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Specific Inhibition of Glycogen Phosphorylase by a Spirodiketopiperazine at the Anomeric position of Glucopyranose

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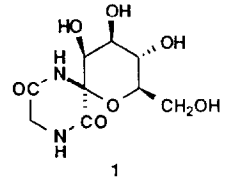
Abstract: A key intermediate bicyclic lactone **6** allows control of the anomeric configuration of a spirodiketopiperazine of glucopyranose **5** which is a specific inhibitor of glycogen phosphorylase, showing no inhibition of α- and β-glucosidases, α- and β-galactosidases, β-N-acetylglucosaminidase, pectinase, xylanase or cellulase.

Diketopiperazines, both naturally occurring[1] and synthetic,[2] are a class of bioactive peptides with a range of potential chemotherapeutic applications.[3] The preceding paper reports the synthesis of the mannose derivative **1**, the first example of a spirodiketopiperazine of a pyranose.[4] In a project to discover efficient inhibitors of glycogen phosphorylase (GPb) as possible therapeutic agents for the treatment of diabetes,[5,6] a number of derivatives of glucose-1-carboxylic acids[7,8] **2** and N-acyl derivatives of β-glucopyranosylamine[9] **3** have been studied; the best inhibitor in these classes of compounds was the β-N-acetyl derivative **3** (R=Me) with a K_i of 32 μM,[10] a 250-fold improvement over β-D-glucose [K_i 7.4 mM].[11] The glucopyranose analogue of hydantocidin **4**, the first reported spirohydantoin of a pyranose, is a more powerful inhibitor of glycogen phosphorylase with a K_i of 3 μM.[12] Molecular modelling studies indicated that the spirodiketopiperazine of glucose **5** would bind strongly to GPb and should be a good inhibitor. This paper reports the synthesis of **5** *via* a bicyclic lactone **6** in which the pyranose ring is already formed and in which the configuration at the anomeric centre has been defined; **5** is a highly specific - though not as strong an - inhibitor of GPb and has no effect on a number of glycosidases.



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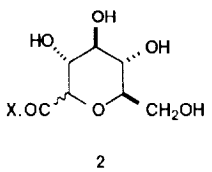

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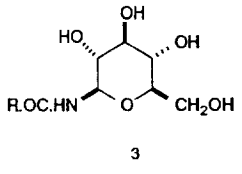

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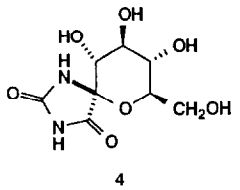

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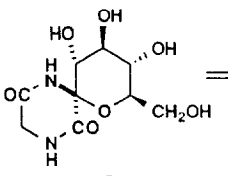

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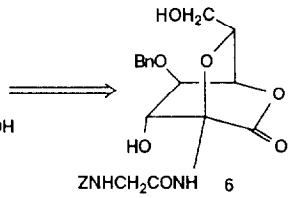
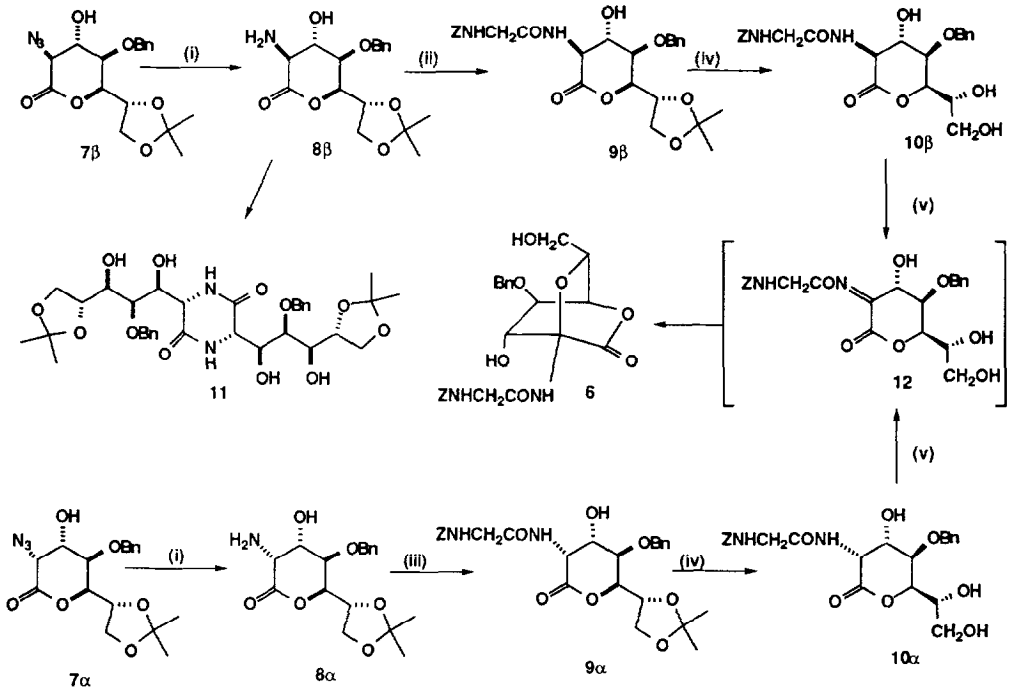


6

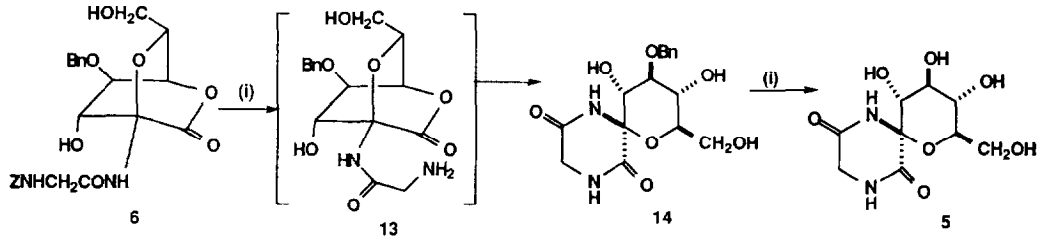
The glucose analogue **5** was prepared from the epimeric azides **7**,[13] *via* the key bicyclic intermediate lactone **6** [Scheme 1] in which the pyranose ring is already formed and the stereochemistry at the anomeric position has been defined. Hydrogenation of the more readily available *glucoheptono*azide 7β with palladium black in ethyl acetate gave the amine **8β** which was coupled by reaction with ZglyOH, dicyclohexylcarbodiimide and 1-hydroxy-benzotriazole in dichloromethane to give **9β** in an overall yield of 27%; the low yield in this sequence is due primarily to the aminolactone **8β** which undergoes relatively easy self condensation to give a compound, the spectral data of which are consistent with the diketopiperazine structure **11**. In contrast, hydrogenation of the epimeric azide **7α** in the

presence of palladium black in ethyl acetate gave the more stable amine **8α** which with ZglyOH, triethylamine and ethyl chloroformate afforded the dipeptide **9α** in an overall yield of 88%. The reasons for the difference in the stabilities of the epimeric aminolactones **8**[14] with respect to self condensation are not clear and studies are in progress to avoid such a low yield step in the route from **7β**.



Scheme 1: (i) H_2, Pd, EtOAc (ii) $ZNHCH_2COOH$, DCC, 1-hydroxybenzotriazole, CH_2Cl_2 (iii) $ZNHCH_2COOH$, Et_3N, ClCOOEt, THF/MeCN (iv) aq. AcOH with a trace of CF_3COOH (v) N-bromosuccinimide, MeCN, NaOAc

Treatment of **9α** and **9β** with aqueous acetic acid in the presence of a trace of trifluoroacetic acid resulted in the removal of the acetonide protecting group to afford the epimeric triols **10α** and **10β** in 82% and 63% yield, respectively. Oxidation of **10α** and **10β** with N-bromosuccinimide gave an imine **12** which spontaneously cyclised by attack of the secondary hydroxyl group of the side chain to give the bicyclic lactone **6**, colourless wax, $[\alpha]_D^{22}$ -15.5 (c 0.33, $CHCl_3$), in 68% and 59% yields, respectively; the yields in this oxidation are significantly better than the analogous cyclisation in the mannose series,[4] probably reflecting the greater stability of the bicyclic structure **6** in which the oxygen functional groups of the diol bridge are *trans* to each other.



Scheme 2: (i) cyclohexene, 10% Pd/C, methanol, heat

Transfer hydrogenation of the bicyclic lactone **6** with cyclohexene in methanol in the presence of 10% palladium on charcoal caused initial removal of the benzyloxycarbonyl protecting group to afford the free amine **13** [Scheme 2]. Spontaneous intramolecular attack of the amine group in **13** onto the lactone carbonyl

group gave the spirodiketopiperazine **14**, m.p. 225-230°C, $[\alpha]_D^{22}$ +39.5 (c 0.40, methanol), in quantitative yield. The anomeric configuration is defined by the bicyclic structure of **6** and only the single anomer **14** is produced; under these conditions, there is no indication of any equilibration to the other anomeric pyranose form, nor to any furanose isomers. The structure of **14** was firmly established by single X-ray crystallographic analysis.[15] Further transfer hydrogenation of **14** gave the required unprotected spiro compound **5**[16] [80% yield from **6**].

GPb activity was measured at pH 6.8 and 30°C in the direction of glycogen synthesis by the release of orthophosphate from the substrate glucose 1-phosphate[5,7], by using four different concentrations of **5** (0.1, 0.2, 0.3, and 0.4 mM) at five different concentrations of glucose 1-phosphate (3, 4, 6, 10, and 20 mM). Lineweaver-Burk plots of the kinetic data obtained indicated that **5** acts as a non-linear competitive inhibitor with respect to glucose 1-phosphate with a K_i value of 59.7 ± 5.3 μM, determined from the intersection on the abscissa from the replot of apparent K_m *versus* inhibitor concentration. Since the concentrations of AMP (1 mM) and glycogen (1%) were saturating, we interpret this as a value for the enzyme-AMP-glycogen-glucodiketopiperazine complex. Glucose, a well-known T state stabilizing effector of the enzyme, acts as a non-linear competitive inhibitor for glucose 1-phosphate with a Hill coefficient *(n)* 1.5 indicating a shift in the equilibrium toward the T state. This parameter (*n*) has a value of 1.6 for **5** with respect to glucose 1-phosphate, indicating that this sugar analogue is as effective as glucose in promoting the T state. **5** (K_i 59.7 μM) is 20 times less effective than its parent spirohydantoin analogue **4** (K_i 3.1 μM).[12] Comparison of inhibition constants indicates a difference in binding energies between **4** and **5** of about 1.8 kcal/mol.

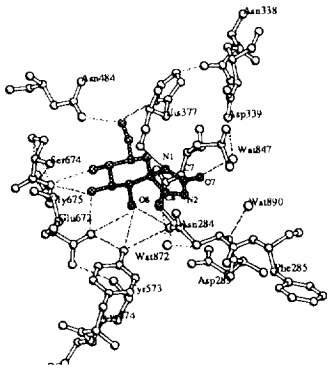


*Fig 1: Binding of the spirohydantoin **4** to GPb.*

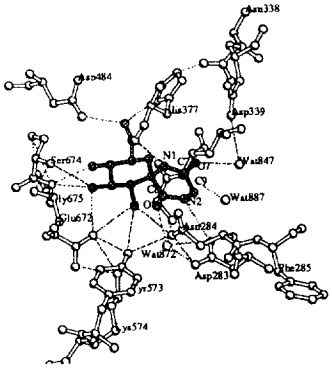


*Fig 2: Binding of the spirodiketopiperazine **5** to GPb.*

From X-ray crystallographic analyses[17] a comparison of the ligand-enzyme bound structures of **4** and **5** shows both ligands to bind in a similar manner to GP*b* (Figs. 1 and 2). The hydrogen bonding network to the peripheral hydroxyls of the glucopyranose moiety of **4** and **5** are analogous to those observed for the α-D-glucose complex. The hydrogen bonds to N1, O7 and O8 are maintained in both complexes with **4** and **5** but are slightly longer in the complex with **5** (N1 ··· O His377, 3.0Å; O7 ··· Wat847, 2.7Å; O9 ··· Wat872, 2.6Å); **5** forms an additional hydrogen bond between N2 and OD1 Asp283 (3.2Å). Examination of the van der Waals contacts with C8 of compound **5** show this atom to be largely in an unfavourable polar environment. Wat890 is 3.5Å away from C8 and is therefore displaced in this complex but is weakly bound in the complex with **4**. In addition, in the complex with **5** the 280s loop (important for maintaining the enzyme in the inactive T state) is held in place in the catalytic site by two hydrogen bonds; one through a water molecule (Wat872) analogous to that observed with **4** and, one additional contact directly to the protein (N2 ··· OD1 Asp283). In the complex with **4**, N2 is just too far away from the side chain of Asp283 (4Å).

The major difference between the two ligands in the complex structures is in the planarity of the spiro rings. The spirohydantoin compound **4** represents a rigid-fused ring structure and is the first of its kind to be complexed with GP*b*. In the complex with **5**, the spiro ring can adopt either a planar or a bent conformation which places C8 and N2 on either side of the mean plane by 0.1Å. It would seem that the additional conformational flexibility found in the complex with **5** leads to an unfavourable entropic gain in energy upon

binding. Conversely, the more rigid spirohydantoin complex gives rise to a favourable conformational energy gain. This may account for the ten-fold difference in inhibition of these two compounds. The effect of conformational entropy on inhibition has been observed in other complexes with GP*b* in which analogous rigid and flexible groups have been engineered resulting in a loss in inhibition in every case for the more flexible compounds.[5]

The glucodiketopiperazine **5** showed a complete absence of any inhibition of a number of glycosidases. Thus, at 2.5 x 10^{-4} M, **5** showed no inhibition of α-glucosidase (Brewers Yeast and rabbit gut mucosa), β-glucosidase (Almonds and rabbit liver and rabbit gut mucosa), α-galactosidase (*E. coli* and green coffee beans), β-galactosidase (*E. coli* and rabbit liver and gut mucosa) or β-N-acetylglucosaminidase (bovine) using 5mM *p*-nitrophenylpyranosides as substrates; **5** also showed no inhibition of pectinase (*Aspergillus niger*), xylanase (*Trichoderma viride*) or cellulase (*A. Niger*) at 1.9 x 10^{-4} M.

Thus, this paper further demonstrates the strategy for the synthesis of spiroderivatives of pyranoses with control of the stereochemistry at the anomeric position of the sugar *via* bicyclic lactones. Although the spirodiketopiperazine **5** is not as good an inhibitor of GPb as the spirohydantoin **4**, the complete lack of inhibition by **5** of a wide range of glycosidases indicates that such materials may have the required specificity for inhibition of GPb without also interfering in the metabolism of other sugar metabolising enzymes. Further studies on analogues of **4** and **5** are in progress with the objective of increasing the potency of inhibition of GPb while maintaining a complete lack of inhibition of glycosidases.[18]

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14. Spectroscopic and/or microanalytical data consistent with each proposed structure have been obtained for all new compounds in this paper.
15. The atomic coordinates for (2R, 3S, 4S, 5R, 6S)-4-Benzyloxy-3,5-dihydroxy-2-hydroxymethyl-7,10-diaza-1-oxaspiro[5.5]-undecan-8,11-dione **14** are available on request from the Cambridge Crytallograhic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW.
16. Selected data for **5**: m.p. 256-258°C (from ethanol/water); $[\alpha]_D^{22}$ +56.4 (c 0.31, methanol); ν_{max}(KBr) 3399 cm^{-1} (b, OH and NH), 1684 cm^{-1} (C=O); δ_H (D_2O): 3.41 (1 H, pt, $J_{2,3} = J_{3,4} \sim 9.5$ Hz, H-3), 3.44 (1 H, d, $J_{4,5} = 9.5$ Hz, H-5), 3.67 (1 H, dd, J = 5.4, J_{gem} = 12.5 Hz, 0.5 CH_2OH), 3.81 (1 H, dd, J = 2.2 Hz, CH_2OH), 3.98 (1 H, ddd, H-2), 3.99, 4.13 (2 H, 2 d, J_{gem} = 18.8 Hz, H-9, H-9'), 4.29 (1 H, pt, H-4); δ_C (D_2O): 44.6 (t, C-9), 61.4 (t, CH_2OH), 69.8, 73.8, 76.0, 77.1 (4 d, C-2, C-3, C-4, C-5), 84.9 (s, C-6), 165.9, 171.1 (2 s, C-8, C-11).
17. The final crystallographic R factor was 20.5% based on 31131 unique reflections collected to 2.4Å resolution. The inhibitor was easily located bound to the catalytic site of GP*b* by difference Fourier synthesis and refined with the program XPLOR using the GP*b*-glucose complex to provide initial phases.

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