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Quantum Mechanical Calculations and Experimental NMR Studies of Esterified Methyl-α-D-Galactopyranoside Derivatives

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Abstract

Theoretical quantum mechanical calculations using density functional theory (DFT) at the B3LYP level have been carried out for esterified methyl-α-D-galactopyranoside derivatives. The predicted NMR characteristics obtained with GIAO method have been compared with ^{1}H, ^{13}C and ^{31}P NMR data for synthesized model compounds. This comparison has shown that the compounds under investigation do not occur in the form of inner salts but in the neutral forms.

Keywords: galactopyranoside derivatives, DFT calculations, NMR

Introduction

Carbohydrate derivatives are of great therapeutic interest as antiviral, anti-cancer and anti-HIV agents [1]. Several bacterial polysaccharides (e.g. lipopolysaccharide (LPS)) contain non-carbohydrate substituents like phosphate, pyrophosphoethanolamine, phosphocholine, acetate and aminoacids (glycine or alanine). The occurrence of phosphate or aminoacid in bacterial LPS has strong influence on the physical properties, antigenic, toxic and the other biological activities of pathogens. The glycine is located at the sugar unite, mainly gluco- or galactopyranose, usually phosphorylated at primary hydroxyl group. The ester groups can migrate over the sugar ring and in equilibrium the most stable isomer predominates. The aim of our paper is the comparison of experimental and theoretical NMR data for phosphorylated glycoconjugates based on methyl α-D-galactopyranoside unit. This compound is analogous with the structure present in the core oligosaccharide of LPS. Search for the common epitopes suitable for construction of an antimicrobial vaccine with desired broad specificity seems to be the proper approach to solve the problem of Gram-negative infections.

Experimental and methods

The synthesis of the model compound was performed using the direct phosphorylation of methyl α-D-galactopyranoside with diphenyl phosphoryl chloride and esterification of Boc-glycine with methyl (α-D-galactopyranosid-1-yl)-6-*O*-diphenylphosphate. In the next step one phenyl group was selectively removed in the hydrogenolisis reaction using Pd/C, ammonium acetate and 2-propanol. The removal of Boc-group - protecting amino acid residue - was carried out in 33% trifluoroacetic acid (TFA). Finally, methyl (3-*O*-glicynyl-α-D-galactopyranosid-1-yl)-6-*O*-phenylphosphate was obtained (Fig.1a) [3]. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian Inova 400 Spectrometer and measured in DMSO and D_2O solutions. Optimized structures of all regarded isolated molecules were calculated by the density functional three-parameter hybrid method (DFT/B3LYP) using the 6-31G* basis set. Subsequently, the magnetic isotropic

shielding tensors were calculated by the GIAO technique. Computations were performed using the GAUSSIAN 03 program package [4]. In NMR experiments nuclear shielding constants σ are measured relative to the reference substance and are given as the chemical shift δ. Calculations, on the other hand, produce the absolute tensors σ and their traces σ_{iso}. The relationship $\delta=\sigma_{ref}-\sigma$ is a good approximation of the relationship between chemical shifts and shieldings. In order to compare ^{1}H, ^{13}C and ^{31}P absolute isotropic shielding with experimental chemical shift, we have used as σ_{ref} the value of the isotropic shielding tensor of the experimental standards, TMS (for ^{1}H and ^{13}C) and phosphoric acid (for ^{31}P), calculated at the same DFT/6-31G* level. The atom numbering in α-D-galactopyranoside ring used during calculations is shown in Fig. 1c.

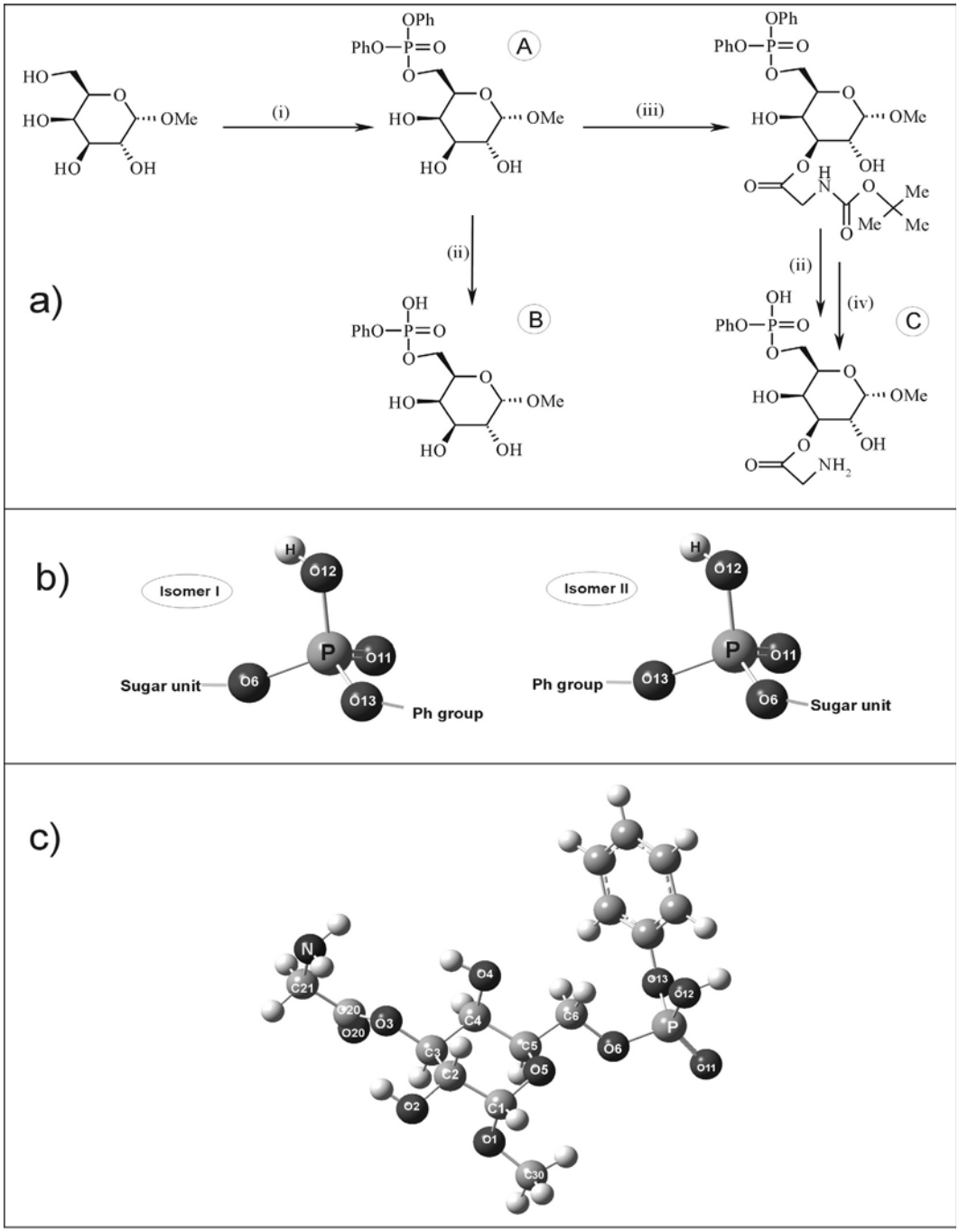

Fig. 1. (a) Synthesis of methyl (3-*O*-glicynyl-α-D-galactopyranosid-1-yl)-6-*O*-phenylphosphate (C). Reagents and conditions: (i) diphenyl phosphoryl chloride, pyridine; (ii) Pd/C, ammonium acetate, 2-propanol; (iii) Boc-glycine, pyridine, DCC, DMAP; (iv) 33% TFA; (b) two possible stereoisomers at the phosphorus atom; (c) optimized structure of methyl (3-*O*-glicynyl-α-D-galactopyranosid-1-yl)-6-*O*-phenylphosphate (compound (C)) with atom numbering

Table 1. Comparison of selected experimental and theoretical chemical shifts δ [ppm]

	methyl (α-D-galactopyranosid-1-yl)-6-*O*-diphenylophosphate A		**methyl (α-D-galactopyranosid-1-yl)-6-*O*-phenylophosphate B**			**methyl (3-*O*-glicynyl-α-D-galactopyranosid-1-yl)-6-*O*-phenylophosphate C**		
	exp.	calculated	exp.	calculated		exp.	calculated	
				isomer I	isomer II		isomer I	isomer II
C1	100,01	98,11	100,03	98,05	97,92	99,30	98,12	98,13
C2	68,72	69,87	68,32	69,64	69,36	68,85	67,49	67,53
C3	69,06	72,65	68,98	72,71	70,77	71,99	77,38	77,34
C4	67,97	72,47	68,02	72,37	71,50	69,10	68,96	68,54
C5	69,01	70,23	69,24	70,17	71,13	69,33	70,08	69,74
C6		69,59	63,24	69,19	69,52	65,22	69,10	67,85
C20	-	-	-	-	-	163,01	163,85	163,80
C21	-	-	-	-	-	40,12	46,39	46,32
C30	54,33	54,16	54,37	54,07	53,62	55,25	53,63	53,51
H1	4,58	4,87	4,51	4,85	4,72	4,79	4,94	4,87
H2	3,72	3,79	3,51	3,76	3,91	3,81	4,18	4,13
H3	3,82	3,83	3,67	3,80	3,56	5,10	5,06	4,92
H4	3,50	3,27	3,46	3,26	2,66	3,75	3,95	3,78
H5	3,58	4,23	3,87	4,10	3,23	3,85	4,41	3,92
H66’	4,32	4,29	4,41	4,20	4,24	3,98-4,15	4,35	4,15
C21/HH’	-	-	-	-	-	3,94	3,29	5,24
N/HH’(PO/H)	-	-	-	-	-		0,40(2,90)	0,37(3,03)
C30/HH’H”	3,17	3,54	3,39	2,86	3,26	3,34	3,49	3,41
PO/H	-	-	3,21	2,87	3,34	-	-	-
P	-10,58	-9,57	-4,18		-5,00	-4,09		-4,11
			-4,36	-5,44		-4,51	-6,07	

Results and discussion

Taking into account the possible linkage sites of the phosphoric acid and glycine radicals, 12 structures for each deprotected compound at the synthesis scheme (Fig. 1a) have been regarded. Moreover, we have assumed the migration possibility of the hydrogen atom of the phosphoric group to the amine group; the emerging molecule has a character of an inner salt (zwitterion). The energy and vibrational frequencies calculations [5] of the studied molecules performed with regard to the above mentioned conditions, not presented here, have shown that we have to be concerned with the neutral form in which the phosphoric group is attached to C6 and glycine group to C3 atoms, respectively. Taking into account the space location of the substituents at the phosphorus atom (see Fig. 1b), two stereoisomers can exist for the compounds B and C; the calculations were carried out for these two isomers.

In Table 1 the calculated chemical shifts of the compound A and two possible isomers of compounds B and C are compared with experimental carbon, proton and phosphorus shifts values. NMR data in Table 1 show the satisfactory agreement between experimental and theoretical chemical shifts both for carbons and protons; the differences between these values for ^{13}C are in the range from 0.2 to 6 ppm. The comparison between calculated and experimental proton shifts is much less decisive because of the small proton shifts values in relation to the carbon ones. From ^{13}C and ^{1}H NMR data we can say that both isomers, I and II, coexist. On the basis of ^{31}P chemical shift data more can be said. For the compound B, two signals, -4.18 ppm and -4.36 ppm, with the integral intensities 94.5 and 5, correspondingly, were ascribed as follows; the former to the

isomer II (δ_{th} = -5.00 ppm) and the latter to the isomer I (δ_{th} = -5.44 ppm). From these data the mole ratio of both isomers can be estimated as 19:1 (isomer II : isomer I). Analogous procedure applied to the compound C (δ_{exp} = -4.09 ppm corresponds to δ_{th} = -4.11 ppm, isomer I, δ_{exp} = -4.51 ppm corresponds to δ_{th} = -6.07, isomer II) gives this estimation as 11: 1. We see, therefore, that the isomer II predominates. Search in the experimental ^{1}H NMR spectra for the signals originating from the NH_3^+ group has given negative results. That can be considered as another evidence that the examined model system does not exist in the inner salt form.

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