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Anti-AIDS Agents 90. Novel C-28 Modified Bevirimat Analogues as Potent HIV Maturation Inhibitors

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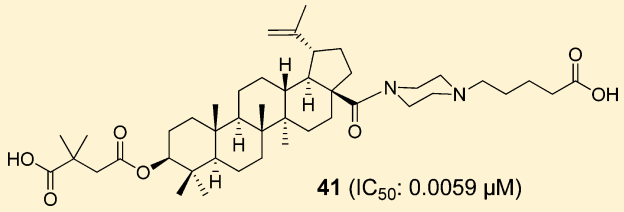
Supporting Information

ABSTRACT: In a continuing study of bevirimat (**2**), the anti-HIV-maturation clinical trials agent, 28 new betulinic acid (BA, **1**) derivatives were designed and synthesized. Among these compounds, **17**, with a C-28 MEM ester moiety, and **22**, with a C-28 ethyl hexanoate, increased the anti-HIV replication activity compared with **2** by 2-fold while compounds **40**, **41**, **48**, and **49**, with C-28 piperazine or piperidine amide substitutions, increased the activity by 3- to 15-fold. The best new compound, **41**, exhibited an anti-HIV IC_{50} of 0.0059 μM compared with 0.087 μM for **2**. All of the active compounds showed only antimaturation effects, as confirmed by TZM-bl assay, in blocking the HIV replication. The results suggest that proper C-28 substitutions can further enhance the antimaturation activity of **2** without any antientry effects. Thus, **41** may serve as a promising new lead for development of anti-AIDS clinical trial candidates.



INTRODUCTION

Although introduction of highly active antiretroviral therapy (HAART) has significantly improved the treatment of HIV/AIDS,[1−4] HIV incidence is still increasing in many countries and regions. Over 2.6 million new infections occurred in 2009 alone, contributing to the current global incidence of 33.3 million.[5] New infections continue to outpace the number of people placed on treatments, and the virus is suppressed rather than eradicated.[6−8] In addition, the efficacy of the treatments is hampered by the emergence of drug-resistant viral strains and severe drug−drug interactions.[9−11] Therefore, novel potent antiretroviral agents with different targets are still urgently needed.

Triterpenes, such as betulinic acid (BA, **1**),[12] represent a promising class of anti-HIV agents with novel mechanisms. In our prior research on modified triterpenes, bevirimat [3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid, **2**] was found to exhibit remarkable anti-HIV-1 activity against primary and drug-resistant HIV-1 isolates (Figure 1).[13,14] Mechanism of action study revealed that **2** blocks the last step of viral gag precursor polyprotein processing from p25 (CA-SP1) to functional p24 (CA), resulting in the production of noninfectious immature HIV-1 particles.[15] Therefore, **2** represents a unique first in a class of anti-HIV compounds termed maturation inhibitors (MIs), and it succeeded in phase I and IIa clinical trials against sensitive viruses during 2007−2009.[14,16−20]

Introduction of a C-28 side chain into a C-3 modified BA-analog (e.g., **2**) can generate a bifunctional HIV inhibitor with both antimaturation and antientry activities.[21] Consequently, in the current study, additional C-28 side chains were investigated.

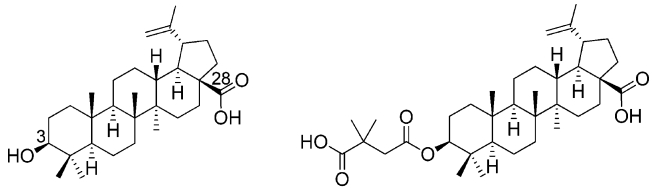


Figure 1.

Interestingly, it was discovered that certain C-28 side chains could significantly enhance the anti-HIV maturation activity of **2** without introducing the second mechanism of antientry effects. Their syntheses, anti-HIV evaluation, and structure−activity relationship (SAR) correlations are reported in this paper.

Design. Prior C-28 modification of **1** focused only on the promotion of anti-HIV entry activity. It was found that C-28 amide functionality, a second aminoalkanoic acid group near the end of the C-28 side chain, and a 7−10 carbon alkane chain between these two amide moieties are important to the enhanced antientry potency.[22] In order to design antimaturation promoting C-28 side chains without an antientry effect, we first synthesized a group of BA derivatives (**3**, **4**, **7**−**15**, and **29**) with a C-28 ester moiety rather than the prior amide functionality. Methoxymethyl (MOM), methoxyethoxymethyl

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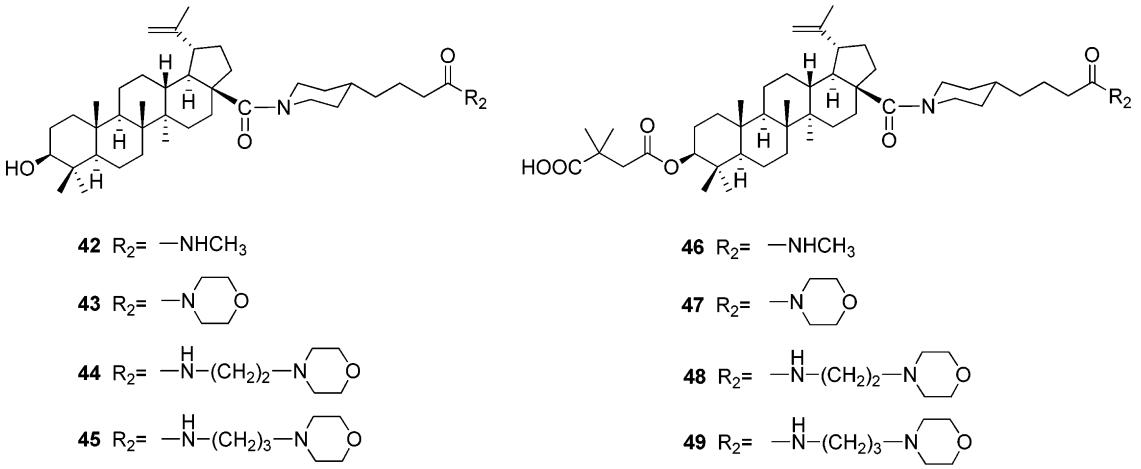
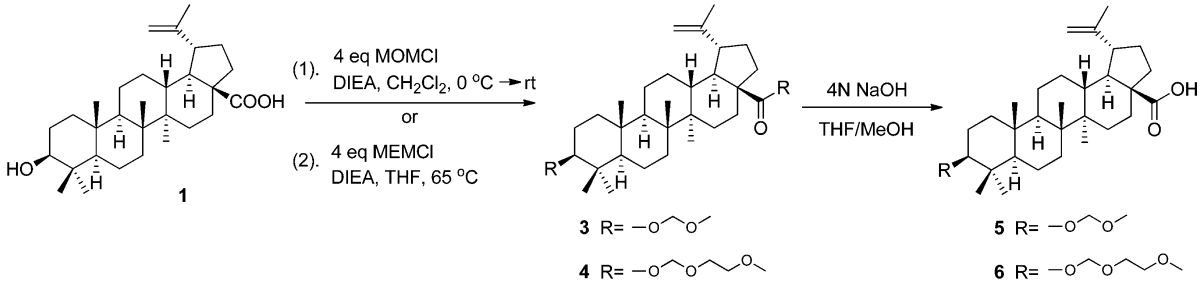
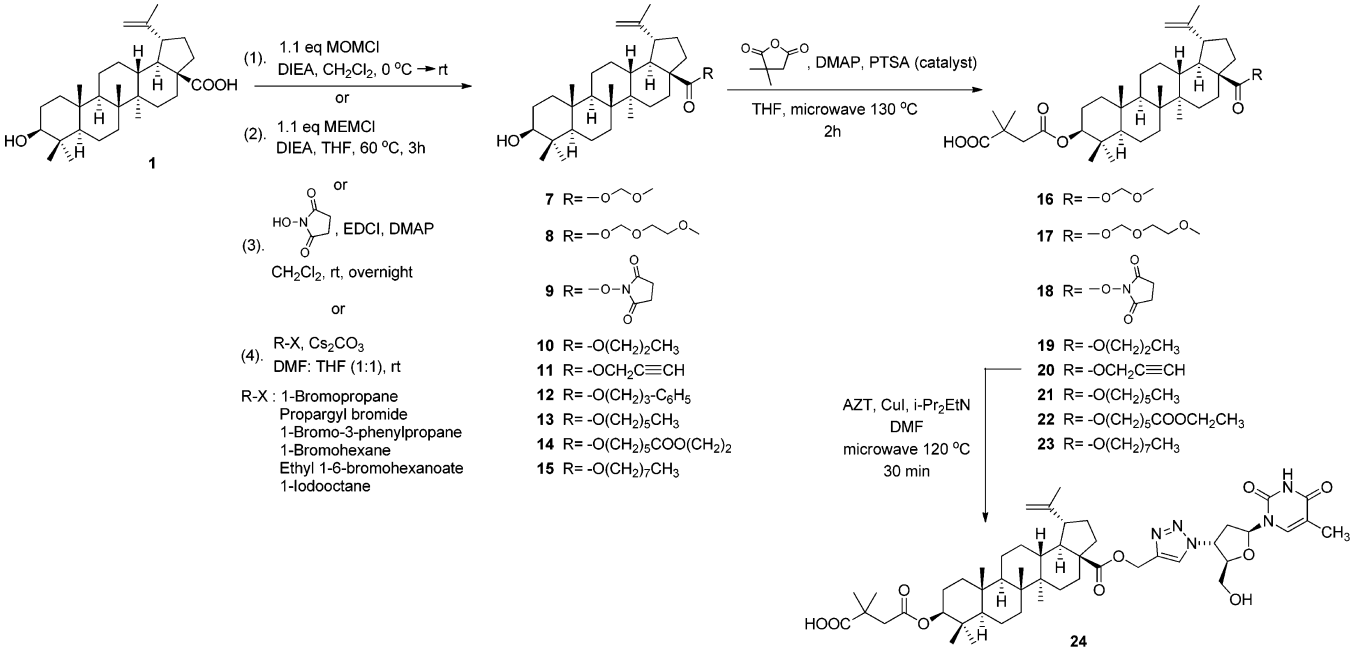


Figure 2.

Scheme 1



Scheme 2



(MEM), and tetraethylene glycol chains were incorporated to improve the hydrophilicity of the compounds (**7**, **8**, and **29**) and to investigate the influence of a polar heteroatom (e.g., oxygen) present within the C-28 side chain, which was originally a pure carbon alkane chain. Bulky substitutions (**9** and **12**) and an unsaturated C-28 chain (**11**) were also synthesized. Different lengths of the C-28 alkane chains with or without a second ester group near the terminus were also investigated (**14** vs **10**, **13**, and **15**). These 28-modified BA derivatives were evaluated for anti-HIV replication activity to determine if these compounds function as HIV-1 entry inhibitors before the C-3 antimaturation pharmacophore, a dimethylsuccinyl side chain, was incorporated to generate the corresponding 28-substituted derivatives (**16**–**24** and **30**) of **2**.

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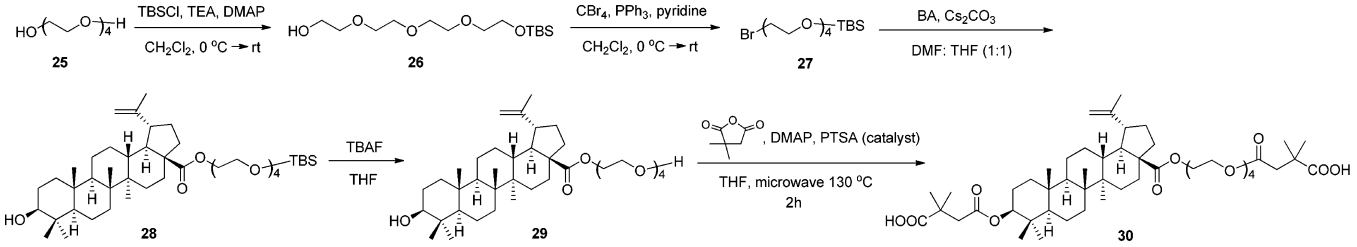
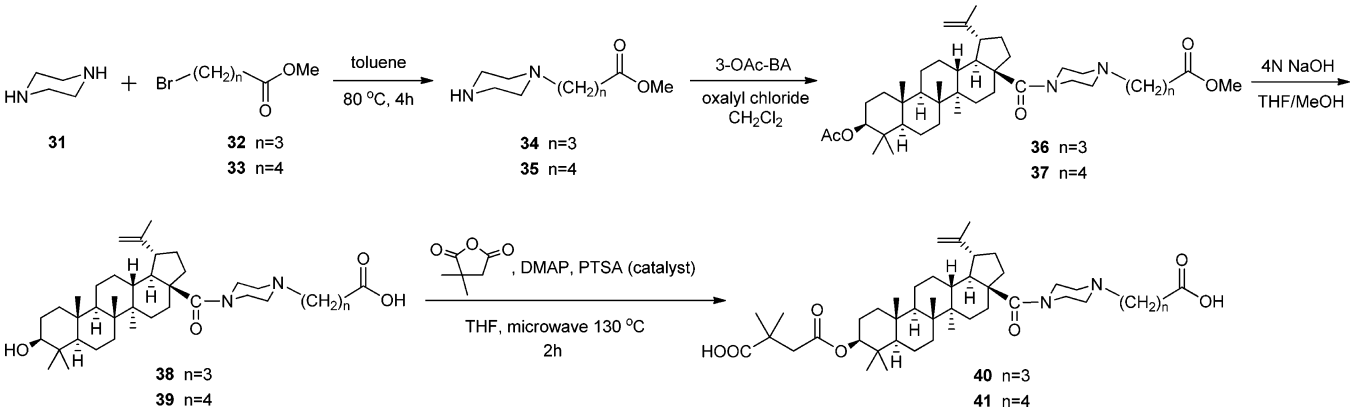
Scheme 3



Scheme 4



In addition, a cyclic secondary amine from piperazinebutyric acid or piperazinepentanoic acid, rather than a primary amine, was used to form the amide bond with the C-28 carboxylic acid of **1** to yield compounds **38** and **39**. In this design, an additional polar heteroatom nitrogen was present within the side chain, and the substitution near the C-28 amide moiety was bulkier, in order to reduce the potential antientry activity and promote the antimaturation effect. The corresponding C-3 succinylated compounds **40** and **41** were then prepared and evaluated in comparison with **2**. Previously synthesized compounds **42**–**49**, with C-28 piperidine side chains (Figure 2),[23] were also tested because of their structural similarity to **38**–**41**. The SAR of the antimaturation promoting activity of C-28 side chains was then established.

CHEMISTRY

The syntheses of BA derivatives **3**–**6** were carried out according to Scheme 1. Four equivalents of MOMCl or MEMCl were reacted with **1** and DIEA in CH_2Cl_2 at room temperature overnight or in THF at 65 °C for 6 h to generate compounds **3** and **4**, respectively, in 83% or 89% yield. Hydrolysis of **3** and **4** using 4 N NaOH provided **5** and **6** in over 90% yield.

Scheme 2 depicts the synthetic pathway to compounds **7**–**24**. Compounds **7** and **8** were obtained by reducing the amount of MOMCl or MEMCl to 1.1 equiv in the reaction mixture. *N*-Hydroxysuccinimide and **1** were reacted in the presence of EDCI and DMAP overnight to furnish **9** in 53% yield. Compounds **10**–**15** (67–98% yield) were obtained by treating **1** with Cs_2CO_3, followed by adding different halogenated alkanes or alkynes. Esterification of **7**–**11** and **13**–**15** with 2,2-dimethylsuccinic anhydride was conducted using microwave conditions rather than conventional oil-bath heating to yield compounds **16**–**23**. A catalytic amount of PTSA and THF as solvent was used instead of pyridine, resulting in yields of 40–55%. Compound **24** was synthesized in 75% yield by click reaction of **20** with AZT in the presence of CuI and *N*,*N*-diisopropylethylamine in DMF using a microwave synthesizer at 120 °C for 30 min.

A tetraethyleneglycol chain was also introduced at the C-28 position of **2**, and compounds **29** and **30** were prepared as described in Scheme 3. Overall, **25** was first protected with TBSCl to yield **26**, which was then converted to bromo-substituted **27** in 85% yield by reaction with CBr_4 using PPh_3 and pyridine. Reaction of **27** with **1** provided **28** in a yield of 72%, and deprotection of **28** using TBAF provided **29**. Compound **30** was prepared by a microwave reaction as described above.

The syntheses of piperazine incorporated BA derivatives **38**–**41** were achieved according to Scheme 4. The C-28 piperazine side chains of **34** and **35** were prepared in toluene at 80 °C. C-3 acetyl protected BA (3-OAc-BA) was prepared previously[23] and was activated by oxalyl chloride before reacting with **34** and **35** to furnish **36** and **37** in 89% and 100% yields, respectively. After hydrolysis, the resulting compounds **38** and **39** were reacted with 2,2-dimethylsuccinic anhydride in a microwave synthesizer to provide compounds **40** and **41** in approximately 40% yield.

RESULTS AND DISCUSSION

The anti-HIV-1 replication activity of BA derivatives **3**–**24**, **29**, **30**, and **38**–**49** was assessed in HIV-1_{NL4-3} infected MT-4 cell lines in parallel with AZT and **2**, and the results are summarized in Table 1. The results indicated that compounds with MOM or MEM substitution at C-3 (**5** and **6**), C-28 (**7** and **8**), or both positions (**3** and **4**) of **1** did not show anti-HIV activity. Similarly, compounds **9**–**15** and **29** with a C-3 hydroxy group and C-28 ester substitutions of different length or size, or with heteroatoms within the side chains, did not show antiviral activity, suggesting that **1** derivatives bearing ester modifications at the C-28 position do not exhibit anti-HIV-1 activity and

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

compd	IC_{50} (μM)	CC_{50} (μM)	TI
AZT	0.030	>37	1248
bevirimat (**2**)	0.087	14.2	171
3	NS[b]	NT[c]	
4	NS[b]	NT[c]	
5	NS[b]	NT[c]	
6	NS[b]	NT[c]	
7	NS[b]	NT[c]	
8	NS[b]	NT[c]	
9	NS[b]	NT[c]	
10	NS[b]	NT[c]	
11	NS[b]	NT[c]	
12	NS[b]	NT[c]	
13	NS[b]	NT[c]	
14	NS[b]	NT[c]	
15	NS[b]	NT[c]	
16	0.17	12.4	70.9
17	0.046	12.1	261.3
18	0.13	11.0	86.2
19	0.088	10.4	118.2
20	0.067	15.3	226.2
21	1.8	14.8	8.3
22	0.056	8.6	153.7
23	NS[b]	NT[c]	
24	0.10	11.0	101.0
29	NS[b]	NT[c]	
30	0.079	8.9	111.3
38	NS[b]	NT[c]	
39	NS[b]	NT[c]	
40	0.011	13.0	1181
41	0.0059	13.2	2237
42	NS[b]	NT[c]	
43	NS[b]	NT[c]	
44	NS[b]	NT[c]	
45	NS[b]	NT[c]	
46	0.24	>13.3	>55.5
47	0.07	10.9	155.8
48	0.035	10.0	286.1
49	0.031	10.0	321.2

[a]All data presented are averages of at least three separate experiments. IC_{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}/IC_{50}. [b]NS: no suppression at the testing concentration (10 μM). [c]NT: not tested.

are not entry inhibitors. The results confirmed that C-3 dimethylsuccinyl substitution is essential to the antimaturation activity of **2**.

Diverse C-28 ester substituted **2** derivatives (**16**–**24** and **30**) were then investigated to establish the SAR of the C-28 side chain toward **2**'s antimaturation potency. Compounds **19**, **21**, and **23**, with the C-28 alkane chain length increasing from propyl to hexyl to octyl, respectively, showed a decreasing activity trend. While **19** (IC_{50} = 0.088 μM) and **2** (IC_{50} = 0.087 μM) exhibited equivalent anti-HIV potency, compound **21** was approximately 20-fold less potent (IC_{50} = 1.8 μM) and **23** showed no inhibition of HIV-1 replication. Compound **20**, with a C-28 prop-2-ynyl moiety, exhibited good antiviral activity (IC_{50} = 0.067 μM), and **22**, with a second ester substitution at the terminus of a C-28 hexyl chain, showed increased antiviral potency (IC_{50} = 0.056 μM). For those compounds with one or two polar oxygen atoms present within their C-28 side chain, **16** (MOM ester) had an anti-HIV IC_{50} of 0.17 μM, while **17** (MEM ester) had an IC_{50} of 0.046 μM and thus was 3.7-fold more potent than **16** and 2-fold more potent than **2**. Compound **30**, with a C-28 tetraethylene glycol side chain terminating in a dimethyl succinyl ester, in addition to the dimethyl succinyl ester at C-3, was equipotent (IC_{50} = 0.079 μM) with **2**. Compounds with bulky C-28 substituents, such as an *N*-succinimide ester (**18**), or conjugated to AZT through a triazole linkage (**24**) displayed slightly reduced anti-HIV-1 activity (IC_{50} of 0.13 and 0.10 μM, respectively) relative to **2**.

To further investigate the effect of C-28 side chains, cyclic secondary amines from piperazinebutyric acid, piperazinepentanoic acid, and piperidinebutyric acid, rather than a primary amine, were used in amidation reactions with the C-28 carboxylic acid of 3-acetylated BA, resulting in **38**, **39**, and **42**–**45**. None of these **1** analogues exhibited anti-HIV replication activity in MT-4 lymphocytes, suggesting that bulkier C-28 amide moieties compromised the antientry effect of C-28 modified BAs.

Subsequently, the C-3 hydroxy group of **38**, **39**, and **42**–**45** was esterified with a dimethylsuccinyl moiety. Among the resulting compounds, **40**, **41**, **48**, and **49** showed significantly increased antiviral activity (IC_{50} values ranging from 0.0059 to 0.035 μM). These results indicate that incorporating a cyclic secondary amide group with an extended side chain at the C-28 position can remarkably enhance the activity of **2**. Specifically, a C-28 amide from piperidinebutyric acid with a second amide group formed from ethylmorpholine (**48**) or propylmorpholine (**49**) increased the antiviral activity of **2** by about 3-fold, with IC_{50} values of 0.035 and 0.031 μM, respectively. The presence of additional polar nitrogen heteroatom within the C-28 amide side chain (from piperazinebutyric or piperazinepentanoic acid) significantly increased the potency by 8- to 15-fold, as shown by compound **40** (IC_{50} = 0.011 μM) and **41** (IC_{50} = 0.0059 μM), respectively.

A TZM-bl assay was used to confirm the mechanism of action of active compounds. This assay measures neutralization in TZM-bl cells as a function of a reduction in Tat-induced luciferase (Luc) reporter gene expression after a single round of virus infection. The TZM-bl cell line is a HeLa cell clone that was engineered to express CD4 and CCR5 and contains integrated reporter gene for firefly luciferase and *E.coli* β-galactosidase under control of an HIV-1 LTR. Expression of the reporter genes is induced in trans by viral Tat protein soon after infection, and the TZM-bl assay can detect HIV-entry inhibitors sensitively. However, because maturation inhibitors block HIV-1 replication only at the last step of the viral life cycle and, thus, still permit the production of immature noninfectious viral particles, the single round TZM-bl assay cannot detect HIV-maturation inhibitors because the expression of reporter gene is not impaired in the first cycle. Therefore, all of the novel active 3,28-modified BAs were further screened in this assay system. As expected, none of the compounds were active in the single round TZM-bl assay, proving that they do not function as HIV entry inhibitors and, thus, their antiviral replication activity should result from an antimaturation mechanism of action.

The following SAR conclusions summarize the effects of the C-28 substitutions to promote the antimaturation activity of **2**. C-28 ester side chains with a three- to six-carbon alkyl chain result in enhanced anti-HIV activity. The presence of a polar

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oxygen atom within the side chain and the introduction of a second ester functionality at the terminus of the C-28 ester chain can moderately enhance the antimaturation activity of **2**, as shown by **17** and **22**. Meanwhile, while **1** analogues with C-28 primary amide substitutions function as antientry agents, **1** analogues with C-28 secondary cyclic amide groups do not. Instead, the latter compounds exhibit significant antimaturation promoting activity. Thus far, compound **41**, which has an additional polar nitrogen heteroatom present in the C-28 amide (piperazinepentanoic acid), showed the best antiviral replication activity acting through an antimaturation mechanism of action.

To evaluate its potential as a drug candidate, **41** was further assessed for its in vitro metabolic stability in pooled human liver microsomes (HLMs). The results revealed that 86.15% of **41** remained intact after 60 min of incubation in HLMs. Compound **41**'s long in vitro half-life ($t_{1/2}$ of 327 min) and low clearance (CL_{int} of 0.0021 mL min^{-1} mg^{-1}) proved that incorporation of C-28 cyclic secondary amide chains would not decrease the stability of **2**, which has a long elimination half-life ($t_{1/2}$ of 56.3−69.5 h) in healthy volunteers.[18]

Overall, new BA derivatives were designed and synthesized in order to investigate the modulating effects of C-28 substitution toward **2**'s antimaturation activity. It was discovered that certain ester or cyclic secondary amide substitutions at C-28 will enhance the antiviral activity of **2**, acting only through an antimaturation effect. The best new compound **41** showed excellent anti-HIV activity with an IC_{50} of 0.0059 μM, 15-fold better than that of **2**. Compound **41** also exhibited good in vitro metabolic stability in HLMs, and therefore, may serve as an attractive new lead for the development of a next generation of maturation inhibitors as anti-AIDS 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 an Inova 400 MHz spectrometer using Me_4Si (TMS) as internal standard. The solvent used was $CDCl_3$, unless otherwise indicated. High resolution mass spectra were measured on a Shimadzu LCMS-IT-TOF with ESI interface. HPLC for purity determinations was conducted using a Shimadzu LCMS with a Grace Alltima 2.1 mm ×150 mm HP C18 5 μm column and a Shimadzu SPD-M20A detector at 205 or 220 nm wavelength. The detailed solvent percentage conditions for each tested compound are listed in the Supporting Information. All target compounds were at least 95% pure. Optical rotations were measured with a Jasco Dip-2000 digital polarimeter at 25 °C at the sodium D line. Thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F-254 plates. For purification of all synthetic BA derivatives, a Grace Reveleris flash system equipped with RevealX detection allowing for multisignal (UV/ELSD) collection to low milligram quantities was used for medium pressure column chromatography. Reveleris Navigator method optimizer and Grace Reveleris flash silica cartridges were employed for high quality separations. Commercial chemicals were obtained from Sigma-Aldrich, Inc.

Syntheses of 3 and 7. Compound **1** (1 equiv), DIEA (4 equiv), and MOMCl (4 equiv for **3** and 1.1 equiv for **7**) were dissolved in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred overnight under N_2, allowing a gradual warming to room temperature. The solution was then diluted with CH_2Cl_2 (20 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds **3** and **7**.

Methoxymethyl 3β-Methoxymethoxylup-20(29)-en-28-oate (3). Yield, 149 mg (83% yield), starting from 179 mg of **1**; white amorphous powder. Mp 99−102 °C. HRMS (ESI−) m/z: 543.3991 ($C_{34}H_{56}O_5$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.28−5.22 (2H, app d, J = 5.6 Hz, -COOCH_2O-), 4.80, 4.67 (1H each, app d, J = 6.4 Hz, -OCH_2O-), 4.73, 4.61 (1H each, s, H-29), 3.48, 3.38 (3H each, s, -OCH_3), 3.08−2.99 (2H, m, H-3, H-19), 1.69 (3H, s, H-30), 0.97, 0.95, 0.93, 0.83, 0.78 (3H each, s, 5 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.9, 16.2, 16.4, 16.5, 18.5, 19.6, 21.1, 24.4, 25.8, 28.2, 29.8, 30.8, 32.2, 34.6, 37.1, 37.3, 38.5, 38.9, 38.9, 41.0, 42.6, 47.1, 49.6, 50.8, 55.7, 56.0, 57.0, 57.9, 85.4, 90.1, 96.2, 109.9, 150.7, 175.8.

Methoxymethyl 3β-Hydroxylup-20(29)-en-28-oate (7). Yield, 190 mg (86% yield), starting from 200 mg of **1**; white amorphous powder. Mp 114−115 °C. HRMS (ESI−) m/z: 499.3745 ($C_{32}H_{52}O_4$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.28−5.22 (2H, app q, J = 6.0, 5.6 Hz, -COOCH_2O-), 4.73, 4.61 (1H each, s, H-29), 3.48 (3H, s, -OCH_3), 3.18 (1H, dd, J = 11.2, 5.2 Hz, H-3), 3.05−2.98 (1H, m, H-19), 2.21−2.30 (2H, m, H-16), 1.69 (3H, s, H-30), 0.97, 0.96, 0.93, 0.82, 0.75 (3H each, s, 5 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.9, 15.6, 16.2, 16.3, 18.5, 19.6, 21.1, 25.7, 27.6, 28.2, 29.8, 29.9, 30.7, 32.2, 34.6, 37.1, 37.4, 38.4, 38.9, 39.0, 40.9, 42.6, 47.1, 49.6, 50.8, 55.6, 56.9, 57.9, 79.1, 90.1, 109.9, 150.6, 175.8.

Syntheses of 4 and 8. Compound **1** (1 equiv), DIEA (4 equiv), and MEMCl (4 equiv for **4** and 1.1 equiv for **8**) were dissolved in THF (5 mL) at room temperature. For **4**, the mixture was stirred at 65 °C under N_2 for 6 h, and for **8**, the mixture was stirred at 60 °C for 3 h. The solution was then diluted with water, extracted with CH_2Cl_2, and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds **4** and **8**.

Methoxyethoxymethyl 3β-Methoxyethoxymethoxylup-20(29)-en-28-oate (4). Yield, 311 mg (89% yield), starting from 252 mg of **1**; white amorphous powder. Mp 102−105 °C. HRMS (ESI +) m/z: 655.4579 ($C_{38}H_{64}O_7$ + Na). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37−5.30 (2H, app dd, J = 6.0, 5.8 Hz, -COOCH_2O-), 4.81, 4.65 (1H each, app d, J = 6.8 Hz, -OCH_2O-), 4.71, 4.58 (1H each, s, H-29), 3.80, 3.71, 3.57, 3.50 (2H each, m, 2 × -OCH_2CH_2O-), 3.38 (6H, s, 2 × -OCH_3), 3.09−3.05 (1H, dd, J = 11.4, 4.4, 4.0 Hz, H-3), 3.01−2.94 (1H, m, H-19), 1.68 (3H, s, H-30), 0.97, 0.93, 0.91, 0.83, 0.77 (3H each, s, 5 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.91, 16.25, 16.38, 16.49, 18.51, 19.59, 21.14, 24.30, 25.78, 28.27, 29.88, 30.76, 32.20, 34.60, 37.10, 37.27, 38.45, 38.86, 38.89, 41.98, 42.64, 47.11, 49.62, 50.79, 55.94, 56.93, 59.26, 59.31, 67.19, 69.70, 71.76, 72.07, 85.33, 89.08, 95.13, 109.88, 150.70, 175.79.

Methoxyethoxymethyl 3β-Hydroxylup-20(29)-en-28-oate (8). Yield, 219 mg (92% yield), starting from 200 mg of **1**; white amorphous powder. Mp 119−121 °C. HRMS (ESI+) m/z: 567.4043 ($C_{34}H_{56}O_5$ + Na). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37−5.30 (2H, app dd, J = 6.4, 6.0 Hz, -COOCH_2O-), 4.73, 4.60 (1H each, s, H-29), 3.80, 3.56 (2H each, t, J = 5.2 Hz, -OCH_2CH_2O-), 3.39 (3H, s, -OCH_3), 3.18 (1H, m, H-3), 3.04−2.98 (1H, m, H-19), 1.68 (3H, s, H-30), 1.00, 0.97, 0.92, 0.82, 0.75 (3H each, s, 5 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.9, 15.6, 16.2, 16.4, 18.5, 19.6, 21.1, 25.8, 27.6, 28.2, 29.9, 30.8, 32.2, 34.6, 37.1, 37.4, 38.4, 39.0, 39.1, 41.0, 42.7, 47.1, 49.6, 50.8, 55.6, 56.9, 59.3, 69.7, 71.8, 79.2, 89.1, 109.9, 150.7, 175.8.

Syntheses of 34 and 35. Piperazine **31** (2 equiv) was dissolved in toluene, and the mixture was heated to 80 °C. Compound **32** or **33** (1 equiv) was added, and the reaction proceeded for 4 h. After the mixture was cooled and filtered, the organic layer was concentrated under reduced pressure. The residue was chromatographed using a silica gel column to yield 500 mg of **34** (50%) and 498 mg of **35** (42%).

Syntheses of 36 and 37. Oxalyl chloride solution (2 M in CH_2Cl_2, 10 mL) was added to 3-OAc-BA (1 equiv)[22] in CH_2Cl_2 (10 mL), and the mixture was stirred for 2 h. After concentration under vacuum, the residual mixture was treated with **34** or **35** (1.6 equiv) and triethylamine (Et_3N, 1.2 equiv) in CH_2Cl_2. The mixture was stirred at room temperature overnight. The solution was then diluted with CH_2Cl_2 (20 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced

pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds **36** and **37**.

Methyl *N*-[3*β*-Acetoxylup-20(29)-en-28-oyl]-4-piperazinebutanoate (36). Yield, 177.5 mg (89% yield), starting from 150 mg of 3-OAc-BA; white amorphous powder. Mp 203−205 °C. HRMS (ESI+) *m*/*z*: 667.5055 ($C_{41}H_{66}N_2O_5$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.72, 4.60 (1H each, s, H-29), 4.47 (1H, dd, *J* = 12.4, 5.6 Hz, H-3), 3.68 (3H, s, -COOCH_3), 3.61−3.52 (4H, m, 28-CON(CH_2CH$_2$)$_2$N-), 3.01 (1H, m, H-19), 2.91−2.76 (4H, m, 28-CON(CH$_2$CH_2)$_2$N-), 2.46 (2H, m, -NCH_2-), 2.05 (3H, s, OCOCH_3), 1.68 (3H, s, H-30), 1.02, 0.99, 0.96, 0.85, 0.81 (3H each, s, 5 × CH_3).

Methyl *N*-[3*β*-Acetoxylup-20(29)-en-28-oyl]-5-piperazinepentanoate (37). Yield, 847 mg (100% yield), starting from 622.8 mg of 3-OAc-BA; white amorphous powder. Mp 217−218 °C. HRMS (ESI+) *m*/*z*: 681.5219 ($C_{42}H_{68}N_2O_5$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.73, 4.60 (1H each, s, H-29), 4.46 (1H, m, H-3), 3.67 (3H, s, -COOCH_3), 3.58−3.47 (4H, m, 28-CON(CH_2CH$_2$)$_2$N-), 3.00 (1H, m, H-19), 2.87−2.76 (4H, m, 28-CON(CH$_2$CH_2)$_2$N-), 2.46 (2H, m, -NCH_2-), 2.06 (3H, s, OCOCH_3), 1.69 (3H, s, H-30), 0.98, 0.97, 0.90, 0.86, 0.83 (3H each, s, 5 × CH_3).

Syntheses of 5, 6, 38, and 39. To a solution of the appropriate ester intermediates **3**−**4** and **36**−**37** (1 equiv) in MeOH (8 mL) and THF (4 mL) was added 4 N NaOH (4 mL). The mixture was stirred overnight and then neutralized with 20% HCl. The solution was dried under vacuum and reconstituted with CH_2Cl_2. The organic layer was washed with brine and dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds **5**−**6** and **38**−**39**.

3*β*-Methoxymethoxybetulinic Acid (5). Yield, 85 mg (93% yield), starting from 100 mg of **3**; white amorphous powder. Mp 117−119 °C. HRMS (ESI−) *m*/*z*: 499.3730 ($C_{32}H_{52}O_4$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.75, 4.62 (1H each, app d, *J* = 6.4 Hz, -OCH_2O-), 4.71, 4.59 (1H each, s, H-29), 3.36 (3H, s, -OCH_3), 3.05−2.96 (2H, m, H-3, H-19), 1.71 (3H, s, H-30), 0.97, 0.95, 0.90, 0.83, 0.77 (3H each, s, 5 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): *δ* 14.7, 16.1, 16.1, 16.3, 18.3, 19.4, 20.9, 24.2, 25.5, 28.0, 29.7, 30.6, 32.2, 34.3, 37.1, 38.4, 38.7, 40.7, 42.4, 47.0, 49.3, 50.5, 55.5, 55.7, 56.4, 77.3, 85.2, 96.0, 109.7, 150.4, 181.8.

3*β*-Methoxyethoxymethoxybetulinic Acid (6). Yield, 118 mg (92% yield), starting from 150 mg of **4**; white amorphous powder. Mp 121−124 °C. HRMS (ESI+) *m*/*z*: 567.4033 ($C_{34}H_{56}O_5$ + Na). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.80, 4.66 (1H each, app d, *J* = 6.8 Hz, -OCH_2O-), 4.70, 4.58 (1H each, s, H-29), 3.69, 3.52 (2H each, t, *J* = 4.4 Hz, -OCH_2CH_2O-), 3.36 (3H, s, -OCH_3), 3.07 (1H, dd, *J* = 12.0, 4.4 Hz, H-3), 3.00 (1H, m, H-19), 1.67 (3H, s, H-30), 0.94, 0.91, 0.89, 0.80, 0.73 (3H each, s, 5 × CH_3).

***N*-[3*β*-Hydroxylup-20(29)-en-28-oyl]-4-piperazinebutyric Acid (38).** Yield, 91 mg (79% yield), starting from 125 mg of **36**; white amorphous powder. Mp 153−154 °C. HRMS (ESI−) *m*/*z*: 609.4646 ($C_{38}H_{62}N_2O_4$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.71, 4.60 (1H each, s, H-29), 3.65−3.49 (4H, m, 28-CON(CH_2CH$_2$)$_2$N-), 3.17 (1H, m, H-3), 3.01 (1H, m, H-19), 2.82−2.68 (4H, m, 28-CON(CH$_2$CH_2)$_2$N-), 2.45 (2H, m, -NCH_2-), 1.69 (3H, s, H-30), 1.01, 0.98, 0.89, 0.84, 0.78 (3H each, s, 5 × CH_3).

***N*-[3*β*-Hydroxylup-20(29)-en-28-oyl]-5-piperazinepentanoic Acid (39).** Yield, 624 mg (85% yield), starting from 800 mg of **37**; white amorphous powder. Mp 159−161 °C. HRMS (ESI−) *m*/*z*: 623.4781 ($C_{39}H_{64}N_2O_4$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.71, 4.58 (1H each, s, H-29), 3.64−3.51 (4H, m, 28-CON(CH_2CH$_2$)$_2$N-), 3.19 (1H, m, H-3), 2.99 (1H, m, H-19), 2.89−2.72 (4H, m, 28-CON(CH$_2$CH_2)$_2$N-), 2.46 (2H, m, -NCH_2-), 1.68 (3H, s, H-30), 0.98, 0.95, 0.92, 0.82, 0.81 (3H each, s, 5 × CH_3).

***N*-Succinimide 3*β*-Hydroxylup-20(29)-en-28-oate (9).** A mixture of **1** (260 mg, 1 equiv), *N*-hydroxysuccinimide (1.5 equiv), EDCI (1.5 equiv), and DMAP (0.1 equiv) was dissolved in CH_2Cl_2 (5 mL) and stirred under N_2 at room temperature overnight. The solution was then diluted with CH_2Cl_2 (20 mL) and washed with brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield 167 mg of **9** (53% yield), a white amorphous powder. Mp 189−191 °C. HRMS (ESI+) *m*/*z*: 576.3656 ($C_{34}H_{51}NO_5$ + Na). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.72, 4.61 (2H, s, H-29), 3.18 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 2.95 (1H, m, H-19), 2.84, 2.82 (4H, d, *J* = 5.2 Hz, -COCH_2CH_2CO-), 2.47−2.42 (1H, m, H-13), 1.69 (3H, s, H-30), 0.98 (6H, s, 2 × CH_3), 0.96, 0.82, 0.75 (3H each, s, 3 × CH_3).

Syntheses of 26 and 27. A mixture of tetraethylene glycol (**25**, 35 g, 5 equiv), TEA (1.5 equiv), and DMAP (0.1 equiv) was dissolved in CH_2Cl_2 (200 mL) at 0 °C. TBSCl (5 g, 1 equiv) in CH_2Cl_2 (50 mL) was added dropwise, and reaction continued overnight. The solution was diluted with CH_2Cl_2 and washed with mildly acidic water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography to yield 50 g of TBS-protected tetraethylene glycol **26** (90%).[24] In the next step, a mixture of **26** (852 mg, 1 equiv) and CBr_4 (2 equiv) was dissolved in CH_2Cl_2 (200 mL) at 0 °C. Pyridine (2.5 mL, 10 equiv) was added, followed by PPh_3 (2 equiv). The mixture was stirred overnight with a gradual warming to room temperature. The suspension was filtered through Celite, and the organic layer was concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield 872 mg of bromo-substituted TBS-protected tetraethylene glycol **27** (85% yield).[25]

Syntheses of 10−15 and 28. Compound **1** (1 equiv) and CS_2CO_3 (3 equiv) were dissolved in a 1:1 mixed solution of DMF and THF (4 mL), and the mixture was stirred for 30 min at room temperature. Bromo- or iodo-substituted compound (RX) or **27** (3 equiv) was then added, and the reaction was carried out overnight at room temperature. Water was added to stop the reaction, and the solution was extracted with EtOAc. The organic layer was washed further with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was chromatographed using a silica gel column to yield the pure target compounds **10**−**15** and **28**.

Propyl 3*β*-Hydroxylup-20(29)-en-28-oate (10). Yield, 56 mg (86% yield), starting from 60 mg of **1**; white amorphous powder. Mp 106−107 °C. HRMS (ESI+) *m*/*z*: 499.4155 ($C_{33}H_{54}O_3$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.73, 4.60 (2H, s, H-29), 4.09 (2H, m, 28-COOCH_2-), 3.18 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 3.01 (1H, m, H-19), 1.68 (3H, s, H-30), 1.01 (6H, s, 2 × CH_3), 0.94 (3H, s, CH_3), 0.91 (3H, m, CH_3), 0.82, 0.76 (3H each, s, 2 × CH_3).

Prop-2′-ynyl 3*β*-Hydroxylup-20(29)-en-28-oate (11). Yield, 47 mg (72% yield), starting from 60 mg of **1**; white amorphous powder. Mp 131−133 °C. HRMS (ESI+) *m*/*z*: 495.3826 ($C_{33}H_{50}O_3$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.73, 4.61 (2H, s, H-29), 4.69, 4.65 (2H, d, *J* = 2.2 Hz, 28-COOCH_2-), 3.18 (1H, m, H-3), 3.01 (1H, m, H-19), 2.43 (1H, t, *J* = 2.2 Hz, -C≡CH), 1.69 (3H, s, H-30), 0.97 (6H, s, 2 × CH_3), 0.93, 0.82, 0.75 (3H each, s, 3 × CH_3).

3′-Phenylpropyl 3*β*-Hydroxylup-20(29)-en-28-oate (12). Yield, 72 mg (96% yield), starting from 60 mg of **1**; white amorphous powder. Mp 169−172 °C. HRMS (ESI+) *m*/*z*: 575.4465 ($C_{39}H_{58}O_3$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.31−7.26, 7.22−7.18 (5H, m, -C$_6$H$_5$), 4.73, 4.61 (2H, s, H-29), 4.09 (2H, m, 28-COOCH_2-), 3.18 (1H, m, H-3), 3.02 (1H, m, H-19), 2.72 (1H, t, *J* = 6.4 Hz, -CH_2C$_6$H$_5$), 1.69 (3H, s, H-30), 0.97, 0.96, 0.91, 0.81, 0.75 (3H each, s, 5 × CH_3).

Hexyl 3*β*-Hydroxylup-20(29)-en-28-oate (13). Yield, 48 mg (67% yield), starting from 60 mg of **1**; white amorphous powder. Mp 124−125 °C. HRMS (ESI+) *m*/*z*: 541.4629 ($C_{36}H_{60}O_3$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.73, 4.59 (2H, s, H-29), 4.07 (2H, m, 28-COOCH_2-), 3.18 (1H, dd, *J* = 11.8, 4.4 Hz, H-3), 3.02 (1H, m, H-19), 1.68 (3H, s, H-30), 0.97 (6H, s, 2 × CH_3), 0.92 (3H, s, CH_3), 0.90 (3H, m, CH_3), 0.82, 0.75 (3H each, s, 2 × CH_3).

Ethyl *O*-[3*β*-Hydroxylup-20(29)-en-28-oyl]-6-hexanoate (14). Yield, 77 mg (98% yield), starting from 60 mg of **1**; white amorphous powder. Mp 191−194 °C. HRMS (ESI+) *m*/*z*: 599.4649 ($C_{38}H_{62}O_5$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* 4.72, 4.59 (2H, s, H-29), 4.16−4.02 (4H, m, 28-COOCH_2-, -COOCH_2CH$_3$), 3.41 (1H, t, *J* = 6.8 Hz, -OH), 3.17 (1H, dd, *J* = 11.8, 4.4 Hz, H-3), 3.00 (1H, m, H-19), 2.31 (2H, m, -CH_2COO-), 1.68 (3H, s, H-30), 1.26 (3H, t, *J* = 7.2 Hz,

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-COOCH$_2$CH$_3$), 0.97 (6H, s, 2 × CH$_3$), 0.91, 0.82, 0.75 (3H each, s, 3 × CH$_3$).

Octyl 3β-Hydroxylup-20(29)-en-28-oate (15). Yield, 70 mg (94% yield), starting from 60 mg of **1**; white amorphous powder. Mp 146−147 °C. HRMS (ESI+) *m/z*: 569.4936 ($C_{38}H_{64}O_3$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (2H, s, H-29), 4.07 (2H, m, 28-COOC*H*$_2$-), 3.17 (1H, dd, *J* = 12.0, 4.0 Hz, H-3), 3.02 (1H, m, H-19), 1.68 (3H, s, H-30), 0.96 (6H, s, 2 × CH$_3$), 0.92 (3H, s, CH$_3$), 0.89 (3H, t, *J* = 6.4 Hz, CH$_3$), 0.82, 0.75 (3H each, s, 2 × CH$_3$).

2-(2-(2-(2-*O*-*tert*-Butyldimethylsilylethoxy)ethoxy)ethoxy)-ethyl 3β-hydroxylup-20(29)-en-28-oate (28). Yield, 142 mg (72% yield), starting from 120 mg of **1**; white amorphous powder. Mp 203−206 °C. HRMS (ESI+) *m/z*: 747.5603 ($C_{44}H_{78}O_7Si$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.62 (2H, s, H-29), 4.25 (2H, m, -COOC*H*$_2$CH$_2$O-), 3.96−3.54 (2H each, m, -COOCH$_2$C*H*$_2$O-, 3 × -OCH$_2$CH$_2$O-), 3.17 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 3.01 (1H, m, H-19), 1.68 (3H, s, H-30), 0.98 (12H, s, CH$_3$, -Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.91, 0.89, 0.82, 0.75 (3H each, s, 4 × CH$_3$), 0.21 (6H, s, -Si(C*H*$_3$)$_2$C-(CH$_3$)$_3$).

2-(2-(2-(2-Hydroxyethoxy)ethoxy)ethoxy)ethyl 3β-hydroxyl-up-20(29)-en-28-oate (29). To a solution of **28** (170 mg, 1 equiv) in THF (5 mL) was added TBAF (1.5 equiv). The mixture was stirred overnight at room temperature. The organic layer was concentrated to dryness under reduced pressure and the residue was chromatographed using a silica gel column to yield 166 mg of **29** (98% yield), a white amorphous powder. Mp 178−180 °C. HRMS (ESI+) *m/z*: 633.4743 ($C_{38}H_{64}O_7$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.61 (2H, s, H-29), 4.25 (2H, m, -COOC*H*$_2$CH$_2$O-), 3.65−3.44 (2H each, m, -COOCH$_2$C*H*$_2$O-, 3 × -OCH$_2$CH$_2$O-), 3.17 (1H, dd, *J* = 11.6, 4.8 Hz, H-3), 3.01 (1H, m, H-19), 1.68 (3H, s, H-30), 0.98, 0.97, 0.89, 0.82, 0.76 (3H each, s, 5 × CH$_3$).

Syntheses of 16−23, 30, 40, and 41. The appropriate BA derivative (1 equiv), 2,2-dimethylsuccinic anhydride (5 equiv), DMAP (1.6 equiv), and PTSA (catalytic amount) was dissolved in THF (1.5 mL), and the mixture was stirred at 130 °C for 2 h using a microwave synthesizer (Biotage). Water was added, and the mixture was then extracted with CH_2Cl_2 (15 mL). The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield pure target compounds **16**−**23**, **30**, **40**, and **41**.

Methoxymethyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (16). Yield, 25 mg (40% yield), starting from 52 mg of **7**; white amorphous powder. Mp 107−109 °C. HRMS (ESI−) *m/z*: 627.4247 ($C_{38}H_{60}O_7$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.11 (1H, br s, -COO*H*), 5.28−5.22 (2H, app d, *J* = 5.8 Hz, -COOC*H*$_2$O-), 4.72, 4.60 (1H each, s, H-29), 4.47 (1H, dd, *J* = 11.2, 5.6 Hz, H-3), 3.48 (3H, s, -OC*H*$_3$), 3.00 (1H, m, H-19), 2.64−2.42 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.30, 1.28 (3H each, s, 2 × CH$_3$-3′), 0.98, 0.97, 0.92, 0.81, 0.75 (3H each, s, 5 × CH$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.9, 16.2, 16.4, 16.7, 18.4, 19.6, 21.1, 23.8, 25.3, 25.7, 25.8, 28.1, 29.9, 30.8, 32.2, 34.5, 37.1, 37.3, 38.0, 38.4, 38.6, 40.7, 41.0, 42.6, 44.9, 47.1, 49.6, 50.7, 55.7, 57.0, 57.9, 81.8, 90.1, 109.9, 150.6, 171.2, 175.8, 182.6.

Methoxyethoxymethyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (17). Yield, 30.5 mg (41% yield), starting from 60 mg of **8**; white amorphous powder. Mp 115−118 °C. HRMS (ESI−) *m/z*: 671.4511 ($C_{40}H_{64}O_8$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 11.16 (1H, br s, -COO*H*), 5.37−5.30 (2H, app d, *J* = 6.0 Hz, -COOC*H*$_2$O-), 4.72, 4.59 (1H each, s, H-29), 4.47 (1H, t, *J* = 7.6 Hz, H-3), 3.80, 3.56 (2H each, t, *J* = 5.2 Hz, -OC*H*$_2$C*H*$_2$O-), 3.42 (3H, s, -OC*H*$_3$), 3.01 (1H, m, H-19), 2.67−2.46 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.29, 1.28 (3H each, s, 2 × CH$_3$-3′), 1.00, 0.97, 0.92, 0.82, 0.76 (3H each, s, 5 × CH$_3$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.9, 16.2, 16.4, 16.7, 18.4, 19.6, 21.1, 23.8, 25.3, 25.7, 25.8, 28.1, 29.9, 30.7, 32.2, 34.5, 37.1, 37.3, 37.9, 38.4, 38.6, 40.7, 41.0, 42.6, 45.0, 47.1, 49.6, 50.7, 55.7, 56.9, 59.3, 69.7, 71.7, 81.8, 89.1, 109.9, 150.6, 171.2, 175.8, 183.0. $[\alpha]_D^{25}$ −13.8° (*c* 0.28, MeOH).

***N*-Succinimide 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (18).** Yield, 17 mg (39% yield), starting from 35 mg of **9**; light yellow amorphous powder. Mp 164−166 °C. HRMS (ESI−) *m/z*: 680.4161 ($C_{40}H_{59}NO_8$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.72, 4.60 (2H, s, H-29), 4.47 (1H, dd, *J* = 11.2, 5.2 Hz, H-3), 2.99 (1H, m, H-19), 2.84, 2.82 (4H, d, *J* = 5.2 Hz, -COC*H*$_2$C*H*$_2$CO-), 2.64−2.41 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.28 (6H, s, 2 × CH$_3$-3′), 0.98 (6H, s, 2 × CH$_3$), 0.91, 0.82, 0.75 (3H each, s, 3 × CH$_3$).

Propyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (19). Yield, 23 mg (46% yield), starting from 40 mg of **10**; white amorphous powder. Mp 109−111 °C. HRMS (ESI−) *m/z*: 625.4465 ($C_{39}H_{62}O_6$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (2H, s, H-29), 4.48 (1H, dd, *J* = 11.0, 4.8 Hz, H-3), 4.09 (2H, m, 28-COOC*H*$_2$-), 3.00 (1H, m, H-19), 2.58−2.39 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.28, 1.27 (3H each, s, 2 × CH$_3$-3′), 1.00, 0.98, 0.93 (3H each, s, 3 × CH$_3$), 0.89 (3H, t, *J* = 6.4 Hz, CH$_3$), 0.82, 0.75 (3H each, s, 2 × CH$_3$).

Prop-2′-ynyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (20). Yield, 28 mg (55% yield), starting from 40 mg of **11**; light yellow amorphous powder. Mp 127−129 °C. HRMS (ESI−) *m/z*: 621.4175 ($C_{39}H_{58}O_6$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (2H, s, H-29), 4.69, 4.66 (2H, d, *J* = 2.4 Hz, 28-COOC*H*$_2$-), 4.47 (1H, dd, *J* = 11.8, 5.6 Hz, H-3), 3.00 (1H, m, H-19), 2.62−2.40 (3H, m, H-2′, -C≡C*H*), 1.69 (3H, s, H-30), 1.30, 1.28 (3H each, s, 2 × CH$_3$-3′), 0.97 (6H, s, 2 × CH$_3$), 0.93, 0.81, 0.75 (3H each, s, 3 × CH$_3$).

Hexyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (21). Yield, 26 mg (52% yield), starting from 40 mg of **13**; white amorphous powder. Mp 118−120 °C. HRMS (ESI−) *m/z*: 667.4931 ($C_{42}H_{68}O_6$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (2H, s, H-29), 4.47 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 4.07 (2H, m, 28-COOC*H*$_2$-), 3.01 (1H, m, H-19), 2.54−2.41 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.31, 1.28 (3H each, s, 2 × CH$_3$-3′), 0.97 (6H, s, 2 × CH$_3$), 0.92 (3H, s, CH$_3$), 0.89 (3H, m, CH$_3$), 0.81, 0.75 (3H each, s, 2 × CH$_3$).

Ethyl *O*-[3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oyl]-6-hexanoate (22). Yield, 28 mg (46% yield), starting from 50 mg of **14**; light yellow amorphous powder. Mp 179−181 °C. HRMS (ESI−) *m/z*: 725.4989 ($C_{44}H_{70}O_8$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.72, 4.59 (2H, s, H-29), 4.48 (1H, t, *J* = 7.8 Hz, H-3), 4.18−4.02 (4H, m, 28-COOC*H*$_2$-, -COOC*H*$_2$CH$_3$), 3.01 (1H, m, H-19), 2.62−2.43 (2H, m, H-2′), 2.31 (2H, m, -C*H*$_2$COO-), 1.68 (3H, s, H-30), 1.31, 1.28 (3H each, s, 2 × CH$_3$-3′), 1.26 (3H, t, *J* = 7.2 Hz, -COOCH$_2$C*H*$_3$), 0.98 (6H, s, 2 × CH$_3$), 0.91, 0.82, 0.75 (3H each, s, 3 × CH$_3$). $[\alpha]_D^{25}$ −16.6° (*c* 0.22, MeOH).

Octyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (23). Yield, 31 mg (51% yield), starting from 50 mg of **15**; white amorphous powder. Mp 136−137 °C. HRMS (ESI−) *m/z*: 695.5248 ($C_{44}H_{72}O_6$ - H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (2H, s, H-29), 4.47 (1H, t, *J* = 7.6 Hz, H-3), 4.07 (2H, m, 28-COOC*H*$_2$-), 3.01 (1H, m, H-19), 2.61−2.40 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.28 (6H, s, 2 × CH$_3$-3′), 0.97 (6H, s, 2 × CH$_3$), 0.92 (3H, s, CH$_3$), 0.89 (3H, t, *J* = 6.4 Hz, CH$_3$), 0.82, 0.75 (3H each, s, 2 × CH$_3$).

2-[2-[2-[2-*O*-(3′,3′-Dimethylsuccinyl)ethoxy]ethoxy]ethoxy]-ethyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (30). Yield, 50 mg (41% yield), starting from 100 mg of **29**; light yellow amorphous powder. Mp 165−167 °C. HRMS (ESI−) *m/z*: 759.5013 ($C_{44}H_{72}O_{10}$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (2H, s, H-29), 4.48 (1H, dd, *J* = 11.4, 4.8 Hz, H-3), 4.26 (2H, m, -COOC*H*$_2$CH$_2$O-), 3.68−3.41 (2H each, m, -COOCH$_2$C*H*$_2$O-, 3 × -OCH$_2$CH$_2$O-), 3.01 (1H, m, H-19), 2.64−2.42 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.30, 1.28 (3H each, s, 2 × CH$_3$-3′), 0.98, 0.97, 0.91, 0.82, 0.76 (3H each, s, 5 × CH$_3$).

***N*-[3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oyl]-4-piperazinebutyric Acid (40).** Yield, 45 mg (53% yield), starting from 70 mg of **38**; light yellow amorphous powder. Mp 127−129 °C. HRMS (ESI−) *m/z*: 737.5109 ($C_{44}H_{70}N_2O_7$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.71, 4.60 (1H each, s, H-29), 4.47 (1H, dd, *J* = 11.6, 4.8 Hz, H-3), 3.65−3.49 (4H, m, 28-CON(C*H*$_2$CH$_2$)$_2$N-), 3.01 (1H, m, H-19), 2.82−2.68 (4H, m, 28-CON(CH$_2$C*H*$_2$)$_2$N-), 2.64−2.40 (3H, m, H-2′, -NC*H*$_2$-), 1.68 (3H, s, H-30), 1.31, 1.28 (3H each, s, 2 × CH$_3$-3′), 1.01, 0.98, 0.91, 0.84, 0.76 (3H each, s, 5 × CH$_3$). $[\alpha]_D^{25}$ −17.5° (*c* 0.28, MeOH).

***N*-[3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oyl]-5-piperazinepentanoic Acid (41).** Yield, 199 mg (55% yield), starting

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from 300 mg of **39**; white amorphous powder. Mp 135−137 °C. HRMS (ESI−) *m/z*: 751.5251 ($C_{45}H_{72}N_2O_7$ − H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.73, 4.60 (1H each, s, H-29), 4.47 (1H, dd, *J* = 11.2, 4.8 Hz, H-3), 3.64−3.51 (4H, m, 28-CON(CH_2CH_2)$_2$N-), 3.00 (1H, m, H-19), 2.89−2.72 (4H, m, 28-CON(CH_2CH_2)$_2$N-), 2.61−2.40 (3H, m, H-2′, -NCH_2-), 1.69 (3H, s, H-30), 1.30, 1.28 (3H each, s, 2 × CH_3-3′), 0.98, 0.97, 0.92, 0.82, 0.75 (3H each, s, 5 × CH_3). $[\alpha]_D^{25}$ −18.4° (*c* 0.30, MeOH).

[1-(3′-Deoxythymidine)-1*H*-1,2,3-triazol-4-yl]methyl 3β-*O*-(3′,3′-Dimethylsuccinyl)lup-20(29)-en-28-oate (24). Compound **20** (25 mg, 1.3 equiv), AZT (1 equiv), CuI (0.2 equiv), and *i*-Pr_2EtN (2 equiv) were dissolved in DMF (1 mL), and the mixture was stirred at 120 °C for 30 min using a microwave synthesizer (Biotage). The solution was diluted with EtOAc and then washed with water, saturated $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated to dryness under reduced pressure. The residue was chromatographed using a silica gel column to yield 20 mg of **24** (75%), a white amorphous powder. Mp 186−189 °C. HRMS (ESI+) *m/z*: 890.5227 ($C_{49}H_{71}N_5O_{10}$ + H). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.31 (1H, br, s, -NH), 7.79 (1H, s, triazol-H), 7.48, 7.44 (1H, d, *J* = 16 Hz, Thy-H), 6.21 (1H, t, *J* = 5.6 Hz, ribose-C*H*-2′), 5.42 (1H, m, ribose-C*H*-4′), 5.28−5.20 (2H, m, 28-COOCH_2-), 4.72, 4.60 (2H, s, H-29), 4.46 (1H, m, H-3), 4.04, 3.80−3.73 (4H, m, ribose-C*H*-5′-CH_2-O*H*), 3.06−2.96 (3H, m, H-19, ribose-CH_2-3′), 2.70−2.56 (2H, m, H-2′), 1.94 (3H, s, Thy-CH_3), 1.67 (3H, s, H-30), 1.30, 1.28 (3H each, s, 2 × CH_3-3′), 1.01 (6H, s, 2 × CH_3), 0.93, 0.81, 0.75 (3H each, s, 3 × CH_3).

HIV-1$_{NL4-3}$ Replication Inhibition Assay in MT-4 Lymphocytes. A previously described HIV-1 infectivity assay was used.[26,27] 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 PI for the p24 antigen capture using an ELISA kit from ZeptoMetrix Corporation (Buffalo, NY). The 50% inhibition concentration (IC_{50}) was defined as the concentration that inhibits HIV-1$_{NL4-3}$ replication by 50%.

Cytotoxicity Assay. A CytoTox-Glo cytotoxicity assay (Promega) was used to determine the cytotoxicity of the synthesized BA derivatives. Mock-infected MT-4 cells were cultured in the presence of various concentrations of the compounds for 2 days. Percent of viable cells was determined by following the protocol provided by the manufacturer. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration that caused a 50% reduction of cell viability.

TZM-bl Assay. Anti-HIV-1 activity was measured as reduction in Luc reporter gene expression after a single round of virus infection of TZM-bl cells. HIV-1 at 200 $TCID_{50}$ and test samples were mixed in a total volume of 100 mL of growth medium in 96-well black solid plates (Corning-Costar). After 48 h of incubation, culture medium was removed from each well and 100 mL of Bright Glo luciferase reagent was added to each culture well. The luciferase activity in the assay wells was measured using a Victor 2 luminometer. The 50% inhibitory dose (IC_{50}) was defined as the sample concentration that caused a 50% reduction in relative luminescence units (RLU) compared to virus control wells after subtraction of background RLU.

Microsomal Stability Assay. Stock solutions of **41**, as well as reference compounds propranolol and terfenadine (1 mg/mL), were prepared by dissolving the pure compound in DMSO and were stored at 4 °C. Before the assay, the stock solution was diluted with ACN to 0.1 mM. For measurement of metabolic stability, all compounds were brought to a final concentration of 1 μM with 0.1 M potassium phosphate buffer at pH 7.4, which contained 0.1 mg/mL human liver microsomes and 5 mM $MgCl_2$. The incubation volumes were 300 μL, and reaction temperature was 37 °C. Reactions were started by adding 60 μL of NADPH (final concentration of 1.0 mM) and quenched by adding 600 μL of ice-cold ACN to stop the reaction at 5, 15, 30, and 60 min time points. Samples at 0 min time point were prepared by adding 600 μL of ice-cold ACN first, followed by 60 μL of NADPH. Incubations of all samples were conducted in duplicate. After quenching, all samples were centrifuged at 12 000 rpm for 5 min at 0 °C. The supernatant was collected, and 30 μL of the supernatant was injected directly onto a Shimadzu LC−MS 2010 system by autosampler. The HPLC−MS analysis was carried out with an electrospray ionization source (ESI). An Alltima C18 column (5 μm, 150 mm × 2.1 mm) was used for HPLC with a gradient elution at a flow rate of 0.3 mL/min. The elution conditions for **41** were ACN (B) in water (A) at 30% for 0−3 min, 95% for 3−9 min, and 30% for 9−12 min. The MS conditions were optimized to detector voltage of +1.7 kV, positive acquisition mode with selected ion monitoring (SIM) of the appropriate molecular weights of the testing compounds. The CDL temperature was 250 °C, and heat block temperature was 200 °C. The neutralizing gas flow was 1.5 L/min. The peak heights of test compounds at different time points were converted to the percentage remaining, and the peak height values at initial time (0 min) served as 100%. The slope of the linear regression from log of the percentage remaining versus incubation time relationships (−*k*) was used to calculate in vitro half-life ($t_{1/2}$) by the formula of in vitro $t_{1/2}$ = 0.693/*k*, regarded as first-order kinetics. Conversion to in vitro clearance (CL_{int} in units of (mL/min)/mg protein) was calculated by the formula:[28] CL_{int} = (0.693/in vitro $t_{1/2}$)[(mL incubation)/(mg of microsomes)]. Fast and moderate metabolizing reference compounds terfenadine and propranolol, respectively, have $t_{1/2}$ of 21.14 and 40.82 min, respectively, in these assay conditions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

ACN, acetonitrile; AIDS, acquired immunodeficiency syndrome; HIV-1, human immunodeficiency virus type 1; BA, betulinic acid; HAART, highly active antiretroviral therapy; MI, maturation inhibitor; P24 (CA), capsid; P25 (CA-SP1), capsid precursor; bevirimat, 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid; AZT, zidovudine; MOMCl, methoxymethyl chloride; MEMCl, methoxyethoxymethyl chloride; DIEA, diisopropylethylamine; THF, tetrahydrofuran; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; DMAP, 4-(dimethylamino)pyridine; TBS, *tert*-butyldimethylsilyl; TEA, triethylaluminum; PTSA, *p*-toluenesulfonic acid; TBAF, tetra-*n*-butylammonium fluoride; HLM, human liver microsome; PI, postinfection

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