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Synthesis and evaluation of D-*gluco*-pyranocyclopropyl amines as potential glucosidase inhibitors

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ABSTRACT

In the recent past sugar-derived cyclopropylamines were proposed as structurally new glycosidase inhibitors. In this Letter we report our efforts in the synthesis of a set of α-glucose configured oxabicyclo[4.1.0] heptanes, based on this hypothesis, bearing an amine substituent on the propyl ring and reveal that their inhibitory potential towards a range of mammalian glucosidases is modest.

Glycosidase inhibitors continue to be of interest both for fundamental and applied biomedical research.[1] The importance of glycosidase and glycosyltransferase inhibitors is underscored by the successful development of Zavesca™ (*N*-butyldeoxynojirimycin, **1**) and Miglitol™ (*N*-hydroxyethyldeoxynojirimycin, **2**) as drugs for the treatment of Gaucher disease[2] and type two diabetes,[3] respectively (Fig. 1). We recently found that the lipophilic iminosugar *N*-adamantanemethyloxypentyl-1-deoxynojirimycin **3** inhibits both human glucosylceramidase and intestinal glycosidases and on the basis of these combined actions presents a new lead towards the development of type two diabetes drugs.[4]

Despite these and other successes there still is ample room for the development of conceptually new classes of glycosidase inhibitory compounds. A potent inhibitor for a given glycosidase activity can normally be designed with confidence by tuning substitution pattern and configuration on a general scaffold, and for this purpose most literature studies make use of carbohydrate mimetic alkaloids.[5] These include polyhydroxylated pyrrolidines, piperidines (deoxynojirimycin, azafagomine) and indolizidines (castanospermine, swainsonine). Such inhibitors however are most often not selective in that multiple glycosidase activities are affected at concentrations needed to downregulate the target glycosidase to the desired level. Interestingly, this also holds true for the glycosidase inhibitor based drugs Miglitol, the therapeutically relevant property of which is based on inhibition of intestinal enzymes but which impairs the action of several subcellular glycosidases as off targets, and Zavesca, whose therapeutic activity relies on glucosylceramide synthase inhibition whereas it also inhibits with considerable potency most subcellular β-glucosidases as well as several intestinal digestive glycosidases. Therefore, more selective glycosidase and glycosyl transferase inhibitors should be of interest for the development of new pharmaceutical leads. It should be noted here that glycosyl transferases are much more elusive targets for inhibition by carbohydrate mimetics than their hydrolytic counterparts.[6] Here it appears that glucosylceramide synthase is the exception as it is the only glycosyl transferase species for which clinically relevant inhibitors have been reported.[3,4,6] Several scaffolds other than carbohydrate mimetic alkaloids are pursued for the development of new glycosidase inhibitors.[7] These include C-glycosides,[8] cyclitols[9] (polyhydroxylated cyclopentane or cyclohexane derivatives, including conduritol epoxides[10]) and functionalised cyclic thio-ethers.[11] In this respect, our attention was drawn to the α-D-glucose configured amine-functionalised oxabicyclo[4.1.0]heptane **4** (Fig. 1), which was recently suggested as a selective inhibitor of retaining α-glycosidases.[12] The rationale behind this idea is the assumption that, upon binding to an α-glucosidase active site, the secondary amine in **4** will point towards the carboxylic acid residue present in the enzyme active site on the α-face of the molecule. Next to binding due to glucose mimicry added stability is then expected to arise from the formation of a salt bridge that would be the result of protonation of the amine by the active site carboxylate. If true, this would then be a distinguishing feature that should allow the molecule to discriminate between α-glucosidases and the corresponding retaining

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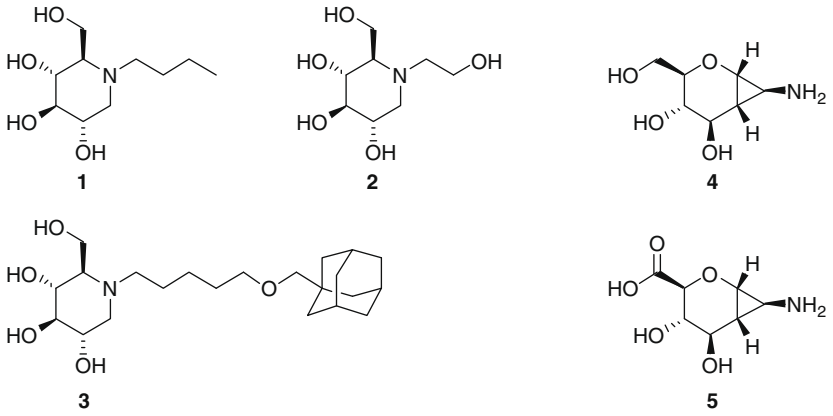


Figure 1. Structures of Zavesca (**1**), Miglitol (**2**), *N*-adamantanemethyloxypentyl-1-deoxynojirimycin (**3**), target compound **4** and sugar amino acid **5**.

β-glucosidases[13] where the carboxylic acid residue resides on the β site of its substrate glycosides. It should however be noted that retaining β-glucosidases do have a carboxylate at the α site of the substrate that might coordinate with the amine or ammonium salt presented by structure **4**. In any case, we decided to verify the hypothesis as put forward by Stick and Stubbs[12] by the synthesis of **4** and some analogues and assessment of their inhibitory potential against both α-glucosidases and β-glucosidases.

We recently reported[14] on the synthesis of glucose-derived oxabicyclo[4.1.0]heptane amino acid (SAA) **5** (Fig. 1) as part of the design of a new class of conformationally constrained sugar amino acids. Protected aminocyclopropanes corresponding to **4** featured as intermediates en route to SAA **5** and we decided to adapt this route of synthesis to generate **4** and a small number of N-alkylated (**6**, **7**) and N-acylated (**8**, **9**) derivatives. The alkyl and acyl chains were selected to arrive at compounds that resemble in substitution pattern most closely *N*-butyldeoxynojirimycin **1** and *N*-adamantanemethyloxypentyl-deoxynojirimycin **3**.[3,4] The acyl substituents were selected to probe the difference, of the (partially) protonated cyclopropyl amines and the neutral species on glycosidase inhibitory potential. In this respect, we note that the pK_a of unsubstituted cyclopropylamine (in its protonated form) is about 9.1 and we expect that our substituted derivatives, although possibly endowed with a slightly lowered pK_a due to the electronegative elements present in the carbohydrate core, are sufficiently basic to be largely protonated at physiological pH. We have previously established the inhibitory potency of several potent but non-selective glucosylceramide synthase inhibitors, as well as several related compounds on a broad panel of human glycosidases, including retaining α- and β-glucosidases.[4,6,15] By comparing this data with the inhibitory potential of compounds **4** and **6–9** we aimed to establish whether carbohydrate-derived amino- and amidocyclopropanes are useful leads for the development of selective glycosidase inhibitors.

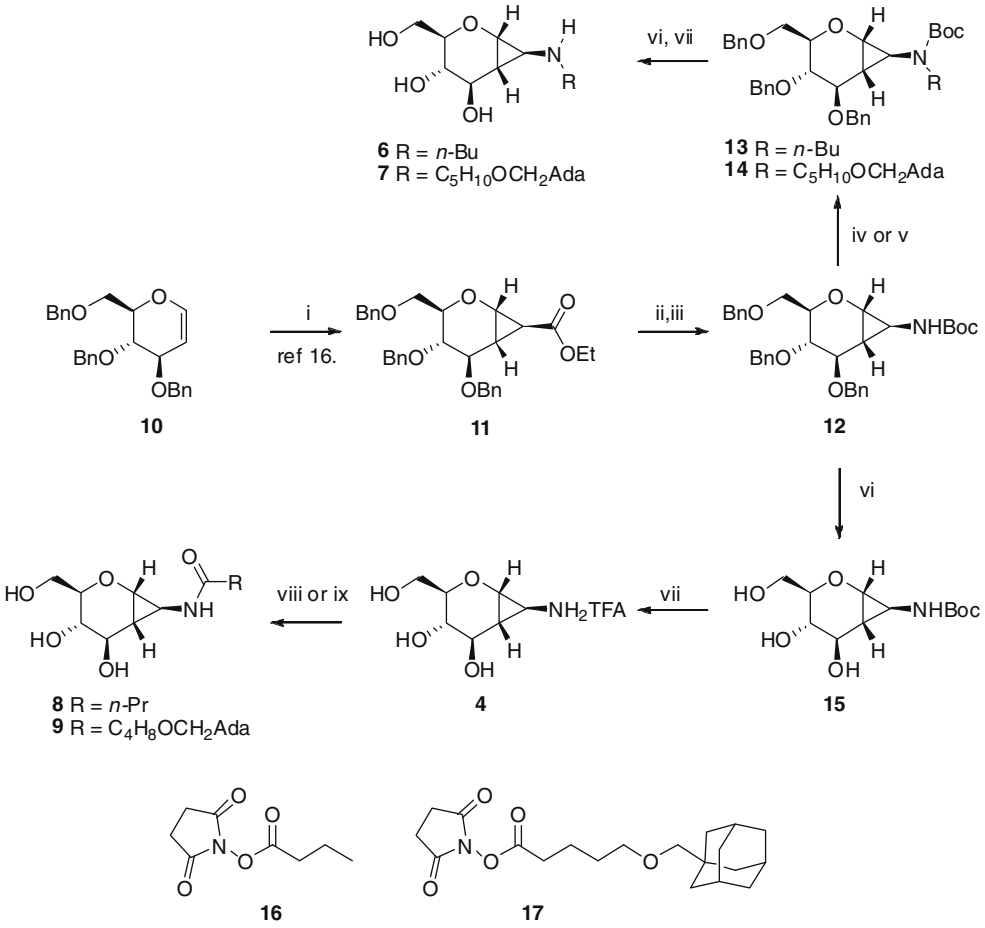
The synthesis of the target compounds is depicted in Scheme 1 and proceeds as follows. Commercially available 3,4,6-tri-*O*-benzyl-D-glucal **10** is treated with ethyl diazoacetate under the agency of rhodium(II) acetate to give cyclopropane adduct **11** in 59% yield and as the single diastereoisomer, as described in the literature.[14] Curtius rearrangement of **11** to *tert*-butoxycarbonyl(Boc)-protected amine **12** was effected by applying the two-step procedure we previously used towards the construction of SAA **5**.[16] Briefly, saponification of the ethyl ester (LiOH, THF, H_2O, MeOH) was followed by addition of diphenylphosphoryl azide and triethylamine in *tert*-butyl alcohol at elevated temperature, leading after work-up to the fully protected cyclopropylamine scaffold **12** in 58% yield over the two steps. N-Alkylation was effected by deprotonation of the NHBoc group in **12** with sodium hydride followed by addition of 1-bromobutane or 5-(adamant-1-yl-methoxy)-1-bromopentane,[6] to give fully protected *N*-butyl derivative **13** and the corresponding adamantanemethoxypentyl derivative **14** in 85% and 73% yield, respectively.

Concomitant deprotection of the benzyl groups and the Boc-group using palladium-catalysed hydrogenation followed by treatment with neat trifluoroacetic acid gave the target compounds **6** and **7** both in a near quantitative yield over the last two steps. Applying the same deprotection conditions with compound **12** gave the parent compound **4** in 98% yield. Compound **4** was acylated with either *N*-hydroxysuccinimidyl-butyrate **16** to give **6** or *N*-hydroxysuccinimidyl-5-(adamantylmethoxy)-pentanoate **17** to give **7**, in 63%, respectively 58% yield.

The inhibitory potency (IC_{50}) of the panel of five aminocyclopropanes **4**, **6**, **7**, **8** and **9** against human lysosomal glucosylceramidase (GBA1), human non-lysosomal glucosylceramidase (GBA2), human lysosomal-α-glucosidase, rice α-glucosidase, *Saccharomyces cerevisiae* α-glucosidase, *Bacillus stearothermophilus* α-glucosidase and sweet almond β-glucosidase was established using standard assays in which we included alkylated deoxynojirimycin derivatives **1** and **3** as well as the natural compound deoxynojirimycin (**18**) for comparison.[17] None of the newly synthesized compounds inhibit the human lysosomal α-glucosidase at concentrations of up to one millimolar (Table 1). This result is in contrast to the inhibitory poten-

Table 1
IC_{50} values in μM[18–20]

Compound	GBA1	GBA2	Human lysosomal α-glucosidase	Rice α-glucosidase	Sacch.cer α-glucosidase	Bac. Stear. α-glucosidase	Sweet almond β-glucosidase
1	400	0.230	0.10	1	20	20	1000
3	0.20	0.001	0.40	0.13	1	0.9	100
4	>1000	>1000	>1000	>1000	>1000	>1000	>1000
6	>1000	>1000	>1000	>1000	>1000	>1000	>1000
7	100	>1000	>1000	>1000	>1000	>1000	>1000
8	>1000	>1000	>1000	>1000	>1000	>1000	>1000
9	400	>1000	>1000	>1000	>1000	>1000	>1000
18	250	20	1.5	0.03	0.5	0.65	250



Scheme 1. Synthesis of cyclopropylamines and -amides **4**, **6**, **7**, **8** and **9**. Reagents and conditions: (i) Rh_2OAc_4, ethyldiazoacetate, CH_2Cl_2, 59%; (ii) 4 M LiOH, THF, MeOH; (iii) diphenylphosphorylazide, Et_3N, *t*BuOH, 4 Å molecular sieves, Δ, 58%; (iv) 1-bromobutane, NaH, DMF, 85%; (v) 5-(adamant-1-yl-methoxy)-1-bromopentane, NaH, DMF, 73%; (vi) Pd/C, H_2, MeOH, HOAc (98%); (vii) TFA, CH_2Cl_2, (quant.); (viii) **16**, DMF, DIPEA, 63%; (ix) **17**, DMF, DIPEA, 58%.

tial of the known derivatives **1**, **3** and **18** which have IC_{50} values ranging from nanomolar to low micromolar. Interestingly, compounds **7** and **9** exert moderate inhibitory activity towards the lysosomal glucosylceramidase GBA1. This retaining β-glucosidase is inhibited at concentrations in the same range of those observed for deoxynojirimycin **18** and *N*-butyldeoxynojirimycin **1**, with the adamantane derivative **7** being slightly more active and selective for GBA1 in this experiment.

Compound **4** and the novel analogs **6–9** do not inhibit the retaining lysosomal α-glucosidase. This means that the hypothesis on which we based the synthesis of compounds **4**–**9** is not sound, at least where it concerns this particular retaining α-glucosidase. A more thorough assessment of the inhibitory activities of these types of compounds towards α-glycosidases from other sources is needed to give a definitive answer on this subject.

Surprisingly, compounds **7** and **9** show selective inhibition of GBA1. Although, their inhibitory properties are not strong, compounds **9** and especially **7** may serve as lead structures for the development of selective inhibitors of retaining β-glucosidases. Besides the synthesis of related derivatives of **7** and **9**, it is very interesting to investigate whether derivatives having the corresponding β-configured oxabicyclo[4.1.0]heptane core will turn out to be more potent inhibitors for this class of glycosidases.

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Supplementary data

Supplementary data (experimental procedures and analytical data for all compounds and enzyme assays) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.10.022.

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