Enzyme Inhibition

The Synthesis of Hybrids of D-Galactose with 1-Deoxynojirimycin Analogues as Glycosidase Inhibitors**

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The synthesis of hybrid molecules, which are made up of two different molecular units, has recently gained importance because many of them exhibit promising physical, chemical, and biological properties as well as being novel architectures.[1] These molecules are derived from either natural products or are a combination of natural products and synthetic compounds that have established or potentially significant properties, for example, compounds that could be used in biological or material science applications. In this context, natural products such as carbohydrates,[2] peptides,[3] steroids,[4] and taxoids[5] have been employed in the preparation of hybrid molecules, and their synthetic counterparts have included anthraquinones,[6] fullerenes,[7] and β-lactams.[8] Combinations are chosen with the expectation that the hybrid molecules will display enhanced or modified properties. Further work on the synthesis of newer hybrid molecules is expected, as is evident from two very important, recent reviews.[1]

The importance of glycobiology in medicinal and biological chemistry is at its height, and there have been several notable contributions in this area from both biologists and chemists.[9] Recent advances in understanding the role of glycosidase inhibitors in treating various diseases, though in their infancy, are significant in the study of glycobiology.[10] Among many glycosidase inhibitors, nojirimycin, 1-deoxynojirimycin, and their analogues have received a large amount of attention.[11] Clearly, hybrid molecules derived from nojirimycin analogues will be of interest from the standpoint of glycosidase inhibition. From the point of view of drug discovery, hybrids of D-glucose and some heterocycles have been designed and synthesized by Smith III, Hirschman, and co-workers.[12] Also, an interesting hybrid molecule that contains carbasugars has recently been found to be a better glycosidase inhibitor than the parent carbasugar.[13] These reports and our interest in the chemistry of glycals[14] and nitroglycals[14f,g] prompted us to explore the synthesis of novel hybrids as possible glycosidase inhibitors.

Herein, we report the synthesis of three novel hybrids of D-galactose with three analogues of 1-deoxynojirimycin: 1-deoxymannonojirimycin, 1-deoxygulonojirimycin, and 1-deoxymannohomonojirimycin, with 3,4,6-tri-*O*-benzyl-2-nitro-D-galactal (**1**) used as the starting point. The importance of 2-nitroglycals in the synthesis of glycopeptides, 2-amino-*O*-glycosides, 2-amino-*C*-glycosides, and other useful molecules has been well demonstrated by Schmidt and co-workers.[14g,15] We have also recently reported the synthesis of D-lividosaminide[14f] from 3-deoxy-2-nitroglucal and 2-amino-*C*-glycosides from 2-nitroglycals.[14g] A general retrosynthetic analysis of the hybrids of D-galactose and analogues of 1-deoxynojirimycin is presented in Scheme 1, which illustrates the importance of 2-nitrogalactal and ring-closing metathesis in our synthetic endeavors. Treatment of 2-nitrogalactal derivative **1**[15] with vinylmagnesium bromide gave a 1:5 mixture of **2a** and **2b** (Scheme 1) in 73% yield, which were readily separated by column chromatography. The minor compound **2a** was found to possess a $^1C_4$ conformation as was revealed by ^{1}H NMR spectroscopic analysis and NOE interaction experiments. The ^{1}H NMR spectrum of the major compound **2b**, on the other hand, indicated that it is a mixture of two inseparable components in a ratio of 1.2:1. However, reduction of this mixture with $LiAlH_4$ followed by protection of the free amine with an *N-tert*-butoxycarboxy (NHBoc) group gave a mixture of three compounds **3a**, **3b**, and **3c** that could be readily separated by chromatography. The structures of compounds **3a** and **3c** were confirmed by ^{1}H NMR spectroscopic analysis, COSY, and NOE interaction experiments as having $^4C_1$ conformations. It was difficult to assign the stereochemical orientations of the vinyl and NHBoc groups on the basis of the ^{1}H NMR spectrum of **3b**; however, the appearance of the *tert*-butyl group as a clean singlet at $\delta = 1.42$ ppm in the spectrum indicated that **3b** is a single isomer, which was also confirmed by its ^{13}C NMR spectrum. However, it was the spectral analysis of the bicyclic compound **7** that provided the basis for the conclusive assignment of the stereochemistry of **3b**.

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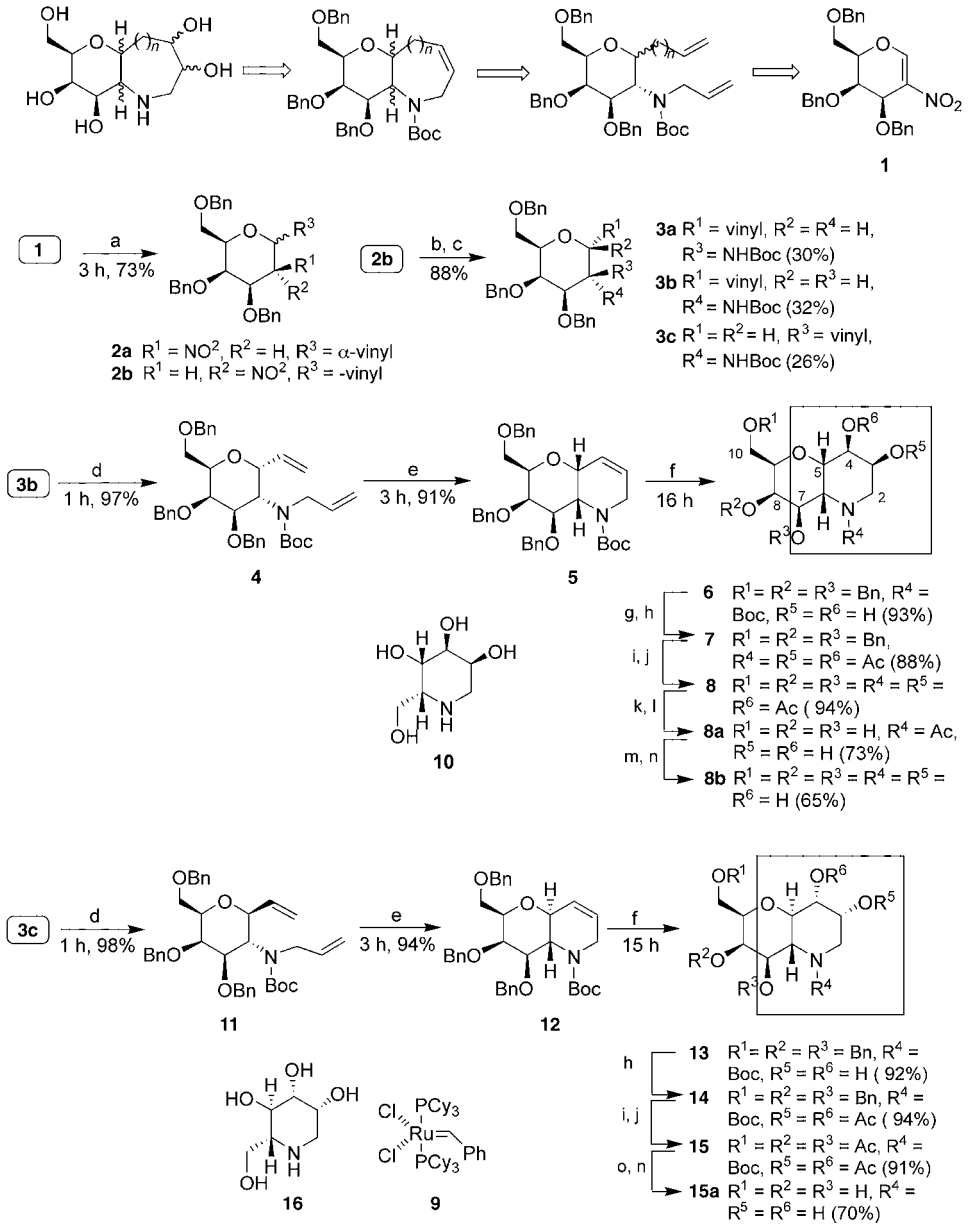


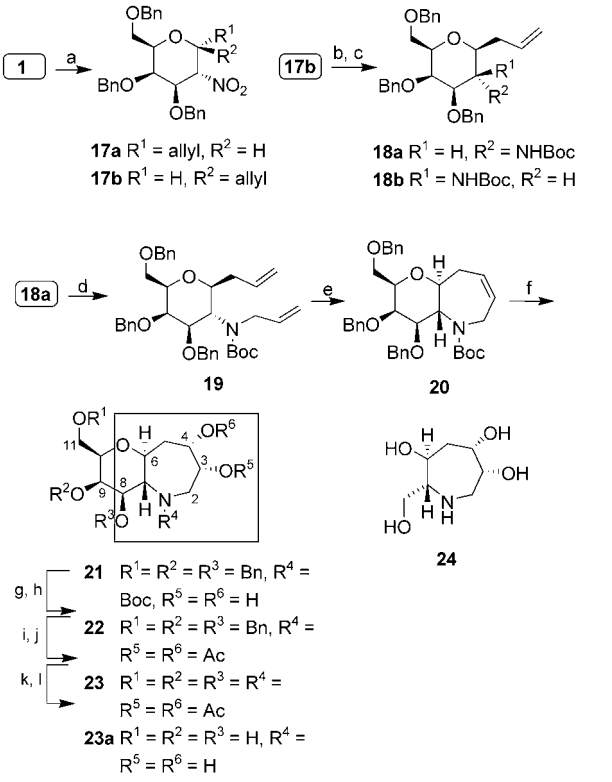
Scheme 1. a) CH_2=CHMgBr, THF, −60 °C–RT; b) $LiAlH_4$, THF, 0 °C–RT, 0.5 h; c) Boc_2O, Et_3N, CH_2Cl_2; d) allylbromide, NaH, DMF, 0 °C–RT; e) Grubbs' catalyst (5 mol %), CH_2Cl_2, reflux; f) OsO_4, NMO, acetone/H_2O/*t*BuOH (1:1:0.4); g) 3 M HCl in EtOAc, RT, 1 h; h) Ac_2O, Et_3N, CH_2Cl_2, RT, 3 h; i) 20 % $Pd(OH)_2$/C, THF, H_2, 8 h; j) Ac_2O, pyridine, 4 h; k) NaOMe, MeOH, RT, 15 min; l) amberlite (H^+) resin (300 mg), MeOH, 10 min; m) 3 M HCl in MeOH, RT, 1 h; n) dowex (OH^-) resin; o) conc. HCl (0.5 mL) in MeOH (3 mL), reflux, 17 h. DMF = *N*,*N*-dimethylformamide, Boc = *tert*-butoxycarbonyl, Bn = benzyl, and Cy = cyclohexyl.

The NHBoc group in **3a** is axially oriented and allylation failed under a variety of conditions possibly because of steric hindrance. However, compound **3b** underwent smooth allylation with allyl bromide in the presence of NaH at 0 °C–room temperature over one hour to give compound **4** in 97 % yield. Ring-closing metathesis of the diene **4** by using the first generation Grubbs' catalyst **9**[16] produced the expected bicyclic compound **5** in 91 % yield. Dihydroxylation of **5** was then performed with OsO_4/*N*-methylmorpholine *N*-oxide (NMO) to form diol **6** in 93 % yield. The *cis*-dihydroxy groups were found to have a β geometry as revealed by ^{1}H NMR spectroscopic analysis and the NOE interaction experiments of the corresponding triacetate **7**. Compound **7** showed significant NOE interaction correlations between H7, H8, and H9; H5 and H6; and H3, H4, and H9 (see the Supporting Information). Hydrogenolysis of the triacetate **7** followed by acetylation was carried out to obtain the hexaacetate **8**, which represents a hybrid molecule of D-galactose and 1-deoxygulonojirimycin (**10**).

Compound **3c** was also transformed into a bicyclic compound **15** (Scheme 1), a hybrid of D-galactose and 1-deoxymannonojirimycin (**16**), by following the same reaction sequence as above. The *cis*-dihydroxy groups of the diol **13** were found to have an α geometry, as confirmed by ^{1}H NMR

spectroscopic analysis and NOE interaction experiments of the corresponding diacetate **14** (see the Supporting Information). Further hydrogenolysis of the diacetate **14** with Pd-$(OH)_2$/C and hydrogen gas followed by acetylation yielded the pentaacetate **15**, whose structure was determined by X-ray crystallographic studies;[17] thus, the structures assigned to compounds **3c** and **13** were further confirmed.

A hybrid molecule containing a homologue of the 1-deoxynojirimycin analogue was prepared from 2-nitrogalactal (**1**; Scheme 2), which was treated with allylzinc bromide at



Scheme 2. a) $CH_2{=}CH{-}CH_2ZnBr$, THF, −60 °C–RT, 3.5 h, 82%; b) $LiAlH_4$, THF, 0 °C–RT, 0.5 h; c) Boc_2O, Et_3N, CH_2Cl_2, RT, 2 h, 78% (over two steps); d) allylbromide, NaH, DMF, 0 °C–RT, 1 h, 98%; e) Grubbs' catalyst (5 mol%), CH_2Cl_2, reflux, 6 h, 94%; f) OsO_4, NMO, acetone/H_2O/*t*BuOH (1:1:0.4), 14 h, 85%; g) 3 M HCl in EtOAc, RT, 1 h; h) Ac_2O, Et_3N, CH_2Cl_2, RT, 3 h, 87% (over two steps); i) Pd-$(OH)_2$/C, H_2, RT, overnight; j) Ac_2O, pyridine, RT, overnight, 98%; k) conc. HCl (0.5 mL) in MeOH (3 mL), reflux, 17 h; l) dowex (OH^-) resin, 75%.

−60 °C. The corresponding 2-nitro-*C*-allyl glycoside was produced in 82% yield as a 1:4 mixture of two isomers **17a** and **17b**, which were readily separated by column chromatography. The minor isomer **17a** was assigned the structure as shown; NOE interaction correlation data (see the Supporting Information) indicated it had a $^1C_4$ conformation. The major *trans* isomer **17b** was reduced with $LiAlH_4$ and the free amine protected as a NHBoc group to give a 1:1 mixture of two isomers **18a** and **18b** in 78% yield. Clearly, epimerization of **17b** had occurred even under mild reaction conditions (0 °C to room temperature). The structures of isomers **18a** and **18b** were confirmed by COSY and NOE interaction experiments.

The *trans* isomer **18a** underwent smooth allylation with allyl bromide in the presence of NaH to give diene **19** in 98% yield. An attempted allylation of **18b** under similar conditions was unsuccessful, even with heating. This observation is not surprising as the orientation of the allyl group at C1 and the NHBoc group at C2 is *cis* and so there may be steric hindrance towards allylation. Ring-closing metathesis of the diene **19** with the Grubbs' catalyst **9** gave the expected bicyclic compound **20** in 94% yield, which was then dihydroxylated with OsO_4/NMO to give **21** in 85% yield. The signal of the anomeric hydrogen atom of **17b** appeared in the ^1H NMR spectrum as a quintet at $\delta = 3.72$–3.75 ppm. However, irridation of the signal for the allylic methylene groups in **17b** at $\delta =$ 2.29 ppm in a homonuclear decoupling experiment resulted in the signal for the anomeric hydrogen atom becoming a doublet with $J = 10.0$ Hz. This result indicated that hydrogen atoms H1 and H2 are diaxially oriented. The structure was further confirmed by NOE interaction experiments that established a *cis* relationship between H1, H3, and H5 (see the Supporting Information). Additionally, the structure was also confirmed by single-crystal X-ray diffraction analysis of the triacetate **22**.[17] The triacetate **22** was hydrogenolyzed and acetylated to obtain the hexaacetate **23**, a hybrid of D-galactose and 1-deoxymannohomonojirimycin (**10**).

The hybrid azasugars **8a**, **8b**, **15a**, and **23a** showed selective inhibition activity towards the α-glycosidases[18] at millimolar concentrations (Table 1). 1-Deoxymannonojirimycin[11a,d] and 1-deoxygulonojirimycin have been recently isolated,[11a,d] however, enzyme inhibition activity of these compounds has been studied with only three glycosidases. Thus, the natural 1-deoxymannonojirimycin inhibits α-galactosidase (coffee beans) at a concentration of 420 μM (IC_{50}).[11d] In our studies with the same galactosidase, the hybrid molecule **15a** of D-galactose with 1-deoxymannonojirimycin showed no inhibition even at 7 mM concentrations (entry 1). However, it showed considerable inhibition of α-glucosidase (rice) at a concentration of 2.20 mM (entry 2). Conversely, the hybrid of D-galactose with 1,4-dideoxyhomomannonojirimy-

Table 1: Inhibitory activity of compounds **8a**, **8b**, **15a**, and **23a**.[a]

Entry	Enzymes	IC_{50} [mM]			
		8a	**8b**	**15a**	**23a**
1	α-galactosidase				
	coffee beans	3.73	4.68	NI	2.00
	almonds	NI	6.22	NI	NI
2	α-glucosidase				
	rice	3.70	7.00	2.20	2.19
	yeast	NI	4.86	NI	ND
3	β-glucosidase				
	almonds	NI	NI	NI	NI
4	α-mannosidase				
	jack beans	6.80	5.20	NI	2.66
5	β-galactosidase				
	bovine liver	ND	NI	4.50	NI
	almonds	NI	5.39	NI	NI

[a] Inhibition studies were carried out at millimolar concentrations, optimal pH of the enzymes, and 37 °C. NI = no inhibition at < 7.00 mM concentration of the inhibitor, ND = not determined.

cin **23a** showed reasonable inhibition of α-galactosidase (coffee beans), α-glucosidase (rice), and α-mannosidase (jack beans; entries 1, 2, and 4) and no inhibition of β-glucosidase and β-galactosidase (entries 3 and 5). This observation indicates that compound **23a** is more specific towards the inhibition of α-glycosidases than β-glycosidases. Likewise, hybrid molecules **8a** and **8b** also moderately inhibit α-glucosidase (rice) and α-galactosidase (coffee beans). These studies indicate that the hybrid molecules **8a**, **8b**, **15a**, and **23a** are indeed moderate inhibitors of various glycosidases. It further suggests that structural variations of these hybrid molecules could promote glycosidase inhibition. We are currently investigating this possibility and will report our results in due course.

In summary, we have synthesized three novel hybrids of D-galactose and 1-deoxynojirimycin analogues that act as glycosidase inhibitors. Furthermore, they represent novel scaffolds for possible use in drug discovery, especially in view of the reports by Smith III, Hirschmann, and co-workers.[12] Also, the presence of five hydroxy groups raises the possibility of generating a library of such hybrid molecules, which enhances the scope of the present syntheses.

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[17] CCDC-239523 and -239524 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif..

[18] All the enzymes (except α-galactosidase, β-galactosidase, and β-glucosidase, which were isolated from almond seeds) and the corresponding substrates were purchased from Sigma Chemicals Co. The inhibition potencies of the sugar–azasugar hybrids **8a**, **8b**, **15a**, and **23a** were evaluated using α-glycosidases. The IC_{50} values obtained are summarized in Table 1.

