Anti-AIDS Agents. 78.[†] Design, Synthesis, Metabolic Stability Assessment, and Antiviral Evaluation of Novel Betulinic Acid Derivatives as Potent Anti-Human Immunodeficiency Virus (HIV) Agents

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In a continuing study of potent anti-HIV agents, seventeen 28,30-disubstituted betulinic acid (BA, **1**) derivatives and seven novel 3,28-disubstituted BA analogues were designed, synthesized, and evaluated for in vitro antiviral activity. Among them, compound **21** showed an improved solubility and equal anti-HIV potency ($EC_{50} = 0.09\ \mu M$) when compared to HIV entry inhibitors **3b** (IC9564, (3*R*,4*S*)-*N*′-[*N*-[3β-hydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoyl]-4-amino-3-hydroxy-6-methylheptanoic acid) and **4** (A43-D, [[*N*-[3β-*O*-(3′,3′-dimethylsuccinyl)-lup-20(29)-en-28-oyl]-7-aminoheptyl]carbamoyl]methane). Using a cyclic secondary amine to form the C-28 amide bond increased the metabolic stability of the derivatives significantly in pooled human liver microsomes. The most potent compounds **47** and **48** displayed potent anti-HIV activity with EC_{50} values of 0.007 and 0.006 μM, respectively. These results are slightly better than that of bevirimat (**2**, 3′,3′-dimethylsuccinylbetulinic acid), which is currently in phase IIb clinical trials. Compounds **47** and **48** should serve as attractive promising leads to develop next generation, metabolically stable, 3,28-disubstituted bifunctional HIV-1 inhibitors as clinical trials candidates.

Introduction

As the world enters the third decade of the AIDS[a] epidemic, this pandemic has rapidly grown into the fourth leading cause of mortality globally.[1] Introduction of highly active antiretroviral therapy (HAART), which employs a combination of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs), has significantly improved the treatment of HIV/AIDS.[2−5] However, the virus is suppressed rather than eradicated by HAART.[6−8] On HAART regimens, multiple drug therapies can lead to increased adverse effects and toxicities due to long-term use and drug−drug interactions.[9,10] Moreover, it inevitably leads to the emergence of multidrug-resistant viral strains.[11] In fact, a significant proportion of newly infected individuals harbor HIV-1 isolates that are resistant to at least one ART.[12,13] Therefore, novel potent antiretroviral agents are needed, with different targets than currently approved drugs and preferably with simplified treatment regimens (fewer pills and less-frequent administration).

Triterpenes, such as betulinic acid (BA, **1**, Figure 1),[14] represent a promising class of anti-HIV agents with novel mechanisms. Two types of BA derivatives have exhibited potent anti-HIV profiles. C-3 esterification of BA led to the discovery of bevirimat (DSB, PA-457, **2**, 3′,3′-dimethylsuccinylbetulinic acid),[15] which is a HIV-1 maturation inhibitor (MI) that blocks cleavage of p25 (CA-SP1) to functional p24 (CA), resulting in the production of noninfectious HIV-1 particles.[16,17] Bevirimat (**2**) is currently in phase IIb clinical trials launched by Panacos Pharmaceuticals, Inc.[18,19] On the other hand, the C-28 side chain was proven to be a necessary pharmacophore for anti-HIV entry activity, as seen with the equipotent diastereomers **3a** (RPR103611, (3*S*,4*S*)-*N*′-[*N*-[3β-hydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoyl]-4-amino-3-hydroxy-6-methylheptanoic acid)[20,21] and **3b** (IC9564, (3*R*,4*S*)-*N*′-[*N*-[3β-hydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoyl]-4-amino-3-hydroxy-6-methylheptanoic acid).[22,23] Mechanism of action studies have revealed that C-28 modified BA derivatives function at a post-binding, envelope-dependent step involved in fusion of the virus to the cell membrane.[24] Recent studies further suggested that **3b** may also function by targeting the V3 loop of gp120, a domain involved in chemokine receptor binding.[25] Although **3a** showed potent antiviral activity in vitro, the clinical development of **3a** by Rhone-Poulenc (now Sanofi-Aventis) was stopped because of poor "pharmacodynamic properties".[26] However, the high potency and novel mechanism of **3a** suggest that further modification of this compound class as HIV entry inhibitors is warranted. Therefore, in the current study, we mainly focused on the modification of BA to maintain the anti-HIV activity while improving pharmacokinetic properties.

Design

Structurally, the triterpenoid skeleton of BA contains three functional groups: C-3 hydroxyl group, C-28 carboxylic acid

[†] For part 77, see the following. Xu, S.; Yan, X.; Chen, Y.; Xia, P.; Yu, D.; Qian, K.; Xia, Y.; Yang, Z. Y.; Morris-Natschke, S. L.; Lee, K. H. Anti-AIDS agents. 77. Synthesis and anti-HIV activity of 2′-monomethyl-4-methyl DCK and 1′-thia-4-methyl DCK analogs. J. Med. Chem., submitted.

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[a] Abbreviations: AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; NRTIs, nucleoside/nucleotide reverse transcriptase inhibitors; NNRTIs, non-nucleoside reverse transcriptase inhibitors; PIs, protease inhibitors; MI, maturation inhibitor; BA, betulinic acid; P24 (CA), capsid; CYPs, cytochrome P450; UGTs, UDP-glucuronosyltransferases; FMO, flavin containing monooxygenase; NBS, *N*-bromosuccinimide; PDC, pyridinium dichromate; HOBt, hydroxybenzotriazole; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; DMAP, 4-(dimethylamino)pyridine; AZT, zidovudine.

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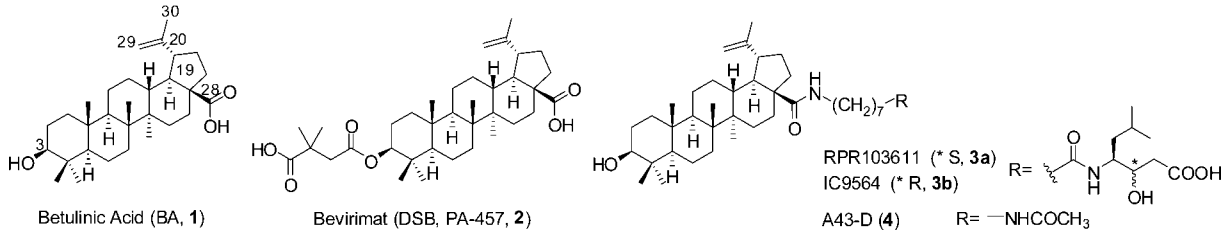
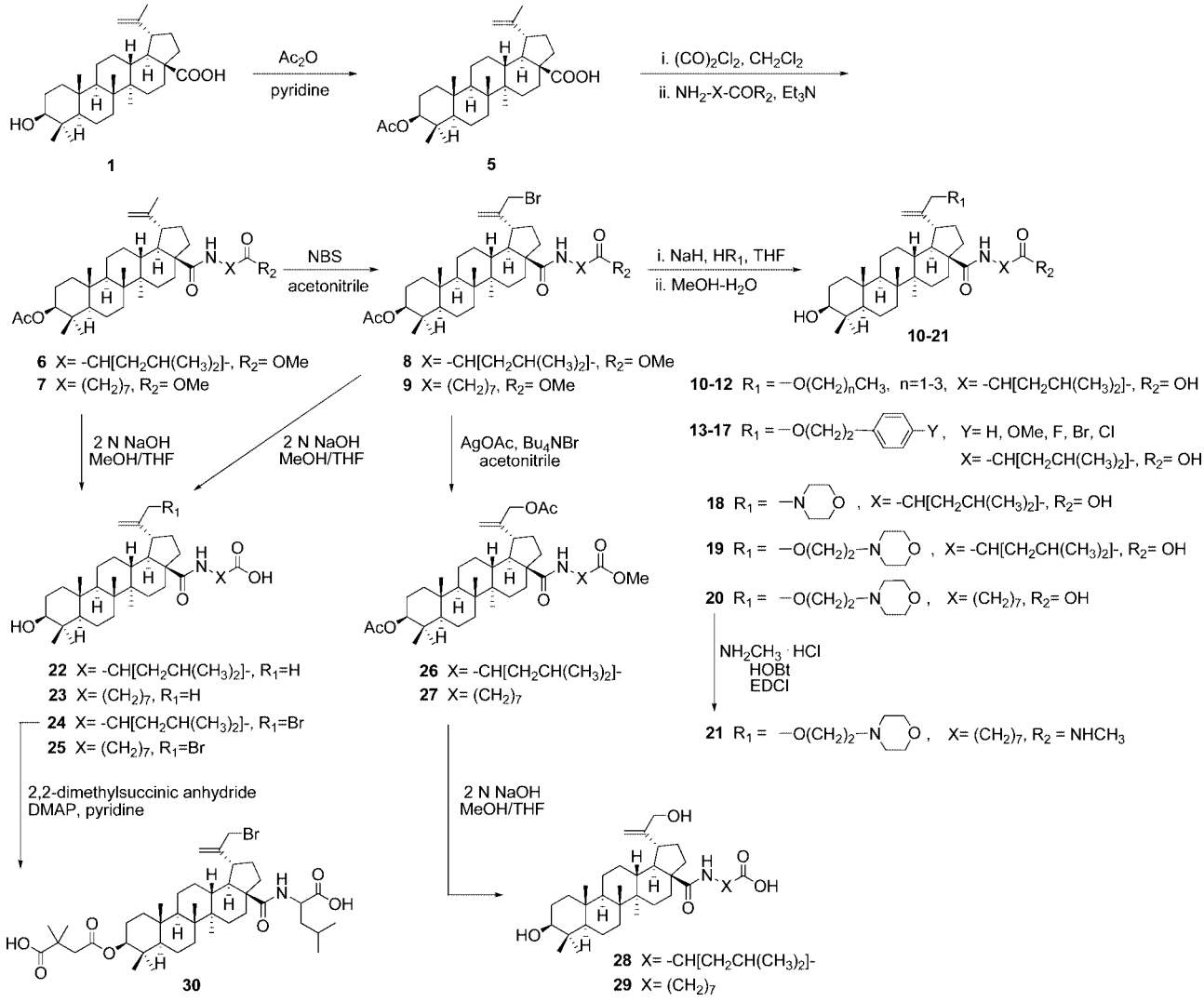


Figure 1

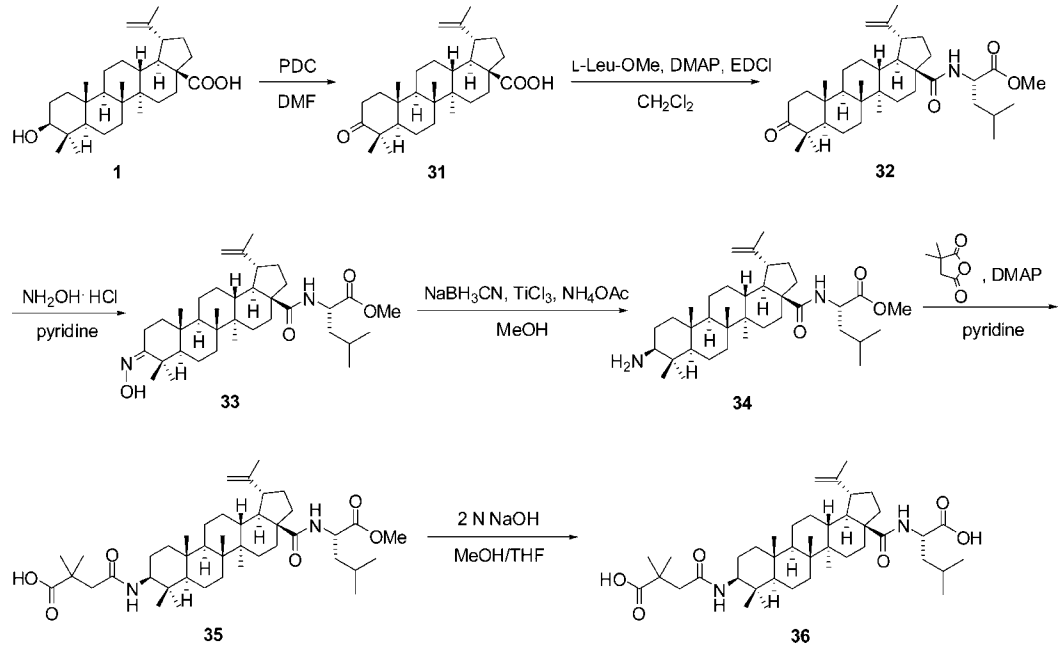
Scheme 1. General Synthetic Route for 28,30-Disubstituted BA Derivatives



group, and C-19 isopropenyl moiety. Because the C-19 isopropenyl group has been less investigated, modification was first carried out on this moiety. Previous research suggested that saturation of the 20(29) double bond did not influence the antiviral activity of the BA derivatives significantly.[15] Therefore, we focused on the modification at the C-30 allyl position in order to better explore the SAR. Some early data suggested that thioether-linked substitution on the C-30 position retained the anti-HIV-1 potency slightly in the resulting BA analogues.[27] In the current study, the bioisosteric oxygen ether linker was chosen to replace the thioether group. Leucine and 8-aminooctanoic acid were proved to improve entry inhibition in our previous study; thus, they were incorporated into the C-28 side chain of the diverse C-30 modified analogues to yield compounds **10**−**30**.

The fact that **2** has suitable pharmocokinetic properties for development, but **3a** does not, led us to speculate that the C-28 side chain, which is critical for anti-HIV entry activity, may be responsible for the poor performance of **3a** in preclinical testing. Indeed, in our study, we observed that C-28 modified BA derivatives, including **4** (A43-D, [[*N*-[3*β*-*O*-(3′,3′-dimethylsuccinyl)-lup-20(29)-en-28-oyl]-7-aminoheptyl]carbamoyl]methane)[28] which is the prior best antientry hit, are less water-soluble. Meanwhile, although **2** has been demonstrated to be metabolized primarily by UGT glucuronidation,[29] there is no report regarding to the metabolism of C-28 modified BA analogues. Therefore, in the present study, in vitro metabolic stability assessment was first carried out in pooled human liver microsomes (BD Biosciences), which contain enzymes including cytochrome P450 (CYPs), UGTs, and FMO, etc. The results revealed that C-28 modified BA

Scheme 2. Synthesis of 3,28-Disubstituted 3β-Amino BA Derivative



derivatives like **4** are quickly metabolized in liver microsomes. Therefore, novel C-28 side chains were designed and synthesized. A cyclic secondary amine (from 4-piperidinebutyric acid), rather than a primary amine, was used to form the critical amide bond with the C-28 carboxylic acid group, which yielded **38**.

Moreover, although BA derivatives with both C-3 ester and C-28 amide side chains exhibit both antientry and maturation activity,[30] they also display some metabolic problems, likely due to the C-28 side chain. Thus, modifications were also carried out on C-28 and C-3 side chains to enhance the stability as well as increase the anti-HIV activity in the 3,28-disubstituted BA analogues, which yielded compounds **36**, **39**, **40**, and **45**−**48**. This article reports the design, syntheses, SAR, and metabolic stability assessment of these novel BA derivatives as potent HIV-1 inhibitors.

Chemistry

Scheme 1 depicts the synthetic pathway of 28,30-disubstituted BA derivatives **10**−**30**. The C-3 β-hydroxyl group of BA was protected as the acetate ester of 3-*O*-Ac-BA (**5**). Compound **5** was reacted with oxalyl chloride in dichloromethane to yield an intermediate acid chloride, which was then treated with leucine methyl ester or 8-aminooctanoic acid methyl ester to furnish **6** and **7**. Allylic bromination of **6** and **7** was carried out using *N*-bromosuccinimide (NBS) in dilute acetonitrile at room temperature to provide 30-bromo BA derivatives **8** and **9**. The bromide group of **8** and **9** was then substituted by a diverse set of nucleophilic compounds to yield **10**−**20**. In this step, the desired nucleophilic compound was first treated with 10 equiv of NaH in THF for 30 min. The 30-bromo **8** or **9** was then reacted with the resulting suspension using a microwave apparatus at 120 °C. After the mixture was cooled, 1 mL of MeOH-H_2O was added to convert the intermediate esters to carboxylic acids by saponification, which furnished the target compounds **10**−**20** in 59−77% yields. Reaction of **20** with methylamine in the presence of HOBt/EDCI in dichloromethane led to **21** in a 69% yield. Saponification of the 30-bromo **8** and **9** with 2 N sodium hydroxide in MeOH/THF yielded the corresponding carboxylic acids **24** and **25**. The previous reported **22** and **23** were also prepared by saponification of the ester intermediates **6** and **7**. Reaction of silver acetate with **8** and **9** in the presence of a catalytic amount of the phase transfer catalyst tetrabutylammonium bromide (Bu_4NBr) in acetonitrile gave diacetoxy esters **26** and **27**, which were then converted to 30-hydroxyl BA derivatives **28** and **29**. The 3,28,30-trisubstituted analogue **30** was acquired by reaction of **24** with 2,2-dimethylsuccinic anhydride in the presence of DMAP in pyridine.

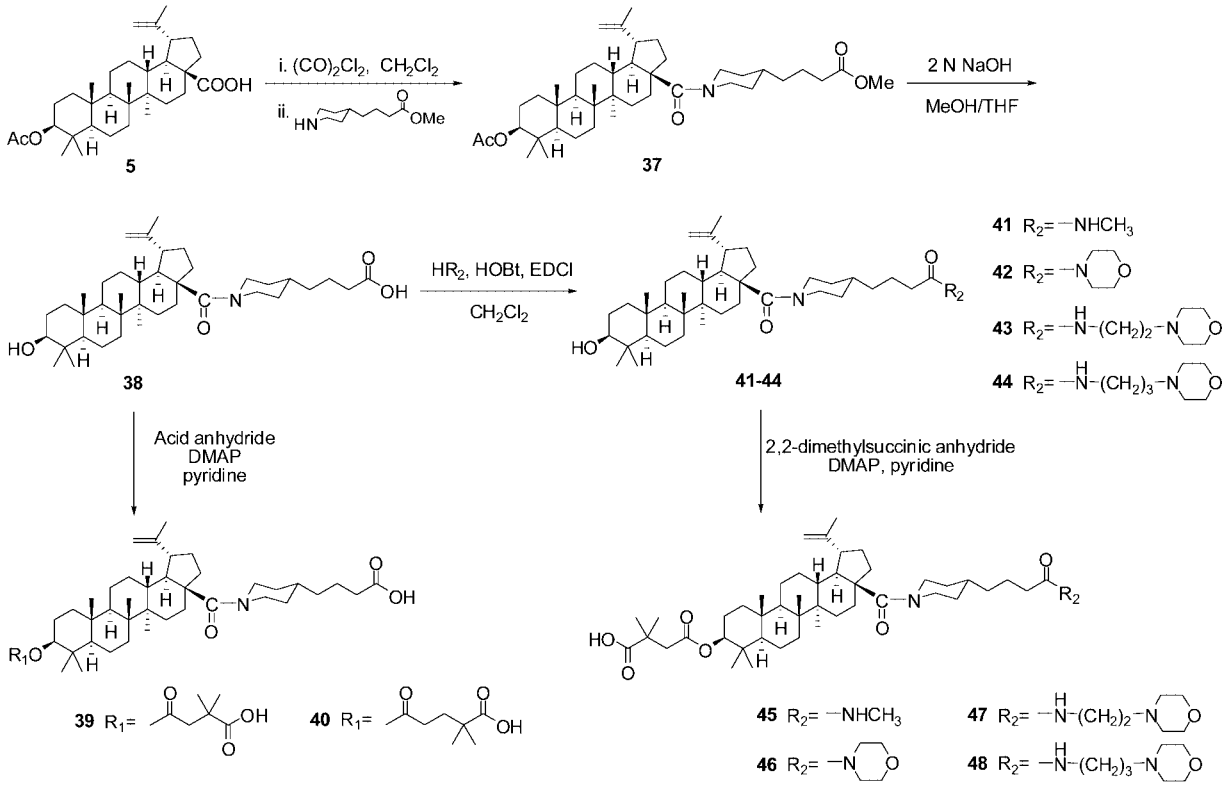
The 3,28-disubstituted 3β-amino analogue **36** was successfully prepared as described in Scheme 2. Oxidation of **1** with 2 equiv of PDC produced the 3-keto-BA **31** (87% yield). L-Leucine methyl ester was reacted with the C-28 carboxylic acid of **31** in the presence of DMAP and EDCI to furnish **32**. The keto moiety of **32** was then converted to an oxime by treatment with hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) in pyridine, which yielded **33** (90% yield).[31] The 3β-amine **34** was readily prepared in 82% yield from oxime **33** by enantioselective reduction of the Schiff base with $TiCl_3$ and $NaCNBH_3$, as reported by Leeds and Kirst.[32] Treatment of **34** with 2,2-dimethylsuccinic anhydride under DMAP in pyridine yielded **35**. Finally, hydrolysis of **35** with 2 N sodium hydroxide furnished the desired 3,28-disubstituted 3β-amino BA analogue **36**.

The syntheses of 3,28-disubstituted 28-piperidine analogues **38**−**48** were carried out according to Scheme 3. The 3-*O*-Ac-BA (**5**) was treated with oxalyl chloride in dichloromethane, followed by reaction with the readily prepared 4-piperidinebutyric acid methyl ester to provide **37** in a 94% yield. Saponification of **37** yielded the desired lead compound **38** quantitatively. Esterification of **38** with 2,2-dimethylsuccinic anhydride and 2,2-dimethylglutaric anhydride under DMAP in pyridine led to **39** and **40** in yields of 55% and 36%, respectively. The syntheses of **41**−**44** were carried out by reacting **38** with different amines in the presence of HOBt and EDCI. Reaction of the 3β-hydroxyl group of **41**−**44** with 2,2-dimethylsuccinic anhydride provided the 3,28-disubstituted target compounds **45**−**48** in yields of 58−81%.

Results and Discussion

The anti-HIV-1 replication activities of the newly synthesized BA derivatives **10**−**30**, **36**, **38**−**40**, and **45**−**48** were assessed in HIV-1_{IIIB} infected MT-2 lymphocytes in parallel

Scheme 3. General Synthetic Route for 3,28-Disubstituted 28-Piperidine BA Analogues



with AZT and **2**. Compounds **21** and **45**−**48** were further evaluated against HIV-1_{NL4-3} in MT-4 cell lines and compared with **3b** and **4**. Because these two antiviral screening systems used slightly different protocols, the results may vary for the same compounds. The bioassay data are summarized in Tables 1 and 2, respectively, and the SAR conclusions are summarized in Figure 2.

Within the 28,30-disubstituted BA derivatives, the C-28 leucine, C-30 substituted analogues **10**−**19**, **24**, and **28** did not inhibit viral replication. Because the monosubstituted C-28 leucine derivative **22** did not exhibit antiviral replication activity either, we postulated that the derivatives did not lose antiviral potency due to the C-30 modification. However, because the presence of different C-30 substitutions did not increase the anti-HIV-1 activity, the C-19 isopropenyl moiety is unlikely to be an activity pharmacophore. Nevertheless, considering that BA derivatives with antientry necessary C-28 lipophilic side chains are less water-soluble, C-30 allylic modification may still be useful to influence pharmacokinetic properties, such as hydrophilicity and solubility.

The introduction of a free hydroxyl group at C-30 in **29** reduced the antiviral activity relative to **23** by severalfold to an EC_{50} value of 14.8 μM. This result suggested that a hydrogen bond donor is not tolerated near the C-19 isopropenyl group, a conclusion that is also supported by prior data that a primary amine substituent at C-30 is unfavorable.[27] In comparison, analogues with 2-morpholinoethoxy (**20**) and bromide (**25**) moieties at C-30 retained the antiviral activity of **23**. Specifically, the 28-aminooctanoic acid derivatives **23**, **20**, and **25** showed antiviral EC_{50} values of 3.3, 1.8, and 2.3 μM against HIV-1_{IIIB}, respectively. These results demonstrated that the C-30 position of BA can accommodate some diverse ethers without decreasing the anti-HIV potency. Moreover, the introduction of the morpholinoethoxy moiety in **20**, which reduced the log *P* value of **23** from 9.79 to 8.26 (calculated by ACD/LogP DB software), resulted in an increase in the derivative's solubility, confirming that the C-30 position may serve as a good place to incorporate water-solubilizing moieties.

Table 1. Anti-HIV-1 Replication Activities in HIV-1_{IIIB} Infected MT-2 Cell Lines[a]

compd	EC_{50} (μM)	CC_{50} (μM)	TI
AZT	0.056	1870	33392
2	0.011	40	3636
10	NS	40.7	
11	NS	25.3	
12	NS	24.9	
13	NS	21.7	
14	NS	16.7	
15	NS	24.4	
16	NS	20.8	
17	27.8	34.6	1.2
18	NS	27.5	
19	26.6	35.8	1.4
20	1.8	24.8	13.8
21	0.09	22.3	250
22	NS	29.8	
23	3.3	33.4	10.1
24	NS	26.8	
25	2.3	36.9	16.1
28	NS	30.7	
29	14.8	40.7	2.8
30	0.011	33.2	3022
36	13.2	35.9	2.7
38	21.72	41.0	1.9
39	0.015	20.8	1389
40	0.23	33.2	145
45	0.067	33.3	497
46	0.011	19.2	1748
47	0.007	17.3	2473
48	0.006	18.5	3087

[a] All data presented are averages of at least three separate experiments performed by Panacos Pharmaceuticals Inc., Gaithersburg, MD. EC_{50}: concentration that inhibits HIV-1_{IIIB} replication by 50%. CC_{50}: concentration that inhibits mock-infected MT-2 cell growth by 50%. TI = CC_{50}/EC_{50}. NS: no suppression at concentrations below the CC_{50}.

Table 2. Anti-HIV-1 Replication Activities in HIV-1_{NL4-3} Infected MT-4 Cell Lines[a]

compd	EC_{50} (μM)	CC_{50} (μM)	TI
3b	0.089	>10	>112.4
4	0.092	>10	>150
21	0.09	>10	>138
45	0.24	>10	>41.7
46	0.07	8.8	121.4
47	0.035	8.5	283.9
48	0.031	8.6	245.7

[a] All data presented are averages of at least two separate experiments performed by Dr. Chin-Ho Chen, Duke University, NC. EC_{50}: concentration that inhibits HIV-1_{NL4-3} replication by 50%. CC_{50}: concentration that inhibits mock-infected MT-4 cell growth by 50%. TI = CC_{50}/EC_{50}. NS: no suppression at the testing concentration (10 μM).

Analogue **21** with methylamine linked to the terminal carboxylic acid of **20** exhibited potent anti-HIV-1 activity with an EC_{50} value of 0.09 μM and TI of 250 against both HIV-1_{IIIB} and HIV-1_{NL4-3} variants. These data are similar to those with the prior best entry inhibitor **4** (EC_{50} = 0.10 μM, TI > 100). This result indicates that the amide moiety near the end of the C-28 side chain is necessary for enhanced antiviral potency. Interestingly, **21** differs from **4** in the direction of the terminal amide linkage ($-CONHCH_3$ in **21** and $-NHCOCH_3$ in **4**). Compound **21** showed a significantly reduced log *P* value of 7.5 compared with the lead compound **23** and prior best hit **4**.

Analogue **30** with a 3′,3′-dimethylsuccinyl side chain linked to the C-3 β-hydroxyl group of **24** showed extremely potent antiviral activity with an EC_{50} value of 0.011 μM and TI of 3.0 $\times$ 10^3, which are similar to those of **2** (EC_{50} = 0.011 μM, TI > 3.6 $\times$ 10^3). This result suggests that the presence of small substitutions on C-30 of BA does not harm the high anti-HIV-1 potency of **2**. Thus, incorporation of polar groups into the C-30 position may also help to improve the hydrophilicity of 3,28-disubstituted BA derivatives.

Because an amide is generally more stable in vivo than an ester moiety, we also synthesized 3,28-disubstituted 3β-amino BA derived analogue **36**. Its C-3 side chain is similar to that of **2** except for a C-3 amide rather than ester bond. However, the antiviral activity of **36** against HIV-1_{IIIB} decreased significantly to an EC_{50} value of 13.2 μM, suggesting that bioisosteric replacement of the C-3 ester bond with an amide moiety is not tolerated.

Results from the metabolism study revealed that changing the C-28 side chain from 8-aminooctanoic acid (**23**) to 4-piperidinebutyric acid (**38**) could significantly increase the in vitro metabolic stability. Specifically, approximately 50% of **23** disappeared after around 35 min of incubation with pooled human liver microsomes. A similar result was found with buspirone ($t_{1/2}$ = 31 min), an established fast-metabolized drug used as reference in the same experiment, suggesting that **23** was degraded quite easily in the assay system. Comparatively, it took about 125 min to lose 50% of the newly designed analogue **38**, indicating a much longer half-life (Table 3). This result might be due to the increased steric hindrance at the C-28 pharmacophore of **38** so that the amide bond would be less available to metabolic enzymes. Thus, **38** represents a more stable lead for the development of C-28 modified BA derived HIV-1 entry inhibitors and 3,28-disubstituted bifunctional inhibitors.

Table 3. In Vitro Metabolic Stability of Compounds **23** and **38**[a]

	percentage of remaining parent compounds, $X \pm$ SD (%)	
incubation time (min)	compd **23**	compd **38**
0	100.0	100.0
5	93.4 $\pm$ 2.5	101.7 $\pm$ 4.7
15	70.7 $\pm$ 1.5	97.6 $\pm$ 6.2
30	61.9 $\pm$ 10.1	81.4 $\pm$ 8.6
60	40.1 $\pm$ 1.6	62.7 $\pm$ 4.3
120	7.9 $\pm$ 4.2	53.8 $\pm$ 3.8

[a] Data presented are averages of two separate experiments.

From the bioassay data, we discovered that compound **39** with the C-3 side chain of **2** incorporated into **38** showed very potent anti-HIV-1 activity with an EC_{50} value of 0.015 μM and TI of 1.4 $\times$ 10^3, proving that the presence of a bulky amide moiety near C-28 does not reduce the antiviral potency of **2**. Compound **40**, with a 4′,4′-dimethylglutaryl rather than 3′,3′-dimethylsuccinyl C-3 ester side chain, had a higher antiviral EC_{50} value (0.23 μM), indicating the importance of the positioning of the dimethyl substitution in the C-3 modification of BA. However, compound **38** itself showed decreased antiviral activity compared with **23**, likely due to the slightly reduced length of the new C-28 side chain compared with the previous 8-aminooctanoic chain (**23**). Indeed, the BA derivative with 7-aminoheptanoic acid as the C-28 side chain showed 16-fold decreased anti-HIV activity compared with **23**,[20] confirming the importance of the length of the C-28 side chain.

Compounds **47** and **48**, which contain 3′,3′-dimethylsuccinyl at C-3 and a morpholine ring at the end of C-28 side chain that is separated from the terminal amide bond by a short alkyl spacer (two or three methylenes), exhibited extremely potent anti-HIV-1 replication activity against HIV-1_{IIIB} with EC_{50} values of 0.007 and 0.006 μM, respectively, which are almost 2-fold better than that of **2**. These two compounds were also 2- to 3-fold more potent than entry inhibitors **3b** and **4** in the anti-

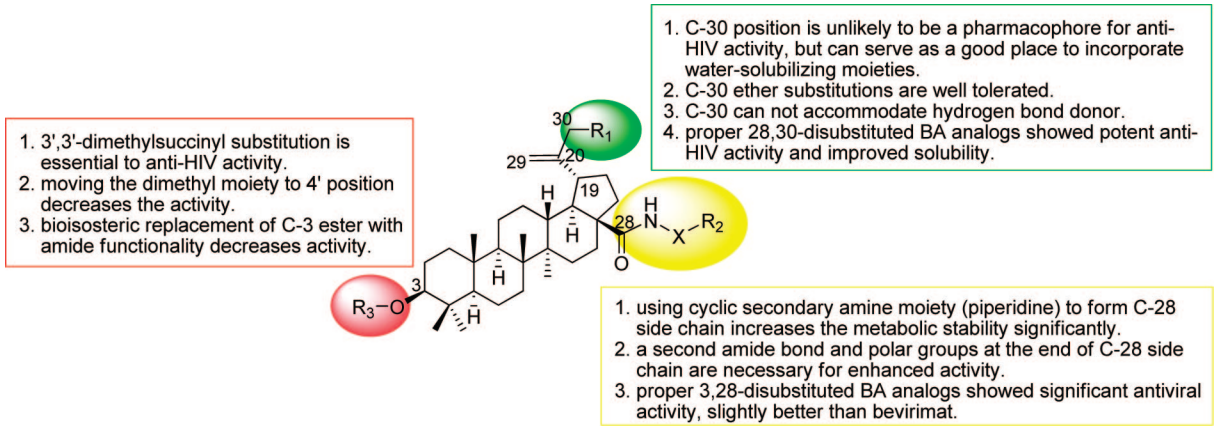


Figure 2. Summary of SAR of BA analogues.

HIV screening against HIV-1_{NL4-3}. Compound **46**, with morpholine directly involved in the amide bond, and **2** had equivalent anti-HIV-1_{IIIB} potency (EC_{50} = 0.011 μM). Compound **45** with methylamine at the end of C-28 showed a slightly decreased antiviral potency (EC_{50} = 0.067 μM) against HIV-1_{IIIB} compared with **2**. These results further confirm the importance of the length of the C-28 side chain to the enhanced antiviral potency in 3,28-disubstituted BA analogues. The better potency of **47** and **48** indicates that the activity of **2** can be increased with proper C-28 substitution. Moreover, because the C-28 side chains in all prior potent HIV inhibitors terminate in a free carboxylic acid or amide, the success of **47** and **48** also demonstrates that other polar groups at the end of this side chain can also increase antiviral potency.

In conclusion, diverse 28,30-disubstituted BA analogues were synthesized in our study. We discovered that a hydrogen bond donor is not tolerated near the C-19 isopropenyl moiety. Otherwise, C-30 substitution did not significantly influence the anti-HIV-1 activity of BA derivatives. Therefore, the C-30 position serves as a good place to incorporate water-solubilizing moieties to increase the hydrophilicity. The resulting analogue **21** showed a good solubility as well as equal potency against HIV-1 compared with the previous best antientry hit **4**. Using a cyclic secondary amine moiety (piperidine) rather than a primary amine to form the C-28 amide bond significantly increased the metabolic stability of the derivatives in pooled human liver microsome assessment. Subsequent introduction of a second amide bond at the carboxylic terminus of this metabolically stable C-28 side chain and introduction of the 3′,3′-dimethylsuccinyl side chain at the C-3 position resulted in the discovery of **47** and **48**, which showed extremely potent antiviral activity slightly better than that of **2**. They should serve as attractive promising leads for the development of a next generation of BA derived 3,28-disubstituted HIV-1 inhibitors, as clinical trial candidates.

Experimental Section

Chemistry. The melting points were measured with a Fisher Johns melting apparatus without correction. ^{1}H NMR spectra were measured on a 300 MHz Varian Gemini 2000 spectrometer using Me_4Si (TMS) as internal standard. The solvent used was $CDCl_3$ unless otherwise indicated. Mass spectra were measured on Shimadzu LCMS-2010 (ESI-MS). High resolution mass spectra (HRMS) were measured on Shimadzu LCMS-IT-TOF with ESI interface. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Target compounds were analyzed for C, H and gave values within ±0.4% of the theoretical values. HPLC for purity determinations were conducted using Shimadzu LCMS-2010 with a Grace Alltima 2.1 mm × 100 mm HP C18 3 μm column and a Shimadzu SPD-M20A detector at 200 nm wavelength. Two different solvent systems for HPLC purity analyses were as follows: (1) solvent B was acetonitrile, and solvent A was water with or without 0.1% formic acid; (2) solvent B was methanol, and solvent A was water with or without 0.1% formic acid. The flow rate was 0.3 mL/min. The isocratic HPLC mode was used, and the specific solvent percentage conditions for each tested compound were listed in the Supporting Information. All target compounds possessed a purity of at least 95% as determined by elemental analysis or by HPLC−UV−MS. Optical rotations were measured with a Jasco Dip-2000 digital polarimeter at 20 °C at the sodium D line. Thin-layer chromatography (TLC) and preparative thin-layer chromatography (PTLC) were performed on Merck precoated silica gel 60 F-254 plates. Flash+ and CombiFlash systems (Teledyn-Isco) were used as medium pressure column chromatography. Silica gel (200−400 mesh) from Aldrich, Inc., was used for column chromatography. All other chemicals were obtained from Aldrich, Inc.

3-*O*-Acetylbetulinic Acid (5). A mixture of **1** (2.1 g), pyridine (1.5 mL), and acetic anhydride (Ac_2O, 20 mL) was stirred at room temperature overnight until it became homogeneous. The mixture was then poured into ice-cold water (30 mL) and stirred for 20 min. The crude product was filtered off and purified on a silica-gel column to yield 1.98 g (87% yield) of pure **5**, white amorphous powder. Mp 289−291 °C. MS (ESI−) *m*/*z*: 497.38 (M^- − H) for $C_{32}H_{50}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.74, 4.61 (1H each, s, H-29), 4.47 (1H, dd, J = 9.9, 5.9 Hz, H-3), 3.01 (1H, m, H-19), 2.05 (3H, s, $OCOCH_3$), 1.69 (3H, s, H-30), 0.97, 0.93, 0.86, 0.84, 0.83 (3H each, s, 5 × CH_3).

Syntheses of BA Derivatives 6, 7, and 37. Oxalyl chloride solution (2 M in CH_2Cl_2, 10 mL) was added to **5** (1 equiv) in CH_2Cl_2 (10 mL) and stirred for 2 h. After concentration under vacuum, the residual mixture was treated with leucine methyl ester (1.6 equiv), 8-aminooctanoic acid methyl ester (1.6 equiv), or 4-piperidinebutyric acid methyl ester (1.6 equiv) and triethylamine (Et_3N, 1.2 equiv) in CH_2Cl_2. The mixture was stirred at room temperature overnight until no starting material was observed by TLC. The solution was then diluted with CH_2Cl_2 (20 mL) and washed three times with brine and distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds.

Methyl *N*-[3β-acetoxy-lup-20(29)-en-28-oyl]leucinate (6): 1.15 g (80.5% yield) starting from 1 g of **5**; white amorphous powder. Mp 230−232 °C. MS (ESI+) *m*/*z*: 626.48 (M^+ + H), 648.47 (M^+ + Na) for $C_{39}H_{63}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.87 (1H, d, J = 8 Hz, −CON*H*−), 4.72, 4.59 (1H each, s, H-29), 4.64 (1H, m, −NHC*H*−), 4.49 (1H, t, J = 8 Hz, H-3), 3.73 (3H, s, $-COOCH_3$), 3.05 (1H, m, H-19), 2.10−2.20 (1H, m, H-13), 2.04 (3H, s, $OCOCH_3$), 1.68 (3H, s, H-30), 1.01 (6H, s, leucine moiety $-(CH_3)_2$), 0.97 (6H, s, 2 × CH_3), 0.89, 0.84, 0.83 (3H each, s, 3 × CH_3).

Methyl *N*-[3β-acetoxy-lup-20(29)-en-28-oyl]-8-aminooctanoate (7): 643 mg (98% yield) starting from 500 mg of **5**; light-yellow amorphous powder. Mp 104−105 °C. MS (ESI+) *m*/*z*: 654.5 (M^+ + H) for $C_{41}H_{67}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.57 (1H, t, J = 6 Hz, −CON*H*−), 4.73, 4.60 (1H each, s, H-29), 4.45 (1H, m, H-3), 3.67 (3H, s, $-COOCH_3$), 3.30−3.08 (3H, m, H-19, $-CONHCH_2-$), 2.50 (1H, m, H-13), 2.31 (2H, t, J = 7 Hz, $-CH_2COOCH_3$), 2.05 (3H, s, $OCOCH_3$), 1.68 (3H, s, H-30), 0.97, 0.94 (3H each, s, 2 × CH_3), 0.85, 0.84, 0.81 (3H each, s, 3 × CH_3).

Methyl *N*-[3β-acetoxy-lup-20(29)-en-28-oyl]-4-piperidinebutanoate (37): 1.02 g (94% yield) starting from 800 mg of **5**; white amorphous powder. Mp 195−197 °C. MS (ESI+) *m*/*z*: 666.5 (M^+ + H) for $C_{42}H_{67}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.72, 4.57 (1H each, s, H-29), 4.47 (1H, dd, J = 11.1, 5.7 Hz, H-3), 3.67 (3H, s, $-COOCH_3$), 3.67−3.47 (4H, m, 28-CON(CH_2CH_2)$_2$CH−), 2.99 (1H, m, H-19), 2.31 (2H, t, J = 7 Hz, $-CH_2COOCH_3$), 2.05 (3H, s, $OCOCH_3$), 1.68 (3H, s, H-30), 0.96 (6H, s, 2 × CH_3), 0.94, 0.82, 0.75 (3H each, s, 3 × CH_3).

Syntheses of BA Derivatives 8 and 9. A mixture of *N*-bromosuccinimide (1.1 equiv) and **6** or **7** (1 equiv) in acetonitrile (ACN, 30 mL) was stirred at room temperature until the starting material was not observed by TLC. The mixture was concentrated to dryness under reduced pressure and chromatographed over silica gel to yield pure target compounds.

Methyl *N*-[3β-acetoxy-30-bromo-lup-20(29)-en-28-oyl]leucinate (8): 297 mg (66% yield) starting from 400 mg of **6**; light-yellow amorphous powder. Mp 127−129 °C. MS (ESI+) *m*/*z*: 704.4 (M^+ + H), 706.4 (M^+ + H) for $C_{39}H_{62}BrNO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.81 (1H, d, J = 8 Hz, −CON*H*−), 5.11, 5.05 (1H each, s, H-29), 4.65 (1H, m, −NHC*H*−), 4.45 (1H, t, J = 8 Hz, H-3), 4.00 (2H, s, H_2-30), 3.72 (3H, s, $-COOCH_3$), 3.10 (1H, m, H-19), 2.50−2.32 (1H, m, H-13), 2.05 (3H, s, $OCOCH_3$), 1.02 (6H, s, leucine moiety $-(CH_3)_2$), 0.99 (6H, s, 2 × CH_3), 0.89, 0.86, 0.85 (3H each, s, 3 × CH_3).

Methyl *N*-[3*β*-acetoxy-30-bromo-lup-20(29)-en-28-oyl]-8-aminooctanoate (9): 402 mg (72.5% yield) starting from 360 mg of **7**; light-yellow amorphous powder. Mp 99−101 °C. MS (ESI+) *m/z*: 732.4 ($M^+ + H$), 734.4 ($M^+ + H$) for $C_{41}H_{66}BrNO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.59 (1H, t, J = 6 Hz, −CON*H*−), 5.13, 5.04 (1H each, s, H-29), 4.47 (1H, t, J = 8.1 Hz, H-3), 4.00 (2H, s, H_2-30), 3.67 (3H, s, −$COOCH_3$), 3.41−3.09 (3H, m, H-19, −CONHCH_2−), 2.46 (1H, m, H-13), 2.31 (2H, t, J=7.5 Hz, −C$H_2$$COOCH_3$), 2.04 (3H, s, $OCOCH_3$), 0.97, 0.93 (3H each, s, 2 × CH_3), 0.89, 0.84, 0.83 (3H each, s, 3 × CH_3).

Syntheses of BA Derivatives 10−20. NaH (60% in mineral oil) was washed three times with hexane. A solution of appropriate nucleophilic compound (8 equiv) and NaH (10 equiv) in anhydrous THF (1.5 mL) was stirred under dry nitrogen at room temperature for 30 min. The 30-bromo BA derivative **8** or **9** (1 equiv) was then added into the system. The mixture was heated using microwave (Biotage) at 120 °C for 30 min. After the mixture was cooled to room temperature, 1 mL of MeOH−H_2O was added into the mixture and stirred to transform the intermediate esters to carboxylic acids by saponification. The mixture was neutralized with 10% HCl and dried under vacuum and reconstituted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds.

***N*-[3*β*-Hydroxy-30-ethoxy-lup-20(29)-en-28-oyl]leucine (10):** 22 mg (59% yield) starting from 40 mg of **8**; white amorphous powder. Mp 128−130 °C. MS (ESI−) *m/z*: 612.4 (M^- − H) for $C_{38}H_{63}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.88 (1H, d, J = 8 Hz, −CON*H*−), 4.93, 4.92 (2H, br s, H-29), 4.63−4.58 (1H, m, −NHC*H*−), 3.90 (2H, s, H_2-30), 3.47 (2H, m, 30-OC$H_2$$CH_3$), 3.18 (1H, dd, J = 11.1, 5.4 Hz, H-3), 2.99 (1H, m, H-19), 2.50−2.32 (1H, m, H-13), 1.00 (9H, br s, 30-$OCH_2C$$H_3$, leucine moiety −$(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.89, 0.86, 0.85 (3H each, s, 3 × CH_3).

***N*-[3*β*-Hydroxy-30-propoxy-lup-20(29)-en-28-oyl]leucine (11):** 23 mg (60% yield) starting from 40 mg of **8**; white amorphous powder. Mp 116−117 °C. MS (ESI−) *m/z*: 626.5 (M^- − H) for $C_{39}H_{65}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.13 (1H, br s, −CON*H*−), 4.91, 4.90 (2H, br s, H-29), 4.52 (1H, m, −NHC*H*−), 3.90 (2H, s, H_2-30), 3.36 (2H, t, J = 6.9 Hz, 30-OC$H_2$$CH_2CH_3$), 3.18 (1H, dd, J = 11.1, 5.4 Hz, H-3), 2.99 (1H, m, H-19), 0.96, 0.94, 0.92, 0.89 (15H, m, 30-$O(CH_2)_2C$$H_3$, leucine moiety −$(CH_3)_2$, CH_3-23, 24), 0.82, 0.81, 0.79 (3H each, s, 3 × CH_3).

***N*-[3*β*-Hydroxy-30-butoxy-lup-20(29)-en-28-oyl]leucine (12):** 10 mg (37% yield) starting from 30 mg of **8**; yellow amorphous powder. Mp 104−105 °C. MS (ESI−) *m/z*: 640.2 (M^- − H) for $C_{40}H_{67}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.01 (1H, br s, −CON*H*−), 4.90, 4.88 (2H, br s, H-29), 4.58 (1H, m, −NHC*H*−), 3.89 (2H, s, H_2-30), 3.37 (2H, m, 30-OC$H_2$$(CH_2)_2CH_3$), 3.17 (1H, m, H-3), 3.01 (1H, m, H-19), 0.99 (9H, br s, 30-$O(CH_2)_3C$$H_3$, leucine moiety −$(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.86, 0.84, 0.81 (3H each, s, 3 × CH_3).

***N*-[3*β*-Hydroxy-30-phenethoxy-lup-20(29)-en-28-oyl]leucine (13):** 37 mg (77% yield) starting from 50 mg of **8**; light-yellow amorphous powder. Mp 155−157 °C. MS (ESI−) *m/z*: 688.4 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.68−7.62 (2H, m, H ar-3′), 7.28−7.20 (3H, m, H ar-2′, 4′), 5.97 (1H, br s, −CON*H*−), 4.91, 4.90 (2H, br s, H-29), 4.44 (1H, m, −NHC*H*−), 3.93 (2H, s, H_2-30), 3.64 (2H, t, J = 7.2 Hz, 30-OC$H_2$$CH_2$Ph), 3.17 (1H, dd, J = 11.1, 5.4 Hz, H-3), 2.91 (1H, m, H-19), 2.57 (2H, m, 30-$OCH_2C$$H_2$Ph), 0.95 (12H, s, leucine moiety −$(CH_3)_2$, CH_3-23, 24), 0.89, 0.78, 0.74 (3H each, s, 3 × CH_3). Anal. ($C_{44}H_{67}O_5N$·$2H_2O$) C, H, O.

***N*-[3*β*-Hydroxy-30-(4′-methoxyphenethoxy)-lup-20(29)-en-28-oyl]leucine (14):** 46 mg (64% yield) starting from 70 mg of **8**; light-yellow amorphous powder. Mp 128−129 °C. MS (ESI−) *m/z*: 718.5 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.27, 7.16−7.13, 6.85−6.82 (5H, m, H ar-2′, 3′, 4′), 5.97 (1H, br s, −CON*H*−), 4.91, 4.89 (H each, br s, H-29), 4.48 (1H, m, −NHC*H*−), 3.93 (2H, s, H_2-30), 3.79 (3H, s, ar-OCH_3), 3.60 (2H, t, J = 7.2 Hz, 30-OC$H_2$$CH_2$Ph(*p*-$OCH_3$)), 3.17 (1H, dd, J = 11.1, 5.4 Hz, H-3), 2.85 (1H, t, J = 7.5 Hz, H-19), 2.39 (3H, m, 30-$OCH_2C$$H_2$Ph(*p*-$OCH_3$), H-13), 0.95, 0.93, 0.90 (15H, s, leucine moiety −$(CH_3)_2$, 3 × CH_3), 0.79, 0.75 (3H each, s, 2 × CH_3). Anal. ($C_{45}H_{69}O_6N$·$4^1/_2H_2O$) C, H, O.

***N*-[3*β*-Hydroxy-30-(4′-fluorophenethoxy)-lup-20(29)-en-28-oyl]leucine (15):** 24 mg (40% yield) starting from 60 mg of **8**; light-yellow amorphous powder. Mp 102−104 °C. MS (ESI−) *m/z*: 706.4 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.16−6.80 (5H, m, H ar-2′, 3′, 4′), 5.96 (1H, br s, −CON*H*−), 4.90, 4.89 (2H, br s, H-29), 4.48 (1H, m, −NHC*H*−), 3.92 (2H, s, H_2-30), 3.61 (2H, t, J = 7.2 Hz, 30-OC$H_2$$CH_2$Ph(*p*-F)), 3.17 (1H, m, H-3), 2.87 (1H, t, J = 7.5 Hz, H-19), 2.36−2.10 (3H, m, 30-$OCH_2C$$H_2$Ph(*p*-F), H-13), 0.95, 0.88 (15H, s, leucine moiety −$(CH_3)_2$, 3 × CH_3), 0.75, 0.73 (3H each, s, 2 × CH_3). Anal. ($C_{44}H_{66}O_5NF$·$3^1/_2H_2O$) C, H, O.

***N*-[3*β*-Hydroxy-30-(4′-bromophenethoxy)-lup-20(29)-en-28-oyl]leucine (16):** 18 mg (41% yield) starting from 40 mg of **8**; light-yellow amorphous powder. Mp 127−129 °C. MS (ESI−) *m/z*: 706.4 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56−7.28 (5H, m, H ar-2′, 3′, 4′), 5.96 (1H, br s, −CON*H*−), 4.91, 4.90 (2H, br s, H-29), 4.48 (1H, m, −NHC*H*−), 3.91 (2H, s, H_2-30), 3.60 (2H, t, J = 7.0 Hz, 30-OC$H_2$$CH_2$Ph(*p*-Br)), 3.17 (1H, dd, J = 11.0, 5.6 Hz, H-3), 2.89 (1H, t, J = 7.5 Hz, H-19), 2.39 (1H, m, 30-$OCH_2C$$H_2$Ph(*p*-Br)), 0.96 (12H, s, leucine moiety −$(CH_3)_2$, 2 × CH_3), 0.82, 0.79, 0.75 (3H each, s, 3 × CH_3). Anal. ($C_{44}H_{66}O_5NBr$·$2H_2O$) C, H, O.

***N*-[3*β*-Hydroxy-30-(4′-chlorophenethoxy)-lup-20(29)-en-28-oyl]leucine (17):** 16 mg (38% yield) starting from 40 mg of **8**; light-yellow amorphous powder. Mp 119−121 °C. MS (ESI−) *m/z*: 706.4 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.18−6.87 (5H, m, H ar-2′, 3′, 4′), 5.96 (1H, br s, −CON*H*−), 4.91, 4.90 (2H, br s, H-29), 4.48 (1H, m, −NHC*H*−), 3.91 (2H, s, H_2-30), 3.62 (2H, t, J = 6.8 Hz, 30-OC$H_2$$CH_2$Ph(*p*-Cl)), 3.17 (1H, dd, J = 11.0, 5.6 Hz, H-3), 2.87 (1H, t, J = 7.5 Hz, H-19), 2.36−2.06 (3H, m, 30-$OCH_2C$$H_2$Ph(*p*-Cl), H-13), 0.96 (15H, s, leucine moiety −$(CH_3)_2$, 3 × CH_3), 0.81, 0.76 (3H each, s, 2 × CH_3). Anal. ($C_{44}H_{66}O_5NCl$·H_2O) C, H, O.

***N*-[3*β*-Hydroxy-30-morpholino-lup-20(29)-en-28-oyl]leucine (18):** 22 mg (41% yield) starting from 60 mg of **8**; white amorphous powder. Mp 98−100 °C. MS (ESI−) *m/z*: 653.5 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.61 (1H, d, J = 6 Hz, −CON*H*−), 4.92, 4.90 (H each, s, H-29), 4.63−4.58 (1H, m, −NHC*H*−), 3.72 (4H, m, 30-$N(CH_2C$$H_2)_2O$), 3.17 (1H, dd, J = 11.1, 5.4 Hz, H-3), 3.00 (3H, m, H-19, H_2-30), 2.53 (4H, m, 30-$N(C$$H_2CH_2)_2O$), 2.42 (1H, m, H-13), 0.96 (6H, s, leucine moiety −$(CH_3)_2$), 0.92 (6H, s, 2 × CH_3), 0.86, 0.81, 0.75 (3H each, s, 3 × CH_3). Anal. ($C_{40}H_{66}O_5N_2$·$2H_2O$) C, H, O.

***N*-[3*β*-Hydroxy-30-(2′-morpholinoethoxy)-lup-20(29)-en-28-oyl]leucine (19):** 26 mg (56% yield) starting from 50 mg of **8**; white amorphous powder. Mp 89−91 °C. MS (ESI−) *m/z*: 697.4 (M^- − H). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.61 (1H, d, J = 8 Hz, −CON*H*−), 4.92, 4.90 (H each, s, H-29), 4.59 (1H, m, −NHC*H*−), 3.94 (2H, s, H_2-30), 3.72 (4H, m, $-N(CH_2C$$H_2)_2O$), 3.58 (2H, t, J = 5.7 Hz, 30-OC$H_2$$CH_2$-morpholine), 3.18 (1H, dd, J = 11.4, 4.6 Hz, H-3), 3.01 (1H, m, H-19), 2.60 (2H, t, J = 5.4 Hz, 30-$OCH_2C$$H_2$-morpholine), 2.53 (4H, m, $-N(C$$H_2CH_2)_2O$), 1.00 (6H, s, leucine moiety −$(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.89, 0.85, 0.80 (3H each, s, 3 × CH_3). Anal. ($C_{42}H_{70}O_6N_2$·H_2O) C, H, O.

***N*-[3*β*-Hydroxy-30-(2′-morpholinoethoxy)-lup-20(29)-en-28-oyl]-8-aminooctanoic acid (20):** 51 mg (64% yield) starting from 80 mg of **9**; white amorphous powder. Mp 111−112 °C. MS (ESI−) *m/z*: 725.5 (M^- − H) for $C_{44}H_{74}N_2O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.61 (1H, d, J = 8 Hz, −CON*H*−), 4.91, 4.90 (H each, s, H-29), 3.94 (2H, s, H_2-30), 3.72 (4H, m, $-N(CH_2C$$H_2)_2O$), 3.58 (2H, t, J = 5.7 Hz, 30-OC$H_2$$CH_2$-morpholine), 3.18 (3H, m, −CONHCH_2−, H-3), 3.01 (1H, m, H-19), 2.60 (2H, t, J = 5.4 Hz, 30-$OCH_2C$$H_2$-morpholine), 2.53 (4H, m, $-N(C$$H_2CH_2)_2O$), 2.28 (2H, t, J = 7.5 Hz, -CH_2COOH), 0.96 (6H, s, 2 × CH_3), 0.92, 0.81, 0.75 (3H each, s, 3 × CH_3).

Syntheses of BA Derivatives 26 and 27. A solution of 30-bromo BA derivative **8** or **9** (1 equiv), silver acetate (AgOAc, 2 equiv) and tetrabutylammonium bromide (Bu_4NBr, 0.2 equiv) in acetonitrile (1.5 mL) was heated using microwave at 100 °C for 25 min.

The precipitant was filtered, and the solution was concentrated to dryness under vacuum. The residue was chromatographed over a silica gel column to yield the pure diacetoxy intermediates **26** and **27**.

Methyl *N*-[3*β*,30-diacetoxy-lup-20(29)-en-28-oyl]leucinate (26): 80 mg (68% yield) starting from 120 mg of **117**; off-white amorphous powder. Mp 201−203 °C. MS (ESI+) *m/z*: 684.5 ($M^+ + H$) for $C_{41}H_{65}NO_7$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.69 (1H, br s, −CON*H*−), 4.97, 4.94 (2H, d, *J* = 9, H-29), 4.58−4.52 (3H, m, −NHC*H*−, H_2-30), 4.45 (1H, t, *J* = 8 Hz, H-3), 3.72 (3H, s, $-COOCH_3$), 3.10 (1H, m, H-19), 2.50−2.32 (1H, m, H-13), 2.08 (6H, s, 2 × $OCOCH_3$), 1.05 (6H, s, leucine moiety $-(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.89, 0.82, 0.81(3H each, s, 3 × CH_3).

Methyl *N*-[3*β*,30-diacetoxy-lup-20(29)-en-28-oyl]-8-aminooctanoate (27): 77.8 mg (69.5% yield) starting from 80 mg of **118**; white amorphous powder. Mp 167−169 °C. MS (ESI+) *m/z*: 712.5 ($M^+ + H$) for $C_{43}H_{69}NO_7$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.60 (1H, t, *J* = 4.6 Hz, −CON*H*−), 4.94, 4.90 (1H each, s, H-29), 4.56 (2H, s, H_2-30), 4.45 (1H, t, *J* = 7 Hz, H-3), 3.66 (3H, s, $-COOCH_3$), 3.41−3.09 (3H, m, H-19, $-CONHCH_2-$), 2.46 (1H, m, H-13), 2.31 (2H, t, *J* = 7.5 Hz, $-CH_2COOCH_3$), 2.05 (6H, s, 2 × $OCOCH_3$), 0.97, 0.96, 0.85, 0.81, 0.80 (3H each, s, 5 × CH_3).

Syntheses of BA Derivatives 22−25, 28, 29, 36, and 38. To a solution of the appropriate ester intermediates **6**−**9**, **26**, **27**, **35**, and **37** (1 equiv) in MeOH (8 mL) and THF (4 mL) was added 2 N NaOH (4 mL). The mixture was stirred overnight and then neutralized with 20% HCl. The solution was dried under vacuum and reconstituted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds.

***N*-[3*β*-Hydroxy-lup-20(29)-en-28-oyl]leucine (22):** 27 mg (100% yield) starting from 30 mg of **6**; white amorphous powder. Mp 243−244 °C. MS (ESI−) *m/z*: 568.42 ($M^- - H$) for $C_{36}H_{59}NO_4$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.86 (1H, d, *J* = 8 Hz, −CON*H*−), 5.11, 5.02 (1H each, s, H-29), 4.65 (1H, m, −NHC*H*−), 3.17 (1H, dd, *J* = 9.7, 5.4 Hz, H-3), 3.10−3.03 (1H, m, H-19), 1.68 (3H, s, H-30), 1.00 (6H, s, leucine moiety $-(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.83, 0.80, 0.79 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −17.2 ° (*c* 1.40, $CHCl_3$).

***N*-[3*β*-Hydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoic acid (23):** 37 mg (100% yield) starting from 40 mg of **7**; white amorphous powder. Mp 110−112 °C. MS (ESI−) *m/z*: 596.5 ($M^- - H$) for $C_{38}H_{63}NO_4$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.60 (1H, br s, −CON*H*−), 4.73, 4.60 (1H each, s, H-29), 3.21−3.09 (4H, m, H-3, H-19, $-CONHCH_2-$), 2.31 (2H, t, *J* = 6.9 Hz, $-CH_2COOH$), 2.10−2.20 (1H, m, H-13), 1.68 (3H, s, H-30), 0.97 (6H, s, 2 × CH_3), 0.85, 0.79, 0.75 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −3.6 ° (*c* 0.19, $CHCl_3$). $[\alpha]_D^{25}$ −8.48 ° (*c* 0.20, MeOH).

***N*-[3*β*-Hydroxy-30-bromo-lup-20(29)-en-28-oyl]leucine (24):** 102 mg (100% yield) starting from 110 mg of **8**; white amorphous powder. Mp 102−104 °C. MS (ESI−) *m/z*: 646.41, 648.39 ($M^- - H$) for $C_{36}H_{58}BrNO_4$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.86 (1H, d, *J* = 8 Hz, −CON*H*−), 5.11, 5.02 (1H each, s, H-29), 4.65 (1H, m, −NHC*H*−), 3.90 (2H, s, H_2-30), 3.17 (1H, dd, *J* = 9.7, 5.4 Hz, H-3), 3.10−3.03 (1H, m, H-19), 1.00 (6H, s, leucine moiety $-(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.83, 0.80, 0.79 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −10.5 ° (*c* 0.15, MeOH).

***N*-[3*β*-Hydroxy-30-bromo-lup-20(29)-en-28-oyl]-8-aminooctanoic acid (25):** 44 mg (95% yield) starting from 50 mg of **9**; white amorphous powder. Mp 119−122 °C. MS (ESI−) *m/z*: 674.4 ($M^- - H$) for $C_{38}H_{62}BrNO_4$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.60 (1H, br s, −CON*H*−), 5.13, 5.04 (1H each, s, H-29), 4.00 (2H, s, H_2-30), 3.21−3.09 (4H, m, H-3, H-19, $-CONHCH_2-$), 2.34 (2H, m, $-CH_2COOH$), 0.96 (6H, s, 2 × CH_3), 0.92, 0.82, 0.81 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −16.5 ° (*c* 0.22, MeOH).

***N*-[3*β*,30-Dihydroxy-lup-20(29)-en-28-oyl]leucine (28):** 24 mg (98% yield) starting from 30 mg of **26**; white amorphous powder. Mp 145−148 °C. MS (ESI−) *m/z*: 584.5 ($M^- - H$) for $C_{36}H_{59}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.89 (1H, br s, −CON*H*−), 4.91, 4.90 (1H each, s, H-29), 4.68 (1H, m, −NHC*H*−), 4.12 (2H, s, H_2-30), 3.17 (1H, dd, *J* = 11.2, 5.6 Hz, H-3), 3.01 (1H, m, H-19), 2.34 (1H, m, H-13), 1.10 (6H, s, leucine moiety $-(CH_3)_2$), 0.99 (6H, s, 2 × CH_3), 0.86, 0.83, 0.80 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −39.3 ° (*c* 0.35, MeOH).

***N*-[3*β*,30-Dihydroxy-lup-20(29)-en-28-oyl]-8-aminooctanoic acid (29):** 50 mg (80% yield) starting from 58 mg of **27**; white amorphous powder. Mp 135−137 °C. MS (ESI−) *m/z*: 612.5 ($M^- - H$) for $C_{38}H_{63}NO_5$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.61 (1H, br s, −CON*H*−), 4.94, 4.90 (1H each, s, H-29), 4.12 (2H, s, H_2-30), 3.25−3.15 (3H, m, H-3, $-CONHCH_2-$), 3.01 (1H, m, H-19), 2.34 (2H, t, *J* = 7.6 Hz, $-CH_2COOH$), 2.06 (1H, m, H-13), 0.97, 0.96 (3H each, s, 2 × CH_3), 0.92, 0.82, 0.75 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −143.3 ° (*c* 0.10, MeOH).

***N*′-[3*β*-*N*-(3′,3′-Dimethylsuccinyl)-lup-20(29)-en-28-oyl]leucine (36):** 24 mg (98%) starting from 25 mg of **35**; white amorphous powder. Mp 248−250 °C. MS (ESI−) *m/z*: 695.5 ($M^- - H$) for $C_{42}H_{68}N_2O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.87 (1H, d, *J* = 7.6 Hz, −CON*H*−), 4.72, 4.58 (1H each, s, H-29), 4.64 (1H, m, −NHC*H*−), 3.59 (1H, m, H-3), 2.99 (1H, m, H-19), 2.64−2.42 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.30, 1.26 (3H each, s, 2 × CH_3-3′), 1.00 (6H, s, leucine moiety $-(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.89, 0.86, 0.85 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −16.1 ° (*c* 0.28, MeOH).

***N*-[3*β*-Hydroxy-lup-20(29)-en-28-oyl]-4-piperidinebutyric acid (38):** 190 mg (100%) starting from 200 mg of **37**; white amorphous powder. Mp 145−146 °C. MS (ESI−) *m/z*: 608.4 ($M^- - H$) for $C_{39}H_{63}NO_4$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 4.72, 4.57 (1H each, s, H-29), 3.67−3.47 (4H, m, 28-CON(CH_2CH_2)$_2$CH−), 3.19 (1H, m, H-3), 2.99 (1H, m, H-19), 2.31 (2H, t, *J* = 8.4 Hz, $-CH_2COOH$), 1.68 (3H, s, H-30), 0.96 (6H, s, 2 × CH_3), 0.94, 0.82, 0.81 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −22.7 ° (*c* 0.33, MeOH).

Synthesis of BA Derivatives 21 and 41−44. A solution of **20** or **38** (1 equiv), EDCI (2 equiv), *N*-hydroxybenzotriazole (HOBt, 1 equiv), Et_3N (0.05 mL), and the appropriate amine (2 equiv) in anhydrous CH_2Cl_2 (8 mL) was stirred at room temperature overnight until the starting material was not observed by TLC. The solution was diluted with CH_2Cl_2 (20 mL) and washed three times with brine and distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield pure target compounds.

***β*-Hydroxy-30-(2′-morpholinoethoxy)-lup-20(29)-en-28-oyl]-8-aminooctanoyl]aminomethane (21):** 21 mg (69% yield) starting from 30 mg of **20**; white amorphous powder. Mp 106−107 °C. MS (ESI+) *m/z*: 740.5 ($M^+ + H$) for $C_{45}H_{77}N_3O_5$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.61 (2H, m, 2 × −CON*H*−), 4.92, 4.90 (H each, s, H-29), 3.94 (2H, s, H_2-30), 3.72 (4H, m, −N(CH_2CH_2)$_2$O), 3.58 (2H, t, *J* = 5.7 Hz, 30-OCH_2CH_2-morpholine), 3.28−3.14 (3H, m, $-CONHCH_2-$, H-3), 3.01 (1H, m, H-19), 2.81 (3H, d, *J* = 4.8 Hz, $-CONHCH_3$), 2.60 (2H, t, *J* = 5.4 Hz, 30-OCH_2CH_2-morpholine), 2.53 (4H, m, −N(CH_2CH_2)$_2$O), 2.16 (2H, t, *J* = 7.5 Hz, $-CH_2CONHCH_3$), 0.96 (6H, s, 2 × CH_3), 0.92, 0.81, 0.75 (3H each, s, 3 × CH_3).

***N*′-[*N*-[3*β*-Hydroxy-lup-20(29)-en-28-oyl]-4-piperidinebutanoyl]-aminomethane (41):** 46 mg (100% yield) starting from 50 mg of **38**, off-white amorphous powder. Mp 202−204 °C. MS (ESI+) *m/z*: 623.5 ($M^+ + H$) for $C_{40}H_{66}N_2O_3$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 5.44 (1H, br s, −CON*H*CH_3), 4.69, 4.54 (1H each, s, H-29), 3.58−3.54 (4H, m, 28-CON(CH_2CH_2)$_2$CH−), 3.16 (1H, m, H-3), 2.95 (1H, m, H-19), 2.78 (3H, d, *J* = 4.8 Hz, $-CONHCH_3$), 2.16 (2H, t, *J* = 7.5 Hz, $-CH_2CONHCH_3$), 1.66 (3H, s, H-30), 0.93 (6H, s, 2 × CH_3), 0.92, 0.90, 0.79 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −10.7° (*c* 0.19, MeOH).

***β*-Hydroxy-lup-20(29)-en-28-oyl]-4-piperidine-butanoyl]morpholine (42):** 53 mg (87% yield) starting from 50 mg of **38**, white amorphous powder. Mp 132−133 °C. MS (ESI+) *m/z*: 679.5 ($M^+ + H$) for $C_{43}H_{70}N_2O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): *δ* 4.69, 4.54 (1H each, s, H-29), 3.66−3.60 (8H, m, 28-CON(CH_2CH_2)$_2$CH−, −CON(CH_2CH_2)$_2$O), 3.42 (4H, m, −CON(CH_2CH_2)$_2$O), 3.14 (1H, m, H-3), 2.95−2.60 (1H, m, H-19), 2.27 (2H, t, *J* = 9.2 Hz, $-CH_2CON(CH_2CH_2)_2O$), 1.65 (3H, s, H-30), 0.93 (3H each, s, 2

× CH_3), 0.91, 0.83, 0.79 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −20.0° (*c* 0.31, MeOH).

***N′*-[*N*-[3*β*-Hydroxy-lup-20(29)-en-28-oyl]-4-piperidine-butanoyl]-2-aminoethylmorpholine (43):** 54 mg (91% yield) starting from 55 mg of **38**, white amorphous powder. Mp 114−116 °C. MS (ESI+) *m/z*: 722.6 ($M^+ + H$), 744.5 ($M^+ + Na$) for $C_{45}H_{75}N_3O_4$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 6.79 (1H, br, s, −CON*H*CH_2−), 4.68, 4.53 (1H each, s, H-29), 3.69−3.67 (8H, m, 28-CON(C*H*$_2$CH$_2$)$_2$CH−, −CH_2N(CH$_2$C*H*$_2$)$_2$O), 3.31 (2H, m, −CONHC*H*$_2$−), 3.17 (1H, m, H-3), 2.95−2.60 (1H, m, H-19), 2.43 (6H, m, −C*H*$_2$N(C*H*$_2$CH$_2$)$_2$O), 2.17 (2H, t, *J* = 7.5 Hz, −C*H*$_2$CONHCH$_2$−), 1.63 (3H, s, H-30), 0.93 (3H each, s, 2 × CH_3), 0.90, 0.79, 0.72 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −10.6° (*c* 0.15, MeOH).

***N′*-[*N*-[3*β*-Hydroxy-lup-20(29)-en-28-oyl]-4-piperidine-butanoyl]-3-aminopropylmorpholine (44):** 54 mg (90% yield) starting from 50 mg of **38**, white amorphous powder. Mp 122−124 °C. MS (ESI+) *m/z*: 736.6 ($M^+ + H$) for $C_{46}H_{77}N_3O_4$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.93 (1H, br, s, −CONHC*H*$_2$−), 3.15 (1H, m, H-3), 2.95−2.60 (1H, m, H-19), 2.47−2.41 (6H, m, −C*H*$_2$N(C*H*$_2$CH$_2$)$_2$O), 2.16 (2H, t, *J* = 7.5 Hz, −C*H*$_2$CONHCH$_2$−), 1.65 (3H, s, H-30), 0.93 (3H each, s, 2 × CH_3), 0.91, 0.79, 0.72 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −7.5° (*c* 0.18, MeOH).

3-Deoxybetulinic Acid (31). To a solution of **1** (2 g, 1 equiv) in DMF was added pyridium dichromate (PDC, 2 equiv). The mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with EtOAc (30 mL), and the precipitate was filtered through a short pack of Florisil. The solution was washed with 20% HCl and distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under vacuum. The residue was chromatographed using a silica gel column to yield 1.68 g (87%) of pure **31**, white powder. Mp 246−248 °C. MS (ESI−) *m/z*: 453.3 ($M^- - H$) for $C_{30}H_{46}O_3$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 4.72, 4.58 (1H each, s, H-29), 3.09 (1H, m, H-19), 2.41−2.26 (2H, m, H-2), 1.69 (3H, s, H-30), 0.98, 0.97, 0.96, 0.92, 0.89 (3H each, s, 5 × CH_3).

Methyl *N*-[3-Deoxy-lup-20(29)-en-28-oyl]leucinate (32). A solution of **31** (500 mg, 1 equiv), DMAP (0.6 equiv), and EDCI (1.6 equiv) in CH_2Cl_2 was stirred at 0 °C for 30 min. Leucine methyl ester (1.6 equiv) and Et_3N (1 equiv) was then added into the system and stirred at room temperature overnight. The mixture was diluted with CH_2Cl_2 (20 mL) and washed with brine. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated to dryness under vacuum. The residue was chromatographed using a silica gel column to yield 372 mg (58%) of pure **32**, white amorphous powder. Mp 191−193 °C. MS (ESI+) *m/z*: 582.5 ($M^+ + H$) for $C_{37}H_{59}NO_4$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.87 (1H, d, *J* = 8.4 Hz, −CON*H*−), 4.70, 4.59 (1H each, s, H-29), 4.64 (1H, m, −NHC*H*−), 3.73 (3H, s, −$COOCH_3$), 3.05 (1H, m, H-19), 2.41−2.26 (2H, m, H-2), 1.68 (3H, s, H-30), 1.06, 1.02 (3H each, s, leucine moiety −$(CH_3)_2$), 0.98 (3H, s, CH_3), 0.96 (6H, s, 2 × CH_3), 0.92, 0.89 (3H each, s, 2 × CH_3).

Methyl *N*-[3-Oxime-lup-20(29)-en-28-oyl]leucinate (33). A solution of **32** (230 mg, 1 equiv) and hydroxylamine hydrochloride (4 equiv) in pyridine (10 mL) was heated at 50 °C for 2 h. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 and washed three times by 20% HCl and brine. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated to dryness under vacuum. The residue was chromatographed using a silica gel column to yield 235 mg (90%) of pure **33**, white amorphous powder. Mp 213−215 °C. MS (ESI+) *m/z*: 582.5 ($M^+ + H$) for $C_{37}H_{59}NO_4$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.87 (1H, d, *J* = 8.4 Hz, −CON*H*−), 4.70, 4.59 (1H each, s, H-29), 4.64 (1H, m, −NHC*H*−), 3.73 (3H, s, −$COOCH_3$), 3.05 (1H, m, H-19), 2.20−2.15 (2H, m, H-2), 1.67 (3H, s, H-30), 1.05, 1.02 (3H each, s, leucine moiety −$(CH_3)_2$), 0.98 (3H, s, CH_3), 0.96 (6H, s, 2 × CH_3), 0.94, 0.92 (3H each, s, 2 × CH_3).

Methyl *N*-[3*β*-Amino-lup-20(29)-en-28-oyl]leucinate (34). To a solution of **33** (100 mg, 1 equiv) and ammonium acetate (15 equiv) in MeOH was added sodium cyanoborohydride ($NaCNBH_3$, 20 equiv) under nitrogen atmosphere. The mixture was cooled to 0 °C, and 15% aqueous titanium trichloride ($TiCl_3$, 3 equiv) was added dropwise over 45 min. The mixture was stirred at room temperature overnight and then treated with 2 N NaOH until pH 10. The solution was dried under vacuum, and the residual aqueous solution was extracted with CH_2Cl_2 and washed with distilled water until pH 7 was attained. The organic layer was then dried over anhydrous Na_2SO_4 and concentrated to dryness under vacuum. The residue was chromatographed using a silica gel column to yield 80 mg (82%) of pure **34**, white amorphous powder. Mp 135−137 °C. MS (ESI+) *m/z*: 582.5 ($M^+ + H$) for $C_{37}H_{59}NO_4$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.86 (1H, d, *J* = 7 Hz, −CON*H*−), 4.72, 4.58 (1H each, s, H-29), 4.64 (1H, m, −NHC*H*−), 3.73 (3H, s, −$COOCH_3$), 3.05 (1H, m, H-19), 2.44 (1H, m, H-3), 2.10−1.90 (1H, m, H-13), 1.68 (3H, s, H-30), 0.97 (9H, s, CH_3-23, leucine moiety−$(CH_3)_2$), 0.96, 0.94, 0.93, 0.92 (3H each, s, 4 × CH_3).

Synthesis of BA Derivatives 30, 35, 39, 40, 45−48. A solution of the appropriate BA analogue (1 equiv), DMAP (1.5 equiv), and the appropriate acid anhydride (5 equiv) in anhydrous pyridine (1.5 mL) was stirred at 160 °C for 2 h using microwave (Biotage). The reaction mixture was diluted with EtOAc (15 mL) and washed three times with 20% HCl solution and distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield pure target compounds.

***N*-[3*β*-*O*-(3′,3′-Dimethylsuccinyl)-30-bromo-lup-20(29)-en-28-oyl]leucine (30):** 20 mg (32% yield) starting from 50 mg of **24**; light-yellow amorphous powder. Mp 105−107 °C. MS (ESI−) *m/z*: 774.5 ($M^- - H$) for $C_{42}H_{66}BrNO_7$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.86 (1H, d, *J* = 8 Hz, −CON*H*−), 5.11, 5.02 (1H each, s, H-29), 4.65 (1H, m, −NHC*H*−), 4.54 (1H, dd, *J* = 11.2, 5.7 Hz, H-3), 3.90 (2H, s, H_2-30), 2.99 (1H, m, H-19), 2.64−2.42 (2H, m, H-2′), 1.30, 1.26 (3H each, s, 2 × CH_3-3′), 1.00 (6H, s, leucine moiety −$(CH_3)_2$), 0.96 (6H, s, 2 × CH_3), 0.87, 0.86, 0.81 (3H each, s, 3 × CH_3).

Methyl*N′*-[3*β*-*N*-(3′,3′-dimethylsuccinyl)-lup-20(29)-en-28-oyl]leucinate (35): 37 mg (38% yield) starting from 80 mg of **34**; white amorphous powder. Mp 187−189 °C. MS (ESI+) *m/z*: 711.5 ($M^+ + H$), 733.4 ($M^+ + Na$) for $C_{43}H_{70}N_2O_6$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.69 (1H, br s, −CON*H*−), 4.70, 4.58 (1H each, s, H-29), 4.62 (1H, m, −NHC*H*−), 3.73 (3H, s, −$COOCH_3$), 3.59 (1H, m, H-3), 2.99 (1H, m, H-19), 2.64−2.42 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.30, 1.26 (3H each, s, 2 × CH_3-3′), 1.09 (6H, s, leucine moiety −$(CH_3)_2$), 0.97 (6H, s, 2 × CH_3), 0.92, 0.82, 0.80 (3H each, s, 3 × CH_3).

***N*-[3*β*-*O*-(3′,3′-Dimethylsuccinyl)-lup-20(29)-en-28-oyl]-4-piperidinebutyric acid (39):** 20 mg (41% yield) starting from 50 mg of **38**, white amorphous powder. Mp 116−118 °C. MS (ESI+) *m/z*: 738.6 ($M^+ + H$). MS (ESI−) *m/z*: 736.5 ($M^- - H$) for $C_{45}H_{71}NO_7$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 4.72, 4.57 (1H each, s, H-29), 4.47 (1H, t, *J* = 7.5, H-3), 3.65−3.50 (4H, m, 28-CON(C*H*$_2$CH$_2$)$_2$CH-), 2.98 (1H, m, H-19), 2.64−2.42 (2H, m, H-2′), 2.30 (2H, t, *J* = 7.2 Hz, −C*H*$_2$COOH), 1.68 (3H, s, H-30), 1.27, 1.25 (3H each, s, 2 × CH_3-3′), 0.95, 0.93, 0.84, 0.83, 0.82 (3H each, s, 5 × CH_3). $[\alpha]_D^{25}$ −27.7° (*c* 0.30, MeOH).

***N*-[3*β*-*O*-(4′,4′-Dimethylglutaryl)-lup-20(29)-en-28-oyl]-4-piperidinebutyric acid (40):** 12 mg (38% yield) starting from 30 mg of **38**, white amorphous powder. Mp 143−145 °C. MS (ESI+) *m/z*: 752.4 ($M^+ + H$), (ESI−) *m/z*: 750.4 ($M^- - H$) for $C_{46}H_{73}NO_7$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 4.72, 4.57 (1H each, s, H-29), 4.47 (1H, t, *J* = 7.5, H-3), 3.65−3.50 (4H, m, 28-CON(C*H*$_2$CH$_2$)$_2$CH−), 3.00 (1H, m, H-19), 2.35−2.30 (4H, m, H-2′, −C*H*$_2$COOH), 1.68 (3H, s, H-30), 1.27, 1.25 (3H each, s, 2 × CH_3-3′), 0.96 (6H, s, 2 × CH_3), 0.89, 0.86, 0.82 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −23.1° (*c* 0.20, MeOH).

***N′*-[*N*-[3*β*-*O*-(3′,3′-Dimethylsuccinyl)-lup-20(29)-en-28-oyl]-4-piperidinebutanoyl]aminomethane (45):** 17 mg (48% yield) starting from 30 mg of **41**, white amorphous powder. Mp 166−169 °C. MS (ESI+) *m/z*: 751.6 ($M^+ + H$) for $C_{46}H_{74}N_2O_6$. ^1H NMR (300 MHz, $CDCl_3$): *δ* 5.43 (1H, br s, −CON*H*CH_3), 4.69, 4.54 (1H each, s, H-29), 4.47 (1H, t, *J* = 7.5, H-3), 3.68−3.60 (4H, m, 28-CON(C*H*$_2$CH$_2$)$_2$CH−), 2.98 (1H, m, H-19), 2.79 (3H, d, *J* = 3.4 Hz, −CONHC*H*$_3$), 2.67−2.62 (2H, m, H-2′), 2.14 (2H, t, *J* = 6.8

Hz, $-CH_2CONHCH_3$), 1.65 (3H, s, H-30), 1.30, 1.25 (3H each, s, 2 × CH_3-3′), 0.93, 0.91 (3H each, s, 2 × CH_3), 0.90, 0.80, 0.79 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −25.0 ° (*c* 0.12, MeOH).

***N′*-[*N*-[3*β*-*O*-(3′,3′-Dimethylsuccinyl)-lup-20(29)-en-28-oyl]-4-piperidinebutanoyl]morpholine (46):** 23 mg (55% yield) starting from 35 mg of **42**, off-white amorphous powder. Mp 122−124 °C. MS (ESI+) *m/z*: 807.6 ($M^+ + H$) for $C_{49}H_{78}N_2O_7$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.69, 4.54 (1H each, s, H-29), 4.45 (1H, t, *J* = 6.9, H-3), 3.66−3.61 (8H, m, 28-CON(CH_2CH_2)$_2$CH−, −CON(CH_2CH_2)$_2$O), 3.45−3.42 (4H, m, −CON(CH_2CH_2)$_2$O), 2.99−2.82 (1H, m, H-19), 2.67−2.52 (2H, m, H-2′), 2.28 (2H, t, *J* = 7.8 Hz, $-CH_2CON(CH_2CH_2)_2O$), 1.65 (3H, s, H-30), 1.26 (6H, s, 2 × CH_3-3′), 0.92, 0.90 (3H each, s, 2 × CH_3), 0.79 (6H, s, 2 × CH_3), 0.77 (3H, s, CH_3). $[\alpha]_D^{25}$ −19.1° (*c* 0.41, MeOH).

***N′*-[*N*-[3*β*-*O*-(3′,3′-Dimethylsuccinyl)-lup-20(29)-en-28-oyl]-4-piperidinebutanoyl]-2-aminoethylmorpholine (47):** 14 mg (41% yield) starting from 30 mg of **43**, white amorphous powder. Mp 121−123 °C. MS (ESI+) *m/z*: 850.4 ($M^+ + H$) for $C_{51}H_{83}N_3O_7$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.01 (1H, br, s, −CON*H*CH_2−), 4.70, 4.54 (1H each, s, H-29), 4.45 (1H, t, *J* = 10.2, H-3), 3.81−3.78 (8H, m, 28-CON(CH_2CH_2)$_2$CH−, $-CH_2N(CH_2CH_2)_2O$), 3.48 (2H, m, −CONHCH_2−), 2.85−2.70 (7H, m, $-CH_2N(CH_2CH_2)_2O$, H-19), 2.60−2.52 (2H, m, H-2′), 2.14 (2H, t, *J* = 7.5 Hz, $-CH_2CONHCH_2$−), 1.25 (6H, s, 2 × CH_3-3′), 1.63 (3H, s, H-30), 0.93 (3H each, s, 2 × CH_3), 0.90, 0.79, 0.72 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −18.0° (*c* 0.16, MeOH).

***N′*-[*N*-[3*β*-*O*-(3′,3′-Dimethylsuccinyl)-lup-20(29)-en-28-oyl]-4-piperidinebutanoyl]-3-aminopropylmorpholine (48):** 17 mg (47% yield) starting from 30 mg of **44**, white amorphous powder. Mp 125−127 °C. MS (ESI+) *m/z*: 864.6 ($M^+ + H$) for $C_{52}H_{85}N_3O_7$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.76 (1H, br, s, −CON*H*CH_2−), 4.69, 4.54 (1H each, s, H-29), 4.30 (1H, m, H-3), 3.81−3.67 (8H, m, 28-CON(CH_2CH_2)$_2$CH−, $-CH_2N(CH_2CH_2)_2O$), 3.28 (2H, m, −CONHCH_2−), 2.96 (1H, m, H-19), 2.80−2.73 (6H, m, $-CH_2N(CH_2CH_2)_2O$), 2.60−2.52 (2H, m, H-2′), 2.16 (2H, t, *J* = 7.5 Hz, $-CH_2CONHCH_2$−), 1.65 (3H, s, H-30), 1.25, 1.24 (6H, s, 2 × CH_3-3′), 0.92, 0.90 (3H each, s, 2 × CH_3), 0.80, 0.79, 0.78 (3H each, s, 3 × CH_3). $[\alpha]_D^{25}$ −14.1° (*c* 0.24, MeOH).

In Vitro Metabolic Stability Assessment. Materials. BA derivatives **23** and **38** were synthesized and characterized in our study. NADPH, $MgCl_2$, KH_2PO_4, formic acid, and ammonium acetate were purchased from Sigma-Aldrich. Reference compounds (fast-metabolized, buspirone, propranolol; moderate-metabolized, atenolol; and slow-metabolized, imipramine) were also purchased from Sigma-Aldrich. HPLC-grade acetonitrile and water were purchased from VWR. Pooled human liver microsomes (lot no. 70196) were purchased from BD biosciences (Woburn, MA).

Sample Preparation. Stock solutions of **23** and **38** (1 mg/mL) were prepared by dissolving the pure compound in methanol and stored at 4 °C. For measurement of metabolic stability, four reference compounds as well as test compounds **23** and **38** were brought to a final concentration of 3 μM with 0.1 M potassium phosphate buffer at pH 7.4, which contained 0.2 mg/mL human liver microsome and 5 mM $MgCl_2$. The incubation volumes were 800 μL. Reactions were started by adding 80 μL of NADPH (final concentration of 1.0 mM) and stopped by taking the aliquots over time, then adding to 1.5 volumes of ice-cold acetonitrile. Incubations of all samples were conducted in duplicate. For each sample, 100 μL aliquots were taken out at 0, 5, 15, 30, 60, 120 min time points. After addition of 150 μL of ice-cold acetonitrile, the mixture was centrifuged at 12 000 rpm for 5 min at 0 °C. The supernatant was collected, and 20 μL of the supernatant was directly injected to LCMS. The following controls were also conducted: (1) positive control incubations that contain liver microsomes, NADPH, and the fast-metabolized substrate propranolol; (2) negative control incubations that omit NADPH; (3) baseline control that only contain liver microsomes and NADPH.

HPLC−MS Conditions. Analysis was carried out on Shimadzu LCMS-20 with an electrospray ionization source (ESI). An Alltima C18 5 μm, 150 mm × 2.1 mm column was used with a gradient elution at a flow rate of 1.5 mL/min. The initial elution condition was acetonitrile (B) in water (A, with 0.1% formic acid and 5 mM ammonium acetate) at 55%. After staying at initial condition for 3 min, the concentration of B increased linearly to 90% at 15 min and stayed at 90% for 2 min. The mobile phase was then returned to the initial condition and re-equilibrated for 3 min. The MS conditions were optimized to detector voltage: +1.35 kV; acquisition mode, SIM of the appropriate molecular weights of the testing compounds. The CDL temperature is 200 °C, heat block is 230 °C, and neutralizing gas flow is 1.5 L/min. Samples were injected by autosampler. Electrospray ionization was operated in the positive ion mode. Full-scan spectra were also monitored over the range of 180−1000 *m/z*.

HIV-1_{IIIB} Replication Inhibition Assay in MT-2 Lymphocytes. The evaluation of HIV-1 inhibition was carried out as follows. The human T-cell line, MT-2, 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 first dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mg/mL to generate master stocks with dilutions made into tissue culture media to generate working stocks. The following drug concentrations were routinely used for screening: 100, 20, 4, and 0.8 μg/mL. For agents found to be active, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value could be determined. Test samples were prepared, and to each sample well, was added 90 μL of media containing MT-2 cells at 3 × 10^5 cells/mL and 45 μL of virus inoculum (HIV-1_{IIIB} 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 identical set of samples were added to cells under the same conditions without virus (mock infection) for cytotoxicity determinations (CC_{50} defined below). In addition, AZT and beviri-mat were also assayed during each experiment as positive drug controls. On day 4 postinfection, the assay was terminated and culture supernatants were harvested for p24 antigen ELISA analysis. The compound cytotoxicity was determined by XTT using the mock-infected sample wells. The detailed procedure was described previously.[33,34] If a test sample inhibited virus replication and was not cytotoxic, its effects were reported in the following terms: EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; CC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; therapeutic index (TI), the ratio of the CC_{50} to EC_{50}.

HIV-1_{NL4-3} Replication Inhibition Assay in MT-4 Lymphocytes. A previously described HIV-1 infectivity assay was used.[30,35] A 96-well microtiter plate was used to set up the HIV-1_{NL4-3} replication screening assay. NL4-3 variants at a multiplicity of infection (MOI) of 0.01 were used to infect MT4 cells. Culture supernatants were collected on day 4 postinfection for the p24 antigen capture using an ELISA kit from ZeptoMetrix Corporation (Buffalo, NY).

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Supporting Information Available: Additional information on compound purity, high-resolution mass spectral data, HPLC analysis results, and elemental analysis data of the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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