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Flexible synthesis and biological evaluation of novel 5-deoxyadenophorine analogues

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Abstract—Adenophorine and its 5-deoxy analogue have been identified as natural iminosugars with efficient glycosidase inhibitory effects. The syntheses and biological evaluation of two new series of 5-deoxyadenophorine analogues in their racemic form are reported. The compounds **12e** and **13d** bearing a C_{11} and C_7 alkyl chain, respectively, were found to be potent inhibitors of the β-glucosidase from almond with K_i near to 60 μM. The compounds **13a**,**d** which possess a 3,4-*cis* stereochemistry were efficient on glucosidases but also on the β-galactosidase, what was not observed with the 3,4-*trans* series **12**.

Iminosugars have been the subject of particular attention in recent years because they were found to be inhibitors not only of glycosidases[1] but also of a range of crucial enzymes such as glycosyltransferases,[2] glycogen phosphorylases,[3] and metalloproteinases.[4] Their structural resemblance to carbohydrates enabled them to be designed as potential tools for the modulation of carbohydrate-processing enzymes. Thus, digestive glycosidases, participating in the regulation of carbohydrate absorption in the small intestine and considered a serious target for type II diabetes treatment, were shown to be inhibited by several natural or synthetic iminosugars. As an example, 1-deoxynojirimycin **1** (DNJ—Fig. 1), first synthesized by Paulsen and Todt[5] and later isolated from the roots of mulberry trees, was rapidly characterized as a potent inhibitor of glucosidases in vitro. Its low efficiency in vivo was solved by the development of substituted analogues, leading to an *N*-hydroxyethyl-DNJ **2** called Miglitol™ and commercialized today by Glaxo to treat type II diabetes.

Therefore, N-alkylation of iminosugars, such as DNJ **1** or its deoxy analogues fagomine **3** and isofagomine **4**[6], was proposed as a good approach to access novel inhibitors with enzyme specificity. The change in potency and in enzyme selectivity of these lipophilic N-substituted polyhydroxylated piperidine derivatives seemed to be dependent not only on the nature of the *N*-alkyl side-chain but also on the biologically active conformation of the iminosugar. In the search for more potent and more specific inhibitors targeting endoplasmic reticulum (ER) α-glucosidase I implicated in several viral processes, including human immunodeficiency virus (HIV) and hepatitis B virus,[7] *N*-butyl-1-deoxynojirimycin **5** was shown to be a more effective viral agent than its parent DNJ **1**. A possible explanation for this difference in potency was proposed by Asano et al., who showed using ^{1}H NMR studies that N-alkylation of DNJ **1** led to a conformational change (the C_6 OH axial conformation) of the molecule and consequently enhanced the specificity of inhibition of ER α-glucosidase I.[8] Furthermore, *N*-butyl-DNJ **5** was also characterized as an inhibitor of ceramide glycosyltransferase (CGT), an enzyme involved in glycosphingolipid biosynthesis that has been approved as a good candidate for the treatment of Gaucher disease, a severe lysosomal storage disorder.[9] Recently, Butters and co-workers found that lengthening the *N*-alk(en)yl side-chain from C_4 to C_{18}

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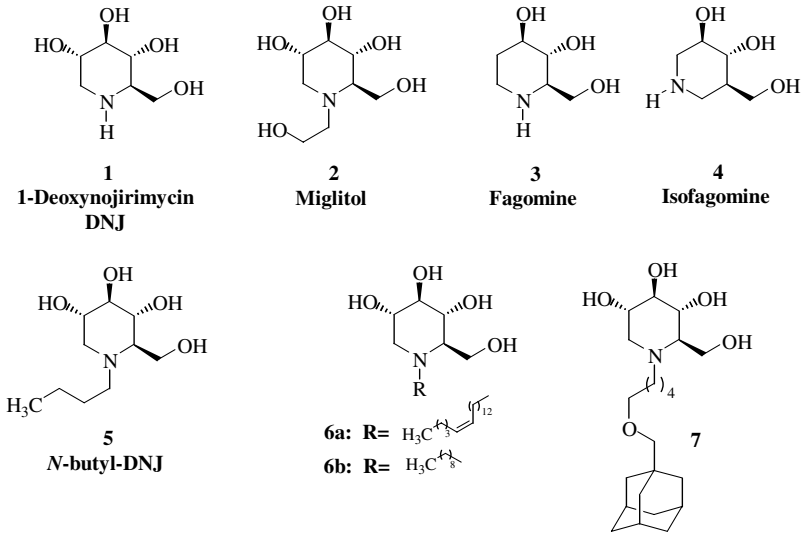


Figure 1. N-Alkylated iminosugars.

improved iminosugar retention in the cell and therefore enhanced the CGT inhibitory efficacy.[10] As observed, the lipophilic *N*-*cis*-13-octadecenyl-DNJ **6a** did not inhibit ER oligosaccharide-processing enzyme glycosidases in culture HL60 cells,[11] probably due to the accessibility of these enzymes located in the ER lumen in comparison with the CGT known to be located at the cytosolic side surface of the various Golgi subfractions.[12] Similar observations were previously made by Aerts and co-workers with compound *N*-[(5-adamantane-1-yl-methoxy)pentyl]-DNJ **7**, which is a nanomolar inhibitor (IC_{50}: 1.7 nM) of non-lysosomal glucosylceramidase but acted on α-glucosidases at a concentration not far from the micromolar (IC_{50}: 0.87 mM).[13] Another approach to favour CGT specificity was to modify one carbon configuration on the *N*-butyl-DNJ **5** piperidine skeleton, to afford the galactono analogue *N*-butyl-DGJ, which lost activity towards glucosidases but not to CGT.[14]

It is clear that both stereochemistry modification and the introduction of lipophilic substituents by simple *N*-alkyl(en)ation on iminosugars such as DNJ could be interesting strategies to design more efficient enzyme inhibitors.

Recently, Martin and co-workers have synthesized and studied a range of DNJ derivatives bearing the lipophilic alkyl chain not only on the nitrogen atom of the piperidine ring but also on the 1-*C* position of the iminosugar. With mono-alkylated compounds, the best activities were obtained on α-glucosidase (isomaltase) with the 1-*C*–C_9 derivative **8a** (IC_{50}: 3.5 nM) (Fig. 2) compared to the *N*–C_9 analogue **6b** (IC_{50}: 230 nM) (Fig. 1).[15] Furthermore, the *N*–C_8 and 1-*C*–C_8 dialkylated compound recently prepared by the same group from chiral pool L-sorbose was characterized as a glucosylceramide synthase inhibitor (IC_{50}: 174 μM) and was expected to be a weaker inhibitor of glucosidase than DNJ.[16] Extending their work on iminosugars to α-1-C-substituted dideoxynojirimycin derivatives (1-C-alkyl fagomine analogues), they proposed a strategy starting from tri-*O*-benzyl-D-glucal to access, in eight steps, a key bicyclic aziridine intermediate **9** (Fig. 2). This bicyclic compound reacted with various heteroatomic nucleophiles (amine, thiol, carboxylate or phosphate) to give the corresponding α-1-C-substituted fagomine derivatives. However, introducing an α-1-*C*-alkyl chain with organometallic nucleophiles via this aziridine intermediate **9** failed to give the desired products. This was achieved with moderate yield by treatment of the relatively unstable α-1-*C*-iodomethyl derivative of fagomine with nPr_2CuLi to give **10** (Fig. 2).[17] It should be pointed out that α-1-C-substituted analogues of DNJ **1** or fagomine **3**, such as adenophorine **11** or 5-deoxya denophorine (+)-**12a** (Fig. 2), have recently been isolated from plants.[18]

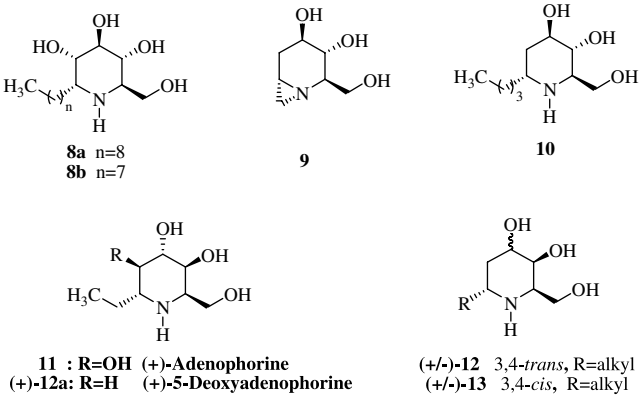


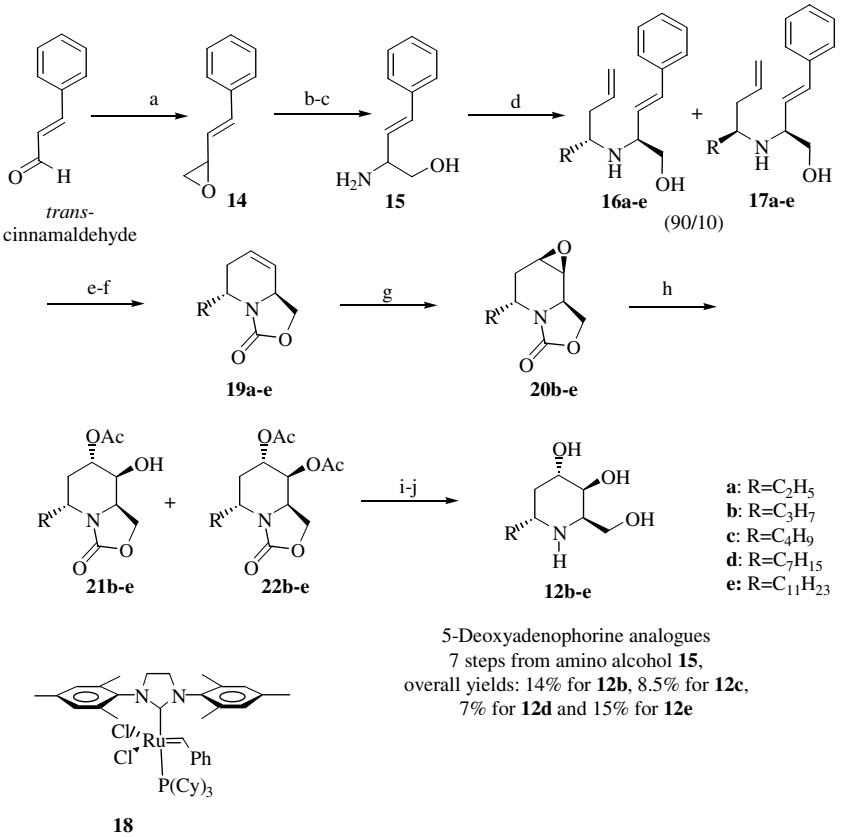
Figure 2. C-Alkylated iminosugars.

The investigations of our group into new strategies to access 1,6-disubstituted piperidines have led us recently to publish the first total synthesis of 1-*O*-β-D-glucopyranosyl-5-deoxyadenophorine and its aglycon congener (+)-**12a**.[19] In the present work, we extend this original and flexible synthesis to the production of a series of 5-deoxyadenophorine analogues (±)-**12** and (±)-**13** (Fig. 2) and to the evaluation of their activities towards

selected glycosidases. For this preliminary screening, the syntheses were carried out on racemic series.

α-1-*C*-Alkyl iminosugars **12b–e** and **13a**,**d** were prepared from the common 1-*C*-alkyl tetrahydropyridine intermediate **19**. Starting from commercial *trans*-cinnamaldehyde, a methylenation reaction was performed using Corey reagent[20] $Me_3S^+I^-$ to give epoxide **14** in 95% yield (Scheme 1). Amino alcohol **15** was then obtained in 69% yield for the two steps by regioselective nucleophilic ring-opening of the epoxide **14** with sodium azide and subsequent reduction of the azide intermediate with triphenylphosphine in the presence of water.[21] This modest yield resulted from successive recrystallizations of the amino alcohol **15**, which was contaminated by triphenylphosphine oxide. In parallel, we performed this reduction with $SnCl_2{\cdot}H_2O$ but without better results (40% yield). At this stage, we introduced, in a one-pot process, the allyl and alkyl side-chains by condensation of the amino alcohol **15** with the selected aldehydes to give the corresponding imines. These were then treated with two equivalents of allylmagnesium bromide to furnish, with good diastereoselectivities, the diethylenic *trans*-amino alcohols **16a–e**. The minor *cis*-isomers **17a–e** (around 10% yield) present with the *trans*-isomers could be separated in the following steps. It should be noted that the formation of the major *trans*-derivative **16a** is in accord with previous work from our group.[19,22] It is well documented in the literature that RCM is ineffective with free amine due to chelations with ruthenium.[23] Nevertheless, these reactions could be carried out in acidic media to form an ammonium derivative without complexation ability. In these conditions, diethylenic aminoalcohol exposed to Grubbs' catalyst gave the desired compounds contaminated by residual ruthenium species. In fact, amino alcohols act as ligands so to avoid this problem we protected the amine and hydroxyl functions as oxazolidinone. Thus, amino alcohols **16a–e** were treated with carbonyldiimidazole in the presence of triethylamine to give the corresponding oxazolidinone derivatives. These were subjected to RCM reaction in the presence of second generation Grubbs' catalyst **18** in refluxing dichloromethane to afford the *trans*-compounds **19a–e** in very good yields (72–88%) (Scheme 1).

The *trans*-3,4 iminosugar series was obtained by functionalization of the selected intermediates **19b–e** in a three-step process (Scheme 1). Epoxidation of the double bound with *m*-CPBA proceeded with good diastereoselectivity (85/15 dr) in favour of the desired *endo* isomers **20b–e**, as already noted in our previous work.[19] Opening these epoxides with acetic acid allowed regioselective access to the monoacetates **21b–e** with some diacetate derivatives **22b–e** (80/20 ratio) provided by esterification of **21** in the reaction conditions. The mixture of **21** and **22** was directly treated with potassium carbonate in methanol to give quantitatively the corresponding diols and subsequent hydrolysis of the oxazo-



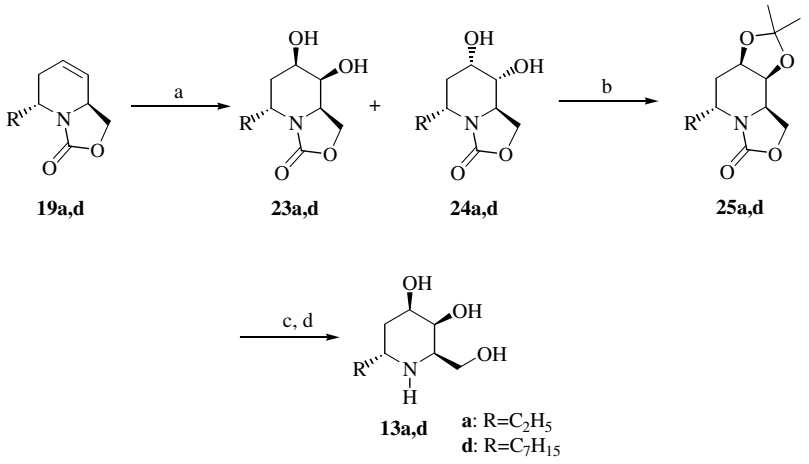
Scheme 1. Reagents and conditions: (a) $Me_3S^+I^-$, NaH, DMSO/THF, 0 °C, 30 min then rt, 40 min, 95%; (b) NaN_3, acetone/H_2O, reflux, 2 h, 94%; (c) PPh_3, THF, rt, overnight then H_2O, 24 h, 73%; (d) RCHO, $MgSO_4$, THF, rt, 12 h, then allylmagnesium bromide, THF, Et_2O, −78 °C to −10 °C, 6 h, 52–65%; (e) CDI, Et_3N, DCM, 18 h, 69–88%; (f) **18**-[Ru] (5 mol %), DCM, reflux, 1 h, 72–88%; (g) *m*-CPBA, NaH_2PO_4, DCM, 0 °C to rt, 72 h, 54–93%; (h) AcOH, 100 °C, 17 h; (i) K_2CO_3, MeOH, rt, 3 h, 50–70% for two steps; (j) 8 N aq NaOH, MeOH, 95 °C, 24 h, 66–74%.

lidinone with aqueous NaOH solution in methanol led to the desired compounds **12b–e** in 37–47% yield (three steps) after flash chromatography purification.

The *cis*-3,4 iminosugar series was obtained starting from the common precursors **19a**,**d** in a four-step process (Scheme 2). Stereoselective dihydroxylation of ethylenic compounds **19a**,**d** under Upjohn conditions afforded a mixture of *cis*-diastereoisomers **23a**,**d** and **24a**,**d** in a 70/30 ratio. At this point, the use of a bulky osmium reagent like AD-mix-α increased the diastereoselectivity (80/20) in favour of compound **23**. However, these *cis*-diols could be separated in their acetonide forms after an additional protection step carried out with 2,2-dimethoxypropane in dichloromethane. Pure compounds **25a**,**d** were isolated in around 70% yield. Subsequent cleavage of the acetal and carbamate protective groups, in classical acidic and basic conditions, respectively, led to the target molecules **13a**,**d** in 43% overall yield starting from tetrahydropyridine derivatives **19a**,**d**.

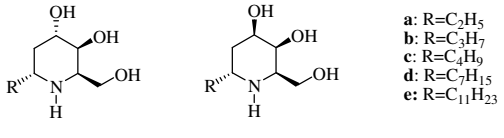
The preliminary biological assays[24] were investigated in order to reveal the potency of these novel iminosugars **12** and **13** on a range of α- and β-glycosidases and to study the influence of the length of the alkyl chain on the inhibition of these selected enzymes. It must be noted that every compound tested here is in a racemic mixture. Nevertheless, the relative stereochemistry of these analogues can be regarded as that corresponding to 5-deoxyadenophorine (Fig. 2) for compounds **12b–e** and its 4-epimer for compounds **13a**,**d**.

With iminosugars **12b–e** showing the 3,4-*trans*-diol stereochemistry, evaluation on α- and β-glucosidases or galactosidases and α-mannosidase revealed that only the glucosidases were affected by these compounds (Table 1). The inhibitory effects on β-glucosidase were clearly observed with a 1 mM concentration of the presumed inhibitors **12** bearing the longest alkyl side-chain C_7 and C_{11}, compounds **12d** and **e**, respectively. The activities were weaker on α-glucosidase while no activity



Scheme 2. Reagents and conditions: (a) AD-mix-α, $(DHQ)_2PHAL$, $MeSO_2NH_2$, *t*-BuOH/H_2O, 0 °C to rt, 12 h; (b) DMP, APTS, DCM, rt, 3 h, 72% for **25a** and 65% for **25d** in two steps; (c) 2 N aq HCl, THF, rt, 12 h; (d) 8 N aq NaOH, MeOH, 95 °C, 24 h, 60% for **13a** and 67% for **13d** in two steps.

Table 1. Inhibitory activities of (±)-5-deoxy analogues of adenophorine **12b–e** and of some 4-epimers **13a**,**d**



Enzyme/inhibitor	**12b**	**12c**	**12d**	**12e**	**13a**	**13d**	(+)-**12a**[b]
α-Glucosidase/yeast	NI[a]	NI	NI	196 μM	123 μM	154 μM	NI[c]
β-Glucosidase/almond	NI	NI	83%	58 μM	NI	61 μM	NI
α-Galactosidase/green coffee	NI	NI	NI	NI	721 μM	141 μM	6.4 μM
β-Galactosidase/*Aspergillus*	NI	NI	NI	NI	NI	NI	34 μM[d]
α-Mannosidase/almond	NI	NI	NI	NI	NI	NI	—

Percentage of inhibition at 1 mM concentration or K_i when measured, in optimal conditions.
[a] NI, no inhibitory effect, less than 40% inhibition at 1 mM.
[b] IC_{50} values of natural (+)-5-deoxyadenophorine (+)-**12a**, taken from Ref. 25.
[c] α-Glucosidase from rice.
[d] β-Galactosidase from bovine liver.

was noted on galactosidases and α-mannosidase. The K_i was evaluated for compound **12e** and was found to be 196 μM on α-glucosidase and 58 μM on β-glucosidase (Table 1). For these 1-*C*-alkyl iminosugars in their α anomeric form, it could be surprising to observe such activity on β-glucosidase. However, Compain and Co-workers recently published similar results for α-1-*C*-alkyl-1-deoxynojirimycin derivatives and, with α-1-*C*-octyl-1-deoxynojirimycin **8b** (Fig. 2), they found inhibition activities not far from 25 μM on α-glucosidase (yeast) and β-glucosidase (sweet almond).[15] On the other hand, Asano and co-workers stated that natural 5-deoxyadenophorine (+)-**12a** had no inhibition effects on a series of α-glucosidases and a β-glucosidase (almond) (Table 1).[25] All these results could indicate that the long α-1-*C*-alkyl chain of our iminosugar **12e** induces a complementary effect in the recognition of these potential inhibitors, especially in the β-glucosidase enzymatic site.

(+)-5-Deoxyadenophorine (+)-**12a** was also found to have a potent effect on α-galactosidase (IC_{50}: 6.4 μM, Table 1) and β-galactosidase (IC_{50}: 34 μM, Table 1), but this was not observed with compounds (±)-**12b–e** in our experiments. Surprisingly, the 4-*epi* analogues (±)-**13** revealed some activity on α-galactosidase (K_i = 721 and 141 μM for compounds **13a** and **d**, respectively) and a structure–activity relationship depending on the length of the alkyl chain seemed to be observed. Compound **13d**, bearing the C_7 alkyl side-chain, was also one of the best inhibitors we have found to date on β-glucosidase with a K_i value of 61 μM. In this *cis*-3,4 iminosugar series, it should be pointed out that glycosidase activity appeared with an iminosugar bearing a short alkyl side-chain (compound **13a**) but this was not observed in the *trans*-3,4 series. Unfortunately, no marked specificity was observed.

In this work, we have developed an efficient and flexible synthesis of novel 5-deoxyadenophorine analogues **12b–e** and **13a**,**d** in seven steps from the common amino alcohol **15** and with good overall yields. The preliminary structure–activity relationship study has shown a dependence of the inhibitory activity upon the 2,3-3,4 *cis*/*trans* or *cis*/*cis* stereochemistry of the molecules and a dependence of the potency upon the length of the alkyl side-chain. Therefore, β-galactosidase activity was only observed with the *cis*-3,4 derivatives **13a** and **13d** but the inhibitory effect was more efficient on β-glucosidase with compounds bearing a C_7 (**13d**: K_i = 61 μM) or a C_{11} (**12e**: K_i = 58 μM) alkyl side-chain. The *trans*-3,4 series also showed a marked structure–activity relationship dependent on the length of the 1-*C*-alkyl side-chain with α-glucosidase but the results only began to be interesting with compound **12e** with the 1-*C*–C_{11} substituent. However, these original iminosugars with a lipophilic alkyl chain could be designed as potential ceramide glycosyltransferase inhibitors as already demonstrated in the literature. Now, we are focusing our work on the synthesis of enantiomerically pure forms of compounds **12** and **13** starting from both enantiomers of Garner's aldehyde and on the introduction of lipophilic and exotic side-chains at the anomeric position of these 1-deoxyiminosugars.

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