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# BINDING AND CATALYSIS BY YEAST ALDOSE REDUCTASE: A SUBSTRATE-ANALOG APPROACH WITH NEW ALDOSE DERIVATIVES

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**Abstract**: 5-Deoxy-D-xylofuranose derivatives and a range of new 5,6-dideoxy analogs of D-glucofuranose bearing azido or fluoro substituents were synthesised and employed as substrates of the NADH-dependent aldehyde reduction catalysed by yeast aldose reductase. In terms of catalytic efficiencies, these products proved to be superior to the parent compounds. 

A detailed investigation on the contributions of noncovalent enzyme/substrate interactions to the catalytic efficiency of aldose reductase (EC 1.1.1.21) from the yeast *Candida tenuis* revealed that 5-deoxy-D-xylofuranose (**4a**) is an approximately 100-times better substrate of the enzyme than D-xylose.[1] It was assumed that this observation was largely due to the approximately 100-times higher proportion of the free aldehyde species in aqueous solution of **4a** compared with D-xylose.[1] Since aldose sugars such as D-xylose or D-glucose bind poorly to aldose reductase - apparent substrate binding constants ($K_m$) are in the range of 0.1 to 1 mol/L - tight-binding derivatives could be very useful tools for studying the catalytic properties of the enzyme. It was the aim of this work (i) to synthesise a number of derivatives of D-xylose and D-glucose by replacing the hydroxyl groups at C-5, C-5 and C-6, respectively, and (ii) to derive, from initial velocity measurements, the kinetic parameters for the aldose reductase-catalysed NADH-dependent reduction of these derivatives. Comparison of the kinetic parameters for the aldose derivatives with those for the parent compounds is expected to yield valuable information pertaining to the binding energy derived from noncovalent enzyme/substrate interactions. The yeast aldose reductase is particularly suited for such studies, because in contrast to the counterpart enzymes from mammalian sources,[2] interactions of this enzyme with nonreacting portions of the aldehyde play an important role for transition state stabilisation.[1] As with previous studies,[1,3] the aldose reductase from the xylose-utilising yeast *Candida tenuis* was employed. This aldose reductase has a dual coenzyme specificity, catalysing aldehyde reduction by utilising NADPH and NADH with similar catalytic efficiencies.[3] It is highly useful in the coenzyme-dependent synthesis of sugar alcohols, and, consequently, detailed knowledge on structure-reactivity relationships is important for potential synthetic applications of this enzyme.

The following derivatives were considered useful: 5-deoxy-D-xylofuranose (**4a**)[4], 5-azido-5-deoxy-D-xylofuranose (**4b**)[5], 5-deoxy-5-fluoro-D-xylofuranose (**4c**)[6] and homologs of 5,6-dimodified D-glucofuranoses.

The synthesis of derivatives **4a-c** is outlined in ref 7.

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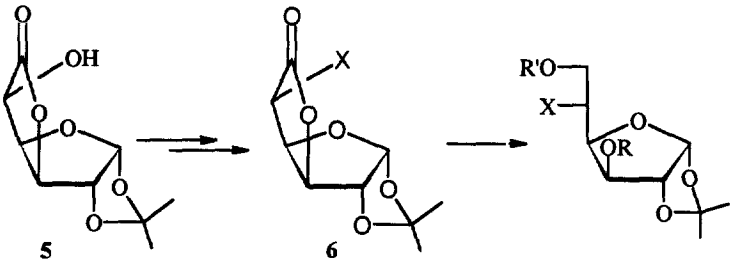
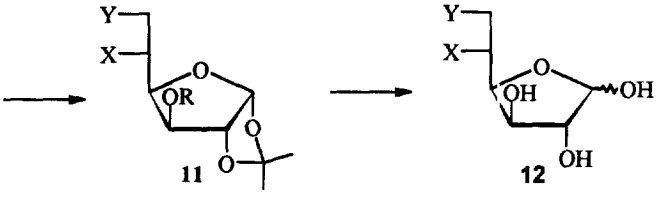
**Scheme 1**




**6a**: X=$N_3$
**6b**: X=H
**6c**: X=F

**7**: R'=R=H X=$N_3$, H, F
**8**: R'=Tr, R=H, X=$N_3$, H, F
**9**: R'=Tr, R=Ac, X=$N_3$, H, F
**10**: R'=H, R=Ac, X=$N_3$, H, F




**11a**: X=H, Y=H, R=Ac
**11b**: X=H, Y=F, R=Ac
**11c**: X=H, Y=$N_3$, R=Ac
**11d**: X=F, Y=F, R=Ac
**11e**: X=F, Y=H, R=Ac
**11f**: X=F, Y=$N_3$, R=Ac
**11g**: X=$N_3$, Y=$N_3$, R=Ac
**11h**: X=$N_3$, Y=F, R=Ac

**12a**: X=H, Y=H
**12b**: X=H, Y=F
**12c**: X=H, Y=$N_3$
**12d**: X=F, Y=F
**12e**: X=F, Y=H
**12f**: X=F, Y=$N_3$
**12g**: X=$N_3$, Y=$N_3$
**12h**: X=$N_3$, Y=F

The general strategy for the preparation of 5,6-dimodified D-glucofuranoses was as follows. Modification of C-5, with overall retention of configuration, was achieved according to literature procedures by employing 1,2-*O*-isopropylidene-α-D-glucofuranurono-6,3-lactone (**5**), yielding the D-*gluco* configured 5-azidodeoxy compound **6a**[10] and 5-deoxyfluoro compound **6c**.[11] Deoxy analog **6b** is accessible either *via* a modified Barton-McCombie-deoxygenation[12] protocol or by using dibromotriphenylphosphorane and excess triphenylphosphane.[13] After reduction of the lactone[10,11] ($NaBH_4$ in methanol at 0°C) the attempted modifications of C-6 by sulfonating the primary hydroxyl group regiospecifically with tosyl chloride in pyridine failed due to unexpected intramolecular 6,3-anhydro ring formation. Consequently, a protection-deprotection sequence had to be conducted. In a one-pot procedure, OH-6 of the diol **7** was protected as the tritylether **8** followed by acetylation of OH-3 to give acetate **9**. After removal of the trityl group[14] ($BF_3$·diethylether complex in dichloromethane at 0°C), OH-6 in **10** was sulfonylated (trifluoromethanesulfonic anhydride in

dichloromethane/pyridine). Subsequent displacement of the leaving group with hetero substituents ($NaN_3$ or NaI in DMF and dried tetrabutylammonium fluoride in acetonitrile, respectively) furnished fully protected 5,6-dimodified glucofuranoses **11a-h** in high yields. The deoxy products were obtained by catalytic reduction of the corresponding iodo compounds (Raney-Ni/$H_2$). Deprotection with ion exchange resin Amberlite IR 120[$H^+$] in acetonitrile/water mixtures yielded the anomeric mixtures of the free glucofuranoses **12**.[15]

5,6-Dideoxy-D-xylohexofuranose (**12a**) was prepared by the modification of a known method.[16]

**Table 1. Kinetic parameters for the NADH-dependent aldehyde reduction catalysed by aldose reductase from *Candida tenuis*.** Reactions were carried out at 25 °C in 50 mmol/L potassium phosphate buffer, pH 7.0. The coenzyme was saturated at 220 µmol/L, that is approx 9 x $K_{m\ NADH}$.

| Substrate | $k_{cat}$ ($s^{-1}$) | $K_m$ (mM) | $k_{cat}/K_m$ ($M^{-1}\ s^{-1}$) | $-\Delta\Delta G$ (kJ/mol)[a] |
|---|---|---|---|---|
| D-xylose | 16.4 | 87 | 189 | - |
| **4a** | 21.6 | 0.93 | 23,226 | 11.9 |
| **4b** | n.d. | n.d. | 1,690 | 5.4 |
| **4c** | 22.3 | 0.65 | 34,308 | 12.9 |
| D-glucose | n.d. | n.d. | 21 | - |
| **12a** | 20.6 | 0.53 | 38,868 | 18.6 |
| **12d** | 21.4 | 1.4 | 15,286 | 16.3 |

[a] calculated according to $\Delta\Delta G = -RT \ln [(k_{cat}/K_m)_{derivative}/(k_{cat}/K_m)_{parent}]$, by using 298.15 for $T$ and 8.314 J/mol for $R$; n.d., not determined; $k_{cat}$ values are based on a molecular mass of the aldose reductase monomer of 43 kDa (Ref. 3).

Kinetic parameters for the NADH-dependent reduction of some derivatives are shown in Table 1. The determination of the catalytic constant ($k_{cat}$), which requires saturation of the enzyme with substrate (Å 5 x $K_m$), was not possible in all cases. With D-glucose, the apparent $K_m$ is greater than 1 mol/L. The apparent $K_m$ of **4b** is larger than 20 mM, and limited availability of **4b** precluded saturation with substrate in the experiment. However, the values for $k_{cat}/K_m$ with these substrates were obtained from the part of the Michaelis-Menten curve where the reaction rate is a linear function of the substrate concentration. Judging from $k_{cat}/K_m$ values in Table 1, it is clear that derivatives **4a-4c** as well as **12a** and **12d** are much better substrates than the corresponding parent compounds. The effect on $k_{cat}/K_m$ results from a very large decrease in $K_m$ and a smaller but significant increase in $k_{cat}$, comparing data for **4a** and **4c** as well as D-xylose. In the $^1$H-NMR-spectra of compounds **12** a small signal of the proton of the free aldehyde (around 10ppm) was found. Therefore, it was evident that the open-chain tautomers were present in a concentration range near the limit of detection (1-5%). Compared with D-glucose, the amount of this tautomer is increased by approximately three orders of magnitude.[17] This fact almost certainly accounts for the marked decrease in apparent binding constants, and consequently higher catalytic efficiencies in the enzymatic reaction.

The results presented underline the utility of using substrate analogues for studying yeast aldose reductase with respect to reactant binding and catalysis. They support the view that aldose reductases bind aldose sugars in the free aldehyde form and, themselves, provide no catalytic assistance to the ring opening reaction. In agreement with our findings, Grimshaw[18] described mammalian aldose reductase as an enzyme reagent that traps the open chain form by NADPH-dependent reduction the free aldehyde form of D-glucose in aqueous solution.

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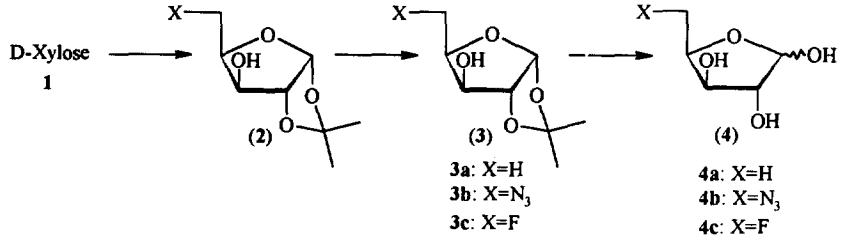
7. 5-modified D-xylose derivatives were prepared from D-xylose 1 by standard carbohydrate methodology: A one-pot procedure[8] gave 1,2-*O*-isopropylidene-α-D-xylofuranose (**2**). Modifications at C-5 were achieved by regioselective displacement of the primary hydroxyfunction in **2** with triphenylphosphine-*N*-bromosuccinimide (TPP-NBS)[9] followed by nucleophilic substitution ($NaN_3$ in DMF for **3a**) or catalytic dehalogenation (Raney-Ni/$H_2$ for **3b**). Compound **3c** was obtained by addition of a solution of **2** in dichloromethane to diethylaminosulfur trifluoride (DAST) at -30°C. Acidic deprotection yielded the known anomeric mixtures of the xylofuranoses **4a**,[4] **4b**,[5] and **4c**.[6] respectively.

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