X-Ray Crystallographic Analysis of 2,6-Anhydro-*N*-methyl-D-*glycero*-D-*ido*-heptonamide: the First Example of a Simple Glucose Analogue with a Skew Boat Structure

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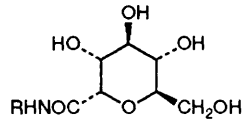
Single crystal X-ray crystallographic analysis of 2,6-anhydro-*N*-methyl-D-*glycero*-D-*ido*-heptonamide and a crystallographic binding study with glycogen phosphorylase show an unexpected skew boat conformation for the glucose analogue.

Several glucose analogues have been synthesized and used in a three-dimensional (3-D) crystallographic binding study with the large regulatory enzyme, glycogen phosphorylase (GP).[1] The knowledge of the 3-D structures of GP[2] and the glucose–GP complex[3] have been used as models for the design of glucose analogues that may be effective in lowering blood glucose levels. It is known that glucose itself is a weak competitive inhibitor of GP (K_i = 1.7 mmol dm^{-3}) and in a self-regulatory system helps lower blood glucose levels, by inhibition of glycogen degradation and promotion of glycogen synthesis. It has been postulated that glucose analogues with greater inhibition of GP may result in more effective regulatory agents than glucose.[1,4] Among other potential inhibitors, binding of 2,6-anhydro-*N*-methyl-D-*glycero*-D-*ido*-heptonamide **1a** with GP was investigated.

The methylamide **1a** was prepared from the protected hydroxymethyl compound **2**.[5] Oxidation of the primary alcohol **2** under Swern conditions, followed by treatment of the resulting aldehyde with bromine in aqueous methanol,[6]



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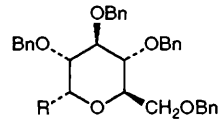
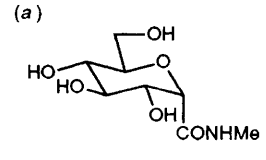
a; R = Me
b; R = H
c; R = NH_2



2; R = CH_2OH
3; R = CO_2Me
4; CONHMe



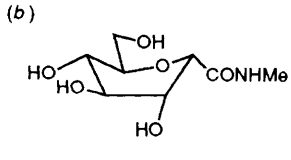


Fig. 1 Conformations of **1a** in (*a*) $^4C_1$ chair and (*b*) skew-boat

gave the methyl ester **3**† in 81% yield. Reaction of the ester **3** with aqueous methylamine and sodium acetate in methanol gave the protected amide **4** (68% yield) from which the benzyl protecting groups were removed by hydrogenolysis in the presence of palladium to give **1a** in 80% yield (45% overall yield from **2**).

Owing to well-defined protocols,[1] in both X-ray crystallographic data collection and processing, analyses of the inhibitor–phosphorylase complexes are relatively straightforward.‡ The starting model for the glucose ligand was generated using ideal bond and torsion angles for the standard $^4C_1$ chair conformation which places the α-carboxamido substituent axial [Fig. 1(*a*)]. Fitting of compound **1a**, in the $^4C_1$ chair conformation to the difference Fourier electron density map calculated with respect to the native protein, led to an unfavourable fit in the region of the C-1 substituents. The best fit showed equatorial substitution at C-1. After several unsuccessful attempts to fit the model, **1a** was sent for single crystal structure analysis.§ The result showed that the compound existed in a skew-boat conformation [Fig. 1(*b*)], in which the αC-1 substituents are approximately in an equatorial conformation. Comparison of the ring torsion angles for **1a** with those expected for α-D-glucose in a $^4C_1$ chair geometry[7] (see Table 1) indicates that the ring torsion angles C(1)–C(2)–C(3)–C(4), C(2)–C(3)–C(4)–C(5), C(4)–C(5)–O(5)–C(1) and C(5)–O(5)–C(1)–C(2) are all highly distorted from the expected values, thus confirming a skewed conformation. The crystal structure of the phosphorylase–glucose analogue complex refined satisfactorily with **1a** in a skew-boat conformation‡ and the structure was consistent with the observed electron density. Fig. 2 shows the single X-ray crystal structure of **1a** and Fig. 3 shows this structure superimposed on the 2.3 Å resolution calculated difference Fourier map of the bound ligand–protein complex. The conformation of compound **1a** does not appear to be the result of a crystal packing effect since the skew-boat geometry is observed both in the single X-ray crystal structure and when the glucose analogue is bound to phosphorylase. Distortion of the chair to the skew-boat conformation leads to fewer hydrogen bonds between **1a** and the enzyme than between glucose and the enzyme. Kinetics studies (N. G. Oikonomakos, unpublished results) show **1a** is a poorer inhibitor than glucose (K_i compound **1a** = 37 mmol dm^{-3}, K_i α-D-glucose = 1.7 mmol dm^{-3}) consistent with these observations. Searches in both the Cambridge Structural Database and the Chemical Abstract Database have shown **1a** to be the first reported skew-boat structure of an unprotected glucose analogue or derivative.

The rationale behind the unusual ring geometry is not clear. Carbohydrate conformation is influenced by stereoelectronic

Table 1 Ring torsion angles for (i) 2,6-anhydro-*N*-methyl-D-*glycero*-D-*ido*-heptonamide **1a** and (ii) α-D-glucose

	(i)	(ii)
O(5)–C(1)–C(2)–C(3)	−59.7(3)	54.1(2)
C(1)–C(2)–C(3)–C(4)	26.9(3)	−51.3(1)
C(2)–C(3)–C(4)–C(5)	31.1(3)	53.3(1)
C(3)–C(4)–C(5)–O(5)	−64.8(3)	−57.5(1)
C(4)–C(5)–O(5)–C(1)	33.7(3)	62.2(2)
C(5)–O(5)–C(1)–C(2)	27.0(3)	−60.9(2)

† All new compounds reported in this paper have microanalytical and spectroscopic data consistent with the proposed structures. Selected data for the new compounds.

For **1a**: m.p. 187–189 °C; $[\alpha]_D^{20}$ +63.9 (*c*, 0.29 in MeOH); ν_{max}/cm^{-1} (KBr): 3300br (OH, NH), 1636 (C=O); δ_H (500 MHz, D_2O): 2.67 (3H, s, Me), 3.36 (1H, m), 3.65–3.74 (5H, m), 4.37 (1H, d, $J_{1,2}$ 5.2 Hz, H-1); δ_c (CD_3OD): 25.3 (q) 61.8 (t) 70.5, 72.1, 73.4, 74.1, 78.8 (5 × d) 173.5 (s, C=O).

For **3**: oil, $[\alpha]_D^{20}$ + 72.7 (*c*, 1.38 in $CHCl_3$); ν_{max}/cm^{-1} (film): 1741 (C=O); δ_H (500 MHz, $CDCl_3$): 3.63–3.73 (3H, m), 3.76 (3H, s, Me), 3.86 (1H, dd, *J* 6.7, 8.9 Hz), 4.12 (1H, t, *J* 8.7 Hz), 4.40–4.49 (1H, m), 4.51 (2H, m), 4.61 (1H, d, *J* 12.2 Hz), 4.66–4.72 (3H, m), 4.79 (1H, d, *J* 11.0 Hz), 4.83 (1H, d, *J* 11.0 Hz), 4.90 (1H, d, *J* 11.0 Hz), 7.17–7.38 (20H, m); δ_C ($CDCl_3$): 51.8 (q) 68.8, 73.4, 73.5 (3 × t) 73.6, 74.6 (2 × d) 75.4, 76.6 (2 × t) 77.1, 78.1, 81.6, 127.8, 128.0, 128.1, 128.6 (7 × d) 138.0, 138.2, 138.5, 138.9 (4 × s) 170.9 (s, C=O).

For **4**: m.p. 80–81 °C; $[\alpha]_D^{20}$ + 27.9 (*c*, 0.29 in $CHCl_3$); ν_{max}/cm^{-1} (KBr): 3365 (N–H), 1661 (C=O); δ_H (500 MHz, $CDCl_3$): 2.81 (3H, d, *J* 4.9 Hz), 3.57 (1H, dd, *J* 3.9, 8.1 Hz), 3.67 (1H, dd, *J* 6.2, 10.4 Hz), 3.70–3.75 (2H, m), 4.17–4.20 (2H, m), 4.40–4.46 (2H, m), 4.51–4.65 (6H, m), 4.68 (1H, d, *J* 11.7 Hz), 7.01 (1H, br s, NH), 7.19–7.90 (20H, m, ArH); δ_C ($CDCl_3$): 25.6 (q) 69.4, 72.4 (2 × t) 73.1 (d) 73.5, 74.0 (2 × t) 74.1, 75.9, 76.3, 77.5, 128.0, 128.2, 128.5, 128.6 (8 × d) 138.0, 138.2 (2 × s) 170.7 (s, C=O).

‡ *Crystallographic binding study for* **1a**: Crystals of T state GP*b* were grown as detailed previously.[10] Prior to data collection, T state GP*b* crystals were soaked for 1 h in a buffered solution [10 mmol dm^{-3} *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, 0.1 mmol dm^{-3} ethylenediaminetetraacetic acid pH 6.7] containing approximately 100 mmol dm^{-3} of compound **1a**. Data collection (to 2.3 Å resolution) was performed on a Nicolet IPC multiwire area detector using a Rigaku RU-200 rotating anode X-ray source, with a graphite monochromator, operating at 50 kV, 50 mA. The detector was placed 16 cm away from the crystal and at a swing angle of 22°. Data frames of 0.2° oscillation were collected with 100 s exposure time. The data frames were processed with the XENGEN package of programs to produce a scaled set of structure factors, which were then used to generate difference Fourier electron density maps (with respect to the native protein) using the CCP4 package of crystallographic programs. Total number of reflections measured was 75 943. Number of unique reflections was 33 834. Number with $I/\sigma > 10$ was 32 309. Merging *R* factor = 0.075 and mean fractional isomorphous difference in structure factor amplitudes from the native GP*b* was 0.119. A difference map was calculated and examined using the molecular graphics program FRODO implemented on an Evans and Sutherland PS390 graphics terminal. Final refinement of the phosphorylase–ligand complex was performed on a Convex C210 using X-PLOR energy and crystallographic least-squares minimization. Individual atomic *B*-factor refinement was performed in the final cycles resulting in a final *R* = 0.183. In the refinement the glucose analogue was included with the skew-boat conformation§ and torsion angles for the glucopyranose ring were restrained with force constants 500 kcal per ° mol^{-1} in order to maintain ring geometry. Further details will be published elsewhere.

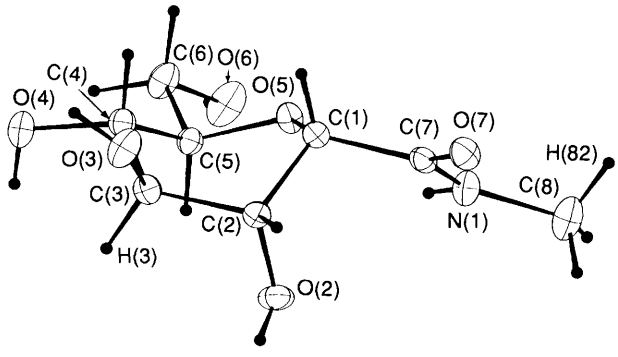


Fig. 2 View of the single X-ray crystal structure of **1a**, showing the ring atoms in a skew-boat conformation

§ *Crystal data* for **1a**: $C_8H_{15}O_6N$, *M* = 220.2, monoclinic, space group $P2_1$, *a* = 6.870(1), *b* = 7.359(1), *c* = 9.691(1) Å, β = 96.85(1)°, D_c = 1.50 g cm^{-3}, *Z* = 2. Linear absorption coefficient = 10.72 cm^{-1} (Mo-Kα), λ = 0.71069 Å, Graphite monochromator, crystal size = 0.15 × 0.15 × 0.30 mm. Data were collected on an Enraf-Nonius CAD4 diffractometer in ω–2θ mode. Number of reflections measured was 1327. Number of unique reflections was 1202. Number with $I > 3\sigma(I)$ was 896. The merging *R* factor = 0.0274 and the azimuthal absorption correction was, max : min = 1.31 : 1.16. The structure was solved with SHELXS86, refined with CRYSTALS, and drawn with CAMERON. Hydroxy hydrogen atoms were found by difference syntheses, others were placed geometrically. Secondary extinction coefficient was refined. Final *R* factor = 0.0338, weighted *R* factor = 0.0363. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

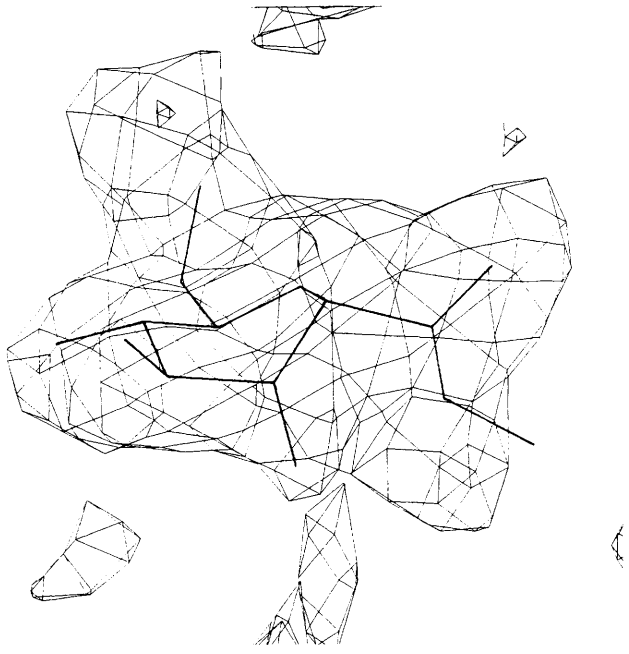


Fig. 3 Single X-ray crystal structure of **1a** superimposed with the calculated difference Fourier map for the bound ligand–GP*b* complex. Contour level is approximately three standard deviations of the map.

effects such as the anomeric effect.[8] This usually results in the α substituent of glucose analogues being placed in an axial orientation, but steric effects as shown with C-glycosides may also be important.[9] Phosphorylase–ligand binding studies with both the unsubstituted amide **1b** and the hydrazide **1c** showed that these compounds exhibited usual $^4C_1$ chair geometry when bound to phosphorylase, while compounds with more bulky substituents (*e.g.* R = benzyl) exhibited a skew-boat conformation. It is concluded that the skew-boat conformation is accessible to the amido derivatives of D-*glycero*-D-*ido*-heptonic acid but that the conformation is influenced in a subtle way by the nature of the substituent groups.

In summary, this paper reports the first X-ray crystallographic analysis of a simple unprotected glucose analogue which has a skew boat conformation both in the single crystal structure and when bound to phosphorylase and, indicates the need for caution with any assumption that C-glycoside analogues are likely to occur in the $^4C_1$ chair conformation. Studies on **1a** and results of other potential GP inhibitors will be reported in detail elsewhere.

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