Chemical Modification of Oleanene Type Triterpenes and Their Inhibitory Activity against HIV-1 Protease Dimerization

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Oleanolic acid derivatives with different lengths of 3-*O*-acidic acyl chains were synthesized and evaluated for their inhibitory activity against HIV-1 protease. The lengths of the acidic chains were optimized to 6 and 8 carbons. Changing a 3-ester bond to an amide bond or dimerization of the triterpenes retained their inhibitory activity against HIV-1 protease. Introduction of an additional acidic chain to C-28 of oleanolic acid increased the inhibitory activity appreciably, though a derivative with only one acidic chain linked at C-28 also showed potent activity against HIV-1 protease.

The inhibitory mechanism was proved directly by size exclusion chromatography to be inhibition of dimerization of the enzyme polypeptides. The ester bonds of the triterpene derivatives were found to be stable to lipase under mild alkaline conditions.

Key words HIV-protease; triterpene; oleanolic acid; sophoradiol; inhibition

Being essential for the maturation of the AIDS virus, HIV protease (PR) has been regarded as one of the most promising targets for the development of anti-AIDS agents. Inhibitors of this enzyme such as saquinavir, ritonavir and indinavir have been used clinically as potent anti-AIDS drugs. However, their usage is limited by the emergence of drug resistant viral strains, severe side effects, drug-drug interactions, moderate bioavailability and short plasma half-life.[1] Most of the drugs are of high cost, require frequent dosing and/or food restriction. A new generation of PR inhibitors with lower price, superior pharmacokinetics, fewer drug interactions, excellent tolerability and convenient dosing schedule are needed. For this purpose, we have conducted a search for HIV-1 PR inhibitors of a new structural-type or new inhibitory mechanism. In the present paper, we report on a series of non-peptide HIV-1 PR inhibitors with a novel mechanism of dimerization inhibition.

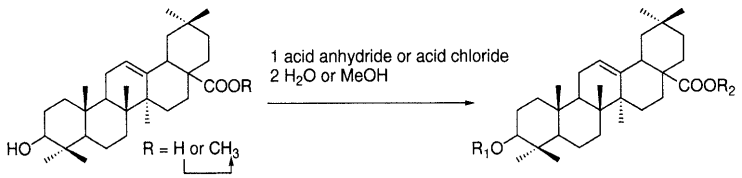
We previously reported that ursolic acid, oleanolic acid and betulinic acid as well as their dicarboxylic acid hemiesters showed potent inhibitory activity of HIV PR. Glutaric acid hemiesters of these triterpenes with the longest 3-acyl side chain showed the most potent activity,[2] which prompted us to synthesize longer 3-acyl derivatives of triterpenes to improve the activity. The present experiment was designed to find the optimized length of the 3-*O*-acyl chain, and to investigate the effects of other substituents at position 3 and an additional acidic chain at other positions of the triterpene skeleton on their inhibitory activity against HIV-1 PR. In addition, the inhibitory mechanism and the stability of the triterpene derivatives were also investigated. For economic reasons, oleanolic acid, which is abundant in nature and is cheap in price was used as a starting material for the synthesis of most of the derivatives.

Results and Discussion

Chemistry 3-*O*-Acyl derivatives **11** and **14** were prepared by treatment of oleanolic acid with the corresponding acid anhydride; Compounds **7**—**10**, **12** and **13** were obtained by heating oleanolic acid and its methyl ester with the appropriate acid chloride followed by treatment with H_2O or, in the case of **12** and **13**, with MeOH.

Referring to the methods used by Sun *et al.* in the synthesis of betulin derivatives,[3] oleanolic acid and its methyl ester were oxidized with pyridinium chlorochromate (PCC) to give 3-oxo compounds, **15** and **16**. Treatment of the 3-oxo compounds with NH_2OH in pyridine yielded the hydroxyimino compounds **17** and **18**. Reduction of **18** with $TiCl_3$ and $NaCNBH_3$ yielded 3β- and 3α-amino compounds, **19** and **20**.

Treatment of **17** and **18** with adipoyl chloride yielded the



7	R_1 = $CO(CH_2)_4COOH$	R_2 = H
8	R_1 = $CO(CH_2)_4COOH$	R_2 = CH_3
9	R_1 = $CO(CH_2)_6COOH$	R_2 = H
10	R_1 = $CO(CH_2)_8COOH$	R_2 = H
11	R_1 = $COCH_2C(CH_3)_2CH_2COOH$	R_2 = H
12	R_1 = $CO(CH_2)_4COOCH_3$	R_2 = H
13	R_1 = $CO(CH_2)_4COOCH_3$	R_2 = CH_3
14	R_1 = $CO(CH_2)_4CH_3$	R_2 = H

Chart 1. Synthesis of Oleanolic Acid Derivatives with Different Chain Lengths at C-3

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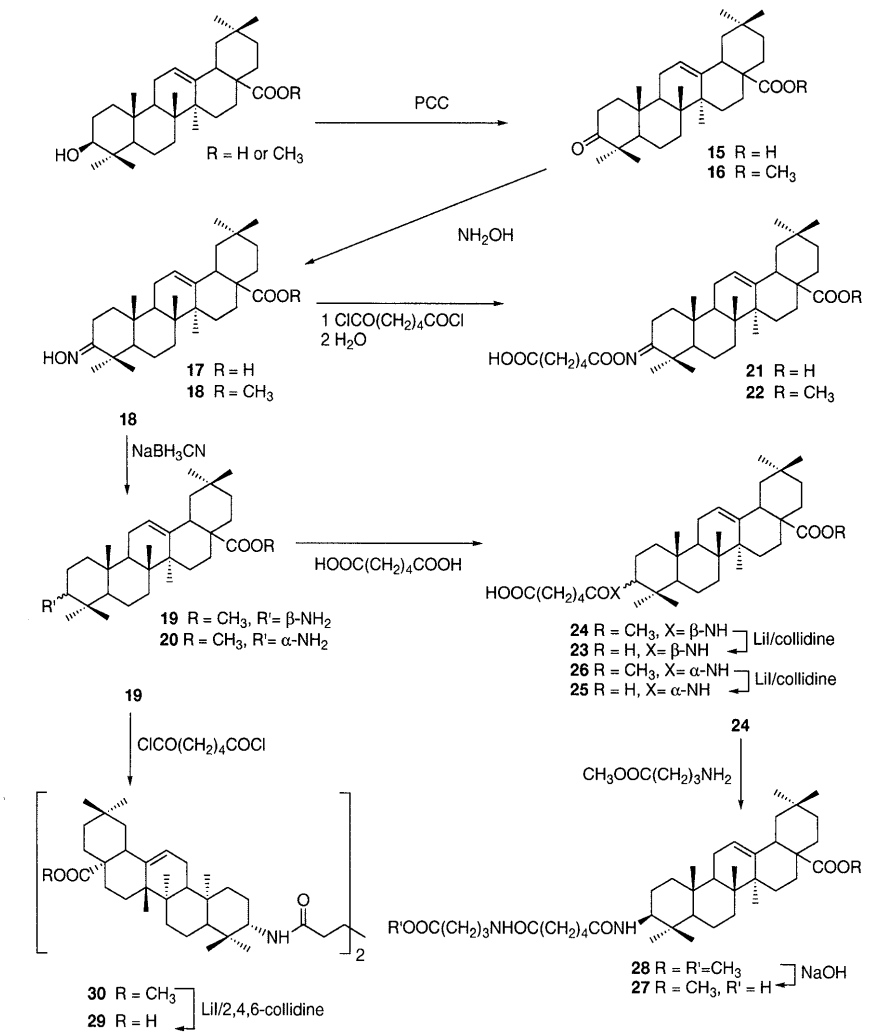


Chart 2. Synthesis of Oleanolic Acid Derivatives with Different Bond and Chain Natures at C-3

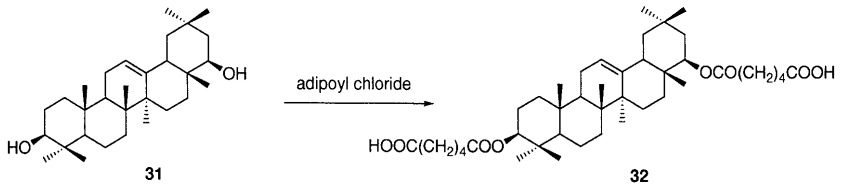


Chart 3. Synthesis of Oleanene Derivative with Two Acidic Chains at C-3 and C-22

respective acyl derivatives, **21** and **22**. Amides **24** and **26** were obtained by condensation of the amino compounds, **19** and **20**, with adipic acid in the presence of *N,N'*-dicyclohexyl carbodiimide (DCC). Hydrolysis of the sterically hindered 28-methyl ester proved to be very difficult. Several methods were tried and finally lithium iodide and 2,4,6-collidine was employed to halogenolyze[4] **24** and **26** to the corresponding 28-free acid compounds **23** and **25**. One more amide group was introduced to compound **24** to yield **28** by condensation of **24** with 4-amino-*n*-butyric acid methyl ester using a DCC-4-dimethylaminopyridine (DMAP) method. Saponification of **28** with aqueous sodium hydroxide in a methanol/THF mixture yielded compound **27** with a free carboxy group at the 3-acyl chain.

A dimeric compound, **30**, was obtained by treatment of **19** with adipoyl chloride. Halogenolysis of **30** yielded the 28-free acid dimeric compound **29**.

Treatment of sophoradiol (**31**) with adipoyl chloride afforded the diadipoylated compound **32**.

Referring to the method used by Evers *et al.* in the synthesis of betulinic acid derivatives,[5] oleanolic acid was first converted to its acetyl ester, which was then treated with oxalyl chloride to give acid chloride. Condensation of methyl 6-aminohexanoate with acetyl oleanolic acid chloride followed by saponification yielded the 28-acidic chain compound **33**. Treatment of **33** with adipoyl chloride afforded the 3, 28-diacidic chain compound **34**.

Structure and Activity Relationship The inhibitory activity of the synthesized triterpenes against HIV-1 PR was evaluated by quantitative analysis of the cleavage of a synthetic substrate by HPLC. Tables 1—3 show 50% inhibitory concentrations (IC_{50}) of the triterpene derivatives against HIV-1 PR.

As reported previously,[2] of the dicarboxylic acid

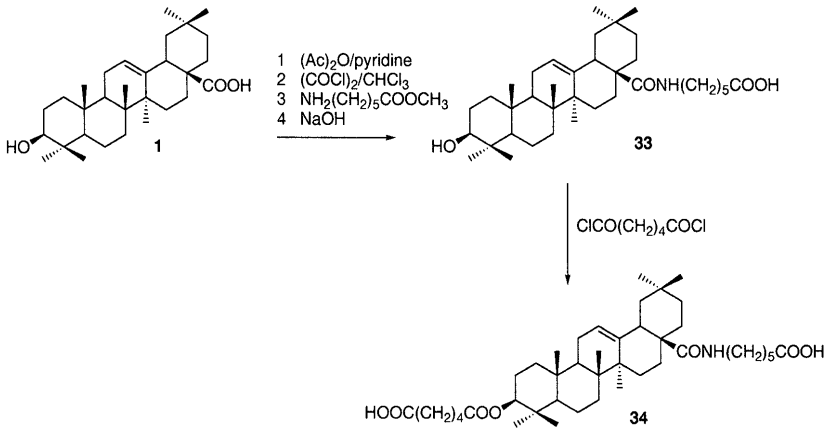


Chart 4. Synthesis of Oleanene Derivatives with Acidic Chain(s) at C-28 as Well as at Both C-3 and C-28

hemiesters of oleanolic acid, when the 3-acyl chains were within five carbons, the HIV-1 PR inhibitory activity increased as the lengths of the 3-acyl chains increased. The activity of glutaryl hemiester of oleanolic acid (IC_{50} 4 μM) was increased 2-fold as compared to oleanolic acid (IC_{50} 8 μM).

Lengthening the 3-acyl chain by one or three methylene units (**7**, **9**) led to increases in inhibitory activity, with an IC_{50} value of 3.0 μM for both compounds. Further extension of the acyl chain by two additional methylene units (**10**) led to slightly decreased inhibitory activity (IC_{50} 4.0 μM). Compounds with two methyl groups substituted at the acyl chain (**11**) showed similar activity as that of compounds with a straight acyl chain (**6**).

Methylation either of the 28- or of the 3-acyl carboxylic acid (**8**, **12**, respectively) decreased the inhibitory activity (IC_{50} 7.5 and 5.6 μM, respectively). Methylation of both carboxylic acid residues in the triterpene derivative led to complete loss of activity (**13**, IC_{50}>20 μM).

Changing a 3-hydroxyl of oleanolic acid to an oxo or hydroxyimino group retained the inhibitory activity (**15**, **17**). Replacing a 3-hydroxyl of 28-methyl oleanolate to an oxo (**16**) or amino group (**19**, **20**) did not ameliorate the poor activity. However, 3-hydroxyimino-olean-12-en-28-oic acid methyl ester (**18**) exhibited two-fold increased activity as compared to methyl oleanolate.

Introduction of an acidic chain to the 3-hydroxyimino and amino compounds significantly increased the inhibitory activity whether the 28-carboxy group was free or methylated (**22**—**26**, IC_{50} of 2—4 μM) (a 3-acylated hydroxyimino compound with a free carboxy group at position 28 (**21**) is an exception, which exhibited the same activity as that of the non-acylated compound (**17**)).

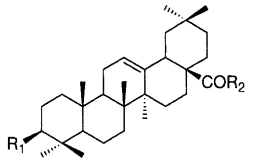
Introduction of a second amide group into the acyl chain of compound **24** was detrimental to the activity (**27**, IC_{50}= 6.0 μM). Its methylated derivative (**28**), like most of the other compounds without a free carboxy group in their structures, showed no activity at all.

The inhibitory activity of the dimeric compound was similar to that of the monomer. No inhibitory activity was observed in the 28-methylated dimer (**30**) without free carboxy groups in its structure. The dimeric compound (**29**) with two free carboxy groups in its structure showed inhibitory potency comparable to that of the monomer compound (**23**).

The above results indicated clearly that oleanolic acid with an acidic chain of six carbons linked at its position 3 exhibited potent inhibitory activity against HIV-1 PR. Consequently, we considered introducing an additional acidic chain to the oleanene skeleton. Sophoradiol (**31**, an aglycone of kaikasaponin III[6]) is an oleanene type triterpene with two hydroxy groups at positions 3 and 22. The distance between these two hydroxy groups (12.321 Å) is quite similar to that of the 3-OH and 17-COOH in the structure of oleanolic acid (12.409 Å), as calculated with Chem 3D models. Acylation of **31** with adipoyl chloride yielded 3, 22-di-*O*-adipoyl-sophoradiol (**32**) which showed more than 8 fold more activity than the parent triterpene (**32** IC_{50} 2.3 *vs.* **31** IC_{50} 18.8 μM).

Encouraged by the positive result of the diadipoylated derivative (**32**), we tried to synthesize another oleanolic acid derivative, **34**, with two acidic chains linking at both positions 3 and 28. As expected, this compound demonstrated more potent inhibitory activity against HIV-1 PR, with an IC_{50} value of 1.7 μM. Compound **33**, with only one acidic

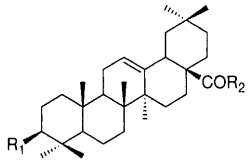
Table 1. Anti-HIV-1 Protease Activity of Oleanolic Acid Derivatives with Various Chain Lengths at C-3



R_1	R_2=–OH		R_2=–OCH_3	
	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
–OH	**1**[a]	8	**2**[a]	20
–OCOCOOH	**3**[a]	20		
–$OCOCH_2COOH$	**4**[a]	8		
–$OCO(CH_2)_2COOH$	**5**[a]	4		
–$OCO(CH_2)_3COOH$	**6**[a]	4		
–$OCO(CH_2)_4COOH$	**7**	3.0	**8**	7.5
–$OCO(CH_2)_6COOH$	**9**	3.0		
–$OCO(CH_2)_8COOH$	**10**	4.0		
–$OCOCH_2C(CH_3)_2CH_2COOH$	**11**	3.8		
–$OCO(CH_2)_4COOCH_3$	**12**	5.6	**13**	>20
–$OCO(CH_2)_4CH_3$	**14**	>20		

a) Reported previously.[2]

Table 2. Anti-HIV-1 Protease Activity of Oleanolic Acid Derivatives with Different Bond and Chain Natures at C-3



R_1	R_2=–OH		R_2=–OCH_3	
	Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
β-OH	**1**[a]	8	**2**	20
=O	**15**	5.5	**16**	20
=NOH	**17**	5.5	**18**	9.5
β-NH			**19**	>20
α-NH			**20**	>20
=NOCO$(CH_2)_4$COOH	**21**	5.5	**22**	4.0
β-NHCO$(CH_2)_4$COOH	**23**	3.0	**24**	3.0
α-NHCO$(CH_2)_4$COOH	**25**	2.1	**26**	3.5
β-NHCO$(CH_2)_4$CONH$(CH_2)_3$COOH			**27**	6.0
β-NHCO$(CH_2)_4$CONH$(CH_2)_3$COOCH$_3$			**28**	>20
β-NHCO$(CH_2)_4$CONH-β-oleanolic acid 28-R_2	**29**	3.3	**30**	>20

a) Reported previously.[2]

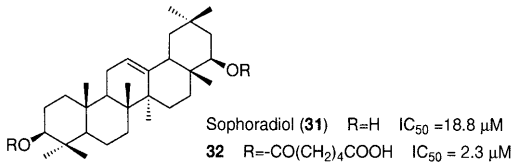


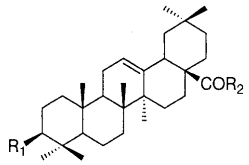
Fig. 1. Anti-HIV-1 Protease Activity of Oleanene Type Triterpenes with Hydroxyls or Acidic Chains at C-3 and C-22

chain linked at position **28**, was also obtained and tested for its activity. This **33** showed an inhibitory activity as potent as that of **34**, more than 4 times the potency of the parent triterpene, oleanolic acid. This can be explained by the highly steric hindrance of the 28-carboxylic acid in oleanolic acid hampering the hydrogen bond formation of oleanolic acid with enzyme, though the exact amino acid interacting is not clear at present. A 28-long acidic chain in compound **33** greatly reduced the steric hindrance, thus enabling this compound to form a hydrogen bond with an enzyme stronger than that of oleanolic acid.

A computer modeling study has predicted that esterification of the 3-OH of triterpene with dicarboxylic acids could improve their binding with HIV-1 PR.[7] The present results confirmed this presumption and showed that an acidic chain at position 28 could enhance the interaction with HIV-1 PR even more strongly than that at position 3 (**33** IC_{50} 1.7 *vs.* **7** IC_{50} 3.0 μM).

The above results demonstrated clearly that introduction of an acidic chain(s), especially that of six carbons, to the oleanane skeleton could increase the anti-HIV-1 PR activity significantly. To test the stability of the ester bond which links the acidic chain and the triterpene skeleton, two compounds, **7** and **14**, were incubated with lipase in a weak alkaline condition. No hydrolyzed products were detected in either compound even after 18 h incubation. Because the ester bond is known to be more stable under an acidic condition, these triterpene derivatives could be stable in the digestive

Table 3. Anti-HIV-1 Protease Activity of Oleanolic Acid Derivatives with Acidic Chain(s) at Either C-3 or C -28, as Well as at Both C-3 and C-28



	R_1	R_2	IC_{50} (μM)
1[a]	–OH	–OH	8
7	–OCO$(CH_2)_4$COOH	–OH	3.0
33	–OH	–NH$(CH_2)_5$COOH	1.7
34	–OCO$(CH_2)_4$COOH	–NH$(CH_2)_5$COOH	1.7

a) Reported previously.[2]

system.

HIV-1 PR is known to be composed of two identical monomers, which assemble by noncovalent interactions to form the composite active site. This structural peculiarity has provided the possibility of a special inhibitory mechanism, *i.e.* a dimerization inhibition mechanism. A dimerization inhibitor of HIV-1 PR could dissociate the enzyme into the inactive monomer form and thus inhibit the enzyme's activity. Ursolic acid has been analyzed by kinetic study as a dimerization inhibitor of HIV-1 PR.[7] In the present experiment, the dissociation of HIV-1 PR by triterpene compound was monitored directly by size exclusion chromatography. According to an earlier study by Zutshi *et al.*,[8] the molecular weight of HIV-1 PR dimer was about 11000 by size exclusion of a Bachem HIV-1 PR of affinity purified grade. In the present study, two main proteins at retention times of 29.4 and 39.0 min were assigned to HIV-1 PR dimer and monomer, respectively, by interpolation of a standard protein curve (Fig. 2). After incubation with **7**, the peak of the HIV-1 PR monomer was dominant and that of the dimer had disappeared completely. On the other hand, after treatment with an

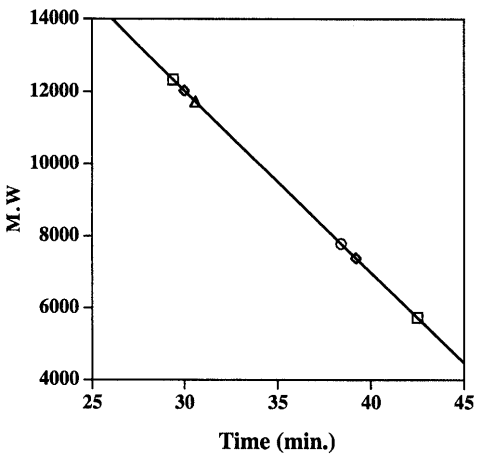


Fig. 2. Size Exclusion Chromatography of HIV-1 Protease (◇), and HIV-1 Protease with Compound **7** (○) and Acetyl Pepstatin (△)

A standard curve was generated using cytochrome C and insulin (□).

active site inhibitor, acetyl pepstatin, the dimer was dominant and the monomer had disappeared. The present experiment clearly demonstrated that the triterpene compound could dissociate the dimeric polypeptides of HIV-1 PR into a monomeric one, *i.e.* triterpene inhibited the activity of HIV-1 PR through the mechanism of dimerization inhibition.

The scaffold of triterpene compounds matches in its volume that of the backbone of a cyclic hexapeptide, and a computer docking study has revealed that some triterpenes could fit well into the hydrophobic interface site of the relaxed HIV-1 PR monomers.[7] Therefore, it is not surprising that structure modification of triterpene compounds could lead to HIV-1 PR inhibitors with the same inhibitory mechanism and similar inhibitory potency as some peptide compounds. Because the triterpene skeleton is more rigid and stable than peptide, it is expected that triterpene derivatives might be more specific to HIV-1 PR and have better pharmacokinetic properties.

In conclusion, we have presented here a number of triterpene derivatives with potent HIV-1 PR inhibitory activity based on a novel mechanism of dimerization inhibition. The potency of most of the derivatives synthesized in the present experiment was comparable to that of peptide compounds reported recently based on the same inhibition mechanism.[8,9] The most potent oleanolic acid derivatives (**33**, **34**) showed more than 4-fold increased activity against HIV-1 PR as compared to oleanolic acid (**33**, **34** IC_{50} 1.7, 1.7 μM *vs.* **1** IC_{50} 8 μM). A derivative of sophoradiol showed more than 8-fold more activity than its parent triterpene (**32** IC_{50} 2.3 μM *vs.* **31** IC_{50} 18.8 μM).

Experimental

General Experimental Procedures Optical rotations were measured with a Jasco DIP-360 automatic polarimeter. IR spectra were measured with a Jasco FT-IR-230 infrared spectrometer. ^{1}H- and ^{13}C-NMR spectra were measured with either a Varian Gemini 300 (^{1}H, 300 MHz; ^{13}C, 75 MHz), Varian Unity 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) or Jeol JNA-LA 400WB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz) NMR spectrometer, with chemical shifts being represented in ppm and TMS used as an internal standard. EIMS were measured with a Jeol JMS-AX 505 HAD mass spectrometer at an ionization voltage of 70 eV. Electrospray ionization (ESI) MS was measured with a Perkin-Elmer SCIEX API-III biomolecular mass analyzer. Preparative HPLC was carried out on a Gilson instrument with a 231LX injector, a 119 UV/VIS detector and a TSK gel ODS-80T_M column (21.5×300 mm, Tosoh Co.).

HIV-1 PR assay kit was purchased from Bachem Bioscience. Sephadex G-50 (Particle sized: 20—80 μM) was purchased from Pharmacia Fine Chemicals AB Uppsala, Sweden. Cytochrome C (from bovine heart), insulin (from bovine pancreas), and lipase (type II, crude, from porcine pancreas) were purchased from Sigma Chemical Co.

General Procedure for Synthesis of Dicarboxylic Acid Esters (7—10, 12, 13) A solution of oleanolic acid (or methyl oleanolate for **8** and **13**, 0.10 mmol), and appropriate acid dichloride (10 equiv. mol) in anhydrous THF (50 ml) was heated for 3—4 h at 70 °C. The reaction mixture was poured into 100 ml of H_2O (or MeOH for **12** and **13**) and allowed to stand for 2 h. The mixture was extracted three times with 150 ml of $CHCl_3$. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on RP-18 and then a silica gel column to afford the respective dicarboxylic acid esters.

3-*O*-Adipoyloleanolic Acid (7) 50% yield [after chromatography on RP-18 with MeOH/H_2O (80—100%) and then on silica gel with MeOH/$CHCl_3$ (0—5%)], a white powder; $[\alpha]_D^{25}$ +58.5° (c=0.07, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.72 (3H, s), 0.80 (6H, s), 0.85 (3H, s), 0.88 (6H, s), 1.09 (3H, s) (7×CH_3), 1.58 (overlapped signals, H-3′, 4′), 2.28 (4H, t-like, H-2′, 5′), 2.77 (1H, dd, J=13.5, 4.1 Hz, H-18), 4.44 (1H, t, J=8.0 Hz, H-3), 5.21 (1H, t-like, H-12); *Anal.* Calcd for $C_{36}H_{56}O_6 \cdot H_2O$: C, 71.72; H, 9.70. Found: C, 72.16; H, 9.23; MS (positive-ESI): 607 $(M+Na)^+$ (50), 585 $(M+1)^+$ (60); (negative-ESI): 583 $(M-1)^-$ (100), 421 (50).

3-*O*-Adipoyloleanolic Acid Methyl Ester (8) 55% yield [after chromatography on RP-18 with MeOH/H_2O (80—100%) and then on silica gel with acetone/hexane (5—20%)], a white powder; $[\alpha]_D^{25}$ +55.9° (c=0.56, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.72 (3H, s), 0.85 (6H, s), 0.90 (3H, s), 0.93 (6H, s), 1.13 (3H, s) (7×CH_3), 1.63 (overlapped signals, H-3′, 4′), 2.36 (4H, t-like, H-2′, 5′), 2.87 (1H, dd, J=13.5, 4.1 Hz, H-18), 3.62 (3H, s, CH_3-28), 4.50 (1H, t, J=7.8 Hz, H-3), 5.28 (1H, t-like, H-12); *Anal.* Calcd for $C_{37}H_{58}O_6$: C, 74.21; H, 9.76. Found: C, 74.18; H, 9.75; MS (EI): 598 $[M]^+$, 552, 538, 452, 262, 203 (base).

3-*O*-Suberoyloleanolic Acid (9) 45% yield [after chromatography on RP-18 with MeOH/H_2O (80—100%) and then on silica gel with MeOH/$CHCl_3$ (0—5%)], a white powder; $[\alpha]_D^{25}$ +59.6° (c=0.13, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.74 (3H, s), 0.86 (6H, s), 0.90 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 1.13 (3H, s) (7×CH_3), 1.36 (overlapped signals, H-4′, 5′), 1.61 (overlapped signals, H-3′, 6′), 2.30 (2H, t, J=7.1 Hz, H-2′), 2.36 (2H, t, J=7.1 Hz, H-7′), 2.81 (1H, dd, J=13.5, 4.1 Hz, H-18), 4.50 (1H, t, J=8.0 Hz, H-3), 5.27 (1H, t-like, H-12); *Anal.* Calcd for $C_{38}H_{60}O_6 \cdot 1.2H_2O$: C, 71.93; H, 9.91. Found: C, 71.64; H, 9.45; MS (positive-ESI): 635 $[M+Na]^+$ (75), 613 $[M+1]^+$ (20), 439 (80); (negative-ESI): 611 $[M-1]^-$ (100), 421 (30).

3-*O*-Sebacoyloleanolic Acid (10) 55% yield [after chromatography on RP-18 with MeOH/H_2O (80—100%) and then on silica gel with MeOH/$CHCl_3$ (0—5%)], a white powder; $[\alpha]_D^{25}$ +56.2° (c=0.79, $CHCl_3$); ^{1}H-HMR ($CDCl_3$) δ 0.75 (3H, s), 0.85 (3H, s), 0.86 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 1.13 (3H, s) (7×CH_3), 1.31 (8H, s, H-4′, 5′, 6′, 7′), 1.60 (overlapped signals, H-3′, 8′), 2.29 (2H, t, J=7.5 Hz, H-2′), 2.34 (2H, t, J=7.1 Hz, H-9′), 2.81 (1H, dd, J=13.5, 4.1 Hz, H-18), 4.50 (1H, t, J=8.0 Hz, H-3), 5.27 (1H, t-like, H-12); *Anal.* Calcd for $C_{40}H_{64}O_6 \cdot H_2O$: C, 72.91; H, 10.10. Found: C, 72.53; H, 9.76; MS (positive-ESI): 663 $[M+Na]^+$ (80); (negative- ESI): 639 $[M-1]^-$ (100), 421 (50).

3-*O*-(6′-*O*-Methyladipoyl)oleanolic Acid (12) 55% yield [after chromatography on RP-18 with MeOH/H_2O (80—100%) and then on silica gel with acetone/hexane (5—20%)], a white powder; $[\alpha]_D^{25}$ +60.8° (c=0.17, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.75 (3H, s), 0.83 (3H, s), 0.85 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.94 (3H, s), 1.13 (3H, s) (7×CH_3), 1.64 (overlapped signals, H-3′, 4′), 2.32 (4H, q-like, H-2′, 5′), 2.82 (1H, dd, J=13.5, 4.1 Hz, H-18), 3.67 (3H, s, CH_3-6′), 4.50 (1H, t, J=7.8 Hz, H-3), 5.27 (1H, t-like, H-12); *Anal.* Calcd for $C_{37}H_{58}O_6 \cdot 0.5H_2O$: C, 73.11; H, 9.78. Found: C, 72.87; H, 9.53; MS (EI): 598 $[M]^+$, 552, 438, 351, 248 (base), 203.

3-*O*-(6′-*O*-Methyladipoyl)oleanolic Acid Methyl Ester (13) 60% yield [after chromatography on RP-18 with MeOH/H_2O (80—100%) and then on silica gel with acetone/*n*-hexane (5—20%)], a white powder; $[\alpha]_D^{25}$ +60.1° (c=0.84, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.72 (3H, s), 0.85 (6H, s), 0.90 (3H, s), 0.93 (6H, s), 1.13 (3H, s) (7×CH_3), 1.64 (overlapped signals, H-3′, 4′), 2.32 (4H, q-like, H-2′, 5′), 2.85 (1H, dd, J=13.5, 3.4 Hz, H-18), 3.62 (3H, s, CH_3-28), 3.67 (3H, s, CH_3-6′), 4.49 (1H, t, J=8.0 Hz, H-3), 5.28 (1H, t-like, H-12); MS (EI): 612 $[M]^+$, 552, 452, 349, 262 (base), 203 (base).

3-*O*-(3′,3′-Dimethylglutaryl)oleanolic Acid (11) A solution of oleanolic acid (200 mg, 0.44 mmol), 4-dimethylaminopyridine (1000 mg, 8.19 mol; 200 mg, 1.64 mmol), and 3′,3′-dimethylglutaryl anhydride (1000 mg, 7.04 mmol; 220 mg, 1.55 mmol) in anhydrous pyridine (10 ml) was refluxed for

36 h. The reaction mixture was concentrated under reduced pressure to dryness. The residue was chromatographed on an RP-18 column eluted with MeOH–H_2O to afford **11** as a white powder (150 mg, 57.1% yield): $[\alpha]_D^{25}$ +52.4° (c=0.97, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.72 (3H, s), 0.81 (3H, s), 0.87 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.95 (3H, s), 1.08 (3H, s), 1.11 (3H, s), 1.15 (3H, s) (9×CH_3), 2.28 (1H, d, J=12.9), 2.36 (1H, d, J=15.9 Hz), 2.54 (1H, d, J=15.9), 2.75 (1H, d, J=12.9 Hz) (H-2′,4′), 2.80 (1H, br d, J=13.2 Hz, H-18), 4.51 (1H, dd, J=5.7, 9.9 Hz, H-3), 5.27 (1H, t-like, H-12); *Anal.* Calcd for $C_{37}H_{58}O_6 \cdot 0.5H_2O$: C, 73.11; H, 9.78. Found: C, 73.06; H, 9.63; MS (negative-ESI): 597 $[M-1]^-$ (100), 455 (30).

3-*O*-Hexanoyloleanolic Acid (14) A mixture of oleanolic acid (30 mg, 0.066 mmol) and hexanoic anhydride (0.5 ml, 2.2 mmol) in 1 ml of pyridine was stirred overnight at room temperature. The mixture was concentrated to dryness and chromatographed on an RP-18 column eluted with 80—100% MeOH to afford **14** (11 mg, 30.2% in yield) as a white powder; ^{1}H-NMR ($CDCl_3$) δ 0.75 (3H, s), 0.85 (3H, s), 0.86 (3H, s), 0.90 (3H, s), 0.90 (3H, t, J=7.5 Hz), 0.93 (3H, s), 0.94 (3H, s), 1.13 (3H, s) (8×CH_3), 2.29 (2H, t, J=7.5 Hz, H-2′), 2.81 (1H, dd, J=13.5, 3.4 Hz, H-18), 4.50 (1H, t, J=7.8 Hz, H-3), 5.27 (1H, t-like, H-12).

3-Oxo-olean-12-en-28-oic Acid (15) To a solution of oleanolic acid (1781 mg, 3.9 mmol) in acetone–CH_2Cl_2 (10 ml) was added PCC (2527 mg, 11.7 mmol). After stirring for 6 h at room temperature, the mixture was diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness and then subjected to silica gel column chromatography eluted with *n*-hexane–acetone (95 : 5) to give **15** as a colorless crystalline powder: (1016 mg, 57.3% in yield); $[\alpha]_D^{25}$ +73.6° (c=0.26, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.81 (3H, s), 0.91 (3H, s), 0.93 (3H, s), 1.03 (3H, s), 1.05 (3H, s), 1.08 (3H, s), 1.15 (3H, s) (7×CH_3), 2.39 (1H, ddd, J=3.7, 6.8, 16.0 Hz, H_a-2), 2.55 (1H, ddd, J=7.6, 11.2, 16.0 Hz, H_b-2), 2.84 (1H, dd, J=13.9, 3.9 Hz, H-18), 5.30 (1H, t, J=3.4 Hz, H-12).

3-Oxo-olean-12-en-28-oic Acid Methyl Ester (16) To a solution of methyl oleanolate (480 mg, 1 mmol) in CH_2Cl_2 (5 ml) was added PCC (320 mg, 1.5 mmol). After stirring for 1.5 h at room temperature, the mixture was diluted with Et_2O and filtered through a short Florisil column. The column was then washed with 300 ml of Et_2O. The combined Et_2O eluates were concentrated under vacuum to give **16** as a colorless crystalline powder: 99% yield; $[\alpha]_D^{25}$ +98.2° (c=0.10, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.78 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 1.04 (6H, s), 1.09 (3H, s), 1.14 (3H, s) (7×CH_3), 2.36 (1H, ddd, J=3.6, 6.9, 15.4 Hz, H_a-2), 2.55 (1H, ddd, J=7.1, 11.3, 15.4 Hz, H_b-2), 2.87 (1H, dd, J=13.7, 3.5 Hz, H-18), 3.63 (3H, s, $COOCH_3$), 5.30 (1H, t-like, H-12); *Anal.* Calcd for $C_{31}H_{48}O_3$: C, 79.44; H, 10.32. Found: C, 79.92; H, 10.16; MS (EI): 468 $[M]^+$, 408, 262, 203 (base).

3-Hydroxyimino-olean-12-en-28-oic Acid (17) A solution of **15** (841 mg, 1.9 mmol) and hydroxylamine hydrochloride (346 mg, 4.9 mmol) in pyridine (2 ml) was heated for 4 h at 50 °C. After cooling to room temperature, the reaction mixture was concentrated under vacuum to dryness and then purified with RP-18 (MeOH, 90—100%) to yield a white powder (445 mg, 49.7% yield): $[\alpha]_D^{25}$ +283.8° (c=0.10, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 0.82 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 1.02 (3H, s), 1.05 (3H, s), 1.13 (3H, s), 1.14 (3H, s) (7×CH_3), 2.17 (1H, ddd, J=6.0, 12.6, 15.3 Hz, H-2_a), 2.85 (1H, dd, J=13.2, 3.9 Hz, H-18), 3.05 (1H, ddd, J=3.3, 5.1, 15.3 Hz, H-2_b), 5.29 (1H, t-like, H-12); *Anal.* Calcd for $C_{30}H_{47}O_3N \cdot 0.1H_2O$: C, 76.42; H, 10.09; N, 2.97. Found: C, 76.17; H, 10.01; N, 2.84.

3-Hydroxyimino-olean-12-en-28-oic Acid Methyl Ester (18) A solution of **16** (450 mg, 0.96 mmol) and hydroxylamine hydrochloride (300 mg, 4.3 mmol) in pyridine (5 ml) was heated for 2 h at 50 °C. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (75 ml) and washed with 10% HCl (3×50 ml). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The product was crystallized from diethyl ether and CH_2Cl_2 to yield a white powder (441 mg, 95%): $[\alpha]_D^{25}$ +26.2° (c=0.53, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.76 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 1.03 (3H, s), 1.06 (3H, s), 1.11 (3H, s), 1.16 (3H, s) (7×CH_3), 2.15 (1H, ddd, J=5.8, 12.8, 15.4 Hz, H_a-2), 2.86 (1H, dd, J=13.7, 4.3 Hz, H-18), 3.08 (1H, ddd, J=3.6, 5.1, 15.4 Hz, H_b-2), 3.63 (3H, s, CH_3O-28), 5.29 (1H, t, J=3.5 Hz, H-12); *Anal.* Calcd for $C_{31}H_{49}O_3N \cdot 0.2H_2O$: C, 76.40; H, 10.22; N, 2.87. Found: C, 76.43; H, 10.11; N, 2.76; MS (EI): 483 $[M]^+$, 262, 203 (base).

3β-Amino-olean-12-en-28-oic Acid Methyl Ester (19) and 3α-Amino-olean-12-en-28-oic Acid Methyl Ester (20) Sodium cyanoborohydride (0.58 g, 18 mmol) was added to a methanol solution of **18** (400 mg, 0.83 mmol) and ammonium acetate (0.74 g, 13.6 mmol) under argon atmosphere. The solution was chilled in ice water, and 15% aqueous titanium trichloride (2.4 ml, 2.8 mmol) was added dropwise over 20 min. The mixture was stirred at room temperature for 12 h and then adjusted with 2 N sodium hydroxide to pH=10. The aqueous solution was extracted with CH_2Cl_2 (300 ml×2), and the organic layer was washed with distilled water and concentrated to dryness. The crude products were subjected to neutral Al_2O_3 chromatography, and eluted with MeOH/$CHCl_3$ (0—20%) to give **19** (310 mg) and **20** (60 mg).

Compound **19**: A white powder, 79.8% yield; $[\alpha]_D^{25}$ +52.8° (c=1.00, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.72 (3H, s), 0.73 (3H, s), 0.88 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.94 (3H, s), 1.13 (3H, s) (7×CH_3), 2.32 (1H, dd, J=11.3, 4.4 Hz, H-3), 2.85 (1H, dd, J=13.2, 4.1 Hz, H-18), 3.62 (3H, s, CH_3O-28), 5.28 (1H, t-like, H-12); *Anal.* Calcd for $C_{31}H_{51}O_2N \cdot H_2O$: C, 76.34; H, 10.95; N, 2.98. Found: C, 76.12; H, 10.31; N, 2.78. MS (EI): 469 $[M]^+$ (base), 454, 413, 262, 203 (base).

Compound **20**: A white powder, 15.4% yield; $[\alpha]_D^{25}$ +58.1° (c=0.55, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.72 (3H, s), 0.87 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 0.93 (3H, s), 1.15 (3H, s) (7×CH_3), 2.60 (1H, br s, H-3), 2.85 (1H, dd, J=13.8, 4.1 Hz, H-18), 3.62 (3H, s, CH_3O-28), 5.28 (1H, t, J=3.6 Hz, H-12); *Anal.* Calcd for $C_{31}H_{51}O_2N$: C, 79.26; H, 10.94; N, 2.98. Found: C, 79.34; H, 10.83; N, 2.72. MS (EI): 469 $[M]^+$ (base), 454, 413, 262, 203.

3-(*N*-Adipoylimino)olean-12-en-28-oic Acid (21) A solution of **17** (20 mg, 0.04 mmol) and adipoyl dichloride (100 μl, 0.55 mmol) in anhydrous THF (10 ml) was heated for 0.5 h at 60 °C. The reaction mixture was poured into 100 ml of H_2O, allowed to stand for 1 h and then concentrated under reduced pressure. The residue was chromatographed on RP-18 (H_2O–MeOH) and then silica gel (*n*-hexane–acetone 9 : 1—7 : 3) to afford a white powder (9.0 mg); $[\alpha]_D^{25}$ +41.3° (c=0.26, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, CD_3OD) δ 0.83 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 1.03 (3H, s), 1.14 (6H, s), 1.25 (3H, s) (7×CH_3), 1.58 (overlapped signals, H-3′, 4′), 2.32 (2H, t, J=6.6 Hz, H-2′), 2.47 (2H, t, J=6.6 Hz, H-5′), 2.37 (1H, overlapped signals, H-2_a), 2.85 (1H, dd, J=13.5, 4.1 Hz, H-18), 2.91 (1H, dt, J=3.3, 15.3 Hz, H-2_b), 5.29 (1H, t-like, H-12); MS (negative-ESI): 596 $(M-1)^-$.

3-(*N*-Adipoylimino)olean-12-en-28-oic Acid Methyl Ester (22) A solution of **18** (18 mg, 0.04 mmol), adipic acid (54 mg, 0.37 mmol) and DCC (20 mg, 0.10 mmol) in anhydrous THF (2 ml) was stirred at room temperature for 3 h. The reaction mixture was concentrated to dryness and purified by ODS column chromatography, then eluted with MeOH–H_2O (1 : 1—100 : 0) to afford a white powder (22.0 mg, 97.3% yield); $[\alpha]_D^{25}$ +26.1 (c=0.50, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.76 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 1.02 (3H, s), 1.13 (6H, s), 1.26 (3H, s) (7×CH_3), 2.39 (2H, t, J=6.6 Hz, H-2′), 2.46 (2H, t, J=6.6 Hz, H-5′), 2.30 (1H, m, H-2_a), 2.88 (1H, dd, J=14.5, 4.8 Hz, H-18), 2.91 (1H, dt, J=3.3, 15.3 Hz, H-2_b), 3.63 (3H, s, CH_3O-28), 5.29 (1H, t-like, H-12); MS (negative-ESI): 610 $[M-1]^-$.

3β-(*N*-Adipoylamino)olean-12-en-28-oic Acid Methyl Ester (24): A solution of **19** (50 mg, 0.11 mmol), adipic acid (73 mg, 0.50 mmol) and DCC (30 mg, 0.15 mmol) in THF (10 ml) was stirred at room temperature for 4 h. The mixture was concentrated to dryness under vacuum, chromatographed on RP-18, then eluted with MeOH/H_2O (6 : 4—1 : 0) to obtain compound **24** as a white powder (54 mg, 84.5% in yield); $[\alpha]_D^{25}$ +45.6° (c=1.2, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 0.72 (3H, s), 0.77 (3H, s), 0.87 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 1.13 (3H, s) (7×CH_3), 1.68 (overlapped signals, H-3′, 4′), 2.22 (2H, t, J=6.7 Hz, H-2′), 2.37 (2H, t, J=6.5 Hz, H-5′), 2.85 (1H, dd, J=14.0, 4.5 Hz, H-18), 3.62 (3H, s, $COOCH_3$), 3.67 (1H, ddd, J=4.5, 9.2, 12.5 Hz, H-3), 5.28 (1H, t-like, H-12), 5.46 (1H, d, J=9.2 Hz, NH); *Anal.* Calcd for $C_{37}H_{59}O_5N \cdot H_2O$: C, 72.16; H, 9.98; N, 2.34. Found: C, 72.63; H, 9.37; N, 2.09. MS (EI): 597 $(M)^+$, 538, 336, 262, 203 (base).

3β-(*N*-Adipoylamino)olean-12-en-28-oic Acid (23) and 3α-(*N*-Adipoylamino)olean-12-en-28-oic Acid (25) Compound **24** or **26** (25 mg, 0.04 mmol) in 2.0 ml of freshly distilled collidine was heated under reflux in an atmosphere of argon with dehydride lithium iodide[10] (50 mg) for 8 h. The reaction mixture was cooled, poured into water (5 ml), acidified to pH 4 with dilute hydrochloric acid, and extracted with ether. The ether layer was washed with 5% $Na_2S_2O_3$, dried over Na_2SO_4 and evaporated to give a solid, which was purified by SiO_2 column chromatography, eluted with $CHCl_3$–MeOH (100 : 0—90 : 10) and RP-18 column chromatography with MeOH–H_2O (50—80%) to give **23** (8 mg, yield 31.0%) and **25** (7 mg, yield 30.0%) as white powder.

Compound **23**: ^{1}H-NMR δ 0.77 (3H, s), 0.78 (3H, s), 0.87 (3H, s), 0.90 (6H, s), 0.93 (3H, s), 1.15 (3H, s) (7×CH_3), 1.65 (overlapped signals, H-3′, 4′), 2.21 (2H, t-like, H-2′), 2.33 (2H, t-like, H-5′), 2.83 (1H, dd, J=14.0, 4.5 Hz, H-18), 3.63 (1H, m, H-3), 5.27 (1H, t-like, H-12), 5.87 (1H, d, J=8.1 Hz, NH); *Anal.* Calcd for $C_{36}H_{57}O_5N \cdot H_2O$: C, 71.84; H, 9.88; N, 2.33. Found: C, 71.36; H, 9.36; N, 2.25. MS (positive-ESI): 606 $[M+Na]^+$ (90), 584 $[M+1]^+$ (75); (negative-ESI): 582 $[M-1]^-$ (100), 421 (45).

Compound **25**: $[\alpha]_D^{25}$ +28.5° (c=0.41, $CHCl_3$); ^{1}H-NMR ($CDCl_3$–CD_3OD 8 : 1) δ 0.79 (3H, s), 0.84 (3H, s), 0.91 (3H, s), 0.94 (3H, s), 0.95 (6H, s), 1.19 (3H, s) (7×CH_3), 2.24 (2H, t, J=6.8 Hz, H-2′), 2.34 (2H, t, J=6.8 Hz, H-5′), 2.83 (1H, br d, J=10.5 Hz, H-18), 3.81 (1H, br s, H-3), 5.28 (1H, t-like, H-12); MS (negative-ESI): 582 $[M-1]^-$ (100), 421 (20).

3α-(N-Adipoylamino)olean-12-en-28-oic Acid Methyl Ester (26) A solution of **20** (32 mg, 0.07 mmol), adipic acid (37 mg, 0.25 mmol) and DCC (30 mg, 0.15 mmol) in THF (5 ml) was stirred at room temperature for 3 h. The mixture was concentrated to dryness under vacuum and chromatographed on RP-18 with MeOH–H_2O (6 : 4—1 : 0) to obtain compound **26** as a white powder (27 mg, 66.3% yield). $[\alpha]_D^{25}$ +32.0° (c=0.40, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.73 (3H, s), 0.84 (3H, s), 0.90 (3H, s), 0.93 (3H, s), 0.95 (6H, s), 1.18 (3H, s) (7×CH_3), 1.68 (overlapped signals, H-3′, 4′), 2.25 (2H, t, J=6.8 Hz, H-2′), 2.38 (2H, t, J=6.2 Hz, H-5′), 2.87 (1H, dd, J=13.6, 4.1 Hz, H-18), 3.61 (3H, s, $COOCH_3$), 3.84 (1H, br d, J=9.5, H-3), 5.29 (1H, t, J=3.6 Hz, H-12), 5.79 (1H, d, J=9.5 Hz, NH); *Anal.* Calcd for $C_{37}H_{59}O_5N$: C, 74.33; H, 9.95; N, 2.34. Found: C, 74.17; H, 9.91; N, 2.25. MS (EI): 597 $[M]^+$, 538, 336, 262, 203 (base).

3β-[*N*-(3″-Carboxypropylamino)adipo-6′-yl]amino-olean-12-en-28-oic Acid Methyl Ester (27) To a solution of **28** (7.6 mg, 0.01 mmol) in a 1 : 1.5 mixture of CH_3OH and THF (2.5 ml) was added aqueous NaOH (4 N, 0.6 ml). After stirring for 5 h and concentrating in vacuo, the residue was suspended in distilled water. The suspension was acidified with 4 N HCl to pH 3. The mixture was passed through a PR-18 column, washed with H_2O and then MeOH to give compound **27** as a white powder: yield 67.3% (5.0 mg), $[\alpha]_D^{25}$ +49.4° (c=0.11, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 0.72 (3H, s), 0.78 (3H, s), 0.87 (3H, s), 0.89 (3H, s), 0.90 (3H, s), 0.92 (3H, s), 1.13 (3H, s) (7×CH_3), 1.66 (overlapped signals, H-3′, 4′), 1.87 (overlapped signals, H-2″), 2.25 (4H, br s, H-2′, 3″), 2.41 (2H, t, J=6.4 Hz, H-5′), 2.87 (1H, dd, J=14.1, 4.1 Hz, H-18), 3.37 (2H, br s, H-1″), 3.62 (3H, s, $COOCH_3$), 3.62 (overlapped signal, H-3), 5.28 (1H, t, J=3.2 Hz, H-12), 5.66 (1H, d, J=9.5 Hz, NH), 6.48 (1H, t-like, NH); MS (negative-ESI): 681 $(M-1)^+$.

3β-[*N*-(3″-Methoxycarbonylpropylamino)adipo-6′-yl]amino-olean-12-en-28-oic Acid Methyl Ester (28) A solution of **24** (43 mg, 0.072 mmol), 4-aminobutyric acid methyl ester hydrochloride (76.5 mg, 0.50 mmol), 4-dimethylamino-pyridine (13 mg, 0.09 mmol) and DCC (30 mg, 0.15 mmol) in THF (10 ml) was heated for 3 h at 70 °C. The reaction mixture was cooled and acidified to pH 3 with diluted hydrochloric acid. After being concentrated to dryness, the mixture was chromatographed on RP-18 with MeOH–H_2O (6 : 4—1 : 0) to obtain compound **28** as a white powder: yield 63.9% (32 mg), $[\alpha]_D^{25}$ +43.6° (c=1.00, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.72 (3H, s), 0.77 (3H, s), 0.87 (3H, s), 0.90 (6H, s), 0.92 (3H, s), 1.13 (3H, s) (7×CH_3), 1.66 (overlapped signals, H-3′, 4′), 1.83 (overlapped signals, H-2″), 2.20 (4H, br s, H-2′, 3″), 2.37 (2H, t, J=7.0 Hz, H-5′), 2.85 (1H, dd, J=13.6, 3.4 Hz, H-18), 3.29 (2H, dt, J=6.7, 6.0 Hz, H-1″), 3.62 (3H, s, CH_3O-28), 3.68 (3H, s, CH_3O-4″), 3.68 (overlapped signal, H-3), 5.28 (1H, t-like, H-12), 5.45 (1H, d, J =9.9 Hz, NH), 6.06 (1H, t-like, NH); MS (EI): 696 $[M]^+$ (40), 435 (95), 203 (base).

***N*^{1},*N*^{6}-Bis(28-carboxyolean-12-en-3-yl) Adipic Acid Diamide (29)** Under an atmosphere of argon, compound **30** (10 mg, 0.01 mmol) in freshly distilled collidine (0.5 ml) was heated with dehydride lithium iodide (16 mg) under reflux for 9 h. The reaction mixture was cooled, poured into water (5 ml), acidified to pH 4 with dilute hydrochloric acid, and extracted with ether. The ether layer was extracted three times with 5% $Na_2S_2O_3$, dried over Na_2SO_4 and evaporated to give a solid, which was purified by a SiO_2 column with $CHCl_3$–MeOH (98 : 2) to give **29** (3 mg) as a white powder, 30.0% yield. ^{1}H-NMR ($CDCl_3$–CD_3OD 2 : 1): δ 0.77 (6H, s), 0.79 (6H, s), 0.87 (6H, s), 0.90 (12H, s), 0.93 (6H, s), 1.15 (6H, s) (14×CH_3), 1.61 (overlapped signals, H-3′, 4′), 2.20 (4H, t-like, H-2′,5′), 2.83 (2H, dd, J=13.2, 4.1 Hz, H-18, 18″), 3.66 (2H, overlapped with H_2O signal, H-3, 3″), 5.27 (2H, t-like, H-12, 12″); MS (negative-ESI): 1019 $[M-1]^-$ (3).

***N*^{1},*N*^{6}-Bis(28-methoxycarbonylolean-12-en-3-yl) Adipic Acid Diamide (30)** Adipoyl chloride (18.3 μl, 0.1 mmol) was added to a solution of compound **19** (40 mg, 0.085 mmol) in THF (5 ml). The mixture was stirred at room temperature for 1.5 h and then partitioned with H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness and subjected to neutral Al_2O_3 chromatography, with MeOH/$CHCl_3$ (0—10%) to give **30** (12 mg) as a white powder, 26.9% in yield. $[\alpha]_D^{25}$ +45.9° (c=0.6, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.72 (6H, s), 0.76 (6H, s), 0.87 (6H, s), 0.89 (12H, s), 0.92 (6H, s), 1.13 (6H, s) (14×CH_3), 1.66 (overlapped signals, H-3′,4′), 2.20 (4H, t-like, H-2′,5′), 2.85 (2H, dd, J=13.2, 4.1 Hz, H-18, 18″), 3.62 (6H, s, OCH_3-28, 28″), 3.66 (2H, dt, J=9.9, 4.8 Hz, H-3, 3″), 5.28 (2H, t-like, H-12, 12″), 5.39 (2H, d, J=9.9 Hz, NH); *Anal.* Calcd for $C_{68}H_{108}O_6N_2 \cdot 2H_2O$: C, 75.23; H, 10.40; N, 2.58. Found: C, 75.58; H, 10.15; N, 2.47. MS (positive-ESI): 1049 $[M+1]^+$ (10).

Olean-12-ene-3β,22β-diol (Sophoradiol, 31) 3-*O*-[α-L-Rhamnopyranosyl-(1→2)-β-D-galactopyranosyl-(1→2)-β-D-glucuronopyranosyl]-sophoradiol (kaikasaponin III, 150 mg)[6] was refluxed with 7% HCl in H_2O–EtOH (1 : 1, 50 ml) for 4 h. The reaction mixture was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was concentrated to dryness. The residue was chromatographed on an ODS column (H_2O–MeOH 1 : 1—0 : 1), and purified with HPLC (ODS column, 80—100% MeOH), and SiO_2 preparative TLC (benzene–EtOAc 9 : 1) to give sophoradiol (**31**, 15 mg, 20.9% yield). $[\alpha]_D^{24}$ +77.5° (c=0.51, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.79 (3H, s), 0.88 (3H, s), 0.91 (3H, s), 0.95 (3H, s), 0.98 (3H, s), 1.00 (3H, s), 1.04 (3H, s), 1.12 (3H, s) (8×CH_3), 2.10 (1H, br d, J=11.2 Hz, H-18), 3.22 (1H, dd, J=5.1, 11.0 Hz, H-3), 3.44 (1H, t, J=5.1Hz, H-22), 5.25 (1H, t, J=3.6 Hz, H-12); MS (EI): 442 $(M)^+$ (15), 424 (15), 406 (8), 234 (100).

3,22-Di-*O*-adipoylsophoradiol (32) Compound **31** was treated with adipoyl chloride as described for **7** to yield **32** (5 mg, 45.3%). $[\alpha]_D^{24}$ +57.8° (c=0.29, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.81 (3H, s), 0.87 (6H, s), 0.89 (3H, s), 0.97 (6H, s), 0.98 (3H, s), 1.15 (3H, s) (8×CH_3), 2.19 (1H, br d, J=10.5 Hz, H-18), 2.35 (8H, m, H-2′, 5′, 2″, 5″), 4.51 (1H, t, J=7.8 Hz, H-3), 4.66 (1H, t-like, H-22), 5.26 (1H, t-like, H-12); MS (positive-ESI): 721 $[M+Na]^+$ (100); (negative-ESI): 697 $[M-1]^-$ (100), 587 (10), 569 (30).

***N*-[3β-Hydroxylolean-12-en-28-oyl]-6-aminohexanoic Acid (33)** To a solution of 3-*O*-acetyloleanolic acid[2] (400 mg, 0.80 mmol) in $CHCl_3$ was added oxalyl chloride (0.3 ml, 3.42 mmol). The reaction mixture was stirred at room temperature for 20 h and the solvent was removed. Cyclohexane (20 ml) was added to the residue, and the mixture was evaporated to dryness. This was repeated twice to yield crude 3β-acetoxylolean-12-en-28-oyl chloride.

To a suspension of 6-aminohexanoic acid (500 mg) in 20 ml of MeOH–benzene (5 : 1) was added 4 ml of 10% trimethylsilidiazomethane (TMS–CHN_2) in hexane. The mixture was stirred at room temperature for 2 h, concentrated to dryness and partitioned with H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness to yield methyl 6-aminohexanoate (150 mg).

A CH_2Cl_2 solution (50 ml) of the above prepared methyl 6-aminohexanoate (150 mg, 1.03 mmol) and crude 3β-acetoxyolean-12-en-28-oyl chloride was stirred in the presence of triethylamine (0.3 ml, 2.15 mmol) at room temperature overnight. The reaction mixture was concentrated under vacuum and partitioned with H_2O and $CHCl_3$. The $CHCl_3$ layer was concentrated to dryness and chromatographed over ODS with H_2O–MeOH. The MeOH eluate was further purified by SiO_2 column chromatography with hexane–AcOEt (9 : 1—8 : 1) to give methyl *N*-[3β-acetoxyolean-12-en-28-oyl]-6-aminohexanoate as white powder (150 mg, 30.0% in yield). $[\alpha]_D^{25}$ +37.4° (c=0.28, $CHCl_3$); ^{1}H-NMR ($CDCl_3$): δ 0.76 (3H, s), 0.86 (3H, s), 0.87 (3H, s), 0.91 (6H, s), 0.94 (3H, s), 1.17 (3H, s) (7×CH_3), 2.05 (3H, s, CH_3CO), 2.31 (2H, t, J=7.2 Hz H-5′), 2.50 (1H, br d, J=12.6 Hz, H-18), 2.99 (1H, m, H_a-1′), 3.36 (1H, m, H_b-1′), 3.67 (3H, s, 6′-OCH_3), 4.49 (1H, t, J=7.8 Hz, H-3), 5.37 (1H, t-like, H-12), 5.93 (1H, t-like, NH); MS (positive-ESI): 648 $[(M+Na)^+$, 90], 626 $[(M+1)^+$, 100].

A solution of methyl *N*-[3β-acetoxylolean-12-en-28-oyl]-6-aminohexanoate (130 mg, 0.21 mmol) and aqueous NaOH (4 N, 1.2 ml) in CH_3OH–THF (1 : 1.5, 5 ml) was stirred for 18 h at room temperature and then concentrated *in vacuo*. The residue was suspended in water, treated with 4 N HCl to pH 3 and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O to pH 6 and concentrated to dryness to give **33** (118 mg, 99.0% in yield). $[\alpha]_{23}^D$ +47.2° (c=1.38, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.76 (3H, s), 0.79 (3H, s), 0.90 (9H, s), 0.99 (3H, s), 1.16 (3H, s) (7×CH_3), 2.35 (2H, t, J=7.5 Hz, H-5′), 2.48 (1H, br d, J=10.5 Hz, H-18), 3.00 (1H, m, H_a-1′), 3.23 (1H, dd, J=5.1, 10.2 Hz, H-3), 3.36 (1H, m, H_b-1′), 5.38 (1H, t-like, H-12), 5.99 (1H, t-like, NH); MS (positive-ESI): 592 $[(M+Na)^+$, 100], 570 $[M+H]^+$ (100); *Anal.* Calcd for $C_{36}H_{59}O_4N \cdot 2H_2O$: C, 71.36; H, 10.48; N, 2.31. Found: C, 71.30; H, 9.78; N, 2.26. (negative -ESI): 568 $[M-H]^-$ (100).

***N*-[3β-*O*-Adipoylolean-12-en-28-oyl]-6-aminohexanoic Acid (34)** Compound **33** (50 mg, 0.09 mmol) was treated with adipoyl chloride as described for **7** to yield **34** (22 mg, 35.9%). $[\alpha]_D^{25}$ +39.9° (c=0.69, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 0.76 (3H, s), 0.86 (6H, s), 0.90 (6H, s), 0.94 (3H, s), 1.16 (3H, s) (7×CH_3), 2.35 (6H, t, J=7.2 Hz H-2′, 5′, 5″), 2.48 (1H, br d, J=10.5 Hz, H-18), 3.01 (1H, m, H_a-1′), 3.35 (1H, m, H_b-1′), 4.50 (1H, t, J=7.8 Hz, H-3), 5.38 (1H, t-like, H-12), 6.00 (1H, t-like, NH); MS (positive-ESI): 698 $[M+H]^+$ (100), 552 (100); (negative-ESI): 696 $[M-H]^-$ (100).

HIV-1 Protease Assay The same HIV-1 PR assay kit and method were used as described previously.[2]

Lipase Assay Four tubes, each containing 0.9 ml of a lipase-buffer solution (0.4 mg/ml, pH 7.7 at 37 °C) and 75 μl of 1.2 mM compound **7** or **14**

(DMSO) were incubated at 37 °C for 1, 5, and 18 h, respectively. The mixture was then extracted with $CHCl_3$ (3×6 ml). The $CHCl_3$ layer was washed with 1 ml H_2O and then concentrated to dryness. The residues were co-chromatographed on silica gel TLC ($CHCl_3$–acetone 20 : 1) with compounds **7** and **14**, and oleanolic acid. All the samples from 1, 5, and 18 h of incubation displayed only one spot, corresponding to the starting materials, **7** or **14**. Even a trace of the hydrolyzed product, oleanolic acid, could not be detected in any reaction mixture.

Size Exclusion Chromatography The chromatography was done at 4 °C using a column of Sephadex G-50, equilibrated with a buffer containing 20 mM phosphate, 1 mM DTT, and 1 mM EDTA at pH 5.5. A flow rate of 0.52 ml/min was maintained, and the eluant was monitored with a fluorospectrometer (for insulin at 302 nm, λ_{ex}=226 nm; for PR at 365 nm, λ_{ex}= 226 nm) or a UV/VIS spectrometer for cytochrome C (413 nm). A standard curve was generated using insulin (M.W. 5733) and cytochrome C (M.W. 12327). Fifty microliters of 0.07 mg/ml HIV PR solution was loaded on the column, and the molecular weight of the eluent peaks was calculated from the standard curve. Similarly, 50 μl of 0.07 mg/ml HIV PR solution was preincubated with either 1 μl of a 5 mM solution of **7** (DMSO) or 1 μl of a 0.15 mM solution of acetylpepstatin (DMSO) for 2 h at room temperature (20 °C). This solution was loaded on the column and the molecular weight of the eluting species calculated.

References and Notes

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