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α-1-*C*-Alkyl-1-deoxynojirimycin derivatives as potent and selective inhibitors of intestinal isomaltase: remarkable effect of the alkyl chain length on glycosidase inhibitory profile

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Abstract—A series of α- and β-1-*C*-alkyl-1-deoxynojirimycin derivatives was prepared and evaluated as glycosidase inhibitors. Biological assays showed a marked dependence of the selectivity and potency of the inhibitors upon the position of the alkyl chain (α-1-*C*-, β-1-*C*- or *N*-alkyl derivatives). In addition, the efficiency of α-1-*C*-alkyl-1-deoxynojirimycin derivatives as intestinal isomaltase inhibitors increases with the length of the alkyl chain. The strongest inhibition was found for α-1-*C* -nonyl-1-deoxynojirimycin with an IC_{50} = 3.5 nM (25× more potent inhibitor than the shorter chain homologue carrying a C_8 chain). These results demonstrate that subtle changes in the aglycon fragment may result in remarkable enzyme specificity.

1. Introduction

Glycosidases form a widespread group of enzymes responsible for the hydrolysis of the carbohydrate glycosidic bond. These enzymes are found in all organisms and are involved in numerous biological processes, ranging from the trimming of cell- and viral-surface oligosaccharides to the lysosomal catabolism of glycoconjugates. Since glycoside cleavage is a biologically prevalent process, glycosidase inhibitors constitute leads for the development of new therapeutic agents in a wide range of diseases such as diabetes, viral infections and lysosomal storage disorders.[1] Iminosugars are a very important class of carbohydrate-processing enzyme inhibitors[2] and are known to display potent activity towards glycosidases.[3] The main drawback associated with the use of such 'azasugars' is their lack of selectivity as α- or β-glycosidase inhibitors, which may lead to detrimental side effects in therapeutic applications. One major challenge is therefore to identify iminosugar-based inhibitors that specifically modulate the activity of a given glycosidase of therapeutic interest. This goal may be achieved by a better understanding of the mechanism of action of glycosidases[4] and by the careful design of structure–activity relationship studies. An important structural feature shared by most iminosugars of therapeutic interest[5] is *N*-alkylation. For example, *N*-butyl-1-deoxynojirimycin (Zavesca™) has been approved as a drug for the treatment of Gaucher's disease[6] since 2003 and *N*-nonyl derivatives of 1-deoxynojirimycin or of 1-deoxygalactonojirimycin constitute a promising class of antiviral agents.[7] In connection with our recent work on iminosugar *C*-glycosides,[8] we synthesized a range of lipophilic derivatives of 1-deoxynojirimycin bearing an alkyl chain attached to the pseudo-anomeric position.[9] Measurements with glycosidases were made in order to assess their aglycon specificity with respect to the length and the position of the alkyl chain (α-1-*C*-, β-1-*C*- or *N*-alkyl derivatives). We focused our biological evaluation on intestinal glucosidases since they represent valuable targets for the management of type 2 diabetes as it has been demonstrated recently by the introduction of new drugs based on this concept[10] such as acarbose (Glucobay™), voglibose (Basen™) and miglitol (Glyset™).

2. Synthesis of 1-*C*-alkyl-1-deoxynojirimycin analogues

α-1-*C*-Alkyl-1-deoxynojirimycin derivatives **3a**–**c** were synthesized according to the versatile strategy we

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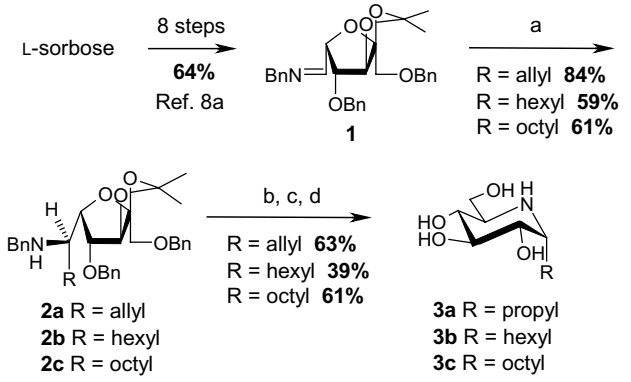
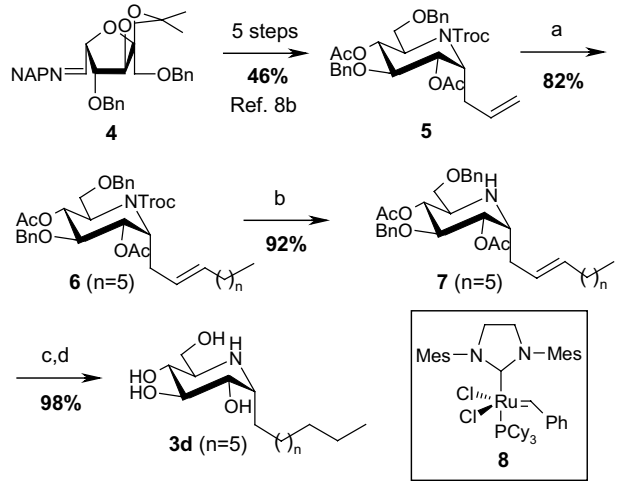


Scheme 1. Reagents and conditions: (a) AllMgBr, hexylMgBr or octylMgBr (3 equiv), Et_2O, 0–20 °C, 24 h. (b) TFA/H_2O (9/1), 30 h. (c) $NaBH_3CN$ (4 equiv), AcOH (1 equiv), MeOH, 30 h. (d) H_2, Pd/C, HCl 5 N cat., MeOH, 24 h.

recently developed.[8a] Chain extension of imine **1** with allyl-, hexyl- or octylmagnesium bromide proceeded in good yields and high diastereoselectivity (Scheme 1). Deprotection of the acetal function in aqueous TFA, followed by intramolecular reductive amination and hydrogenation of the resulting piperidinols afforded the expected nojirimycin *C*-glycosides **3a–c** in good yields after purification by chromatography on ion-exchange resin [Dowex 1-X2, (OH^- form)].[11]

α-1-*C*-Nonyl-1-deoxynojirimycin was prepared by another route using a cross-metathesis reaction as the key step (Scheme 2).

In connection with our recent work,[8b] we were indeed interested in exploring the reactivity of properly functionalized iminosugars towards various types of olefins. Reaction of α-1-*C*-allyl-1-deoxynojirimycin derivative **5**[8b] with 1-octene in the presence of Grubbs' catalyst **8** provided the expected iminoglycolipid **6** in 82% yield



Scheme 2. Reagents and conditions: (a) 1-octene (5 equiv), Grubbs' catalyst **8** (12 mol%), CH_2Cl_2, Δ, 72 h. (b) Zn (30 equiv), Et_2O/AcOH (2/1), 4 h. (c) Na, MeOH, 4 h. (d) H_2, Pd/C, HCl 5 N cat., MeOH, 48 h. NAP = 2-naphthylmethyl.

and with excellent stereoselectivity, as the (*E*)-stereoisomer was the only product detected by NMR spectroscopy.[12]

After removal of the protecting groups and reduction of the double bond, α-1-*C*-nonyl-1-deoxynojirimycin (**3d**) was obtained in 90% yield from **6**. Finally, α-1-*C*-butyl-1-deoxynojirimycin **3e** and its β-epimer **3f** were prepared according to the synthetic strategy we already reported.[8a]

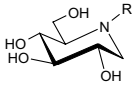
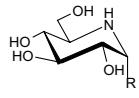
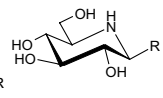
3. Biological evaluation[13,14]

3.1. Results

Having prepared the iminoglycolipids **3**, we first investigated the influence of the position of the alkyl chain on glycosidase inhibition by comparing the IC_{50} values obtained for *N*-butyl-1-deoxynojirimycin, α- and β-1-*C*-butyl-1-deoxynojirimycin. The results reported in Table 1 indicate clearly that a simple 1,2-shift of the alkyl chain from the endocyclic nitrogen to the pseudo-anomeric carbon having a β-configuration is detrimental to the inhibition towards α-glycosidases. β-1-*C*-Butyl-1-deoxynojirimycin is indeed a weak inhibitor of α-glycosidases and is devoid of activity towards β-glycosidases and trehalase.

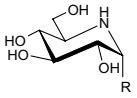
In contrast, IC_{50} values found for α-1-*C*-butyl-1-deoxynojirimycin are higher but relatively close to those obtained for its *N*-butyl analogue with the exception of rat intestinal isomaltase. The IC_{50} value of 150 nM obtained for this latter enzyme is improved by an 18-fold

Table 1. Evaluation of *N*- or 1-*C*-butyl-1-deoxynojirimycin derivatives as glycosidase inhibitors

Enzyme	IC_{50} (μM)			
				
	R = H DNJ	R = C_4H_9 *N*-Bu-DNJ	R = C_4H_9 **3e**	R = C_4H_9 **3f**
α-Glucosidase				
Rice	0.05[a]	0.42	11	61
Yeast	190[b]	NI[d]	465	NI
Maltase[e]	0.36[c]	2.1[c]	12	210
Sucrase[e]	0.21[c]	58[c]	3.6	155
Isomaltase[e]	0.3[c]	2.7[c]	0.15	115
β-Glucosidase				
Sweet almond	81[b]	NI	NI	NI
Caldocellum saccharolyticum	55	NI	NI	NI
Trehalase				
Porcine kidney	40	NI	780	NI

[a] Taken from Ref. 15.
[b] Taken from Ref. 16.
[c] Taken from Ref. 17.
[d] NI: less than 50% inhibition at 1000 μM.
[e] From rat intestine.

Table 2. Evaluation of α-1-*C*-alkyl-1-deoxynojirimycins **3a–e** as glycosidase inhibitors

Enzyme	IC_{50} (μM)				
					
	R = C_3H_7 **3a**	R = C_4H_9 **3e**	R = C_6H_{13} **3b**	R = C_8H_{17} **3c**	R = C_9H_{19} **3d**
α-Glucosidase					
Rice	15	11	0.8	0.59	1.5
Yeast	630	465	52	25	56
Maltase[a]	43	12	3.5	1.9	4.0
Sucrase[a]	39	3.6	1.2	1.5	2.5
Isomaltase[a]	**0.53**	**0.15**	**0.31**	**0.09**	**0.0035**
β-Glucosidase					
Sweet almond	NI[b]	NI	12	27	150
Caldocellum saccharolyticum	ND[c]	NI	NI	110	80

[a] From rat intestine.
[b] NI: less than 50% inhibition at 1000 μM.
[c] Not determined.

factor and is comparable to that of 1-deoxynojirimycin (DNJ).

These results encouraged us to explore further the activity of α-1-*C*-alkyl-1-deoxynojirimycins since it seemed possible to reach selective inhibition with this family of compounds. A range of nojirimycin α-*C*-glycoside derivatives bearing a linear alkyl chain (C_3, C_4, C_6, C_8 and C_9) was thus evaluated as glycosidase inhibitors (Table 2). We were pleased to observe for some enzymes a remarkable correlation between the chain length and the inhibition values. With the exception of the C_6-analogue **3b**, the potency and selectivity of α-1-*C*-alkyl-1-deoxynojirimycin as intestinal isomaltase inhibitor increased regularly with the length of the alkyl chain. Remarkably, the simple extension of the alkyl chain by only one CH_2 (from octyl to nonyl) is sufficient to promote a 25-fold increase of activity. To our knowledge, α-1-*C*-nonyl-1-deoxynojirimycin (**3d**) is the most potent and selective inhibitor of intestinal isomaltase reported to date, with an IC_{50} of 3.5 nM.[18] In addition, iminosugar **3d** shows 45,000-fold and 570-fold more potent inhibition towards isomaltase than acarbose and voglibose, respectively.[19]

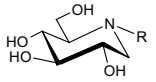
In light of the results obtained for α-1-*C*-alkyl derivatives **3c–d**, a series of *N*-alkyl analogues **9a–d** was synthesized[20,21] and evaluated as glucosidase inhibitors to verify the decisive role of the lipophilic chain position (Table 3). These *N*-alkyl-1-deoxynojirimycin derivatives were found to be less efficient and less selective as isomaltase inhibitors than the parent α-*C*-glycosides **3**. These results further underline α-1-*C*-alkyl-1-deoxynojirimycins as potent and selective inhibitors of intestinal isomaltase and validate the preliminary study performed with the iminosugars bearing a butyl chain (Table 1).

3.2. Discussion

Rat intestinal isomaltase is a retaining α-glucosidase that hydrolyses α-1,6-branching links in α-glucans (α-limit dextrins).[22] Crystallographic studies associated with site-directed mutagenesis have provided structural basis to understand the mechanism of this family of enzymes (oligo-1,6-glucosidases–EC 3.2.1.10).[19] Three carboxylic acid residues (Glu or Asp) have been clearly identified as essential catalytic-binding site residues, consistent with a double-displacement mechanism. In the first step, one of the carboxylic group protonates the glycosidic oxygen. In their anionic carboxylate form, the second one generates a covalent glycosyl-enzyme complex and the third one activates the water hydroxylating the anomeric position.

The detrimental effect of the β-alkyl chain in nojirimycin *C*-glycoside analogues may be rationalized by unfavourable interactions with one of the catalytic carboxylate residues. The affinity of *N*-alkyl- or α-1-*C*-alkyl-1-deoxynojirimycin derivatives for intestinal isomaltase may be

Table 3. Evaluation of *N*-alkyl-1-deoxynojirimycins **9a–d** as glycosidase inhibitors

Enzyme	IC_{50} (μM)			
				
	R = C_3H_7 **9a**	R = C_6H_{13} **9b**	R = C_8H_{17} **9c**	R = C_9H_{19} **9d**
α-Glucosidase				
Rice	2.0	0.70	0.08	0.08
Yeast	NI[b]	NI	NI	NI
Maltase[a]	7.0	3.7	1.5	1.3
Sucrase[a]	2.0	0.45	0.66	0.66
Isomaltase[a]	1.4	0.45	0.28	0.23
β-Glucosidase				
Sweet almond	900	350	34	150
Caldocellum saccharolyticum	100	450	160	80

[a] From rat intestine.
[b] NI: less than 50% inhibition at 1000 μM.

explained in terms of an aglycon-binding site having an extended hydrophobic region.[18] The lipophilic chain is supposed to mimic the hydrophobic face[23] of the monosaccharide unit α-glucosylated at O-6. However, an alkyl chain at C-1 with the appropriate α-configuration seems to be better positioned for suitable interactions with the putative lipophilic pocket.

Another marked dependence of the selectivity and potency upon the length of the alkyl chain is exemplified by inhibition values observed for configuration-retaining β-glucosidase from almond. IC_{50} values in the μM range were found for α-1-*C*-hexyl-and α-1-*C*-octyl-1-deoxynojirimycin whereas the parent propyl and butyl analogues were devoid of inhibitory activity (Table 2). Further extension of the alkyl chain, from octyl to nonyl, resulted in a 6-fold decrease of activity. The IC_{50} value obtained for α-*C*-hexyl-1-deoxynojirimycin (**3b**) is quite surprising in light of the fact that 1-deoxynojirimycin derivatives display generally weak inhibition towards configuration-retaining β-glucosidases such as the one from almond compared to azasugars having nitrogen instead of the 'anomeric' carbon. Various structure–activity relationship studies, using mainly amino carbasugar probes, have revealed the positive effect of a hydrophobic aglycone on β-glucosidase inhibition.[1b] This effect may be sufficient to partially compensate for the unsuitable position of the nitrogen atom in 1-deoxynojirimycin derivatives and may explain that **3d** displays relatively good inhibitory activity towards β-glucosidase from almond.

4. Conclusion

In conclusion, a range of α- and β-1-*C*-alkyl-1-deoxynojirimycin derivatives have been synthesized and evaluated as glycosidase inhibitors. This structure–activity relationship study showed a marked dependence of the selectivity and potency upon the position and the length of the alkyl chain. The best result was obtained with α-1-*C*-nonyl-1-deoxynojirimycin (**3d**), a potent and selective inhibitor of rat intestinal isomaltase (IC_{50} 3.5 nM, an inhibitor 25-fold more potent than the lower α-1-*C*-octyl homologue). These findings demonstrate that subtle changes in the iminosugar aglycone region may result in remarkable enzyme specificity. Application of this principle to glycosidases of therapeutic relevance is currently under investigation in our laboratory.

Acknowledgements

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