), 4.49 (1H, dd, J = 9.9, 5.4 Hz, H-3), 5.34 (1H, s, H-19), 5.88 (1H, d, J = 8.1 Hz, -CO*NH*-). Anal. ($C_{42}H_{69}NO_6$), C 73.59, H 10.20, N 1.99.

***N*-[3β-*O*-(3′,3′-Dimethylsuccinyl)-olean-18-en-28-oyl]-L-leucine (20).** Compound **20** was obtained in 30% yield **30**; white amorphous powder; mp 223−224 °C; MS (ESI−) *m/z*: 696.8 ($M^- - 1$) for $C_{42}H_{67}NO_7$. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.76, 0.77, 0.83, 0.85, 0.93 (6H), 1.03 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 0.99 (6H, d, J = 7.2 Hz, -CH(CH_3)$_2$-28 side chain), 1.28, 1.31 (3H each, s, 2 × CH_3-3′), 4.52 (1H, dd, J = 8.1, 4.8 Hz, H-3), 4.68 (1H, m, −NHC*H*-), 5.37 (1H, s, H-19), 6.32 (1H, d, J = 8.1 Hz, -CO*NH*-). Anal. ($C_{42}H_{67}NO_7$), C 71.98, H 9.72, N 2.01.

***N*-[3β-*O*-(3′,3′-Dimethylsuccinyl) olean-18-en-28-oyl]-aminoundecanoic Acid (21).** Compound **21** was obtained in 48% yield from compound **31**; white amorphous powder; mp 131−132 °C; MS (ESI+) *m/z*: 790.6 ($M^+ + Na$) for $C_{47}H_{77}NO_7$. ^{1}H NMR (500 MHz) δ 0.76, 0.80, 0.85, 0.86, 0.96, 0.98, 1.00 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 1.26, 1.29 (3H each, s, 2 × CH_3-3′), 4.54 (1H, dd, J = 11.5, 5.0 Hz, H-3), 5.32 (1H, s, H-19), 5.87 (1H, dd, J = 8.5, 3.5 Hz, -CO*NH*-). Anal. ($C_{47}H_{77}NO_7$), C 73.12, H 9.95, N 1.76.

***N*-[3β-*O*-(2′,2′-Dimethylsuccinyl)-olean-18-en-28-oyl]-aminoundecanoic Acid (22).** Compound **22** was obtained in 10% yield from compound **31** as a dia-isosteric isomer of **21**, white amorphous powder; mp 109−111 °C; MS (ESI+) *m/z*: 790.6 ($M^+ + Na$) for $C_{47}H_{77}NO_7$. ^{1}H NMR (500 MHz) δ 0.76, 0.76, 0.86, 0.95, 0.96, 0.98, 1.00 (3H each, s, CH_3-23, 24, 25, 26, 27, 29 and 30), 1.27, 1.31 (3H each, s, 2 × CH_3-2′), 4.40 (1H, dd, J = 12.0, 4.5 Hz, H-3), 5.32 (1H, s, H-19), 5.85 (1H, t, J = 7.5 Hz, -CO*NH*-). Anal. ($C_{47}H_{77}NO_7$), C 73.36, H 10.10, N 1.78.

HIV Growth Inhibition Assay in H9 Lymphocytes. The evaluation of HIV-1 inhibition was carried out as follows using H9 lymphocytes. The human T-cell line, H9, was maintained in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum supplemented with L-glutamine at 5% CO_2 and 37 °C. Test samples were prepared as described previously,[6] and to each sample well was added 90 μL of media containing H9 cells at 3 × 10^5 cells/mL and 45 μL of virus inoculum (HIV-1 IIIIB isolate) containing 125 $TCID_{50}$. Control wells containing virus and cells only (no drug) and cells only (no virus or drug) were also prepared. A second set of samples were prepared identical to the first and were added to cells under identical conditions without virus (mock infection) for toxicity determinations (IC_{50} defined below). In addition, AZT was also assayed during each experiment as a positive drug control. On days 1 and 4 postinfection (PI), spent media were removed from each well and replaced with fresh media. On day 6 PI, the assay was terminated, and culture supernatants were harvested for analysis of virus replication by p24 antigen capture. The compound toxicity was determined by XTT using the mock-infected sample wells. If a test sample inhibited virus replication and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; and the Therapeutic index (TI), the ratio of the IC_{50} to EC_{50}.

Anti-HIV Assay in MT4 Cells. A previously described HIV-1 infectivity assay was used in the experiments.[13] A 96-well microtiter plate was used to set up the HIV-1 replication assay. HIV-1 at a multiplicity of infection (MOI) of 0.01 was used to infect MT4 cells. Culture supernatants were collected on day 4 postinfection for the p24 assay using an ELISA kit from ZeptoMetrix Corporation (Buffalo, New York).

Cell Fusion Assay. A protocol modified from a previously described fusion assay was used in this study.[9] TZM cells that expressed luciferase upon fusion with envelope-expressing COS cells were used as fusion partners. The fusion assays were performed by transfecting monkey kidney cells (COS) with an expression vector pSRHS that contained the HIV-1 NL4-3 envelope genes. Electroporation was performed to express the HIV-1 envelope on COS cells. Briefly, COS cells (10^6) in culture medium were incubated with 2 μg of the envelope expression vector on ice for 10 min. Electroporation was performed using a gene pulser (BioRad,

Hercules, CA) with capacitance set at 950 μF and voltage at 150 V. The transfected COS cells were cultured for 1 day before mixing with TZM cells. TZM cells (10×10^4) were incubated with COS cells (10^4) in 96-well flat-bottomed plates (Costar) in a 100 μL culture medium. Compounds to be tested at various concentrations in 10 μL of culture medium were incubated with the cell mixtures at 37 °C for 24 h. Luciferase activity was quantified using a Biotek luminometer.

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Supporting Information Available: Elemental analysis data for compounds **16**−**22**. This material is available free of charge via the Internet at http://pubs.acs.org.

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