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Anti-AIDS Agents 81. Design, Synthesis and Structure-Activity Relationship Study of Betulinic Acid and Moronic Acid Derivatives as Potent HIV Maturation Inhibitors†

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Abstract

In our continuing study of triterpene derivatives as potent anti-HIV agents, different C-3 conformationally restricted betulinic acid (BA, **1**) derivatives were designed and synthesized in order to explore the conformational space of the C-3 pharmacophore. 3-*O*-Monomethylsuccinyl-betulinic acid (MSB) analogs were also designed to better understand the contribution of the C-3′ dimethyl group of bevirimat (**2**), the first-in-class HIV maturation inhibitor, which is currently in phase IIb clinical trials. In addition, another triterpene skeleton, moronic acid (MA, **3**) was also employed to study the influence of the backbone and the C-3 modification towards the anti-HIV activity of this compound class. This study enabled us to better understand the structure-activity relationships (SAR) of triterpene-derived anti-HIV agents, and led to the design and synthesis of compound **12** (EC_{50}: 0.0006 μM), which displayed slightly better activity than **2** as a HIV-1 maturation inhibitor.

Introduction

It is estimated that at the end of 2008, approximately 33.2 million people were infected worldwide with human immunodeficiency virus (HIV), the etiologic cause of acquired immunodeficiency syndrome (AIDS). Each year, around 2.7 million more people become infected with HIV and 2 million die of AIDS.[1] Considering that the development of a safe and effective HIV vaccine is still in the future,[2] the current research continues to focus on the disease treatment by chemical anti-HIV agents. Although significant progress has been made since the introduction of highly active antiretroviral therapy (HAART),[3, 4] which employs a combinational use of nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs), it has also led to some serious problems, including increased adverse effects and the emergence of multi-drug-resistant viral strains.[5–7] Drug-resistant virus is then involved in HIV transmission, and more than 25% of newly infected individuals harbor HIV-1 isolates that are resistant to at least one ART.[8] Therefore, novel potent antiretroviral

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Supporting Information Available: Additional information on compound purity, high-resolution mass spectral 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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agents with different targets than currently approved drugs may hold particular promise in addressing issues of current therapies.

Our prior modification study on betulinic acid (BA, **1**) resulted in the discovery of bevirimat [3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid, **2**].[9] Bevirimat exhibits extremely potent antiviral activity against HIV-1 primary isolates and several drug-resistant virus, and represents a unique first in a class of anti-HIV compounds termed maturation inhibitors (MIs).[10, 11] MIs block 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. Most importantly, MIs retain their high anti-HIV potency against different viral strains that are resistant to current ARTs, including AZT (NRTI), Nevirapine (NNRTI), and Indinavir (PI). Compound **2** has recently succeeded in Phase IIb clinical trials and is in preparation for Phase III clinical trials.[12–14]

To summarize our prior structure-activity relationship (SAR) study of **2** and other 3- *O*-acyl-BA derivatives, we know that C-3 ester substitution is important to the enhanced antiviral activity.[9, 15] Within the C-3 side chain, the proper length, a terminal carboxylic acid, and C-3′ dimethyl substitution contribute to antiviral potency. However, the C-3 ester groups of prior BA analogs have mainly contained freely rotatable alkyl chains. Therefore, in the present study, five C-3 conformationally restricted BA analogs (**4–8**) were synthesized in order to explore the conformational space of the C-3 pharmacophore. Two 3-*O*-monomethylsuccinyl betulinic acid (MSB) derivatives (**9–10**) and compound **11** were further designed to investigate how the methyl substituents on the C-3 side chain impact the antiviral potency.

Meanwhile, moronic acid (MA, **3**), which was isolated from Brazilian propolis, also exhibited promising anti-HIV activity as a lead compound with an EC_{50} of 0.1μg/mL.[16] However, insertion of the same 3′,3′-dimethylsuccinyl side chain found in **2** at the 3β position of morolic acid (**30**), the C-3 ketone reduced analog of **3**, unexpectedly did not increase the antiviral activity.[16] Because **2** and **30** share similar 3D conformational space of their triterpene skeletons, this unexpected result led us to further investigate possible modifications on analogs of **3** and **30**.

Detailed SAR of triterpene-derived HIV-1 maturation inhibitors has been established from the current study. This analysis led to the design and synthesis of compound **12** with slightly better activity than **2**.

Design

Because the presence of C-3 substitution is critical to the anti-HIV-1 activity of BA derivatives, five conformationally restricted 3-*O*-acyl-BA analogs (**4**–**8**) (Figure 1) were first synthesized and evaluated, in order to further explore the conformational space of this pharmacophore. The C-3′ dimethyl group was also moved towards the 4′-position in a C-3 glutaryl-substituted compound (**11**) (Figure 1) to study the influence of different positioning of the methyl groups. In addition, although we know that the presence of the C-3′ dimethyl within the C-3 side chain is vital to anti-HIV-1 activity, it is still unclear which, if either, C-3′ methyl group of **2** contributes more toward activity. Therefore, 3-*O*-monomethylsuccinyl betulinic acid (MSB) derivatives (**9–10**) (Figure 1) were designed and synthesized.[17] Their antiviral activities were then evaluated *in vitro* against HIV-1_{IIIB} replication in MT-2 cell lines. Diverse substitutions (**13–21**) (Scheme 4) were also incorporated into **30** to study their influence on the antiviral potency and the impact of the triterpene skeleton itself on the antiviral activity. After reviewing the initial promising bioassay results, compound **12** (Figure 1) was then designed to try to improve the antiviral potency of **2**.

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Chemistry

Compounds **4** and **5** were synthesized by reaction of the corresponding cycloalkanedicarboxylic acid with the C-3 β-hydroxyl group of **1** in the presence of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDCI) and dimethylaminopyridine (DMAP), resulting in yields of 26% and 35%, respectively (Scheme 1). Compounds **6–8** and **11** were synthesized according to Scheme 2. Reaction of the corresponding acid anhydride with **1** furnished the target compounds in yields of 35–55%.

The synthesis of MSB analogs was reported before in Ref [17]. As shown in Scheme 3, the hydroxyl of 3*R*-bromo-2-methylpropanol (**22a**) was first protected by as a *tert*-butyldimethylsilyl (TBDPS) moiety to yield **23a** quantitatively. The bromide group was then replaced with a cyano moiety (**24a**), which was reduced to an aldehyde moiety (**25a**),[18] and consequentially oxidized to a carboxylic acid (**26a**). Reaction of **1** with **26a** led to esterification of the 3β-hydroxy group of **1** to provide **27a**. After cleavage of the TBDPS group with TBAF in THF, the primary hydroxyl of **28** was oxidized to a carboxylic acid in the presence of 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO) and $PhI(OAc)_2$ to give 3-*O*-(3′*R*-methylsuccinyl)-betulinic acid (3′*R*-MSB, **9**).[19] The 3′*S*-MSB isomer (**10**) was synthesized using the same method starting with 3*S*-bromo-2- methylpropanol (**22b**).

Scheme 4 depicts the synthesis of MA-analogs. Compound **3** was first treated with $NaBH_4$ to yield 3β-hydroxyl group of **30**. Different acyl chloride was then reacted with **30** in the presence of Et_3N to furnish 3-*O*-acyl-MA analogs **13–21**.

The synthesis of **12** started from the commercially available 2-ethyl-2- methylsuccinic acid (**31**), which was stirred in trifluoroacetic anhydride (TFAA) to form 2-ethyl-2-methylsuccinic anhydride (**32**). The reaction of **32** with **1** furnished 3-*O*-(3′-ethyl-3′-methylsuccinyl) betulinic acid (**12**) in 55% yield (Scheme 5).

Results and Discussion

The newly synthesized conformationally restricted 3-*O*-acyl-BA analogs (**4–8**) were first tested in acutely $HIV\text{-}1_{IIIB}$ infected MT-2 cell lines, and compared to **2** and AZT. The data are listed in Table 1. Only the 2-methylmaleic acid substituted BA derivative (**8**) showed moderate anti-HIV-1 activity with a TI of 1.4×10^2 and an EC_{50} of 0.18 μM. A pendant cyclopentyl or cyclohexyl ring within the C-3 side chain reduced the antiviral potency of the derivatives (**4–7**) significantly. One possible reason for the reduced or abolished activity of these compounds is that the pendant ring moieties are locked into a conformation that is not the bioactive one. Alternatively, steric hindrance at the 2′-position [as was seen with 3-*O*-(2′, 2′-dimethylsuccinyl) BA, which had an EC_{50} of only 2.7 μM][9] may be a contributing factor to the loss in potency. This hindrance may impart an unfavorable interaction with either the binding site, resulting in reduced affinity, or the triterpene template, leading to an unfavorable C-3 side chain conformation.

In contrast, 3-*O*-(4′,4′-dimethylglutaryl)-betulinic acid (**11**) had an antiviral EC_{50} of 0.048 μM and a TI of 8.7×10^2. This result confirms that the dimethyl moiety is essential to the anti-HIV-1 potency of 3-*O*-acyl-BA analogs. Although moving the dimethyl substitution to the 2′-position was highly detrimental, moving the dimethyl group to the 4′-position of the C-3 side chain was well tolerated and significantly increased the antiviral activity, compared with that of the unesterified compound **1**. However, the anti- HIV-1 activity of **11** was still 20-fold less than that of **2**, indicating that the 3′-position, rather than 4′-position, of the C-3 side chain remains the optimal substitution position. Overall, results of the conformationally restricted compounds (**4–8**) and **11** stimulated our interest in further study of the importance

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of the C-3′ dimethyl moiety and the exact contribution of both groups to the anti-HIV-1 potency of **2**.

The MSB analogs **9** and **10** were then synthesized and evaluated in parallel with **2** and AZT against viral replication in HIV-1_{IIIB} infected MT-2 cell lines. A 1:1 mixture (**33**) of **9** and **10** was also tested. The anti-HIV-1 activity data of these derivatives are also listed in Table 1. Among the MSB derivatives, compound **10** with 3′*S*-methyl showed very potent antiviral activity with a TI of 3.8×10^3 and EC_{50} of 0.0087 μM, which is comparable to that of **2** (EC_{50}: 0.0013 μM). Compound **9** with 3′*R*-methyl exhibited only moderate anti-HIV-1 activity with a TI of 3.7×10^2 and EC_{50} of 0.12 μM. The antiviral activity (EC_{50}: 0.016 μM) of the mixture (**33**) of the two stereoisomers fell in between those of **9** and **10**. This result indicates that the two C-3′ methyl groups in the C-3 ester side chain contribute differently to the extremely potent anti-HIV-1 activity of this compound class. We postulate that interaction of the 3′*S*-methyl group with the viral target might be essential to the anti-HIV-1 activity of **2**. The interaction of the 3′*R*-methyl group within the target is less significant, but still necessary, since **2**, with dimethyl substitution at C-3′ position, is slightly more potent than **10**.

In order to further elucidate the mechanism of action, the production of virus from **10**-treated HIV-1 wild-type (NL4-3) or resistant variants transfected Hela cells were subjected to characterization. Radioimmunoprecipitation analyses revealed that, like **2**, **10** also functions as a maturation inhibitor. In detail, **10** specifically inhibited the conversion of p25 (CA-SP1) to p24 (CA) in both cell and virion lysates (Figure 3 showing the results from virion lysate), which led to defective Gag processing and production of morphologically abnormal, non-infectious virion particles.

The anti-HIV replication activity of newly synthesized MA analogs **13–21** were tested against HIV-1_{IIIB} and summarized in Table 1 as well. These compounds have diverse substitutions at the 3β position of analog **30** to explore the conformational space to enhance the antiviral potency. However, none of them showed significant anti-HIV activity. Nevertheless, although the insertion of the 3′,3′-dimethylsuccinyl side chain into the 3β of **30** did not follow the general trend of increasing the activity, the current study indicates that a terminal polar moiety, such as a carboxylic acid group, may still be necessary for the compounds to exert their antiviral replication activity. It also proves that the triterpene skeleton itself plays an important role in the enhanced anti-HIV activity of BA derivatives.

As stated above, our investigation of the C-3′ chiral center of the anti-HIV-1 clinical trials agent **2** showed that the 3′*S*-methyl group is the major contributor to enhanced anti- HIV-1 activity, while the 3′*R*-methyl group is less important but still necessary. Accordingly, we postulated that the latter effect may be due to insufficient interaction or a slightly different positioning of the 3′*S* or 3′*R* moiety. To confirm our hypothesis, one of the methyl groups was further enlarged to an ethyl group to give the 3′-ethyl-3′-methyl substitution found in **12**, which is a mixture of 3′*R* and 3′*S* isomers. Compound **12** was evaluated and compared with **2** and AZT in a HIV-1_{IIIB} infected MT-2 cell line. It showed very potent antiviral activity with an EC_{50} of 0.0006 μM and TI of 6.1×10^4, which was more active than **33**, the mixture of **9** and **10**. Indeed, its potency was slightly better than those of **10** and **2**.

To identify which isomer is the active component, the two C-3′ diastereoisomers (**12a** and **12b**) of **12** were separated by using recycling preparative HPLC (JAI LC-918). After four cycles of separation, the two isomers were obtained successfully (Figure 2). Their purities were ascertained by analytical HPLC. By comparing the ^{1}H-NMR spectra and the optical rotation data of **2**, **9**, **10**, **12a**, and **12b**, we assigned **12a** as the 3′*S* isomer and **12b** as the 3′*R* isomer. The antiviral activities of **12a** and **12b** were determined in HIV-1_{NL4-3} infected

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MT-4 lymphocytes, where **12a** was active and **12b** showed no antiviral activity. This result indicates that within the C-3 pharmacophore of BA-derived maturation inhibitors, the 3′*S* substitution is critical to the anti-HIV activity. Modification on this site may further increase the antiviral potency of the current maturation inhibitors.

To conclude our SAR investigation of the C-3 modification on triterpene-derived anti-HIV agents, we found that no conformationally restricted 3-*O*-acyl BA analog (**4**–**8**) showed significant antiviral activity, indicating that C-3′ dimethyl substitution of the succinyl side chain is crucial to the high potency of **2**. A C-3 terminal polar moiety, such as carboxylic acid, is also very important to the antiviral activity, as was proven by MA analogs **13**–**21**. Further SAR study of the C-3′ chiral center of the newly designed MSB analogs (**9**–**10**) revealed that the 3′*S*-methyl group of **2** is the major contributor to the compound's enhanced anti-HIV-1 activity. This result led us to design and synthesize compound **12**, which has an enlarged C-3′ substituent (methylethyl instead of dimethyl). As we anticipated, **12** showed extremely potent antiviral activity, and was slightly better than **2**. Further investigation confirmed that **12a**, the 3′*S* isomer, is the active compound. This result provides us with better SAR information to further design the next generation of potent BA derived HIV-1 maturation inhibitors.

Experimental Section

Chemistry

The melting points were measured with a Fisher Johns melting apparatus without correction. ^{1}H NMR spectra were measured on a 300MHz Varian Gemini 2000 spectrometer or 500MHz Inova 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 and LCMS-IT/TOF (ESI-MS). HPLC for purity determinations were conducted using Shimadzu LCMS-2010 with a Grace Alltima 2.1mm × 150mm HP C18 5μ column or a 2.1mm × 100mm HP C18 3μ column, and a Shimadzu SPD-M20A detector at 205 nm wavelength. Two different solvent systems for HPLC purity analyses were as follows: 1) acetonitrile:water = 80:20, 2) MeOH:water = 90:10. The isocratic HPLC mode was used and the flow rate was 0.3 mL/min. All target compounds were at least 95% pure, as determined 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) was performed on Merck precoated silica gel 60 F-254 plates. Flash+™ and CombiFlash systems were used as medium pressure column chromatography. All other chemicals were obtained from Aldrich, Inc.

Synthesis of BA derivatives 4 and 5

A solution of **1** (1 eq), EDCI (8 eq), DMAP (2 eq) and the proper cycloalkanedicarboxylic acid (5 eq) in anhydrous CH_2Cl_2 (8 mL) was stirred at rt 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 the pure target compounds.

3*β*-*O*-[(2′*R*, 3′*R*)-3′-Carboxycyclopentanecarbonyl]-betulinic acid (4)

26% yield (starting with 150 mg of **1**); white amorphous powder. Mp 228–230 °C. MS (ESI −) *m/z*: 595.4 ($M^- - H$) for $C_{37}H_{56}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.70, 4.57 (1H each, s, H-29), 4.45 (1H, dd, *J* = 7.8, 5.7 Hz, H-3), 3.20 (1H, m, H-19), 3.01, 2.88 (1H each, m, H-3′, H-2′), 1.85–2.06 (6H, m, 3 × CH_2, H-4′, 5′, 6′), 1.65 (3H, s, H-30), 0.94 (6H, s, 2 × CH_3), 0.85, 0.81, 0.75 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ −15.29 ° (c = 0.17, MeOH).

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3*β*-*O*-[(2′*R*, 3′*R*)-3′-Carboxycyclohexanecarbonyl]-betulinic acid (5)

35% yield (starting with 150 mg of **1**); white amorphous powder. Mp 233–235 °C. MS (ESI −) *m/z*: 609.4 (M^- − H) for $C_{38}H_{58}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.71, 4.59 (1H each, s, H-29), 4.46 (1H, dd, *J* = 10.2, 5.1 Hz, H-3), 2.98 (1H, m, H-19), 2.89, 2.71 (1H each, m, H-3′, H-2′), 1.84–2.16 (8H, m, 4 × CH_2, H-4′, 5′, 6′, 7′), 1.66 (3H, s, H-30), 0.94 (9H, s, 3 × CH_3), 0.81, 0.79 (3H each, s, 2 × CH_3). $[\alpha]^{20}_D$ −27.00 ° (c = 0.20, MeOH).

Synthesis of BA derivatives 6–8 and 11

A solution of **1** (1 eq), DMAP (2 eq) and the proper acid anhydride (5 eq) in anhydrous pyridine (1.5 mL) was stirred at 160 °C for 2 h using microwave. The reaction mixture was diluted with EtOAc (20 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 the pure target compounds.

3*β*-*O*-(3′-Carboxycyclohex-5′-enecarbonyl)-betulinic acid (6)

40% yield (starting with 100 mg of **1**); white amorphous powder. Mp 178–180 °C. MS (ESI −) *m/z*: 607.4 (M^- − H) for $C_{38}H_{56}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.70 (2H, m, H-5′, 6′), 4.72, 4.60 (1H each, s, H-29), 4.48 (1H, m, H-3), 3.02 (1H, m, H-19), 2.83, 2.72 (1H each, m, H-3′, H-2′), 2.32–2.27 (4H, m, 2 × CH_2, H-4′, 7′), 1.68 (3H, s, H-30), 1.01, 0.97 (3H each, s, 2 × CH_3), 0.89, 0.83, 0.81 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ +14.17 ° (c = 0.18, MeOH).

3*β*-*O*-[3′-Carboxybicyclo[2.2.1]hept-5′-enecarbonyl]-betulinic acid (7)

35% yield (starting with 100 mg of **1**); white amorphous powder. Mp 165–167 °C. MS (ESI −) *m/z*: 619.4 (M^- − H) for $C_{39}H_{56}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.73 (2H, m, H-5′, 6′) 4.71, 4.59 (1H each, s, H-29), 4.45 (1H, m, H-3), 3.01 (1H, m, H-19), 2.95, 2.76 (1H each, m, H-3′, H-2′), 2.52-2.39 (4H, m, 2 × CH_2, H-4′, 7′), 1.67 (3H, s, H-30), 0.96 (6H, s, 2 × CH_3), 0.91, 0.85, 0.82 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ +24.00 ° (c = 0.10, MeOH).

3*β*-*O*-[(*Z*)-3′-Carboxybut-2-enoyl]-betulinic acid (8)

55% yield (starting with 150 mg of **1**); red amorphous powder. Mp 169–171 °C. MS (ESI−) *m/z*: 567.4 (M^- − H) for $C_{35}H_{52}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.89 (1H, s, H-2′), 4.70, 4.58 (1H each, s, H-29), 4.46 (1H, m, H-3), 2.96 (1H, m, H-19), 1.93 (3H, s, CH_3-3′), 1.66 (3H, s, H-30), 0.94, 0.93, 0.90 (3H each, s, 3 × CH_3), 0.80, 0.79 (3H each, s, 2 × CH_3). $[\alpha]^{20}_D$ +9.41 ° (c = 0.17, MeOH).

3*β*-*O*-(4′,4′-Dimethylglutaryl)-betulinic acid (11)

53% yield (starting with 100 mg of **1**); white amorphous powder. Mp 228–230 °C. MS (ESI −) *m/z*: 597.4 (M^- − H) for $C_{37}H_{58}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.73, 4.61 (1H each, s, H-29), 4.45 (1H, dd, *J* = 9.0, 5.2 Hz, H-3), 3.00 (1H, m, H-19), 2.32 (2H, dd, *J* = 6.6, 5.4 Hz, H-2′), 2.16 (1H, m, H-13), 1.69 (3H, s, H-30), 1.22, 1.21 (3H each, d, *J* = 6 Hz, 2 × CH_3-4′), 0.97, 0.91 (3H each, s, CH_3-23, 24), 0.86, 0.85, 0.83 (3H each, s, CH_3-25, 26, 27). $[\alpha]^{20}_D$ −0.77 ° (c = 0.13, MeOH).

Synthesis of 23a and 23b

A solution of **22a** or **22b** (1 eq), imidazole (1.1 eq) and TBDPSCl (1.1 eq) in dry DMF was stirred at rt, until the starting material was not observed by TLC. The reaction mixture was diluted with EtOAc and washed with 20% HCl solution and distilled water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness under reduced

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

(3*R*-Bromo-2-methylpropoxy)(tert-butyl)diphenylsilane (23a)

2.03 g (100%) yielded from **22a**; colorless oil. MS (ESI+) *m/z*: 391.2 (M^+ + H), 413.2 (M^+ + Na) for $C_{20}H_{27}BrOSi$.

(3*S*-Bromo-2-methylpropoxy)(tert-butyl)diphenylsilane (23b)

1.44 g (100%) yielded from **22b**; colorless oil. MS (ESI+) *m/z*: 391.1 (M^+ + H), 413.1 (M^+ + Na) for $C_{20}H_{27}BrOSi$.

Synthesis of 24a and 24b

To a solution of **23a** or **23b** (1 eq) in dry DMSO (10 mL) was added sodium cyanide (3 eq). The mixture was stirred at 120 °C for 2 h until the starting material was not observed. After cooling to rt, the reaction was diluted with diethyl ether (30 mL) and washed with 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.

4-(*tert*-Butyldiphenylsilyloxy)-3*R*-methylbutanenitrile (24a)

1.70g (93%) yield starting with **23a**; colorless oil. MS (ESI+) *m/z*: 338.2 (M^+ + H) for $C_{21}H_{27}NOSi$.

4-(*tert*-Butyldiphenylsilyloxy)-3*S*-methylbutanenitrile (24b)

1.163 g (93%) yield starting with **23b**; colorless oil. MS (ESI+) *m/z*: 338.2 (M^+ + H) for $C_{21}H_{27}NOSi$.

Synthesis of 25a and 25b

To a solution of **24a** or **24b** (1 eq) in anhydrous toluene (10 mL) was added diisobutylaluminium hydride (DIBALH, 1.1 eq) dropwise at 0 °C under argon. After stirring for 30 min, ice water was added slowly followed by 10% HCl and aqueous saturated potassium tartrate. Stirring was continued for 15 min at 0 °C and then the aqueous layer was extracted three times with CH_2Cl_2. 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.

4-(*tert*-Butyldiphenylsilyloxy)-3*R*-methylbutanal (25a)

1.02 g (95%) yield starting with **24a**; colorless oil. MS (ESI+) *m/z*: 341.2 (M^+ + H) for $C_{21}H_{28}O_2Si$.

4-(*tert*-Butyldiphenylsilyloxy)-3*S*-methylbutanal (25b)

1.047 g (90%) yield starting with **24a**; colorless oil. MS (ESI+) *m/z*: 341.2 (M^+ + H) for $C_{21}H_{28}O_2Si$.

Synthesis of 26a and 26b

To a solution of **25a** or **25b** (1 eq) in dry DMSO (1.0 mL) was added $NaH_2PO_4 \cdot H_2O$ (0.8 eq in 2.0 mL H_2O) and 80% $NaClO_2$ (1.5 eq in 2.0 mL H_2O) dropwise over 5 min. The mixture was stirred overnight. The reaction was quenched with saturated NH_4Cl solution and extracted with EtOAc. The organic layer was washed with brine, dried over anhydrous

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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.

4-(*tert*-Butyldiphenylsilyloxy)-3*R*-methylbutanoic acid (26a)

700 mg (100%) yield starting with **25a**; colorless oil. MS (ESI−) *m/z*: 355.2 (M^- − H) for $C_{21}H_{28}O_3Si$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.68-7.62 (4H, m, H ar-2′), 7.45-7.33 (6H, m, H ar-3′, 4′), 3.59, 3.43 (2H, dd, *J* = 7.6, 1.5 Hz, H-4), 2.61-2.30 (3H, m, H-2, H-3), 1.05 (9H, s, $SiC(CH_3)_3$), 0.95 (3H, d, *J* = 10 Hz, H-5).

4-(*tert*-Butyldiphenylsilyloxy)-3*S*-methylbutanoic acid (26b)

830 mg (76%) yield starting with **25b**; colorless oil. MS (ESI−) *m/z*: 355.2 (M^- − H) for $C_{21}H_{28}O_3Si$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.68-7.62 (4H, m, H ar-2′), 7.45-7.33 (6H, m, H ar-3′, 4′), 3.58, 3.42 (2H, dd, *J* = 7.6, 1.5 Hz, H-4), 2.60-2.30 (3H, m, H-2, H-3), 1.05 (9H, s, $SiC(CH_3)_3$), 0.95 (3H, d, *J* = 10 Hz, H-5).

Synthesis of 27a and 27b

A solution of **1** (1 eq), EDCI (8 eq), DMAP (2 eq) and **26a** or **26b** (5 eq) in anhydrous CH_2Cl_2 (5 mL) was stirred at rt overnight, until the starting material was not observed by TLC. The solution was diluted with CH_2Cl_2 (15 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.

3*β*-*O*-[4′-(*tert*-Butyldiphenylsilyloxy)-3′*R*-methylbutanoyl]-betulinic acid (27a)

32% yield starting with 340 mg BA, white powder. Mp 179–181 °C. MS (ESI−) *m/z*: 793.5 (M^- − H) for $C_{51}H_{74}O_5Si$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.68-7.62 (4H, m, H ar-2′), 7.45-7.33 (6H, m, H ar-3′, 4′), 4.72, 4.60 (1H each, s, H-29), 4.45 (1H, m, H-3), 3.58, 3.42 (2H, m, H-4′), 2.87–2.95 (2H, m, H-19, H-3′), 2.64-2.42 (2H, m, H-2′), 1.69 (3H, s, H-30), 1.28, 1.26 (3H, d, *J* = 6 Hz, CH_3-3′), 1.01 (9H, s, $SiC(CH_3)_3$), 0.96 (6H, s, 2 × CH_3), 0.89, 0.85, 0.82 (3H each, s, 3 × CH_3).

3*β*-*O*-[4′-(*tert*-Butyldiphenylsilyloxy)-3′*S*-methylbutanoyl]-betulinic acid (27b)

48% yield starting with 170 mg BA, white powder. Mp 186-187 °C. MS (ESI−) *m/z*: 793.5 (M^- − H) for $C_{51}H_{74}O_5Si$. ^1H NMR (300 MHz, $CDCl_3$): δ 7.68-7.62 (4H, m, H ar-2′), 7.45-7.33 (6H, m, H ar-3′, 4′), 4.73, 4.60 (1H each, s, H-29), 4.51 (1H, dd, *J* = 11.1, 4.8 Hz, H-3), 3.60-3.42 (2H, m, H-4′), 2.96–3.03 (1H, m, H-19), 2.86–2.93 (1H, m, H-3′), 2.73, 2.69 (1H each, dd, *J* = 11.2, 5.7, 4.8 Hz, H-2′), 1.69 (3H, s, H-30), 1.27, 1.25 (3H, d, *J* = 6 Hz, CH_3-3′), 1.02 (9H, s, $SiC(CH_3)_3$), 0.97, 0.94, 0.86, 0.85, 0.81 (3H each, s, CH_3- 23, 24, 25, 26, 27).

Synthesis of 28 and 29

A solution of **27a** or **27b** (1 eq) and TBAF (1.0 M in THF, 3 eq) in anhydrous THF was stirred at 0 °C for 1.5 h. The mixture was then allowed to warm to rt and stirred until there was no starting material detected by TLC. The reaction was diluted with 15 mL of CH_2Cl_2 and washed with saturated NH_4Cl solution and brine. 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.

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3*β*-*O*-(4′-Hydroxy-3′*R*-methylbutanoyl)-betulinic acid (28)

100% yield from **27a**, white powder. Mp 201–203 °C. MS (ESI−) *m/z*: 555.4 (M^- − H) for $C_{35}H_{56}O_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.73, 4.60 (1H each, s, H-29), 4.54 (1H, dd, *J* = 9.9, 5.7 Hz, H-3), 3.55, 3.42 (2H, m, H-4′), 3.01 (1H, m, H-19), 2.87–2.95 (1H, m, H-3′), 2.70-2.42 (2H, m, H-2′), 1.68 (3H, s, H-30), 1.27, 1.25 (3H, d, *J* = 6 Hz, CH_3-3′), 0.93 (6H, s, 2 × CH_3), 0.85, 0.84, 0.81 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ +15.50 ° (c = 0.12, MeOH).

3*β*-*O*-(4′-Hydroxy-3′*S*-methylbutanoyl)-betulinic acid (29)

100% yield from **27b**, white powder. Mp 229–231 °C. MS (ESI−) *m/z*: 555.4 (M^- − H) for $C_{35}H_{56}O_5$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.73, 4.60 (1H each, s, H-29), 4.51 (1H, dd, *J* = 12.5, 5.6 Hz, H-3), 3.58, 3.43 (2H, m, H-4′), 2.97–3.01 (1H, m, H-19), 2.83–2.92 (1H, m, H-3′), 2.70, 2.62 (1H each, dd, *J* = 16.2, 6.3, 4.8 Hz, H-2′), 1.69 (3H, s, H-30), 1.27, 1.25 (3H, d, *J* = 6 Hz, CH_3-3′), 1.01, 0.96, 0.86, 0.85, 0.81 (3H each, s, CH_3-23, 24, 25, 26, 27). $[\alpha]^{20}_D$ −16.10 ° (c = 0.11, MeOH).

Synthesis of 9 and 10

To a solution of **28** or 2**9** (1 eq) in CH_2Cl_2 (5 mL) was added TEMPO (0.1 eq), and $PhI(OAc)_2$ (1.5 eq). The mixture was stirred at rt until the starting material was not observed by TLC. The reaction was filtered through thin silica gel pad and eluted with CH_2Cl_2 and washed with saturated NH_4Cl solution and brine. 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.

3*β*-*O*-(3′*R*-Methylsuccinyl)-betulinic acid (9)

75% yield from **28**, white amorphous powder. Mp 268–271 °C. MS (ESI−) *m/z*: 569.38 (M^- − H) for $C_{35}H_{54}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.73, 4.60 (1H each, s, H-29), 4.54 (1H, dd, *J* = 9.9, 5.7 Hz, H-3), 3.01 (1H, m, H-19), 2.87-2.95 (1H, m, H-3′), 2.84, 2.42 (1H each, dd, *J* = 15.9, 8.3, 5.6, H-2′), 2.10–2.20 (1H, m, H-13), 1.69 (3H, s, H-30), 1.28, 1.26 (3H, d, *J* = 6 Hz, CH_3- 3′), 0.97 (6H, s, 2 × CH_3), 0.86, 0.83, 0.80 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ +13.00 ° (c = 0.05, MeOH).

3*β*-*O*-(3′*S*-Methylsuccinyl)-betulinic acid (10)

88% yield from **29**, white amorphous powder. Mp 279–281 °C. MS (ESI−) *m/z*: 569.38 (M^- − H) for $C_{35}H_{54}O_6$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.73, 4.60 (1H each, s, H-29), 4.51 (1H, dd, *J* = 11.1, 4.8 Hz, H-3), 2.96–3.03 (1H, m, H-19), 2.86–2.93 (1H, m, H-3′), 2.70, 2.61 (1H each, dd, *J* = 16.2, 6.3, 4.8 Hz, H-2′), 2.06–2.16 (1H, m, H-13), 1.69 (3H, s, H-30), 1.27, 1.25 (3H, d, *J* = 6 Hz, CH_3-3′), 0.97, 0.94, 0.86, 0.85, 0.81 (3H each, s, CH_3-23, 24, 25, 26, 27). $[\alpha]^{20}_D$ − 12.88 ° (c = 0.08, MeOH).

Synthesis of 12

2-Ethyl-2-methylsuccinic acid (50 mg, 4 eq) was stirred in TFAA at rt for 3 h until the reaction mixture become homogenous. The solution was then concentrated to dryness under reduced pressure to yield 2-ethyl-2-methylsuccinic anhydride (**32**). Compound **32** was reacted without further purification with **1** (36 mg, 1 eq) and DMAP (19 mg, 2 eq) in anhydrous pyridine (1.5 mL), stirring at 160 °C for 2 h in microwave. The reaction mixture was diluted with EtOAc (10 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 30 mg (55%) of **12**; white amorphous powder.

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3*β*-*O*-(3′*S*-Ethylmethylsuccinyl)-betulinic acid (12a)

Mp 186–187 °C. MS (ESI−) *m/z*: 597.4 ($M^- - H$) for $C_{37}H_{58}O_6$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.71, 4.59 (1H each, s, H-29), 4.48 (1H, m, H-3), 3.00 (1H, m, H-19), 2.75, 2.52 (1H each, d, *J* = 15.5, H-2′), 1.66 (3H, s, H-30), 1.34-1.32 (2H, m, CH_2-3′), 1.23 (3H, s, CH_3-3′), 1.18 (2H, m, CH_3-3″), 0.94 (6H, s, 2 × CH_3), 0.85, 0.84, 0.78 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ −9.00 ° (c = 0.10, MeOH).

3*β*-*O*-(3′*R*-Ethylmethylsuccinyl)-betulinic acid (12b)

Mp 179–181 °C. MS (ESI−) *m/z*: 597.4 ($M^- - H$) for $C_{37}H_{58}O_6$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.71, 4.58 (1H each, s, H-29), 4.51 (1H, dd, *J* = 10.2, 6.4 Hz, H-3), 2.99 (1H, m, H-19), 2.98, 2.26 (1H each, d, *J* = 15.5, H-2′), 1.67 (3H, s, H-30), 1.35 (2H, m, CH_2-3′), 1.25 (3H, s, CH_3-3′), 1.19 (3H, m, CH_3-3″), 0.98, 0.95 (3H each, s, 2 × CH_3), 0.86, 0.82, 0.79 (3H each, s, 3 × CH_3). $[\alpha]^{20}_D$ +9.57 ° (c = 0.07, MeOH).

Synthesis of 30

To a solution of **3** (1 g, 1eq) in anhydrous THF was added sodium borohydride (209 mg, 2.5 eq). The reaction was neutralized with 10% HCl after stirring in rt for 4 h, and extracted with EtOAc. 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 905 mg (90%) of **30**; white amorphous powder. Mp 197–199 °C. MS (ESI−) *m/z*: 455.5 ($M^- - H$) for $C_{30}H_{47}O_3$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.15 (1H, s, H-19), 3.19 (1H, dd, *J* = 10.8, 5.6 Hz, H-3), 0.97, 0.96 (3H each, s, H-29, H-30), 0.95 (6H, s, H-24, H-26), 0.84 (3H, s, H-25), 0.75, 0.74 (3H each, s, H-23, H-27).

Synthesis of MA derivatives 13–21

To a solution of **30** in dry CH_2Cl_2 were added triethylamine (50 μL) and corresponding acyl chloride (50 μL). The reaction was stirred at rt for 30 min. The mixture was diluted with CH_2Cl_2 and washed with 10% HCl. 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.

3*β*-*O*-(2′-Methylbutyryl)-moronic acid (13)

73% yield (starting with 20 mg of **1**); white amorphous powder. Mp 237–239 °C. MS (ESI −) *m/z*: 539.4 ($M^- - H$) for $C_{35}H_{56}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (1H, s, H-19), 4.47 (1H, dd, *J* = 10.0, 5.6 Hz, H-3), 2.35 (1H, dd, *J* = 10.0, 6.0 Hz, H-2′), 1.96 (2H, m, H-3′), 1.17, 1.15 (3H, d, *J* = 6 Hz, CH_3-2′), 0.98, 0.97 (3H each, s, H-29, H-30), 0.91 (3H, m, H-4′), 0.88, 0.84 (6H), 0.82 (3H each, s, H-26, H-25, H-24, H-23), 0.77 (3H, s, H-27).

3*β*-*O*-Propionyl-moronic acid (14)

57% yield (starting with 11.2 mg of **1**); white amorphous powder. Mp 210–211 °C. MS (ESI −) *m/z*: 511.5 ($M^- - H$) for $C_{33}H_{52}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.175 (1H, s, H-19), 4.48 (1H, dd, *J* = 9.9, 6.6 Hz, H-3), 2.32 (2H, q, *J* = 7.2 Hz, H-2′), 1.14 (3H, t, *J* = 7.8 Hz, H-3′), 0.99, 0.98 (3H each, s, H-29, H-30), 0.88 (6H), 0.84 (6H), 0.77, (3H each, s, H-26, H-25, H-24, H-23, H-27).

3*β*-*O*-Cyclobutancarbnyl-moronic acid (15)

58% yield (starting with 12.2 mg of **1**); white amorphous powder. Mp 231–233 °C. MS (ESI −) *m/z*: 537.4 ($M^- - H$) for $C_{35}H_{54}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (1H, s, H-19), 4.48 (1H, dd, *J* = 11.7, 4.8 Hz, H-3), 3.12 (1H, quintet, *J* = 8.4 Hz, H-2′), 2.34-2.13,

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2.04-1.86 (4H, m, H-3′, H-5′),1.62-1.27 (2H, m, H-4′), 0.99, 0.98, 0.97, 0.88, 0.84 (6H), 0.77 (3H each, s, H-29, H-30, H-25, H-26, H24, H- 23, H-27).

3β-*O*-Valeroryl-moronic acid (16)

84% yield (starting with 13.5 mg of **1**); white amorphous powder. Mp 197–199 °C. MS (ESI −) *m/z*: 539.4 ($M^- - H$) for $C_{35}H_{56}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (1H, s, H-19), 4.48 (1H, dd, *J* = 10.6, 6.0 Hz, H-3), 2.30 (2H, t, *J* = 6.0 Hz, H-2′), 1.76 (2H, m, H-3′), 0.99, 0.98, 0.97, 0.88, 0.84 (6H), 0.77, (3H each, s, H-29, H-30, H-26, H-25, H-24, H-23, H-27).

3β-*O*-Isovaleroryl-moronic acid (17)

54% yield (starting with 12.2 mg of **1**); white amorphous powder. Mp 134–136 °C. MS (ESI −) *m/z*: 539.4 ($M^- - H$) for $C_{35}H_{56}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (1H, s, H-19), 4.48 (1H, dd, *J* = 11.6, 5.6 Hz, H-3), 2.17 (2H, d, *J* = 10.0, H-2′), 1.63 (1H, m, H-3′), 0.99 (6H), 0.97 (6H), 0.95, 0.88, 0.84, 0.835, 0.77, (3H each, s, 2 × H-4′, H-29, H-30, H-26, H-25, H-24, H-23, H-27).

3β-*O*-Cyclopetanecarbonyl-moronic acid (18)

75% yield (starting with 20 mg of **1**); white amorphous powder. Mp 209–210 °C. MS (ESI −) *m/z*: 551.4 ($M^- - H$) for $C_{36}H_{56}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (1H, s, H-19), 4.48 (1H, dd, *J* = 10.6, 5.6 Hz, H-3), 3.13 (1H, quintet, *J* = 6.0 Hz, H-2′), 2.36-2.13, 2.06-1.89 (4H, m, H-3′, H-6′),1.78-1.19 (4H, m, H-4′, H-5′), 1.00, 0.98, 0.97, 0.88, 0.84 (6H), 0.77 (3H each, s, H-29, H-30, H-25, H-26, H24, H-23, H-27).

3β-*O*-Butyryl-moronic acid (19)

71% yield (starting with 20 mg of **1**); white amorphous powder. Mp 242–244 °C. MS (ESI −) *m/z*: 525.5 ($M^- - H$) for $C_{34}H_{54}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.18 (1H, s, H-19), 4.48 (1H, dd, *J* = 10.0, 6.0 Hz, H-3), 2.28 (2H, t, *J* = 6.0 Hz, H-2′), 1.76 (2H, m, H-3′), 1.00, 0.98 (3H each, s, H-29, H-30), 0.97 (3H, t, *J* = 6.0 Hz, H-4′), 0.92, 0.88, 0.84 (6H), 0.77, (3H each, s, H-26, H-25, H-24, H-23, H-27).

3β-*O*-Isobutyryl-moronic acid (20)

66% yield (starting with 20 mg of **1**); white amorphous powder. Mp 206–207 °C. MS (ESI −) *m/z*: 525.4 ($M^- - H$) for $C_{34}H_{54}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.18 (1H, s, H-19), 4.46 (1H, dd, *J* = 10.0, 6.0 Hz, H-3), 2.54 (1H, septet, *J* = 6.0 Hz, H-2′), 1.19, 1.17, 1.16, 1.15 (6H, d, *J* = 3.0 Hz, H-3′, CH_3-2′), 0.98 (9H), 0.88, 0.85, 0.84, 0.77 (3H each, s, H-29, H-30, H-25, H-26, H24, H-23, H-27).

3β-*O*-Trimethylacetyl-moronic acid (21)

34% yield (starting with 25 mg of **1**); white amorphous powder. Mp 103–105 °C. MS (ESI −) *m/z*: 539.5 ($M^- - H$) for $C_{35}H_{56}O_4$. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.17 (1H, s, H-19), 4.46 (1H, dd, *J* = 10.0, 5.4 Hz, H-3), 1.15 (9H, s, 3 × CH_3-2′), 0.98, 0.97, 0.96, 0.88, 0.84 (6H), 0.77 (3H each, s, H-29, H-30, H-25, H-26, H24, H-23, H-27).

HIV-1_{IIIB} Replication Inhibition Assay in MT-2 Cell Lines.[17, 20, 21]

The evaluation of HIV-1 inhibition was carried out as follows using MT-2 lymphocytes. Test samples were first dissolved in dimethyl sulfoxide (DMSO). 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

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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 toxicity determinations (IC_{50} defined below). In addition, AZT and **2** were also assayed during each experiment as positive drug controls. On day 1 PI (post-infection), 140 μL fresh cell specific media was added to each cell. On day 4 PI, the assay was terminated and culture supernatants were harvested for p24 antigen ELISA analysis. The compound toxicity was determined by XTT using the mock-infected sample wells. If a test sample inhibited virus replication and was not toxic, its effects were reported in the following terms: EC_{50}, the concentration of the test sample that was able to suppress HIV replication by 50%; IC_{50}, the concentration of test sample that was toxic to 50% of the mock-infected cells; and therapeutic index (TI), the ratio of the IC_{50} to EC_{50}.

HIV-1 Maturation Inhibition Assay.[10]

Hela cells were transfected with pNL4-3 or mutated HIV-1 virus (A1V or L231M) and cultured in the absence or presence of **10**. Two days post-transfection, cells were metabolically labeled for 2h with ^{35}S-Met/Cys. Cell lysates were prepared, and virions were pelleted by ultracentrifugation. Cell and viral lysates were immunoprecipitated with HIV-Ig. Western blotting was then performed,[22, 23] and p25 and p24 are indicated.

HIV-1_{NL4-3} Replication Inhibition Assay in MT-4 Lymphocytes.[21]

A previously described HIV-1 infectivity assay was used. 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, New York).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AIDS	acquired immunodeficiency syndrome
HIV-1	human immunodeficiency virus type 1
BA	betulinic acid
MA	moronic acid
HAART	highly active antiretroviral therapy
NRTI	nucleoside/nucleotide reverse transcriptase inhibitor
NNRTI	non-nucleoside reverse transcriptase inhibitor
PI	protease inhibitor
MI	maturation inhibitor

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P24 (CA)	capsid
P25 (CA-SP1)	capsid precursor
Bevirimat	3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid
MSB	3-*O*-monomethylsuccinyl betulinic acid
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride
DMAP	4-(dimethylamino)pyridine
TBDPS	tert-butyldimethylsilyl
TBAF	tetra-n-butylammonium fluoride
THF	tetrahydrofuran
TEMPO	2,2,6,6-tetramethyl-1-piperidinyloxy
$PhI(OAc)_2$	(diacetoxyiodo) benzene
$NaBH_4$	Sodium borohydride
Et_3N	triethylamine
TFAA	trifluoroacetic anhydride
AZT	zidovudine
PI	post-infection

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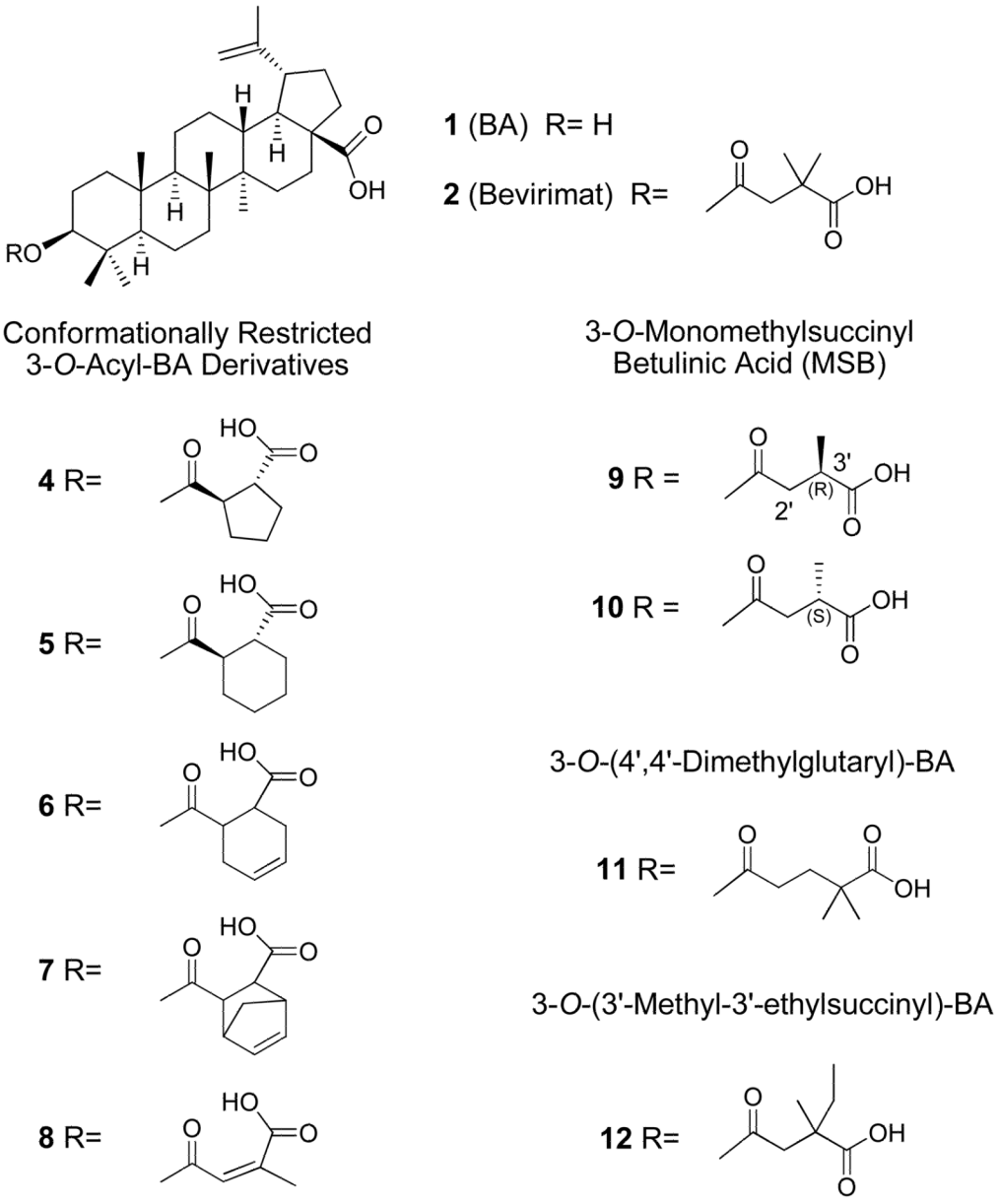

Figure 1.
Structures of MSB analogs and other 3-*O*-acyl BA analogs

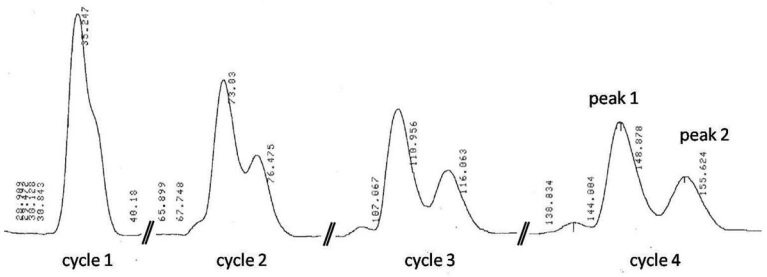

Figure 2. Isolation of the two isomers of compound 12 by JAI LC-918 recycling preparative HPLC

Mobile phase: 85% Acetonitrile in water (0.1% TFA); Flowrate: 4.0 mL/min; Detector: Refractive Index (RI); Column: Alltima 10mm × 250mm C18 5μ. Peak 1 is **12a** (3′*S* isomer); and peak 2 is **12b** (3′*R* isomer).

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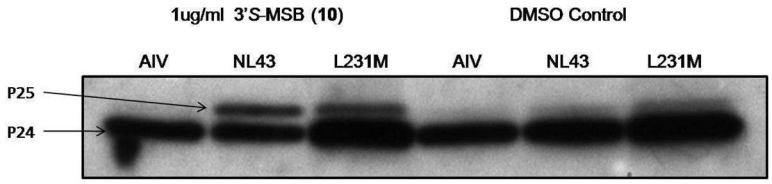
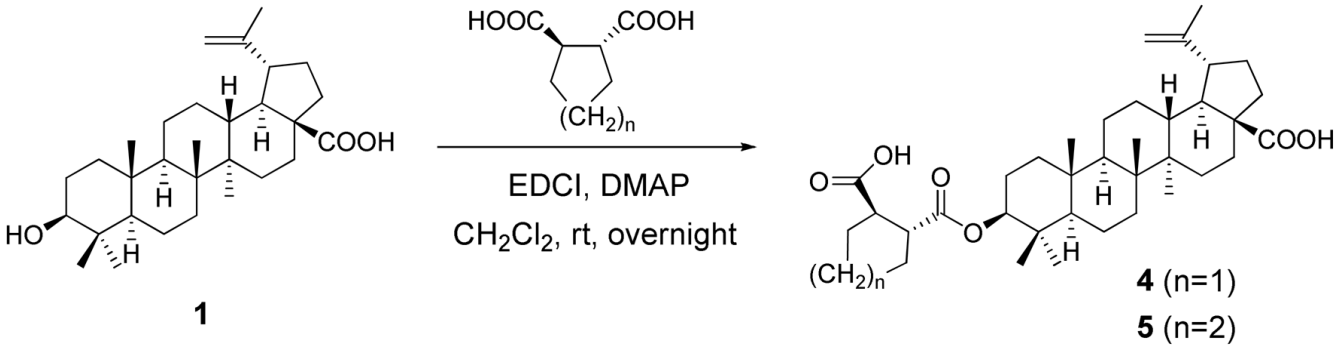


Figure 3. Effect of compound 10 on virus particle production and Gag processing in virion lysate
Note the accumulation of p25 in the presence of **10**. A1V is a CA-SP1 mutant resistant to **2**. L231M is a PI-resistant viral mutant.



Scheme 1.
Synthesis of compounds 4 and 5.

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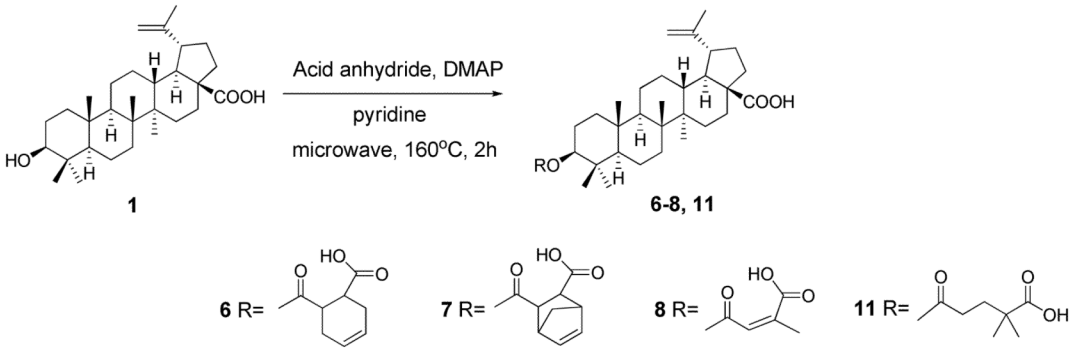
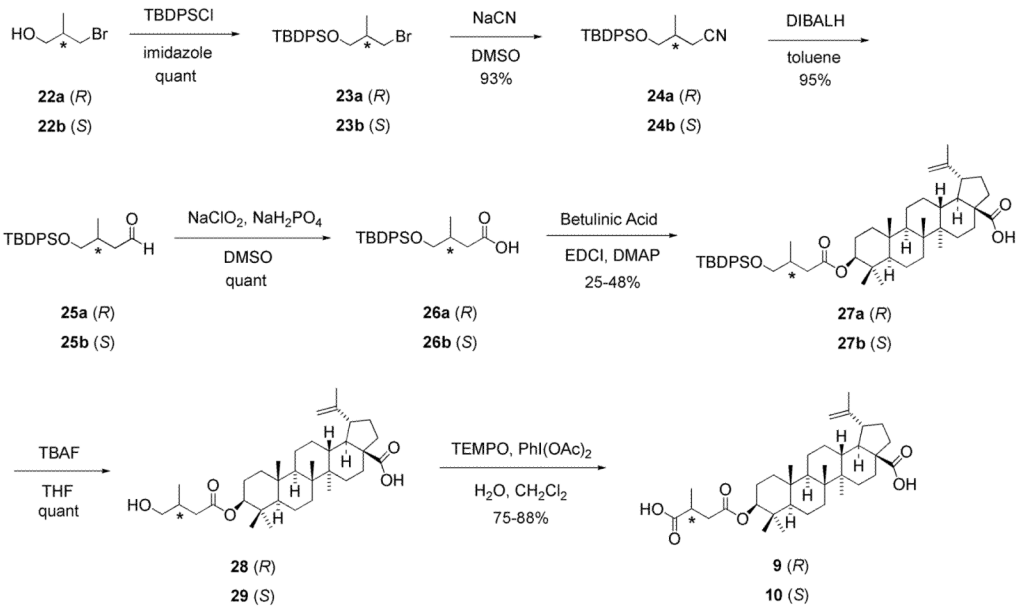


Scheme 2.
Syntheses of compounds 6–8 and 11.



Scheme 3.
Total syntheses of compounds 9 and 10.

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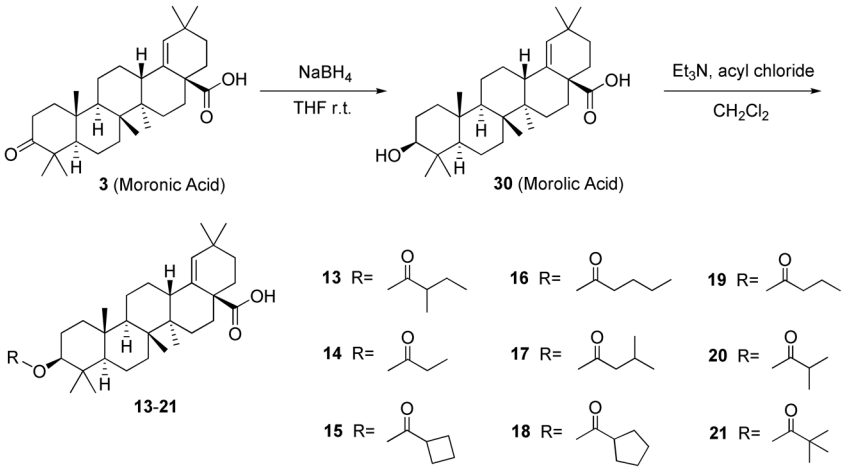

Scheme 4.
Synthesis of MA analogs 13–21.

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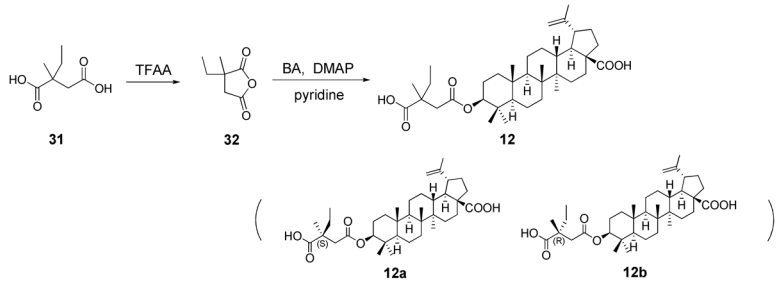

Scheme 5.
Synthesis of compound 12.

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Table 1

Anti-HIV-1 replication activities for BA and MA derivatives in acutely infected MT-2 cell lines[a]

Compd	EC_{50} (μM)	IC_{50} (μM)	Therapeutic Index
AZT	0.034	1,870	55,000
2	0.0013	42.78	32,907
4	2.2	41.89	19
5	17.3	40.93	2.4
6	NS	41.06	–
7	NS	40.27	–
8	0.18	42.51	236.2
9	0.12	43.83	365.3
10	0.0087	32.78	3,768
11	0.048	41.75	869.8
12	0.0006	36.41	60,683
13	NS	> 10	–
14	NS	> 10	–
16	NS	> 10	–
17	NS	> 10	–
18	NS	> 10	–
19	NS	> 10	–
20	NS	> 10	–
21	NS	> 10	–
33 (9+10)	0.016	44.93	2,808

[a] All data presented are averages of at least two separate experiments performed by Panacos Pharmaceutical Inc. EC_{50}: concentration that inhibits HIV-1 replication by 50%. IC_{50}: concentration that inhibits mock-infected cell growth by 50%. TI = IC_{50}/EC_{50}. NS: no suppression at the testing concentration.