Synthetic Iminosugar Derivatives as New Potential Immunosuppressive Agents

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Abstract: Several iminosugar derivatives were synthesized, and their effects on the secretion of IL-4 and IFN-γ from the mouse splenocytes were examined. The effects on membrane expression of other T cell-associated molecules (CD3, CD4, CD8) and B cell-associated molecules (CD19) were also investigated. The experimental data demonstrated that synthetic iminosugars hold potential as immunosuppressive agents.

The search to find better immunosuppressants in transplantation has been stimulated by the need to improve transplant survival and by the reduction of the toxicity of current agents. The main immunosuppressive agents currently used in organ transplantation, such as cyclosporin A (CyA), tacrolimus, mycophenolate mofetil, and sirolimus, have significant side-effects including nephrotoxicity, neurotoxicity, infection, cancer, new-onset posttransplant diabetes mellitus, hyperlipidemia, and hypertension.[1−5] At present there is no antidote for the toxicity of these transplant drugs. Although many treatment options for organ transplant patients have been developed,[6] there remains a great need for effective and safe immunosuppressive agents.

Iminosugars, also called the "sugar-shaped alkaloids", are carbohydrate analogues in which the ring oxygen has been replaced by nitrogen and are found to be widespread in plants and microorganisms.[7] They are frequently found to be potent inhibitors of many carbohydrate-processing enzymes involved in important biological systems.[8−10] These unique molecules promise a new generation of iminosugar-based medicines in a wide range of diseases such as diabetes,[11] viral infections,[12] tumor metastasis,[13] and lysosomal storage disorders.[14] However, these iminosugar derivatives as immunosuppressive agents are less explored, and little is known about their inhibition effects on immune system responses. So far only castanospermine (CAST), an indolizidine alkaloid derived from the Australian rainforest plant Castanaospermum australe,[15] was found to exhibit some immunosuppressive activity.[16−18] We report herein the synthesis of several iminosugar derivatives **1**−**5** (Figure 1) and their effects on the secretion of IL-4 and IFN-γ from the mouse splenocytes. Furthermore, the effects on membrane expression of other T cell-associated molecules (CD3, CD4, CD8) and B cell-associated molecules (CD19) were investigated.

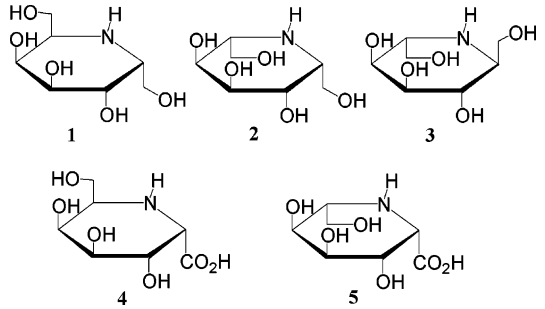
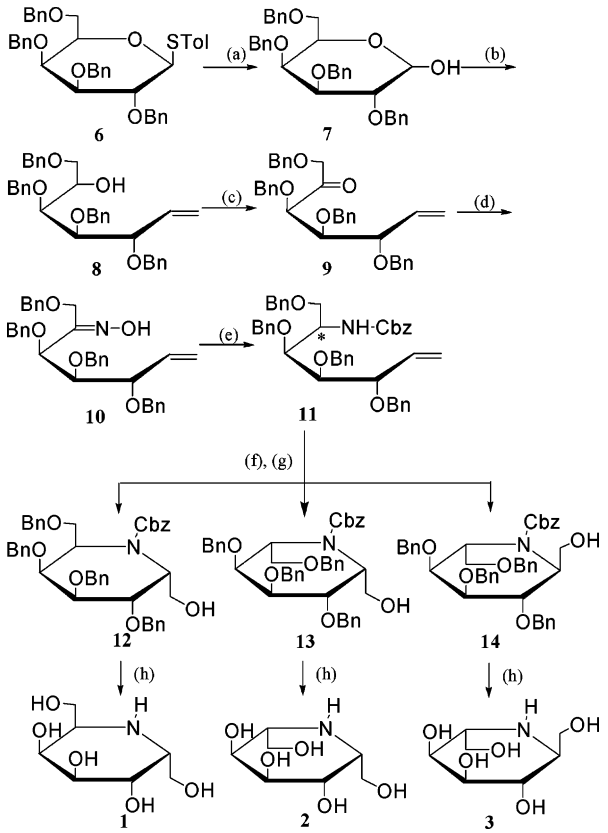


Figure 1. Structures of the iminosugar derivatives **1**−**5**.

Scheme 1[a]



[a] Reagents and conditions: (a) NBS, acetone−water, 75 °C, 97%; (b) $Ph_3P{=}CH_2$, THF, 80 °C, 79%; (c) PCC, CH_2Cl_2, 80%; (d) $NH_2OH{\cdot}HCl$, $KHCO_3$, 85 °C, 96%; (e) $LiAlH_4$, Et_2O, r.t.; then CbzCl, K_2CO_3, THF, 93%; (f) $Hg(OAc)_2$, THF, 85 °C; then KCl/H_2O/$CHCl_3$, r.t.; (g) $NaBH_4$, O_2, DMF, r.t., 65%; (h) H_2/Pd/C, HOAc:H_2O:THF = 4:2:1, r.t., 92−97%.

The synthesis of iminosugars **1**, **2**, and **3** was achieved in six steps starting from a D-galactose derivative **7**[19] by way of a chain extension−amination−cyclization sequence (Scheme 1). The key synthetic step involved an intramolecular amidomercuration reaction.

As shown in Scheme 1, tetra-*O*-benzyl-D-galactopyranose (**7**), easily prepared from the thiogalactoside **6**,[20] underwent Wittig methylenation by modified Martin's procedure[21] to give the corresponding heptenitol **8** in high yield. Oxidation of **8** with PCC produced the ketone

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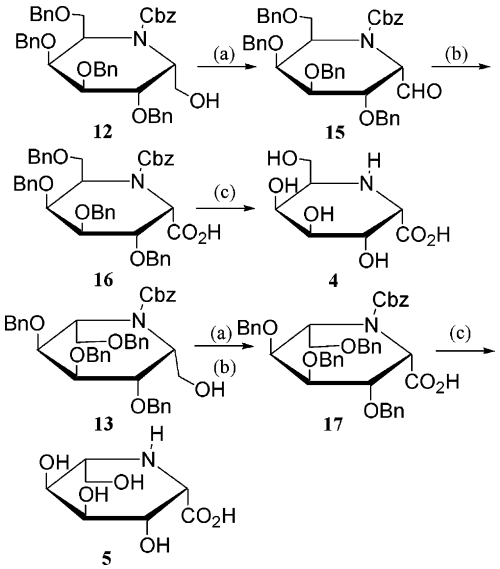
Scheme 2[a]



[a] Reagents and conditions: (a) PCC, 4 Å MS, CH_2Cl_2, r.t., 86%; (b) $NaClO_2$, NaH_2PO_4, CH_3CN-H_2O, H_2O_2, r.t., 60%; (c) H_2/Pd−C, HOAc:H_2O:THF= 4:2:1, r.t., 92% for **4**, 98% for **5**.

9 which was readily converted to the oxime **10** by treatment with hydroxylamine hydrochloride in the presence of potassium bicarbonate. Although it was reported[22] that the reduction of the oxime in the glucose derivative gave preponderantly the corresponding D-*gluco* aminoheptenitol, we found in the galactose case it was different. Reduction of the oxime **10** followed by protection for the amino group afforded **11** as a chromatographically inseparable mixture of L-*altro* and D-*galacto* epimers in excellent yield (ratio ~1.5:1). With the mixture in hand, the mercury-mediated cyclization of unsaturated amidoalditols was attempted. Upon treatment with mercuric acetate, after ligand exchange with the saturated KCl and reductive oxygenation using $NaBH_4-O_2$, we fortunately obtained the three separable cyclized isomeric alcohols **12**, **13**, and **14** in 26%, 29%, and 10% isolated yield, respectively. Theoretically, in this step four possible stereoisomers could be formed. But we isolated only three isomers presumably due to the stereoselectivity of aminomercuration for the D-*galacto* amidoalditol derivative. It can be also explained by the outcome of the cyclized products (the ratio of **13** + **14** to **12** is 39% to 26%, that is 1.5 to 1 in accord with the ratio of the starting material **11**). Finally, catalytic hydrogenolysis of **12**, **13**, and **14** over Pd−C in the mixed solvent of acetic acid, water, and THF gave the corresponding iminosugar **1**, **2**, and **3** in the form of acetate. The structure identification of iminosugars **1**−**3** and their precursors **12**−**14** was based on the analysis of their 1D (^1H, ^{13}C) and 2D NMR spectroscopy (COSY, HSQC, gHMBC, NOESY) (see the Supporting Information), and structures of **1** and **2** were further confirmed by comparison with the published data.[21,23] Although **1** and **2** are known compounds used as α-galactosidase inhibitors, by use of the modified procedure we prepared iminosugars **1**−**3** at the same time in an efficient way which involved in less synthetic steps.

The corresponding carboxylic acid derivatives **4** and **5** of iminosugars **1** and **2** were also synthesized (Scheme 2). The alcohol **12** was treated with PCC to give the aldehyde **15** in 86% isolated yield. Oxidation of **15** with sodium chlorite provided the acid **16**. Full deprotection of **15** over Pd−C in acetic acid led to the iminosugar derivative **4** in the form of acetate. Likewise, the L-altro epimer **5** was prepared from the alcohol **13** in the same manner.

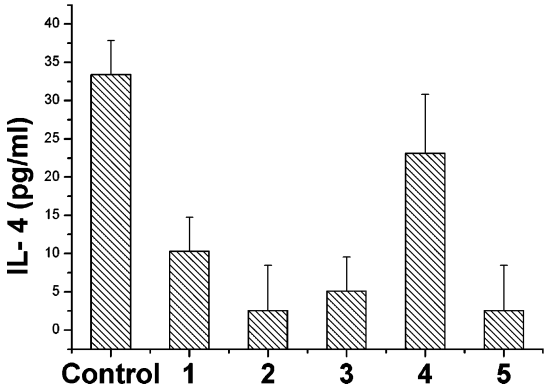


Figure 2. The inhibition effects on the secretion of IL-4 in mouse by the five compounds at 25 μM. Data are means ± SEM of at least three independent experiments, $p < 0.05$.

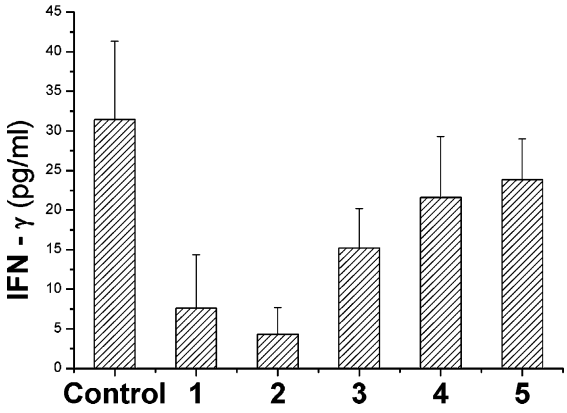


Figure 3. The inhibition effects on the secretion of IFN-γ in mouse by the five compounds at 25 μM. Data are means ± SEM of at least three independent experiments, $p < 0.05$.

To assay the effects of the five synthetic iminosugars **1**−**5** (in the form of acetate) on the secretion of cytokines from the splenocytes in mouse, the spleen cells were induced by Concanavalin A with 25 μM concentration of the compounds at 37 °C, 5% CO_2 for 72 h. The secretion of IL-4 was detected from the supernatant of spleen cells by the use of mice ELISA kit. Compared to the control, the levels of IL-4 secretion were reduced 69.2%, 92.3%, 84.6%, 30.8%, and 92.3% when including 25 μM of **1**, **2**, **3**, **4**, and **5**, respectively (Figure 2). It was found that among the five synthetic iminosugar derivatives, compounds **5** and **2** displayed the strongest inhibition ability to the IL-4 secretion.

The assay of the secretion of IFN-γ from splenocytes was similar to the assay of IL-4. The supernatant of spleen cells were detected by mice IFN-γ ELISA kit. The level of IFN-γ secretion were reduced 75.9%, 86.2%, 51.6%, 31.2%, and 24.2% when including 25 μM of compound **1**, **2**, **3**, **4**, and **5** respectively (Figure 3). The reduction efficiency of iminosugar **2** is the strongest among the five compounds.

T cells and B cells are two major types of lymphocytes. CD3 and CD19 molecules are hallmarks on the T cells and B cells, respectively. T cells contain two classes of

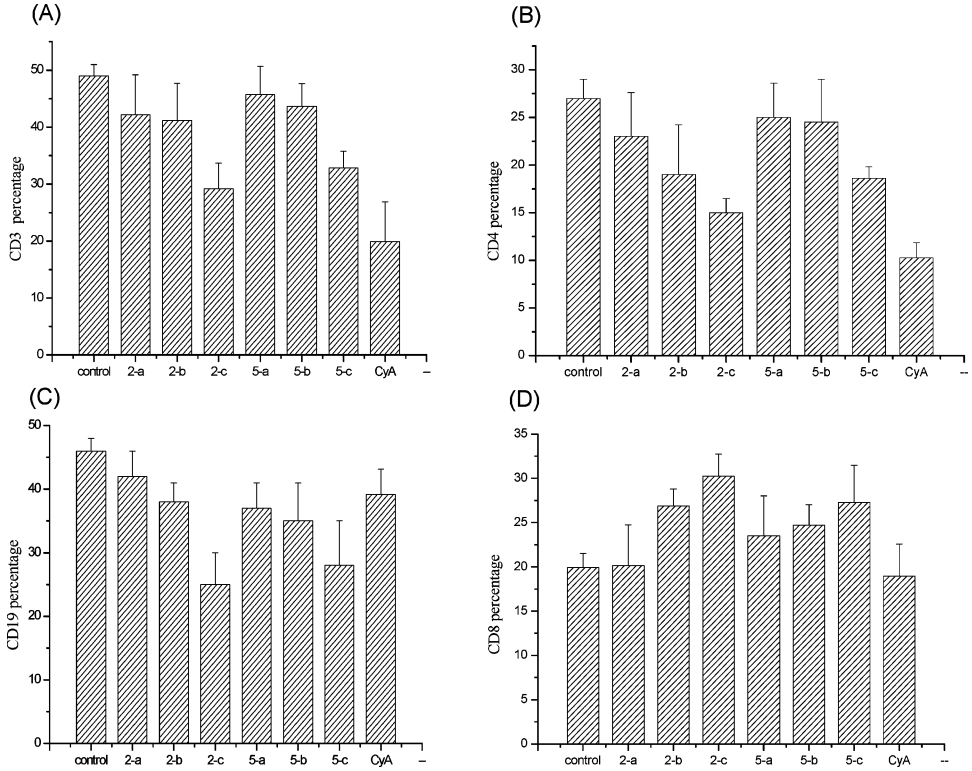


Figure 4. Effects of the compound **2** and **5** on the expression of CD3 (A), CD4 (B), CD8 (D), and CD19 (C) in mouse splencytes. CyA was used as 30 μM concentration in the test; a, b, c represented as three different concentrations respectively: 10 μM, 30 μM, and 90 μM. Data are means ± SEM of at least three independent experiments.

T lymphocytes, T helper cells, which contain CD4 markers, and T cytotoxicity cells, which contain CD8 markers. The effects of these iminosugars on the expression of CD3, CD4, CD8, and CD19 were measured by the flow cytometer (FCM).[24] We chose compound **2** and **5**, due to their strongest inhibition effects on the secretion of cytokines in mice, to further measure the effects on the expression of major immune molecules on the lymphocytes. It was first found that both compounds **2** and **5** had inhibition effects on CD3, hallmarks on the surfaces of T cells, CD4, markers of CD4+T cells, and CD19 molecules which were hallmarks on the surfaces of B cells (Figure 4 A, B, C); however, they had stimulating effects on CD8 (Figure 4 D). Compound **2** had even higher effects on all of these molecules than compound **5** did. These results suggested that the synthetic compound **2** and **5** had specific inhibition effects on the CD4+T cells and B cells, while CyA had the strongest inhibition effects on CD3 and CD4 molecules, and had no effects on CD19 and CD8 molecules.

The 50% inhibitory concentrations (IC_{50}) was determined by the tetrazolium chlorimetric reduction assay (MTT assay), which measures the mitochondrial dehydrogenase activity of surviving cells. IC_{50} of these five compounds (**1**, **2**, **3**, **4**, and **5**) (in the form of acetate) and CyA to mouse splenocytes proliferation by 72 h MTT assay were 519 μM, 332 μM, 272 μM, 340 μM, 346 μM, and 19.4 μM, respectively. These results showed that these five synthetic compounds were much less toxic than CyA to mouse PBMCs.

It is well-known iminosugars mimicking the structures of monosaccharides inhibit glycosylation processing enzymes because of a structural resemblance to the sugar moiety of the natural substrate and might have enormous therapeutic potential in many diseases. However, the effects of iminosugars on immune system and the application as immunosuppressants have been less investigated. To explore the potential application of this class of compounds as immunosuppressive agents, D-galacto- and L-altro- iminosugar derivatives were designed and synthesized in an efficient way. Furthermore, the effects of the synthetic compounds **1**−**5** on the secretion of cytokines and immune molecules surfaces expression were examined.

CD4+T helper (Th) cells usually control proliferation and differentiation, whereas CD8+T cytotoxic (Tc) cells usually kill aberrant cells. CD4+T cells recognize the antigen peptide and MHC II complex and are activated as Th1 and Th2 cells. Th1 cells secrete IFN-γ and IL-2, while Th2 cells secrete IL-4, IL-5, IL-6, and IL-10. IFN-γ mediate Th1 type of cellular immune response, while IL-4 and IL-5 stimulate B cells to secrete antibodies.[25] The ratio of the two Th cell types, Th1 and Th2, is closely correlated with the outcome of many diseases. Th1 responses predominate in organ-specific autoimmune disorders, acute allograft rejection, and in some chronic inflammatory disorders. In contrast, Th2 responses predominate in Omann's syndrome, transplantation tolerance, chronic graft-versus-host disease, systemic sclerosis, and allergic diseases.

These iminosugars may have different mechanisms to inhibit cytokine secretion and CD markers expression from CyA (form complex with cyclophilin). CyA selectively inhibits the transcription and expression of IL-2 gene and mainly inhibits CD4 Th cells, while iminosugars are carbohydrate mimetics and are potent inhibitors of many carbohydrate-processing enzymes. All cytokines and CD molecules are N-glycoproteins. The

immunosuppressive effects by these iminosugars may hold possibly the N-glycoprotein-processing inhibition effects on these glycoproteins. The data present here demonstrated that compared with the synthetic D-galacto type of iminosugars, the L-altro type of iminosugars had stronger reduction effects on the immune system. The cytokines (e.g. IFN-γ) secreted by the Th1 subset act primarily in cell-mediated response, whereas those (e.g. IL-4) secreted by the Th2 subset function mostly in B-cell activation and humoral response. The compound **2** had the strongest inhibition effects on both secretion of IFN-γ and IL-4 among these compounds and therefore might hold reduction efficiency to both humoral response and cell-mediated immune system. On the other hand, compound **5** had specifically the strongest effects on the secretion of IL-4 and only minor inhibition effects on the secretion of IFN-γ and therefore might have inhibition efficiency to the Th2-mediated humoral immune reaction. Both compound **2** and **5** inhibited the expression of CD3, CD4, and CD19, so that they may specifically inhibit the function of CD4+T cells and B cells. However, compounds **2** and **5** had some stimulating effects on the expression of CD8 molecules. The mechanisms of these differences are not well-known.

Our study first found that L-altro iminosugars **2** and **5** are much less toxic than CyA, which is a well-known immunosuppressive drug. Our results demonstrated that synthetic iminosugars, especially the L-altro type of iminosugars, hold the potential as immunosuppressive agents. In summary, the described findings may open a new avenue in the development of a new class of drugs possessing immunosuppressive activity.

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Supporting Information Available: Full experimental procedures and characterization data for all compounds. This material is available free of charge via Internet at http://pubs.acs.org.

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