Synthesis of Substituted Chiral Piperazones Resembling Aza-sugars

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

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

It has long been known that compounds resembling the oxocarbonium ion of a monosaccharide are potent inhibitors of glycosidases. 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. This compound inhibits yeast α-glucosidase and almond β-glucosidase with $K_i$ values of 86 and 0.11 μM, respectively. We have under-
taken 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 inexpens-
ive D-glucosamine hydrochloride (4) with a protected amino acid, deprotection and reductive amination (Scheme 1).

To obtain 1 we had to react 4 with protected glycine, reduce the hemiacetal, cleave the polyl 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-benzoxycarbonylglycine 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
even though some of the α-anomer was obtained crystalline.

The hemiacetal 7 was reduced with NaBH₄ in EtOH
to give the pentitol, which was then allowed to react with NaIO₄ to give the aldehyde hydrate 8 in 75% yield.
Catalytic hydrogenation of 8 with palladium on carbon
and hydrogen resulted in cleavage of the benzoxycar-
bonyl (CBz) group and subsequent reductive amination
gave 1 in 92% yield.

Alternatively 7 was subjected to direct catalytic hydro-
genation 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 NaIO₄ to the aldehyde
followed by reduction with NaBH₄ 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 glyco-
sidases. 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 isofagomine (6) and piperazone (1) to β-glucosidase.

Scheme 1. Enantioselective synthesis of new piperazines: (a) NaOMe; (b) HOBT, DCC; (c) NaBH₄; (d) NaIO₄; (e) H₂, Pd–Carbon; (f) (tert-BuO₂)₂O, NaOH; (g) TFA, CH₂Cl₂.

<table>
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<tr>
<th>Enzyme/compound</th>
<th>6 (Ref. 8)</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>α-Glucosidase</td>
<td>86</td>
<td>No inhibition</td>
<td>No inhibition</td>
<td>No inhibition</td>
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<tr>
<td>β-Glucosidase</td>
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<td>5278</td>
<td>5100</td>
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<td>(almonds)</td>
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<tr>
<td>Isomaltase</td>
<td>7.2</td>
<td>No inhibition</td>
<td>No inhibition</td>
<td>No inhibition</td>
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<tr>
<td>(baker’s yeast)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>—</td>
<td>6880</td>
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<td>&gt;10000</td>
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<td>(E. Coli)</td>
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<td>4310</td>
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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. 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. 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 $^1H$ NMR spectra were recorded on Varian instrument Gemini 200. D$_2$O was used as the solvent with DHO ($^1H$ NMR: $\delta$ 4.7) and (CH$_3$)$_2$SO ($^1H$ NMR: $\delta$ 2.52. $^{13}$C NMR: $\delta$ 40.4) as references. With CDCl$_3$ as the solvent (CH$_3$)$_3$Si and CHCl$_3$ ($^{13}$C NMR: $\delta$ 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$^\circ$C.

2-4H-$N$-(Benzyloxycarbonyl) glycaminido]-2-deoxy-L-glyceraldehyde hydrate (8). To a solution of 2-[N-(benzyloxy carbonyl) glycaminido]-2-deoxy-D-glucose (7) 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$_2$SO$_4$. 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 x 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$_2$Cl$_2$ as the eluent. Yield of 8 (hydrate): 1.80 g (75%, white solid). $[a]_D^{25}+1.4^\circ$ (c 2, CHCl$_3$). MS (EI) $m/z$ 298 ($M^+$). $^1H$ NMR (200 MHz, CDCl$_3$): $\delta$ 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$_3$): $\delta$ 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-(benzyloxy carbonyl) glycaminido]-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). $[a]_D^{25}+3.1^\circ$ (c 1.1, H$_2$O). MS (EI) $m/z$ 130 ($M^+$). $^1H$ NMR (200 MHz, $D_2$O): $\delta$ 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, $D_2$O): $\delta$ 168.9, 64.7, 52.2, 46.7, 44.6.

(6S)-4-[tert-Butoxycarbonyl]-6-4H-[R,R,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-benzyloxycarbonyl-
glycinamido-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 H₂O-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 CH₂Cl₂ as the eluent. Yield of 9: 0.93 g (58%). [α]D²⁰ = 11.6° (c 2.1, MeOH). MS (EI) m/z 220 (M⁺ - BOC). ¹H NMR [200 MHz, (CD₃)₂SO]: δ 3.28-4.14 (m, 10 H), 1.44 (s, 9 H). ¹³C NMR [200 MHz, (CD₃)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). (6S)-4-(tert-Butyloxycarbonyl)-6-[(1R,2S,3R)-1,2,3,4-tetrahydroxybutyl]piperazin-2-one (9, 0.10 g, 0.31 mmol) was stirred in a mixture of CH₂Cl₂-TFA (1:1, 5 ml) for 30 min and evaporated to dryness. Yield of 3: 0.10 g (100%, clear oil). [α]D²⁰ = 2.9° (c 1.0, H₂O). MS (EI) m/z 220 (M⁺). ¹H NMR (200 MHz, D₂O): δ 3.50-4.06 (m, 9 H), 3.24-3.39 (m, 1 H). ¹³C NMR (200 MHz, D₂O): 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-butyloxycarbonyl)-6-[(1R,2S,3R)-1,2,3,4-tetrahydroxybutyl]piperazin-2-one (9, 0.41 g, 1.3 mmol) in H₂O (25 ml), was added sodium periodate (0.80 g, 3.7 mmol). After being stirred for 45 min, the mixture was extracted with CHCl₃ (3×20 ml). The combined organic phases were evaporated to dryness, redissolved in a mixture of H₂O-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 CHCl₃ (3×20 ml). The combined organic phases were dried (MgSO₄), evaporated to dryness and purified by flash chromatography on silica using 5% methanol in CH₂Cl₂ as eluent. The product was stirred in a mixture of CH₂Cl₂-TFA (1:1, 5 ml) for 30 min and evaporated to dryness. Yield of 2: 0.19 g (63%). [α]D²⁰ = −19° (c 1.4, H₂O). MS (EI) m/z 130 (M⁺). ¹H NMR (200 MHz, D₂O): δ 3.78-3.86 (m, 2 H), 3.48-3.71 (m, 4 H), 3.26-3.40 (m, 1 H). ¹³C NMR (200 MHz, D₂O): 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-nitrophosphoryl β-D-glucopyranoside, 2-nitrophosphoryl β-D-galactopyranoside or 4-nitrophosphoryl β-D-mannopyranoside, 0.1 ml of a solution 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 nitrophosphoryl glycoside concentrations. The other four samples contained no inhibitor, but various nitrophosphoryl 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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References

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