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Phenyl galactopyranosides – ^{13}C CPMAS NMR and conformational analysis using genetic algorithm

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ABSTRACT

Structural analyses of four compounds (phenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (**1**), phenyl β-D-galactopyranoside (**2**), phenyl 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranoside (**3**) and phenyl α-D-galactopyranoside (**4**)) have been performed using solid-state ^{13}C MAS NMR spectroscopy and theoretical methods. Conformational analysis involved grid search and genetic algorithm (GAAGS). Low-energy conformers found by GAAGS were further optimized by DFT and chemical shifts were calculated using GIAO/DFT approach. ^{13}C CPMAS NMR chemical shift of carbon C2 is indicative of the glycoside torsional angle. Separated or merged resonances of C2 and C6 suggest free rotation of phenyl ring in the solid phase.

1. Introduction

Aryl galactosides occur widely in nature [1,2]. Their diverse biological activities make them attractive targets for synthesis [3] as well as for structural investigation [4,5]. Among them, aryl glycosides, galactosides seem to be very important due to existence of galactoside-specific recognition system in mammalian liver [6–8]. Phenyl galactosides could be of potential target compounds for pharmacy as leads in designing bioactive ligands for drugs. Some of them were used in cancer therapy as a efficient prodrugs so-called antibody directed prodrug therapy [9,10]. According to Ghosh et al. β-galactoside phosphoramide mustard prodrug might have good potential in increasing antitumor selectivity in cancer therapy [11]. Saccharides linked to proteins and lipids cover a large fraction of the surface area of most cells. Many of saccharides are involved in specific recognition processes. To understand their biological function in detail it is necessary to have information about their three dimensional (3D) structure, as well as glycosidic linkage. Knowledge about the 3D structure of oligosaccharides also has medical applications [12].

3,5-Substituted phenyl galactosides were synthesised as leads in designing effective cholera toxin antagonists and subjected to crystallographic studies [13]. Fluorophenyl-β-D-galactopyranosides are responsive to the action of β-galactosidase, the product of the lacZ gene. Liberation of the aglycon caused by the enzyme can be followed by ^{19}F NMR and yields information on gene transfection [14,15]. p-Nitrophenyl α- and β-D-galactopyranosides were used as a substrates for investigation of the respective galactosidases activity [16,17].

Mixtures of gluco- and galactopyranoside derivatives with ibandronate monosodium salt were designed to prepare co-crystals with improved intestinal absorption of the bisphosphonate. The solid mixtures were studied by FT-Raman, FT-NIR and ^{31}P CPMAS NMR. Only phenyl β-D-galactopyranoside yielded potential co-crystals with ibandronate probably due to *cis*-orientation of phenoxy moiety. However, contrary to expectation, the evaluated co-crystals showed relatively low bioavailability [18]. Binding of α- and β-D-galactopyranosides with different hydrophobic aglycones to lactose permease of *E. coli* was compared [18]. The most potent new compound appeared to be *m*-nitrophenyl-α-D-galactopyranoside. Nitro- or methyl-substituted phenyl α-D-galactopyranosides bind with significantly higher affinities than β-D-galactopyranosides. The crystal structures of the most promising compounds were determined in order to understand the basis for affinity differences. The data suggest that the primary interaction between the permease and hydrophobic aglycones is directed toward the carbon atom bonded to the anomeric oxygen. The different positioning of this carbon atom in α- or β-D-galactopyranosides may provide a rationale for the characteristic binding preference of the permease for α-anomers. Therefore, conformational analysis of α- and β-D-galactopyranosides with aromatic substituents is desirable.

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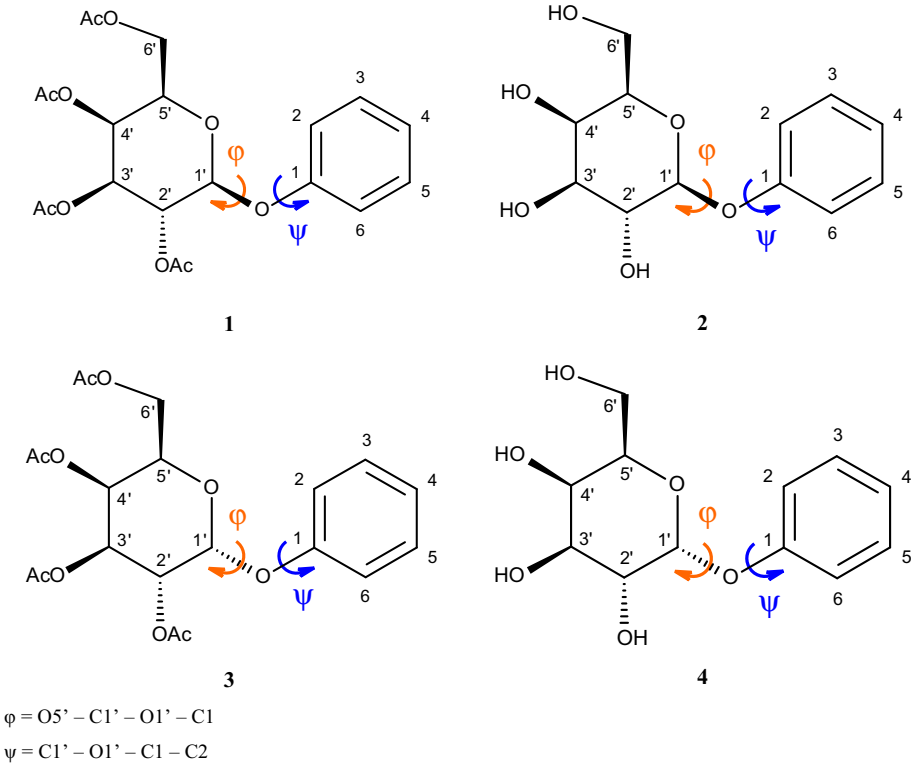

φ = O5' – C1' – O1' – C1

ψ = C1' – O1' – C1 – C2

Fig. 1. Chemical structure of phenyl *O*-galactosides **1**–**4** with atom numbering.

^{13}C cross polarization (CP) magic angle spinning (MAS) NMR spectroscopy has become a powerful technique for structural studies of all kinds of solids including single crystals, crystalline and amorphous powder [14,19–21]. However, there are only few studies on phenyl glycosides by ^{13}C CPMAS NMR. Some solid state NMR data of aryl glycosides containing p-nitrophenyl [22,23] or α-tocopheryl aglycon [24] have been reported.

As a part of larger project, a series of peracetylated and deacetylated α/β phenyl *O*-galactosides **1**–**4** were obtained (see Fig. 1) and subjected to conformational analysis and solid-state NMR studies. Experimental and theoretical methods were applied in tandem: high-resolution solid-state ^{13}C NMR spectroscopy and *ab initio* calculations of NMR shielding constants contribute to our understanding of crystal structures [25]. There are many reports showing that solid-state NMR supported by calculations of shielding constants are used as a verification methods [26,27].

2. Experimental

Phenyl *O*-galactosides **1**–**4** were prepared accordingly to the commonly known procedure [28–30] starting from a mixture α/β peracetylated galactose in a yield 65–70%. The fraction of pure α- and β-anomers **1** and **3** were obtained by column chromatography (hexane–ethyl acetate 15:1, v/v) performed on Merck silica gel (70–230 mesh). Deacetylation **1** and **3** were performed using procedure of Herzig et al. [20] (MeOH, KCN, rt) in yield 95–98%. All compounds **1**–**4** gave solution ^{1}H and ^{13}C NMR spectra identical with those described in literature **1**, **2** [31,32] and **3**, **4** [33] respectively.

Cross polarization magic angle spinning (CPMAS) ^{13}C NMR were recorded on a Bruker DSX-400 spectrometer at 100.16 MHz. Powder samples were spun at 10 kHz in a 4 mm ZrO_2 rotor, contact time of 2 or 4 ms, a repetition time of 6 s and a spectral width of 20 kHz were used for accumulation of 700–900 scans. Chemical shifts were calibrated indirectly through the glycine C = O signals recorded at 176.3 ppm relative to TMS.

^{1}H, ^{13}C, HSQC and DFQ NMR spectra for $CDCl_3$ or CD_3OD solutions were obtained using a Bruker 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield from TMS.

Conformational analysis was performed using genetic algorithm-assisted grid search method (GAAGS), which combines the standard grid search technique with genetic algorithm [34]. It encompassed preparation of potential energy maps of compounds **1**–**4** with respect of values of torsion angles around glycosidic bond. One of the problem with creating such a maps is associated with 'multiple minimum problem' which may arise from the fact that different arrangement of the pendant groups may have significant influence on the value of the potential energy of structure in particular point of the map [35]. This problem can be overcome by evaluating the most optimal orientation of the pendant groups with respect to the galactose ring prior to final optimization of the structure. In the GAAGS method this is realized by genetic algorithm. This method was successfully used in [34,36]. The specially developed software was adapted to cooperate with TINKER 6.3 molecular mechanics package [37]. Potential energy maps were created using MMFF94 molecular mechanics force field [38]. Maps were prepared with Gnuplot software [39]. Calculations were carried out at two different values of dielectric constant: ε = 1.0 and 4.0. This was aimed at finding out how weakening of electrostatic interactions affects the optimal arrangements of pendant groups. Higher value of dielectric constant mimics the more polar surrounding of the molecule. The most optimal structures determined by means of GAAGS method were subjected to further optimization using DFT methods.

Gaussian 09 package was used for DFT calculations [40]. Calculations were carried out using B3LYP hybrid functional with 6-31+G(d,p) basis set. The X-ray molecular geometry of **1** was included for DFT calculations. The position of hydrogen atoms was optimized for the structure with frozen heavy atoms.