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Theoretical and experimental studies on methyl α-D-glucopyranoside derivatives

E. Chełmecka [a,*], K. Pasterny [a], M. Gawlik-Jędrysiak [b,c], W. Szeja [c], R. Wrzalik [a]

[a] University of Silesia, Institute of Physics, Uniwersytecka 4, 40-007 Katowice, Poland
[b] Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Weigla 12, 53-114 Wrocław, Poland
[c] Silesian Technical University, Department of Chemistry, B. Krzywoustego 8, 44–100 Gliwice, Poland

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Abstract

Theoretical quantum mechanical calculations using density functional theory (DFT) at the B3LYP level have been carried out for synthesized esterified methyl α-D-glucopyranoside derivatives. The predicted NMR characteristics obtained with GIAO method are compared with ^{1}H, ^{13}C and ^{31}P experimental NMR data, whereas calculated vibrational frequencies have been confronted with experimental Raman spectra. This comparison has permitted to state that the compounds under investigation do not occur in the form of a zwitterion but in the neutral form as well as to determine the most favourable stereoisomer.

Keywords: Phosphorylated saccharides; Glucose derivatives; DFT calculations; NMR and Raman

1. Introduction

The study of carbohydrates within biological systems has illustrated that they are involved in a number of fundamental biological functions such as cell–cell recognition and cell-external agent interactions. These interactions can initiate beneficial biological events, such as immune response, as well as detrimental disease processes, such as inflammation, viral and bacterial infections [1]. It was found that several core oligosaccharides of bacterial lipopolysaccharides (LPS), besides the common structural elements such as heptose, glucose, galactose, Kdo contain also non-carbohydrate groups: phosphate, pyrophosphoethanolamine, phosphoethanolamine, phosphocholine, acetate, and glycine [2]. The glycine is located at the sugar unit, mainly gluco- or galactopyranose, usually phosphorylated at primary hydroxyl group [3]. The ester groups can migrate over the sugar ring and in equilibrium the most stable isomer predominates. Understanding the biological role of non-carbohydrate substituents in bacterial LPS is not easy, because they are acid and basic-labile and are easily lost during isolation of native LPS from bacterial mass. For this reason chemical synthesis of compound with structure similar to native core oligosaccharide is necessary. Synthetic oligosaccharides and glycoconjugates provide materials for correlating structure with function [4]. The aim of our research is to predict the structure of synthesized sugar derivatives based on methyl α-D-glucopyranoside unit, using theoretical computational methods and to compare the results with spectroscopic data.

2. Experimental

2.1. Synthetic procedure (Fig. 1)

2.1.1. Methyl (α-D-glucopyranosid-1-yl)-6-O-diphenylphosphate (*1*)

Methyl α-D-glucopyranosid was dissolved in a pyridine in the presence of DMAP, and cooled for 0.5 h to −4 °C, in the next step, diphenyl phosphoryl chloride (1.1 equiv)

* Corresponding author. Fax: +48 32 588431.
E-mail address: chelmeck@us.edu.pl (E. Chełmecka).

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was added. The reaction was stirred over night in temperature range −4 °C to room temperature, then poured into water and extracted three times with methylene chloride. The combined organic layers were washed twice with water, dried over anhydrous Na_2SO_4 and concentrated in vacuo with toluene. The residue was purified on silica gel (eluent: ethyl acetatae)[5]. $[\alpha]_D^{20}$ 62.1 (*c* 0.9, $CHCl_3$).

*2.1.2. Methyl (α-D-glucopyranosid-1-yl)-6-O-phenylphosphate (**2**)*

Methyl (α-D-glucopyranosid-1-yl)-6-*O*-phenylphosphate was obtained using the new highly efficient hydrogenolysis method with ammonium formate as a hydrogen source and palladium on carbon as the catalyst, which will be published elsewhere [6]. $[\alpha]_D^{20}$ 61.9 (*c* 0.5, H_2O).

*2.1.3. Methyl (3-O-(N-Bocglicynyl)-α-D-glucopyranosid-1-yl)-6-O-diphenylphosphate (**3**)*

Methyl (α-D-glucopyranosid-1-yl)-6-*O*-diphenylphosphate, Boc-glycine (2 equiv.) and DMAP were dissolved in a methylene chloride and cooled for 0.5 h to −4 °C. In the next step, DCC (2 equiv.) was added, the reaction was carried out 2 h at −4 °C. The progress of reaction was monitored by TLC (chloroform–methanol 15:1). After filtration, the solution was concentrated in vacuo and the residue was purified on silica gel (eluent: chloroform) [7].

2.1.4. Methyl (3-O-(N-Bocglicynyl)-α-D-glucopyranosid-1-yl)-6-O-phenylphosphate

Methyl (3-*O*-(*N*-Bocglicynyl)-α-D-glucopyranosid-1-yl)-6-*O*-phenylphosphate was obtained by the same method as compound (**2**) [6].

*2.1.5. Methyl (3-O-glicynyl-α-D-glucopyranosid-1-yl)-6-O-phenylphosphate (**4**)*

The removal of Boc-group, protecting amino acid residue, was carried out in 33% trifluoroacetic acid (TFA) for 2 h. The progress of reaction was monitored by TLC (toluene–ethyl acetate–ethanol 2:2:1). Finally, the mixture was lyophilized [8].

2.2. Techniques

^1H, ^{13}C and ^{31}P NMR spectra were recorded on a Varian Inova 400 Spectrometer and measured in $CDCl_3$ and D_2O solutions at room temperature. Both proton and carbon signals were referenced to TMS and phosphorus signals were referenced to H_3PO_4.

Raman measurements were made using Lab-Ram dispersive spectrometer with the sample at room temperature. Argon-ion laser light 514.5 nm was used to excite the spectra. The power at the sample was below 10 mW. The polariser, half-wave plate and analyzer were used to control incident and scattering polarizations. To eliminate the Rayleigh line the scattered light was passed through a holographic notch filter with cut-off 100 cm^{-1}. The intensity was measured using Peltie-cooled CCD detector (1064 × 256 pixels).

3. Method of calculations

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. Computations were performed using the Gaussian 03 program package [9]. The optimized geometries were used as input for NMR and harmonic vibrational frequency calculations. The gauge-invariant atomic orbitals (GIAO) method [10–12] was used for prediction of DFT nuclear shielding. In NMR experiments, nuclear shielding σ 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 ^1H, ^{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 ^1H and ^{13}C) and phosphoric acid (for ^{31}P), calculated at the same DFT/6-31G* level.

The atom numbering used for the present calculations is shown in Fig. 2a; according to X, Y, Z substituents we have to do with compounds (**1**), (**2**) or (**4**), shown on the synthesis scheme (Fig. 1), which are the subject of our investigations. Oxygen atoms have the same number as carbon atoms which they are linked to, except those atoms from the phosphoric group that are denoted as O11, O12 and O13. Carbon atoms in glycine radical are denoted as C20 and C21. Taking into account the space distribution of the substituents at the phosphorus atom (Fig. 2b), for compounds (**2**) and (**4**) two stereoisomers, denoted in the following as isomer **I** and **II**, can exist. Considering the presence of the glycine group in (**4**), we have assumed the migration possibility of the hydrogen atom from the phosphoric to amino group (Fig. 3); the emerging molecule has a character of an inner salt (zwitterion, betaine).

4. Results and discussion

Theoretically calculated energies for isomers **I** and **II**, both in neutral and zwitterionic forms of compound (**4**), differ very little [13,14] thus their coexistence is probable. Therefore, possible isomers of all regarded compounds, (**1**), (**2**) and (**4**), will be subjected to further analysis exploiting theoretical and experimental data.

4.1. Molecular geometry

Selected DFT calculated structural parameters of investigated molecules are listed in Table 1. Both the bond lengths and angles within the sugar ring for (**1**), (**2**) and (**4**) are close each other and comparable to experimental data for α-D-glucose [15]. For the neutral forms the insertion of the glicydyl group causes noticeable elongation of

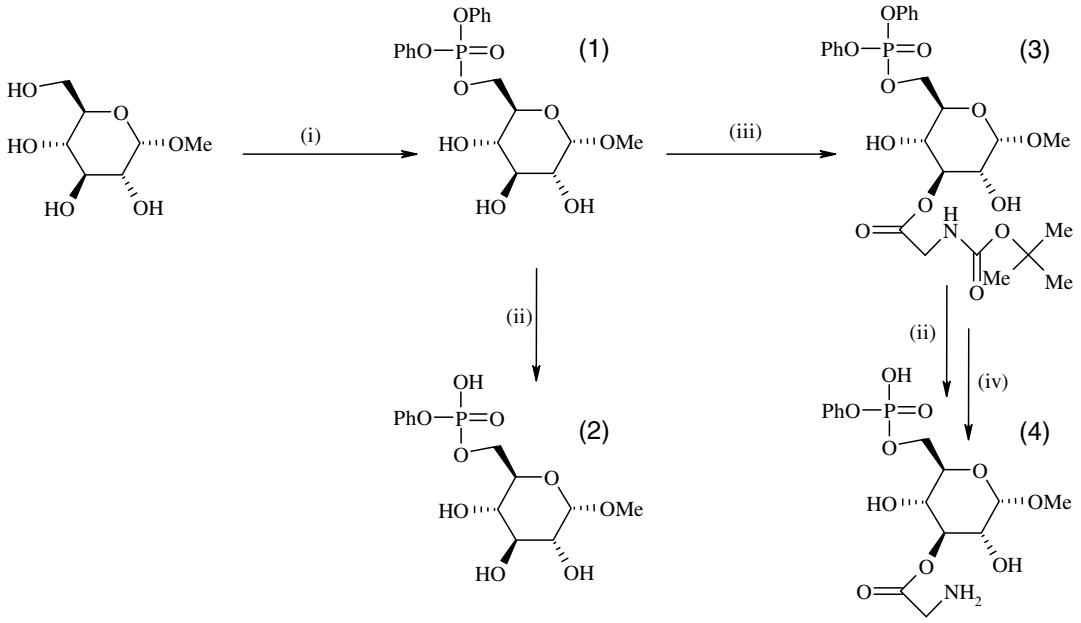

Fig. 1. Synthesis of methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylphosphate. Reagents and conditions: (i) diphenyl phosphoryl chloride, pyridine; (ii) Pd/C, ammonium acetate; (iii) Boc-glycine, methylene chloride, DCC, DMAP; (iv) 33% TFA.

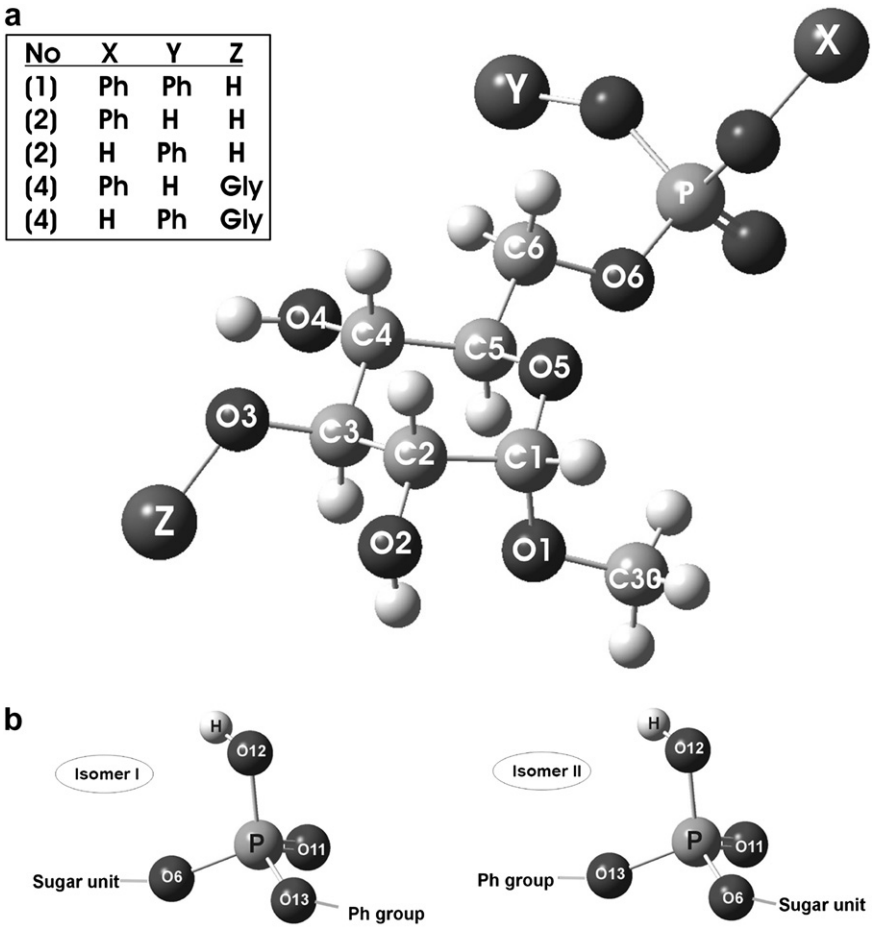

Fig. 2. (a) Atom numbering used for all regarded molecules, (b) two possible stereoisomers at phosphorus atom.

the C3–O3 bond (by about 0.025 Å) and inconsiderable elongation of C1–C2, C1–O1 and C2–C3 bonds, whereas C4–O4 and O5–C1 distances are slightly shortened. For both isomers of (**4**) the possibility of proton migration has been considered that relates with the changes of O···H···N distances and affects the shape of optimized

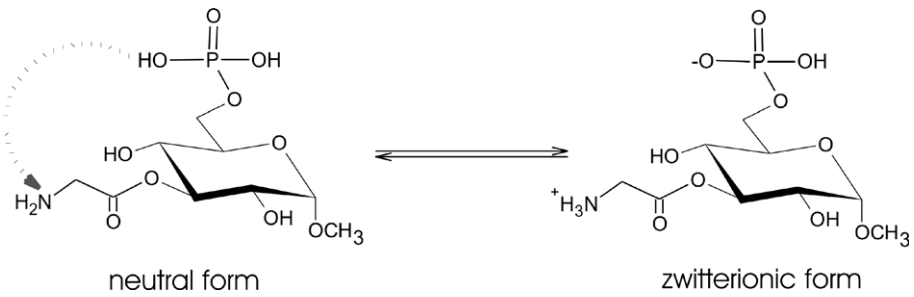

Fig. 3. The scheme for the reaction of the zwitterion formation.

molecules (see Fig. 4). In the case of H···N distance we observe its decrease from about 12 Å in the neutral form to 1 Å in the zwitterionic form that means the proton transfer from the acid to amino group. Besides these changes we also observe for the betaine form the increase in the C21–N, C5–C6, C3–O3 and C4–O4 and C20–C21 bond lengths by 0.005, 0.003, 0.002 and 0.018 Å, respectively, whereas O3–C20 distance is shorter by about 0.012 Å. The length of the ester bond P–O6 is sensitive to the ionization and increases by 0.06 or 0.07 Å according to the stereoisomer. Calculations for the neutral isomers have given the value equal 1.600 Å which is comparable with that reported by Katti at al. [16] for G6P monobarium salt.

The angle differences between neutral isomers of (**4**) and those of (**2**) and (**1**) are clear for O4–C4–C5, O3–C3–C4 and C2–C3–O3 only. In the case of (**4**) the first two mentioned angles are smaller by about 5° and 4°, respectively, when comparing with corresponding angles for (**2**) and (**1**), while the last angle is greater by about 3°; the differences for other angles are negligible. The comparison of calculated angles for betaine and neutral forms of (**4**) shows more significant differences, C3–C4–C5 angle for zwitterionic form is greater by about 10° and O3–C3–C4 is smaller by 4°.

The C5–C6–O6–P dihedral angle indicates the differences originating from the stereoisomerism at the phosphorus atom for (**2**) and (**4**); its calculated value for isomer **I** is −178.2° and −180.0° for (**4**) and (**2**), respectively, and for isomer **II** 137.2° and 138.2°. Considering the possible rotation about the exocyclic C5–C6 bond, the O6–C6–C5–O5 angle is the most regarded dihedral angle for sugar derivatives [17]. From our calculations follows that for (**1**), (**2**) and neutral forms of (**4**) this angle takes the synclinal (+*sc*) value (in the range 70–78°), whereas for zwitterionic forms its value is antiplanar (*ap*, −163 to −168°). Considerable differences between betaine and neutral forms of (**4**) occur within the glicydyl group as a result of the proton migration mentioned above.

4.2. NMR studies

Experimental and calculated chemical shifts for all compounds under investigations are presented in Table 2. For (**1**) measurements have been carried out in $CDCl_3$ solution while for (**2**) and (**4**) in D_2O. The agreement between calculated and experimental proton and carbon chemical shifts is quite satisfactory, somewhat greater differences appear for carbon atoms of aromatic ring. An increase of the C6 chemical shift by about 6 ppm in comparison with methyl α-D-glucopyranosid indicates that the phosphoric group is attached to C6 atom.

The analysis of the fine structure of the spectra for (**1**) has given the coupling constants: $^2J_{P,C(ipso)} = 7.02$ Hz, $^2J_{P,C6} = 5.85$ Hz, $^3J_{P,C5} = 6.04$ Hz and $^3J_{H1,H2} = 3.30$ Hz.

For (**2**) we succeeded in obtaining following coupling constants: $^3J_{H4,H5} = 9.77$ Hz, $^3J_{H2,H3} = 9.53$ Hz, $^3J_{H3,H4} = 9.28$ Hz, $^3J_{H1,H2} = 3.67$ Hz, $^2J_{P,C6} = 5.51$ Hz, $^3J_{P,C5} = 7.55$ Hz. In the case of (**4**) the $^3J_{H1,H2}$ value has been determined as 3.6 Hz. This value indicates that the configuration of saccharides studied is the α one.

For (**4**) we observe an increase in the chemical shift for C3 to 77.18 ppm in comparison with 73.9 ppm for (**1**). For H3 the chemical shift changes from 3.72 ppm for (**1**) to 5.22 ppm for (**4**). For neighbouring C2 and C4 atoms the chemical shift decreases, whereas for the next ones these values are greater than in the case of (**1**). These facts indicate that the glicydyl group is substituted to the C3 carbon atom. From calculation for (**4**) significant difference of chemical shifts for C3 (about 10 ppm) between isomers in neutral and zwitterionic forms is clearly seen and the theoretically predicted values for neutral form are in accordance with experimental ones. Similar situation concerns chemical shifts for C4 although in this case the difference is smaller; zwitterionic forms exhibit greater chemical shifts by about 5 ppm in comparison with neutral ones. Practically the same can be said with regard to the chemical shift for C20. In this case, however, as opposed to C4 and C3, the chemical shifts are greater for neutral forms by about 6–7 ppm and are also in agreement with experiment.

In the proton NMR spectra for (**4**) the signal at 3.41 ppm was observed which has been assigned to the PO/H acid proton. The signal originating from the NHH′ group in the vicinity of 0.4 ppm has not been observed, probably it is vanishing among the noise signal. Simultaneously, the signal in the vicinity of 7 ppm which could originate, according to the calculations, from the betaine form (NH_3^+) has not been registered either. Indeed, in the neighbourhood of 7 ppm have been observed multiplets but their integral analysis did not confirm the presence of

Table 1
Selected DFT optimized structure parameters of methyl (α-D-glucopyranosid-1-yl)-6-*O*-diphenylophosphate (**1**), methyl (α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**2**) and methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**4**)

	(**1**)	(**2**)		(**4**)				α-D-glucose
		Isomer **I**	Isomer **II**	Isomer **I**	Isomer **II**	Isomer **I** ion	Isomer **II** ion	Exp. [15]
Bond length (Å)								
C1–C2	1.533	1.533	1.533	1.539	1.540	1.540	1.540	1.539
C1–O1	1.396	1.396	1.396	1.405	1.404	1.410	1.409	1.400
C2–C3	1.528	1.527	1.526	1.534	1.534	1.527	1.529	1.529
C2–O2	1.412	1.412	1.413	1.409	1.409	1.412	1.412	1.421
C3–C4	1.530	1.530	1.530	1.525	1.528	1.523	1.522	1.523
C3–O3	1.422	1.422	1.423	1.447	1.447	1.468	1.467	1.425
C4–C5	1.539	1.539	1.539	1.637	1.536	1.535	1.534	1.534
C4–O4	1.431	1.431	1.430	1.419	1.422	1.440	1.442	1.430
C5–O5	1.430	1.429	1.430	1.431	1.421	1.433	1.432	1.431
O5–C1	1.428	1.427	1.428	1.416	1.417	1.410	1.411	1.430
C5–C6	1.516	1.516	1.518	1.516	1.517	1.545	1.545	1.517
C6–O6	1.446	1.446	1.449	1.445	1.447	1.434	1.437	1.424
O6–P	1.598	1.598	1.599	1.595	1.600	1.661	1.673	
O3–C20	–	–	–	1.359	1.359	1.348	1.347	
C20–C21	–	–	–	1.518	1.518	1.536	1.535	
C20–O20	–	–	–	1.209	1.210	1.204	1.205	
C21–N	–	–	–	1.454	1.400	1.504	1.507	
O···H	–	–	–	0.972	0.971	1.706	1.532	
H···N	–	–	–	11.723	12.217	1.073	1.103	
Angles (deg)								
O5–C1–O1	112.5	112.5	112.5	113.1	113.1	113.4	113.4	111.5
O5–C1–C2	108.9	108.9	109.0	111.2	111.1	112.6	112.5	110.1
O1–C1–C2	109.3	109.3	109.3	103.6	106.5	105.8	105.9	109.3
C1–C2–C3	109.7	109.6	109.6	108.5	108.6	109.2	109.4	111.1
C1–C2–O2	109.9	110.0	110.0	112.1	112.1	110.9	110.9	110.9
O2–C2–C3	112.0	112.0	112.0	113.3	113.2	112.9	112.9	112.2
C2–C3–O3	107.4	107.3	107.0	110.2	110.2	108.6	108.4	108.1
O3–C3–C4	111.5	111.4	111.4	107.2	107.3	102.9	103.2	110.6
C2–C3–C4	111.0	111.0	110.7	111.0	111.0	112.2	112.2	109.8
C3–C4–C5	109.9	110.0	109.7	108.3	108.3	117.8	117.0	111.1
C3–C4–O4	110.5	110.5	110.7	111.8	111.7	107.0	107.8	108.2
O4–C4–C5	113.2	112.9	112.9	108.2	108.2	111.3	110.1	110.9
C4–C5–O5	109.7	109.9	110.2	109.6	109.6	112.1	112.0	108.7
C5–O5–C1	114.1	114.2	114.1	114.6	114.5	115.8	115.5	113.7
C4–C5–C6	112.2	112.0	112.4	112.2	112.3	107.8	108.1	111.5
O5–C5–C6	107.6	107.4	107.6	107.2	107.5	105.4	105.5	108.1
C5–C6–O6	108.2	107.9	108.6	107.4	108.6	109.4	109.3	109.9
C6–O6–P	122.0	122.3	123.0	122.5	121.8	123.9	120.1	
C6–O6–C20	–	–	–	117.2	117.2	118.3	118.1	
O6–C20–C21	–	–	–	110.6	110.6	109.6	109.7	
O6–C20–O20	–	–	–	124.7	124.7	126.6	126.6	
O20–C20–C21	–	–	–	124.5	124.6	123.7	123.6	
C20–C21–N	–	–	–	110.0	110.0	108.0	107.3	
Dihedral angles (deg)								
P–O6–C6–C5	162.0	−180.0	138.2	−178.1	137.2	105.7	116.5	
O6–C6–C5–O5	72.0	73.6	78.7	70.3	72.0	−168.0	−163.5	70.2
O6–C6–C5–C4	−167.2	−165.5	−159.8	−169.2	−167.4	−48.0	−43.9	
C5–O5–C1–O1	58.4	58.5	59.2	60.3	60.2	59.9	59.7	
C5–O5–C1–C2	−62.9	−62.9	−62.9	−59.4	−59.6	−60.3	−60.4	−60.9
C1–O5–C5–C6	−175.9	−176.8	−176.2	−177.1	−176.4	164.5	166.7	−176.5
C1–O5–C5–C4	61.8	61.1	60.9	60.9	61.3	47.4	49.3	62.2
O5–C1–C2–O2	−179.3	−178.9	−178.9	−179.7	179.9	−176.8	−177.5	
O1–C1–C2–O2	57.4	57.8	57.8	56.6	56.3	58.8	58.2	56.9
O5–C1–C2–C3	57.1	57.5	57.5	54.3	54.1	58.1	57.3	54.1
O1–C1–C2–C3	−66.2	−65.7	−65.8	−69.4	−69.5	−66.3	−67.0	−68.7
C1–C2–C3–O3	−176.0	−176.2	−176.7	−173.3	−173.3	−159.6	−159.8	
O2–C2–C3–O3	61.6	61.5	61.0	61.4	61.5	76.5	76.1	63.2
C1–C2–C3–C4	−53.9	−54.2	−54.8	−54.6	−54.5	−46.4	−46.5	−51.3
O2–C2–C3–C4	−176.3	−176.6	−177.2	−179.9	−179.9	−170.4	−170.6	

Table 1 (*continued*)

	(**1**)	(**2**)		(**4**)				α-D-glucose
		Isomer **I**	Isomer **II**	Isomer **I**	Isomer **II**	Isomer **I** ion	Isomer **II** ion	Exp. [15]
C2–C3–C4–O4	178.1	177.6	178.2	175.7	175.7	164.6	164.6	
O3–C3–C4–O4	−62.2	−62.8	−62.5	−63.8	−63.8	−78.7	−78.8	−65.5
C2–C3–C4–C5	52.5	52.2	53.0	56.6	56.7	38.4	39.9	53.3
O3–C3–C4–C5	172.2	171.7	172.3	177.1	177.2	155.0	156.4	
C3–C4–C5–C6	−174.1	−173.1	−174.4	−176.5	−177.2	−152.3	−155.1	
O4–C4–C5–C6	61.8	62.8	61.7	62.2	61.5	83.5	81.3	62.9
C3–C4–C5–O5	−54.6	−53.7	−54.3	−57.4	−57.8	−36.7	−39.2	−57.5
O4–C4–C5–O5	−178.7	−177.7	−178.2	−178.7	−179.1	−160.8	−162.8	
C2–C3–O3–C20	–	–	–	−94.1	−95.2	−153.2	−153.1	
C4–C3–O3–C20	–	–	–	144.9	143.8	87.6	87.8	
C3–O3–C20–O20	–	–	–	−3.5	−3.4	43.8	42.5	
C3–O3–C20–C21	–	–	–	179.9	179.9	−133.3	−133.2	
O3–C20–C21–N	–	–	–	−163.2	−163.8	67.4	73.6	
O20–C20–C21–N	–	–	–	20.2	19.5	−109.9	−102.2	

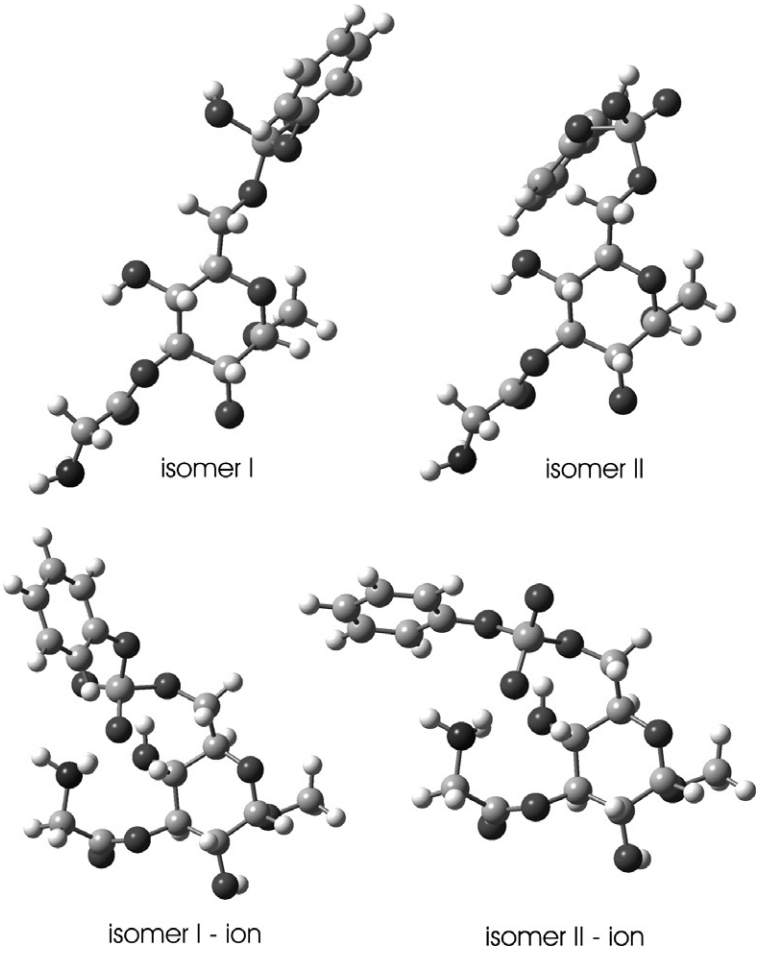

Fig. 4. Optimized structures of methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**4**) isomers.

additional three protons from NH_3^+ group. It allowed us to assign the analyzed signals to the protons of a phenyl group that is in accordance with the calculated values for these protons. The facts mentioned above show that the compound (**4**) occurs in the neutral form.

For all compounds studied the ^{31}P NMR measurements have been performed. For (**1**) only one signal at −10.33 ppm has been registered that agrees sufficiently well with the calculated value (−12.03 ppm). For (**2**) three signals have been observed, namely −1.97, −3.25 and

Table 2
Comparison of experimental and theoretical chemical shifts [ppm] of methyl (α-D-glucopyranosid-1-yl)-6-*O*-diphenylophosphate (**1**), methyl (α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**2**) and methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**4**)

	(**1**)		(**2**)			(**4**)				
	Exp.	Calculated	Exp.	Calculated		Exp.	Calculated			
				Isomer **I**	Isomer **II**		Isomer **I**	Isomer **II**	Isomer **I** ion	Isomer **II** ion
^{13}C										
C1	95.51	98.02	102.13	96.75	97.93	99.16	98.58	98.34	98.96	98.94
C2	71.88	72.42	73.97	72.11	72.51	69.15	71.63	71.50	69.49	69.42
C3	73.92	74.11	75.79	74.68	74.65	77.18	76.13	75.78	85.98	85.39
C4	69.63	77.16	72.03	69.92	71.83	67.03	68.68	68.55	73.42	72.99
C5	70.29	69.90	73.36	71.13	72.11	70.60	70.98	71.54	68.49	68.02
C6	68.22	69.31	67.72	67.67	67.95	64.49	67.98	67.34	67.73	65.03
C20						167.98	165.21	165.34	158.31	159.61
C21						40.11	44.08	44.04	44.61	44.37
C30	55.27	55.20	57.94	53.15	53.70	55.06	53.57	53.27	53.94	53.90
C(ipso)	150.45	145.25	154.58	145.10	144.39	151.79	144.26	143.92	146.37	147.50
C(orto)	120.12	114.95	123.08	113.81	114.15	120.35	114.35	115.78	114.77	112.02
C(meta)	129.84	122.55	132.63	122.40	122.61	129.80	122.58	120.62	121.74	123.29
C(para)	125.52	118.02	127.21	116.91	117.88	124.38	117.84	118.26	115.8	114.96
^{1}H										
H1	4.67	4.74	4.77	4.33	4.68	4.97	4.66	4.54	4.68	4.65
H2	3.37	3.21	3.53	3.14	3.60	3.67	3.27	3.20	3.65	3.59
H3	3.72	3.64	3.66	3.41	3.37	5.22	5.13	5.11	4.98	4.97
H4	3.40	3.21	3.47	3.28	2.87	3.40	3.38	3.35	5.37	5.12
H5	4.46	4.88	3.72–3.80	3.80	3.30	3.75	4.13	3.91	3.79	3.80
H66′	3.34	4.22	4.12–4.30	4.19	4.21	4.02	4.30	4.33	4.16	4.07
O2–H		3.57		0.87	1.43		0.87	0.80	1.04	1.01
O3–H	2.05	1.81		1.77	1.77		0.99	1.18	3.53	4.03
O4–H	1.26	1.62		1.31	−0.60					
C21/HH′						3.86	3.41	3.44	3.16	2.97
N/HH′(PO/H)				(3.21)	(3.11)	(3.41)	0.38(2.91)	0.37(3.32)		
N/H′H″H‴							–	–	7.21	7.17
C30/HH′H″	3.35	3.80	3.40	3.10	3.37	3.43	3.54	3.20	3.45	3.43
C(orto)/H	7.14–7.22	7.13	7.22–7.24	7.34	7.29	7.22	7.25	7.41	7.23	7.09
C(meta)/H	7.27–7.33	7.21	7.39–7.44	7.16	7.20	7.42	7.20	7.20	7.06	7.13
C(para)/H	7.14–7.22	7.06	7.22–7.24	6.95	7.02	7.22	7.04	7.03	6.48	6.83
^{31}P										
P	−10.33	−12.03	−1.97(2.2)	−4.55		−3.97(5.74)		−2.60	−9.32	−3.46
			−3.25(96.2)		−4.93	−4.37(1)	−5.90			
			−10.17(1.6)							

For ^{31}P data the integrated intensities are given in parentheses.

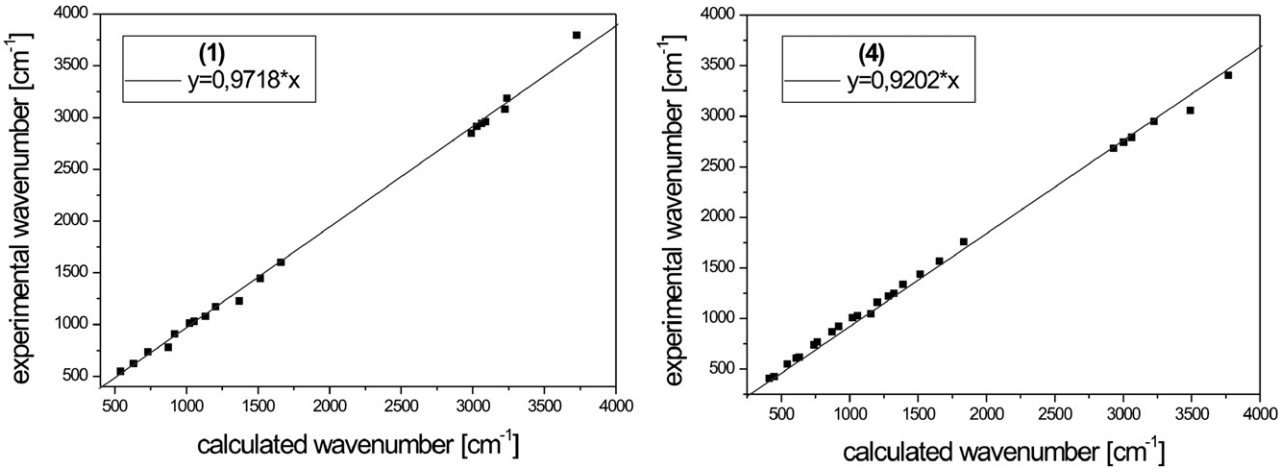

Fig. 5. The correlation between calculated and experimental frequencies for methyl (α-D-glucopyranosid-1-yl)-6-*O*-diphenylophosphate (**1**) and methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**4**).

−10.17 ppm. The last signal has been assigned to (**1**) as to an intermediate product in the synthesis and the small amount of this product can be caused by the incomplete reaction of the sample; the difference between −10.33 and −10.17 ppm chemical shifts can arise, probably, from the change of the solvent during the measurements in (**1**) and (**2**). The two remaining signals have been assigned to the two possible isomers, to the isomer **I** the signal −1.97 ppm and to the isomer **II** the signal −4.93 ppm. Analyzing the integral intensities of these signals, the ratio of both isomers has been estimated as 2:98 (isomer **I**: isomer **II**). In the case of (**4**) we are dealing with the similar situations. The −3.97 ppm signal has been assigned to the neutral form of the isomer **II**, whereas the −4.37 ppm signal to the neutral form of the isomer **I**. The estimated ratio, isomer **I**: isomer **II**, gives this time 15:85. We see, therefore, that in the mixture the isomer **II** predominates.

4.3. Raman studies

Raman measurements were made for (**1**) and (**4**); for (**2**), because of the very strong fluorescence, the spectra have been omitted in the further analysis.

Generally, the differences between calculated vibrational frequencies, both for neutral and zwitterionic isomers, are very small, except for the range below 2800 cm^{-1} where in the case of zwitterionic forms the vibrations arising from the NH_3^+ group appear. Raman spectroscopy is not sensitive enough to the changes connected with the space distri-

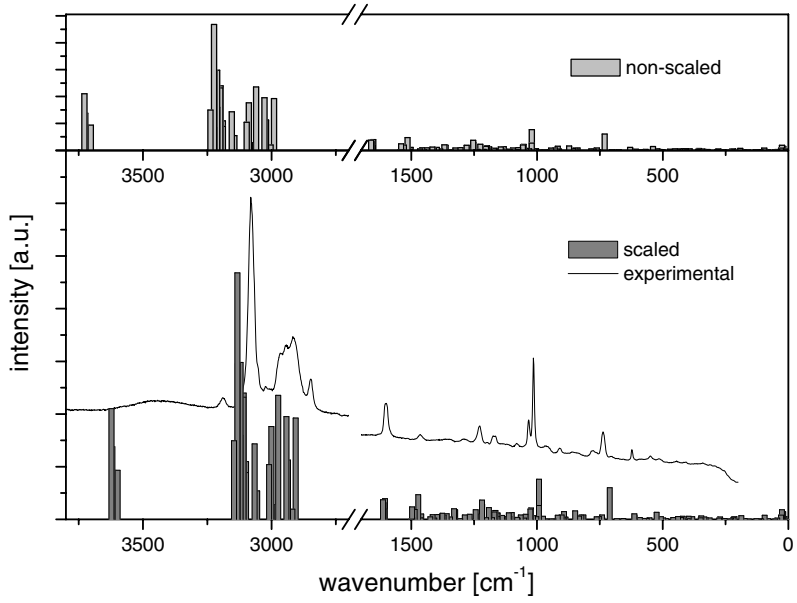

Fig. 6. Comparison of the calculated non-scaled and scaled Raman spectrum of methyl (α-D-glucopyranosid-1-yl)-6-*O*-diphenylophosphate (**1**) with the experiment.

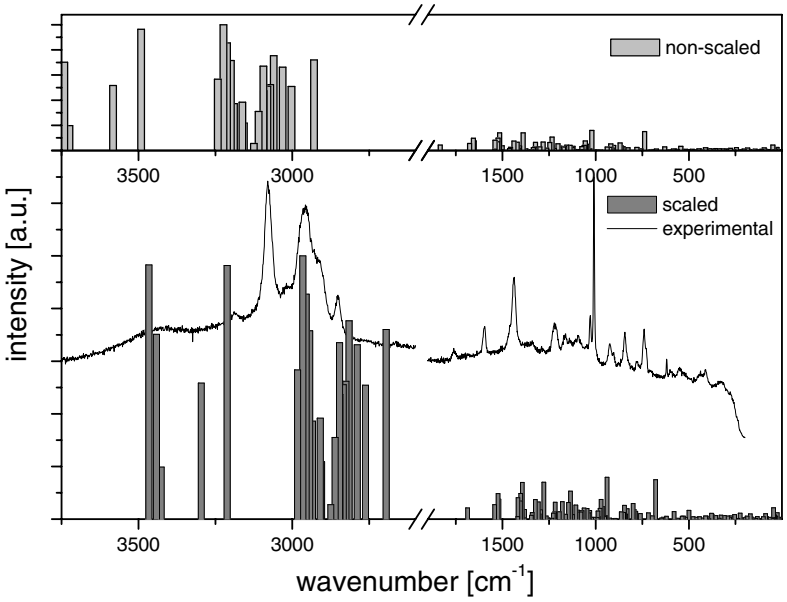

Fig. 7. Comparison of the calculated non-scaled and scaled Raman spectrum of methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylophosphate (**4**) with the experiment.

bution of the substituents at the phosphorus atom and it do not allow to distinguish which isomer has been obtained. Therefore, for (**4**) we have used for the comparison with experimental spectra the theoretically predicted results for the neutral isomer **II**, i.e., for that isomer which, according to the NMR analysis, predominates.

To calculated Raman spectra the scaling procedure has been applied. The common scaling factor $f_{(1)} = 0.97$ for (**1**) and $f_{(4)} = 0.92$ for (**4**) for all frequencies studied was taken from the calibration of the corresponding theoretical DFT results with the most intense experimental bands from the range 3500–500 cm^{-1}. Vibrational assignments were made using the visualization of vibrations by Molekel program and basing on the assignment for D-glucose-6-phosphate [17]. In Fig. 5, as an example, the correlation between calculated and experimental frequencies for (**1**) and (**4**) is presented.

The comparison of the theoretical scaled Raman spectra with the experimental spectra of compounds (**1**) and (**4**) are shown in Figs. 6 and 7, respectively. In the case of both spectra the additional theoretical non-scaled spectra have been inserted. Although the very simple scaling procedure with the only one scaling factor has been applied, the satisfactory agreement between theory and experiment is observed.

We would like to focus here on the most characteristic features of the investigated spectra only. In the phosphorylated saccharides one observes pronounced band at 1200 cm^{-1} originating from the stretching P=O mode [17,18]. For (**1**) this mode correspond to 1229 cm^{-1} and for (**4**) to 1250 cm^{-1}. For (**4**) in opposition to (**1**), without the glicydyl group, the band at 1761 cm^{-1} is observed. The presence of this band can suggest the existence both of C=O and NH_2 groups. It follows from the performed calculations that the band corresponding to NH_3^+ stretching vibrations should appear at 2715 and 2309 cm^{-1} for betaine forms of isomers **I** and **II**, respectively. This band has not been found on the registered spectrum that confirms conclusions drawn from NMR studies about the existence of the compounds considered in the neutral form.

5. Concluding remarks

Methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylphosphate has been obtained using the new highly efficient method of hydrogenolysis with ammonium formate as a hydrogen source and palladium on carbon as a catalyst. This compound and two intermediate products of synthesis have been studied by theoretical DFT method as well as by NMR and Raman spectroscopy. Two types of the optimized stereoisomers can be distinguished according to the space distribution of the substituents at the phosphorus atom. The presence of the glycine group in methyl (3-*O*-glicynyl-α-D-glucopyranosid-1-yl)-6-*O*-phenylphosphate has caused the necessity to take into account the studied molecule in the form of a zwitterion. DFT calculations, using the B3LYP exchange-correlation functional and the 6-31G* basing set, have shown to be a powerful help in the interpretation of NMR and Raman experiments. The comparison of predicted chemical shifts for ^{1}H, ^{13}C and ^{31}P NMR with experiment has permitted to find that the phosphoric and glicydyl groups are attached to C6 and C3 carbon atoms, respectively. The agreement between calculated vibrational frequencies scaled by one factor only and experimental ones is satisfactory. The calculations have also shown that from among two possible stereoisomers one predominates and that the examined compounds occur in the neutral form. This last conclusion is certainly important in searching of common epitopes suitable for

construction of antimicrobial vaccine with desired broad specificity.

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