Anti-AIDS Agents. 34.† Synthesis and Structure−Activity Relationships of Betulin Derivatives as Anti-HIV Agents

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Received June 30, 1998

Succinyl and 3′-substituted glutaryl betulin derivatives showed stronger anti-HIV activity and higher therapeutic index (TI) values than their dihydrobetulin counterparts, with ratios of 1.2:1 to 15:1 (cf. **7** and **15**, **9** and **17**, **10** and **18**, **11** and **19**, and **12** and **20**). For various 3′-substituted glutaryl compounds, the order of anti-HIV effects, from strong to weak inhibition, was 3′,3′-dimethyl, 3′-methyl, 3′-ethyl-3′-methyl, followed by 3′,3′-tetramethylene glutaryl derivatives (**10** > **9** > **11** > **12**, **18** > **17** > **19** > **20**). The most potent compound, **10**, has two 3′,3′-dimethylglutaryl groups and displays significant anti-HIV potency with an EC_{50} value of 0.000 66 μM and a TI of 21 515. Results for compounds (**22** and **23**) without a C-3 acyl group confirmed the importance of the C-3 acyl group to the anti-HIV effect. With 3′,3′-tetramethylene glutaryl derivatives, triacyl **29** showed stronger inhibition than diacyl **12**; in contrast, 3′,3′-dimethylglutaryl compounds displayed opposite results. 3-Keto compounds (**35** and **36**) and 2,3-dihydro compounds (**39** and **40**) had EC_{50} values in the range of 4.3−10.0 μM, suggesting that A ring modification led to decreased potency. The reduced activity of amide (**33** and **34**), ester (**41**), and oxime (**42**) analogues suggested that the orientation and linkage of the C-3 acyl side chain play crucial roles in the potent anti-HIV activity. Finally, replacing the C-28 acyl group with a bulky non-carboxylic group produced a less potent compound (**44**). In the study of mechanism of action, our results indicated that fusion is not the primary target for the anti-HIV activity of **10**. It appears to inhibit HIV replication at a late stage of the viral life cycle, i.e., after viral protein synthesis.

Introduction

HIV infection leads to the disease called acquired immunodeficiency syndrome (AIDS), which has been a life-threatening health problem since 1981.[2] Without treatment, an infected patient has a marked reduction in CD4 T lymphocytes, is susceptible to a wide range of opportunistic infections, and ultimately dies. Many strategies, including FDA-approved anti-HIV reverse transcriptase (RT) and protease agents, have been investigated to prevent and/or control the spread of virus infection.[3] Current therapy can effectively control plasma viremia, but the virus is suppressed rather than eradicated in HIV-infected individuals.[4−6] To circumvent the existing therapeutic difficulties, novel compounds with unique modes of actions are urgently needed.

Triterpenes represent a unique class of anti-HIV agents as seen by the discovery of active plant natural products targeting RT (maprounic acid),[7] protease (maslinic acid),[8] or unknown sites (glycyrrhizin, suberosol).[9−13] In our continuing bioactivity-directed isolation of new anti-HIV agents, betulinic acid, purified from the leaves of *Syzigium claviflorum*, had an EC_{50} and therapeutic index (TI) of 1.4 μM and 9.3, respectively, in H9 lymphocytes.[8] Modification at the C-3 position produced 3-*O*-(3′,3′-dimethylsuccinyl)-betulinic acid (**2**), which was 4000-fold more active and had a 2150-fold higher TI than betulinic acid.[15,16] In addition, 3′,3′-dimethylglutaryl or diglycolyl analogues (**3** and **4**) showed good levels of activity. In the preparation of **2**, the 2′,2′-dimethylsuccinyl isomer (**5**) was also generated and separated by HPLC; however, **5** (EC_{50} = 2.7 μM and TI = 5.9) was much less active than **2**. In our mechanism(s) of action study, compounds **2**−**4** mediated potent inhibition via a site other than syncytium formation or HIV-1 RT, requiring at least 3 orders of magnitude higher concentration to completely inhibit HIV induced fusion. These compounds did not affect HIV RT activity under our experimental conditions. Independently, Mayaux and Soler et al. have reported that RPR 103611, the most promising betulinic acid derivative in their

† For part 33, see ref 1.

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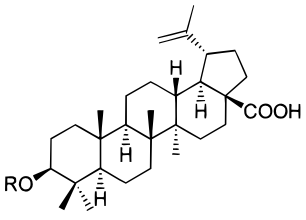
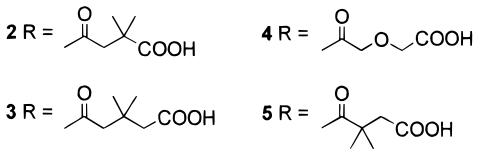
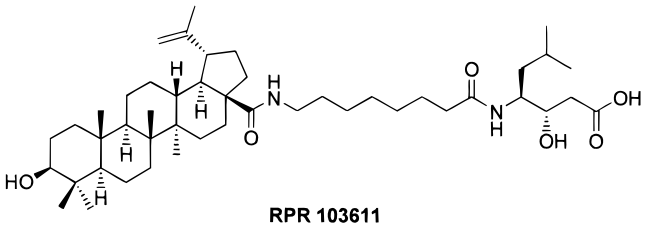
10.1021/jm980391g CCC: $15.00
Published on Web 10/14/1998



Betulinic Acid (**1**) R = H





RPR 103611

studies, interfered with virus replication specifically at syncytium formation.[17−18]

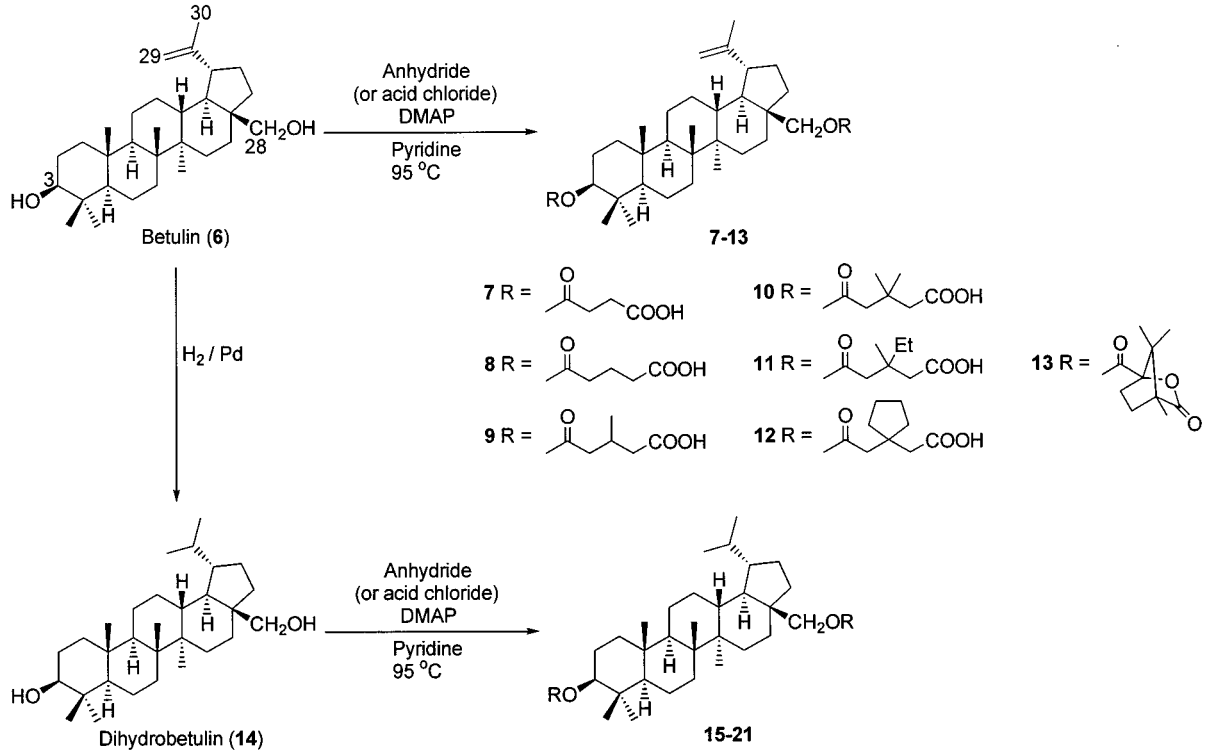
Betulinic acid (**1**) and betulin (**6**), where C-28 is a hydroxymethylene, have similar in vitro pharmacological properties (examples are cyclic AMP-dependent protein kinase and antiinflammatory activities),[20,21] but the latter is 7 times less expensive.[19] In a preliminary study, betulin showed weak anti-HIV activity with an EC_{50} of 23 μM and had a low TI of 1.9; thus, it was 16-fold less potent and had a 5-fold lower TI compared with betulinic acid. However, betulin can be structurally modified in three places to improve its pharmacological profile: the C-3 hydroxy group on the A ring, the C-28 hydroxy group, and the isopropylidene moiety. Therefore, we initiated research on betulin due to its structural similarity with and lower cost than betulinic acid.

Chemistry

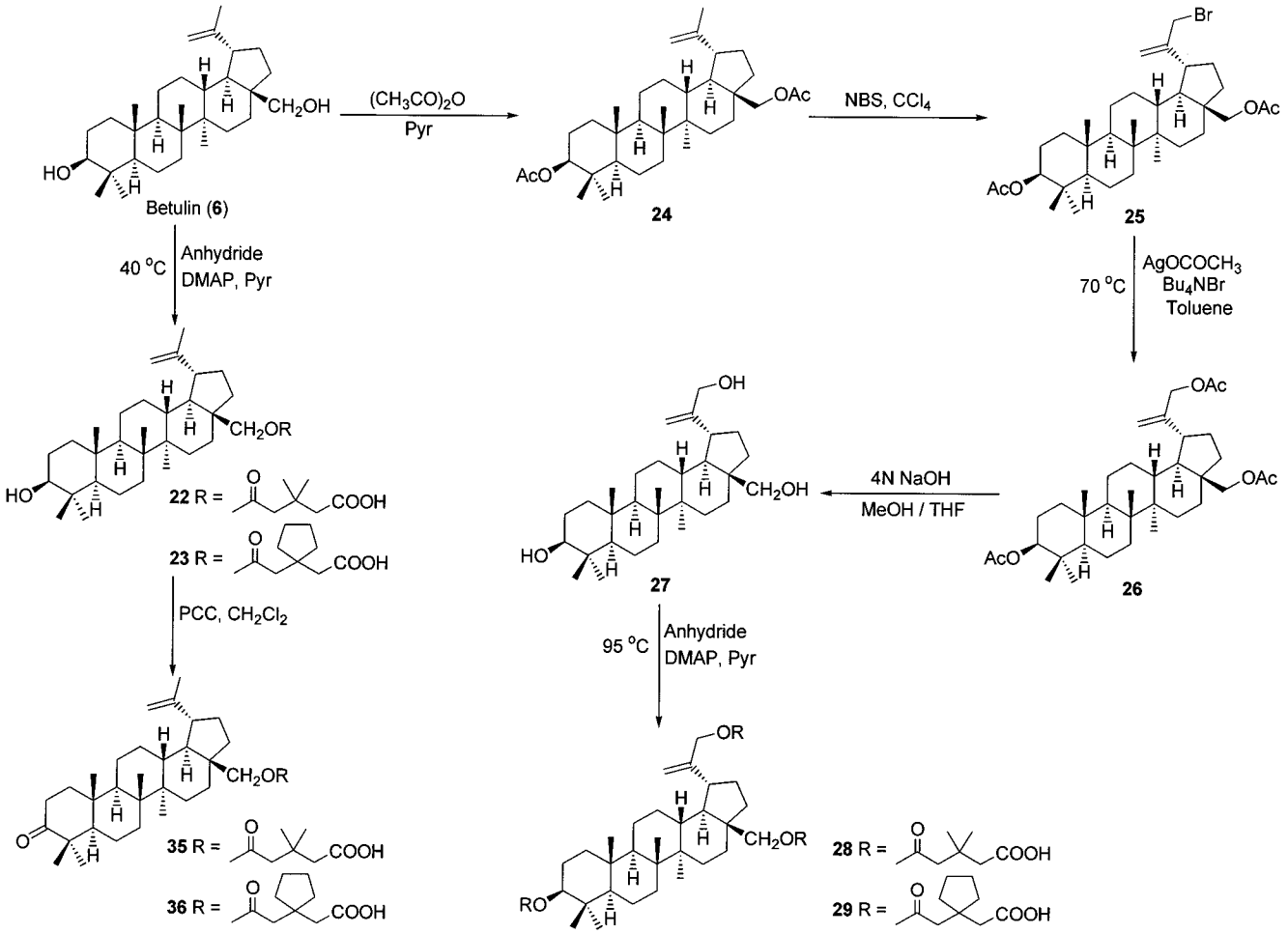
As described in Scheme 1, two acyl groups were introduced at the C-3 and C-28 hydroxy groups of betulin and dihydrobetulin, resulting in a second acylated group in the triterpene molecule compared with **2**−**5**. 3,28-Di-*O*-acyl derivatives (**7**−**13** and **15**−**21**) were successfully obtained by heating triterpenes with an acid anhydride or an acid chloride in the presence of 4-(dimethylamino)pyridine (DMAP) and dry pyridine.[22] A 6-fold excess of each acid anhydride was required in order to obtain diacylated products.

The monoesters **22** and **23** were obtained in 68−71% yield by heating betulin (**6**) at 40 °C with a 2-fold excess of the appropriate anhydride; recovery of betulin was 15−20%. The triol betulin **27** was successfully prepared in four steps by the methods described in ref 23 and shown in Scheme 2. After acylating the two hydroxy groups of betulin, allylic bromination with *N*-bromosuc-

Scheme 1. Synthesis of 3,28-Di-*O*-acylated Betulins and Dihydrobetulins



Scheme 2. Synthesis of 28-Mono-*O*-acyl, 3-Keto-28-mono-*O*-acyl, and 3,28,30-Tri-*O*-acyl Betulins



cinimide (NBS) in carbon tetrachloride afforded the bromo compound **25**. The rate of bromination was accelerated in the presence of the catalyst dibenzylperoxide, but the desired compound and a byproduct had close R_f values, resulting in difficult separation. Transformation of bromide **25** to ester **26** was achieved by heating with silver acetate and the phase transfer catalyst tetrabutylammonium bromide in dry toluene. Finally, alkaline hydrolysis yielded the triol **27**.

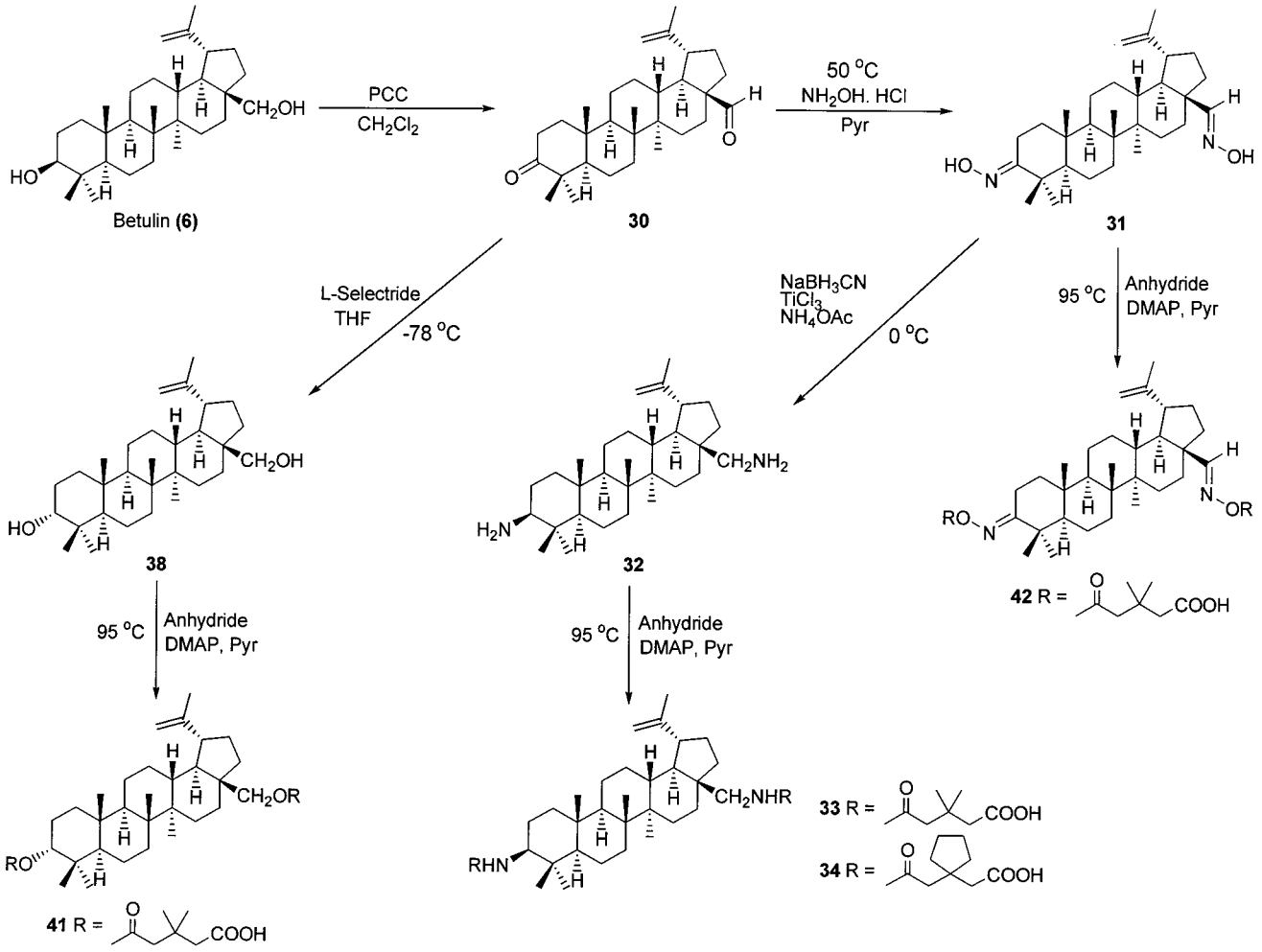
As seen in Scheme 3, oxidation of betulin with 3 equiv of pyridinium chlorochromate (PCC) yielded the keto/aldehyde **30**,[24] which was then converted to oxime **31** by treatment with NH_2OH in pyridine.[25] Amine **32** was readily prepared from oxime **31** by enantioselective reduction of the Schiff base with $TiCl_3$ and $NaCNBH_3$, as reported by Leeds and Kirst.[26] The triesters **28** and **29** and amides **33** and **34** were obtained by the same methods as for the diacyl analogues, by heating triol **27** and amine **32** at 95 °C with 6- to 10-fold of the corresponding anhydrides. Treatment of **22** and **23** with 1.5 equiv of PCC produced the 3-keto products **35** and **36**, respectively. Reduction of the keto/aldehyde **30** with 15 equiv of L-selectride at −78 °C produced 3-epi-betulin (**38**) in 74% yield and the natural betulin in 21% yield.[27] Under similar reaction conditions, diesters **41** and **42** were prepared from **38** and **31**, respectively. 2,3-Dihydro derivatives (**39** and **40**) were prepared as outlined in Scheme 4. The C-3 OH of **6** was eliminated by Mitsunobu reaction,[28] and the C-28 OH of the resulting **37** was acylated by the method described above for the monoesters. Acylation of **6** with a 1-adamantanylcarbonyl group at room temperature, followed by a 3,3-dimethylglutaryl group at 95 °C, gave **44**.

Results and Discussion

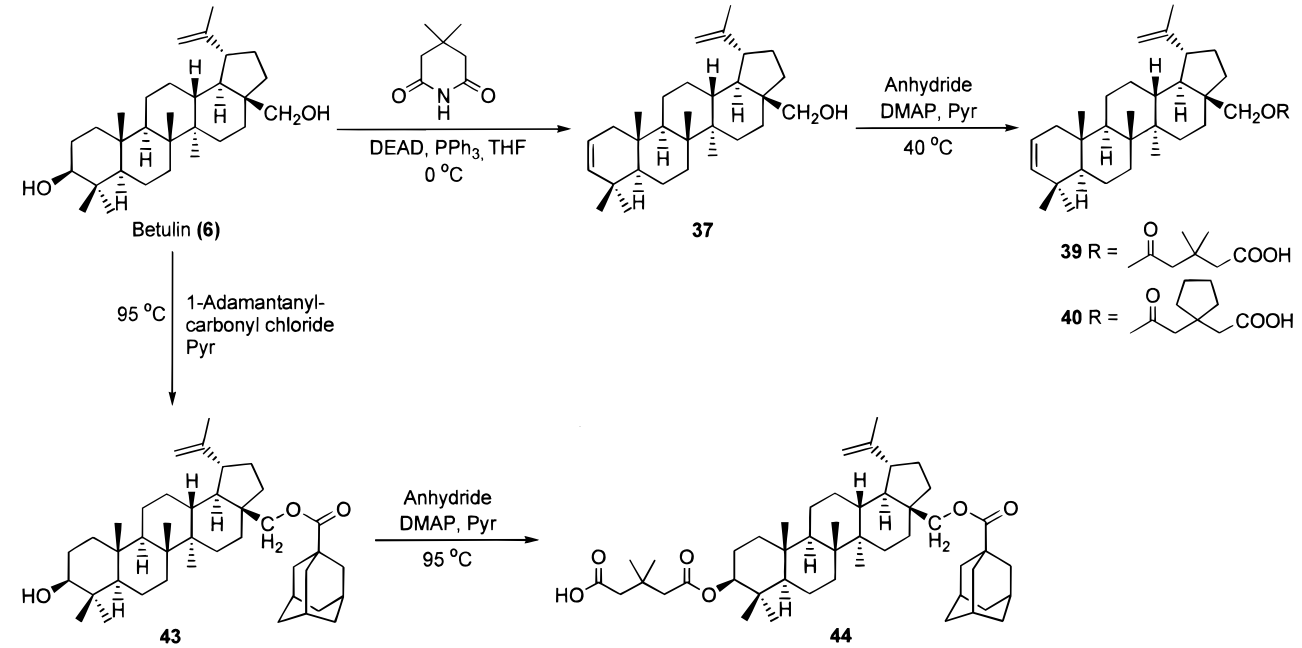
The newly synthesized betulin derivatives were evaluated for anti-HIV activity and cytotoxicity in H9 lymphocytes (see Experimental Section); the results are represented as EC_{50}, IC_{50}, and TI (IC_{50}/EC_{50}) values in Table 1. Due to limited solubility, camphanoyl derivatives **13** and **21** could not be examined and were excluded from the discussion.

With the succinyl products, the betulin analogue **7** had a slightly greater inhibitory effect ($EC_{50} = 3.8\ \mu M$) than the dihydrobetulin congener **15** ($EC_{50} = 4.7\ \mu M$). In contrast, opposite results occurred in the pair of unsubstituted glutaryl compounds. The dihydrobetulin derivative **16** was 2.4-fold more potent and had a 2.5-fold higher TI than that of the betulin derivative **8**. Substituted glutaryl compounds (**9**−**12** and **17**−**20**) had only slightly lower IC_{50} values, in the range of 10.6−21.6 μM, than those of the unsubstituted compounds (**8** and **16**). However, the former compounds showed significant anti-HIV activity with EC_{50} values in the nanomolar range and, thus, were more potent than the unsubstituted compounds. Furthermore, with these 3′,3″-substituted glutaryl compounds, betulin analogues **9**−**12** showed stronger inhibitory activity than their

Scheme 3. Synthesis of 3,28-Di-*O*-acyl, 3-Epi, and Oxime Betulins and 3,28-Di-N-acyl Amino-betulins



Scheme 4. Synthesis of 2,3-Dihydro and Adamantanylcarbonyl Betulins



corresponding dihydrobetulin analogues **17**−**20**; the ratio of their EC_{50} values ranged from 1:7 to 1:15 (cf. **9** and **17**, **10** and **18**, **11** and **19**, and **12** and **20**). Due to similar cellular toxicity (IC_{50}) of each pair, the ratios of their TI values ranged from 7:1 to 15:1. Invariably, in both series, **10** and **18** with 3′,3′- and 3″,3″-dimethyl groups produced better potency than **9** and **17** with 3′,3″-monomethyl substitution. As the 3′- and 3″-substituted moieties became bulky, as in **12** and **20**, the anti-HIV activity decreased. Thus, dimethyl groups at

Table 1. Anti-HIV Activity of Betulin Derivatives in Acutely Infected H9 Lymphocytes[a]

compd	IC_{50}[b] (μM)	EC_{50}[b] (μM)	TI	compd	IC_{50}[b] (μM)	EC_{50}[b] (μM)	TI
1	13.0	1.4	9.3	**23**	13.3	2.1	6.1
2	>7.0	<0.000 35	>20 000	**24**	>189.8	>189.8	<1
3	4.54	0.0023	1,974	**25**	–	–	–
4	11.7	0.01	1,172	**26**	25.0	10.2	2.5
5	15.9	2.7	5.9	**27**	>140.5	50.8	2.8
6	43.7	23	1.9	**28**	17.5	0.045	389
7	35.3	3.8	9.3	**29**	6.99	0.0054	1190
8	25.8	3.8	6.8	**30**	49.0	45.6	1.1
9	20.7	0.0039	5308	**31**	5.47	1.07	5.1
10	14.2	0.000 66	21 515	**32**	0.52	1.07	5.1
11	18.4	0.0053	3476	**33**	20.6	0.57	36.6
12	20.5	0.077	267	**34**	15.0	4.81	3.1
13	–[c]	–	–	**35**	29.2	10.0	2.9
14	–	–	–	**36**	12.8	4.3	3.0
15	28.8	4.7	6.2	**37**	31.9	11.9	2.7
16	26.3	1.6	17	**38**	12.3	3.62	3.4
17	19.2	0.059	325	**39**	28.3	5.4	5.2
18	10.6	0.0047	2253	**40**	36.2	8.5	4.3
19	18.7	0.075	248	**41**	33.6	>13.8	2.4
20	21.6	0.58	37	**42**	15.4	4.57	3.4
21	–	–	–	**43**	–	–	–
22	28.2	3.6	7.8	**44**	>133.9	2.98	>44.8
AZT	500	0.015	33 333				

[a] See Anti-HIV Assay subsection in the Experimental Section for experimental procedures. [b] All data represented are an average of at least two experiments. [c] (–) Not tested due to limited solubility in DMSO.

the 3′ position are required for maximal activity. Compound **10** was most potent (EC_{50} = 0.000 66 μM) and had a remarkable TI (21 515).

Monoacyl betulins **22** and **23**, which contain substituted glutaryl groups only at the C-28 position, had EC_{50} values in the range of 2.1−3.6 μM and were 6- to 11-fold more active than betulin (**6**). Therefore, the C-28 acyl group could increase the anti-HIV activity. Triacylated compounds **28** and **29** displayed potent anti-HIV activity with EC_{50} values of 0.045 and 0.0054 μM, respectively. The tetramethylene derivative **29** was 8- and 14-fold more potent than the 3′,3′-dimethylglutaryl **28** and the corresponding diacylated **12**. In contrast, the triacylated 3′,3′-dimethylglutaryl derivative **28** was 68-fold less active than diacylated **10**.

Bioisosteric replacement is a valuable approach in drug design and can produce compounds with similar biological activity. In the current study, bioisosteric replacement in **10** and **12** resulted in amide derivatives **33** and **34**, respectively. The dimethylglutaryl analogue **39** was more active than the tetramethylene analogue **34**, consistent with the results of the diesters (**10** and **12**). However, the amide compounds did not show stronger inhibition than the esters, and due to similar cellular toxicity of the amides and esters, the TI values of amides **33** and **34** dropped significantly. These results demonstrated that the amide linkage had a detrimental effect on anti-HIV activity.

A 6-fold increased activity was observed with 3-keto betulinic acid,[16] but addition of a 3-ketone to betulin derivatives did not show the expected results. Compounds **35** and **36** were slightly less effective as HIV inhibitors than their corresponding nonketone derivatives **22** and **23**. Applying the Mitsunobu reaction to **6** produced 2,3-dihydro **37**, which had 2-fold enhanced activity. Monoacyl dihydro compounds **39** and **40** were slightly more potent than unacylated **37**, but these results along with those of the 3-hydroxy and 3-keto monoester analogues showed that the C-3 acyl chain was crucial for HIV inhibition.

3-Epi-betulin (**38**) and oxime-betulin (**31**) had EC_{50} values of 3.62 and 1.07 μM, respectively. Side chains were introduced to potentially improve the activity and TI. However, the diacyl compounds (**41** and **42**) displayed lower inhibition with EC_{50} values of >13.8 and 4.57 μM, respectively. With an adamantyl group incorporated at the C-28 position, the EC_{50} value of **44** dropped to 2.98 μM. Compared with the extremely potent **10**, these data suggested that the linkage and orientation of the C-3 acyl side chain as well as the C-28 acyl group play an important role in the potent anti-HIV activity.

The above results showed that acylation only at the C-28 position did not result in significant anti-HIV activity. However, compounds with acyl side chains at both C-3 and C-28 positions reached optimal activity. A third chain at the C-30 position gave better inhibition with a tetramethyleneglutaryl derivative, but the potency dropped in the dimethylglutaryl compound. Activity was affected by the type of side chain linkage; ester linkage and 3β-configuration resulted in the most impressive EC_{50} as well as TI values. Incorporating a non-carboxylic and bulky group at the C-28 position gave a less active compound.

In our studies, **10** with 3′,3′-dimethylglutaryl groups demonstrated the strongest activity and highest TI. Betulin derivative **10** was ca. 3-fold more potent and had a 10-fold higher TI than the corresponding betulinic acid derivative **3**. Compared with **2**, **10** was 2-fold less potent but had an equivalent TI and the merit of lower cost and easier preparation. This compound demonstrates another successful discovery of a potent anti-HIV agent through suitable lead modification.

In the mechanism(s) of action studies, **10** was examined for its ability to block HIV-1 entry into cells, since another triterpene derivative, RPR 103611, has been identified as a fusion inhibitor.[17,18] At a concentration as high as 34 μM, **10** did not significantly affect syncytia formation in a fusion assay (Table 2). The effects of higher concentrations of **10** were not evaluated due to

Table 2. Effects of **10** in Anti-HIV H9 Cell, Fusion, and MAGI Assays

compd	H9 Cells[a] EC_{50} (μM)	fusion assay[b] EC_{100} (μM)	MAGI assay[c] EC_{50} (μM)
10	0.00066	>34[d]	0.36
AZT	0.015	–[e]	0.037
DP178	–	0.001	–

[a] See Anti-HIV Assay subsection in the Experimental Section for experimental procedures. [b] See Fusion Assay subsection in the Experimental Section for experimental procedures. [c] See MAGI Assay subsection in the Experimental Section for experimental procedures. DP178 is a fusion inhibitor and used as a control. [d] At a concentration of 34 μM, compound **10** inhibited HIV-induced syncytia by 15%. [e] (–) Not tested.

the appearance of cytotoxicity. In the same assay, a known antifusion agent, DP178,[29] completely blocked syncytia formation at 0.001 μM. These results suggested that **10** did not block HIV-1 entry. In an effort to identify the drug-sensitive phase of the HIV-1 life cycle, **10** was then evaluated in the MAGI assay (multinuclear activation of a galactosidase indicator assay).[31] This assay can detect HIV-1 infection (appearance of blue cells) if the virus completes a major portion of its life cycle, which includes viral entry, reverse transcription, integration, transcription, and protein synthesis. Synthesis of the HIV-1 transactivation protein, tat, is the end point of the MAGI assay. Therefore, anti-HIV agents that inhibit the virus replication at a stage earlier than viral protein synthesis will block the formation of blue cells. For example, the HIV reverse transcriptase inhibitor AZT had an EC_{50} of 0.036 μM in the MAGI assay. This inhibitory concentration is comparable to that (0.015 μM) obtained in a H9 cell-based anti-HIV-1 assay (Table 2). On the other hand, the EC_{50} of **10** in the MAGI assay was 0.36 μM, which is much higher than the EC_{50} (0.000 66 μM) determined in the H9 cell-based anti-HIV assay. These results suggest that the primary site(s) of action of **10** is not at the early stages of the virus life cycle.

Experimental Section

The melting points were measured with a Fisher-Johns melting point apparatus and are uncorrected. The proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectra were obtained using a Bruker AC-300 NMR spectrometer. All chemical shifts are reported in ppm from the internal standard Me_4Si (TMS). Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Optical rotations were measured with a Jasco DIP-1000 polarimeter. Thin-layer chromatography (TLC) was carried out on Merck precoated silica gel 60 F-254 plates. EM Kieselgel 60 (230–400 mesh ASTM) was used for column chromatography. All new target compounds were characterized by optical rotation, ^{1}H NMR, and elemental analyses.

General Procedure for Synthesizing Diacyl Derivatives (7–13 and 15–21). A solution of betulin or dihydrobetuin (0.5 mmol), 4-(dimethylamino)pyridine (1 equiv mol), and an appropriate anhydride (6 equiv mol) in anhydrous pyridine (3–5 mL) was heated overnight at 95 °C until the starting material was not observed by TLC. The reaction time ranged from 2 to 16 h. The reaction mixture was diluted with 20 mL of EtOAc and washed three times with 50 mL of 20% HCl solution. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed using a silica gel column to afford the product.

3,28-Di-*O*-succinyl-betulin (7): yield 56% (after chromatography with *n*-hexane/EtOAc [3.5:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +19.7 (c = 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84, 0.85, 0.86, 0.99, 1.04 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.70 (3H, s; 20-CH_3), 2.39–2.47 (1H, m; H-19), 2.59–2.68 (8H, m; H_2-2′, 2″, 3′, 3″), 3.89, 4.32 (1H each, both d, J = 11.0 Hz; H_2-28), 4.50 (1H, dd, J = 6.8, 9.2 Hz; H-3), 4.60, 4.69 (1H each, both br s; H_2-29). Anal. ($C_{38}H_{58}O_8$·H_2O) C, H.

3,28-Di-*O*-glutaryl-betulin (8): yield 69% (after chromatography with *n*-hexane/EtOAc [3:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +19.8 (c = 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (9H), 0.95, 1.01 (3H each, except 0.82, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.66 (3H, s; 20-CH_3), 2.35–2.42 (9H, m; H_2-2′, 2″, 4′, 4″, H-19), 3.84, 4.27 (1H each, both d, J = 11.0 Hz; H_2-28), 4.46 (1H, dd, J = 4.9, 10.7 Hz; H-3), 4.57, 4.67 (1H each, both br s; H_2-29). Anal. ($C_{40}H_{62}O_8$) C, H.

3,28-Di-*O*-(*RS*-3′-methylglutaryl)-betulin (9): yield 83% (after chromatography with $CHCl_3$/acetone [9:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +16.9 (c = 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81 (9H), 0.94, 1.00 (3H each, except 0.81, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.02 (6H, d, J = 5.8 Hz; 3′-CH_3 and 3″-CH_3), 1.65 (3H, s; 20-CH_3), 2.20–2.44 (11H, m; H_2-2′, 2″, 3′, 3″, 4′, 4″, H-19), 3.82, 4.25 (1H each, both d, J = 11.0 Hz; H_2-28), 4.45 (1H, dd, J = 4.9, 10.7 Hz; H-3), 4.55, 4.65 (1H each, both br s; H_2-29). Anal. ($C_{42}H_{66}O_8$) C, H.

3,28-Di-*O*-(3′,3′-dimethylglutaryl)-betulin (10): yield 75% (after chromatography with $CHCl_3$/acetone [19:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +21.9 (c = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84, 0.85, 0.86, 0.97, 1.03 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.14 (12H, s; 3′-$(CH_3)_2$, 3″-$(CH_3)_2$), 1.68 (3H, s; 20-CH_3), 2.42–2.50 (9H, m; H_2-2′, 2″, 4′, 4″, H-19), 3.86, 4.30 (1H each, both d, J = 11.1 Hz; H_2-28), 4.49 (1H, dd, J = 5.2, 11.4 Hz; H-3), 4.59, 4.69 (1H each, both br s; H_2-29). Anal. ($C_{44}H_{70}O_8$·$^1/_2H_2O$) C, H.

3,28-Di-*O*-(*RS*-3′,3′-methylethylglutaryl)-betulin (11): yield 94% (after chromatography with *n*-hexane/EtOAc [6:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +13.2 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85, 0.86, 0.91, 0.98, 1.04 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.09 (6H, s; 3′-CH_3 and 3″-CH_3), 1.69 (3H, s; 20-CH_3), 2.41–2.57 (9H, m; H_2-2′, 2″, 4′, 4″, H-19), 3.87, 4.30 (1H each, both d, J = 11.0 Hz; H_2-28), 4.52 (1H, dd, J = 4.6, 11.0 Hz; H-3), 4.60, 4.70 (1H each, both br s; H_2-29). Anal. ($C_{46}H_{74}O_8$·$^1/_2H_2O$) C, H.

3,28-Di-*O*-(3′,3′-tetramethyleneglutaryl)-betulin (12): yield 86% (after chromatography with *n*-hexane/EtOAc [8:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +13.9 (c = 0.99, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85, 0.86 (6H), 0.98, 1.04 (3H each, except 0.86, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.69 (3H, s; 20-CH_3), 2.45 (1H, dt, J = 5.8, 10.6 Hz; H-19), 2.52–2.59 (8H, m; H_2-2′, 2″, 4′, 4″), 3.88, 4.29 (1H each, both d, J = 11.1 Hz; H_2-28), 4.51 (1H, dd, J = 5.0, 10.8 Hz; H-3), 4.60, 4.70 (1H each, both br s; H_2-29). Anal. ($C_{48}H_{74}O_8$·H_2O) C, H.

3,28-Di-*O*-(–)-camphanoyl-betulin (13): yield 91% (preparative TLC with *n*-hexane/EtOAc [4:1]), a white powder; mp 144–147 °C; $[\alpha]^{25}_D$ +12.7 (c = 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86, 0.89 (6H), 0.97 (6H), 0.99, 1.04, 1.07 (6H), 1.11, 1.12 (3H each, except 0.89, 0.97, 1.07, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3, 6 × CH_3 from (*S*)-camphanoyl), 1.69 (3H, s; 20-CH_3), 2.37–2.49 (3H, m; H-19, 2 × CH from (*S*)-camphanoyl), 4.01, 4.42 (1H each, both d, J = 11.0 Hz; H_2-28), 4.63 (1H, t, J = 8.1 Hz; H-3), 4.60, 4.70 (1H each, both br s; H_2-29). Anal. ($C_{50}H_{74}O_8$) C, H.

Dihydrobetulin (14): yield 94%, a colorless powder mp 248–250 °C; $[\alpha]_D$ –11.6 (c = 0.50, $CDCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76, 0.77 (3H each, both d, J = 3.4 Hz; 20-$(CH_3)_2$), 0.83, 0.85, 0.96, 0.97, 1.03 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 3.20 (1H, dd, J = 5.3, 11.0 Hz; H-3), 3.30, 3.78 (1H each, both d, J = 11.0 Hz; H_2-28). Anal. Calcd for ($C_{30}H_{52}O_2$) C, H.

3,28-Di-*O*-succinyl-dihydrobetulin (15): yield 43% (after chromatography with $CHCl_3$/acetone [12:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ –9.2 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.75, 0.82 (3H each, both d, J = 6.6 Hz; 20-$(CH_3)_2$), 0.84, 0.85, 0.86, 0.99, 1.04 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 2.59–2.68 (8H, m; H_2-2′, 2″, 3′, 3″), 3.89, 4.32 (1H each, both d, J = 11.0 Hz; H_2-28), 4.50 (1H, dd, J = 6.8,

9.2 Hz; H-3), 4.60, 4.69 (1H each, both br s; H_2-29). Anal. ($C_{38}H_{62}O_8 \cdot {}^1/_2H_2O$) C, H.

3,28-Di-*O*-glutaryl-dihydrobetulin (16): yield 68% (after chromatography with *n*-hexane/EtOAc [3:1]), an amorphous powder; $[\alpha]^{25}_D$ −10.0 (*c* = 0.68, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.75, 0.82 (3H each, both d, *J* = 6.6 Hz; 20-$(CH_3)_2$), 0.82 (6H), 0.84, 0.93, 1.02 (3H each, except 0.82, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.90−2.00 (4H, m; H_2-3′, 3″), 2.35−2.44 (8H, m; H_2-2′, 2″, 4′, 4″), 3.81, 4.26 (1H each, both d, *J* = 11.1 Hz; H_2-28), 4.47 (1H, dd, *J* = 5.3, 10.4 Hz; H-3). Anal. ($C_{40}H_{64}O_8 \cdot {}^1/_2H_2O$) C, H.

3,28-Di-*O*-(*RS*-3′-methylglutaryl)-dihydrobetulin (17): yield 72% (after chromatography with *n*-hexane/acetone [8:1]), an amorphous powder; $[\alpha]^{25}_D$ −6.3 (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.75, 0.82 (3H each, both d, *J* = 6.6 Hz; 20-$(CH_3)_2$), 0.83 (6H), 0.84, 0.93, 1.03 (3H each, except 0.83, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.04 (6H, d, *J* = 7.0 Hz; 3′-CH_3, 3″-CH_3), 2.22−2.49 (10H, m; H_2-2′, 2″, 4′, 4″, H-3′, H-3″), 3.80, 4.27 (1H each, both d, *J* = 11.0 Hz; H_2-28), 4.48 (1H, dd, *J* = 5.3, 10.4 Hz; H-3). Anal. ($C_{42}H_{68}O_8$) C, H.

3,28-Di-*O*-(3′,3′-dimethylglutaryl)-dihydrobetulin (18): yield 81% (after chromatography with $CHCl_3$/acetone [19:1]), an amorphous powder; $[\alpha]^{25}_D$ −15.0 (*c* = 0.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77, 0.84 (3H each, both d, *J* = 6.7 Hz; 20-$(CH_3)_2$), 0.85, 0.86 (6H), 0.95, 1.04 (3H each, except 0.86, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.14 (12H, s; 3′-$(CH_3)_2$, 3″-$(CH_3)_2$), 2.43−2.54 (8H, m; H_2-2′, 2″, 4′, 4″), 3.83, 4.29 (1H each, both d, *J* = 11.0 Hz; H_2-28), 4.52 (1H, dd, *J* = 4.8, 11.0 Hz; H-3). Anal. ($C_{44}H_{72}O_8$) C, H.

3,28-Di-*O*-(*RS*-3′,3′-methylethylglutaryl)-dihydrobetulin (19): yield 84% (after chromatography with *n*-hexane/EtOAc [6:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ −17.6 (*c* = 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.78, 0.85 (3H each, both d, *J* = 6.6 Hz; 20-$(CH_3)_2$), 0.86 (6H), 0.87, 0.91, 1.05 (3H each, except 0.86, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.09 (6H, s; 3′-CH_3, 3″-CH_3), 2.38−2.56 (8H, m; H_2-2′, 2″, 4′, 4″), 3.86, 4.30 (1H each, both d, *J* = 11.0 Hz; H_2-28), 4.52 (1H, dd, *J* = 4.6, 11.0 Hz; H-3). Anal. ($C_{46}H_{76}O_8$) C, H.

3,28-Di-*O*-(3′,3′-tetramethyleneglutaryl)-dihydrobetulin (20): yield 89% (after chromatography with *n*-hexane/EtOAc [8:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ −18.2 (*c* = 0.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.78, 0.85 (3H each, both d, *J* = 6.6 Hz; 20-$(CH_3)_2$), 0.85, 0.87 (6H), 0.96, 1.05 (3H each, except 0.87, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 2.52−2.63 (8H, m; H_2-2′, 2″, 4′, 4″), 3.84, 4.28 (1H each, both d, *J* = 11.1 Hz; H_2-28), 4.51 (1H, dd, *J* = 5.4, 10.3 Hz; H-3). Anal. ($C_{48}H_{76}O_8 \cdot {}^3/_2H_2O$) C, H.

3,28-Di-*O*-(−)-camphanoyl-dihydrobetulin (21): yield 95% (by preparative TLC with *n*-hexane/EtOAc (4:1)), a white powder; mp 153−155 °C; $[\alpha]^{25}_D$ −9.4 (*c* = 0.51, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.78, 0.86 (3H each, both d, *J* = 6.7 Hz; 20-$(CH_3)_2$), 0.88, 0.90 (6H), 0.98 (6H), 0.99, 1.06, 1.07, 1.08, 1.13 (6H) (3H each, except 0.90, 0.98, 1.13, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3, 6 × CH_3 from (*S*)-camphanoyl), 2.38−2.49 (2H, m; 2 × CH from (*S*)-camphanoyl), 3.99, 4.42 (1H each, both d, *J* = 11.0 Hz; H_2-28), 4.65 (1H, t, *J* = 7.9 Hz; H-3). Anal. ($C_{50}H_{76}O_8$) C, H.

General Procedure for Synthesizing Betulin Derivatives (22 and 23, 39 and 40). A solution of starting material (0.5 mmol), 4-(dimethylamino)pyridine (1 equiv mol), and an appropriate anhydride (2 equiv mol) in anhydrous pyridine (3−5 mL) was heated at 40 °C overnight until the starting material disappeared on the TLC. The reaction mixture was diluted with 20 mL of EtOAc and washed with 40 mL of 20% HCl solution three times. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed using a silica gel column to afford the product.

28-*O*-(3′,3′-Dimethylglutaryl)-betulin (22): yield 71% (after chromatography with *n*-hexane/acetone [4:1]), recovered betulin 15%, an off-white amorphous powder; $[\alpha]^{25}_D$ +12.3 (*c* = 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77, 0.83, 0.98 (6H), 1.04 (3H each, except 0.98, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.15 (6H, s; 3′-$(CH_3)_2$), 1.69 (3H, s; 20-CH_3), 2.40−2.48 (5H, m; H-19, H_2-2′, H_2-4′), 3.20 (1H, dd, *J* = 5.2, 10.9 Hz; H-3), 3.87, 4.29 (1H each, both d, *J* = 11.1 Hz; H_2-28), 4.60, 4.70 (1H each, both s; H_2-29). Anal. ($C_{37}H_{60}O_5 \cdot {}^1/_2H_2O$) C, H.

28-*O*-(3′,3′-Tetramethyleneglutaryl)-betulin (23): yield 68% (after chromatography with *n*-hexane/acetone [6:1]), recovered betulin 20%, an off-white amorphous powder; $[\alpha]^{25}_D$ +13.4 (*c* = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77, 0.83, 0.98 (6H), 1.04 (3H each, except 0.98, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.69 (3H, s; 20-CH_3), 2.45 (1H, dt, *J* = 5.6, 10.7 Hz; H-19), 2.58 (4H, s; H_2-2′, H_2-4′), 3.20 (1H, dd, *J* = 5.1, 10.8 Hz; H-3), 3.88, 4.30 (1H each, both d, *J* = 11.0 Hz; H_2-28), 4.60, 4.70 (1H each, both s; H_2-29). Anal. ($C_{39}H_{62}O_5 \cdot {}^1/_2H_2O$) C, H.

3-Deoxy-2,3-dihydro-28-*O*-(3′,3′-dimethylglutaryl)-betulin (39): yield 53% (after chromatography with *n*-hexane/acetone [10:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +26.4 (*c* = 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.84, 0.85, 0.92, 0.97, 1.04 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.13 (6H, s, 3′-$(CH_3)_2$), 1.67 (3H, s, 20-CH_3), 2.38−2.48 (1H, m; H-19), 2.45, 2.45 (each 2H, both s; H_2-2′, H_2-4′), 3.86, 4.28 (1H each, both d, *J* = 11.1 Hz; H_2-28), 4.58, 4.67 (1H each, both br s; H_2-29), 5.34−5.37 (2H, m; H-2, H-3). Anal. ($C_{37}H_{60}O_4$) C, H.

3-Deoxy-2,3-dihydro-28-*O*-(3′,3′-tetramethyleneglutaryl)-betulin (40): yield 77% (after chromatography with *n*-hexane/acetone [22:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +25.7 (*c* = 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85, 0.86, 0.93, 0.97, 1.05 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.67 (3H, s, 20-CH_3), 2.43 (1H, dt, *J* = 5.2, 11.0 Hz; H-19), 2.55 (4H, s; H_2-2′, H_2-4′), 3.88, 4.29 (1H each, both d, *J* = 11.1 Hz; H_2-28), 4.58, 4.68 (1H each, both br s; H_2-29), 5.32−5.42 (2H, m; H-2, H-3). Anal. ($C_{39}H_{62}O_4$) C, H.

3,28-Di-*O*-acetyl-betulin (24). A solution of betulin (1.926 g, 4.35 mmol) in anhydrous pyridine (15 mL) was treated with anhydrous Ac_2O (3.5 mL) and stirred for 6 h. The reaction mixture was diluted with EtOAc (150 mL), and washed with 10% HCl (3 × 150 mL) and saturated $NaHCO_3$ (100 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **24** (2.154 g, 94% yield) as a colorless powder: mp 223−224 °C; $[\alpha]_D$ +23.0 (*c* = 0.46, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81, 0.82 (6H), 0.95, 1.01 (3H each, except 0.82, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.66 (3H, s; 20-CH_3), 2.02, 2.05 (3H each, both s; $OCOCH_3$), 2.42 (1H, dt, *J* = 5.8, 10.8 Hz; H-19), 3.83, 4.23 (1H each, both br d, *J* = 11.1 Hz; H_2-28), 4.45 (1H, dd, *J* = 6.1, 10.0 Hz; H-3), 4.57, 4.66 (1H each, both br s; H_2-29); EI-MS *m*/*z* 526 M^+. Anal. ($C_{34}H_{54}O_4$) C, H.

3,28-Di-*O*-acetyl-30-bromo-betulin (25). A mixture of acetyl ester **24** (2.15 g, 4.08 mmol) and NBS (1.441 g, 8.16 mmol) in CCl_4 (82 mL) was stirred for 24 h at room temperature. The precipitate was filtered, and the filtrate was concentrated and chromatographed over silica gel [hexane/CH_2Cl_2 (4:1 → 3:1)]. The product was recrystallized from hexane to yield a colorless powder, **25** (1.799 g, 73% yield): mp 185 °C (dec); $[\alpha]_D$ +7.4 (*c* = 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81, 0.82 (6H), 0.95, 1.01 (3H each, except 0.82, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 2.01, 2.05 (3H each, both s; $OCOCH_3$), 2.42 (1H, dt, *J* = 5.3, 10.8 Hz; H-19), 3.82, 4.24 (1H each, both br d, *J* = 11.1 Hz; H_2-28), 3.95 (2H, s; H_2-30), 4.44 (1H, dd, *J* = 5.5, 10.5 Hz; H-3), 5.00, 5.11 (1H each, both br s; H_2-29); EI-MS *m*/*z* 605 $[M(^{81}Br)]^+$, 603 $[M(^{79}Br)]^+$, 524 $[M\text{-}Br]^+$. Anal. ($C_{34}H_{53}BrO_4$) C, H.

3,28-Di-*O*-acetyl-30-acetoxybetulin (26). A mixture of bromide **25** (1.696 g, 2.8 mmol), silver acetate (0.935 g, 5.6 mmol), and tetrabutylammonium bromide (180 mg) in anhydrous toluene (20 mL) was heated at 70 °C for 12 h. After the insoluble material was filtered, the solution was concentrated and chromatographed over silica gel [hexane/CH_2Cl_2 (3:2)] to give **26** (1.146 g, 70% yield) as off-white amorphous crystals: $[\alpha]_D$ +7.1 (*c* = 0.31, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81, 0.82 (6H), 0.95, 1.01 (3H each, except 0.82, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 2.02, 2.05, 2.08 (3H each, all s; $OCOCH_3$), 2.35 (1H, dt, *J* = 5.2, 11.0 Hz; H-19), 3.81, 4.22 (1H each, both br d, *J* = 11.1 Hz; H_2-28), 4.44 (1H, dd, *J* = 5.7, 10.5 Hz; H-3),

4.53 (2H, d, J = 2.6 Hz; H_2-30), 4.93 (2H, d, J = 2.7 Hz; H_2-29); ^{13}C NMR ($CDCl_3$) 62.4 (C-28), 65.9 (C-30), 80.8 (C-3), 110.7 (C-29), 148.7 (C-20); EI-MS m/z 584 M^+, 524 $[M\text{-}AcOH]^+$. Anal. ($C_{36}H_{56}O_6 \cdot {}^1/_4H_2O$) C, H.

30-Hydroxy-betulin (27). A solution of **26** (1.023 g, 1.75 mmol) in a mixture of CH_3OH (5 mL), THF (7.5 mL), and 4 N NaOH (2.5 mL) was stirred at room temperature for 24 h. The mixture was acidified with 20% HCl until slightly acidic and concentrated under reduced pressure. The precipitate was filtered, washed with water, and dried. The crude alcohol was chromatographed over silica gel [CH_2Cl_2/THF (14:1)] to give alcohol **27** (0.698 g, 87% yield) as colorless needles: mp 232−234 °C; $[\alpha]_D$ −18.7 (c = 0.45, MeOH/pyridine (1:1)); ^{1}H NMR ($CDCl_3$) δ 0.74, 0.80, 0.95, 0.96, 1.00 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 2.27 (1H, dt, J = 5.4, 10.8 Hz; H-19), 3.16 (1H, dd, J = 5.1, 10.9 Hz; H-3), 3.29, 3.76 (1H each, both br d, J = 10.8 Hz; H_2-28), 4.09 (2H, br s; H_2-30), 4.88, 4.93 (1H each, both br s; H_2-29). Anal. ($C_{30}H_{50}O_3 \cdot {}^1/_4H_2O$) C, H.

3,28-Dideoxy-3,28-dioxo-betulin (30). To a solution of betulin (1.328 g, 3 mmol) in CH_2Cl_2 (13 mL) was added PCC (1.940 g, 9 mmol) at room temperature. After stirring for 1.5 h, the reaction mixture was diluted with Et_2O and filtered through a short pack of Florisil. The residue was washed several times with Et_2O, until it became granular. The filtrate was concentrated in a vacuum and purified by silica gel chromatography [hexane/acetone (9:1)] to give aldehyde **30** (1.138 g, 87% yield) as a colorless gum: yield 87%; $[\alpha]^{25}_D$ +33.8 (c = 0.45, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.90, 0.93, 0.96, 1.00, 1.04 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.68 (3H, s; 20-CH_3), 2.32−2.53 (2H, m; H-2, H-19), 2.85 (1H, dt, J = 5.5, 11.1 Hz; H-2), 4.61, 4.74 (1H each, both br s; H_2-29), 9.65 (1H, s; H-28); EI-MS m/z 438 M^+, 409 $[M\text{-}CHO]^+$. Anal. ($C_{30}H_{46}O_2 \cdot {}^1/_2H_2O$) C, H.

3,28-Dioxime-betulin (31). A solution of keto aldehyde **30** (1.138 g, 2.59 mmol) and hydroxylamine hydrochloride (1.26 g, 18.13 mmol) in pyridine (10 mL) was heated for 2 h at 50 °C. After cooling to room temperature, the reaction mixture was diluted with CH_2Cl_2 (150 mL) and washed with 10% HCl (3 × 100 mL). The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The product was crystallized from diethyl ether and CH_2Cl_2 to yield a colorless powder (941 mg, 2 mmol): yield 78%; $[\alpha]^{25}_D$ +20.0 (c = 0.36, pyridine); ^{1}H NMR ($CDCl_3$ and C_5D_5N) δ 0.90, 0.94, 0.99, 1.02, 1.11 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.67 (3H, s; 20-CH_3), 2.17−2.28 (1H, m; H-2), 2.49 (1H, dt, J = 5.0, 11.2 Hz; H-19), 2.85 (1H, dt, J = 5.4, 14.3 Hz; H-2), 4.58, 4.70 (1H each, both br s; H_2-29), 7.53 (1H, s; H-28); EI-MS m/z 468 M^+, 451 $[M\text{-}OH]^+$. Anal. ($C_{30}H_{48}N_2O_2$) C, H, N.

3β,28-Diamino-betulin (32). To a solution of dioxime **31** (890 mg, 1.9 mmol) and ammonium acetate (1.47 g, 27.2 mmol) in MeOH (150 mL) was added sodium cyanoborohydride (1.7 g, 36 mmol) under N_2 atmosphere. The reaction was cooled to 0−5 °C, and 15% aqueous titanium trichloride (5.85 mL, 5.7 mmol) was added dropwise over 45 min. The mixture was stirred at room temperature for 12 h and then was treated with 2 N sodium hydroxide until pH = 10. The aqueous solution was extracted with CH_2Cl_2 (500 mL × 2) and the organic layer was washed to pH = 7 with distilled water and dried over anhydrous $MgSO_4$. After concentrating to dryness, the crude was subjected to Sephadex G-15 chromatography with an eluent of EtOH to give 586 mg (1.33 mmol) of an off-white amorphous powder: yield 70%; $[\alpha]^{25}_D$ +12.4 (c = 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.79, 0.90, 0.95, 1.00, 1.03 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.66 (3H, s; 20-CH_3), 2.45 (1H, dt, J = 5.3, 11.0 Hz; H-19), 3.57 (1H, br, s; H-3), 3.96, 4.03 (1H each, both d, J = 11.0 Hz; H_2-28), 4.56, 4.67 (1H each, both br s; H_2-29); EI-MS m/z 452 M^+.

General Procedure for Synthesizing Betulin Derivatives (35−36). To a solution of alcohol **22** and **23** (0.5 mmol) in CH_2Cl_2 (3−5 mL) was added PCC (1.5 equiv) at room temperature, and the mixture then became black. After being stirred for 2 h, the reaction mixture containing insoluble solid was diluted with Et_2O (15 mL) and was decanted. The black solid was washed twice with ether and became granular. The organic extracts were combined, filtered through a short pack of Florisil, and concentrated under reduced pressure. The residue was chromatographed using a silica gel column to give the product.

3-Deoxy-3-oxo-28-*O*-(3′,3′-dimethylglutaryl)-betulin (35): yield 72% (after chromatography with *n*-hexane/acetone [4:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +32.4 (c = 0.33, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.94, 1.00, 1.04, 1.08 (6H) (3H each, except 1.08, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.16 (6H, s; 3′-$(CH_3)_2$), 1.69 (3H, s; 20-CH_3), 2.41−2.54 (7H, m; H_2-2, 2′, 4′, H-19), 3.87, 4.30 (1H each, both d, J = 11.0 Hz; H_2-28), 4.61, 4.70 (1H each, both s; H_2-29). Anal. ($C_{37}H_{58}O_5$) C, H.

3-Deoxy-3-oxo-28-*O*-(3′,3′-tetramethyleneglutaryl)-betulin (36): yield 71% (after chromatography with *n*-hexane/acetone [7:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +28.4 (c = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.94, 0.99, 1.04, 1.08 (6H) (3H each, except 1.08, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.69 (3H, s; 20-CH_3), 2.35−2.54 (3H, m; H_2-2, H-19), 2.58 (4H, s; H_2-2′, H_2-4′), 3.88, 4.30 (1H each, both d, J = 11.1 Hz; H_2-28), 4.60, 4.70 (1H each, both s; H_2-29). Anal. ($C_{39}H_{60}O_5$) C, H.

3-Deoxy-2,3-dihydro-betulin (37). To a solution of betulin (443 mg, 1 mmol), triphenylphosphine (1.05 g, 4 mmol), and 3,3-dimethylglutarimide (569 mg, 4 mmol) in dry THF (10 mL) was added dropwise diethylazodicarboxylate (0.63 mL, 4 mmol) (ice bath and N_2 atmosphere). After being stirred for 12 h in room temperature, the mixture was concentrated and subjected to silica gel chromatography with an eluent of *n*-hexane/EtOAc [15:1] to obtain the pure compound: yield 74% (316 mg); $[\alpha]^{25}_D$ +46.5 (c = 0.2, $CHCl_3$); ^{1}H NMR ($CHCl_3$) δ 0.86, 0.87, 0.94, 0.99, 1.05 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.69 (3H, s; 20-CH_3), 2.40 (1H, dt, J = 5.7, 10.6 Hz; H-19), 3.34, 3.82 (1H each, both dd, J = 4.0, 10.7 Hz; H_2-28), 4.58, 4.69 (1H each, both d, J = 1.5 Hz; H_2-29), 5.33−5.43 (2H, m; H-2, H-3). Anal. ($C_{30}H_{48}O \cdot {}^1/_4H_2O$) C, H.

3-Epi-betulin (38). To a cooled (−20 °C) solution of aldehyde **30** (330 mg, 0.75 mmol) in anhydrous THF (10 mL) was added L-selectride (1 M; 5.625 mL), and the solution was stirred for 1 h 45 min. Sodium hydroxide (2 M, 18.75 mL) and hydrogen peroxide (3.75 mL, 30% v/v) were then added, and the mixture was stirred for 1 h. The mixture was evaporated to a low volume and extracted with EtOAc. The organic phase was washed with water, dried over anhydrous Na_2SO_4, concentrated, and chromatographed on silica gel eluting with *n*-hexane/acetone (9:1) to give 73% yield of **38** (244 mg, 0.55 mmol) as an off-white amorphous powder: $[\alpha]^{25}_D$ +27.9 (c = 0.47, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82, 0.83, 0.93, 0.99, 1.02 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.68 (3H, s; 20-CH_3), 2.39 (1H, dt, J = 5.5, 10.7 Hz; H-19), 3.33, 3.80 (1H each, both d, J = 10.9 Hz; H_2-28), 3.39 (1H, t, J = 2.6 Hz; H-3), 4.58, 4.68 (1H each, both d, J = 1.9 Hz; H_2-29). Anal. ($C_{30}H_{50}O_2$) C, H. Further elution gave 70 mg (21%) of 3β-hydroxy compound identical to betulin.

General Procedure for Synthesizing Betulin Derivatives (28 and 29, 33 and 34, 41−44). A solution of starting material (0.5 mmol), (dimethylamino)pyridine (1 equiv mol), and an appropriate anhydride (8−10 equiv mol) or 1-adamantanecarbonyl chloride (2 equiv mol) in anhydrous pyridine (3−5 mL) was heated at 95 °C overnight until the starting material disappeared on the TLC. The reaction time ranged from 2 to 16 h. The reaction mixture was diluted with 20 mL of EtOAc and washed with 50 mL of 20% HCl solution three times. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was chromatographed using silica gel column to afford the product.

3,28,30-Tri-*O*-(3′,3′-dimethylglutaryl)-betulin (28): yield 68% (after chromatography with $CHCl_3$/acetone [10:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ −11.3 (c = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81, 0.82, 0.82, 0.94, 1.00 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.12, 1.13 (18H, both s; CH_3 × 2-3′,3″,3‴), 2.34−2.54 (13H, m; H_2-2′,2″,2‴, H_2-4′,4″,4‴, H-19), 3.77, 4.22 (1H each, both br d, J = 10.8 Hz; H_2-28),

4.45 (1H, dd, J = 5.1, 10.9 Hz; H-3), 4.53 (2H, br s; H_2-30), 4.93 (2H, br s; H_2-29). Anal. ($C_{51}H_{80}O_{12}$) C, H.

3,28,30-Tri-*O*-(3′,3′-tetramethyleneglutaryl)-betulin (29): yield 73% (after chromatography with $CHCl_3$/acetone [12:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ −14.7 (c = 0.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.81, 0.83, 0.83, 0.95, 1.00 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.65−1.66 (24H, m; CH_2 × 2 −3′,3″,3‴), 2.42−2.58 (13H, m; H_2-2′,2″,2‴, H_2-4′,4″,4‴, and H-19), 3.64, 4.23 (1H each, both br d, J = 11.0 Hz; H_2-28), 4.43, 4.64 (1H each, both d, J = 12.1 Hz; H_2-30), 4.48 (1H, dd, J = 5.1, 10.9 Hz; H-3), 4.96 (2H, br s; H_2-29). Anal. ($C_{30}H_{50}O_3$·$^1/_2H_2O$) C, H.

3,28-Di-*N*-(3′,3′-dimethylglutaryl)-betulin (33): yield 76% (after chromatography with $CHCl_3$/acetone [15:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +14.5 (c = 0.33, $CHCl_3$); ^{1}H NMR (C_5D_5N) δ 0.73, 0.85, 0.99, 1.05, 1.08 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.34, 1.37 (6H each, both s; 3′-$(CH_3)_2$, 3″-$(CH_3)_2$), 1.73 (3H, s; 20-CH_3), 2.21−2.26 (1H, m; H-19), 2.54−2.86 (8H, m, H_2-2′, 2″, 4′, 4″), 3.34, 3.78 (1H each, both dd, J = 5.8, 13.3 Hz; H_2-28), 4.06 (1H, q, J = 8.5 Hz; H-3), 4.73, 4.87 (1H each, both d, J = 1.9 Hz; H_2-29), 7.81 (1H, d, J = 9.6 Hz; NH), 8.11 (1H, t, J = 5.8 Hz; CH_2NH). Anal. ($C_{44}H_{72}N_2O_6$·H_2O) C, H.

3,28-Di-*N*-(3′,3′-tetramethyleneglutaryl)-betulin (34): yield 65% (after chromatography with *n*-hexane/acetone [6:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +18.5 (c = 0.48, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.76, 0.81, 0.86, 0.94, 1.04 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.66 (3H, s; 20-CH_3), 2.34−2.54 (8H, m, H_2-2′, 2″, 4′, 4″, H-19), 3.01, 3.60 (1H each, both dd, J = 5.7, 13.3 Hz; H_2-28), 3.62−3.71 (1H, m; H-3), 4.58, 4.68 (1H each, both br s; H_2-29), 6.00 (1H, d, J = 9.2 Hz; NH), 6.15 (1H, t; CH_2NH). Anal. ($C_{48}H_{76}N_2O_6$) C, H.

3,28-Di-*O*-(3′,3′-dimethylglutaryl)-3-epi-betulin (41): yield 80% (after chromatography with *n*-hexane/$CHCl_3$/acetone [10:10:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +22.4 (c = 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.85, 0.86, 0.89, 1.02, 1.05 (3H each, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.16 (6H), 1.17, 1.19 (3H each, except 1.16, all s; 3′-$(CH_3)_2$, 3″-$(CH_3)_2$), 1.70 (3H, s; 20-CH_3), 2.43−2.60 (9H, m, H_2-2′, 2″, 4′, 4″, H-19), 3.86, 4.31 (1H each, both d, J = 11.1 Hz; H_2-28), 4.60 (1H, s; H-3), 4.68, 4.71 (1H each, both s; H_2-29). Anal. ($C_{44}H_{70}O_8$·$^1/_2H_2O$) C, H.

3,28-Di-*O*-(3′,3′-dimethylglutaryl)-oximebetulin (42): yield 59% (after chromatography with $CHCl_3$/acetone [13:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +21.7 (c = 0.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.96 (6H), 0.97, 1.13, 1.14 (3H each, except 0.96, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.16 (12H, s; CH_3 × 2−3′, 3″), 1.69 (3H, s; 20-CH_3), 2.33−2.56 (2H, m; H-2a, H-19), 2.61 (8H, s; H_2-2′,2″, H_2-2′,2″), 3.11 (1H, dt, J = 5.3, 14.5 Hz; H-2e), 4.68, 4.78 (1H each, both br s; H_2-29), 7.62 (1H, s; H-28). Anal. ($C_{44}H_{68}N_2O_8$) C, H.

28-*O*-adamantanecarbonyl-betulin (43): yield 88% (after chromatography with *n*-hexane/$CHCl_3$ [1:4]), mp 237 °C; $[\alpha]^{25}_D$ +12.8 (c = 0.32, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.77, 0.83, 0.98 (6H), 1.04 (3H each, except 0.98, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.69 (3H, s; 20-CH_3), 2.46 (1H, dt, J = 5.6, 10.9 Hz; H-19), 3.19 (1H, dd, J = 5.2, 10.8 Hz; H-3), 3.80, 4.26 (1H each, both d, J = 11.0 Hz; H_2-28), 4.59, 4.69 (1H each, both s; H_2-29). Anal. ($C_{41}H_{64}O_3$) C, H.

3-*O*-(3′,3′-Dimethylglutaryl)-28-*O*-adamantanecarbonyl-betulin (44): yield 73% (after chromatography with *n*-hexane/$CHCl_3$/acetone [5:1:1]), an off-white amorphous powder; $[\alpha]^{25}_D$ +11.7 (c = 0.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.86 (6H), 0.87, 0.98, 1.04 (3H each, except 0.86, all s; 4-$(CH_3)_2$, 8-CH_3, 10-CH_3, 14-CH_3), 1.15 (6H, s; 3′-$(CH_3)_2$), 1.70 (3H, s; 20-CH_3), 2.38−2.52 (5H, m, H_2-2′, 4′, H-19), 3.79, 4.27 (1H each, both d, J = 11.0 Hz; H_2-28), 4.52 (1H, dd, J = 4.5, 11.1 Hz; H-3), 4.60, 4.69 (1H each, both s; H_2-29). Anal. ($C_{48}H_{74}O_6$) C, H.

Anti-HIV Assay. The H9 cell was maintained separately in continuous culture with complete medium (RPMI 1640 with 10% fetal calf serum (FCS) supplemented with L-glutamine at 5% CO_2 and 37 °C). Aliquots of this cell line were only used in experiments when in log phase of growth.

Test samples were first dissolved in dimethyl sulfoxide (DMSO). The following were the final drug concentrations routinely used for screening: 100, 20, 4, and 0.8 μg/mL. However, for active agents, additional dilutions were prepared for subsequent testing so that an accurate EC_{50} value can be achieved.

As the test samples were being prepared, both cell lines were infected with HIV-1 (IIIB isolate, $TCID_{50}$ 10^4 IU/mL, at a multiplicity of infection of 0.1−0.01 IU/cell) for 1 h at 5% CO_2 and 37 °C. The cell lines were washed thoroughly to remove unabsorbed virions and resuspended at 4 × 10^5 cells/mL in complete medium. Aliquots (1 mL) were placed in wells of 24-well culture plates containing an equal volume of test compounds. AZT was also assayed during each experiment as a positive drug control. Each test compound had its toxicity assessed by determining the number of compound-exposed uninfected cells that remained after 4 days at 5% CO_2 and 37 °C. Cell-free supernatants were collected on day 4 for use in p24 antigen ELISA assay. P24 antigen is a core protein of HIV and, therefore, it was an indirect measure of virus present in the supernatants. The p24 antigen assay used a HIV-1 anti-p24 specific monoclonal antibody as the capture antibody coated on 96-well plates. Following a sample incubation period, rabbit serum containing antibodies for HIV-1 p24 was used to tag any p24 "captured" onto the microtiter well surface. Peroxidase conjugated goat anti-rabbit serum was then used to tag HIV-1 p24 specific rabbit antibodies that have complexed with captured p24. The presence of p24 in test samples was then revealed by addition of substrate. P24 in the culture medium was quantitated against a standard curve containing known amounts of p24. Toxicity was determined by performing cell counts by a counter on cells that had either received culture medium (no toxicity) or test sample or AZT.

If a test sample had suppressive capability and was not toxic, its effects were reported in the following terms: IC_{50}, the concentration of test sample which is toxic to 50% of the mock-infected H9 cells; EC_{50}, the concentration of the test sample which is able to suppress HIV replication by 50%; and TI, the ratio of IC_{50} to EC_{50}.

Fusion Assay. Cell fusion assays were performed as previously described in ref 30. Molt-4 cells (7 × 10^4) were incubated with HIV-1 IIIB chronically infected CEM cells (10^4) in 96-well half-area flat-bottomed plates (Costar) in 100 μL culture medium. Test compounds at various concentrations in 10 μL of culture medium were incubated with the cell mixtures at 37 °C for 24 h. Within this time period, giant cell formation could be seen evenly dispersed throughout the surface of each well. These cells had a diameter in excess of 5-fold that of CEM or chronically infected CEM and appeared in numbers that were proportional to the inoculation of infected cells. The efficiency of the process was such that 10−50 infected cells were required to score as a fusion event. Multinucleated syncytia were enumerated by microscopic examination of the entire contents of each well.

MAGI Assay. MAGI assays were performed according to the procedures from Kimpton et al.[31] Hela-CD4/β-gal cells were plated on a 96-well plate at 10 000 cells/well and cultured in DMEM medium containing 500 μg/mL of G418 and 250 μg/mL of hygromycin for 1 day. The cells were infected with virus dilutions in the presence of various concentrations of anti-HIV agents and incubated for 2 days at 37 °C. The infected cells were stained to blue by adding X-gal at 0.4 mg/mL to the culture. The cells were fixed with a solution containing 1% formaldehyde and 0.2% glutaraldehyde before staining. The number of infected cells was counted under an inverted microscope or by an alpha imager. An anti-HIV compound that inhibits 50% of virus infection is defined by its ability to reduce the number of infected cells by 50%, for example from 100 blue cells to 50 blue cells.

Acknowledgment. We are grateful to Dr. Susan Morris-Natschke for valuable suggestions in preparing this paper. This investigation was supported by Grant AI-33066 form the National Institute of Allergies and Infectious Diseases awarded to K. H. Lee.

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JM980391G