Alkylated and bicyclic sugar amino acids : synthesis and applications

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6 D-*Gluco*-pyranocyclopropyl Amines and Amides

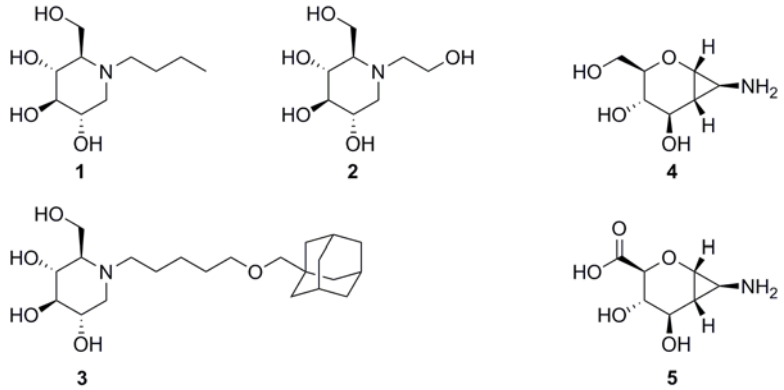
Synthesis and Evaluation as Potential α-Glucosidase Inhibitors

Introduction

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 (Figure 1). It was recently found that the lipophilic iminosugar *N*-adamantanemethyloxy-pentyl-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 the substitution pattern and configuration on a general scaffold, and for this purpose most literature studies describe the 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.

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



Interestingly, this also holds true for two glycosidase inhibitor based drugs. Although the therapeutically relevant activity of Miglitol, is based on inhibition of intestinal enzymes, the compound also impairs the action of several subcellular glycosidases as off targets. Analogously, Zavesca, whose therapeutic activity relies on glucosylceramide synthase inhibition, also inhibits most subcellular β-glucosidases as well as several intestinal digestive glycosidases with considerable potency. 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 have been 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** (Figure 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 primary 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 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, it was decided to verify the hypothesis as put forward by Stick and co-workers[12] by the synthesis of **4** and some analogues and assessment of their inhibitory potential against both α-glucosidases and β-glucosidases.

The synthesis of glucose-derived oxabicyclo[4.1.0]heptane amino acid **5** (Figure 1) as part of the design of a new class of conformationally constrained sugar amino acids is described in the previous chapter.[14] Protected aminocyclopropanes corresponding to **4** featured as intermediates *en route* to SAA **5** and it was 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]

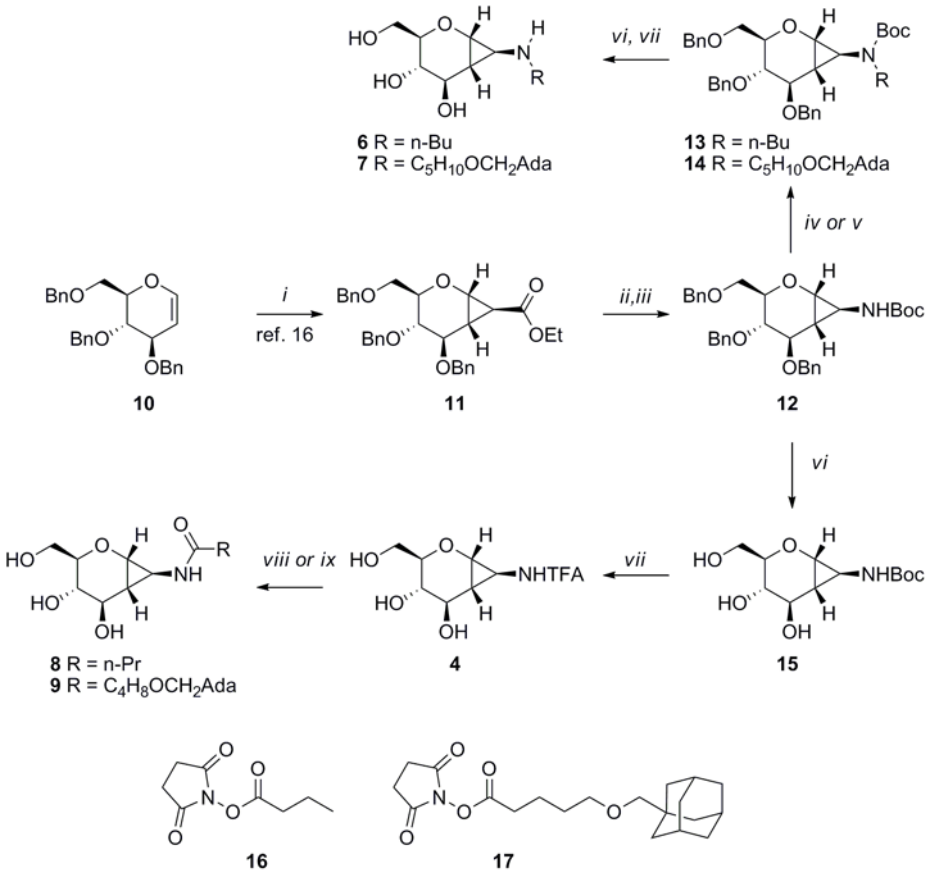
The acyl substituents were selected to probe the difference, of the (partially) protonated cyclopropyl amines and the neutral species on glycosidase inhibitory potential. In this respect, it can be noted that the pKa of unsubstituted cyclopropylamine (in its protonated form) is about 9.1 and it is estimated that the substistituted derivatives, although possibly endowed with a slightly lowered pKa due to the electronegative elements present in the carbohydrate core, are sufficiently basic to be largely protonated at physiological pH. The inhibitory potency of these potent but non-selective glucosylceramide synthase inhibitors was previously established, 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** the aim was to establish whether carbohydrate-derived amino- and amidocyclopropanes are indeed useful leads for the development of selective glycosidase inhibitors.

Results and Discussion

The synthesis of the target compounds is depicted in Scheme 1 and proceeded as follows. Commercially available 3,4,6-tri-*O*-benzyl-D-glucal **10** was treated with ethyl diazoacetate under the agency of rhodium(II) acetate to give cyclopropane adduct **11** in 59% yield and as the single diastereoisomer, essentially as described in the literature.[14] Curtius rearrangement of **11** to *tert*-butoxycarbonyl protected amine **12** was effected by applying the same two-step procedure used in the construction of SAA **5** in chapter 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 cycloproylamine scaffold **12** in 58% yield over the two steps. The *N*-alkylated derivatives **6** and **7** were prepared as follows. Deprotonation of the NHBoc group in **12** with sodium hydride was 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.

Scheme 1. Synthesis of cyclopropylamines and –amides **4,6,7,8** and **9**

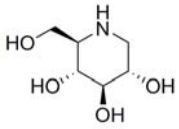


Reagents and conditions: [**i**] $Rh_2(OAc)_4$, ethyldiazoacetate, CH_2Cl_2, 59% [**ii**] 4M LiOH, THF, MeOH [**iii**] diphenylphosphorylazide, Et_3N, tBuOH, 4 Å molecular sieves, Δ, 58% [**iv**] 1-bromobutane, NaH, DMF, 85% [**v**] 5-(adamant-1-yl-methoxy)-1-bromopentane, NaH, DMF, 73% [**vi**] Pd/C, H_2, MeOH, HOAc 98% [**vii**] TFA, CH_2Cl_2, (quant.) [**viii**] **16**, DMF, DIPEA, 63% [**ix**] **17**, DMF, DIPEA, 58%

Concomitant deprotection of the benzyl groups and the Boc-group using palladium catalyzed 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 **8** or *N*-hydroxysuccinimidyl-5-(adamantylmethoxy)-pentanoate **17** to give **9**, in 63% respectively 58% yield.

Figure 2. Structure of 1-deoxynojirimicin (**18**).



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The inhibitory potency (IC_{50}) of the panel of five aminocyclopropanes **4**, and **6**-**9** against human lysosomal glucosylceramidase (GBA1), human non-lysosomal glucosylceramidase (GBA2), human lysosomal-α-glucosidase, rice α-glucosidase, *S. cerevisiae* α-glucosidase, *B. stearothermophilus* α-glucosidase and sweet almond β-glucosidase was established using standard assays in which alkylated deoxynojirimycin derivatives **1**, **3** as well as the natural compound, deoxynojirimycin (DNM, **18**, Figure 2) itself were included for comparison.[17]

Table 1: IC $_{50}$ values in μM

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

As can be seen from Table 1, none of the compounds inhibit the human lysosomal α-glucosidase at concentrations of up to one millimolar. This result is in contrast to the inhibitory potential of deoxynojirimycin **18** and its derivatives **1** and **3**, which display IC_{50} values ranging from nanomolar to low micromolar. Interesingly, the two adamantine bearing compounds, **7** and **9**, do exert moderate inhibitory activity towards the lysosomal glucosylceramidase, GBA1. This retaining β-glucosidase is inhibited at concentrations in the range of those observed for deoxynojirimycin **18** and *N*-

butyldeoxynojirimycin **1**, with the adamantane derivative **7** slightly more active than the other compounds.

Conclusion

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.

Experimental section

All reactions described were performed under an argon atmosphere and at ambient temperature unless stated otherwise. Dichloromethane was distilled from P_2O_5 prior to use. *t*-Butanol was stored on molecular sieves (4Å) for at least 36 hr prior to use. All reagents were purchased from Sigma-Aldrich or Acros and were used as received. Molecular sieves used in reactions (4Å, rods) were activated by heating in a flask over an open flame under high vacuum. Reactions were monitored by TLC analysis using TLC aluminium sheets (Merck, Silica gel 60, F_{254}) with detection by spraying with a solution of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (25 g/L) and $(NH_4)_4Ce(SO_4)_4{\cdot}2H_2O$ (10 g/L) in 10% aqueous H_2SO_4 followed by charring. Column chromatography was performed on 60Å silica gel (40-63 μm). High resolution spectra were recorded with a Finnigan LTQ Orbitrap Mass spectrometer. ^{1}H- and ^{13}C-APT-NMR spectra were recorded with either a Bruker DMX-400 (400/100 MHz) or a Bruker AV-400 (400/100 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as an internal standard for ^{1}H NMR and either $CDCl_3$ or CD_3OD for ^{13}C NMR. Coupling constants (*J*) are given in Hz. Optical rotations were measured with a Propol automatic polarimeter (λ= 589 nm) and IR (ATR-IR) spectra were recorded with a Shimadzu FTIR-8300 spectrometer and are reported in cm^{-1}.

***tert*-Butyl (1S,3R,4S,5R,6S,7S)-4,5-bis(benzyloxy)-3-(benzyloxymethyl)-2-oxabicyclo[4.1.0]heptan-7-yl carbamate (12).**

(1S,3R,4S,5R,6S,7S)-ethyl 4,5-bis(benzyloxy)-3-(benzyloxymethyl)-2-oxabicyclo[4.1.0]heptane-7-carboxylate (**11**)[16] (2.30 g, 4.77 mmol) was taken up in a mixture of THF and methanol (50 mL, 5:1 v/v). To this solution LiOH (5 mL, 4 M aq.) was added and the reaction mixture was stirred overnight at room temperature after which TLC analysis indicated full conversion of the ester into a more polar product. The solution was acidified to pH 2 with 1 M HCl. After dilution of the mixture with ethyl acetate (100 mL) the mixture was washed saturated aqueous NaCl (50 mL). The aqueous fraction was washed once with a fresh batch of ethyl acetate (50 mL). Both organic

layers were combined, dried on Na_2SO_4, filtered and concentrated *in vacuo*. The crude acid was coevaporated twice with toluene (30 mL) and taken up in *tert*-butanol (100 mL) To this solution were added freshly activated molecular sieves (4Å, rods, tablespoon), triethylamine (0.7 mL, 5 mmol) and diphenylphosphorylazide (1.1 mL, 5.0 mmol). The mixture was brought to a gentle reflux under an argon atmosphere for 24 h. The solution was filtered, concentrated *in vacuo* and taken up in ethyl acetate (100 mL). This solution was washed with 1M HCl (100 mL) and saturated aqueous $NaHCO_3$ (100 mL). The organic layer was dried on Na_2SO_4 and concentrated *in vacuo*. Chromatography of the residue on silica (0 →25% ethyl acetate in light petroleum) yielded the product **12** (1.51 g, 2.77 mmol, 58%) as a white wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.39-7.22 (m, 15H), 4.90-4.85 (m, 1H), 4.77-4.67 (m, 3H), 4.56-4.44 (m, 3H), 3.76-3.70 (m, 2H), 3.66-3.61 (m, 1H), 3.56-3.51 (m, 3H), 2.65 (d, *J* = 3.6 Hz, 1H), 1.43 (s, 9H), 1.22 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 155.8, 138.5, 138.2, 128.2, 128.1, 127.8, 127.5, 127.4, 79.5, 77.0, 76.7, 75.9, 73.2, 71.1, 69.9, 55.8, 34.1, 28.3, 25.1; $[\alpha]_D$ = +22.8 (c = 0.17, MeOH); Molecular weight calculated for $(C_{33}H_{39}NO_6+Na)^+$ = 568.2670, mass found: 568.2667; IR = cm^{-1} 3350 (m), 2860 (w), 2360 (w), 1680(vs), 1515 (s), 1450 (m), 1360 (m), 1245 (m), 1170 (m), 1100 (vs), 1025 (m), 730 (vs), 695 (vs).

***tert*-Butyl (1S,3R,4S,5R,6R,7S)-4,5-dihydroxy-3-(hydroxymethyl)-2-oxabicyclo[4.1.0]heptan-7-yl carbamate (15).**

Compound **12** (835 mg, 1.53 mmol) was taken up in methanol (20 mL). Under an argon flow, 10% palladium on carbon (~80 mg) and a few drops of acetic acid (glacial) were added to the mixture. The reaction vessel was purged with hydrogen gas and the mixture was vigorously stirred for 4 hours when TLC analysis indicated full conversion of the starting material into a significantly more polar product. The reaction mixture was filtered and taken to dryness. Chromatography of the residue on silica (0→20% MeOH in $CHCl_3$) yielded the product (414 mg, 1.50 mmol, 98%) as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ = 3.64 (dd, *J* = 11.6, *J* = 5.6 Hz, 1H), 3.57-3.51 (m, 2H), 3.42, (d, *J* = 7.6 Hz, 1 H), 3.27-3.18 (m, 3H), 2.55 (m, 1H), 1.31 (s, 9H), 0.96 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ = 158.8, 80.5, 79.8, 71.8, 71.5, 63.2, 56.9, 34.2, 28.7, 27.14; $[\alpha]_D$ = +49.2 (c = 0.17, MeOH); Molecular weight calculated for $(C_{12}H_{21}NO_6+Na)^+$ = 298.1261, mass found: 298.1262; IR = cm^{-1} 3385 (w), 3100 (b), 2975 (w), 1680 (vs), 1500 (s), 1440 (m), 1360 (m), 1240 (m), 1165 (m), 1080 (vs), 1035 (vs), 860 (w), 735(w).

(1S,3R,4S,5R,6R,7S)-4,5-Dihydroxy-3-(hydroxymethyl)-2-oxabicyclo[4.1.0]heptan-7-aminium–trifluoro-acetate (4).

Compound **15** (382 mg, 1.39 mmol) was taken up in CH_2Cl_2 and trifluoroacetic acid (10 mL, 1/1 v/v) and stirred for 30 minutes. The solution was taken to dryness and coevaporated with toluene (2 x 5 mL) to yield the product (415 mg, 1.39 mmol, quant.) as a pale yellow oil. ^{1}H NMR (400 MHz, D_2O) δ 3.71 (d, J = 8.0, 1H), 3.63-3.57 (m, 2H), 3.44 (dd, *J* = 12, J = 2.4, 1H), 3.31 (dt, *J* = 6.8, J = 2.8, 1H), 3.24 (t, J = 6.8 1H), 2.76 (d, J = 4.4, 1H) 1.31 (m, 1 H); ^{13}C NMR (100 MHz, D_2O): δ = 78.5, 69.6, 68.5, 61.2, 52.0, 31.1, 22.8; $[\alpha]_D$ = +27.5 (c = 0.09, water); Molecular weight calculated for $(C_7H_{13}NO_4+H)^+$ = 176.0917, mass found: 176.0917; IR = cm^{-1} 3290 (b), 1670 (vs), 1540 (w), 1435 (w), 1185 (vs), 1130 (vs), 1030 (m), 920 (w), 840 (m), 800 (m), 725 (s).

(1S,3R,4S,5R,6R,7S)-N-butyl-4,5-dihydroxy-3-(hydroxymethyl)-2-oxabicyclo[4.1.0]heptan-7-aminium - trifluoroacetate (6).

Compound **12** (253 mg, 0.46 mmol) was taken up in DMF (5 mL). The solution was cooled to 0°C and in small portions were added 1-bromobutane (250 µl, 2.3 mmol) and sodium hydride (102 mg, 2.55 mmol, 60% in mineral oil). When TLC analysis revealed complete conversion of the starting material , the reaction mixture was poured into 0.1 M HCl (50 mL) and extracted with diethylether (3 x 50 mL) the organic fractions were pooled, dried on Na_2SO_4, filtered and concentrated in vacuo. Silica gel chromatography (0-30% ethyl acetate in light petroleum) yielded the product (235 mg, 0.39 mmol, 85%) as a white wax.

Compound **13** (201 mg, 0.33 mmol) was taken up in methanol (10 mL). Under an argon flow, 10% palladium on carbon (~50 mg) and a few drops of acetic acid (glacial) were added to the mixture. The reaction vessel was purged with hydrogen gas and the mixture was vigorously stirred for 4 hours when TLC analysis indicated full conversion of the starting material into a significantly more polar product. The reaction mixture was filtered and concentrated *in vacuo*. The residue was taken up in CH_2Cl_2 and trifluoroacetic acid (10 mL, 1/1 v/v) and stirred for 30 minutes. The solution was taken to dryness and coevaporated with toluene (2 x 5 mL) to yield the product (115 mg, 0.33mmol, quant.) as a pale yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ = 3.91 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 3.85-3.78 (m, 2H), 3.67 (dd, *J* = 12 Hz, *J* = 2.8 Hz, 1H), 3.13 (t, *J* = 7.6 Hz, 2H), 3.07 (dd, *J* = 4.8 Hz, *J* = 1.6 Hz, 1H), 1.68 (m, 2H), 1.54-1.50 (m, 1H), 1.47 (m, 2H), 1.01 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ = 79.3, 70.9, 68.8, 62.5, 52.9, 48.9, 38.8, 29.1, 24.2, 20.8, 13.8; $[\alpha]_D$ = +30.1 (c = 0.13, MeOH); Molecular weight calculated for $(C_{11}H_{21}NO_4+H)^+$ = 232.1543, mass found: 232.1545; IR = cm-1 3350 (b), 2360 (s), 2340 (m), 1675 (m), 1650 (m), 1200 (m), 1145 (m), 530 (vs).

(1S,3R,4S,5R,6R,7S)-4,5-dihydroxy-3-(hydroxymethyl)-N-(5- (adamant-1-yl)-methoxy pentyl)-2-oxa-bicyclo[4.1.0]heptan-7-aminium trifluoroacetate (7).

Compound **12** (261 mg, 0.48 mmol) was taken up in DMF (5 mL). The solution was cooled to 0°C and in small portions were added 5-(adamant-1-yl-methoxy)-1-bromopentane (635 mg, 2.0 mmol) and sodium hydride (107 mg, 2.67 mmol, 60% in mineral oil). When TLC analysis revealed complete conversion of the starting material , the reaction mixture was poured into 0.1 M HCl (50 mL) and extracted with diethylether (3 x 50 mL). The organic fractions were pooled, dried on Na_2SO_4, filtered and concentrated *in vacuo*. Silica gel chromatography (0→30% ethyl acetate in light petroleum) yielded the product (273 mg, 0.35 mmol, 73 %) as a white wax.

Compound **14** (223 mg, 0.29 mmol) was taken up in methanol (10 mL). Under an argon flow, 10% palladium on carbon (~50 mg) and a few drops of acetic acid (glacial) were added to the mixture. The reaction vessel was purged with hydrogen gas and the mixture was vigorously stirred for 4 hours when TLC analysis indicated full conversion of the starting material into a significantly more polar product. The reaction mixture was filtered and concentrated *in vacuo*. The residue is taken up in CH_2Cl_2 and trifluoroacetic acid (10 mL, 1/1 v/v) and stirred for 30 minutes. The solution was taken to dryness and coevaporated with toluene (2 x 5mL) to yield the product (150 mg, 0.29 mmol, quant.) as a pale yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ = 3.93 (dd, *J* = 8.4 Hz, *J* = 1.2 Hz, 1H), 3.83-3.77 (m, 2H), 3.65 (dd, *J* = 12.4 Hz, *J* = 2.8 Hz, 1H), 3.49-3.38 (m, 4H), 3.12 (t, *J* = 7.6 Hz, 2H), 3.05 (dd, *J* = 4.8 Hz, *J* = 1.6 Hz, 1H), 2.97 (s, 2H), 1.94 (s, 3H), 1.77-1.47 (m, 19 H); ^{13}C NMR (100 MHz, CD_3OD) δ = 83.0, 79.11, 72.0, 70.9, 68.9, 62.5, 52.9, 49.0, 70.8, 38.7, 38.3, 35.1, 30.1, 29.7, 26.8, 24.3, 24.1; $[\alpha]_D$ = +27.4 (c = 0.17, MeOH); Molecular weight calculated for $(C_{23}H_{39}NO_5+H)^+$ = 410.2901, mass found: 410.2899; IR = cm^{-1} 3370 (b), 2900 (m), 2850 (m), 2360 (w), 1670 (s), 1655 (m), 1460 (w), 1200 (m), 1140 (m), 530 (vs).

***N*-((1S,3R,4S,5R,6R,7S)-4,5-Dihydroxy-3-(hydroxymethyl)-2-oxabicyclo[4.1.0]heptan-7-yl)butyramide (8).**

Compound **4** (152 mg, 0.53 mmol) was taken up in DMF (6 mL). To this solution were added DIPEA (350 µL, 2.0 mmol) and *N*-hydroxysuccinimidyl-butyrate (130 mg, 0.7 mmol). The reaction was stirred for 16 hours and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography yielded the product (82 mg, 0.33 mmol 63%) as a colorless wax. ^{1}H NMR (400 MHz, CD_3OD) δ = 3.78 (dd, *J* = 12 Hz, *J* = 6.4 Hz, 1H), 3.72 (dd, *J* = 7.6 Hz, *J* = 3.2 Hz, 1H), 3.66 (dd, *J* = 12 Hz, *J* = 2.8 Hz), 3.59 (dd, *J* = 8 Hz, *J* = 1.6 Hz, 1H), 3.93-3.43 (m, 1H), 3.37-3.30 (m, 1H), 2.87 (dd, *J* = 4.4 Hz, *J* = 1.2 Hz), 2.11 (t, *J* = 7.2 Hz, 2H), 1.60 (m, 2H), 1.11 (m, 1H), 0.91 (t, *J* = 7.6 Hz, 3 H); ^{13}C NMR (100 MHz, CD_3OD) δ = 177.3, 79.7, 71.5, 71.2, 62.9, 56.3, 38.6, 33.4, 26.7, 20.2, 13.9; $[\alpha]_D$ = +32.1

(c = 0.13, MeOH); Molecular weight calculated for $(C_{11}H_{19}NO_5+H)^+$ = 246.1336, mass found: 246.1337; IR = cm^{-1} 3260 (b), 2360 (m), 1635 (s), 1540 (m), 1420 (wb), 1210 (m), 1060 (w), 530 (vs).

***N*-((1S,3R,4S,5R,6R,7S)-4,5-Dihydroxy-3-(hydroxymethyl)-2-oxabicyclo[4.1.0]heptan-7-yl) 5-(adamant-1-yl-methoxy) pentanamide (9).**

5-(adamant-1-yl-methoxy)-pentanoic acid (2.66 g, 10 mmol) taken up in CH_2Cl_2 (50 mL) was treated with *N*-hydroxysuccinimide (1.59 g, 14.5 mmol) and EDC.HCl (2.63 g, 13.5 mmol) and stirring was continued for 16 h. The reaction mixture was washed with saturated brine (50 mL) and extracted with CH_2Cl_2 (50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. Purification of the residue by silica gel column chromatography yielded the *N*-hydroxysuccinimidyl-5-(adamantylmethoxy)-pentanoate (3.20 g, 8.79 mmol, 88%) as a colorless wax. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.28 (s, 2H), 3.41 (dd, *J* = 6 Hz, 2H), 2.95 (s, 2H), 2.83 (b, 4H), 2.66 (dd, *J* = 7.6 Hz, 2H), 1.95 (b, 3H), 1.85-1.52 (m, 16 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 169.12, 168.58, 81.89, 70.59, 39.66, 37.18, 34.01, 30.65, 28.55, 28.23, 25.54, 21.56.

Compound **4** (148 mg, 0.51 mmol) was taken up in DMF (6 mL). To this solution were added DIPEA (350 µL, 2.0 mmol) and *N*-hydroxysuccinimidyl-5-(adamant-1-yl-methoxy)-pentanoate (260 mg, 0.72 mmol). The reaction was stirred for 16 hours and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography yielded the product (127 mg, 0.30 mmol, 58%) as a colorless wax. ^{1}H NMR (400 MHz, CD_3OD) δ = 3.77 (dd, *J* = 12 Hz, *J* = 6.4 Hz, 1H), 3.72 (dd, *J* = 7.2 Hz, *J* = 2.8 Hz, 1H), 3.65 (dd, *J* = 11.6 Hz, *J* = 2.8 Hz, 1H), 3.58 (dd, *J* = 8.0Hz, *J* = 1.6 Hz, 1H), 3.42-3.34 (m, 4H), 2.96 (s, 2H), 2.87 (dd, *J* = 4.4 Hz, *J* = 1.2 Hz, 1H), 2.16 (t, *J* = 7.2 Hz, 2H), 1.94 (s, 3H), 1.80-1.61 (m, 8H), 1.60-1.50 (m, 8H), 1.09 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ = 177.3, 83.1, 79.8, 72.2, 71.6, 71.3, 63.0, 56.4, 40.8, 38.3, 36.6, 35.2, 33.5, 30.2, 29.8, 26.8, 23.8; $[\alpha]_D$ = +30.7 (c = 0.14, MeOH); Molecular weight calculated for $(C_{23}H_{37}NO^6+H)^+$ = 424.2694, mass found: 424.2692; IR = cm^{-1} 3350 (b), 2900 (m), 2850 (m), 2360 (w), 2340 (m), 1655 (m), 1460 (w), 1115 (m), 535 (vs).

Enzyme activity assays.

The compounds were assayed on their inhibitory activity towards the enzymes GBA1, GBA2 and lysosomal α-glucosidase, rice α-glucosidase, *S. cerevisiae* α-glucosidase, *B. stearothermophilus* α-glucosidase and sweet almond β-glucosidase. Briefly, GBA1 activity was measured using recombinant enzyme and 4-methylumbelliferyl-β-glucoside as substrate at conditions earlier described.[18] GBA2 was measured using enzyme-containing membrane preparations from Gaucher spleen and 4-methylumbelliferyl-β-glucoside as substrate at conditions earlier described.[19] Lysosomal α-glucosidase was measured using purified enzyme from human urine and 4-methylumbelliferyl-α-glucoside as substrate.[20] The other glycosidases were purchased from Sigma and the activity of these was measured using either 4-methylumbelliferyl-β-glucoside or p-nitrophenyl- α-glucoside, as advised by the supplier.

References

(1) (a) Martin, O. R.; Compain, P. *Iminosugars: From synthesis to therapeutic applications* Wiley-VCH, 2007. (b) Stütz, A. E. *Iminosugars as Glycosidase Inhibitors: Nojirimycin and Beyond* Wiley-VCH, 1999. (c) Heightman T. D., Vasella A. T. *Angew. Chem. Int. Ed.* **1999**, *38*, 750-770.

(2) Cox, T.; Lachmann, R.; Hollak, C.; Aerts, J.; van Weely, S.; Hrebicek, M.; Platt, F.; Butters, T.; Dwek, R.; Moyses, C.; Gow, I.; Elstein, D.; Zimran, A. *Lancet* **2000**, *355*, 1481-1485.

(3) Scott, L. J.; Spencer, C. M. Drugs **2000**, *59*, 521-549.

(4) Aerts, J. M.; , Ottenhoff, R.; Powlson, A. S.; Grefhorst, A.; van Eijk, M.; Dubbelhuis, P. F.; Aten, J.; Kuipers, F.; Serlie, M. J.; Wennekes, T.; Sethi, J. K.; O'Rahilly, S.; Overkleeft, H. S. *Diabetes*, **2007**, *56*, 1341-1349.

(5) (a) Winchester, B.; Fleet, G. W. J. *Glycobiology* **1992**, *2*, 199-210. (b) Asano, N.; Nash, R. J.; Molyneux, R. J.; Fleet, G. W. J. *Tetrahedron: Asymm.* **2000**, *11*, 1645-1680. (c) Asano, N. *Glycobiology* **2003**, *13*, 93R-104R (d) Watson, A. A.; Fleet, G. W. J.; Asano, N.; Molyneux, R. J.; Nash, R. J. *Phytochem.* **2001**, *56*, 265-295.

(6) Wennekes, T.; van den Berg, R. J. B. H. N.; Donker, W.; van der Marel, G. A.; Donker, W.; van der Marel, G. A.; Strijland, A.; Aerts, J. M. F. G.; Overkleeft, H. S. *J. Org. Chem.* **2007**, *72*, 1088-1097.

(7) (a) Compain, P.; Martin, O. R. *Bioorg. Med. Chem.* **2001**, *9*, 3077-3092. (b) Compain, P.; Martin, O. R. *Curr. Top. Med. Chem.* **2003**, *3*, 541-560. (c) Arjona, O.; Gomez, A. M.; Lopez, J. C.; Plumet J. *Cherm. Rev.* **2007**, *109*, 1919-2036. (d) Bols, M. *Acc. Chem. Res.* **1998**, *31*, 1-8. (e) Lillelund, V. H.; Jensen, H. H.; Liang, X. F.; Bols, M. *Chem. Rev.* **2002**, *102*, 515-553.

(8) (a) Compain, P.; Martin, O. R.; Boucheron, C.; Godin, G.; Yu, L.; Ikeda, K.; Asano, N. *ChemBioChem* **2006**, *7*, 1356-1359. (b) Godin, G.; Compain, P.; Martin, O. R. *Org. Lett.* **2003**, *5*, 3269-3272. (c) Godin, G.; Compain, P.; Masson, G.; Martin, O. R. *J. Org, Chem.* **2002**, *67*, 6960-6970.

(9) Gomez, A. M.; Moreno, E.; Valverde, S.; Lopez J. C. *Eur. J. Org, Chem.* **2004**, 1830-1840. (b) Gomez, A. M.; Moreno, E.; Valverde, S.; Lopez J. C. *Tetrahedron Lett.* **2002**, *43*, 5559-5562. (c) Berecibar, A.; Grandjean, C.; Siriwardena, A *Chem. Rev.* **1999**, *99*, 779-844.

(10) (a) Legler, G. *Methods Enzymol.* **1977**, *46*, 368-381. (b) Lee, K. J.; Boyd, S. A.; Radin, N. S. *Carbohydr. Res.* **1985**, *144*, 148-154. (c) Falshaw, A.; Hart, J. B.; Tyler, P. C. *Carbohydr. Res.*, **2000**, *329*, 301-308. (d) Tatsuta, K.; Niwata, Y.; Umezawa, K.; Toshima, K.; Nakata, M. *Tetrahedron Lett.*, **1990**, *31*, 1171-1172. (e) Hansen, F. G.; Bundgaard, E.; Madsen, R. *J. Org, Chem.* **2005**, *70*, 10139-10142.

(11) (a) Svansson, L.; Johnston, B. D.; Gu, J. H.; Patrick, B.; Pinto, B. M. *J. Am. Chem. Soc.* **2000**, *122*, 10769-10775. (b) Ghavami, A.; Johnston, B .D.; Pinto B.M. *J. Org. Chem.* **2001**, *66*, 2312-2317. (c) Kumar, N. S.; Pinto, B. M. *J. Org. Chem.* **2006**, *71*, 1262-1264.

(12) Stick, R. V.; Stubbs, K. A. *J. Carbohydr. Chem.*, **2005**, *24*, 529-547.

(13) (a) Aguilar-Moncayo, M.; Gloster, T. M.; Turkenburg, J. P.; Garcia-Moreno, M. I.; Ortiz, M. C.; Davies, G. J.; Garcia Fernandez, J. M. *Org. Biomol. Chem.* **2009**, *7*, 2738-2747. (b) Aguilar-Moncayo, M.; Mellet, C.; Ortiz, M. C.; Garcia Fernandez, J. M.; Garcia-Moreno, M. I. *J. Org. Chem.* **2009**, *74*, 3595-3598. (c) Moreno-Clavijo, E.; Carmona, A. T.; Vera-Ayoso, Y.; Moreno-Vargas, A. J.; Bello, C.; Vogel, P.; Robina, I. *Org. Biomol. Chem.* **2009**, *7*, 1192-1200. (d) Doddi, V. R.; Kancharla, P. K.; Reddy, Y. S.; Kumar, A.; Vankar, Y. D. *Carbohydr. Res.* **2009**, *344*, 606-612. (e) Aguilar, M.; Gloster, T. M.; Garcia-Moreno, M. I.; Ortiz, M. C.; Davies, G. J.; Llebaria, A.; Casas, J.; Egido-Gabas, M.; Garcia Fernandez, J. M. *ChemBioChem* **2008**, *9*, 2612-2618. (f) Behr, J.-B.; Pearson, M. S. M.; Bello, C.; Vogel, P.; Plantier-Royon, R. *Tetrahedron Asymm.* **2008**, *19*, 1829-1832. (g) Li, H.; Liu, T.; Zhang, Y.; Favre, S.; Bello, C.; Vogel, P.; Butters, T. D.; Oikonomakos, N. G.; Marrot, J.; Bleriot, Y. *ChemBioChem* **2008**, *9*, 253-260. (h) Kumar, K. S.; Ajish; C.; Vinod, D.; Dhavale, D. D. *Org. Biomol. Chem.* **2008**, 6, 703-711. (i) Kumar, K. S.; Ajish, C.; Vinod, D.; Puranik, V. G.; Dhavale, D. D. *Eur. J. Org. Chem.* **2007**, *29*, 4895-4901. (j) Lysek, R.; Favre, S.; Vogel, P.

Tetrahedron **2007**, *63*, 6558-6572. (k) Lysek, R.; Vogel, P. *Tetrahedron* **2006**, *62*, 2733-2768. (l) Pabba, J.; Vasella, A. *Helv. Chim. Acta* **2006**, *89*, 2006-2019. (m) Buser, S.; Vasella, A. *Helv. Chim. Acta* **2006**, *89*, 614-621. (n) Li, H.; Schuetz, C.; Favre, S.; Zhang, Y.; Vogel, P.; Sinay, P.; Bleriot, Y. *Org. Biomol. Chem.* **2006**, *4*, 1653-1662. (o) Wrodnigg, T. M.; Stuetz, A. E.; Tarling, C. A.; Withers, S. G. *Carb. Res.* **2006**, *341*, 1717-1722. (p) Li, H.; Schuetz, C.; Favre, S.; Zhang, Y.; Vogel, P.; Sinay, P.; Bleriot, Y. *Org. Biomol. Chem.* **2006**, *4*, 1653-1662. (q) Shanmugasundaram, B.; Vasella, A. *Helv. Chim. Acta* **2005**, *88*, 2593. (r) Magdolen, P.; Vasella, A. *Helv. Chim. Acta* **2005**, *88*, 2454-2469. (s) Cardona, F.; Moreno, G.; Guarna, F.; Vogel, P.; Schuetz, C.; Merino, P.; Goti, A. *J. Org. Chem.* **2005**, *70*, 6552-6555. (t) Li, H.; Bleriot, Y.; Mallet, J-M.; Rodriguez-Garcia, E.; Vogel, P.; Zhang, Y.; Sinay, P. *Tetrahedron Asymm.* **2005**, *16*, 313-319. (u) Verdoucq, L.; Moriniere, J.; Bevan, D. R.; Esen, A.; Vasella, A.; Henrissat, B.; Czjze, M. *J. Biol. Chem.* **2004**, *279*, 31796-31803. (v) Lysek, R.; Vogel, P. *Helv. Chim. Acta* **2004**, *87*, 3167-3181. (w) Li, H.; Bleriot, Y.; Mallet, J-M.; Zhang, Y.; Rodriguez-Garcia, E.; Vogel, P.; Mari, S.; Jimenez-Barbero, J.; Sinay, P. *Heterocycles* **2004**, *64*, 65-74. (x) Popowycz, F.; Gerber-Lemaire, S.; Schutz, C.; Vogel, P. *Helv. Chim. Acta* **2004**, *87*, 800-810. (y) Li, H.; Bleriot, Y.; Chantereau, C.; Mallet, J-M.; Sollogoub, M.; Zhang, Y.; Rodriguez-Garcia, E.; Vogel, P.; Jimenez-Barbero, J.; Sinay, P. *Org. Biomol. Chem.* **2004**, *2*, 1492-1499. (z) Bluechel, C.; Ramana, C. V.; Vasella, A. *Helv. Chim. Acta* **2003**, *86*, 2998-3036. (ai) Kapferer, P.; Birault, V.; Poisson, J-F.; Vasella, A. *Helv. Chim. Acta* **2003**, *86*, 2210-2227. (aii) Bleriot, Y.; Giroult, A.; Mallet, J-M.; Rodriguez, E.; Vogel, P.; Sinay, P. *Tetrahedron Asymm.* **2002**, *13*, 2553-2565. (aiii) Lorthiois, E.; Meyyappan, M.; Vasella, A. *Chem. Commun.* **2000**, *19*, 1829-1830. (aiv) Cardona, F.; Goti, A.; Picasso, S.; Vogel, P.; Brandi, A. *J. Carb. Chem.* **2000**, *19*, 585-601. (av) Panday, N.; Canac, Y.; Vasella, A. *Helv. Chim. Acta* **2000**, *83*, 58-79. (avi) Granier, T.; Gaiser, F.; Hintermann, L.; Vasella, A. *Helv. Chim. Acta* **1997**, *80*, 1443-1456.

(14) Risseeuw, M. D. P.; van der Marel, G.A.; Overkleeft, H.S.; Overhand, M. *Tetrahedron: Asymmetry*, **2009**, *20*, 945-951 and Chapter 5.

(15) Overkleeft, H. S.; Renkema, G. H.; Neele, J.; Vianello, P.; Hung, I. O.; Strijland, A.; van der Burg, A. M.; Koomen, G. J.; Pandit, U. K.; Aerts, J. M. F. G. *J. Biol. Chem.* **1998**, *273*, 26522-26527.

(16) Timmers, C. M.; Leeuwenburgh, M. A.; Verheijen, J. C.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron: Asymm.* **1996**, *7*, 49-52.

(17) (a) Paulsen, H. *Angew. Chem. Int. Ed. Engl.* **1966**, *5*, 495-510. (b) Paulsen, H.; Sangster, I.; Heyns, K. *Chem. Ber.* **1967**, *100*, 802-815. (c) Inouye, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron* **1968**, *24*, 2125-2144 (d) Yagi, M.; Kouno, T.; Aoyagi, Y.; Murai, H. *J. Agric. Chem. Soc. Japan.* **1976**, *50*, 571-572. (e) Murao, S.; Miyata, S. *Agric. Biol. Chem.* **1980**, *44*, 219-221.

(18) Aerts, J. M. F. G.; Donker-Koopman, W. E.; van der Vliet, M. K.; Jonsson, L. M. V.; Ginns, E. I.; Murray, G. J.; Barranger, J. A.; Tager, J. M.; Schram, A. W. *Eur. J. Biochem.* **1985**, *150*, 565-574.

(19) van Weely, S.; Brandsma, M.; Strijland, A., Tager, J. M.; Aerts, J. M. F. G. *Biochim. Biophys. Acta* **1993**, *1181*, 55-62.

(20) Aerts, J. M.; Hollak, C.; Boot, R.; Groener, A. *Phil. Trans. R. Soc. London, Ser. B* **2003**, *358*, 905-914.