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Synthesis of Substituted Chiral Piperazones Resembling Aza-sugars

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(6*R*)-6-(Hydroxymethyl)piperazin-2-one (**1**), (6*S*)-6-(hydroxymethyl)piperazin-2-one (**2**) and (6*S*)-6-[(1*R*, 2*S*, 3*R*)-1,2,3,4-tetrahydroxybutyl]piperazin-2-one (**3**) have been prepared in optically pure forms starting from D-glucosamine hydrochloride (**4**). The compounds (**1**–**3**) were tested for glycosidase inhibition.

Potent inhibitors of glycosyl-cleaving enzymes offer the opportunity to modulate selectively the crucial metabolism of carbohydrates, and hence open up a large number of potential applications, including the treatment of AIDS,[1] diabetes[2] and tumor metastasis,[3] as well as crop protection. Even though a variety of different glycosidase inhibitors are known,[4] the potential uses of such compounds are largely unexploited.

It has long been known that compounds resembling the oxocarbenium ion of a monosaccharide are potent inhibitors of glycosidases.[5] We have, however, recently discovered that compounds resembling ion **5**, such as the protonated form of the 1-aza-sugar **6**, are also strong inhibitors of these enzymes.[6–8] This compound inhibits yeast α-glucosidase and almond β-glucosidase with K_i values of 86 and 0.11 μM, respectively.[8] We have undertaken a synthesis program to study the scope and limitations of 1-aza-sugars with the aim of discovering new potent inhibitors (Fig. 1).

Among the ideas considered was a radical change of the ring hydroxy groups of **6**. It was proposed that replacement of the 3- and 4-hydroxy groups of **6** with an endocyclic lactam (compound **1**) would result in a compound that could act both as a hydrogen bond acceptor at the 3-position, and as a hydrogen bond donor at the 4-position and thus might interact with enzymes in a similar manner to **6**. Arguments in favor of this proposal were a) that carbonyl groups of amides can form very strong hydrogen bonds and b) that compound **1** with only one chiral center should be more accessible than compounds like **6**. In this paper we report the synthesis of the novel compound **1** and its enantiomer **2** and investigations of their biological activity.

Results and discussion

Our synthesis followed an established route to substituted chiral piperazones that relied on *N*-acylation of inexpensive D-glucosamine hydrochloride (**4**) with a protected amino acid, deprotection and reductive amination[9] (Scheme 1).

To obtain **1** we had to react **4** with protected glycine, reduce the hemiacetal, cleave the polyol by periodate and carry out a reductive amination. To obtain the enantiomer **2** the steps had to be switched so that the glycine adduct was deprotected and subjected to reductive amination before periodate cleavage and reduction. Thus **4** was reacted with *N*-benzyloxycarbonylglycine in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in DMF to give amide **7** in 90% yield. This compound was a mixture of anomers though some of the α-anomer was obtained crystalline.

The hemiacetal **7** was reduced with $NaBH_4$ in EtOH to give the pentitol, which was then allowed to react with $NaIO_4$ to give the aldehyde hydrate **8** in 75% yield. Catalytic hydrogenation of **8** with palladium on carbon and hydrogen resulted in cleavage of the benzyloxycarbonyl (CBz) group and subsequent reductive amination gave **1** in 92% yield.

Alternatively **7** was subjected to direct catalytic hydrogenation using palladium on carbon and hydrogen, which also led to cleavage of the CBz group and subsequent reductive amination. After BOC-protection of the amine, the tetraol **9** was obtained in 58% yield. The intermediate **3** was obtained pure from **9** in quantitative yield by removal of the BOC group with trifluoroacetic acid (TFA). Reaction of **9** with $NaIO_4$ to the aldehyde followed by reduction with $NaBH_4$ in EtOH and removal of the BOC group with TFA gave the monool **2** in 63% yield (Table 1).

Compounds **1**–**3** were tested for inhibition of glycosidases. All were either weak competitive inhibitors having K_i values above 1 mM or did not inhibit the enzymes at all. It was noteworthy that **1** was the stronger of the three inhibitors suggesting that this compound does fit better into the active site of the enzymes, because

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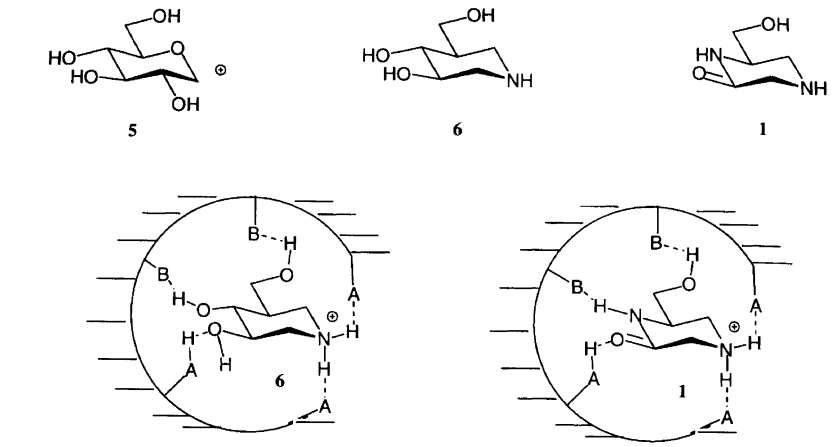
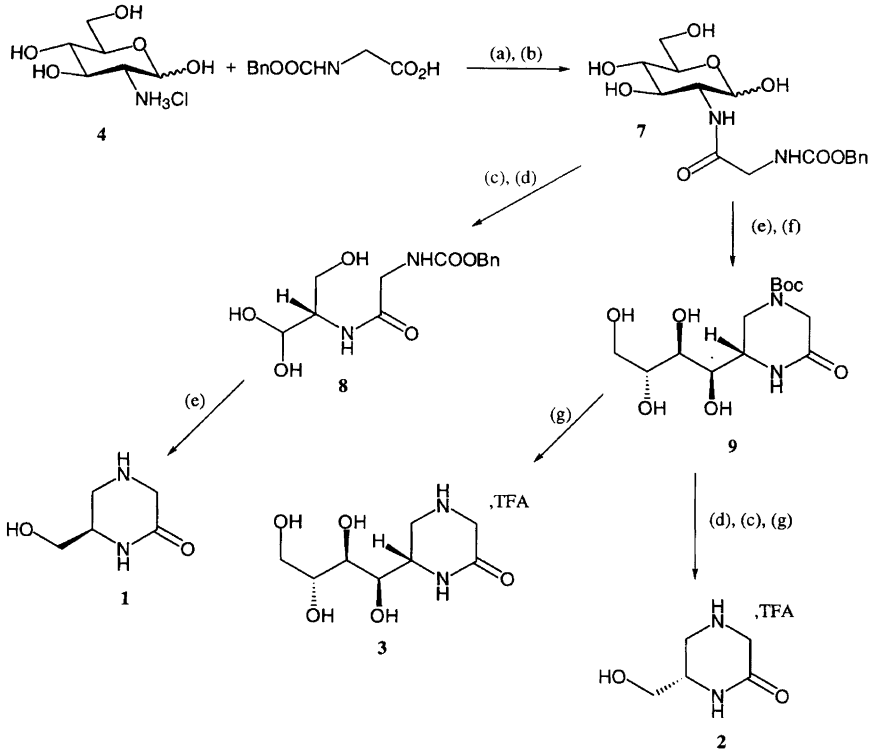


Fig. 1. Proposed binding of isofagomin (**6**) and piperazone (**1**) to β-glucosidase.



Scheme 1. Enantioselective synthesis of new piperazones: (a) NaOMe; (b) HOBT, DCC; (c) $NaBH_4$; (d) $NaIO_4$; (e) H_2, Pd–Carbon; (f) (*tert*-$BuO_2C)_2O$, NaOH; (g) TFA, CH_2Cl_2.

Table 1. K_i values (in μM) of novel compounds towards glycosidases.

Enzyme/compound	**6** (Ref. 8)	**1**	**2**	**3**
α-Glucosidase (baker's yeast)	86	No inhibition	No inhibition	No inhibition
β-Glucosidase (almonds)	0.11	5278	5100	>10000
Isomaltase (baker's yeast)	7.2	No inhibition	No inhibition	No inhibition
β-Galactosidase (E. Coli)	—	6880	>10000	>10000
β-Mannosidase (snail)	—	4310	NR	NR

it has the correct stereochemistry at C-6. Similarly it was observed that **3**, which should fit least well into the active site, was also the poorest inhibitor. The very poor inhibition of **1** compared with **6** suggests that the 3- and 4-hydroxy groups of **6** are crucial for its binding to glycosidases, and also indicates that the 3-hydroxy group acts as a hydrogen bond donor. Recent work on fluorinated 1-aza-sugars confirms this.[10] Thus aza-sugar **10** was a much more potent glycosidase inhibitor than the fluorinated analogue **11**, which suggested that the 3-OH of **10** acted as a hydrogen bond donor in its binding. Similarly aza-sugar **12** was a stronger inhibitor than the fluoro derivative **13**.[11] Therefore similar binding of **6** to the enzymes might be expected (Fig. 2).

Conclusions

In this paper we have synthesised a number of new chiral piperazones in optically pure form that have a high degree of structural resemblance to 1-aza-sugars such as isofagomine (**6**). The new compounds were relatively poor inhibitors of glycosidases suggesting that the 3- and 4-OH groups, in general, are crucial for binding of inhibitors to these enzymes.

Experimental

General. ^{13}C NMR and ^{1}H NMR spectra were recorded on Varian instrument Gemini 200. D_2O was used as the solvent with DHO (^{1}H NMR: δ 4.7) and $(CH_3)_2SO$ (^{1}H NMR: δ 2.52. ^{13}C NMR: δ 40.4) as references. With $CDCl_3$ as the solvent $(CH_3)_4Si$ and $CHCl_3$ (^{13}C NMR: δ 76.93) were used as references. Mass spectra were obtained on a VG TRIO-2 instrument. Melting points are uncorrected. Optical rotations were measured on a Perkin Elmer 141 polarimeter. Concentrations were performed on a rotary evaporator at a temperature below 40 °C.

*2-[N-(Benzyloxycarbonyl)glycinamido]-2-deoxy-*D*-glucose* (**7**). To a solution of 2-amino-2-deoxyglucose hydrochloride (**4**, 5.47 g, 0.025 mol) in DMF (120 ml) at 0 °C, sodium methoxide (1.37 g, 0.025 mol) was added. The reaction was stirred for 10 min, after which *N*-(benzyloxycarbonyl)glycine (5.00 g, 0.025 mol), 1-hydroxybenzotriazole (HOBT) (6.85 g, 0.050 mol) and DCC (6.28 g, 0.025 mol) were added, and the mixture was stirred for another 24 h. The precipitated DCU was filtered off, and the filtrate was poured into diethyl ether (800 ml) and left in the refrigerator overnight, whereby a solid residue was formed. The crude product was collected by filtration and recrystallized form ethanol. Yield of pure **7** (α-anomer): 1.25 g (16%). Evaporation of the mother liquor afforded another 7.0 g (**7**, 74%, mixture of anomers) of product as a white foam. M.p. 176–179 °C; $[\alpha]_D^{22} = +19.5°$ (*c* 1.0; H_2O). ^{1}H NMR [200 MHz, $(C^2H_3)_2SO$]: δ 7.25–7.50 (m, 5 H), 6.51 (d, 1 H), 5.04 (s, 2 H), 4.42–4.98 (m, 3 H), 3.30–3.74 (m, 5 H), 3.04–3.21 (m, 1 H). ^{13}C NMR [200 MHz, $(C^2H_3)_2SO$]: δ 169.3, 156.7, 137.7, 128.1, 128.0, 126.6, 90.9, 72.4, 71.3, 70.8, 65.7, 61.4, 54.6, 43.5.

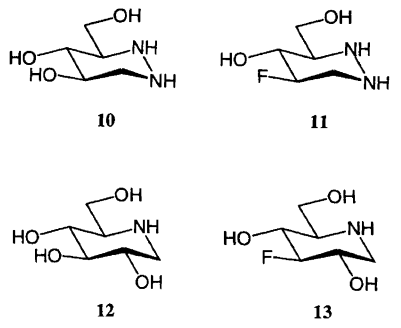


Fig. 2. Azafagomine and 1-deoxynojirimycin analogues.

*2-[N-(Benzyloxycarbonyl)glycinamido]-2-deoxy-*L*-glyceraldehyde hydrate* (**8**). To a solution of 2-[*N*-(benzyloxycarbonyl)glycinamido]-2-deoxy-D-glucose (**7**, 3.0 g, 8.1 mmol) in ethanol (150 ml) was added sodium borohydride (0.46 g, 12.1 mmol). The reaction was followed by TLC and stopped after 30 min. The solution was filtered through Celite and evaporated to dryness, redissolved in water (150 ml) and neutralised with 1 M H_2SO_4. Sodium periodate (5.2 g, 24.3 mmol) was added and the reaction was stirred for 1 h. The reaction volume was reduced to approximately 50 ml under reduced pressure and extracted with $CHCl_3$ (8 × 50 ml). The combined organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was purified by flash chromatography on silica with 5–10% methanol in CH_2Cl_2 as the eluent. Yield of **8** (hydrate): 1.80 g (75%, white solid). $[\alpha]_D^{22}$ −1.4° (*c* 2, $CHCl_3$). MS (EI) *m/z* 298 (M^+). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.20–7.34 (m, 5 H), 6.36 (m, 1 H), 5.03 (s, 2 H), 3.56–4.68 (m, 3 H), 3.29 (s, 2 H). ^{13}C NMR (200 MHz, $CDCl_3$): δ 171.4, 157.8, 136.6, 129.0, 128.7, 128.5, 97.7, 67.6, 61.8, 55.5, 44.8.

(6R)-6-Hydroxymethylpiperazin-2-one (**1**). To a solution of 2-[*N*-(benzyloxycarbonyl)glycinamido]-2-deoxy-L-glyceraldehyde hydrate (**8**, 110 mg, 0.37 mmol) in methanol (50 ml) was added 10% Pd on carbon (50 mg). The mixture was hydrogenated for 24 h at 50 atm, filtered through Celite and evaporated to dryness. Yield of **1**: 46 mg (96%, clear oil). $[\alpha]_D^{22}$ +3.1° (*c* 1.1, H_2O). MS (EI) *m/z* 130 (M^+). ^{1}H NMR (200 MHz, D_2O): δ 3.46–3.68 (m, 3.5 H), 3.28–3.34 (m, 1.5 H), 2.96–3.08 (m, 1 H), 2.64–2.78 (m, 1 H). ^{13}C NMR (200 MHz, D_2O): δ 168.9, 64.7, 52.2, 46.7, 44.6.

*(6S)-4-(*tert*-Butoxycarbonyl)-6-[(1R,2S,3R)-1,2,3,4-tetrahydroxybutyl]piperazin-2-one* (**9**). Sodium methoxide (0.27 g, 5.0 mmol) and hydroxylamine hydrochloride (0.35 g, 5.0 mmol) were stirred in methanol (5 ml) for 10 min and filtered. The remaining solution was diluted

to 50 ml with methanol and 2-(*N*-benzyloxycarbonylglycinamido-2-deoxy-D-glucose (**7**, 1.85 g, 5.0 mmol) was added together with 10% Pd on carbon (150 mg). The mixture was hydrogenated at 50 atm overnight, filtered through Celite and evaporated to dryness. The resulting oil was dissolved in a mixture of H_2O–dioxane (1 : 1, 50 ml) and 1 M NaOH (10.0 ml) and di-*tert*-butyl dicarbonate (2.18 g, 10.0 mmol) were added. After being stirred for 16 h the mixture was evaporated to dryness and purified by flash chromatography on silica with 10–30% methanol in CH_2Cl_2 as the eluent. Yield of **9**: 0.93 g (58%). $[\alpha]_D^{22}$ +11.6° (*c* 2.1, MeOH). MS (EI) *m/z* 220 (M^+ − BOC). ^{1}H NMR [200 MHz, $(C^2H_3)_2SO$]: δ 3.28–4.14 (m, 10 H), 1.44 (s, 9 H). ^{13}C NMR [200 MHz, $(C^2H_3)_2SO$]: δ 166.6, 153.6, 79.9, 71.5, 71.3, 69.6, 63.7, 53.8, 47.1, 42.2, 28.8.

(6S)-6-[(1R,2S,3R)-1,2,3,4-Tetrahydroxybutyl]piperazin-2-one trifluoroacetate (**3**). (6*S*)-4-(*tert*-Butoxycarbonyl)-6-[(1*R*,2*S*,3*R*)-1,2,3,4-tetrahydroxybutyl]-piperazin-2-one (**9**, 0.10 g, 0.31 mmol) was stirred in a mixture of CH_2Cl_2–TFA (1 : 1, 5 ml) for 30 min and evaporated to dryness. Yield of **3**: 0.10 g (100%, clear oil). $[\alpha]_D^{22}$ −2.9° (*c* 1.0, H_2O). MS (EI) *m/z* 220 (M^+). ^{1}H NMR (200 MHz, D_2O): δ 3.50–4.06 (m, 9 H), 3.24–3.39 (m, 1 H). ^{13}C NMR (200 MHz, D_2O): δ 168.7, 74.0, 73.4, 72.2, 65.5, 54.1, 46.6, 44.5.

(6S)-6-(Hydroxymethyl)piperazin-2-one, trifluoroacetate (**2**). To a solution of (6S)-4-(*tert*-butoxycarbonyl)-6-[(1*R*,2*S*,3*R*)-1,2,3,4-tetrahydroxybutyl]piperazin-2-one (**9**, 0.41 g, 1.3 mmol) in H_2O (25 ml), was added sodium periodate (0.80 g, 3.7 mmol). After being stirred for 45 min, the mixture was extracted with $CHCl_3$ (3 × 20 ml). The combined organic phases were evaporated to dryness, redissolved in a mixture of H_2O–ethanol (1 : 3, 25 ml), and sodium borohydride (0.071 g, 1.8 mmol) was added. After being stirred for 30 min, the mixture was neutralised with 1 M HCl and extracted with $CHCl_3$ (3 × 20 ml). The combined organic phases were dried ($MgSO_4$), evaporated to dryness and purified by flash chromatography on silica using 5% methanol in CH_2Cl_2 as the eluent. The product was stirred in a mixture of CH_2Cl_2–TFA (1 : 1, 5 ml) for 30 min and evaporated to dryness. Yield of **2**: 0.19 g (63%). $[\alpha]_D^{22}$ −1.9° (*c* 1.4, H_2O). MS (EI) *m/z* 130 (M^+). ^{1}H NMR (200 MHz, D_2O): δ 3.78–3.86 (m, 2 H), 3.48–3.71 (m, 4 H), 3.26–3.40 (m, 1 H). ^{13}C NMR (200 MHz, D_2O): δ 168.9, 64.7, 52.2, 46.6, 44.5.

Measurements of glycosidase inhibition. Each glycosidase assay was performed by preparing four 2 ml samples in cuvettes consisting of 1 ml sodium phosphate buffer (0.1 M) of pH 6.8, 0.2–0.8 ml of a 1.0 or 10 mM solution of either 4-nitrophenyl α-D-glucopyranoside, 4-nitrophenyl β-D-glucopyranoside, 2-nitrophenyl β-D-galactopyranoside or 4-nitrophenyl β-D-mannopyranoside, 0.1 ml of a solution of either the potential inhibitor or water, and distilled water to a total volume of 1.9 ml. Two times four of the samples contained the potential inhibitor at two fixed concentrations, but with varying nitrophenyl glycoside concentrations. The other four samples contained no inhibitor, but various nitrophenyl glycoside concentrations. Finally the reaction was started by adding 0.1 ml of a diluted solution of either α-glucosidase from baker's yeast (EC 3.2.1.20, Sigma G-5003), β-glucosidase from almonds (EC 3.2.1.21, Sigma G-0395), isomaltase from yeast (EC 3.2.1.10, Sigma I-1256), β-galactosidase from E. Coli (EC 3.2.1.23, Sigma G-6008) or β-mannosidase from snail (EC 3.2.1.25, Sigma M-9400), and the formation of 4-nitrophenol was followed for 2 min at 26 °C by measuring absorbance at 400 nm. Initial rates were calculated from the slopes of each of the eight reactions and used to construct two Hanes plots: one with and without inhibitor. From the two Michaelis–Menten constants (K_m) thus obtained the inhibition constant (K_i) was calculated.

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