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Divergent Synthesis of 4-*epi*-Fagomine, 3,4-Dihydroxypipecolic Acid, and a Dihydroxyindolizidine and Their β-Galactosidase Inhibitory and Immunomodulatory Activities

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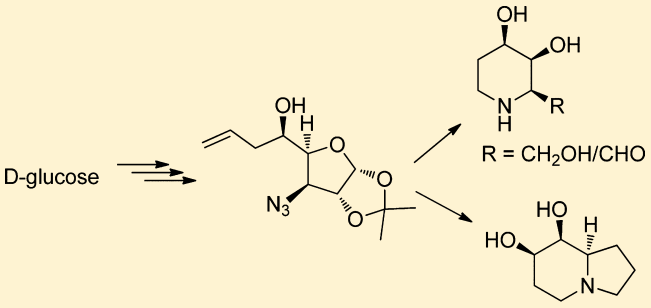
Supporting Information

ABSTRACT: A divergent asymmetric synthesis of the titled iminosugars has been formulated starting from a chiral homoallyl alcohol as the versatile intermediate. The homoallyl alcohol was prepared by a highly diastereoselective Barbier reaction on a D-glucose-derived aldehyde. The protection of its hydroxyl function followed by reductive ozonolysis of the olefin and a subsequent one-pot three-step protocol involving a Staudinger reaction, reductive amination, and benzyloxy carbonyl protection yielded an important bicyclic furanopiperidine derivative. This was converted to the target compounds by following standard reactions. Among the synthesized compounds, 4-*epi*-fagomine (**2b**) was the best β-galactosidase inhibitor, and it also prevented LPS-mediated activation of Raw 264.7 macrophage cells. Its congener, 3,4-dihydroxypipecolic acid (**4b**) also showed similar trends in its cytokine- and enzyme-inhibitory properties at a low concentration (10 μM) but was proinflammatory at higher concentrations. The bicyclic compound dihydroxyindolizidine (**21**) reduced the proinflammatory cytokine (IL-1β and TNF-α) levels in the LPS-activated Raw 264.7 cells without showing any enzyme-inhibition activity.



INTRODUCTION

Aliphatic nitrogen-containing piperidines and pyrrolidines with polyhydroxy pendants, also known as iminosugars, have attracted significant interest among chemists and biologists.[1] Because of their structural similarity with sugars, polyhydroxylated heterocycles can act as potent substrate mimics for a variety of glycosidases; hence, they have found importance as diagnostic and therapeutic agents. Some of the well-known naturally occurring iminosugars include 1-deoxynojirimycin (**1**),[2a] fagomine (**2a**),[2a] isofagomine (**3**),[2a−c] and hydroxypipecolic acids (**4a** and **4b**)[2d] as well as their alkyl derivatives such as compound **5**,[2e,f] castanospermine (**6**),[2g] swainsonine (**7**),[2h] and so forth (Figure 1).

Various stereomers of nojirimycin and its analogues are naturally occurring. Its trihydroxy congener D-fagomine **2a** has been isolated from the seeds of the Japanese buckwheat *Fagopyrum esculentum* austral Moench as well as *Castanospermum australe* (Leguminosae), whereas two other stereomers **2c** and **2d** occur in *Xanthocersis zambesiaca* leaves and roots.[2i] The number and stereochemical disposition of their hydroxyl groups largely decides their selectivity in inhibiting different glycosidases as well as their therapeutic potential.[3a−d] For example, compounds **6** and **7** and their derivatives possess antiviral, antitumor, and immunomodulatory activities.[4a−d] Likewise, compounds **1**, **2a**, and **3** show inhibitory activity against human α-glucosidase, mammalian α-glucosidase/β-galactosidase, and liver glycogen phoshorylase.[2a] Interestingly, non-natural structural isomer 4-*epi*-fagomine **2b** is a good inhibitor of lysosomal α-galactosidase A in Fabry lymphoblasts.[4e,f] Pipecolic acid and its derivatives possess anesthetic,[5a] NMDA antagonist,[5b] anticoagulant,[5c] and glycosidase inhibitory activities.[5d] Additionally, the unnatural, nonproteinogenic amino acids are potential water-soluble auxiliaries for use in native chemical ligation (NCL).[6] Furthermore, the pipecolic acid unit is often used as a ring-expanded homologue of proline for conformational and ligand-binding studies in biologically active peptides and foldamers.[7]

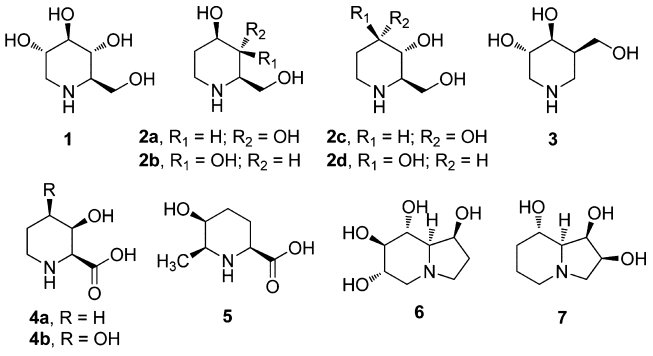


Figure 1. Chemical structures of some iminoalditols, bicyclic alkaloids, and pipecolic acids.

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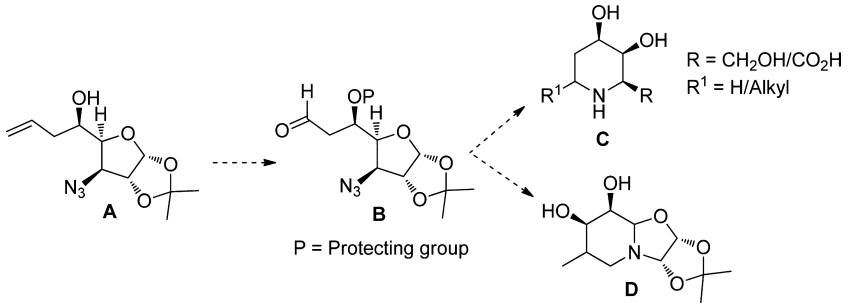
Scheme 1. Diversity-Oriented Approach to the Syntheses of Iminosugars



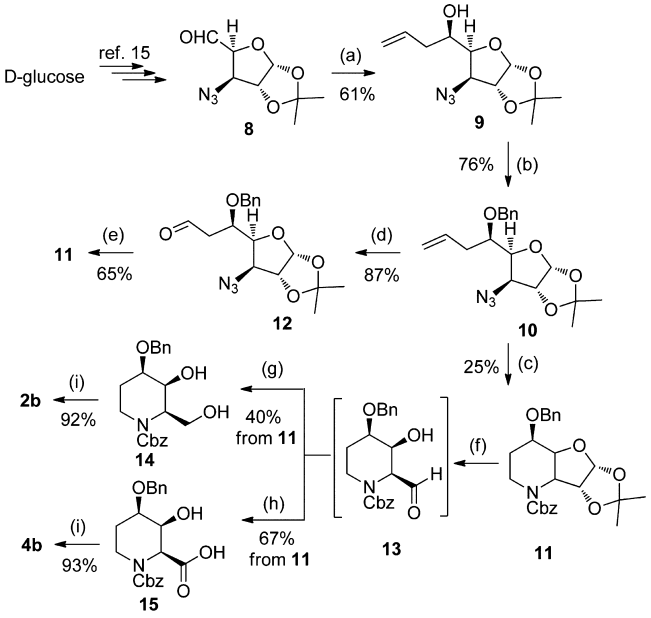
All of these properties make them interesting synthesis targets. Because of their limited natural occurrence, increasing the availability of these molecules with different substitution patterns and stereochemistry would help to establish the structure–activity relationships that are important for their biological effects. Hence, a number of different hydroxyl-substituted and ring-expanded iminosugar analogues were synthesized over the past decades using carbohydrate and noncarbohydrate substrates as well as biocatalytic routes.[8] However, these target-specific syntheses may not meet the demand of skeletal and stereochemical diversities required for discovery of new drugs. More flexible, universal, and efficient methods leading to several targets with shorter synthesis routes are desired. Designing a common strategy for the synthesis of various bioactive molecules always has a vital significance in organic synthesis. To this end, diversity-oriented synthesis (DOS)-based protocols that use the same precursor to produce compounds with both structural and stereochemical diversities are appealing.[9] As a part of our interest in the polyhydroxy piperidines,[10] presently we have attempted to devise such a DOS strategy for this class of iminosugars.

We envisioned an entity of type **A** (Scheme 1) as a versatile intermediate for easy access to these compounds. This design was surmised while keeping in mind the general substitution patterns of azasugars. Cleavage of the alkene function of **A** would generate azido aldehyde **B**, which, as such or after converting to a ketone, can be subjected to reductive amination to furnish various polyhydroxy piperidine compounds **C**. However, the aldehydes derived from the acetonide moiety can be homologated and subsequently cyclized to afford bicyclic alkaloid skeleton **D**. To illustrate the synthesis strategy, we formulated a diastereoselective synthesis of the type **A** intermediate and used it for the concise total syntheses of 4-*epi*-fagomine **2b**, pipecolic acid analogue **4b**, and bicyclic iminoalditol **18**. Among these, pipecolic acid and its derivatives are attractive targets.[11] The glycosidase inhibitory activity of pipecolic acid derivatives can be tuned by altering the substitutions, as reflected in the moderate activity of a synthetic alkyl derivative of the acid.[12] Hence, we have synthesized **4b** as a representative compound of this class because very few syntheses of a dihydroxypipecolic acid are reported.[13] Bicyclic indolizidine alkaloid **18** has been designed as a hybrid of naturally occurring lentiginosine and swainsonine for studying its glycosidase inhibitory property.

RESULTS AND DISCUSSION

During the past several years, our group has used the furanose form of D-glucose as the template in asymmetric syntheses.[8g,10,14] The present synthesis started with known azido aldehyde **8**, prepared in 50–57% overall yield from D-glucose, by a reported procedure involving six steps with chromatographic purification in all of the steps.[15] We are routinely synthesizing **8** on the ~25 g scale. As shown in Scheme 2, a Zn-mediated Barbier-type reaction of aldehyde **8** with allyl bromide in moist THF afforded homoallylic alcohol **9** as the major isomer in a 95:5 diastereomeric ratio (determined from the ^{1}H NMR of the reaction mixture).[16] The observed selectivity in favor of **9** could be due to the weak chelating effect of allyl zinc with the furanose ring oxygen and carbonyl group of **8**. Danishefsky et al. have reported that the reactive conformation in a xylose derivative similar to **8** exhibits a dihedral angle of 157.4° between the carbonyl group and the C–O bond, which makes α-face attack the most favorable in the absence of any strong chelation.[17] This was also validated by the fact that the

Scheme 2. Synthesis of 4-*epi*-Fagomine and a Pipecolic Acid Analogue[a]



[a](a) Allyl bromide, Zn, THF, 0 to 20°C, and 7 h. (b) BnBr, NaH, THF, 0 to 25 °C, and 4 h. (c) (i) O_3, CH_2Cl_2, −78 °C, then PPh_3, −78 to 25 °C, 12 h; (ii) $NaBH_3CN$, MeOH, and AcOH (cat.); (iii) CbzCl, $NaHCO_3$, MeOH, 0 to 25 °C, and 4 h. (d) O_3, CH_2Cl_2, −78 °C, then Me_2S, −78 to 25 °C, and 24 h. (e) (i) PPh_3, MeOH, 0 to 25 °C, and 1 h; (ii) $NaBH_3CN$, MeOH, AcOH (cat.), 0 °C, and 2 h, then 25 °C, and 8 h; (iii) CbzCl, $NaHCO_3$, MeOH, 0 to 25 °C, and 6 h. (f) (i) TFA–H_2O (3:2), 0 °C, and 1 h; (ii) $NaIO_4$, acetone–water (4:1), 0 °C, and 30 min. (g) $NaBH_4$, MeOH–H_2O (9:1), 0 °C, and 20 min. (h) NaH_2PO_4, $NaClO_2$, 30% H_2O_2, CH_3CN, 0 to 25 °C, and 7 h. (i) H_2 (80 psi), 10% Pd/C, MeOH, 25 °C, and 12 h.

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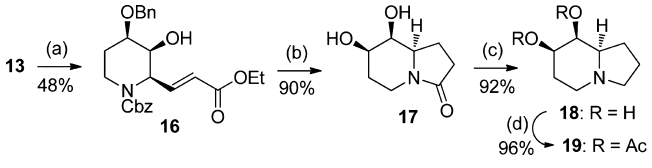
reaction of the Grignard reagent/allyl trimethyl silyl−BF_3 with a xylose derivative yielded the 5S-isomer predominantly.

Reaction of **9** with BnBr in THF in the presence of NaH as the base afforded 5-*O*-benzyl derivative **10** in 76% yield. 5-*O*-Benzyl homoallyl substrate **10** was transformed to key bicyclic compound **11** in a one-pot four-step process in moderate (25%) yield. This involves the ozonolysis of **10** followed by reduction of the resultant malozonide and azide functionalities with PPh_3 to an intermediate, which undergoes reductive amination with $NaBH_3CN$ followed by protection with benzyl chloroformate. To improve the yield of **11**, we carried out the ozonolysis of **10** and reduced the ozonide with Me_2S to isolate aldehyde **12** in 87% yield. Subsequent reduction of the azide functionality of **12** followed by in situ reductive amino cyclization and Cbz protection afforded **11** in 65% yield over three steps. Besides improving the yield of **11**, the second strategy was more advantageous for the following reason. We found considerable degradation of compounds **9** and **10** on storage even at a low temperature, which is possibly due to their azide and olefin functionalities. In contrast to this, aldehyde **12** is stable and can be stored for >1 month at 0 °C. The 2D-ROESY study of **11** showed a long-range coupling of H-5 with H-4 and H-3, revealing its 5R configuration (Figure S11). This also confirmed the assigned configuration of the newly generated center of **9**.

With important precursor **11** in hand, we completed the synthesis of 4-*epi*-fagomine **2b**. Thus, the 1,2-acetonide functionality in **11** was carefully deprotected using trifluoroacetic acetic acid (TFA)−water (3:2), and the intermediate diol was subjected to oxidative cleavage with $NaIO_4$ to afford N-protected amino aldehyde **13**, which is a common intermediate for the synthesis of both **2b** and **4b**. In view of its instability, without purification crude amino aldehyde **13** was subjected to $NaBH_4$ reduction in MeOH−water (9:1) to yield **14** in 40% yield over three steps from **11**. In the final step, catalytic hydrogenation of **14** under pressure (80 psi) over 10% Pd/C in MeOH at room temperature afforded 4-*epi*-fagomine **2b** as a semisolid in 92% yield. The analytical and spectral data of **2b** were found to be in agreement with the reported data.[18] For the synthesis of pipecolic acid derivative **4b**, the aldehyde group in **13** was oxidized with $NaClO_2$, 30% H_2O_2, and NaH_2PO_4 to afford corresponding acid **15** in 67% yield over three steps from **11**. Catalytic hydrogenation of **15** as above gave new dihydroxy pipecolic acid analogue **4b** (Scheme 2).[12]

The synthesis (Scheme 3) of bicyclic iminoalditol **18** commenced from aldehyde **13**, which upon a Wittig−Horner reaction with triethyl phosphonoacetate in the presence of NaH in THF afforded E-ester **16** as the only isolated product.[19] Although of no consequence to the synthesis, the E geometry of the ester was confirmed from the ^{1}H NMR spectrum.

Scheme 3. Synthesis of Dihydroxy Indolizidine[a]



[a](a) $(Eto)_2P(O)CH_2CO_2Et$, NaH, THF, −40 °C, 10 min, −20 °C, and 5 h. (b) $HCOONH_4$, 10% Pd−C, MeOH, reflux, and 4 h. (c) $LiAlH_4$, THF, 0 °C to reflux, and 2 h. (d) Ac_2O, Py, DMAP, 0 to 30 °C, and 6 h.

Catalytic hydrogenation of ester **16** with $HCOONH_4$ over 10% Pd/C in MeOH under reflux directly furnished amide **17** via the simultaneous deprotection of the alcohol and amine functions, saturation of the alkene moiety, and reductive amino cyclization. Reduction of amide **17** with $LiAlH_4$ proceeded uneventfully to give amine **18**. This was further acetylated to furnish diacetate **19** in 96% yield.

Conformational Studies. It is known that 1-deoxycastanospermine adopts a $^8C_5$ (**I**) conformation, whereas its 8a-epi-isomer takes an $^5C_8$ (**II**) conformation (Figure 2.).

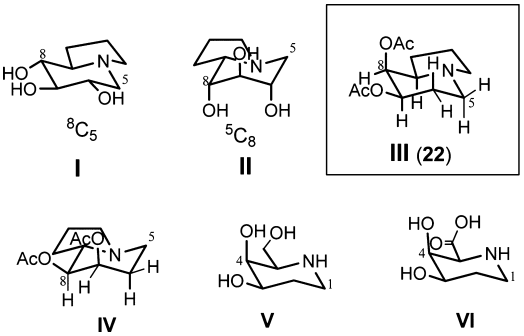


Figure 2. Conformation of indolizidines and piperidines.

Hence, it was of interest to establish the conformation of new indolizidine **18** in solution. As a hybrid of natural lentiginosine and swainsonine, compound **18** can exist in conformation **III** or **IV**. Because the ^{1}H NMR spectroscopic pattern of **18** did not provide much conformational information, it was converted to acetate **19** to analyze its conformation using 2D-COSY spectroscopy. The ddd at δ 4.85 with J = 12.0, 4.9, and 3.2 Hz in the ^{1}H NMR spectrum of **19** was assigned to its H7 proton. The large coupling constant of 12.0 Hz suggested a diaxial orientation of the H7a and H6a protons. The small coupling constant of 4.9 Hz was attributed to an axial−equatorial coupling between H7a and H6e, whereas the smallest coupling constant of 3.2 Hz pointed to an axial−equatorial relation between H7a and H8e protons. Taken together, the 2D-COSY results revealed that new indolizidine(s) **19** (and **18**) adopt(s) the $^8C_5$ conformation (**III**), which is a favored conformation of bicyclic-D-iminosugars. From this, the most common $^4C_1$ conformation was assigned for **2b** and **4b**.

Biological Activities. None of compounds **2b**, **4b**, and **18** (10, 50, 100, 250, and 500 μM) inhibited the proliferation of the human non-small-cell lung cancer (A549), osteosarcoma (U2-OS), and histiocytic lymphoma (U937) cells significantly, as revealed by the MTT reduction assay.[20] At a much higher concentration (5 mM), only **4b** showed a 27% inhibition of the proliferation of A549 cells. Insulin resistance, a major characteristic of numerous metabolic disorders such as viral or bacterial infections, diabetes, and cancer, is induced resulting from alterations in several glycoproteins. Earlier, increased serum levels of β-galactosidase and β-hexosaminidase were observed in diabetic rats,[21a] and these levels were reduced by insulin.[21b] These findings ascertained the key roles of these enzymes in diabetes that was also confirmed in diabetic patients.[21c] Hence, we assessed the β-D-galactosidase inhibitory property of compounds **2b**, **4b**, and **18** at different concentrations (1.7, 3.4, 5.1, 6.8, and 8.5 mg/mL), and the results are summarized in Table 1.

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Table 1. Concentration-Dependent β-D-Galactosidase Inhibitory Property of 2b, 4b, and 18[a]

	enzyme inhibition concentration (mg/mL)				
compound	1.7	3.4	5.1	6.8	8.5
2b	33.0[b]	32.3[b]	41.0[b]	49.3[b]	50.8[b]
4b	0	4.9	24.4[b]	−8.3	−22.1[b]
18	5.9	3.9	−0.4	−2.2	−5.4

[a]Enzyme inhibition is expressed in percentage, considering the control value as zero. The data (mean ± SEM) of three independent experiments, each repeated three times were analyzed by paired *t* test and one-way analysis of variance (ANOVA). [b]$p < 0.001$ compared to control.

Compound **2b** showed a dose-dependent enzyme inhibition at up to 6.8 mg/mL; beyond this concentration it reached a plateau. Even at a low concentration (1.7 mg/mL), it showed good (33%) inhibition that increased to ∼50% at 6.8 mg/mL (46.3 mM). Compound **4b** showed moderate activity (24.4%) at 5.1 mg/mL (31.7 mM), whereas bicyclic iminoalditol **18** was almost inactive. In general, monocyclic azasugars resembling D-galactose are good inhibitors of β-galactosidase.[22] Consistent with this, compounds **2b** and **4b** showed moderate-to-good β-galactosidase inhibitory property, whereas bicyclic iminosugar **18** was ineffective. Our results with 4-*epi*-fagomine **2b** and its oxidized analogue **4b** are noteworthy given the reported weak β-galactosidase inhibition by D-3,4-*epi*-fagomine and D-3-*epi*-fagomine analogues.[23a] The poor glycosidase inhibition by bicyclic azasugars was reported previously.[23b,c] To the best of our knowledge, the biphasic β-galactosidase inhibitory property of compounds **4b** and **18** is unprecedented among the azasugars. Only some aliphatic alcohols and the phytoestrogen genistein were reported to show similar behavior.[24a,b]

Cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 may modulate glucose homeostasis and alter insulin resistance.[25] Hence, the effect of compounds **2b**, **4b**, and **18** on cytokine production was examined using lipopolysaccharide (LPS)-treated RAW 264.7 macrophage cells as the model system. Endotoxins such as LPS induce cytokine production via macrophage activation to contribute to the

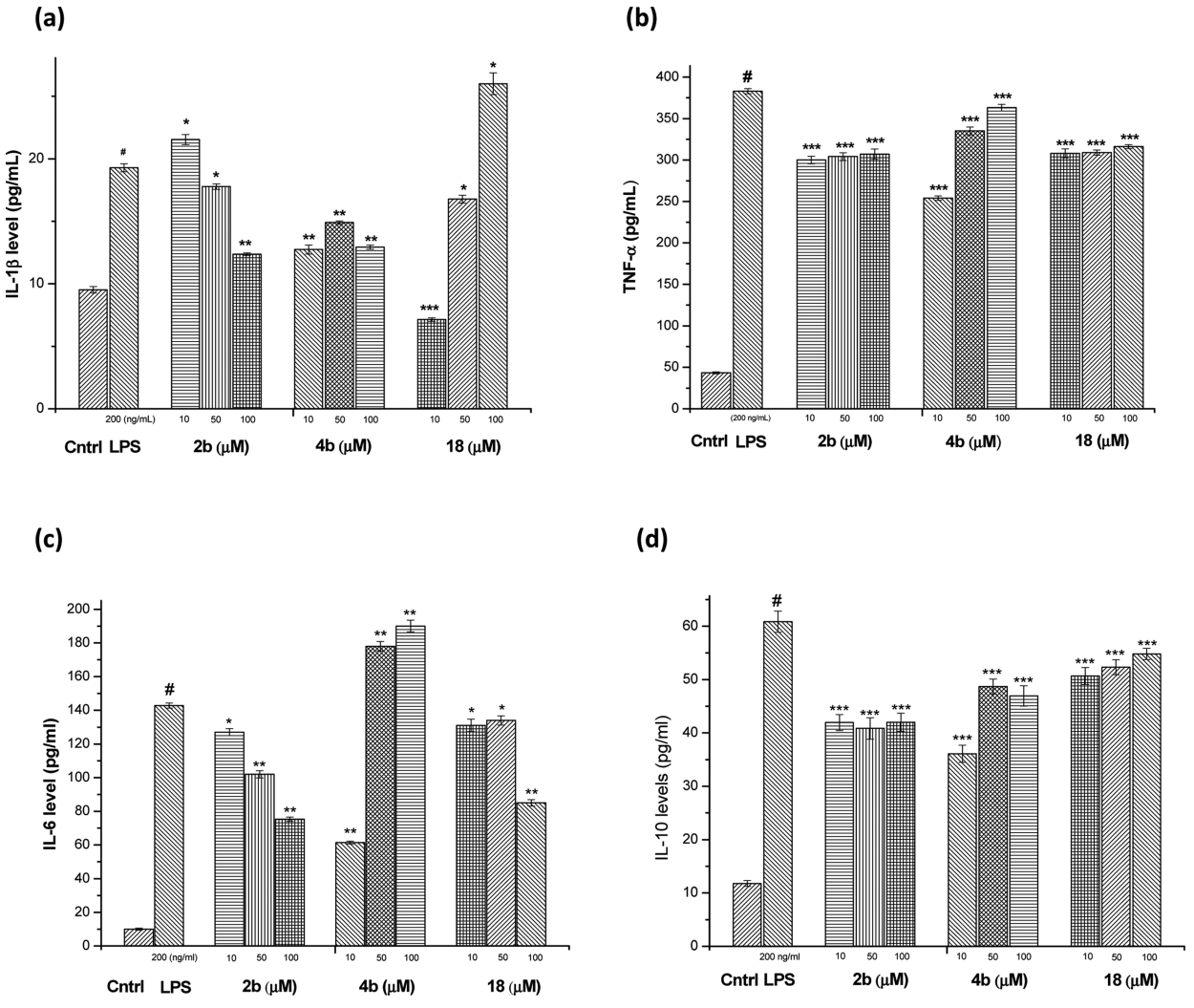

Figure 3. Concentration-dependent effect of the test compounds on LPS-stimulated production of Th1 and Th2 cytokines in RAW 264.7 macrophage cells. The cells (untreated or pretreated with different concentrations of test compounds for 1 h) were incubated with LPS (200 ng/mL) for 16 or 24 h, and the cytokines were analyzed by ELISA. The values are the mean ± SEM of three independent experiments. #$p < 0.001$ compared to normal and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ compared to LPS treatment. The cells that were not treated with LPS or the compounds are designated as Cntrl.

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inflammatory process.[26a] Under in vivo conditions, LPS stimulates macrophages that play a central role in immune response.[26b] Macrophages express Th1 cytokines TNF-α, interferon (INF)-γ, and the ILs (IL-6, IL-2, and IL-1β) as well as Th2 cytokines IL-4 and IL-10. The balance between Th1 and Th2 cytokines defines the nature of the immune response.

Initially we carried out time-dependent experiments to determine the time point at which the maximum concentration of the individual cytokines after the LPS treatment is present (complete data not shown). The optimum time points were 16 h for TNF-α and IL-1β and 24 h for IL-6 and IL-10. LPS treatment increased the levels of TNF-α (8.8-fold), IL-1β (2-fold), IL-6 (14.3-fold), and IL-10 (5.2-fold) significantly compared to the levels produced by the control cells. Compounds **2b** and **18** suppressed an increase in the TNF-α level by ∼20−22% at all of the doses tested. Compound **4b** (10 μM) suppressed an increase in the TNF-α level by 33.7% but was much less effective at higher concentrations. Compound **2b** showed a dose-dependent suppression of the IL-1β level, with the maximum reduction (35.2%) at 100 μM. Compound **4b** suppressed the IL-1β level by 33.9% at all of the tested concentrations. However, compound **18** showed a biphasic behavior, suppressing the IL-1β level by 63% at 10 μM, but its efficacy was reduced thereafter. With regard to IL-6, compound **2b** showed a dose-dependent suppression (12−47%), whereas **18** was effective only at 100 μM and reduced the cytokine level by 40%. Compound **4b** suppressed the IL-6 level by 57% at 10 μM, augmenting it thereafter. Both compounds **2b** and **4b** suppressed the IL-10 level by ∼30−33% at all of the tested concentrations, but **18** was much less effective (10−12% suppression). The results are shown in Figure 3a−d. In separate experiments, the treatment of the cells with LPS (200 ng/mL), with the compounds alone (10, 50, and 100 μM), or with combinations of these did not alter the cell viability, as revealed by an MTT assay (data not shown). This indicated that the suppression of the cytokine levels by the compounds was unrelated to their cytotoxicity. Although cytokine imbalance is associated with diabetes, the precise roles of the Th1 and Th2 cytokines is far from clear.[27] Circulating levels of IL-6 are elevated in type-2 diabetic patients, are correlated with insulin sensitivity, and may predict the development of diabetes.[28ac] However, IL-10 accelerated the development of diabetes in nonobese diabetic (NOD) mice in some studies[29a] but prevented diabetes in others.[29b]

It was observed that compound **2b** reduced the Th1 and Th2 cytokines, suggesting a suppression of LPS-induced macrophage activation. This may reduce the immune cell-mediated beta cell destruction that plays the most vital role in type 2 diabetes. On the basis of these results along with its β-galactosidase inhibitory property, compound **2b** may be a potential antidiabetic agent. Compound **4b** showed similar activity at a low concentration (10 μM) but induced several pro-inflammatory cytokines at the higher concentrations. However, compound **18** showed poor inhibition of glycosidase activity and IL-10 level but reduced the levels of the proinflammatory cytokines IL-1β and TNF-α. This property may be advantageous in managing lysosomal storage diseases by chaperone-mediated therapy, an inexpensive alternative to enzyme replacement therapy (ERT).[30]

CONCLUSIONS

Overall, chiral homoallylic alcohol **9** was conceived as an important DOS intermediate and synthesized via a highly diastereoselective allylation of a D-glucose-derived aldehyde. Its versatility was established by converting it to two monocyclic iminosugars (4-*epi*-fagomine and 4,5-dihydroxypipecolic acid) and a new bicyclic iminosugar (dihydroxyindolizidine) in 16, 27, and 16% overall yields, respectively. The $^{8}C_{5}$ conformation of the dihydroxyindolizidine was confirmed by ^{1}H NMR and 2D-COSY experiments. Previously, we found a poor glycosidase inhibitory property for the L-iminosugars.[14] Hence, presently we synthesized the D-iminosugars using the β-azido epimer of **9**. However, the other stereomers of alcohol **9** can be easily synthesized using our approach. We also demonstrated that **2b** can suppress β-galactosidase activity and the level of cytokines that are associated with diabetes. Interestingly, oxidation of its $-CH_2OH$ group to $-CO_2H$, as in **4b**, reduced its efficacy and even reversed its pharmacological behavior at higher concentrations. Further in vivo studies are required to establish the antidiabetic properties of **2b**.

EXPERIMENTAL SECTION

(1*R*)-1-[(3a*R*,5*S*,6*S*,6a*R*)-6-Azido-2,2-dimethyltetrahydrofuro-[2,3-*d*][1,3]dioxol-5-yl]but-3-en-1-ol (9). To a stirred solution of azido aldehyde **8** (5.0 g, 23.4 mmol) in THF (60 mL) were added allyl bromide (4.46 mL, 51.6 mmol) and aqueous saturated NH_4Cl (3 mL) at 0 °C. The reaction mixture was brought to 20 °C and stirred for 7 h. After the reaction was complete (indicated by TLC), the reaction mixture was filtered and evaporated in vacuo to give a residue that was purified by column chromatography (silica gel, 0−10% EtOAc/hexane) to afford **9** (3.65 g, 61%, colorless thick liquid) as the major product. R_f = 0.48 (15% EtOAc/hexane). $[\alpha]_D^{25}$ −14.1 (*c* 3.33, $CHCl_3$). IR (film): 2104, 1640 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 3H), 1.49 (s, 3H), 1.98 (broad s, 1H, D_2O exchangeable), 2.21−2.29 (m, 1H), 2.65 (m, 1H), 3.86 (m, 1H), 4.03 (dd, *J* = 3.3, 8.7 Hz, 1H), 4.11 (d, *J* = 3.3 Hz, 1H), 4.62 (d, *J* = 3.9 Hz, 1H), 5.18 (m, 1H), 5.24 (m, 1H), 5.78−5.93 (m, 1H), 5.86 (d, *J* = 3.9 Hz, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.9, 26.2, 38.9, 65.8, 67.9, 71.1, 83.0, 104.5, 111.8, 118.6, 133.5. Anal. Calcd for $C_{11}H_{17}N_3O_4$: C, 51.76; H, 6.71; N, 16.46. Found: C, 51.62; H, 6.75; N, 16.63.

(3a*R*,5*S*,6*S*,6a*R*)-6-Azido-5-[(1*R*)-1-(benzyloxy)but-3-en-1-yl]-2,2-dimethyltetrahydrofuro[2,3-*d*][1,3]dioxole (10). To a cooled (0 °C) hexane-washed suspension of NaH (1.51 g, 31.4 mmol, 60% suspension in oil) was dropwise added **9** (3.65 g, 14.3 mmol) in THF (50 mL). After bringing the mixture to room temperature and stirring for 10 min, the mixture was cooled to 0 °C, and BnBr (1.87 mL, 15.7 mmol) and Bu_4NI (cat.) were added. The mixture was brought to room temperature and stirred for an additional 4 h. The reaction was quenched with aqueous saturated NH_4Cl (3 mL), and the mixture was concentrated in vacuo and extracted with EtOAc (3 × 15 mL). The organic extract was washed with H_2O (2 × 10 mL) and brine (1 × 5 mL) and dried. Solvent removal followed by column chromatography (silica gel, 5% EtOAc/hexane) of the residue furnished **10** (3.71 g, 76%) as a colorless thick liquid. R_f = 0.59 (EtOAc/hexane, 1:9). $[\alpha]_D^{25}$ −19.3 (*c* 1.55, $CHCl_3$). IR (film): 2104, 1641 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.31 (s, 3H), 1.46 (s, 3H), 2.36−2.45 (m, 1H), 2.68−2.77 (m, 1H), 3.79 (dt, *J* = 4.5, 9.0 Hz, 1H), 4.07 (d, *J* = 3.0 Hz, 1H), 4.19 (dd, *J* = 3.0, 9.3 Hz, 1H), 4.52 (d, *J* = 11.5 Hz, 1H), 4.63 (d, *J* = 3.6 Hz, 1H), 4.72 (d, *J* = 11.5 Hz, 1H), 5.21 (m, 2H), 5.86 (d, *J* = 3.6 Hz, 1H), 5.87−6.01 (m, 1H), 7.28−7.38 (m, 5H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.3, 26.5, 34.7, 66.1, 71.4, 75.5, 79.3, 83.2, 104.4, 112.1, 118.2, 127.7, 128.0, 129.3, 133.2, 138.0. Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.70; H, 6.53; N, 12.34.

(3*R*)-3-[(3a*R*,5*S*,6*S*,6a*R*)-6-Azido-2,2-dimethyltetrahydrofuro-[2,3-*d*][1,3]dioxol-5-yl]-3-(benzyloxy)propanal (12). Ozone was bubbled through a cooled (−78 °C) solution of **10** (3.71, 10.7 mmol)

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in CH_2Cl_2 (150 mL) until the mixture attained a persistent blue color. After removing the excess ozone by Ar purging, Me_2S (3.96 mL, 53.61 mmol) was added to the reaction mixture, and the mixture was stirred at room temperature for 24 h. After concentration in vacuo, the residue was purified by column chromatography (silica gel, 5% EtOAc/hexane) to afford aldehyde **12** (3.21 g, 87%) as a colorless thick liquid. R_f = 0.59 (10% EtOAc/hexane). $[\alpha]_D^{25}$ −4.6 (*c* 1.05, $CHCl_3$). IR (film): 2102, 1720 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.30 (s, 3H), 1.48 (s, 3H), 2.76−2.82 (m, 1H), 2.89−2.96 (m, 1H), 4.07 (d, *J* = 2.9 Hz, 1H), 4.21 (dd, *J* = 3.0, 9.3 Hz, 1H), 4.24−4.27 (m, 1H), 4.60 (ABq, *J* = 11.5 Hz, 2H), 4.65 (d, *J* = 4.0 Hz, 1H), 5.84 (d, *J* = 3.6 Hz, 1H), 7.26−7.38 (m, 5H), 9.82 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 26.1, 26.4, 46.3, 65.8, 72.9, 76.7, 80.3, 83.3, 104.3, 112.2, 127.7, 127.9, 128.4, 137.4, 200.1. Anal. Calcd for $C_{17}H_{21}N_3O_5$: C, 58.78; H, 6.09; N, 12.10. Found: C, 58.89; H, 5.93; N, 12.34. ESI−MS: $[M + Na]^+$ calcd for $C_{17}H_{21}N_3O_5$, 370.14 Da; found, 370.03 Da.

(3a*R*,4a*S*,5*R*,8a*S*,8b*R*)-*N*-Benzyloxycarbonyl-5-(benzyloxy)-2,2-dimethyloctahydro[1,3]dioxolo[4,5]furo[3,2-*b*]pyridine (11). To a cooled (0 °C) and stirred solution of **12** (3.21 g, 9.33 mmol) in MeOH (140 mL) was added Ph_3P (2.50 g, 9.33 mmol). The reaction mixture was gradually brought to room temperature and stirred for 1 h. The reaction was cooled to −20 °C, acetic acid (cat.) was added, the reaction was stirred for 40 min, and $NaCNBH_3$ (0.70 g, 11.10 mmol) was added in portions (10 min). After stirring at same temperature for 2 h followed by stirring at room temperature for 8 h, the reaction mixture was cooled to 0 °C, and $NaHCO_3$ (2.35 g, 27.90 mmol) in H_2O (15 mL) and benzyl chloroformate (0.19 mL) were added successively. The reaction was stirred at room temperature for 6 h, concentrated in vacuo, and extracted with $CHCl_3$ (3 × 15 mL). The combined organic extracts were washed with H_2O (3 × 10 mL) and brine (1 × 5 mL) and dried. Solvent removal followed by column chromatography (silica gel, 5% EtOAc/hexane) of the residue furnished **11** (2.71 g, 65% in three steps) as a colorless thick liquid. R_f = 0.39 (20% EtOAc/hexane). $[\alpha]_D^{25}$ −54.5 (*c* 2.15, $CHCl_3$). IR (film): 1697, 1454 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.32 (s, 3H), 1.52 (s, 3H), 1.90 (m, 2H), 3.30 (broad s, 1H), 3.64 (m, 1H), 3.72 (broad s, 1H), 4.35 (broad d, *J* = 5.2 Hz, 1H), 4.59−4.64 (m, 4H), 5.14 (ABq, *J* = 12.3 Hz, 2H), 5.92 (d, *J* = 3.4 Hz, 1H), 7.28−7.40 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 24.3, 26.6, 27.1, 38.4, 61.2, 67.4, 70.6, 72.0, 74.9, 85.2, 105.1, 112.0, 127.6, 127.7, 127.9, 128.0, 128.4, 136.2, 137.8, 155.8. Anal. Calcd for $C_{25}H_{29}NO_6$: C, 68.32; H, 6.65; N, 3.19. Found: C, 68.16; H, 6.77; N, 3.05. ESI−MS: $[M + Na]^+$ calcd for $C_{25}H_{29}NO_6$, 462.19 Da; found, 462.17 Da.

(2*R*,3*S*,4*R*)-*N*-Benzyloxycarbonyl-4-(benzyloxy)-2-(hydroxymethyl)piperidin-3-ol (14). A solution of **11** (0.24 g, 0.54 mmol) in TFA−H_2O (2.50 mL, 3:2) was stirred at 0 °C for 1 h. TFA was removed azeotropically with toluene in vacuo to afford the hemiacetal as a thick liquid (2.53 g). To a cooled (0 °C) solution of the crude hemiacetal in acetone/water (5.00 mL, 9:1) was added $NaIO_4$ (0.13 g, 0.6 mmol). After stirring for 30 min, excess $NaIO_4$ was decomposed with ethylene glycol (0.10 mL), the mixture was concentrated in vacuo, and the residue was extracted with $CHCl_3$ (3 × 10 mL) to obtain crude α-amino aldehyde **13** (0. 235 g) as a thick liquid.

To a cooled (0 °C) and stirred solution of **13** in 90% aqueous MeOH (10 mL) was added $NaBH_4$ (0.02 g, 0.55 mmol). After the completion of the reaction (20 min, indicated by TLC), aqueous saturated NH_4Cl (1 mL) was added, and the mixture was concentrated in vacuo and extracted with $CHCl_3$ (3 × 15 mL). The combined organic extracts were washed with H_2O (2 × 10 mL) and brine (1 × 5 mL) and dried. Solvent removal followed by column chromatography (silica gel, 0−15% EtOAc/hexane) of the residue furnished diol **14** (40% from **11** in three steps) as a colorless thick liquid. R_f = 0.35 (40% EtOAc/hexane). $[\alpha]_D^{25}$ −43.3 (*c* 2.8, $CHCl_3$). IR (film): 1672, 1435 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.58−1.67 (m, 3H, partially D_2O exchangeable), 1.99 (broad d, *J* = 13.4 Hz, 1H), 2.77 (broad s, 1H, D_2O exchangeable), 3.35 (broad s, 1H), 3.83 (broad s, 1H), 3.88 (broad s, 1H), 3.98 (broad s, 2H), 4.33 (broad s, 1H), 4.53 (d, *J* = 11.7 Hz, 1H), 4.69 (d, *J* = 11.7 Hz, 1H), 5.15 (ABq, *J* = 12.6 Hz, 2H), 7.28−7.39 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 27.3, 35.6, 56.7, 60.3, 67.4, 69.5, 71.8, 75.5, 127.5, 127.8, 127.9, 128.0, 128.5, 128.6, 136.4, 137.7, 156.0. Anal. Calcd for $C_{21}H_{25}NO_5$: C, 67.91; H, 6.78; N, 3.77. Found: C, 67.83; H, 6.61; N, 3.88. ESI−MS: $[M + Na]^+$ calcd for $C_{21}H_{25}NO_5$, 394.16 Da; found, 394.09 Da.

(2*R*,3*S*,4*R*)-2-(Hydroxymethyl)piperidine-3,4-diol (4-*epi*-Fagomine) (2b). A solution of **14** (0.150 g, 0.40 mmol) and 10% Pd/C (0.025 g) in MeOH (8 mL) was stirred under an H_2 atmosphere at 80 psi for 12 h at 25 °C. The catalyst was filtered through a pad of Celite 545. Solvent evaporation afforded **2b** (0.055 g, 92%) as a semisolid. R_f = 0.51 (40% MeOH/$CHCl_3$). $[\alpha]_D^{25}$ +11.7 (*c* 1.8, H_2O); lit.[18a] $[\alpha]_D^{22}$ +10.2 (*c* 1.4, H_2O); lit.[18b] $[\alpha]_D^{25}$ +10.4 (*c* 1.4, H_2O). IR (film): 3350, 1708 cm^{-1}. ^1H NMR (700 MHz, D_2O): δ 1.80−1.85 (m, 2H), 2.75−2.79 (m, 1H), 2.93 (t, *J* = 6.6 Hz, 1H), 3.20−3.24 (m, 1H), 3.70−3.79 (m, 2H), 3.82−3.87 (m, 1H), 4.01 (s, 1H). ^{13}C NMR (125 MHz, D_2O): δ 26.6, 42.6, 59.1, 61.1, 67.2, 69.3. Anal. Calcd for $C_6H_{13}NO_3$: C, 48.97; H, 8.90; N, 9.52. Found: C, 48.73; H, 8.97; N, 9.70. ESI−MS: $[M + H]^+$ calcd for $C_6H_{13}NO_3$, 148.09 Da; found, 148.13 Da.

(2*S*,3*S*,4*R*)-*N*-Benzyloxycarbonyl-4-(benzyloxy)-3-hydroxypiperidine-2-carboxylic Acid (15). To a stirred solution of amino aldehyde **13** (obtained from **11** (0.59 g, 1.4 mmol)) in CH_3CN (10 mL) was added a solution of NaH_2PO_4 (0.04 g, 0.27 mmol) in H_2O (2 mL) and 30% H_2O_2 (0.21 mL, 1.55 mmol). The mixture was cooled to 0 °C, and $NaClO_2$ (0.21 g, 2.18 mmol) in H_2O (4.5 mL) was added dropwise over a period of 0.5 h. The reaction mixture was stirred at 15 °C and monitored for the evolution of gas with a bubbler connected to the apparatus. After 6 h, the reaction was decomposed by the addition of a small amount of Na_2SO_4 (0.25 g) and extracted with EtOAc (3 × 10 mL). Evaporation of the solvent followed by column chromatography (silica gel, 5% MeOH/$CHCl_3$) of the residue gave **15** (0.23 g, 67% from **11**) as a sticky gum. R_f = 0.60 (20% MeOH/$CHCl_3$). $[\alpha]_D^{25}$ −2.5 (*c* 1.1, $CHCl_3$). IR (film): 3352, 1702 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ 1.76 (m, 1H), 1.91 (m, 1H), 3.50−3.66 (m, 1H), 3.88 (broad s, 1H), 3.90−4.02 (m, 2H, one proton D_2O exchangeable), 4.61 (ABq, *J* = 12.1 Hz, 2H), 5.10−5.25 (m, 4H), 7.28−7.42 (m, 10H), 8.10 (s, 1H, D_2O exchangeable). ^{13}C NMR (125 MHz, $CHCl_3$): δ 28.2, 36.6, 53.4, 67.9, 70.9, 71.8, 72.3, 127.2, 127.6, 127.9, 128.1, 128.4, 128.5, 136.1, 137.8, 168.1, 173.1. Anal. Calcd for $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.59; H, 6.28; N, 3.83. ESI−MS: $[M + Na]^+$ calcd for $C_{21}H_{23}NO_6$, 408.14 Da; found, 408.05 Da.

(2*S*,3*S*,4*R*)-3,4-Dihydroxypiperidine-2-carboxylic Acid (3,4-Dihydroxypipecolic Acid) (4b). Following the same procedure as for **2b**, compound **15** (0.18 g, 0.46 mmol) was catalytically hydrogenated to obtain **4b** (0.072 g, 93%) as a white solid. R_f = 0.46 (30% MeOH/$CHCl_3$). mp: 245−247 °C. $[\alpha]_D^{25}$ +7.5 (*c* 2.1, H_2O). IR (KBr): 3374, 2934, 1048 cm^{-1}. ^1H NMR (500 MHz, D_2O): δ 1.90−2.05 (m, 2H), 3.04−3.15 (td, *J* = 4.3, 12.8 Hz, 1H), 3.49 (d, *J* = 12.6 Hz, 1H), 3.95−4.01 (m, 1H), 4.18 (s, 1H), 4.48 (s, 1H). ^{13}C NMR (125 MHz, D_2O): δ 23.5, 41.5, 60.3, 66.8, 67.1, 169.3. Anal. Calcd for $C_6H_{11}NO_4$: C, 44.72; H, 6.88; N, 8.69. Found: C, 44.65; H, 6.68; N, 8.81. ESI−MS: $[M + Na]^+$ calcd for $C_6H_{11}NO_4$, 184.06 Da; found, 184.05 Da, 206.01 Da [+2Na].

Ethyl (2*E*)-3-[(2*R*,3*S*,4*R*)-1-Benzyloxycarbonyl-4-benzyloxy-3-hydroxypiperidin-2-yl]prop-2-enoate (16). To a cooled (−40 °C) and stirred suspension of hexane-washed NaH (0.21 g, 2.52 mmol, 60% suspension in oil) was dropwise added triethyl phosphonoacetate (0.55 mL, 2.74 mmol). After 10 min, amino aldehyde **13** (obtained from **11** (0.85 g, 2.29 mmol)) in THF (10 mL) was added into the mixture. After 10 min, the mixture was gradually warmed to −20 °C and stirred until the completion of the reaction (∼5 h, indicated by TLC). The mixture was treated with aqueous saturated NH_4Cl (2 mL) and extracted with EtOAc (3 × 10 mL). The organic extract washed with H_2O (2 × 10 mL) and brine (1 × 5 mL) and dried. Solvent removal in vacuo followed by column chromatography of the residue (silica gel, 25% EtOAc/hexane) afforded pure **16** (0.48 g, 48%) as a colorless thick liquid. R_f = 0.30 (25% EtOAc/hexane). $[\alpha]_D^{25}$ −5.9 (*c* 3.4, $CHCl_3$). IR (film): 3355, 2890, 1693, 1681 cm^{-1}. ^1H NMR (700 MHz, $CDCl_3$): δ 1.25 (t, *J* = 7.1 Hz, 3H), 1.59 (m, 2H, partially D_2O exchangeable), 1.99 (m, 1H), 3.13 (t, *J* = 12.2 Hz, 1H), 3.77 (broad s, 1H), 3.86 (d, *J* = 2.3 Hz, 1H), 3.95 (m, 1H), 4.17 (q, *J* = 7.1 Hz, 2H), 4.42 (d, *J* = 11.9 Hz, 1H), 4.65 (d, *J* = 11.9 Hz, 1H), 5.02 (m, 1H),

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5.11 (s, 2H), 5.86 (d, J = 14.9 Hz, 1H), 7.17 (dd, J = 14.8, 6.1 Hz, 1H), 7.23−7.39 (m, 10H). ^{13}C NMR (175 MHz, $CDCl_3$): δ 14.2, 26.9, 33.9, 56.0, 60.3, 67.9, 69.5, 71.4, 73.1, 122.2, 127.0, 127.2, 127.9, 128.1, 128.4, 128.5, 128.6, 136.3, 137.8, 142.5, 155.3, 166.1. Anal. Calcd for $C_{25}H_{29}NO_6$: C, 68.32; H, 6.65; N, 3.19. Found: C, 68.44; H, 6.52; N, 3.31. ESI−MS: $[M + Na]^+$ calcd for $C_{25}H_{29}NO_6$, 462.18 Da; found, 462.17 Da.

(7*R*,8*S*,8a*R*)-7,8-Dihydroxyhexahydroindolizidine-3-(2*H*)-one (17). To a solution of **16** (0.28 g, 0.63 mmol) in anhydrous MeOH (20 mL) was added HCO_2NH_4 (0.20 g, 3.15 mmol) and 10% Pd/C (30 mg). The reaction mixture was refluxed for 4 h and filtered through Celite 540. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (silica gel, 5% MeOH/$CHCl_3$/NH_3 (1drop)) to afford **17** (0.10 g, 90%) as white crystals. R_f = 0.30 (10% MeOH/$CHCl_3$). mp: 162−163 °C. $[\alpha]_D^{25}$ + 6.1 (c 1.8, CH_3OH). IR (KBr): 3357, 1658 cm^{-1}. ^{1}H NMR (500 MHz, CD_3OD): δ 1.58 (m, 1H), 1.74 (m, 1H), 2.06 (m, 2H), 2.29 (m, 1H), 2.41 (m, 1H), 2.73 (m, 1H), 3.67 (m, 3H), 4.00 (ddd, J = 13.2, 5.2, 1.6 Hz, 1H). ^{13}C NMR (175 MHz, CD_3OD): δ 18.3, 25.7, 30.1, 37.1, 60.1, 69.8, 70.1, 175.8. Anal. Calcd for $C_8H_{13}NO_3$: C, 56.13; H, 7.65; N, 8.18. Found: C, 56.01; H, 7.72; N, 8.29. ESI−MS: $[M + H]^+$ calcd for $C_8H_{13}NO_3$, 172.09 Da; found, 172.08 Da.

(7*R*,8*S*,8a*R*)-Octahydroindolizine-7,8-diol (18). To a cooled (0 °C) and stirred suspension of $LiAlH_4$ (0.048 g, 1.27 mmol) in THF (3 mL) was added **17** (0.087 g, 0.50 mmol) in THF (15 mL). The reaction mixture was slowly brought to room temperature and refluxed for 2 h. The reaction was quenched with EtOAc (5 mL) and aqueous saturated NH_4Cl (1 mL), the mixture was filtered through Celite 540, and the resultant thick liquid was purified by column chromatography (silica gel, 5% MeOH/$CHCl_3$) to afford **18** (73 mg, 92%) as a white solid. R_f = 0.20 (40% MeOH/$CHCl_3$). mp: 126−127 °C. $[\alpha]_D^{25}$ + 3.31 (c 3.2, H_2O). IR (KBr): 3366, cm^{-1}. ^{1}H NMR (500 MHz, D_2O): δ 2.0−2.25 (m, 6H), 3.06−3.12 (m, 2H), 3.43 (m, 1H), 3.62−3.67(m, 2H), 4.04 (m, 1H), 4.23 (d, J = 2.1 Hz, 1H). ^{13}C NMR (125 MHz, D_2O): δ 20.1, 23.1, 24.9, 48.4, 52.1, 66.2, 68.0. Anal. Calcd for $C_8H_{15}NO_2$: C, 61.12; H, 9.62; N, 8.91. Found: C, 61.25; H, 9.54; N, 9.13. ESI−MS: $[M + H]^+$ calcd for $C_8H_{15}NO_2$, 158.12 Da; found, 158.16 Da.

(7*R*,8*S*,8a*R*)-Octahydroindolizine-7,8-diylacetate (19). To a cooled (0 °C) and stirred solution of **18** (0.050 g, 0.32 mmol) in pyridine (1 mL) were added Ac_2O (1 mL) and DMAP (cat.). The reaction mixture was warmed to room temperature, stirred for 6 h, concentrated in vaccuo, and extracted with EtOAc (3 × 5 mL). The resultant thick liquid was purified by column chromatography (silica gel, 5% MeOH/$CHCl_3$) to afford **19** (73 mg, 96%) as a thick liquid. R_f = 0.70 (25% MeOH/$CHCl_3$). $[\alpha]_D^{25}$ + 9.00 (c 1.1, H_2O). IR ($CHCl_3$): 1720 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.55−1.59 (m, 1H), 1.70−1.86 (m, 4H), 2.01 (s, 3H), 2.04−2.09 (m, 3H), 2.18 (s, 3H), 2.14−2.20 (m, 1H), 3.13 (t, J = 8.5 Hz, 1H), 3.19 (d, J = 10.8 Hz, 1H), 4.85 (ddd, J = 12.0, 4.9, 3.2 Hz, 1H), 5.40 (broad s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 21.0, 21.3, 24.8, 25.8, 49.5, 53.3, 64.5, 67.8, 71.8, 170.4, 171.2. Anal. Calcd for $C_{12}H_{19}NO_4$: C, 59.73; H, 7.94; N, 5.81. Found: C, 59.88; H, 7.84; N, 5.99. ESI−MS: $[M + H]^+$ calcd for $C_{12}H_{19}NO_4$, 242.13 Da; found, 242.13 Da.

Cell Culture. Human cancer cells (A549, U2OS, and U937) and murine macrophage cells (RAW 264.7) were cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL). Cells were grown at 37 °C under an atmosphere of 5% CO_2.

β-Galactosidase Inhibition Assay. To the reaction mixtures (total volume 350 μL) containing various concentrations (1.7−8.5 μM, 100 μL) of the test compounds and 4-nitrophenyl-β-D-galactopyranoside (200 μL, 4 mg/mL) in Z buffer taken in a 96-well microplate was added commercially available β-D-galactosidase from *E. coli* (50 μL). The mixtures were incubated for 16 min at room temperature, and the absorbance at 405 nm (indicating the release of 4-nitrophenol) was recorded every min for 16 min with a microplate reader (Aviso MQX 200, BioTek Winooski, VT). For the control sample, the test compounds were omitted. The β-galactosidase activity was determined using a standard plot of 4-nitrophenol, and the values are expressed as the mean ± SEM of three independent experiments, with each experiment repeated three times. The data were analyzed by a paired t test and one-way analysis of variance (ANOVA).

Cytokine Estimation. The levels of TNF-α, IL-1β, IL-6, and IL-10 in the culture supernatants were measured using ELISA kits according to the manufacturer's instructions. Briefly, Raw 264.7 cells (1 × 10^6/well) untreated or pretreated with different concentrations of the test compounds for 1 h were incubated with LPS (200 ng/mL) for 16 h to assay TNF-α and IL-1β levels and for 24 h to estimate IL-6 and IL-10 levels. The culture medium was collected, and the concentrations of the cytokines in each sample were determined using standard curves. For the estimation of TNF-α and IL-1β, the culture supernatants were diluted 1:10 by sample diluents provided in the kit. The values are expressed as the mean ± SEM of three independent experiments, with each experiment repeated three times. The data were analyzed by a paired t test and one-way analysis of variance (ANOVA).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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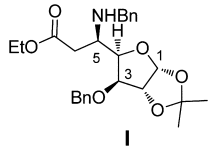

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