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Synthesis and conformational analysis of a simplified inositol-model of the *Streptococcus pneumoniae* 19F capsular polysaccharide repeating unit

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ABSTRACT

Carbohydrate mimics have been studied for a long time as useful sugar substitutes, both in the investigation of biological events and in the treatment of sugar-related diseases. Here we report further evaluation of the capabilities of inositols as carbohydrate substitutes. The conformational features of an inositol-model of a simplified repeating unit corresponding to the capsular polysaccharide of *Streptococcus pneumoniae* 19F has been evaluated by computational analysis, and compared to the native repeating unit. The inositol mimic was synthesized, and its experimental spectroscopic data allowed for verification of the theoretical results.

1. Introduction

Synthesis of carbohydrate analogues has always attracted a great deal of interest, especially since the discovery that carbohydrates play fundamental roles in almost every major biological process [1–5]. Two types of sugar mimics, namely carbasugars [6–8] and azasugars [9] (Fig. 1), characterized by the replacement of the ring oxygen atom with a methylene group or a nitrogen atom respectively, have been widely investigated. The latter have been thoroughly studied as sugar analogues [10–15], with two compounds available on the market, as a treatment for type 1 Gaucher disease (Zavesca®) [16–19] and a treatment for type II diabetes (Glyset®) [20–22]. Alternatively, carbasugars have been studied as substitutes of enhanced biostability, e.g. for inhibition of Ser/Thr protein kinase [23], the transporter protein sodium-glucose cotransporter 2 (SGLT2) [24], and heparanase [25]. In this context, the interesting aspect of azasugars is related to the protonation of the nitrogen atom at physiological pH, and its resulting resemblance to the glycosyl cation that fits into the glycosidase active site. For carbasugars, the main advantage derives from the increased chemical stability gained by replacing the acetal anomeric group with a much more stable secondary alcohol.

In this area, we considered inositols as potential new mimics. Inositols are a family of natural compounds characterized by a polyhydroxy cyclohexane frame, and are involved in several biological functions, e.g. as second messengers when in their polyphosphate form [26–28]. The possibility to use sugars as potential inositol mimics has been investigated to a certain extent [29,30], while the converse substitution has not.

We reported the first attempt at using inositols as sugar mimics by preparing D-pinitol and comparing its properties to those of L-rhamnopyranose [31]. We now focused our attention on a specific case, namely the substitution of the rhamnopyranoside residue of a trisaccharide structure with an inositol analogue. Trisaccharide **4** (Fig. 2) is the capsular polysaccharide repeating unit of *Streptococcus pneumoniae* 19F. An anomeric phosphodiesteric bridge connects two repeating units and represents the point of lability of the whole structure [32,33]. In view of preparing synthetic vaccines based on a modified more stable capsular polysaccharide, which recently gave promising results for *Neisseria meningitidis* A [34], we previously studied the carbasugar analogue of the repeating unit

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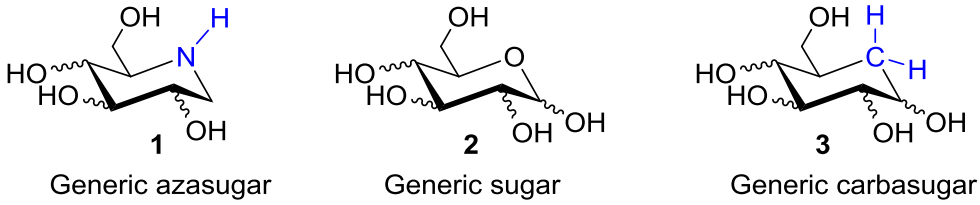

Fig. 1. Generic sugar (**2**), and respective aza- (**1**) and carba- (**3**) analogues.

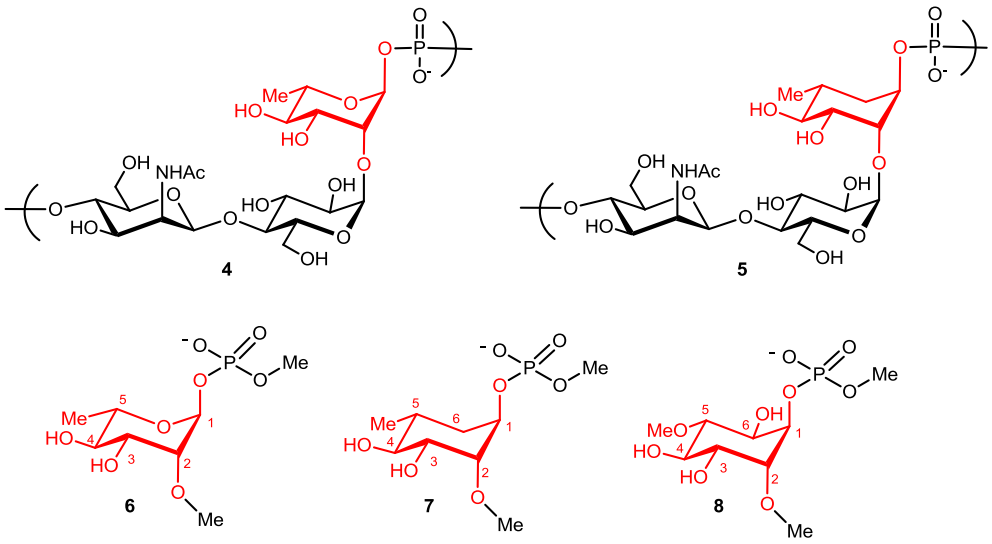

Fig. 2. Repeating unit of the capsular polysaccharide (CPS) of *Streptococcus pneumoniae* 19F (**4**), trisaccharide mimic of the repeating unit of the CPS of *S. pneumoniae* 19F (**5**), simplified *S. pneumoniae* 19F CPS repeating unit (**6**), simplified carba-mimic of *S. pneumoniae* 19F CPS repeating unit (**7**), and simplified inositol-mimic of *S. pneumoniae* 19F CPS repeating unit (**8**).

(**5**) [35] beginning our investigation from the simplified model **7** [36].

Here, we continue our exploration of the possible use of inositols as sugar mimics by analyzing the conformational behaviour of the simplified inositol-model of repeating unit **8** in comparison to that of the simplified repeating unit **6** and of the already reported carba-analogue **7**.

2. Results and discussion

To determine the similarities between **8** and **6** (Fig. 2), an extensive exploration of the conformational space of the two compounds was first carried out through DFT calculations at the B3LYP/6-31G(d) level. A high number of starting geometries, differing in the $^1C_4$ or $^4C_1$ conformations of the ring, and in the orientation of all the substituents, were prepared and optimized. The energies of the conformers obtained were recalculated in water by single point calculations, at the same level as above, using a self-consistent reaction field (SCRF) method, based on the polarizable continuum model (PCM); their percentage contributions to the overall population were determined at 298 K through the Boltzmann equation. The values of torsional angles, relative energies and percentage contributions of the most populated conformations of compounds **6** and **8** (Fig. 2), are reported in Tables 1 and 2. The corresponding three-dimensional plots are shown in Fig. 3.

In the case of **6**, the $^4C_1$ conformations do not give any significant contribution to the population as, in agreement with previous studies [36], they are more than 4 kcal/mol higher in energy than the preferred $^1C_4$ conformer **6A**. Instead, in the case of **8**, the most stable $^4C_1$ conformer **8G** is only 1.52 kcal/mol higher in energy than the global minimum **8A**; however, the overall contribution of the $^4C_1$ conformations remains low (about 7%).

To get a concise picture of the conformational preferences, and to allow for an easy comparison between **6** and **8** (Fig. 2), the percentages relative to a particular arrangement (T, G, mG) at the glycosidic bond for **8** and at the C1 bond for **6**, and to the orientation of the C2- and C5-methoxy groups, are reported in Table 3.

The conformational behaviour of the two compounds presents some differences deriving from their structural diversity. In compound **8** (Fig. 2) the dihedral angle ϕ shows an almost complete preference for the mG orientation, due to the intramolecular

Table 1
Torsional angles (°), relative energies (kcal/mol), and equilibrium percentages (%) of the preferred conformations of compound **6**.

	Ring conf.	ϕ[a]	ψ_1[b]	ψ_2[c]	τ_1[d]	Erel	%
6A	$^1C_4$	−69	−150	72	35	0.00	49.9
6B	$^1C_4$	−154	68	69	27	0.68	15.8
6C	$^1C_4$	−80	−144	72	−52	0.92	10.6
6D	$^1C_4$	−89	88	−143	33	1.04	8.6
6E	$^1C_4$	−148	73	67	−28	1.35	5.1
6F	$^1C_4$	−163	63	−158	31	2.02	1.7
6G	$^1C_4$	−60	−47	−79	35	1.85	2.2
6H	$^1C_4$	−58	−38	110	35	1.99	1.7
others							4.3

[a] ϕ: O–C1–O–P.
[b] ψ_1: C1–O–P–O.
[c] ψ_2: O–P–O–CH_3.
[d] τ_1: H2–C2–O–CH_3.