Stereoselective Galactosyl Transfer to *cis*-Cyclohexa-3,5-diene-1,2-diol

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Diastereoisomeric β-galactosides of *cis*-cyclohexa-3,5-diene-1,2-diol were prepared by galactosyl transfer from lactose catalysed by the β-galactosidase of *Escherichia coli*.

The ability of microorganisms to dihydroxylate aromatic substrates is well documented. The archetypal example is the dihydroxylation of benzene by strains of *Pseudomonas putida* to give *cis*-cyclohexa-3,5-diene-1,2-diol **1** (Scheme 1).[1] When the plane of symmetry in the diol is destroyed by the presence of a substituent, optically active products are obtained.[2]

The potential of these diols as chiral building blocks in organic synthesis has been recognised. The simplest member of the series, the diol **1**, has been used in the synthesis of polyphenylene,[3] (±)-,[4] (+)-,[5] and (−)-pinitol,[5] and *myo*-inositol-1,4,5-triphosphate.[6] Since the precursor **1** is not chiral, synthesis of the pinitol enantiomers required a chem-

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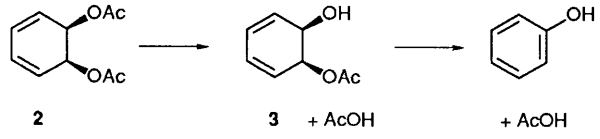
Scheme 1



2 3 + AcOH + AcOH

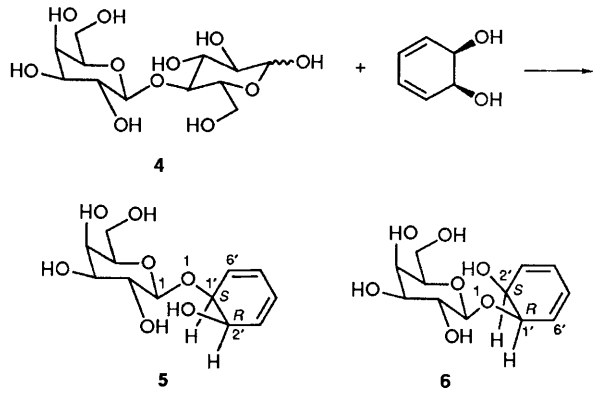
Scheme 2

ical resolution step. The *myo*-inositol-1,4,5-triphosphate was synthesised in racemic form. The demonstrated usefulness of the diol **1** in synthesis prompted us to investigate methods for converting it into a chiral building block that could be used without the need for subsequent resolution. The obvious method to try would be the '*meso* trick,'[7] that is, stereoselective enzymatic hydrolysis of a diacetate **2** of the diol (Scheme 2). However, it was found that although selective hydrolysis occurred, the intermediate monoester **3** rapidly underwent elimination of acetic acid to give phenol as the sole product (Scheme 2).[8]

Accordingly, to produce a stereoselectively modified derivative of the diol **1**, glycosyl transfer[9] was investigated, since this would give a product that would not so readily undergo elimination. Galactosyl transfer, using the β-galactosidase of *Escherichia coli*, with lactose **4** as galactosyl donor gave initially a mixture of isomeric diols in a ratio of 9 : 1 (~80% diastereoisomeric excesss) (Scheme 3). On continued incubation, the amount of total galactoside formed passed through a maximum and the diastereoisomeric ratio declined to 3 : 2. These changes are attributable to hydrolysis of the product galactosides: the fastest-formed diastereoisomer is more susceptible to hydrolysis than the minor diastereoisomer. The diastereoisomeric excess of the product is, therefore, dependent on incubation time. Hydrolysis, however, is relatively slow compared to the initial rate of galactosyl transfer, and isolation of products by HPLC† before extensive hydrolysis has occurred is relatively simple. Experiments with the pure diastereoisomers showed that intramolecular transfer of galactose did not occur.

To optimise overall yields, galactosyl transfer was carried out with an excess of the diol **1** and the product was isolated at the point at which almost all of the lactose had been consumed. The mixture of diastereoisomers (3 : 2) was separated by HPLC† to give a major isomer (A) with the longer retention time, and a minor isomer (B) in yields of 13 and 8% respectively based on lactose.† To determine relative configurations, nuclear Overhauser enhancement (NOE) studies were carried out. A significant NOE was observed between H-1 and H-6′ with galactoside A, but no corresponding effect was observed with galactoside B.

† To a solution of the diol **1** (0.67 g, 6 mmol) and lactose (0.5 g, 1.5 mmol) in phosphate buffer (0.1 mol dm^{-3}; pH 7.3; 5 ml) was added β-galactosidase (Sigma grade X, 100 U, at $t = 0$ h and 100 U at $t = 3$ h). The mixture was incubated at 25 °C for 48 h, at which time all of the lactose had been consumed. Excess of acceptor was removed by extraction (EtOAc) and the product galactosides were isolated as a diastereoisomeric mixture by HPLC [Magnasil 5H aminopropyl column, 25 cm × 4 mm, isocratic elution with MeCN–H_2O (77 : 23)]. The galactoside mixture was separated into the component diastereoisomers by further HPLC purification using the same column but with the solvent mixture MeCN–H_2O (90 : 10). From the mixture of galactosides (87 mg) was obtained galactoside A (54 mg) and galactoside B (32 mg), $[\alpha]_D$ 41.7° (*c* 0.3, H_2O) and 0.6° (*c* 0.23, H_2O) respectively. The pentaacetates had M^+ at *m/z* 502.1925 and 502.1925 respectively by chemical ionisation mass spectrometry (NH_3 ionisation). $C_{22}H_{32}NO_{12}$ requires: 502.1915.



4 5 6

Scheme 3

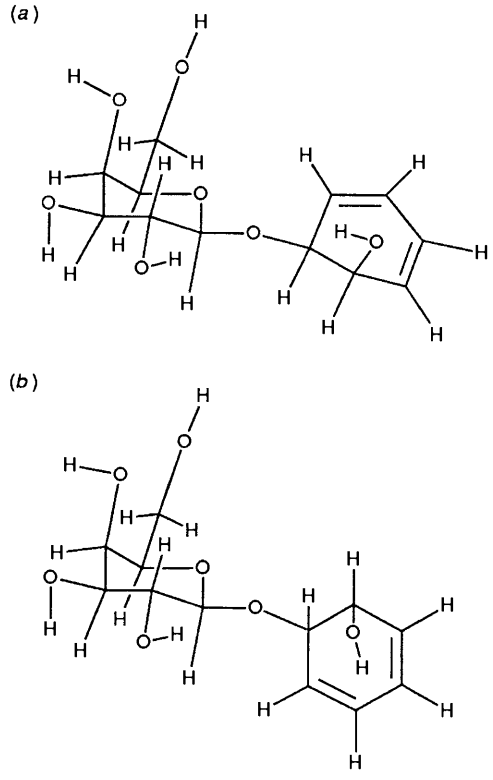


Fig. 1 Minimum energy conformations of (*a*) β-galactoside **5**, and (*b*) β-galactoside **6** of *cis*-cyclohexa-3,5-diene-1,2-diol

The diastereoisomeric structures were minimised using the molecular mechanics programme PCMODEL.[10] To ensure that all reasonable energy minima had been located, The MULTOR routine was used to generate a series of conformations by 60° rotation steps about the C-1–O-1 bond. For each of these conformations, a second set was generated by 60° rotation steps about O-1–C-1′ bond. From the 36 conformations thus generated, 13 physically meaningful conformations were minimised for the (1′*S*)-galactoside **5**, and twelve for the (1′*R*)-galactoside **6**. These minimisations were carried out in two ways: first, permitting intramolecular hydrogen bonding and, secondly, with intramolecular hydrogen bonding prevented to simulate the situation in aqueous solution. In both cases, the lowest energy conformation for the (1′*R*)-galactoside **6** was the one with the smallest 1-H–6′-H distance (2.2 Å) (Fig. 1*b*). For the (1′*S*)-galactoside **5**, the lowest energy conformation, with intramolecular hydrogen bonding permitted, had a 1-H–6′-H distance of 2.5 Å. However, this conformation was stabilised by a hydrogen bond between the hydrogen atom of the 2′-hydroxy group and the ring oxygen of

the galactose component. When this possibility was prevented, this conformer was found to have an energy 8.9 kJ mol^{-1} above that of the minimum energy conformer (Fig. 1*a*) which had a 1-H–6′-H distance of 3.9 Å. Accordingly, by relating these results to the NOE data, it was concluded provisionally that galactoside B was the (1′*S*,2′*R*)-β-galactoside **5** and that galactoside A was the (1′*R*,2′*S*)-β-galactoside **6**.

Further investigations are in hand to explore diastereoselectivity in reactions of the diene system in these glycosides.

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10 Serena Software, Bloomington, Indiana, USA.