Azasugar-Based MMP/ADAM Inhibitors as Antipsoriatic Agents

Hideki Moriyama,[†,‡] Takahiro Tsukida,*[†,‡,||] Yoshimasa Inoue,[‡] Kohichi Yokota,[‡] Kohichiro Yoshino,[‡] Hirosato Kondo,[‡,||] Nobuaki Miura,[†] and Shin-Ichiro Nishimura*[,§]

Japan Bioindustry Association, Hokkaido Collaboration Center N-21, W-12, Kita-Ku, Sapporo 001-0021, Japan, R&D Laboratories, Nippon Organon K.K., 1-5-90, Tomobuchi-cho, Miyakojima-ku, Osaka 534-0016, Japan, and Division of Biological Sciences, Graduate School of Science, Hokkaido University, N-21, W-11, Kita-Ku, Sapporo 001-0021, Japan

Received September 3, 2003

As a part of synthetic studies on MMP (matrix metalloproteinase)/ADAM (a disintegrin and metalloproteinase) inhibitors, we have preliminarily communicated that azasugar-based compound **1a** exhibited a potential inhibitory activity on some metalloprotease-catalyzed proteolytic reactions. To find promising candidates for the topical treatment of psoriasis, we investigated stability in aqueous solution of compound **1a** and its derivative **1b** and then optimized the P1′ substuent (**2**−**5**). In the present study, we synthesized novel derivatives of compound **1a** and evaluated their inhibitory activity toward MMP-1, -3, and -9, TACE, and HB-EGF shedding, from a viewpoint of versatility of azasugars as a functional scaffold. As a result, it was found that compound **1b** demonstrated desirable inhibitory activity as an antipsoriatic agent, and some of the derivatives showed selective inhibitory activity. In addition, it was found that compound **1b** exhibited a significant therapeutic effect on a mouse TPA-induced epidermal hyperplasia model. Therefore, compound **1b** could become a promising candidate as a practical antipsoriatic agent.

Introduction

Psoriasis is a complex inflammatory skin disease and affects 2% of the adult population. The nature of the disease is characterized by hyperproliferation of epidermal keratinocytes.[1,2] Several systemic and topical therapies are available such as methotrexate, retinoid, cyclosporine, glucocorticoid, and analogues of 1,25-dihydroxyvitamine D_3 [1,25$(OH)_2D_3$], but the effects of these agents are not sufficient.[2] In addition, safety concerns prevent widespread application. Although the etiology of psoriasis has not been clearly elucidated, it was demonstrated that the characterized proliferation of keratinocytes is induced by a number of growth factors, such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), amphiregulin (AR),[3] and heparin-binding EGF-like growth factor (HB-EGF).[4−7] Among these growth factors, recently, it has been reported that HB-EGF might be the most important for the proliferation of keratinocytes.[8] These epidermal growth factors were synthesized as membrane-anchored precursors, which are converted to the soluble growth factors.[9] Several studies have suggested that some zinc-dependent metalloproteinases are involved in the processing of these membrane-anchored precursors.[8,10] Moreover, it has been recently reported that ADAM12 might be one of most important enzymes to shed HB-EGF from its precursor protein, proHB-EGF.[11] Therefore, inhibitors of HB-EGF production in epidermis, namely ADAM12 inhibitors, are expected to be effective therapeutic agents for the treatment of skin diseases such as psoriasis caused by the proliferation of keratinocytes. It has already been reported that *N*-{DL-[2-(hydroxylaminocarbonyl)methyl]-4-methylpentanoyl}-L-3-(2′-naphthyl)alanine 2-aminoethylamide (TAPI), a peptide-based MMP inhibitor, inhibited the proteolytic cleavage of HB-EGF precursor.[12] Moreover, Tokumaru et al. have reported that peptide-type MMP inhibitors suppressed the proliferation of keratinocytes both in in vitro and in vivo models.[8] Therefore, MMP inhibitors would be potential lead compounds for the development of antipsoriatic agents.[13]

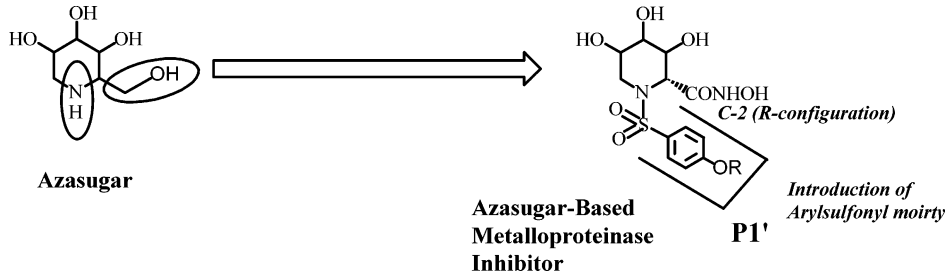
On the other hand, there have been considerable efforts in the design and synthesis of MMP/ADAM inhibitors, and a lot of cyclic small molecule inhibitors have been reported and showed blocking activity against metalloproteinases.[14] Recently, we reported novel metalloproteinase inhibitors based on an azasugar scaffold.[15] An *R*-configuration of the hydroxamic acid at the C-2 position and the introduction of an arylsulfonyl moiety at the N-1 position would become crucial for practical design of the potential metalloproteinase inhibitors (Chart 1).[15b] The azasugar compounds designed were expected to have improved solubility, due to the hydroxyl group at 3-, 4-, and 5-positions. In addition, control of stereochemistry of the azasugar derivatives would be facile, because they were prepared from L-threitol, L-gulono-1,4-lactone, L-glucono-1,5-lactone, and D-gulonic γ-lactone, commercial available starting materials.[15]

In this series, we found that compound **1a** bearing the 2*R*,3*R*,4*R*,5*S*-configuration exhibited potent inhibitory activity toward MMP-1, -3, and -9 and TACE (TNF-α converting enzyme, ADAM17).[15a] Next, our interest has been focused on the discovery of promising

* To whom correspondence should be addressed. T.T.: Tel: +81 6 6401 8199, fax: +81 6 6401 1371; e-mail: takahiro.tsukida@shionogi.co.jp. S.-I.N.: Tel: +81 11 706 9043, fax: +81 11 706 9042; e-mail: shin@glyco.sci.hokudai.ac.jp.
† Japan Bioindustry Association.
‡ Nippon Organon K.K.
§ Hokkaido University.
|| Current address: Manufacturing Technology R&D Laboratories, Shionogi & Co., Ltd., 1-3 Kuise Terajima 2-chome, Amagasaki, Hyogo 660-0813 Japan.

10.1021/jm0304313 CCC: $27.50
Published on Web 03/13/2004

Chart 1. Practical Design of Novel Azasugar-Based Metalloproteinase Inhibitor



candidates for the topical treatment of psoriasis. For this purpose, we investigated stability of compound **1a** in aqueous solution. This property is important for topical application, because the drug must be in a solution for a long time. As a result, a stereoisomer **1b** was designed, and then modification of P1′-substuent of **1b** was investigated (Figure 1).

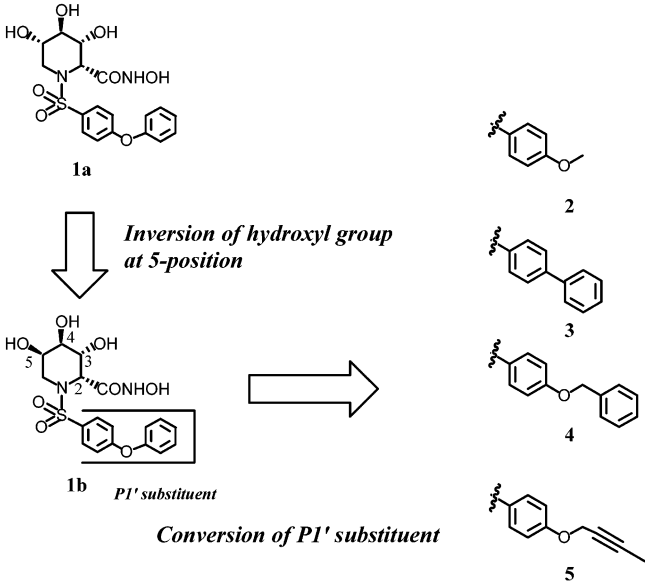


Figure 1. Modification of azasugar-based metalloproteinase inhibitor **1a**.

In this paper, we report the systematic syntheses of compound **1a** analogues and their inhibitory activity toward MMP-1, -3, and -9, TACE, and shedding of HB-EGF. In addition, compound **1b** was subjected to in vivo assay by using TPA-induced hyperplasia in murine skin, as a model of psoriasis.

Chemistry

At first, to verify whether azasugar derivative **1a** would become a promising candidate for the topical treatment of psoriasis, we investigated stability in aqueous solution of compound **1a**. Compound **1a** was dissolved and tested in solutions of pH 4, pH 7.4, and pH 9 and distilled water. Unfortunately, compound **1a** was proved to be unstable in neutral solution, pH 7.4 solution after 1 week, because it was hydrolyzed to the corresponding carboxylic acid **1a′** (Table 1). One of the expected mechanisms of hydrolysis for azasugar-based compound **1a** was postulated as follows: the hydroxamic acid moiety might be hydrolyzed by the equatorial hydroxyl group at the 5-position of compound **1a** together with H_2O in the solution, to produce corresponding carboxylic acid **1a′** (Figure 2). If the stereochemistry of the hydroxyl group at the 5-position would be inverted, such as in compound **1b**, hydrolysis of the hydroxamic acid would be diminished, compared to **1a**. In fact, compound **1b** was tested under the same conditions. Interestingly, as we predicted, stability of compound **1b** in aqueous solution after 1 week was dramatically improved as shown in Table 1.[16]

Table 1. Stability of Azasugar Derivatives **1a**,**b** in Aqueous Solution

	% compd remaining after 1 week[a]			
compd	pH 4	pH 7.4	pH 9	distilled water
1a	98.7	75.4	83.6	96.1
1b	102.1	102.5	104.8	100.0

[a] The details of the assay are described in the Experimental Section.

Next, we focused on the optimization of the inhibitory activity against MMPs and TACE and shedding of HB-EGF by synthesizing new analogues of compound **1b**. For this purpose, we investigated some modifications of the arylsulfonyl moiety of compound **1b**.

Figure 1 shows a straightforward strategy of a new type of analogue of sugar-mimetic metalloproteinase inhibitors such as compound **1b**. Scheme 1 shows synthetic routes of these new compounds.

The azide intermediate **7**,[17] which was readily prepared from D-mannono-1,4-lactone **6**,[18] was hydrogenated in the presence of 10% Pd−C, and then the corresponding amine was reacted with substituted arylsulfonyl chlorides **8i**−**iv** to give compounds **9i**−**iv** in 75−85% yields (Scheme 1). The terminal isopropylidene groups in compounds **9i**−**iv** were selectively cleaved by using cerium chloride heptahydrate and a catalytic amount of oxalic acid in acetonitrile[19] to provide diols **10i**−**iv** in 67−78% yields, respectively. The primary hydroxyl groups of diols **10i**−**iv** were selectively mesylated by the treatment with mesyl chloride at −40 °C in dichloromethane to afford mesylates **11i**−**iv** in moderate yields. The intramolecular cyclization of compounds **11i**−**iv** in the presence of potassium carbonate gave compounds **12i**−**iv**. After the removal of the benzyl group of compound **12iv** by hydrogenolysis in the presence of 10% Pd−C, the corresponding phenol derivative was condensed with 1-bromo-2-butyne in the presence of potassium carbonate to afford compound **12v** in good yield. Compounds **12ii**−**v** were subjected to the aminolysis by the treatment with 50% hydroxylamine aqueous solution in the presence of sodium cyanide in methanol to afford compounds **13ii**−**v** in 35−65% yields, respectively. Finally, 3,4-*O*-isopropylidene groups in **13ii**−**v** were cleaved by treating with Muromac, H^+-form, to provide the target compounds **2**−**5** in 55−81%

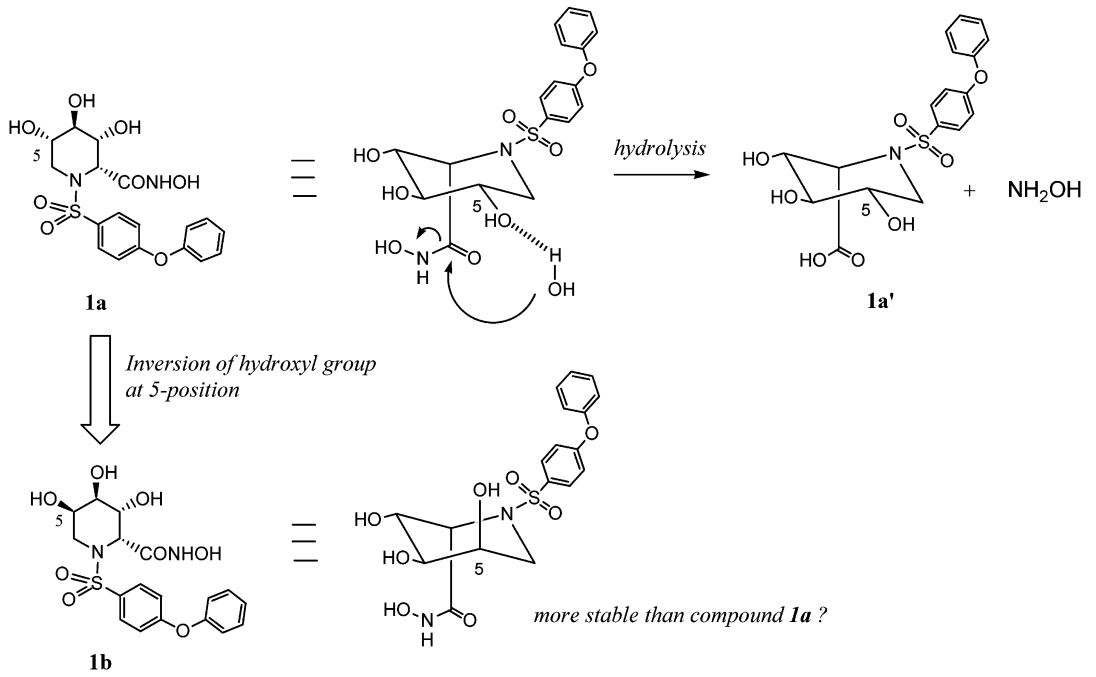
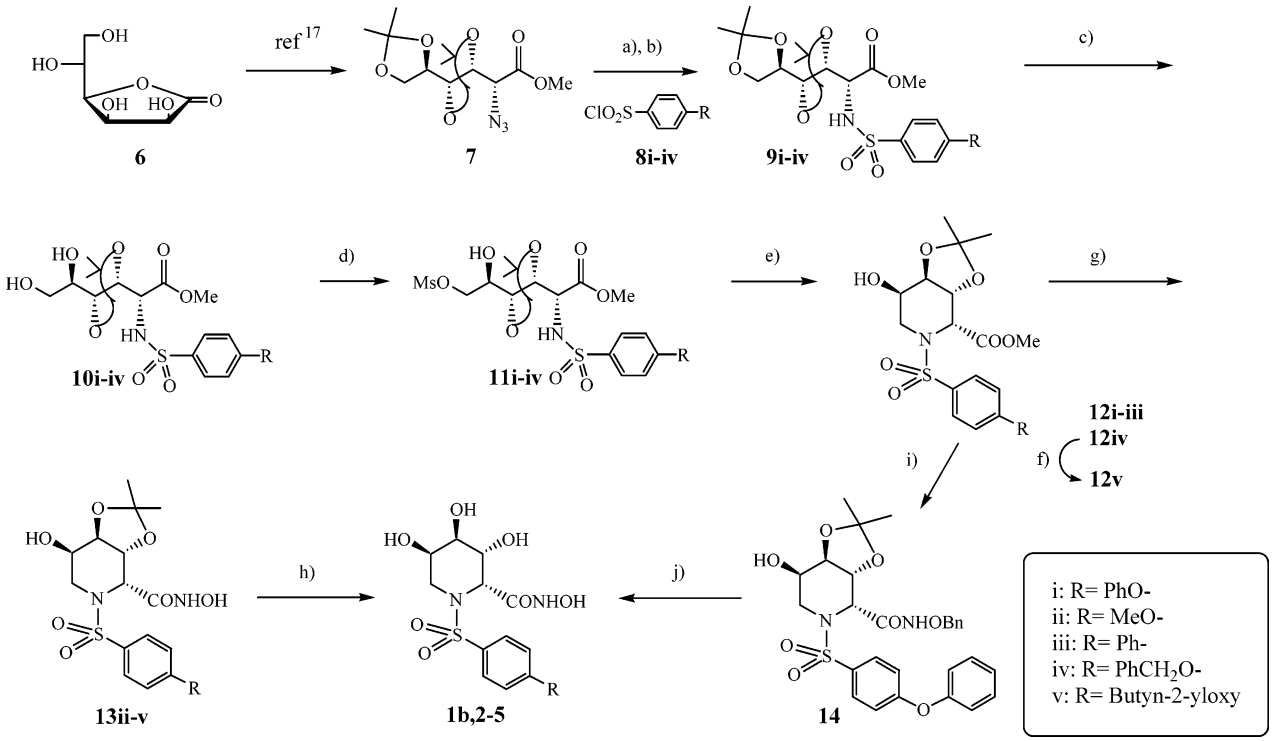


Figure 2. Expected mechanism of hydrolysis for azasugar-based compound **1a**.

Scheme 1[a]



[a] (a) 10% Pd−C/H_2, EtOAc; (b) **8i−iv**, 4-DMAP, DMF, 75−85% from **7**; (c) cerium chloride heptahydrate, oxalic acid, CH_3CN, 67−78%; (d) methanesulfonyl chloride, Et_3N, CH_2Cl_2, 33−86%; (e) K_2CO_3, DMF, 68−92%; (f) (i) 10% Pd−C/H_2, EtOAc, (ii) 1-bromo-2-butyne, K_2CO_3, CH_3CN, 83%; (g) 50% aq NH_2OH, NaCN, MeOH, 35−65%; (h) Muromac, 55−81%, (i) (i) 1 N NaOH, (ii) NH_2OBn, WSC, HOBt, DMF, 73%; (j) (i) Muromac, MeOH, (ii) 10% Pd−C/H_2, 69%.

yields, respectively. On the other hand, compound **12i** was subjected to hydrolysis by 1 N sodium hydroxide solution, followed by the condensation with NH_2OBn in the presence of WSC and HOBt, to give compound **14** in 91% yield. After deprotection of an isopropylidene group of the compound **14** using Muromac, the corresponding triol derivative was hydrogenated in the presence of 10% Pd−C to afford a target compound **1b**.

Biological Activity

Inhibitory activity against TACE, shedding of HB-EGF, and MMPs (MMP-1, MMP-3, MMP-9) of compound **1**−**5** were summarized in Table 2. Compound **1b** with a 2*R*,3*R*,4*R*,5*R*-configuration, having improved stability in aqueous solution after 1 week, exhibited similar and satisfactory inhibition toward MMPs, TACE,

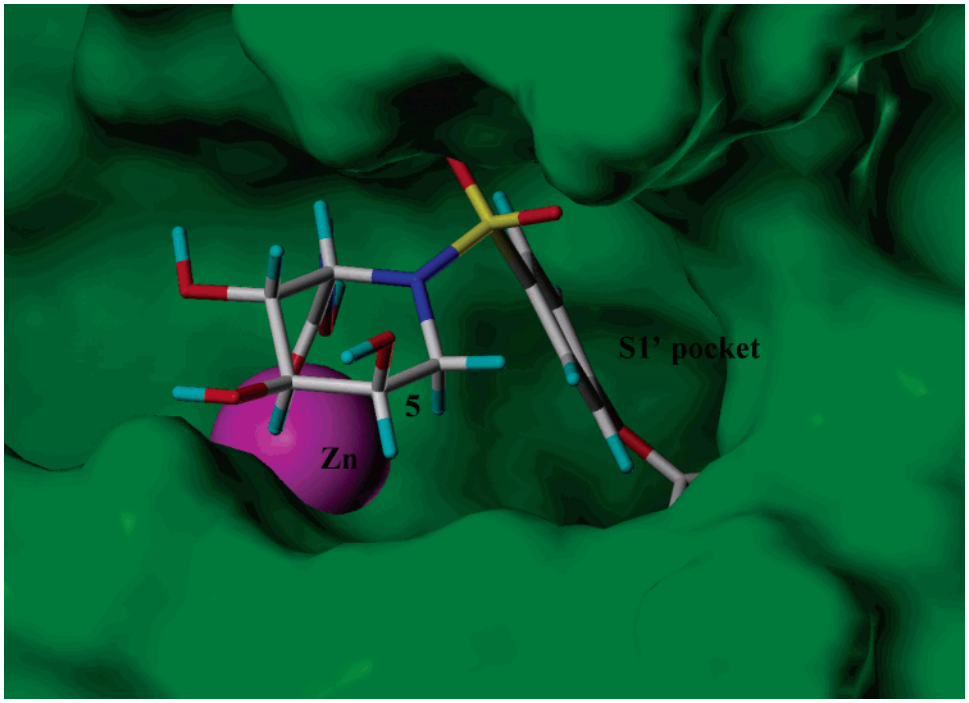


Figure 3. Docking study of sugar-mimetic compound **1b** with MMP-3.

Table 2. Inhibitory Activity of Azasugar Derivatives **1**−**5** against MMPs, TACE, and Shedding of HB-EGF

compd	rMMP-1 K_i (nM)[a]	rMMP-3 K_i (nM)[a]	rMMP-9 K_i (nM)[a]	TACE K_i (nM)[a]	shedding of HB-EGF: IC_{50} (μM)[a]
1a	8.0	0.51	0.06	2.3	0.35
1b	5.3	0.35	0.097	6.2	0.34
2	26	2.0	2.0	15	0.45
3	162	50	47	21	21
4	>850	2.1	7.4	1.7	0.084
5	128	3.3	14	0.53	0.028

[a] The details of the assay are described in the Experimental Section. All values represent the mean of two determinations.

and shedding of HB-EGF, compared to compound **1a** having a 2*R*,3*R*,4*R*,5*S*-configuration. This result suggests that stereochemistry at the 5-position of azasugar compounds might not be essential for inhibitory activity against metalloproteinases. Interestingly, compound **2**, **4**, and **5**, having a methoxy, benzyloxy, and butyn-2-yloxy moiety, respectively, showed similar or higher inhibitory activity toward shedding of HB-EGF, compared to compound **1b**, suggesting that the S1′ pocket of the enzyme responsible for the shedding of HB-EGF is deeper than that of MMP-1.[13b] In addition, it was also found that compound **4** appears to become a selective MMP/ADAM inhibitor, over MMP-1, because the 4-benzyloxybenzenesulfonylamide unit of **4** would not be accommodated to the shallow S1′ pocket in MMP-1.[20] However, in the case of biphenyl derivative **3**, inhibitory activity against metalloproteinases was dramatically decreased.

In addition, the binding model of **1b** with MMP-3 was constructed using the published crystal structure of MMP-3 with a sulfonamide derivative (PDB code: 1D8F).[14b] As shown in Figure 3, the hydroxamic acid moiety was found to be tightly bound with the zinc ion, and the phenoxybenzene unit was placed in the S1′ pocket. One of the oxygen atoms of the sulfonamide was positioned at hydrogen bond distance from the main chain Leu-164 and Ala-165, like that of other sulfonamide-based inhibitors.[14b] As shown in this model, it was also suggested that the axial hydroxyl group at the 5-position was not directed to the surface of the protein. Considering the similar potent inhibitory activity of **1a** and **1b**, it was indicated that the hydroxyl group at the 5-position is not important for desirable interaction with MMP-3.

In Vivo Assay: Effects on Mice TPA-Induced Epidermal Hyperplasia. To evaluate the antipsoriatic activity of compounds synthesized in this study, we investigated the effects of these inhibitors on TPA(12-*O*-tetradecanoylphorbol 13-acetate)-induced hyperplasia in murine skin, one of the psoriatic model.[21] It is well-known that topical application of phorbol esters induce cutaneous inflammation and epidermal hyperproliferation.[21] As shown in Figure 4, TPA induced a significant increase in epidermal thickness, compared with acetone treatment. Topical application of compound **1b** dose-dependently suppressed TPA-induced inflammation at a dose of 1−100 μg/site. This result indicates that compound **1b** would regulate EGF production to suppress phorbol-induced inflammation and epidermal hyperplasia. Considering the potent topical antiinflammatory activity, it was suggested that topical use of compound **1b** was a promising candidate for the treatment of psoriasis.

In conclusion, to find promising candidates for the topical treatment of psoriasis, the aqueous stability of compound **1a** and its derivative **1b** and the structural modification of the P1 -substituent were investigated. In this study, the compound **1a** analogues were systematically synthesized and their inhibitory activity toward MMP-1, -3, and -9, TACE, and shedding of HB-EGF were discussed. As a result, it was found that compound **1b**, having an improved stability in aqueous solution, exhibited a similar potent in vitro profile, compared to compound **1a**, and its derivative **4** showed a selective inhibitory profile over MMP-1. In addition, it was also suggested that compound **1b** was effective on the mice

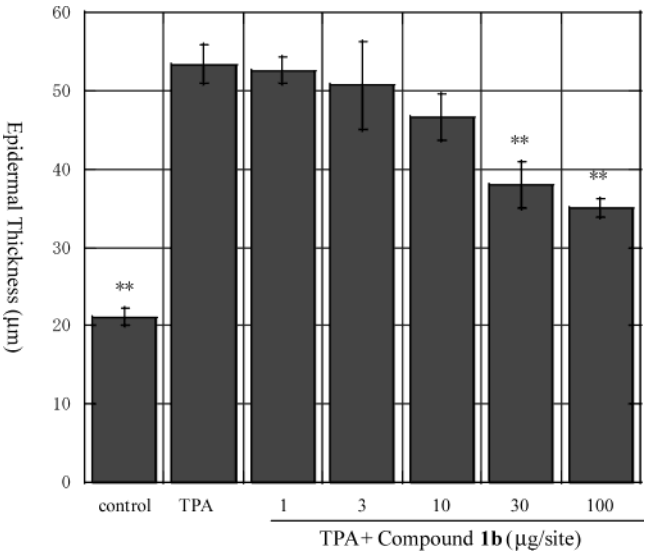


Figure 4. Effect of azasugar-based derivative **1b** on mice TPA-induced epidermal hyperplasia. Data represent the mean ± standard error of five animals. Significant difference from TPA treated group. ** $p < 0.01$ (student's *t*-test). [a] See Experimental Section for details.

TPA-induced epidermal hyperplasia model. Moreover, the binding model of **1b** with MMP-3 demonstrated that the stereochemistry of the hydroxyl group at the 5-position would not contribute to the desirable interaction with MMP-3. Therefore, it was strongly concluded that compound **1b** could become a potential candidate as a practical antipsoriatic agent.

Experimental Section

General Methods and Materials. All commercially available starting materials and solvents were reagent grade. Melting points were uncorrected. ^{1}H NMR spectra were measured at 250 MHz on a Brucker DPX-250 spectrometer or 500 MHz on a Brucker DPX-500 spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent. TPA was purchased from Sigma (St. Louis, MO). Mass spectra were determined on a PerSeptive Biosystems Voyager-DE RP spectrometer or a Brucker BIFLEX III spectrometer. Optical rotations were measured at 20 °C on a Perkin-Elmer Polarimeter 343. The results of elemental analysis are within ±0.4% of the calculated values unless otherwise noted.

Measurement of the Stability of Sugar-Based Inhibitors. Standard solutions were prepared as follows: 5 mg of compounds **14a** and **14b** was dissolved in MeOH (10 mL), respectively. Standard solutions (100 μL) were added to the flask and then dried under nitrogen atmosphere. To the resulting residue was added pH 4, pH 7.4, or pH 9 solution or distilled water (5 mL). One milliliter of each solution was enclosed in ampules. The ampules were incubated in Air Bath Unit A (Taiyo) at 37 °C for 0 or 168 h. At the time of 0 or 168 h, MeOH (1 mL) was added to the ampules. The mixture was analyzed by HPLC (L-column ODS 2.1 × 150 mm). Sample concentration after 0 and 168 h was evaluated by peak area of HPLC (three times). Percent compound remaining after 168 h was calculated by the following equation:

$$\%\ \text{compound remaining} = \frac{\text{average sample concentration after 168 h}}{\text{average sample concentration after 0 h}} \times 100$$

General Procedure for the Preparation of 9i−iii: (2*R*,4′*S*,4″*R*,5*R*)-(4-Phenoxybenzenesulfonylamino)-[2′,2′,2″,2″-tetramethyl-[4′,4″]bi([1,3]dioxolanyl)-5′-yl]acetic Acid Methyl Ester (9i). A solution of compound **7**[17] (30.1 g, 95.30 mmol) and 10% Pd−C (4.3 g) in ethyl acetate (300 mL) was hydrogenated for 4 h at 40 °C, under 4 kgf/cm^2 pressure. The catalyst was removed by filtration, the filtrate was evaporated in vacuo, and the residue was dissolved in DMF (390 mL). To the solution were added 4-DMAP (18.1 g, 148.1 mmol) and 4-phenoxybenzenesulfonyl chloride **8i** (34.5 g, 128.4 mmol) in ice bath, and the reaction mixture was stirred for overnight at room temperature. The mixture was diluted with ethyl acetate, and it was washed with 1 N HCl, sat. $NaHCO_3$ aq., H_2O and brine, successively. The organic layer was dried with $MgSO_4$, and solvent was removed under reduced pressure. The resulting residue was purified by MPLC (ethyl acetate/*n*-hexane; 1:3 → 2:3), to obtain **9i** (38.3 g) in 77% yield as a colorless amorphous. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.33 (s, 3H), 1.37 (s, 3H), 1.40 (s, 3H), 1.48 (s, 3H), 3.57 (s, 3H), 3.85−4.3 (m, 6H), 5.46 (d, 1H, J = 10.5 Hz), 6.95−7.1 (m, 4H), 7.15−7.3 (m, 1H), 7.35−7.5 (m, 2H), 7.7−7.85 (m, 2H).

(2*R*,4′*S*,4″*R*,5*R*)-(4-Methoxybenzenesulfonylamino)-[2′,2′,2″,2″-tetramethyl-[4′,4″]bi([1,3]dioxolanyl)-5′-yl]acetic Acid Methyl Ester (9ii). (Yield 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.32 (s, 3H), 1.37 (s, 3H), 1.40 (s, 3H), 1.49 (s, 3H), 3.53 (s, 3H), 3.86 (s, 3H), 3.9−4.03 (m, 2H), 4.03−4.13 (m, 2H), 4.13−4.3 (m, 3H), 5.45 (d, 1H, J = 10.4 Hz), 7.00 (d, 2H, J = 8.9 Hz), 7.76 (d, 2H, J = 8.9 Hz).

(2*R*,4′*S*,4″*R*,5*R*)-(Biphenyl-4-sulfonylamino)-[2′,2′,2″,2″-tetramethyl-[4′,4″]bi([1,3]dioxolanyl)-5′-yl]acetic Acid Methyl Ester (9iii). (Yield 85%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.33 (s, 3H), 1.38 (s, 3H), 1.41 (s, 3H), 1.50 (s, 3H), 3.50 (s, 3H), 3.9−4.05 (m, 2H), 4.05−4.15 (m, 1H), 4.19 (dd, 1H, J = 8.6, 6.3 Hz), 4.25−4.35 (m, 2H), 5.53 (d, 1H, J = 10.5 Hz), 7.37−7.45 (m, 1H), 7.45−7.53 (m, 2H), 7.55−7.63 (m, 2H), 7.71 (d, 2H, J = 8.4 Hz), 7.89 (d, 2H, J = 8.4 Hz).

General Procedure for the Preparation of 10i−iv: (1″*R*,2*R*,4′*R*,5′*S*)-[5′-(1″,2″-Dihydroxyethyl)-2′,2′-dimethyl-[1,3]dioxolan-4′-yl]-(4-phenoxybenzenesulfonylamino)-acetic Acid Methyl Ester (10i). To a solution of compound **9i** (25.81 g, 49.54 mmol) in acetonitrile (300 mL) were added cerium chloride heptahydrate (36.92 g, 99.08 mmol) and oxalic acid (223 mg, 2.48 mmol), and the mixture was stirred for 70 min at room temperature. Sodium carbonate was added to the mixture. The reaction mixture was neutralized and filtered with a Celite pad, and the Celite pad was washed with ethyl acetate. The filtrate and washings were combined and evaporated in vacuo. The resulting residue was purified by MPLC (ethyl acetate/*n*-hexane; 2:3 → 3:1) to obtain **10i** (16.0 g) in 67% yield as a syrup, and starting material **9i** (7.0 g) was recovered. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.35 (s, 3H), 1.42 (s, 3H), 2.61 (bs, 1H), 3.61 (s, 3H), 3.65−3.95 (m, 3H), 4.05−4.25 (m, 1H), 4.25−4.4 (m, 2H), 5.46 (d, 1H, J = 8.6 Hz), 6.95−7.1 (m, 4H), 7.15−7.3 (m, 1H), 7.3−7.5 (m, 2H), 7.7−7.85 (m, 2H).

(1″*R*,2*R*,4′*R*,5′*S*)-[5′-(1″,2″-Dihydroxyethyl)-2′,2′-dimethyl-[1,3]dioxolan-4′-yl]-(4-methoxybenzenesulfonylamino)-acetic Acid Methyl Ester (10ii). (Yield 74%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.35 (s, 3H), 1.42 (s, 3H), 2.33 (bs, 1H), 2.83 (bs, 1H), 3.56 (s, 3H), 3.75 (dd, 1H, J = 11.3, 4.2 Hz), 3.78−3.82 (m, 1H), 3.82−3.9 (m, 4H), 4.17 (t, 1H, J = 7.4 Hz), 4.25−4.4 (m, 2H), 5.5 (bs, 1H), 6.98 (d, 2H, J = 8.9 Hz), 7.77 (d, 2H, J = 8.9 Hz).

(1″*R*,2*R*,4′*R*,5′*S*)-(Biphenyl-4-sulfonylamino)-[5′-(1″,2″-dihydroxyethyl)-2′,2′-dimethyl[1,3]dioxolan-4′-yl]acetic Acid Methyl Ester (10iii). (Yield 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.36 (s, 3H), 1.44 (s, 3H), 2.20 (s, 1H), 2.74 (bs, 1H), 3.53 (s, 3H), 3.7−3.8 (m, 1H), 3.8−3.85 (m, 1H), 3.85 (m, 1H), 3.85−3.95 (m, 1H), 4,18 (dd, 1H, J = 7.9, 7.1 Hz), 4.33−4.45 (m, 2H), 5.57 (bs, 1H), 7.38−7.45 (m, 1H), 7.45−7.55 (m, 2H), 7.55−7.63 (m, 2H), 7.71 (d, 2H, J = 8.5 Hz), 7.90 (d, 2H, J = 8.5 Hz).

(1″*R*,2*R*,4′*R*,5′*S*)-(4-Benzyloxybenzenesulfonylamino)-[5′-(1″,2″-dihydroxyethyl)-2′,2-dimethyl[1,3]dioxolan-4′-yl]acetic Acid Methyl Ester (10iv). (Yield 67% from **6**). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.37 (s, 3H), 1.48 (s, 3H), 2.17 (s, 1H), 2.69 (d, 1H, J = 6.7 Hz), 3.55 (s, 3H), 3.7−4.0 (m, 3H), 4.0−4.25 (m, 2H), 4.25−4.45 (m, 2H), 5.18 (s, 2H), 5.45 (d, 1H, J = 11.1 Hz), 7.05 (d, 2H, J = 8.9 Hz), 7.25−7.5 (m, 5H), 7.78 (d, 2H, J = 8.9 Hz).

General Procedure for the Preparation of 11i−iv: (1″*S*,2*R*,4′*R*,5′*S*)-[5′-(1″-Hydroxy-2″-methanesulfonyloxyethyl)-2′,2′-dimethyl[1,3]dioxolan-4′-yl]-(4-phenoxybenzenesulfonylamino)acetic Acid Methyl Ester (11i). To a solution of compound **10i** (25.0 g, 51.98 mmol) and triethylamine (5.79 g, 57.18 mmol) in dichloromethane (430 mL) was added methanesulfonyl chloride (6.25 g, 54.57 mmol) in dichloromethane (20 mL) at −40 °C, and the mixture was stirred for 1 h at the same temperature. The reaction mixture was diluted with chloroform, and the organic layer was washed H_2O and dried with $MgSO_4$. The solvent was removed under reduced pressure, and the resulting residue was purified by MPLC (ethyl acetate/cyclohexane; 35:65 → 2:3 → 1:1) to obtain **10i** (13.5 g) in 46% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.34 (s, 3H), 1.41 (s, 3H), 2.91 (d, 1H, J = 6.5 Hz), 3.13 (s, 3H), 3.61 (s, 3H), 3.85−4.0 (m, 1H), 4.05−4.28 (m, 2H), 4.28−4.4 (m, 2H), 4.54 (dd, 1H, J = 11.1, 2.5 Hz), 5.47 (d, 1H, J = 9.5 Hz), 6.95−7.1 (m, 4H), 7.15−7.3 (m, 1H), 7.35−7.5 (m, 2H), 7.7−7.85 (m, 2H).

(1″*S*,2*R*,4′*R*,5′*S*)-[5′-(1″-Hydroxy-2″-methanesulfonyloxyethyl)-2′,2′-dimethyl[1,3]di-oxolan-4′-yl]-(4-methoxybenzenesulfonylamino)acetic Acid Methyl Ester (11ii). (Yield 73%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.35 (s, 3H), 1.41 (s, 3H), 3.14 (s, 3H), 3.59 (s, 3H), 3.87 (s, 3H), 3.93 (bs, 1H), 4.1−4.18 (m, 2H), 4,21 (d, 1H, J = 6.6 Hz), 4.25−4.4 (m, 2H), 4.53 (dd, 1H, J = 11.1, 2.4 Hz), 5.47 (bs, 1H), 6.97 (d, 2H, J = 8.9 Hz), 7.76 (d, 2H, J = 8.9 Hz).

(1″*S*,2*R*,4′*R*,5′*S*)-(Biphenyl-4-sulfonylamino)-[5′-(1″-hydroxy-2″-methanesulfonyloxyethyl)-2′,2′-dimethyl[1,3]-dioxolan-4′-yl]acetic Acid Methyl Ester (11iii). (Yield 33%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.35 (s, 3H), 1.43 (s, 3H), 2.87 (d, 1H, J = 7.6 Hz), 3.14 (s, 3H), 3.56 (s, 3H), 3.94 (bs, 1H), 4.20 (t, 1H, J = 8.1 Hz), 4.25−4.4 (m, 3H), 4.56 (d, 1H, J = 11.1 Hz), 5.51 (d, 1H, J = 9.3 Hz), 7.38−7.45 (m, 1H), 7.45−7.53 (m, 2H), 7.55−7.65 (m, 2H), 7.72 (*d*, 2H, J = 7.4 Hz), 7.89 (d, 2H, J = 7.4 Hz).

(1″*S*,2*R*,4′*R*,5′*S*)-(4-Benzyloxybenzenesulfonylamino)-[5′-(1″-hydroxy-2″-methanesulfonyloxyethyl)-2′,2′-dimethyl[1,3]dioxolan-4′-yl]acetic Acid Methyl Ester (11iv). (Yield 86%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.37 (s, 3H), 1.44 (s, 3H), 2.88 (d, 1H, J = 8.0 Hz), 3.16 (s, 3H), 3.59 (s, 3H), 3.94 (s, 1H), 4.05−4.25 (m, 3H), 4.25−4.45 (m, 2H), 4.56 (d, 1H, J = 11.1 Hz), 5.15 (s, 2H), 5.44 (d, 1H, J = 9.6 Hz), 7.06 (d, 2H, J = 8.9 Hz), 7.3−7.5 (m, 5H), 7.78 (d, 2H, J = 8.9 Hz).

General Procedure for the Preparation of 12i−iv: (3a*R*,4*R*,7*R*,7a*R*)-7-Hydroxy-2,2-dimethyl-5-(4′-phenoxybenzenesulfonyl)hexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Methyl Ester (12i). To a solution of **11i** (13.5 g, 24.12 mmol) in DMF (320 mL) was added potassium carbonate (4.00 g, 28.95 mmol), and the reaction mixture was stirred for 70 min at 45 °C. The reaction mixture was diluted with ethyl acetate, and it was washed with H_2O and brine (×2) and then dried with $MgSO_4$. The organic layer was evaporated in vacuo, and the resulting residue was purified by MPLC (ethyl acetate/cyclohexane; 2:3) to obtain **12i** (10.3 g) in 92% yield. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.35 (s, 3H), 1.44 (s, 3H), 2.23 (s, 1H), 3.65 (s, 3H), 3.65−3.75 (m, 1H), 3.82 (dd, 1H, J = 9.8, 2.5 Hz), 4.0−4.2 (m, 2H), 4.3−4.4 (m, 1H), 5.05 (d, 1H, J = 6.3 Hz), 6.95−7.1 (m, 4H), 7.15−7.25 (m, 1H), 7.35−7.45 (m, 2H), 7.7−7.8 (m, 2H).

(3a*R*,4*R*,7*R*,7a*R*)-7-Hydroxy-2,2-dimethyl-5-(4′-methoxybenzenesulfonyl)hexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Methyl Ester (12ii). (Yield 68%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.31 (s, 3H), 1.44 (s, 3H), 2.23 (s, 1H), 3.55−3.75 (m, 4H), 3.75−3.9 (m, 4H), 4.0−4.15 (m, 2H), 4.37 (s, 1H), 5.06 (d, 1H, J = 6.3 Hz), 6.96 (d, 2H, J = 8.8 Hz), 7.75 (d, 2H, J = 8.8 Hz).

(3a*R*,4*R*,7*R*,7a*R*)-5-(Biphenyl-4′-sulfonyl)-7-hydroxy-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Methyl Ester (12iii). (Yield 87%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.35 (s, 3H), 1.44 (s, 3H), 2.26 (s, 1H), 3.62 (s, 3H), 3.74 (d, 1H, J = 13.8 Hz), 3.84 (d, 1H, J = 9.7 Hz), 4.05−4.2 (m, 2H), 4.39 (s, 1H), 5.10 (d, 1H, J = 6.2 Hz), 7.38−7.45 (m, 1H), 7.45−7.53 (m, 2H), 7.55−7.63 (m, 2H), 7.71 (d, 2H, J = 7.6 Hz), 7.88 (d, 2H, J = 7.6 Hz).

(3a*R*,4*R*,7*R*,7a*R*)-5-(4′-Benzyloxybenzenesulfonyl)-7-hydroxy-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Methyl Ester (12iv). (Yield 72%). ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.37 (s, 3H), 1.46 (s, 3H), 3.62 (s, 3H), 3.72 (d, 1H, J = 13.9 Hz), 3.85 (dd, 1H, J = 9.8, 2.4 Hz), 4.05−4.2 (m, 2H), 4.39 (s, 1H), 5.08 (d, 1H, J = 6.3 Hz), 5.15 (s, 2H), 7.05 (d, 2H, J = 8.9 Hz), 7.3−7.5 (m, 5H), 7.77 (d, 2H, J = 8.9 Hz).

(3a*R*,4*R*,7*R*,7a*R*)-5-(4′-But-2′-ynyloxybenzenesulfonyl)-7-hydroxy-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Methyl Ester (12v). A solution of compound **12iv** (5.8 g, 12.15 mmol) and 10% Pd−C (800 mg) in ethyl acetate (200 mL), was hydrogenated for 2 h at 40 °C, under 3kgf/cm^2 pressure. The catalyst was removed by filtration, the filtrate was evaporated in vacuo, and the residue was dissolved with acetonitrile (70 mL). 1-Bromo-2-butyne (2.4 g, 18.23 mmol) and potassium carbonate (2.52 g, 18.23 mmol) were added to the solution, and the reaction mixture was stirred for 30 min at 90 °C. The mixture was diluted with ethyl acetate, it was washed with H_2O and brine, the organic layer was dried with $MgSO_4$, and solvent was removed under reduced pressure. The resulting residue was purified by MPLC (ethyl acetate/*n*-hexane; 1:2 → 1:1) to obtain **39v** (4.4 g) in 83% yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.38 (s, 1H), 1.46 (s, 3H), 1.8−1.95 (m, 3H), 2.14 (t, 1H, J = 1.0 Hz), 3.65 (s, 3H), 3.72 (d, 1H, J = 13.9 Hz), 3.85 (dd, 1H, J = 9.8, 2.5 Hz), 4.05−4.2 (m, 2H), 4.39 (s, 1H), 4.65−4.75 (m, 2H), 5.08 (d, 1H, J = 6.4 Hz), 6.95−7.1 (m, 2H), 7.7−7.85 (m, 2H).

General Procedure for the Preparation of 13ii−v: (3a*R*,4*R*,7*R*,7a*R*)-7-Hydroxy-2,2-dimethyl-5-(4′-methoxybenzenesulfonyl)hexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Hydroxy Amide (13ii). Hydroxylamine (50% aq) (12 mL) was added to a solution of compound **39ii** (2.09 g, 5.21 mmol) and sodium cyanide (255 mg, 5.21 mmol) in MeOH (60 mL), and the solution was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo, and the resulting residue was purified by MPLC ($CHCl_3$/MeOH: 50:1 → 30:1). The resulting fraction was lyophilized to afford **13ii** (1.06 g) in 50% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.24 (s, 3), 1.30 (s, 3H), 3.65 (d, 1H, J = 12.9 Hz), 3.7−3.82 (m, 2H), 3.84 (s, 3H), 4.13 (dd, 1H, J = 9.7, 2.2 Hz), 4.20 (s, 1H), 4.54 (d, 1H, J = 6.2 Hz), 5.22 (d, 1H, J = 3.9 Hz), 7.07 (d, 2H, J = 8.9 Hz), 7.72 (d, 2H, J = 8.9 Hz), 8.93 (s, 1H), 10.63 (s, 1H); Anal. ($C_{16}H_{22}N_2O_8S\cdot0.45H_2O$) C, H, N.

(3a*R*,4*R*,7*R*,7a*R*)-5-(Biphenyl-4′-sulfonyl)-7-Hydroxy-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Hydroxy Amide (13iii). (Yield 65%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.05 (s, 3H), 1.30 (s, 3H), 3.71 (d, 1H, J = 12.4 Hz), 3.75−3.9 (m, 2H), 4.15 (dd, 1H, J = 9.7, 2.3 Hz), 4.23 (s, 1H), 4.62 (d, 1H, J = 6.3 Hz), 5.29 (d, 1H, J = 3.9 Hz), 7.4−7.48 (m, 1H), 7.48−7.55 (m, 2H), 7.76 (d, 2H, J = 7.4 Hz), 7.84 (s, 4H), 8.98 (d, 1H, J = 1.5 Hz), 10.78 (d, 1H, J = 1.2 Hz); Anal. ($C_{21}H_{24}N_2O_7S\cdot0.80H_2O$) C, H, N.

(3a*R*,4*R*,7*R*,7a*R*)-5-(4′-But-2′-ynyloxybenzenesulfonyl)-7-hydroxy-2,2-dimethylhexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Hydroxy Amide (13v). (Yield 35%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.25 (s, 3H), 1.30 (s, 3H), 1.75−1.9 (m, 3H), 3.67 (d, 1H, J = 13.3 Hz), 3.7−3.85 (m, 2H), 4.14 (dd, 1H, J = 9.7, 2.0 Hz), 4.54 (d, 1H, J = 6.2 Hz), 4.75−4.9 (m, 2H), 5.26 (bs, 1H), 7.10 (d, 2H, J = 8.9 Hz), 7.74 (d, 2H, J = 8.9 Hz), 8.96 (s, 1H), 10.75 (s, H); Anal.($C_{19}H_{24}N_2O_8S$) C, H, N.

(3a*R*,4*R*,7*R*,7a*R*)-7-Hydroxy-2,2-dimethyl-5-(4′-phenoxybenzenesulfonyl)hexahydro[1,3]dioxolo[4,5-*c*]-4-carboxylic Acid Benzyloxyamide (14). To a solution of compound **12i** (10.2 g, 22.0 mmol) in MeOH−1,4-dioxane (30−150 mL) was added aq 1 N NaOH (55 mL), and reaction mixture was stirred for 70 min at room temperature. The reaction mixture was neutralized with 5% citric acid and was extracted with ethyl acetate. The organic layer was washed with H_2O and brine and dried with $MgSO_4$. The solvent was

concentrated in vacuo. To a solution of the resulting residue were added WSC (5.48 g, 28.6 mmol), HOBt (3.86 g, 28.6 mmol) in DMF (200 mL), a solution of *O*-benzylhydroxylamine hydrochloride (4.56 g, 28.6 mmol), and DIEA (3.70 g, 28.6 mmol) in DMF (50 mL), and the reaction mixture was stirred for 2.5 h at room temperature. Then additional amounts of WSC (299 mg), HOBt (211 mg), *O*-benzylhydroxylamine hydrochloride (248 mg), and DIEA (202 mg) were added to the reaction mixture. The mixture was diluted with ethyl acetate. The organic layer was washed with aq 0.5 N HCl, sat. aq $NaHCO_3$, H_2O, and brine, successively, and then dried with $MgSO_4$. The solvent was removed under reduced pressure. The resulting residue was purified by MPLC (ethyl acetate/cyclohexane; 1:1) to obtain **14** (8.90 g) in 73% yield as a colorless powder. ^{1}H NMR (250 MHz, $CDCl_3$) δ: 1.28 (s, 3H), 1.42 (s, 3H), 2.39 (s, 1H), 3.39 (d, 1H, J = 14.5 Hz), 3.61 (d, 1H, J = 9.1 Hz), 4.05−4.25 (m, 1H), 4.28 (s, 1H), 4.83 (d, 1H, J = 11.2 Hz), 4.92 (d, 1H, J = 11.2 Hz), 5.07 (d, 1H, J = 5.4 Hz), 6.95−7.1 (m, 4H), 7.15−7.25 (m, 1H), 7.3−7.5 (m, 7H), 7.8−7.9 (m, 2H), 8.94 (s, 1H); Anal. ($C_{25}H_{26}N_2O_8S\cdot0.70H_2O$) C, H, N.

(2*R*,3*R*,4*R*,5*R*)-3,4,5-Trihydroxy-1-(4′-phenoxybenzenesulfonyl)piperidine-2-Hydroxy Amide (1b). To a solution of compound **14** (8.68 g, 15.65 mmol) in MeOH (25 mL) was added Muromac (19.0 g), and the mixture was stirred for overnight at room temperature. The insoluble material was removed by filtration, and the filtrate was concentrated in in vacuo. The resulting mixture was purified by MPLC ($CHCl_3$/MeOH; 1:0 → 30:1 → 20:1 → 10:1), to obtain the corresponding triol (7.35 g) in 91% yield as a colorless powder. ^{1}H NMR (250 MHz, DMSO-d_6) δ: 3.65−3.90 (m, 5H), 4.15 (d, 1H, J = 6.1 Hz), 4.52 (d, 1H, J = 10.5 Hz), 4.59 (d, 1H, J = 10.5 Hz), 4.65−4.75 (m, 2H), 5.26 (d, 1H, J = 4.3 Hz), 6.95−7.1 (m, 4H), 7.15−7.25 (m, 1H), 7.3−7.45 (m, 7H), 7.78 (d, 2H, J = 8.8 Hz), 11.2 (s, 1H).

To a solution of the triol (6.0 g, 11.66 mmol) in MeOH (180 mL) was added 10% Pd−C (1.3 g), and the reaction mixture was stirred for 2.5 h at 45 °C under a hydrogen atmosphere. Then, the catalyst was removed by the filtration, and the filtrate was concentrated in in vacuo. The resulting mixture was purified by MPLC ($CHCl_3$/MeOH; 20:1 → 10:1 → 5:1) to obtain **1b** (3.76 g) in 76% yield as a colorless powder. mp 103.5−112 °C (dec) ; ^{1}H NMR (250 MHz, DMSO-d_6) δ: 3.5−3.95 (m, 5H), 4.13 (d, 1H, J = 6.5 Hz), 4.55−4.7 (m, 2H), 5.16 (d, 1H, J = 4.3 Hz), 7.04 (d, 2H, J = 8.8 Hz), 7.14 (d, 2H, J = 7.6 Hz), 7.25 (t, 1H, J = 7.3 Hz), 7.4−7.55 (m, 2H), 7.78 (d, 2H, J = 8.8 Hz), 8.76 (s, 1H), 10.56 (s, 1H).; MALDI-TOF MS: 425 $[M + H]^+$, 447 $[M + Na]^+$, 463 $[M + K]^+$; $[\alpha]_D$ 36° (c = 0.1, MeOH); Anal. ($C_{18}H_{20}N_2O_8S\cdot0.90H_2O$) C, H, N.

General Procedure for the Preparation of 2−5: (2*R*,3*R*,4*R*,5*R*)-3,4,5-Trihydroxy-1-(4′-methoxybenzenesulfonyl)piperidine-2-carboxylic Acid Hydroxy Amide (2). To a solution of compound **13ii** (777 mg, 1.93 mmol) in MeOH (40 mL) was added Muromac (6.0 g), and the mixture was stirred overnight at room temperature. The insoluble material was removed by filtration, and the filtrate was concentrated in vacuo. The resulting mixture was purified by MPLC ($CHCl_3$/MeOH; 30:1 → 7:1), and then the fraction was lyophilized to afford **2** (567 mg) in 81% yield. ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.55−3.73 (m, 3H), 3.73−3.90 (m, 5H), 4.10 (d, 1H, J = 6.6 Hz), 4.5−4.65 (m, 2H), 5.12 (bs, 1H), 7.03 (d, 2H, J = 8.9 Hz), 7.72 (d, 2H, J = 8.9 Hz), 8.84 (s, 1H), 10.55 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 48.4, 56.0, 57.0, 66.6, 68.0, 69.8, 114.4, 129.7, 131.7, 162.6, 165.3; MALDI-TOF MS: 385$[M + Na]^+$, 401$[M + K]^+$; Anal. ($C_{13}H_{18}N_2O_8S$) C, H, N.

(2*R*,3*R*,4*R*,5*R*)-1-(Biphenyl-4′-sulfonyl)-3,4,5-trihydroxypiperidine-2-carboxylic Acid Hydroxy Amide (3). (Yield 80%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.6−3.9 (m, 5H), 4.18 (d, 1H, J = 6.7 Hz), 4.64 (d, 1H, J = 5.9 Hz), 4.67 (d, 1H, J = 3.0 Hz), 5.19 (d, 1H, J = 4.5 Hz), 7.4−7.48 (m, 1H), 7.48−7.55 (m, 2H), 7.75 (d, 2H, J = 7.7 Hz), 7.83 (d, 2H, J = 8.5 Hz), 7.87 (d, 2H, J = 8.5 Hz), 8.79 (d, 1H, J = 1.9 Hz), 10.62 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 48.6, 57.1, 66.6, 68.0, 69.8, 127.4, 127.5, 128.2, 128.9, 129.6, 138.8, 139.0, 144.2, 165.3; MALDI-TOF MS: 471$[M + Na]^+$, 487$[M + K]^+$; Anal. ($C_{18}H_{20}N_2O_7S\cdot0.75H_2O$) C, H, N.

(2*R*,3*R*,4*R*,5*R*)-1-(4′-Benzyloxybenzenesulfonyl)-3,4,5-trihydroxypiperidine-2-carboxylic Acid Hydroxy Amide (4). (Yield 34% from **12iv**). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.55−3.67 (m, 2H), 3.70 (d, 1H, J = 12.7 Hz), 3.78 (s, 1H), 3.8−3.9 (m, 1H), 4.09 (d, 1H, J = 6.6 Hz), 4.55−4.65 (m, 2H), 5.0−5.25 (m, 3H), 7.12 (d, 2H, J = 8.8 Hz), 7.3−7.38 (m, 1H), 7.38−7.45 (m, 2H), 7.47 (d, 2H, J = 7.4 Hz), 7.73 (d, 2H, J = 8.8 Hz), 8.75 (s, 1H), 10.55 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 48.4, 57.0, 66.5, 68.0, 69.8, 70.1, 115.1, 128.4, 128.6, 129.0, 131.9, 136.8, 161.7, 165.3; MALDI-TOF MS: 461$[M + Na]^+$, 477$[M + K]^+$; Anal.($C_{19}H_{22}N_2O_8S\cdot1.30H_2O$) C, H, N.

(2*R*,3*R*,4*R*,5*R*)-1-(4′-But-2′-ynyloxybenzenesulfonyl)-3,4,5-trihydroxypiperidine-2-carboxylic Acid Hydroxy Amide (5). (Yield 55%). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 1.8−1.9 (m, 3H), 3.55−3.68 (m, 2H), 3.72 (d, 1H, J = 12.9 Hz), 3.75−3.9 (m, 2H), 4.10 (d, 1H, J = 6.7 Hz), 4.62 (bs, 2H), 4.75−4.9 (m, 2H), 5.14 (bs, 1H), 7.06 (d, 2H, J = 8.9 Hz), 7.74 (d, 2H, J = 8.9 Hz), 8.81 (s, 1H), 10.57 (s, 1H); MALDI-TOF MS: 423$[M + Na]^+$, 439$[M + K]^+$; Anal. ($C_{16}H_{20}N_2O_8S\cdot0.90H_2O$) C, H, N.

Shedding Assay for EGF Receptor Ligand−AP Fusion Protein. Expression vector of HB-EGF fused with human placental alkaline phosphatase (AP) fusion protein that was constructed as described previously was a generous gift from Dr. Higashiyama (School of Medicine, Osaka University, Osaka, Japan). The vector was transfected into HTI080 cells (American Type Culture Collection, Rockville, MD) by lipofection using a lipofectamine system (Gibco/BRL, Gaithersburg, MD) according to the manufacturer's directions. Stable transfectants were selected by growth in G418.

Stable transfectants expressing EGF receptor ligand−AP fusion protein in MEM (containing 10% FCS) as a culture medium were seeded in 96-well plates at a density of 2×10^5 cells/well and incubated for 24 h. The cells were washed with PBS and preincubated with test compounds in MEM (containing 1% DMSO) for 30 min. TPA (60 nM) was added to stimulate inducible processing, and the plate was incubated for 60 min. A 0.1 mL aliquot of the supernatant was transferred to 96-well plates and heated for 10 min at 65 °C in order to inactivate endogenous alkaline phosphatases. A 0.1 mL of substrate solution (1 M diethanolamine, 0.01% $MgCl_2$, 1 mg/mL *p*-nitrophenyl phosphate, pH 9.8) was added to each well, and the plates were incubated for 1−2 h. AP activity was then determined by the measurement of absorbance at 405 nm with a microplate reader. The IC_{50} value was determined with different inhibitor concentrations by using GraphPad Prism, version 3.0 (GraphPad Software, Inc.).

Expression and Purification of Human Recombinant MMPs. DNA fragments coding the catalytic domain of human MMP-1 and human MMP-9 and a DNA fragment coding from the prodomain to the catalytic domain of human MMP-3 were amplified by polymerase chain reaction (PCR) from $_C$DNA of HT1080 cells stimulated with 0.01 μM of TPA. A sequence for the appropriate restriction enzyme site was added to the 5′-end of each PCR primer. Amplified DNA fragments were cloned into a cloning vector and then introduced into a commercially available expression vector containing a His-6 tag sequence at the end of the N-terminus. Recombinant proteins were expressed in *E. coli* cells and purified by Ni-NTA resin (Qiagen, Inc.) and refolded. Recombinant MMP-3 was activated by incubating with 1 mM *p*-aminophenylmercuric acetate for 1 h at 37 °C.

MMP Inhibition Assay.[22] The assays were performed using the fluorogenic substrate MCA-Pro-Leu-Gly-Leu-DPA-Ala- Arg-NH_2 (Peptide Institute, Inc.) at a final concentration of 5 μM (MCA-Arg-Pro-Lys-Pro-Val-Glu-Nva-Trp-Arg-Lys-(Dnp)-NH_2 [Peptide Institute, Inc.] was used for MMP-3). Typical assays were performed as follows. In a well of a 96-well half-area black microplate (COSTAR), an enzyme solution (25 μL) was incubated with 25 μL of a test compound solution (10 mM DMSO stock solution was diluted to the appropriate

concentration with the assay buffer) in 50 mM Tris, 150 mM NaCl, 10 mM $CaCl_2$, and 0.05% Brij-35, pH 7.5, for 10 min at 37 °C. Then the reaction was initiated by adding 50 μL of the substrate solution to the 96-well plate, and incubation was continued for 2 h at 37 °C (3 h for MMP-9). The increase in fluorescence due to cleavage of the substrate was measured with a fluorescence microplate reader (excitation/emission = 320/405 nm, Polarstar; BMG LabTechnologies, Germany). K_i values were calculated by nonlinear regression analysis using the percent inhibition, and K_m values of the substrates for each MMP with the program GraphPad Prism (GraphPad Software, Inc., San Diego, CA).

TACE Inhibition Assay.[23] The DNA fragment coding signal region, prodomain and catalytic domain of TACE, was amplified by polymerase chain reaction from pBluescriptII-TACE clone (cloned a full length cDNA for TACE from human acute monocytic leukemia cell line, THP-1) as the template. The 5′-end of each PCR primer was added to a sequence for the restriction enzyme site, and the FLAG tag sequence was also added to the reverse primer. The amplified DNA fragment was cloned into pFastBac-1 transfer vector (Life Technologies, Rockville, MD), and recombinant bacmid was isolated, purified, and then used to generate baculovirus particle in Sf9 insect cells (Pharmingen, San Diego, CA). Logarithmically growing Sf9 cells were infected with TACE baculovirus at a MOI1. Conditioned media were harvested at 96 h after infection. The recombinant TACE (rTACE) was purified from the medium with an anti-FLAG M2 affinity gel column (Sigma, St. Louis, MO). Purified rTACE had an approximately 90% purity.

The rTACE (final 50ng/mL) was mixed with the compound solution and incubated at 37 °C for 10 min in a reaction buffer (20 mM Tris-HCl (pH 7.5) containing 0.05% Brij-35). The reaction was initiated by addition of 5 μM (final concentration) of fluorescence-quenching peptide substrate (MCA-Pro-Leu-Ala-Glu-Ala-Val-DPA-Arg- Ser-Ser-Ser-Arg-NH_2; Bachem AG, Switzerland), which contained the cleavage site of TNF-a, and the increase of fluorescence intensity (Ex/Em = 320/405 nm) was monitored. K_i values were calculated from the substrate by using GraphPad Prism, version 3.0 (GraphPad Software, Inc., San Diego, CA).

Inhibition Assay for TPA-Induced Epidermal Hyperplasia Model. Backs of mice were shaved by an electric clipper and treated with depilatory cream (Eva cream, Tokyo Tanabe, Tokyo). Three days later, the mice that displayed no evidence of hair regrowth were used for experiments. A total of 20 μL of TPA (1 nM) or vehicle (acetone) was applied to the skin surface of the backs in an area of ca. 1 cm^2 using a micropipet. A minute after TPA application or 1 h prior to TPA application, a test compound or vehicle (acetone) was topically administrated to the same area where it was TPA-treated (day 0). The treatment of the test compound or vehicle was repeated on days 1 and 2. On day 3, the mice were sacrificed, and skin tissues treated with TPA-test compound were removed. The tissue specimen was made and stained with hematoxylin-eosin. The epidermal thickness was measured as the distance from the bottom of the stratum corneum to the bottom of the basal layer using an ocular microscope with a magnification of 400×. The measurement was performed at 10 different fields within a width of 6 mm, and the mean was calculated.

Acknowledgment. The present work was supported by a grant for 'Research and Development on Glyco-cluster Controlling Biomolecules' from the New Energy and Industrial Technology Development Organization (NEDO).

References

(1) Thacher, S. M.; Vasudevan, J.; Tsang, K.-Y.; Nagpal, S.; Chandraratna, R. A. S. New Dermatological Agents for the Treatment of Psoriasis. *J. Med. Chem.* **2001**, *44*, 1.

(2) Ashcroft, D. M.; Po, A. L. W.; Griffiths, C. E. M. Thrapeutic strategy for psoriasis. *J. Clin. Pharm. Ther.* **2000**, *25*, 1.

(3) Shoyab, M.; Plowman, G. D.; McDonald, V. L.; Bradley, J. G.; Todaro, G. J. Structure and function of human amphiregulin: a member of the epidermal growth factor family. *Science* **1989**, *243*, 1074.

(4) (a) Higashiyama, S.; Abraham, J. A.; Miller, J.; Fiddes, J. C.; Klagsbrun, M. A. heparin-binding growth factor secreted by macrophage-like cells that is related to EGF. *Science* **1991**, *251*, 936. (b) Raab, G.; Klagsburn, M. Heparin-binding EGF-like growth factor. *Biochim. Biophys. Acta* **1997**, *1333*, F179−F199.

(5) Cook, P. W.; Piepkorn, M.; Clegg, C. H,; Plowman, G. D.; DeMay, J. M.; Brown, J. R.; Pittekknow, M. R. Transgenic Expression of the Human Amphregulin Gene Induces a Psoriasis-like Phenotype. *J. Clin. Invest.* **1997**, *100*, 2886.

(6) Stoll, S.; Garner, W.; Elder, J. Heparin-binding Ligands Mediate Autocrine Epidermal Growth Factor Receptor Activation In Skin Organ Culture. *J. Clin. Invest.* **1997**, *100*, *1271*.

(7) Piepkorn, M.; Pittekknow, M. R.; Cook, P. W. Autocrine Regulation of Keratinocytes: The Emerging Role of Heparin-Binding, Epidermal Growth Factor-Related Growth Factors. *J. Invest. Dermatol.* **1998**, *111*, 715.

(8) Tokumaru, S.; Higashiyama, S.; Endo, T.; Nakagawa, T.; Miyanaga, J.; Yamamori, K.; Hanakawa, Y.; Ohmoto, H.; Yoshino, K.; Shirakata, Y.; Matsuzawa, Y.; Hashimoto, K.; Taniguti, N. Ectodomain Shedding of Epidermal Growth Factor Receptor Ligands Is Required for Keratinocyte Migration in Cutaneous Wound Healing. *J. Cell. Biol.* **2000**, *151*, 209.

(9) Arribas, J.; Borroto, A. Protein Ectodomain Shedding. *Chem. Rev.* **2002**, *102*, 4627.

(10) Prenzel, N.; Zwick, E,; Daub, H.; Leserer, M.; Abraham, R.; Wallasch, C.; Ullrich, A. EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **1999**, *402*, 884.

(11) Asakura, M.; Kitakaze, M.; Takashima, S.; Liano, Y.; Ishikura, F.; Yoshinaka, T.; Ohmoto, H.; Node, K.; Yoshino, K.; Ishiguro, H.; Asanuma, H.; Sanada, S.; Matsumura, Y.; Takeda, H.; Beppu, S.; Tada, M.; Hori, M.; Higashiyama, S. Cardiac hypertrophy is inhibited by antagonism of ADAM12 processing of HB-EGF: Metalloproteinase inhibitors as a new therapy. *Nature Med.* **2002**, *8*, 35.

(12) Lanzrein, M.; Grarred, O.; Olsnes, S.; Sandvig, K. Diphtheria toxin endocytosis and membrane translocation are dependent on the intact membrane-anchored receptor (HB-EGF receptor): studies on the cell-associated receptor cleaved by a metalloproteinase in phorbol-ester-treated cells. *Biochem. J.* **1995**, *310*, 285.

(13) (a) Sawa, M.; Tsukamoto, T.; Kiyoi, T.; Kurokawa, K.; Nakajima, F.; Nakada, Y.; Yokota, K.; Inoue, Y.; Kondo, H.; Yoshino, K. New Strategy for Antedrug Application: Development of Metalloproteinase Inhibitors as Antipsoriatic Drugs. *J. Med. Chem.* **2002**, *45*, 930. (b) Yoshiizumi, K.; Yamamoto, M.; Miyasaka, T.; Ito, Y.; Kumihara, H.; Sawa, M.; Kiyoi, T.; Yamamoto, T.; Nakajima, F.; Hirayama, R.; Kondo, H.; Ishibushi, E.; Ohmoto, H.; Inoue, Y.; Yoshino, K. Synthesis and Structure−Activity Relationships of 5,6,7,8-Tetrahydropyrido[3,4-b]pyrazine-based Hydroxamic Acids as HB-EGF Shedding Inhibitors. *Bioorg. Med. Chem.* **2003**, *11*, 433.

(14) (a) Zook, S. E.; Dagnino, R. Jr.; Deason, M. E.; Bender, S. L.; Melnick, M. J. Metalloproteinase Inhibitors, Pharmaceutical Compositions Containing Them and Their Pharmaceutical Uses, and Methods and Intermediates Useful For Their Preparation. PCT Int. Appl. WO9720824, 1997. (b) Cheng, M.; De, B.; Pikul, S.; Almsread, N. G.; Natchus, M. G.; Anastasio, M. V.; McPhail, S. J.; Snider, C. E.; Twaio, Y. O.; Chen, L.; Dunaway, M.; Gu, F.; Dowty, M. E.; Mieling, C. E.; Mieling, G. E.; Janusz, M. J.; Wang-Weigand, S. Design and Synthesis of Piperazine-Based Matrix Metalloproteinase Inhibitors. *J. Med. Chem.* **1999**, *42*, 2, A. (c) Almstead, N. G.; Bradley, R. S.; Pikul, S.; De, B.; Natchus, M. G.; Taiwo, Y. O.; Gu, F.; Williams, L. E.; Hynd, B. A.; Janusz, M. J.; Dunaway, C. M.; Mieling, G. E. Design, Synthesis, and Biological Evaluation of Potent Thiazine- and Thiazepine-Based Matrix Metalloproteinase Inhibitors. *J. Med. Chem.* **1999**, *42*, 4547. (d) Whittaker, M.; Floyd, C. D.; Brown, P. Gearing, A. J. T. Design and Therapeutic Application of Matrix Metalloproteinase Inhibitors. *Chem. Rev.* **1999**, *99*, 2735.

(15) (a) Moriyama, H.; Tsukida, T.; Inoue, Y.; Kondo, H.; Yoshino, K.; Nishimura, S.-I. Structure−Activity Relationships of Azasugar Based MMP/ADAM Inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2737. (b) Moriyama, H.; Tsukida, T.; Inoue, Y.; Kondo, H.; Yoshino, K.; Nishimura, S.-I. Design, Synthesis and Evaluation of Novel Azasugar- Based MMP/ADAM Inhibitors. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2741.

(16) Compound **2** bearing methoxy group was also stable in pH 7.4 solution and distilled water after 1 week.

(17) (a) Csuka, R.; Hugener, M.; Vassela, A. A New Synthesis of *N*-Acetylneuraminic Acid. *Helv. Chim. Acta* **1988**, *71*, 609. (b) Lee, B. W.; Jeong, I.-Y.; Yang, M. S.; Choi, S. U.; Park, K. H. A Short and Efficient Synthesis of 2*R*,3*R*,4*R*-3,4-Dihydroxyproline, 1,4-Dideoxy-1,4-imino-L-xylitol, 2*R*,3*R*,4*R*-3,4,5-Trihydroxypipecolic Acid, and 1,5-Dideoxy-1,5-imino-L-iditol. *Synthesis* **2000**, 1305.

(18) D-Mannono-1,4-lactone is commercial available from Tokyo Kasei Kogyo Co., Ltd.

(19) Xiao, X.; Bai, D. An Efficient and Selective Method for Hydrolysis for Acetonide. *Synlett* **2001**, 535.
(20) (a) Yamamoto, M.; Tsujishita, H.; Hori, N.; Ohishi, Y.; Inoue, S.; Ikeda, S.; Okada, Y. Inhibition of Membrane-Type 1 Matrix Metalloproteinase by Hydroxamate Inhibitors: An Examination of Subsite Pocket. *J. Med. Chem.* **1998**, *41*, 1209. (b) Lovejoy, B.; Welch, A. R.; Carr, S.; Luong, C.; Broka, C.; Hendricks, R. T.; Campell, J. A.; Walker, K. A. M.; Martin, R.; Van Wart, H.; Browner, M. F. Crystal structures of MMP-1 and -13 reveal the structural basis for selectivity of collagenase inhibitors. *Nat. Struct. Biol.* **1999**, *6*, 217.
(21) (a) Davidson, A. N.; Backer, R. C. Novel use of Matrix Metalloproteinase Inhibitors. PCT Int. Appl. WO9847494, 1998. (b) Holleran, W. M.; Galardy, R. E.; Gao, W. N.; Levy, D.; Tang, P. C.; Elias, P. M. Matrix Metalloproteinase Inhibitors reduce phorbol ester-induced cutaneous inflammation and hyperplasia. *Arch. Dermatol. Res.* **1997**, *289*, 138.
(22) Nagase, H.; Fields, C. G.; Fields, G. B. Design and Characterization of a Fluorogenic Substrate Selectively Hydrolyzed by Stromelysin 1 (Matrix Metalloproteinase-3). *J. Biol. Chem.* **1994**, *269*(33), 20952.
(23) Van Dyk, D. E.; Marchand, P.; Bruckner, R. C.; Fox, J. W.; Jaffee, B. D.; Gunyuzlu, P. L.; Davis, G. L.; Nurnberg, S.; Convington, M.; Decicco, C. P.; Trzaskos, J. M.; Magolda, R. L.; Copeland, R. A. Comparison of Snake Venom Reprolysin and Matrix Metalloproteinases as Models of TNF-α Converting Enzyme. *Bioorg. Med. Chem. Lett.* **1997**, *7*(10), 1219.

JM0304313