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An expeditious one-pot synthesis of 1,6-dideoxy-*N*-alkylated nojirimycin derivatives and their inhibitory effects on the secretion of IFN-γ and IL-4

Jian Zhou,[a] Yongmin Zhang,[b] Xia Zhou,[c] Jing Zhou,[c] Li-He Zhang,[a] Xin-Shan Ye[a,*] and Xiao-Lian Zhang[c,*]

[a]*The State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Xue Yuan Road #38, Beijing 100083, PR China*
[b]*Ecole Normale Supérieure, Département de Chimie, UMR 8642 CNRS, 24 rue Lhomond, 75231 Paris Cedex 05, France*
[c]*Department of Immunology, State Key Laboratory of Virology, Hubei Province Key Laboratory of Allergy and Immune-Related Diseases, Wuhan University College of Medicine, Wuhan 430071, PR China*

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Abstract—An efficient 'one-pot' approach to the synthesis of 1,6-dideoxy-*N*-alkylated nojirimycin derivatives in good yields and with high stereoselectivity was developed. It was found that the synthetic *N*-alkylated iminosugars showed inhibitory effects on the release of the cytokines IFN-γ and IL-4 from the mouse splenocytes. The preliminary structure–activity relationship was deduced from the activities of *N*-substituted iminosugars. Apart from the cytokine inhibitory activities, a series of glycosidase inhibitory activities were also examined. The present experimental data demonstrated that synthetic iminosugars might hold potential as immunosuppressive agents.
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1. Introduction

The main clinically used immunosuppressive agents, such as cyclosporin A (CyA), tacrolimus, mycophenolate mofetil, and sirolimus, have significant side effects including nephrotoxicity, neurotoxicity, infection, cancer, new onset post-transplant diabetes mellitus, hyperlipidemia, and hypertension.[1–5] Moreover, so far there is no antidote for the toxicity of these organ transplantation drugs. Stimulated by the need to improve transplant survival and by the reduction of the toxicity of current agents, the search to find more effective and safer immunosuppressants is in great demand.

Iminosugars are a family of polyhydroxylated heterocycles containing an endocyclic nitrogen atom. They have been a subject of enduring scientific interest over the last three decades. These compounds are frequently found to be potent inhibitors of many carbohydrate-processing enzymes.[6,7] Since these enzymes are involved in a number of important biological processes,[8–11] iminosugars have tremendous potential as therapeutic agents in a wide range of diseases such as diabetes,[12] viral infections,[13] tumor metastasis,[14] and lysosomal storage disorders.[15,16] The wide range of biological activities displayed by this class of compounds has resulted in many synthetic efforts.[17–19]

Since the information of crystallographic data relating to enzymes and their active sites is only slowly increasing due to the inherent problems experienced with the crystallization of these frequently membrane-bound and sensitive enzymes, rational drug design is still difficult. So it is highly desired to synthesize diversity-oriented small molecules to improve the understanding of structure–activity relationship (SAR) between bio-targets and small molecules. In order to gain rapid access to new iminosugar derivatives of biological interest, a series of *N*-alkylated iminosugar compounds were designed and synthesized by an expeditious 'one-pot' method

Keywords: Iminosugar; One-pot synthesis; Cytokine inhibition; Immunosuppressive agent; Glycosidase inhibitor.
* Corresponding authors. Tel.: +86 10 82801570; fax: +86 10 62014949; e-mail addresses: xinshan@bjmu.edu.cn; zhangxl65@whu.edu.cn

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with high stereoselectivity. Since the effects of iminosugars on immune system and their application as immunosuppressants have been less investigated, and recently we disclosed the iminosugars displayed immunosuppressive activity,[20] inhibitory effects of the synthetic compounds on the secretion of IFN-γ and IL-4 as well as their glycosidase inhibitory activities were evaluated.

2. Results and discussion

2.1. Design

The deoxynojirimycin (DNJ) (**1**) (Fig. 1) family comprises potent inhibitors of the α-glucosidase mediated *N*-linked protein glycosylation. *N*-Alkylated deoxynojirimycin and deoxygalactonojirimycin are inhibitors of the ceramide-specific glucosyltransferase, an enzyme involved in the first step in the glycosylation of many sphingolipids.[21] For instance, *N*-butyl-1-deoxynojirimycin (Zavesta) (**2**) has been approved as medicine for Gaucher disease, a severe lysosomal storage disorder. The iminosugar *N*-hydroxyethyl-DNJ (miglitol) (**3**) is a drug for treatment of type II diabetes.[22] In addition, *N*-alkylated bicyclic compounds such as swainsonine (**4**) also exhibit potent glycosidase inhibitory properties.[23] With regard to the development of new fucosyltransferase inhibitors, many efforts[24] have been devoted to the synthesis and modifications of fuconojirimycin (**5**), but only limited iminosugars displayed fucosidase or fucosyltransferase inhibitory activities.[25] The biological importance has warranted further exploration of *N*-alkylated iminosugars. Considering the high potential of iminosugars as carbohydrate mimetics, the design of a general and efficient synthetic approach to *N*-alkylated iminosugars appears to be an important issue. So far there are few methods available in this area.[26–28] For this purpose, a series of characteristic *N*-alkylated iminosugars, which not only share the basic skeleton of deoxynojirimycin (**1**), but also have a very similar appearance to fuconojirimycin (**5**), were designed and synthesized by an efficient way.

2.2. Chemistry

The preparation of compounds **8a**–**h** is outlined in Scheme 1. The key material alkene **6**, a precursor for the synthesis of *N*-alkylated iminosugars, was obtained from methyl α-D-glucopyranoside through five steps in high overall yield (77%) according to the procedure

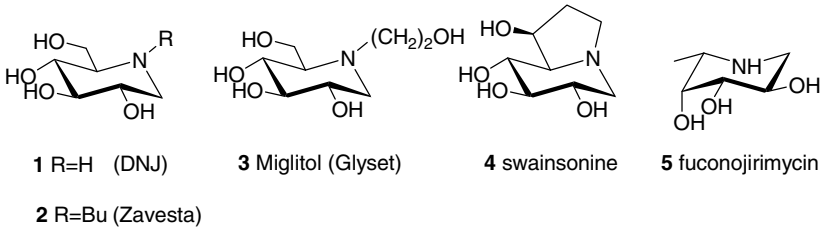
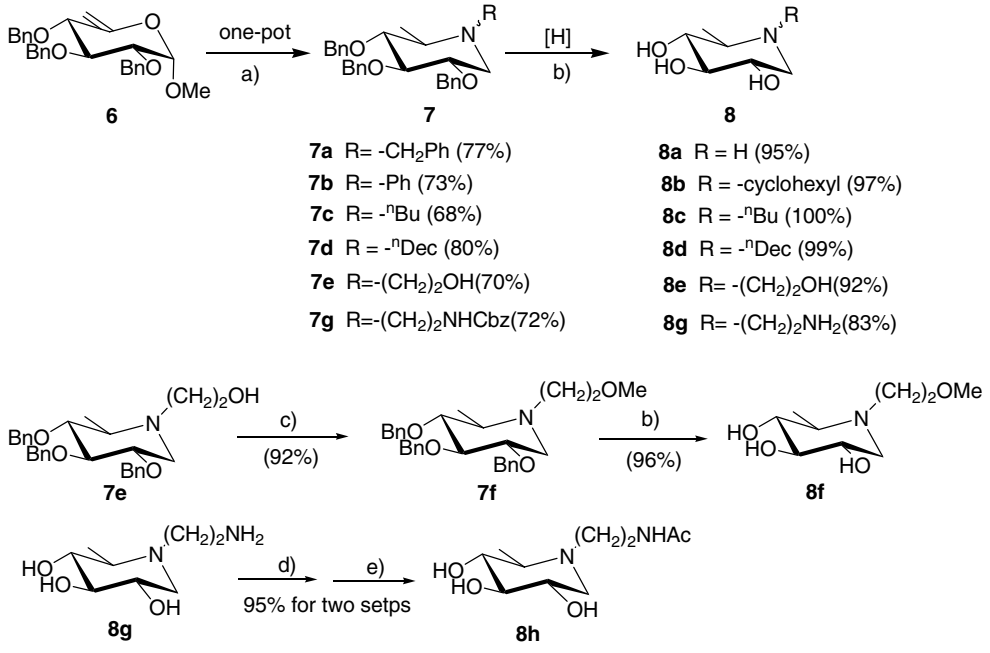


Figure 1.



Scheme 1. Synthesis of iminosugar derivatives. Reagents: (a) CF_3COOH, MeOH then RNH_2, $NaCNBH_3$, CH_3COOH; (b) Pd–C, H_2; (c) MeI, NaH, THF; (d) Ac_2O, Py; (e) MeONa, MeOH.

described previously.[29] The unsaturated compound **6** readily lost the methoxyl aglycon in the presence of catalytic amount of acid to form 1,5-dicarbonyl compound **9** presumedly via the rearrangement of the hydrated intermediate (Scheme 2). It was found some organic acids such as trifluoroacetic acid can greatly accelerate this transformation but inorganic acids such as hydrochloric acid are generally invalid.[30]

With dicarbonyl compound **9** in hand, the iminosugar derivative **7a** was prepared by a well-established double reductive amination approach.[31,32] In order to further simplify the operation procedure, we performed the two reactions in a facile 'one-pot' manner. That is, alkene **6** was treated with trifluoroacetic acid, which was followed by reductive amination with benzylamine and $NaCNBH_3$ without purification of intermediate **9**, yielding iminosugar **7a** in 77% isolated yield. In the same way, iminosugars **7b**–**e** and **7g** were constructed in good yields (Scheme 1). It is noteworthy that unlike the reported approach,[33] in our experiments the reductive amination proceeded in a very stereospecific manner; thus in each operation only one stereoisomer was gained under the reaction conditions we used. The configurations of products were confirmed by their NMR spectra analyses. The coupling constants (J = 9.0–10.0 Hz) and the peak type (triplet) of H-4 NMR signals clearly verified the configurations of each product. The *N*-hydroxyethyl substituted compound **7e** (miglitol derivative) was readily methylated in THF using NaH as a base to yield the corresponding methylate **7f**. Finally, catalytic hydrogenolysis of compounds **7a**–**g** over Pd–C in the mixed solvent of acetic acid, water, and THF provided the corresponding deprotected iminosugars **8a**–**g**. Compound **8h** was obtained via acetylation of *N*-aminoethyl iminosugar **8g**. It is noteworthy that the *N*-phenyl derivative **7b** afforded the product **8b** with the aromatic ring reduced over hydrogenolysis.

2.3. The cytokine inhibitory activity and SAR

To evaluate the effects of the eight synthetic iminosugars **8a**–**h** on the secretion of cytokines from the splenocytes in mouse, the splenocytes were induced by 10 μg/mL of concanavalin A with 60 μM concentration of the compounds at 37 °C, 5% CO_2 for 72 h. The secretion of IFN-γ was detected from the supernatant of spleen cells by the use of mice ELISA kit. Compared to the control, the levels of IFN-γ secretion were reduced 9.0%, 9.1%, 55.5%, 61.0%, 29.0%, 16.0%, 41.0%, and 9.0% when including 60 μM of **8a**–**h**, respectively (Fig. 2). It was found that among the eight synthetic iminosugars, compounds **8c** and **8d** displayed the strongest inhibitory ability to the IFN-γ secretion, whereas compound **8d** even showed comparable inhibitory activity with CyA. As shown in Figure 2, most of the synthetic compounds showed IFN-γ inhibition effects. Compounds **8c** and **8d** with non-polar *N*-side chains showed better inhibition than the polar chain, suggesting that hydrophobic character of the *N*-side chain may be important to the activity. But **8b** with the non-polar substituent did not display significant inhibition, probably due to the steric hindrance of the cyclohexyl substituent. It seems that the carbon atom number of the side chain can be varied based on the fact that both *N*-butyl compound **8c** and *N*-decanyl compound **8d** exhibited better inhibition. Although **8c** and **8f** have similar side chain length, their inhibitory effects are different. Actually, compound **8f** with *N*-polar substituted side chain showed less inhibitory effect.

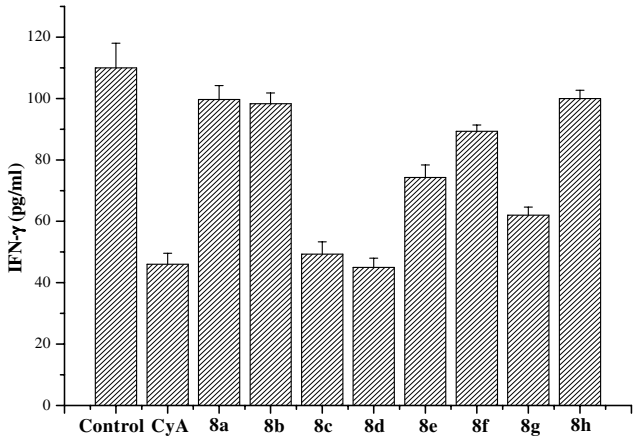
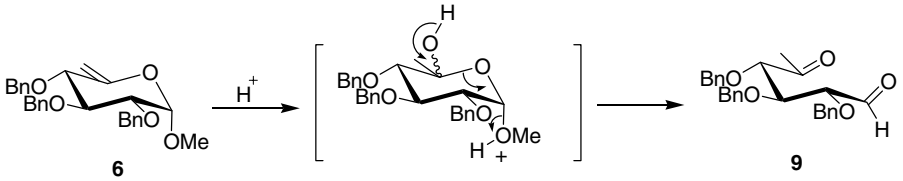


Figure 2. The inhibition effects on the secretion of IFN-γ in mouse by the eight compounds at 60 μM. Data are means ± SEM of at least three independent experiments, $p < 0.05$.

The assay of the secretion of IL-4 from splenocytes was similar to the assay of IFN-γ. The secretion of IL-4 was detected from the supernatant of spleen cells by mice IL-4 ELISA kit. The level of IL-4 secretion was reduced 58.0%, 57.0%, 44.0%, 90.0%, 57.0%, 58.8%, 64.0%, and 59.7% when including 60 μM of compounds **8a**–**h**, respectively (Fig. 3). The reduction efficiency of iminosugar **8d** is the strongest (better than CyA) among the eight compounds. Since all the synthetic compounds showed IL-4 inhibitory activity, it was clear that the intrinsic character of iminosugar is critical to the activity. It was also found the secretion of IL-4 is relatively insensitive to the variety of the *N*-substituents in iminosugars compared with that of IFN-γ. The strongest inhibitor **8d** has a 10-carbon side chain, indicating the



Scheme 2.

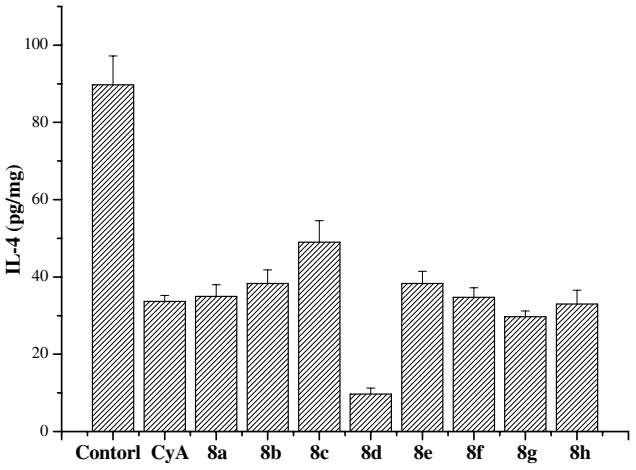


Figure 3. The inhibition effects on the secretion of IL-4 in mouse by the eight compounds at 60 μM. Data are means ± SEM of at least three independent experiments, $p < 0.05$.

hydrophobicity of the side chain could be important to the activity.

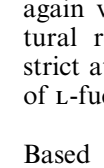
2.4. The glycosidase inhibition and SAR

The good IFN-γ and IL-4 inhibitory activities showed by *N*-alkylated derivatives **8c** and **8d** let us investigate whether there are some correlations between the cytokine inhibition and the glycosidase inhibition. So the inhibitory activities of these iminosugars against a series of glycosidases were evaluated.

As depicted in Table 1, the parent structure 1,6-dideoxynojirimycin (**8a**) showed no inhibition toward the tested glycosidases.[34] Compound **8d** with the best IFN-γ and IL-4 inhibitory activities did not show significant glycosidase inhibition activity at the maximal tested concentration. The *N*-butyl derivative **8c** displayed extensive inhibitory activity against different glycosidases compared to the other *N*-alkyl derivatives. Although no selectivity was observed, it was displayed that the *N*-alkylated iminosugars could enhance the glycosidase inhibition to some extent. The *N*-cyclohexyl derivative **8b**, which had comparable side chain in length to **8c**, did not exhibit glycosidase inhibition. The miglitol analogue **8e** only showed β-glucosidase inhibition. It was anticipated the *N*-alkylated iminosugars could show selective inhibition against different glycosidases by the variation of the *N*-substituted side chains. Compared with **8e**, the methylation derivative **8f** totally lost the glycosidase inhibitory activity, resulting from the sensitive modification of iminosugars. The hydroxyethyl isostere **8g** and its acetylated derivative **8h** were assayed against glycosidases. Neither of them showed significant inhibition. It was also noticeable that the inhibitory activity to α-L-fucosidase did not meet our expectation. The results again verified the conclusion that the minimum structural requirement for inhibition of α-L-fucosidase is strict at C-2, C-3, C-4 positions corresponding to those of L-fucose.[35]

Based on the results of glycosidase inhibition, it is apparent that there is no dramatic correlation between

Table 1. Enzyme inhibition assay: apparent IC_{50} values for compounds **8a–h**



Compound	α-Glucosidase in vitro	β-Glucosidase in vitro	α-Mannosidase in vitro	β-Mannosidase in vitro	α-Galacosidase in vitro	β-Galacosidase in vitro	α-L-Fucosidase in vitro
8a: R = H	ND[a]	NI[b]	NI	NI	NI	NI	ND
8b: R = –cyclohexyl	ND	NI	NI	NI	NI	NI	ND
8c: R = –^{n}Bu	77 μM	540 μM	2 mM	NI	234 μM	ND	3 mM
8d: R = –^{n}Dec	NI	ND	ND	NI	NI	NI	NI
8e: R = –$(CH_2)_2OH$	NI	34 μM	NI	NI	NI	NI	ND
8f: R = –$(CH_2)_2OMe$	NI	NI	NI	NI	NI	NI	ND
8g: R = –$(CH_2)_2NH_2$	ND	NI	NI	NI	NI	NI	NI
8h: R = –$(CH_2)_2NHAc$	ND	>2 mM	NI	NI	NI	ND	NI

[a] NI, inhibition not observed under assay conditions.
[b] ND, not determined. Data are the average of three sets of assay performed.

the cytokine inhibitory activity and glycosidase inhibitory activity. The potential immunosuppressive mechanisms by which iminosugars act still await investigation.

3. Conclusions

We have developed an efficient 'one-pot' approach for the synthesis of 1,6-dideoxy-*N*-alkyl iminosugars with high stereoselectivity and in good yields. The synthetic iminosugars showed cytokines IFN-γ and IL-4 inhibition activity. Especially the compound **8d** displayed the strongest inhibitory effects on both the secretion of IFN-γ and IL-4 among eight synthetic compounds. Compared with the parent 1-deoxynojirimycin (DNJ) and our previously published iminosugars,[20] compound **8d** seemed to be more potent to the inhibition of concanavalin A induced mouse splenocyte proliferation.[36] IFN-γ is a representative of Th1 cytokines which can activate macrophages, NK cells, and cell-mediated immunity (CMI), plus the secretion of certain Ig isotypes, while IL-4 is a representative of Th2 cytokines which tend to favor isotype switching in the humoral immune response. Since the cytokines (e.g., IFN-γ) secreted by the Th1 subset act primarily in cell-mediated immune response, whereas those (e.g., IL-4) secreted by the Th2 subset function mostly in B-cell activation and humoral response, our findings might open a new avenue in the development of a new class of drugs possessing immunosuppressive activity. Using the acquired SAR, future work will focus on the extension of this synthetic strategy to the preparation of other iminosugar derivatives bearing a diverse range of *N*-substituted chains. Our results also showed that there seems to be no direct correlation between the cytokines inhibition and the glycosidases inhibition. The studies on detailed immunosuppressive mechanism of iminosugars will be under investigation.

4. Experimental

4.1. Chemistry

4.1.1. Typical 'one-pot' procedure for the synthesis of 2,3,4-tri-*O*-benzyl-*N*-benzyl-1,6-dideoxynojirimycin (7a). Compound **6**[27] (120 mg, 0.27 mmol) was dissolved in the mixed solvent of CF_3COOH and MeOH (V/V CF_3COOH/MeOH = 1:40, 6 mL). The mixture was heated at 60 °C for 10 min until TLC analysis showed the complete disappearance of the starting material. A slurry of benzylamine (40 μL, 0.38 mmol) in MeOH (1 mL) was treated with acetic acid to adjust the pH value to 5–6, and this solution was added to the reaction mixture over a period of 0.5 h by ice-water bath cooling. A portion of $NaCNBH_3$ (51 mg, 0.81 mmol) was then added, and the mixture was stirred for 18 h at room temperature. The mixture was quenched with 0.5 mL 2 N HCl aqueous solution. After removal of the solvent, the residue was dissolved in 10% Na_2CO_3 (4 mL), and extracted with EtOAc. The EtOAc layers were combined, dried (Na_2SO_4), filtered, and concentrated. The residue was purified by column chromatography on silica gel (petroleum ether/EtOAc 12:1) to provide **7a** (105 mg, 77%) as white solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.31 (d, J = 6.0 Hz, 3H), 1.95 (t, J = 10.5 Hz, 1H), 2.32–2.38 (m, 1H), 2.98 (dd, J = 4.5, 11.0 Hz, 1H), 3.18 (t, J = 9.0 Hz, 1H), 3.24 (d, J = 13.5 Hz, 1H), 3.51 (t, J = 9.0 Hz, 1H), 3.53–3.58 (m, 1H), 4.05 (d, J = 14.0 Hz, 1H), 4.50–4.58 (ABq, J = 11.5 Hz, 2H), 4.62 (d, J = 11.0 Hz, 1H), 4.81 (d, J = 11.0 Hz, 1H), 4.95 (d, J = 11.0 Hz, 1H), 4.96 (d, J = 11.0 Hz, 1H), 7.20–7.33 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.52, 54.28, 56.50, 60.83, 72.45, 75.27, 75.54, 78.52, 84.21, 87.02, 126.90, 127.40, 127.50, 127.58, 127.66, 127.83, 127.86, 128.25, 128.27, 128.35, 128.66, 138.40, 138.54, 138.63, 138.98. HRMS (ESI, positive) for $C_{34}H_{37}NO_3$ Calcd 508.2846 [$(M+H)^+$]. Found: 508.2843.

4.1.2. 2,3,4-Tri-*O*-benzyl-*N*-phenyl-1,6-dideoxynojirimycin (7b). Compound **7b** was prepared from **6** as described in the preparation of **7a**, yielding **7b** (73% yield) as a colorless oil after column chromatography (petroleum ether/EtOAc 15:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.03 (d, J = 6.0 Hz, 3H), 2.84 (t, J = 10.5 Hz, 1H), 3.01–3.06 (m, 1H), 3.30–3.37 (m, 2H), 3.67 (t, J = 8.5 Hz, 1H), 3.78–3.83 (m, 1H), 4.63 (d, J = 11.0 Hz, 1H), 4.66 (s, 2H), 4.84 (d, J = 11.0 Hz, 1H), 4.93 (d, J = 11.0 Hz, 1H), 4.96 (d, J = 11.0 Hz, 1H), 7.04–7.37 (m, 20H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 17.24, 56.38, 59.21, 72.46, 75.08, 79.21, 84.26, 86.29, 123.81, 123.90, 127.49, 127.62, 127.72, 127.87, 128.37, 128.97, 138.38, 138.46, 138.89, 150.67. HRMS (ESI, positive) for $C_{33}H_{35}NO_3$ Calcd 494.2690 [$(M+H)^+$]. Found: 494.2690.

4.1.3. 2,3,4-Tri-*O*-benzyl-*N*-butyl-1,6-dideoxynojirimycin (7c). Compound **7c** was prepared from **6** as described in the preparation of **7a**, yielding **7c** (68% yield) as a colorless oil after column chromatography (petroleum ether/EtOAc 12:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.90 (t, J = 7.5 Hz, 3H, CH_3), 1.18 (d, J = 6.0 Hz, 3H), 1.21–1.29 (m, 2H), 1.33–1.40 (m, 2H), 2.18 (t, J = 11.0 Hz, 1H), 2.28–2.31 (m, 1H), 2.45–2.50 (m, 1H), 2.62–2.68 (m, 1H), 3.04–3.10 (m, 2H), 3.47 (t, J = 9.0 Hz, 1H), 3.58–3.63 (m, 1H), 4.60 (d, J = 11.0 Hz, 1H), 4.65–4.72 (ABq, J = 11.5 Hz, 2H), 4.82 (d, J = 11.0 Hz, 1H), 4.94 (d, J = 11.0 Hz, 1H), 4.96 (d, J = 11.0 Hz, 1H), 7.23–7.35 (m, 15H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.00, 15.89, 20.62, 26.27, 52.25, 54.56, 59.78, 72.73, 75.32, 75.47, 78.93, 84.21, 87.06, 127.41, 127.54, 127.59, 127.77, 127.86, 127.88, 128.28, 128.34, 138.56, 138.98. HRMS (ESI, positive) for $C_{31}H_{39}NO_3$ Calcd 474.3003 [$(M+H)^+$]. Found: 474.2993.

4.1.4. 2,3,4-Tri-*O*-benzyl-*N*-decanyl-1,6-dideoxynojirimycin (7d). Compound **7d** was prepared from **6** as described in the preparation of **7a**, yielding **7d** (80% yield) as white solids after column chromatography (petroleum ether/EtOAc 10:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.88 (t, J = 6.5 Hz, 3H), 1.18 (d, J = 9.0 Hz, 3H), 1.21–1.40 (m, 16H), 2.18 (t, J = 11.5 Hz, 1H), 2.28–2.31 (m, 1H), 2.45–2.50 (m, 1H), 2.61–2.67 (m, 1H), 3.04–3.10 (m, 2H), 3.47 (t, J = 9.0 Hz, 1H), 3.58–

3.62 (m, 1H), 4.60 (d, J = 11.5 Hz, 1H), 4.65–4.72 (ABq, J = 11.5 Hz, 2H), 4.82 (d, J = 11.5 Hz, 1H), 4.93 (d, J = 11.5 Hz, 1H), 4.95 (d, J = 11.5 Hz, 1H), 7.25–7.35 (m, 15H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.11, 15.87, 22.67, 24.00, 27.48, 29.31, 29.55, 29.60, 31.89, 52.60, 54.53, 59.76, 72.77, 75.35, 77.42, 78.90, 84.16, 87.05, 127.45, 127.62, 127.79, 127.90, 128.31, 128.37, 138.55, 138.98. ESI-MS: 558 [M+H$^+$]. Anal. Calcd for $C_{37}H_{51}NO_3$: C, 79.67; H, 9.22; N, 2.51. Found: C, 79.50; H, 9.29; N, 2.42.

4.1.5. 2,3,4-Tri-*O*-benzyl-*N*-hydroxyethyl-1,6-dideoxynojirimycin (7e). Compound **7e** was prepared from **6** as described in the preparation of **7a**, yielding **7e** (70% yield) as white solids after column chromatography (petroleum ether/EtOAc 3:1). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (d, J = 6.0 Hz, 3H), 2.14 (dd, J = 10.0, 11.5 Hz, 1H), 2.32 (dt, J = 4.0, 13.5 Hz, 1H), 2.37–2.41 (m, 1H), 2.43 (br, 1H), 2.97–3.02 (m, 1H), 3.10 (t, J = 9.0 Hz, 1H), 3.13 (dd, J = 4.5, 11.5 Hz, 1H), 3.56–3.62 (m, 2H), 4.60 (d, J = 11.0 Hz, 1H), 4.64–4.72 (ABq, J = 11.5 Hz, 2H), 4.83 (d, J = 11.5 Hz, 1H), 4.94 (d, J = 11.5 Hz, 1H), 4.96 (d, J = 11.5 Hz, 1H), 7.24–7.34 (m, 15H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.33, 52.83, 54.36, 58.52, 61.02, 72.86, 75.36, 75.52, 78.47, 83.90, 86.78, 127.48, 127.63, 127.69, 127.73, 128.31, 128.35, 128.39, 138.33, 138.79. ESI-MS: 462 [M+H$^+$]. Anal. Calcd for $C_{19}H_{35}NO_4$: C, 75.46; H, 7.64; N, 3.03. Found: C, 75.68; H, 7.68; N, 2.96.

4.1.6. 2,3,4-Tri-*O*-benzyl-*N*-methoxyethyl-1,6-dideoxynojirimycin (7f). A suspended mixture of compound **7e** (88 mg, 0.19 mmol) and NaH (9 mg, 0.22 mmol) in THF (5 mL) was vigorously stirred under ice-water bath. MeI (14 μL, 2.2 mmol) was then added to the mixture. The mixture was stirred overnight, and the reaction was quenched with 0.5 mL MeOH. The reaction mixture was concentrated. The syrup was diluted with CH_2Cl_2, washed successively with saturated $NaHCO_3$ and brine. The organic layer was dried and concentrated. The residue was chromatographed on silica gel with petroleum ether–EtOAc (8:1) to give **7f** (83 mg, 92%) as white solids. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (d, J = 6.0 Hz, 3H), 2.29 (t, J = 11.0 Hz, 1H), 2.38 (dd, J = 6.5, 8.5 Hz, 1H), 2.63–2.68 (m, 1H), 2.89–2.95 (m, 1H), 3.10 (t, J = 9.0 Hz, 1H), 3.14 (dd, J = 4.5, 11.5 Hz, 1H), 3.32 (s, 3H), 3.39–3.48 (m, 3H), 3.58–3.63 (m, 1H), 4.60 (d, J = 11.0 Hz, 1H), 4.65–4.71 (ABq, J = 11.0 Hz, 2H), 4.82 (d, J = 11.0 Hz, 1H), 4.93 (d, J = 11.0 Hz, 1H), 4.96 (d, J = 11.0 Hz, 1H), 7.24–7.34 (m, 15H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 16.11, 29.67, 51.55, 55.28, 58.88, 60.30, 69.71, 72.77, 75.35, 75.52, 78.64, 83.91, 86.98, 127.44, 127.60, 127.61, 127.78, 127.89, 128.30, 128.36, 138.49, 138.54, 138.96. HRMS (ESI, positive) for $C_{30}H_{37}NO_4$ Calcd 476.2795 [(M+H)$^+$]. Found: 476.2791.

4.1.7. 2,3,4-Tri-*O*-benzyl-*N*-(*N*′-benzyloxycarbonyl)-aminoethyl-1,6-dideoxynojirimycin (7g). Compound **7g** was prepared from **6** as described in the preparation of **7a** using mono-Cbz protected ethyldiamine,[37] yielding **7g** (72% yield) as white solids after column chromatography (petroleum ether/EtOAc 12:1) separation. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.21 (d, J = 5.5 Hz, 3H), 2.13 (t, J = 10.5 Hz, 1H), 2.34–2.40 (m, 2H), 2.87–2.93 (m, 1H), 3.07–3.10 (m, 2H), 3.17–3.23 (m, 1H), 3.27–3.35 (m, 1H), 3.52 (t, J = 9.0 Hz, 1H, H-4), 3.54–3.60 (m, 1H), 4.62 (d, J = 10.5 Hz, 1H), 4.67 (d, J = 11.5, 14.5 Hz, 1H), 4.72 (d, J = 11.5 Hz, 1H), 4.85 (d, J = 11.0 Hz, 1H), 4.97 (d, J = 11.0 Hz, 1H), 4.99 (d, J = 11.0 Hz, 1H), 5.05 (s, 1H), 5.12–5.17 (m, 2H), 7.24–7.37 (m, 20H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 16.15, 37.92, 50.76, 54.23, 60.77, 66.67, 72.83, 75.34, 75.51, 78.49, 83.92, 86.80, 127.56, 127.62, 127.70, 127.82, 128.13, 128.31, 128.36, 128.39, 128.51, 136.53, 138.36, 138.40, 138.87, 156.31. ESI-MS: 595 [M+H$^+$]. Anal. Calcd for $C_{37}H_{42}N_2O_5$: C, 74.72; H, 7.12; N, 4.71. Found: C, 75.00; H, 7.32; N, 4.55.

4.1.8. 1,6-Dideoxynojirimycin (8a). A mixture of **7a** (37.0 mg, 0.073 mmol) and 10% Pd–C (10.0 mg) in HOAc (2.0 mL), H_2O (1.0 mL), and THF (0.5 mL) was stirred for 24 h under H_2 atmosphere. The catalyst was then removed by filtration through Celite, and the filtrate was concentrated. The residue was subjected to a C-18 reversed-phase column chromatography (H_2O) to give **8a** (in the form of acetate) (13.0 mg, 95%) as solids after lyophilization. The data were in good agreement with those reported by Dhavale et al.[38] ^{1}H NMR (500 MHz, D_2O): δ 1.38 (d, J = 7.0 Hz, 3H), 1.97 (s, 3H), 2.89 (t, J = 12.0 Hz, 1H), 3.08–3.14 (m, 1H), 3.35 (t, J = 10.0 Hz, 1H, H-4), 3.43–3.46 (m, 2H), 3.73–3.75 (m, 1H). ^{13}C NMR (125 MHz, D_2O): δ 15.41, 46.76, 55.84, 68.04, 73.41, 76.77. HRMS (ESI, positive) for $C_6H_{13}NO_3$ Calcd 148.0968 [(M+H)$^+$]. Found: 148.0964.

4.1.9. *N*-Cyclohexyl-1,6-dideoxynojirimycin (8b). Compound **8b** was prepared from **7b** as described in the preparation of **8a**, yielding **8b** (97% yield) as solids after lyophilization. ^{1}H NMR (500 MHz, D_2O): δ 1.03–1.35 (m, 7H), 1.44–1.52 (m, 1H), 1.57–1.63 (m, 2H), 1.73–1.81 (m, 3H), 2.16 (t, J = 11.5 Hz, 1H), 2.46–2.51 (m, 1H), 2.86 (t, J = 11.5 Hz, 1H), 3.04–3.10 (m, 2H), 3.20 (t, J = 9.0 Hz, 1H, H-4), 3.46–3.51 (m, 1H). ^{13}C NMR (125 MHz, D_2O): δ 14.35, 23.20, 25.94, 26.55, 26.80, 31.44, 49.72, 57.54, 58.75, 70.50, 75.97, 78.92. HRMS (ESI, positive) for $C_{12}H_{23}NO_3$ Calcd 230.1751 [(M+H)$^+$]. Found: 230.1744.

4.1.10. *N*-Butyl-1,6-dideoxynojirimycin (8c). Compound **8c** was prepared from **7c** as described in the preparation of **8a**, yielding **8c** (100% yield) in the form of acetate, as solids after lyophilization. ^{1}H NMR (500 MHz, D_2O): δ 0.92 (t, J = 7.0 Hz, 3H), 1.30–1.34 (m, 2H), 1.36 (d, J = 6.0 Hz, 3H), 1.54–1.67 (m, 2H), 1.90 (s, 3H), 2.78 (t, J = 11.5 Hz, 1H), 2.90–3.00 (m, 2H), 3.14 (dt, J = 5.0, 11.5 Hz, 1H), 3.28 (t, J = 9.5 Hz, 1H, H-4), 3.38–3.42 (m, 2H), 3.68–3.73 (m, 1H). ^{13}C NMR (125 MHz, D_2O): δ 13.63, 13.90, 20.23, 25.46, 53.24, 54.36, 61.72, 67.80, 73.71, 77.03. HRMS (ESI, positive) for $C_{10}H_{21}NO_3$ Calcd 204.1594 [(M+H)$^+$]. Found: 204.1587.

4.1.11. *N*-Decanyl-1,6-dideoxynojirimycin (8d). Compound **8d** was prepared from **7d** as described in the preparation of **8a**, yielding **8d** (99% yield) as solids after lyophilization. ^{1}H NMR (500 MHz, CD_3OD): δ 0.89

(t, J = 6.5 Hz, 3H), 1.19 (d, J = 6.0 Hz, 3H), 1.26–1.32 (m, 14H), 1.45–1.48 (m, 2H), 2.12–2.17 (m, 2H), 2.44–2.49 (m, 1H), 2.68–2.74 (m, 1H), 2.93–2.99 (m, 2H), 3.09 (t, J = 9.0 Hz, 1H, H-4), 3.45–3.50 (m, 1H). ^{13}C NMR (125 MHz, CD_3OD): δ 5.45, 6.90, 14.72, 16.07, 19.58, 21.44, 21.62, 21.67, 21.72, 24.05, 44.87, 48.77, 52.77, 61.85, 67.97, 71.18. HRMS (ESI, positive) for $C_{16}H_{33}NO_3$ Calcd 288.2533 [$(M+H)^+$]. Found: 288.2536.

4.1.12. *N*-Hydroxyethyl-1,6-dideoxynojirimycin (8e). Compound **8e** was prepared from **7e** as described in the preparation of **8a**, yielding **8e** (92% yield) as solids after lyophilization. ^{1}H NMR (500 MHz, D_2O): 1.36 (d, J = 6.5 Hz, 3H), 2.77 (t, J = 11.5 Hz, 1H), 2.90–2.94 (m, 1H), 3.00–3.05 (m, 1H), 3.27–3.35 (m, 2H), 3.41 (t, J = 9.5 Hz, 1H, H-4), 3.46 (dd, J = 4.5, 12.0 Hz, 1H), 3.69–3.74 (m, 1H), 3.81–3.91 (m, 2H). ^{13}C NMR (125 MHz, D_2O): δ 14.29, 54.48, 54.96, 56.80, 62.41, 67.81, 73.72, 77.17. HRMS (ESI, positive) for $C_8H_{17}NO_4$ Calcd 192.1230 [$(M+H)^+$]. Found: 192.1228.

4.1.13. *N*-Methoxyethyl-1,6-dideoxynojirimycin (8f). Compound **8f** was prepared from **7f** as described in the preparation of **8a**, yielding **8f** (96% yield) as solids after lyophilization. ^{1}H NMR (500 MHz, D_2O): 1.27 (d, J = 6.5 Hz, 3H), 2.54 (t, J = 11.0 Hz, 1H), 2.62–2.65 (m, 1H), 2.86–2.90 (m, 1H), 3.15–3.22 (m, 2H), 3.27 (dd, J = 7.5, 12.0 Hz, 1H), 3.31 (t, J = 9.5 Hz, 1H, H-4), 3.36 (s, 3H), 3.60–3.65 (m, 2H), 3.68–3.72 (m, 1H). ^{13}C NMR (125 MHz, D_2O): δ 14.84, 51.78, 55.87, 58.83, 61.75, 68.27, 68.59, 74.60, 77.82. HRMS (ESI, positive) for $C_9H_{19}NO_4$ Calcd 206.1387 [$(M+H)^+$]. Found: 206.1380.

4.1.14. *N*-Aminoethyl-1,6-dideoxynojirimycin (8g). Compound **8g** was prepared from **7g** as described in the preparation of **8a**, yielding **8g** (83% yield) as solids after lyophilization. ^{1}H NMR (500 MHz, D_2O): 1.40 (d, J = 6.0 Hz, 3H), 2.85 (t, J = 11.5 Hz, 1H), 2.95–3.01 (m, 1H), 3.24–3.46 (m, 6H), 3.48–3.54 (m, 1H), 3.73 (dt, J = 5.0, 9.5 Hz, 1H). ^{13}C NMR (125 MHz, D_2O): δ 14.18, 34.85, 49.27, 55.05, 62.46, 67.69, 73.63, 76.83. HRMS (ESI, positive) for $C_8H_{18}N_2O_3$ Calcd 191.1390 [$(M+H)^+$]. Found: 191.1388.

4.1.15. *N*-(*N*′-Acetyl)aminoethyl-1,6-dideoxynojirimycin (8h). To solution of **8g** (25 mg, 0.14 mmol) in pyridine (5 mL) was added acetic anhydride (100 μL, 1.12 mmol) under ice-bath cooling. After the mixture was stirred for 5 h, MeOH was added to quench the reaction. The solvent was evaporated under reduced pressure. The residue was co-evaporated with toluene (3 mL) three times, the residue was then dissolved again in MeOH (5 mL). One percentage of catalytic amount of MeONa/MeOH (30% w/v) was added, after 20 min, the mixture was neutralized to pH 7 by Dowex-50 [H^+] resin. The resin was removed by filtration through Celite, and the filtrate was concentrated. The residue was subjected to a C-18 reversed-phase column chromatography (H_2O) to give **8h** (29 mg, 95%) as solids after lyophilization. ^{1}H NMR (500 MHz, D_2O): 1.22 (d, J = 6.0 Hz, 3H), 1.97 (s, 3H, Ac), 2.41–2.47 (m, 2H), 2.75–2.81 (m, 1H), 2.88–2.94 (m, 1H), 3.08 (t, J = 9.0 Hz, 1H), 3.12 (dd, J = 4.5, 11.5 Hz, 1H), 3.25 (t, J = 9.0 Hz, 1H, H-4), 3.35 (t, J = 7.0 Hz, 2H), 3.53–3.58 (m, 1H). ^{13}C NMR (125 MHz, D_2O): δ 15.12, 22.62, 35.51, 50.91, 56.03, 60.53, 69.37, 75.40, 78.33, 174.97. HRMS (ESI, positive) for $C_{10}H_{20}N_2O_4$ Calcd 233.1496 [$(M+H)^+$]. Found: 233.1495.

4.2. Biological assay

4.2.1. General procedure for cytokine inhibition experiments

4.2.1.1. Preparation and cultivation of splenocytes. The spleens from BALB/c mice were taken out in sterile conditions and soaked in non-serum RPMI-1640 cell culture medium. The spleens were grinded using a wire mesh. The cell suspension was filtered with a 200-mesh nylon net. The filtrate of the splenocytes was centrifuged at 2000*g* for 10 min and then the supernatant was removed. The precipitate was dissolved in 5 mL of pH 7.2 Tris–NH_4Cl solution and the cells was incubated at 37 °C for 6–10 min in order to lyse the red cells. Then the cells were centrifuged at 2000*g* for 7 min and the cell pellets were dissolved in RPMI-1640 culture medium with 10% Newborn Calf Serum. Counting cells and adjusting the concentration of cells solution to 5×10^6/mL was done, followed by addition of 5×10^5 cells into each well of 96-well plates for cultivation and assay.

4.2.1.2. Measurement of the secretion of IFN-γ and IL-4 from splenocytes. Subsequently, cells pretreated with 10 μg/mL concanavalin A (type IV) for 4 h were incubated with different concentration of each compound into each well, and incubated at 37 °C, 5% CO_2 for 72 h. The supernatant was collected and centrifuged at 2000*g* for 5 min. The supernatant was collected and stored at −20 °C until the assay. Ninety-six-well plates were coated with anti-mouse IFN-γ and IL-4 MAb in advance (commercial products). Different concentrations of drugs and IL-4 or IFN-γ standards (500, 250, 125, 62.5, 31.25, 15.63 pg/mL) were added into each well, and incubated at 20–25 °C for 120 min. The levels of IFN-γ and IL-4 secreted from immunized mice splenocytes were detected using the cytokine-specific ELISA Kits (R&D). Standard curves were determined using known concentration of the IL-4 or IFN-γ. According to the standard curve, the amount of the samples was then determined.

4.2.2. General procedure for glycoside inhibition experiments.[39,40] Each assay was performed in phosphate and nitrate buffer (25 mM), with *p*-nitrophenyl glycoside derivatives of the sugars except for β-galactosidase assay in which *o*-nitrophenyl β-galactoside was used. The reactions were initiated by the addition of enzyme to a solution of the substrate (0.4–5 mM) in the presence or absence of various concentrations of iminosugars in buffer which had been thermodynamically equilibrated. After the mixture was incubated for 20–25 min at the optimized temperature, the reaction was quenched by the addition of 200 mM Na_2CO_3 solution. The absorbance of the resulting mixture was recorded at 400 nm

(for *p*-nitrophenyl) or 420 nm (for *o*-nitrophenyl). IC_{50} values were determined as a concentration of an iminosugar that inhibits 50% of the enzyme activity.

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