Pergamon

Tetrahedron Letters, Vol. 38, No. 11, pp. 2023-2026, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00223-2

The Design and Synthesis of a Conformationally Constrained Trisaccharide for Probing Carbohydrate-Protein Interactions

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Abstract: *The synthesis of the macrocyclic trisaccharide **II** which is conformationally restricted around its glycosidic linkages and which can adopt a conformation required for binding with the lectin LOLI is described.*

The cornerstone of biological advances this century has been the understanding of the biological interactions of nucleic acids and proteins between either themselves or each other. However, in recent years, the recognition roles played by carbohydrates and glycoconjugates have received much attention.[1] Examples of carbohydrate recognition include embryogenesis, fertilisation, neuronal development, hormonal activities, cell proliferation and their organisation into specific tissues. As many of these processes involve, at some stage, the interaction between proteins and polysaccharides, an understanding of the factors which control these interactions at the molecular level is of prime importance.[2]

There is considerable evidence that the majority of oligosaccharides are flexible and that they exist as many conformational isomers.[3] It has been suggested that the loss of flexibility on binding to a protein may introduce an unfavourable entropic term and this energy term may partly account for the relatively low affinity of carbohydrate-protein binding. This entropic term may be reduced by a process which is called site-directed processing.[4] Thus, an amount of the conformational entropy may already have been lost through the stabilisation of a subset of three-dimensional structures at the protein surface. It is to be expected that the conformational flexibility of carbohydrates has a functional role. For example, flexible oligosaccharides can bind to a protein in a local minimum energy conformation. Furthermore, it may also be anticipated that flexible saccharide ligands bind with relatively low affinities in order to gain an increase in binding rates (thermodynamics *vs.* kinetics).

Recently, the X-ray crystal structure of a biantennary octasaccharide-lectin LOLI complex has been resolved[5] to 2.3 Å. The complex is stabilised by twenty-three hydrogen bonds, fourteen of which are directly between the protein and the saccharide and seven are by way of a water molecule. In addition, fourteen water molecules are involved in indirect interactions, linking the octasaccharide to the protein or to itself. When complexed to the protein, the saccharide adopts an extended conformation and five of the seven φ and ψ values fall into main low-energy wells on calculated φ and ψ energy charts. Pérez *et al.*[6] have performed a systematic conformational analysis for a LOLI-trisaccharide (β-D-Glc*N*Ac*p*-(1-2)-α-D-Man*p*-(1-3)-D-Man) (**I**) complex (the trisaccharide is part of the above mentioned octasaccharide) and the results have been compared with the X-ray crystallographic data. Three families of allowed conformations (50 kJ mol^{-1} energy window) were identified with respective minima LOL1-Min1, LOL1-Min2 and LOL1-Min3. One of the minimum conformations (LOL1-Min3) corresponded with the conformation of the same trisaccharide when it is a part of the octasaccharide in the complex with LOLI.

Herein, we report the preparation of the chemically modified trisaccharide **II** (**Figure 1**) which is conformationally restricted around its glycosidic linkages and which can adopt a conformation required for binding with LOLI.[7] The design of **II** is based on the following concept: an intramolecular hydrogen bond between O2→O6" of the trisaccharide which is present in the saccharide-protein complex is mimicked by a methylene acetal (see **Figure 1**). In this respect, it should be noted that many carbohydrates in protein complexes are stabilised by intramolecular hydrogen bonds.[8] It is to be expected that this type of cyclic trisaccharides will be an important tool for studying the importance of flexibility of saccharides and intramolecular hydrogen bond formation for carbohydrate-protein recognition and complex formation.

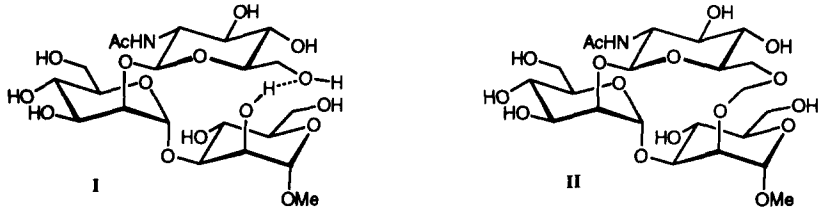
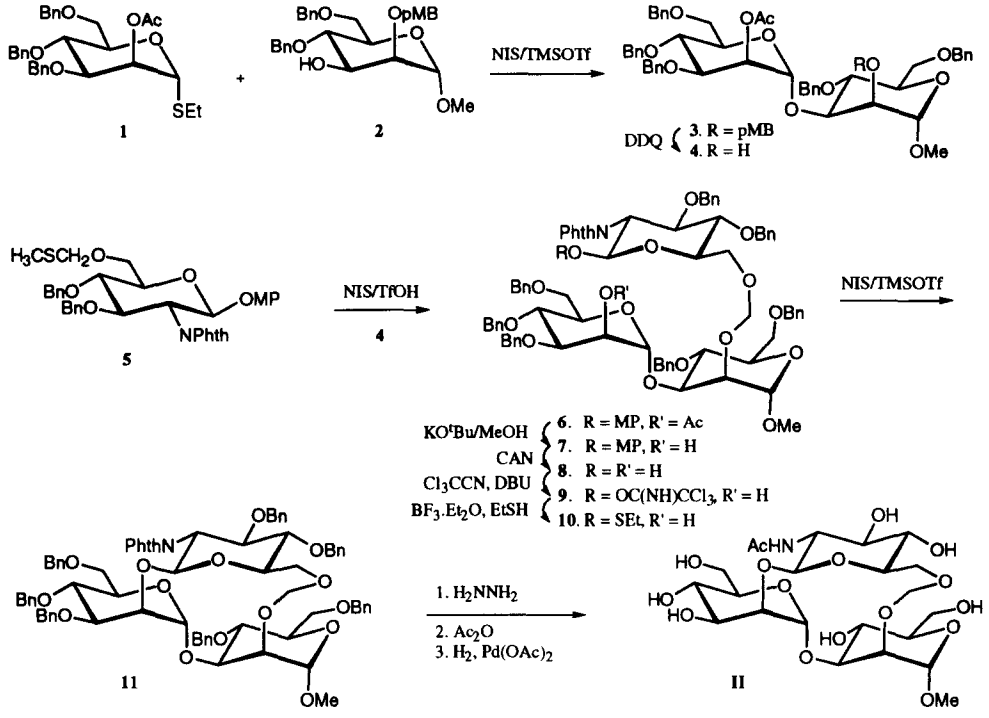


Figure 1: Arbitrary structures of the unconstrained trisaccharide (**I**) and the analogous conformationally constrained saccharide (**II**).

A macrocyclisation is a very challenging aspect of the preparation of the conformationally-restricted trisaccharide **II** and a successful approach is outlined in **Scheme 1**. The cyclisation has been achieved by an intramolecular glycosylation utilising the precursor **10** which had already the methylene acetal linker in place. This approach proved to be more fruitful than macrocyclisation by methylene acetal formation.

The precursor for the macrocyclisation **10** was assembled from the monomeric building blocks **1**, **2**, and **5**. These compounds were readily available by standard protecting group interconversion strategies. Thus, coupling of glycosyl donor **1** with glycosyl acceptor **2** in dichloromethane/ether in the presence of *N*-iodosuccinimide (NIS)/catalytic trimethylsilyl triflate (TMSOTf)[9] afforded the α-linked disaccharide **3** in a good yield (77%). The *p*-methoxybenzyl (pMB) group of **3** was removed by treatment with DDQ to give compound **4** (95%). The methylene acetal functionality was introduced by coupling of the compounds **4** and **5** in the presence of NIS/triflic acid (TfOH) and the required saccharide **6** was obtained in a yield of 96%.[10] Next, methylene linked compound **6** has to be converted into a glycosyl donor being suitable for an intramolecular glycosylation. To this end, the protecting group at the anomeric center of the 2-deoxy-2-phthalimido glycosyl moiety of compound **6** has to be converted into a suitable leaving group and the 2-OH has to be revealed. We selected an anomeric trichloroacetamido functionality as the anomeric leaving group.[11] Such a moiety can be introduced under very mild conditions and activation can be achieved by acid catalysis. Furthermore, this group offers the possibility to be converted into other types of leaving groups. Thus, cleavage of the acetyl group of **6** with potassium *tert*-butoxide in methanol gave **7** and the *p*-methoxyphenyl group (MP) of **7** was removed by reaction with cerium ammonium nitrate (CAN) to afford **8**. Treatment of **8** with trichloroacetonitrile and DBU resulted in the formation of the trichloroacetimidate **9**. The regioselectivity of the latter reaction was achieved by the higher acidity of the anomeric hydroxyl group, however, care had to be taken to avoid di-trichloroacetimidate formation. Unfortunately, TMSOTf-mediated intramolecular glycosylation of **9** gave the macrocyclic compound **11** in a low yield of 10%. However, iodonium-ion mediated glycosylation of the corresponding thioglycoside **10**, derived from the reaction of trichloroacetimide **9** with ethanethiol, gave **11** in an acceptable yield of 34%. It is important to note that the methylene acetal

survived the above discussed manipulations. Apart from cyclic saccharide **11** only hydrolysed material was isolated. Compound **11** was deblocked as follows: conversion of the phthalimido functionality into an NHAc moiety was accomplished by treatment with hydrazine monohydrate followed by acetylation with acetic anhydride and the benzyl ether protecting groups were removed by catalytic hydrogenation over palladium acetate to afford the requisite saccharide **II**.



Scheme 1

The structural integrity of compound **II** was confirmed by NMR spectroscopy and FAB mass spectrometry ($[M+Na]^+$ = 594). The ^1H and ^{13}C NMR signals were unambiguously assigned by two-dimensional homonuclear correlation spectroscopy (COSY, TOCSY). The assignments were aided by hetero-nuclear, proton-carbon chemical shift correlation experiments (H-1 δ 4.61, $J_{1,2}$ = 1.9 Hz, $J_{H\text{-}1,C\text{-}1}$ = 171.4 Hz; H-1' δ 5.78, $J_{1',2'}$ = 2.8 Hz, $J_{H\text{-}1',C\text{-}1'}$ = 172.9 Hz; H-1" δ 4.56, $J_{1",2"}$ = 8.2 Hz, $J_{H\text{-}1",C\text{-}1"}$ = 163.5 Hz; O-CH_2-O δ 4.67 and 4.79, AB, J_{gem} = 6.5 Hz).[12] The vicinal proton coupling constants indicate that the sugar rings adopt chair conformations. The full assignment of the proton spectrum allowed two-dimensional NOE experiments (NOESY) to be carried out. Only two assigned NOEs were relevant for the conformational analysis (between H-1' and H-1" and H-1' and H-3).

The conformational properties of **II** were studied by Monte Carlo multiconformer analyses using molecular mechanics calculations.[13] The global minimum as well as conformations laying within a 50 kJ mol^{-1} energy window were analysed using filtering and clustering analyses. These data were compared with similar data of the unconstrained trisaccharide and it was shown that the conformational space of **II** is significantly reduced but can adopt a conformation as the parent trisaccharide when part of the octasaccharide in the LOLI-glycan complex.

In conclusion, we have described the synthesis of a novel cyclic trisaccharide which will be an important tool to study carbohydrate-protein interactions. The affinity of the lectin for the cyclic ligand **II** will be measured and these data will be compared with similar data for the unconstrained saccharide. These results and full computational data will be presented elsewhere.

Acknowledgement
This work was supported by the European Commission (Training and Mobility Studentship for N.N.)

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13. The structure of constrained trisaccharide **II** was built using MacroModel version 5.0 (F. Mohamadi, N.G.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, G. Caufield, G. Chang, T. Hendrickson, W.C. Still, *J. Comp. Chem.* **1990**, *11*, 440) using a SiliconGraphics Indigo2 Power xz computer. Molecular mechanics calculations were performed with Batchmin version 5.0 on the Indigo computer. The minimisation method was Polak Ribière Conjugate Gradient (PRCG) with the United Atom Amber force field (S.J. Weiner, P.A. Kollman, D.A. Case, U.C. Siggh, C. Ghio, G. Alagona, S. Profeta, P. Weiner, *J. Am. Chem. Soc.* **1984**, *106*, 765; S.J. Weiner, P.A. Kollman, D.T. Nguyen, D.A. Case, *J. Comp. Chem.* **1986**, *7*, 230.) in Batchmin until the RMS-value was below 0.01. Multiconformer analysis was performed with the Monte Carlo Multiple Minimum (MCMM) search (G. Chang, W.C. Guida, W.C. Still, *J. Am. Chem. Soc.* **1988**, *9*, 343) in Batchmin. Each MCMM search contained 10000 cycles, in which 2-4 torsion angles were changed during each cycle. The ring closure bond was located between O and C in the torsion angle C-2—O-2—C—O-6" with an allowable ring closure distance between 1.0 and 4.0 Å (A different closure bond and/or allowable ring closure distances did not result in different conformations.) Conformations within an energy window of 50 kJ mol^{-1} were stored. The torsion angles of the pyranose-rings were maintained during energy minimization and conformational searches. All minimisations were carried out *in vacuo*. Twenty conformations were isolated when filtering on the torsion angle data found for the trisaccharide in the crystallographic complex, allowing ±10°, using the filter-option in MacroModel.

(*Received in UK* 14 *January* 1997; *accepted* 31 *January* 1997)