

Anti-AIDS agents 73: Structure–activity relationship study and asymmetric synthesis of 3-*O*-monomethylsuccinyl-betulinic acid derivatives

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Abstract—3-*O*-3'-(or 2')-Methylsuccinyl-betulinic acid (MSB) derivatives were separated by using recycle HPLC. The structures of four isomers were assigned by NMR and asymmetric synthesis. 3-*O*-3'-*S*-Methylsuccinyl-betulinic acid (3'-*S*-MSB, **4**) exhibited potent anti-HIV activity with an EC₅₀ value of 0.0087 μ M and a TI value of 6.3×10^3 , which is comparable to the data for bevirimat (DSB, PA-457), a current clinical trials drug that was also derived from betulinic acid. The anti-HIV potency of **4** was slightly better than that of AZT.

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Highly active antiretroviral therapy (HAART) combines several anti-HIV drugs with different targets, and its use has significantly improved AIDS treatment.^{1–3} However, it has also led to some serious problems, such as increased toxicities and emergence of multi-drug resistant HIV viral strains.^{4–7} Therefore, new classes of compounds with novel modes of action against HIV are still urgently needed.

We initially identified betulinic acid (BA, **1**) as an anti-HIV lead compound with an EC₅₀ of 1.4 μ M and therapeutic index (TI) of 9.3 from the leaves of *Syzygium claviflorum*.⁸ Our subsequent modification study resulted in the discovery of 3-*O*-(3',3'-dimethylsuccinyl)-betulinic acid (bevirimat, DSB, PA-457, **2**). Bevirimat exhibits extremely potent anti-HIV activity with an EC₅₀ value of <0.00035 μ M and a TI value of >20,000.^{9,10} It was found to inhibit HIV-1 replication by interfering with a previously unidentified viral target, HIV-1 P24/P25 processing, resulting in the production of a noninfectious HIV-1 particle.^{11,12} Therefore, bevirimat represents a unique first in a class of anti-HIV compounds termed maturation inhibitors (MIs). Bevirimat

has recently succeeded in Phase IIa clinical trials and is currently in Phase IIb clinical trials.^{13,14}

Our prior structure–activity relationship (SAR) study of **2** and other 3-*O*-acyl-betulinic acid derivatives suggested that the proper length, a terminal carboxylic acid, and C-3' dimethyl substitution of the C-3 side chain contribute to enhanced anti-HIV activity.^{9,15} However, it is still unclear which, if either, C-3' methyl group within the C-3 side chain of **2** contributes more toward activity. Therefore, 3-*O*-monomethylsuccinyl-betulinic acid (MSB) derivatives were designed to investigate the impact of these methyl groups. This paper reports their synthesis, isolation, structural assignment, and SAR study.

Betulinic acid was treated with 2-methylsuccinic anhydride and DMAP in pyridine at reflux to furnish the 3'-MSB racemic isomers, as well as the undesired 2'-MSB isomers. The separation of the four compounds (**3–6**)¹⁶ was challenging, since they differ only by the presence of a 2' or 3' (*R* or *S*) methyl group, but was successfully achieved by using recycle preparative HPLC (Fig. 1).¹⁷ The purities of **3–6** were ascertained by analytical HPLC (Fig. 2). By comparing the ¹H NMR spectra of the purified isomers with those of **2** and 3-*O*-2',2'-dimethylsuccinyl BA, peaks 1 and 2 were

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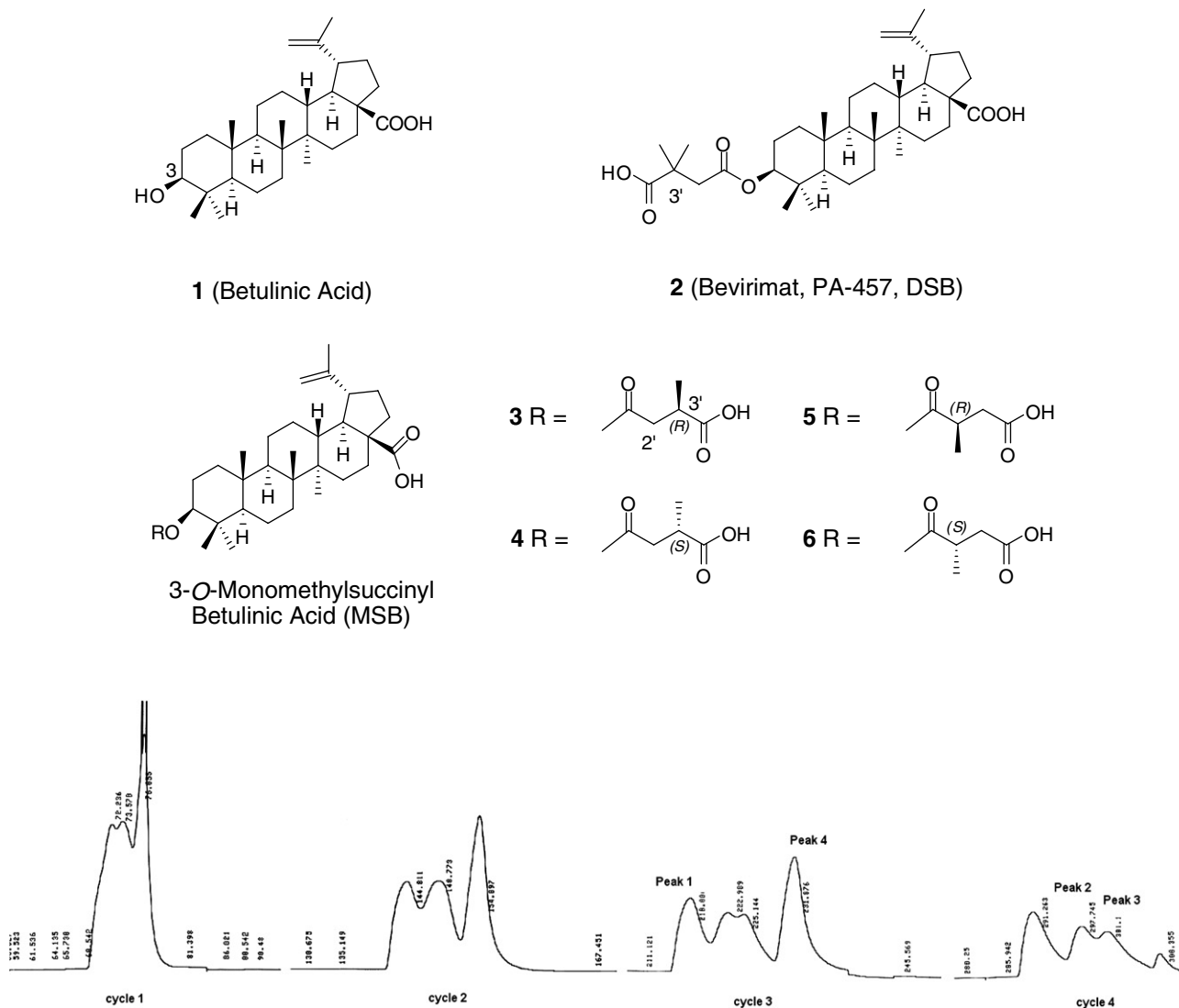


Figure 1. JAI LC-918 recycling preparative HPLC. Mobile phase: 85% ACN 0.1% TFA; flowrate: 5.0 mL/min; detector: refractive index (RI); column: Alltima C18 5 μ m 250 mm \times 22 mm. Peak 1: 3-O-(3'-S-methylsuccinyl)-betulinic acid (4); peak 2: 3-O-(3'-R-methylsuccinyl)-betulinic acid (3); peak 3: 3-O-(2'-S-methylsuccinyl)-betulinic acid (6); peak 4: 3-O-(2'-R-methylsuccinyl)-betulinic acid (5).

assigned as the 3'-MSBs, and peaks 3 and 4 as the 2'-MSBs. However, we still needed to determine the stereochemistry of the C-3' and C-2' chiral centers. Therefore, the 3' *R* and 3' *S* isomers were also prepared through asymmetric synthesis as described below.

As shown in Scheme 1, the hydroxy group of 3*R*-bromo-2-methylpropanol was first protected by reaction with TBDPSCl in DMF. The bromide group was then replaced with an aldehyde group by reaction first with sodium cyanide in DMSO, followed by reduction using DIBALH.¹⁸ The aldehyde was oxidized to a carboxylic acid in the presence of NaClO₂ and NaH₂PO₄ in DMSO to yield **9a**. Reaction of **1** with **9a** in the presence of EDCI and DMAP led to esterification of the β -hydroxy group of **1** with the carboxylic acid of **9a** to provide **10a**. After cleavage of the TBDPS group with TBAF in THF, the primary hydroxy group of **11** was oxidized to a carboxylic acid in the presence of TEMPO, PhI(OAc)₂ in a solution of H₂O–CH₂Cl₂ to give 3-O-(3'-*R*-methyl

succinyl)-betulinic acid (3'-*R*-MSB, **3**). The 3'-*S*-MSB isomer (**4**) was synthesized using the same method starting with 3*S*-bromo-2-methylpropanol. By comparing the HPLC retention times and ¹H NMR spectra of these compounds, we determined that peak 1 is the 3'-*S* diastereoisomer and peak 2 is the 3'-*R* diastereoisomer.

Furthermore, the reaction of 2*R*-methylsuccinic anhydride with betulinic acid yielded the 3'-*R*-MSB (**3**) and 2'-*R*-MSB (**5**) isomers. The HPLC retention time indicated that peak 4 is the 2'-*R* isomer (**5**). Similarly, peak 3 was confirmed as the 2'-*S* isomer (**6**). Thus, the stereochemistry of the 3' or 2' chiral center in **3–6** was fully determined.

Anti-HIV activities of all four synthetic compounds (**3–6**) and **2** were evaluated according to the procedure described in Refs. 19 and 20. The synthetic intermediate **12** and a 1:1 mixture (**13**) of the 3'-*R*- and 3'-*S*-MSB isomers (**3** and **4**) were also tested.

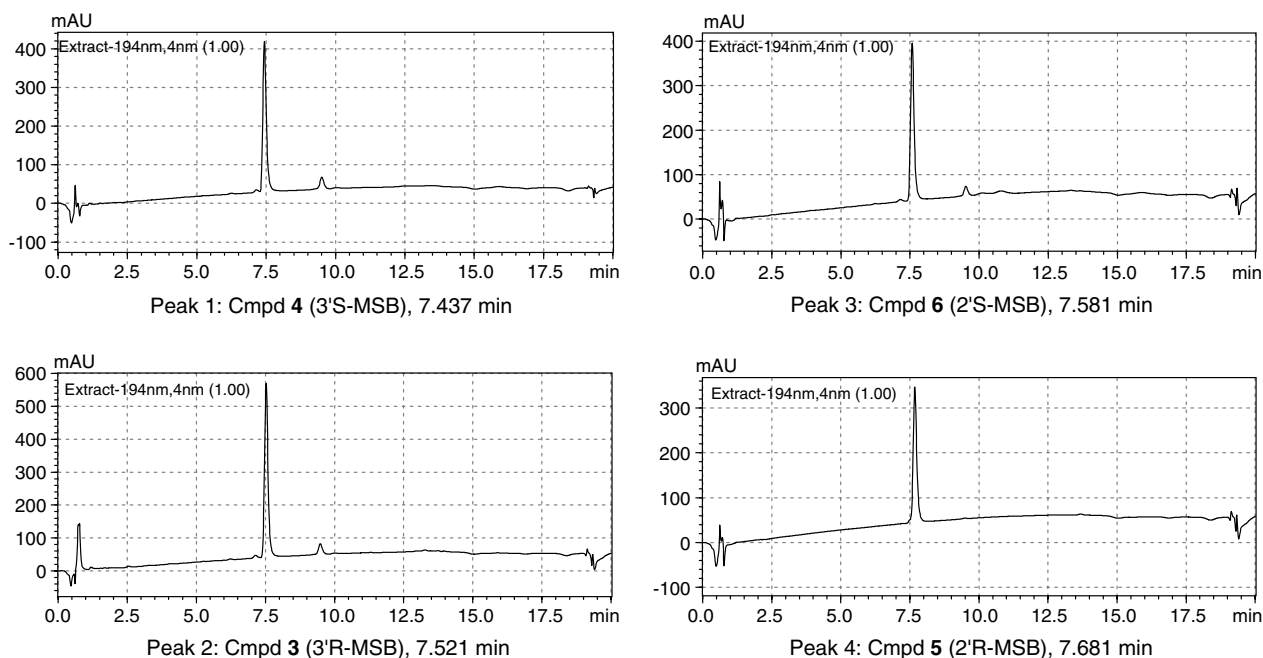
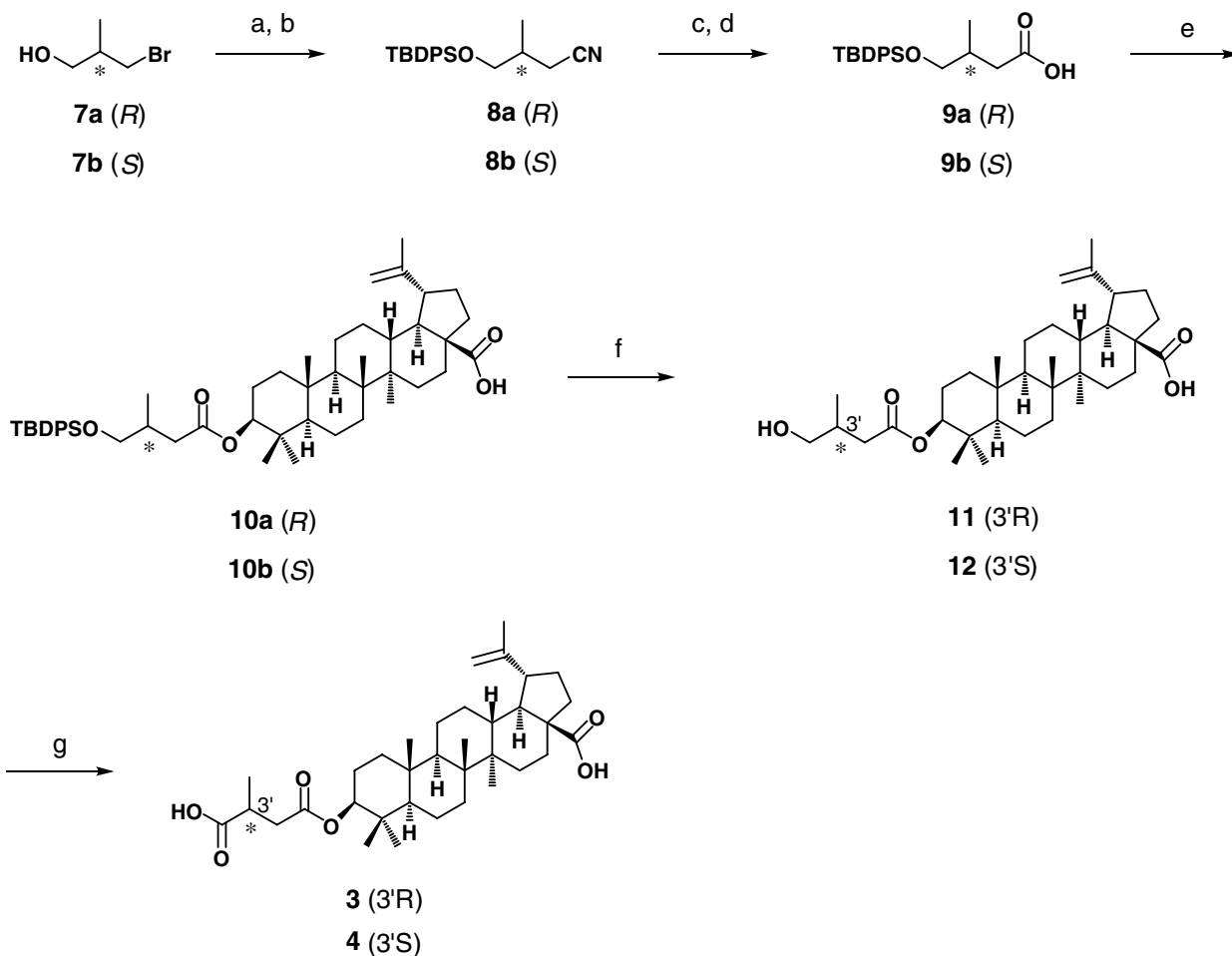


Figure 2. Purity confirmation of the four MSB (3–6) isomers by Shimadzu LC-20AT. Column: Alltima C18 5 μ m 150 mm \times 2.1 mm.



Scheme 1. Reagents and conditions: (a) TBDPSCI, imidazole, quant; (b) NaCN, DMSO, 93%; (c) DIBALH, 95%; (d) NaClO₂, NaH₂PO₄, DMSO, quant; (e) EDCI, DMAP, BA, 25–48%; (f) TBAF, THF, quant; (g) TEMPO, PhI(OAc)₂, H₂O, CH₂Cl₂, 75–88%.

Table 1. Anti-HIV activities for 3-*O*-monomethylsuccinyl-betulinic acid derivatives in HIV-1 infected MT-2 cell line¹⁸

Compound	EC ₅₀ ^a (μM)	IC ₅₀ ^b (μM)	TI (IC ₅₀ /EC ₅₀)
AZT	0.034	1870	55,330
2	0.0013	>42.78	30,555
3	0.12	>43.83	961
4	0.0087	32.78	6274
5	No suppression	>43.83	—
6	0.44	>43.83	100
12	No suppression	>44.93	—
13	0.016	>44.93	5323

^a Concentration that inhibits HIV-1 replication by 50%.^b Concentration that is toxic to 50% of mock-infected MT-2 cells.

The anti-HIV activities of the synthesized compounds are summarized in Table 1. Among these derivatives, the 3'-*S*-MSB isomer (**4**) showed very potent anti-HIV activity with a TI of 6.3×10^3 and EC₅₀ of 0.0087 μM, which is comparable to that of **2** (EC₅₀: 0.0013 μM) and slightly better than that of AZT (EC₅₀: 0.034 μM) in this assay system. The 3'-*R*-MSB isomer (**3**) exhibited only moderate anti-HIV activity with a TI of 9.6×10^2 and EC₅₀ of 0.12 μM. The antiviral activity (EC₅₀: 0.016 μM) of the mixture (**13**) of the two stereoisomers falls in between those of **3** and **4**. This result indicates that the two C-3' methyl groups in the DSB side chain contribute differently to the extremely potent anti-HIV activity of this compound class. We postulate that an interaction of the 3' *S*-methyl group with the viral target might be essential to the anti-HIV 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', is slightly more potent than the 3' *S*-MSB isomer (**4**). In comparison with **3** and **4**, both 2'-MSBs (**5** and **6**) exhibited no or little activity in the same assay. These results agree with our previous SAR study, indicating that methyl substitution at the C-3' position improves the antiviral activity much more significantly than at the C-2' position.

In addition, compound **12**, which differs structurally from **3** only in the replacement of a primary hydroxyl group for a terminal carboxylic acid in the 3-*O*-acyl side chain, had no activity. This result is also consistent with our findings in prior studies that the terminal carboxylic acid is essential for enhanced antiviral activity.¹⁵

In conclusion, 3'-MSBs and 2'-MSBs were prepared in our study to investigate the SAR of the C-3' chiral center of the anti-HIV clinical trials agent bevirimat (**2**). Our results suggest that the 3' *S*-methyl group of **2** is the major contributor to the compound's extremely high anti-HIV potency, since **2** and 3' *S*-MSB (**4**) exhibit comparable anti-HIV activity, while the 3' *R* isomer shows much lower activity.

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- Spectroscopic data for 3-*O*-(3'-*R*-methylsuccinyl)-betulinic acid (**3**): white amorphous powder. Mp 268–271 °C. MS (ESI-) *m/z*: 569.38 (M⁺-H) for C₃₅H₅₄O₆. ¹H NMR (300 MHz, CDCl₃): 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, CH₃-3'), 0.97 (6H, s, 2× CH₃), 0.86, 0.83, 0.80 (3H each, s, 3× CH₃). 3-*O*-(3'-*S*-methylsuccinyl)-betulinic acid (**4**): white amorphous powder. Mp 279–281 °C. MS (ESI-) *m/z*: 569.38 (M⁺-H) for C₃₅H₅₄O₆. ¹H NMR (300 MHz, CDCl₃): 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, CH₃-3'), 0.97, 0.94, 0.86, 0.85, 0.81 (3H each, s, CH₃-23, 24, 25, 26, 27). 3-*O*-(2'-*R*-methylsuccinyl)-betulinic acid (**5**): white amorphous powder. Mp 250–252 °C. MS (ESI-) *m/z*: 569.38 (M⁺-H) for C₃₅H₅₄O₆. ¹H NMR (300 MHz, CDCl₃): δ 4.73, 4.60 (1H each, s, H-29), 4.52 (1H, t, *J* = 7.8 Hz, H-3), 2.90–3.10 (2H, m, H-19, H-2'), 2.67, 2.42 (1H each, dd, *J* = 16.8, 9.6, 4.2, H-3'), 2.13–2.20 (1H, m, H-13), 1.68 (3H, s, H-30), 1.26, 1.23 (3H, d, *J* = 7.2 Hz, CH₃-2'), 0.97 (6H, s, 2× CH₃), 0.86, 0.83, 0.81 (3H each, s, 3× CH₃). 3-*O*-(2'-*S*-methylsuccinyl)-betulinic acid (**6**): white amorphous powder. Mp 256–258 °C. MS (ESI-) *m/z*: 569.38 (M⁺-H) for C₃₅H₅₄O₆. ¹H NMR (300 MHz,

- CDCl₃): δ 4.74, 4.61 (1H each, s, H-29), 4.48 (1H, dd, J = 10.8, 4.8 Hz, H-3), 2.80–3.01 (2H, m, H19, H-2'), 2.76, 2.46 (1H each, dd, J = 16.9, 9.5, 4.7, H-3'), 2.10–2.18 (1H, m, H-13), 1.69 (3H, s, H-30), 1.25, 1.23 (3H, d, J = 6 Hz, CH₃-2'), 0.97, 0.92 (3H each, s, 2 \times CH₃), 0.84–0.86 (9H, m, 3 \times CH₃).
17. Peak 4 (compound **5**) was collected during cycle 3, and peak 1 (compound **4**) during cycle 4. Peak 2 (compound **3**) and peak 3 (compound **6**) were collected together during cycle 4, and subjected again to recycle preparative HPLC, which yielded pure compounds at cycle 7.
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