Struct Chem (2006) 17:337–346
DOI 10.1007/s11224-006-9004-6

ORIGINAL PAPER

Pivaloylated glucoconjugates with heterocyclic oximes

Renata Odžak · Srđanka Tomić · Helena Čičak · Zlatko Mihalić · Biserka Prugovečki · Dubravka Matković-Čalogović

Received: 3 October 2005 / Accepted: 7 February 2006 / Published online: 1 August 2006

Abstract Condensation of tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (**1**) with three heterocyclic oximes: 3-hydroxyiminoquinuclidine (**2**), 4-hydroxyiminomethylpyridine (**3**) and *N*-methyl-2-hydroxyiminomethylimidazole (**4**) leads to the β-*N*-glucoconjugates. Conjugates **6** and **7** were synthesized using aromatic compounds **3** and **4** as the starting material. They were obtained in two isomeric forms (*E* and *Z*) due to the restricted rotation around the oxime's double bond. The presence of *E* and *Z* isomers was proved by comparison of NMR spectra with calculated GIAO/DFT NMR spectra on B3LYP/6-31G(d) level of theory and by X-ray structural analysis of starting oxime reagents. Isomery was not observed in the quinuclidinium glucoconjugate **5**.

Keywords Conjugation · Heterocycles · *N*-glucoconjugates · X-ray structure · DFT calculations

Introduction

The pivaloyl group has been used as an useful protecting group in the synthesis of acylated monosaccharides [1–4] and disaccharides [1, 2]. It can be introduced selectively to produce various partially pivaloylated saccharides, its position in a molecule is easily detectable by ^{1}H NMR, and it can be removed totally or selectively by esterases from mammalian sera [2, 4–6]. Furthermore, the pivaloyl group has been used in carbohydrate chemistry because of its advantageous stereodirecting properties in glycosylation reactions. Thus, only β anomers were obtained in reactions of tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide with simple alcohols such as benzyl alcohol or more complicated structures such as cholesterol [7]. We now report on the synthesis of β-*N*-glucoconjugates of pivaloylated glucose with some heterocyclic oximes. The heterocyclic part of these conjugates were quinuclidinium, pyridinium, or imidazolium oxime, basic structures of many compounds tested in vitro and in vivo as antidotes in poisoning by organophosphorus (OP) compounds such as pesticides and nerve warfare agents [8–10]. The OP compounds inhibit the enzyme acetylcholinesterase (AChE), essentially irreversibly. This causes accumulation of acetylcholine (ACh) which is responsible for many toxic effects. An oxime is considered as an efficient antidote if it has a high reactivation rate constant and if it persists in blood circulation for a long enough period of time. Its toxicity should be low as well. The glucoconjugates on which we report in this work were designed as possible model compounds satisfying these requirements. It was previously found that attachment of a sugar moiety to the oxime derivatives increases the bioavailability of the antidote. Thus, we presume that the sugar moiety might be recognized by sites responsible for sugar transport through the cell membrane leading to the increased permeability of membranes for the quaternary oximes. Furthermore, the previously reported glucoconjugates were less toxic than non-sugar conjugates, and some of them also displayed higher efficacy [11].

R. Odžak · S. Tomić (✉) · H. Čičak · Z. Mihalić
Laboratory of Organic Chemistry, Department of Chemistry,
Faculty of Science, University of Zagreb,
Horvatovac 102a,
HR-10000 Zagreb, Croatia
e-mail: stomic@chem.pmf.hr

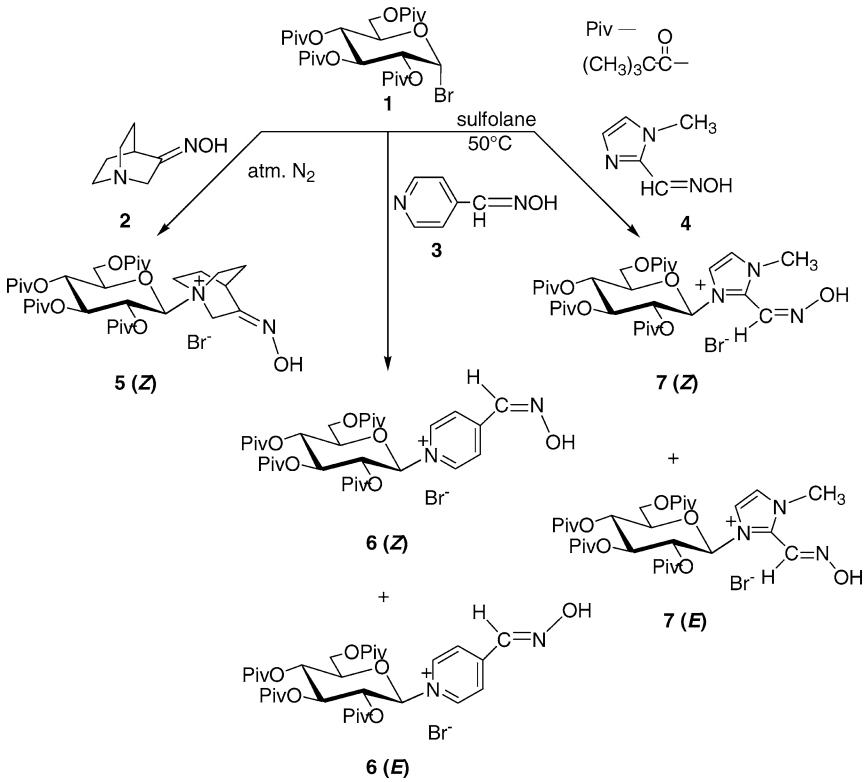
B. Prugovečki · D. Matković-Čalogović
Laboratory of General and Inorganic Chemistry,
Department of Chemistry, Faculty of Science,
University of Zagreb,
Horvatovac 102a,
HR-10000 Zagreb, Croatia

Results and discussion

Synthesis

Glucoconjugates (**5**–**7**) with *N*-glycosyl bond were synthesized from tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (**1**) with three different heterocyclic oximes: 3-hydroxyiminoquinuclidine (**2**), 4-hydroxyiminomethylpyridine (**3**) and *N*-methyl-2-hydroxyiminomethylimidazole (**4**). Pivaloylation of β-D-glucopyranose in pyridine with a 10-fold molar excess of pivaloyl chloride produced penta-*O*-pivaloyl-β-D-glucopyranose [7] which was, after isolation and purification, treated with HBr (33% in glacial acetic acid) to give the starting tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (**1**) [2, 7]. Heterocyclic oxime **3** is commercially available while oximes **2** and **4** were prepared following the previously reported procedure [12–14]. Condensation of equimolar quantities of monosaccharide **1** with heterocyclic oximes **2**–**4** were achieved in sulfolane (tetrahydrotiophene-1,1-dioxide), an aprotic solvent with a high dielectric constant in which both components, the apolar bromide and the polar oxime could be dissolved [11]. All synthesized glucoconjugates were identified by MS, IR, 1D (^{1}H and ^{13}C (APT)) and 2D (HETCOR) NMR spectroscopies, and their purity determined by elemental analyses and optical rotation measurements. The β-configuration of all three conjugates was determined on the basis of ^{1}H NMR spectra in which the anomeric proton was found to be a doublet with a characteristical *J* value of approximately 9 Hz. Nevertheless, the spectra of conjugates **6** and **7** showed presence of two compounds since two sets of signals in ^{1}H and ^{13}C NMR spectra with very similar chemical shifts were observed. Spectra also indicated that two compounds formed in a ratio of 1:3. We presumed that two sets of NMR signals were due to the formation of two isomers, *E* and *Z*, caused by the restricted rotation around the oxime double bond. *E* and *Z* isomers were not observed in the case of the quinuclidinium conjugate **5**. Presence of two isomeric forms was previously reported for the pyridinium oxime **3** which is the starting compound in the synthesis of conjugate **6**. The change of solvent system during the crystallization process led to the separation of *E* and *Z* isomers of oxime **3** which were identified by X-ray structural analysis [15, 16]. However, the X-ray structure showed that only the *E* isomer of the heterocyclic oxime **4** could be isolated in the form adequate for X-ray analysis. On the contrary, X-ray determination of the structure of oxime **2** showed that *Z* isomer was the one which was crystalized. The newly synthesized *N*-glucoconjugates **5**–**7** could not be obtained in a crystaline form suitable for X-ray analysis. We thus attempted to determine the structure of the quinuclidinum conjugate **5** and of the major isomers of the pyridinium and imidazolium compounds **6** and **7** by a different approach. For this purpose, we calculated ^{13}C NMR chemical shifts for both isomers of compounds **5**–**7** by using their model structures optimized on the high level of theory.

Scheme 1



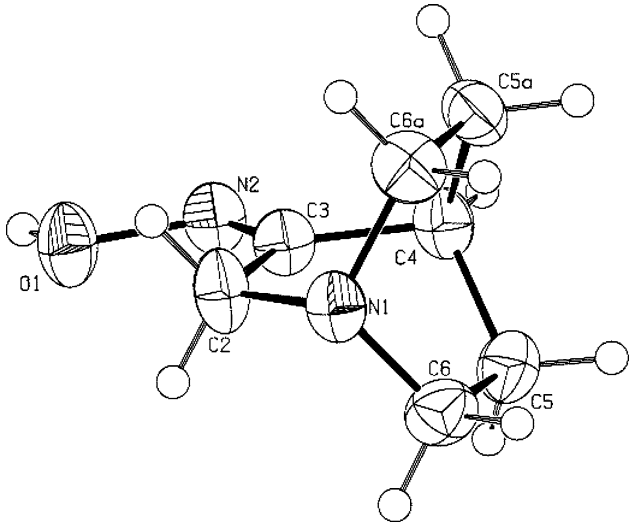


Fig. 1 An ORTEP drawing of **2** with the atom labeling scheme. Ellipsoids are drawn at the 50% probability level

Table 1 Interatomic distances (Å) and angles (°) for **2**

Interatomic distances (Å)	
O1–N2	1.409(3)
N1–C2	1.478(3)
N1–C6 (C6^a)	1.474(2)
N2–C3	1.282(3)
C2–C3	1.504(3)
C3–C4	1.497(3)
C4–C5 (C5^a)	1.527(2)
C5–C6	1.545(2)
Interatomic angles (°)	
C6–N1–C6^a	108.6(2)
C2–N1–C6	108.76(12)
O1–N2–C3	109.15(19)
N1–C2–C3	110.24(19)
N2–C3–C2	126.3(2)
C2–C3–C4	112.44(19)
N2–C3–C4	121.3(2)
C3–C4–C5	107.48(13)
C5–C4–C5^a	108.5(2)
C4–C5–C6	108.66(15)
N1–C6–C5	111.97(15)

^{a}Symmetry code: x, 1/2 − y, z.

Crystal structures of 2 and 4

Single crystal X-ray analysis revealed that the structure of **2** consists of molecules which possess the mirror symmetry (Fig. 1, Table 1). They are connected into endless chains by hydrogen bonds O1–H···N1($x-0.5, y, 1.5-z$) of 2.765(3) Å (Fig. 2, Table 2). The quinuclidinium cage consists of three 6-membered rings which all approach the ideal boat conformation. It was found that the molecule is a *Z* isomer. The same isomer was also found in 3-methoxyiminoquinuclidinium chloride, the only structure solved till now of an oxime-substituted quinuclidine [17].

The crystal structure of **4** consists of two independent molecules in the asymmetric unit (Fig. 3). The molecules are connected into chains by hydrogen bonds O11 − H···N21 ($0.5+x$, $y-0.5$, z) and O21–H···N11 of 2.718(2) and 2.723(3) Å, respectively (Fig. 4, Table 3).

The bond distances (Table 4) in both molecules are comparable, and both are *E* isomers. The planarity of the molecules can be indicated by torsion angles N12–C11–C15–N13 and N22–C21–C25–N23 of −3.4(3)° and −0.9(3)°, respectively. The C11–N11 and C21–N21 bonds (1.323(2) and 1.326(2) Å) are significantly shorter than bonds C11–N12 and C21–N22 (1.357(2) and 1.353(2) Å), indicating localization of the double bond. Similar values were found in 2-hydroxyiminomethyl-1-benzylimidazole (1.322(3) and 1.357(3) Å) which is also an *E* isomer [18]. When a methyl substituent was introduced at the other imidazole nitrogen atom the two C–N bond lengths became similar since the double bond and the charge are both

Fig. 2 Hydrogen bonding in **2**

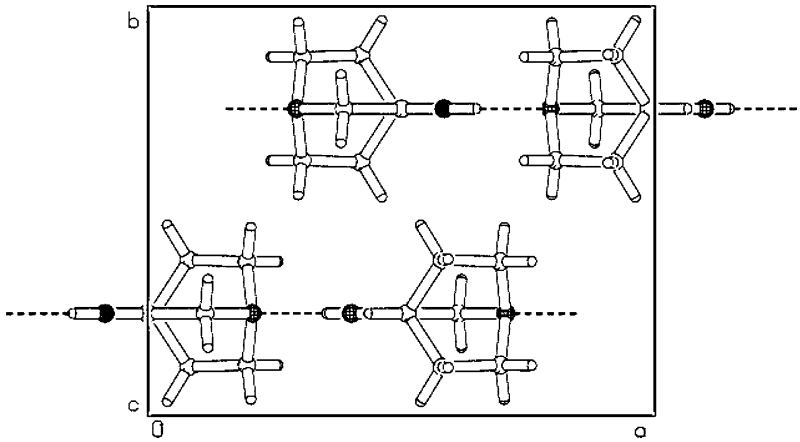


Table 2 Hydrogen bonding in **2**

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
O1–H1···N1[a]	0.82	1.95	2.765(3)	179
O1–H1···N1[b]	0.82	1.95	2.765(3)	179

[a]Symmetry code: $-1/2+x, 1/2-y, 3/2-z$.

[b]Symmetry code: $-1/2+x, y, 3/2-z$.

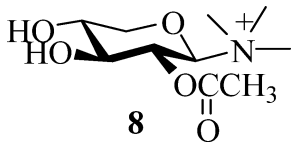
delocalized. Interestingly, after substitution, rotation about the C21–C25 bond occurred (using the atom numbering scheme of the present structure) orienting the oxime nitrogen toward the methyl-substituted nitrogen. The isomer remained *E* [18].

The OP antidotes possess a carbonyl-oxygen, or some related oxygen-containing functional group, which may be important in binding to the enzyme. For the choline and acetylcholine structures the average separation between the quaternary-nitrogen and carbonyl-oxygen is 4.7(3) Å with the range from 4.35 to 5.11 Å [19]. The oxime-oxygen to nitrogen distances have also been investigated [18]. In **2** the O1 to N1 distance is 4.088 (3) Å. In **4** the distance between the oxime-oxygen O11 to the imidazole nitrogen atoms N11 and N12 are 4.584(3) and 4.339(2) Å, while those between O21 to N21 and N22 amount to 4.605(3) and 4.332(2) Å, respectively.

Computational details

Molecular structures

Quality of calculated nuclear magnetic shielding results depend on the quality of the structural data. Since experimental structures are seldom available for the molecules of interest, energy optimized geometries are frequently used [20]. We have chosen model structure **8** for representing the examined *N*-glucoconjugates **5–7** in order to see the difference in shielding between experimentally observed oxime *E* and *Z* isomers of the β anomers since the experiment showed lack of α anomers:



8

Different conformations were examined and fully optimized using the Gaussian 03 program package [21, 22] at the B3LYP/6-31G(d) level of theory [23–26]. Vibrational frequencies calculated with the same model chemistry were used to confirm that the obtained stationary points were minima. The most stable molecular structures and relative energies according to the more stable isomer of each pair are presented in Fig. 5.

Nuclear magnetic shielding calculations

The ^{13}C NMR chemical shifts were calculated for B3LYP/6-31G(d) optimized geometries by the GIAO method (*gauge including atomic orbitals*) [27–29] implemented in the Gaussian 03 suite of programs at the same level of theory. These chemical shifts were referenced to as CH_4, calculated with GIAO method at the HF/6-31G(d) level of theory. The procedure of subtracting the calculated magnetic shielding for the nuclei of interest from the reference compound shielding was automatically performed by the GaussView program [30]. Calculated and appropriate experimental ^{13}C NMR chemical shifts are shown in Tables 5–7.

Interpretation of experimental data for *N*-glucoconjugate with quinuclidinium oxime as the heterocyclic part (**5**) is straightforward. In its NMR spectra there is no signal splitting, which suggests lack of the oxime isomerisation.

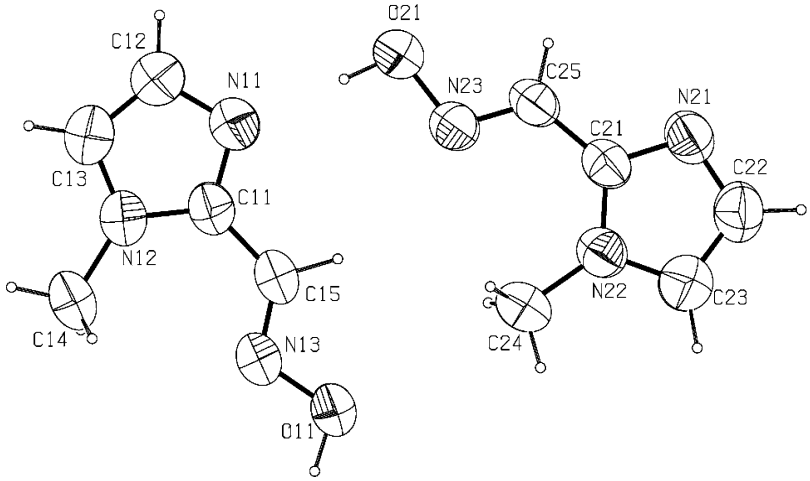


Fig. 3 An ORTEP drawing of **4** with the atom labeling scheme. Ellipsoids are drawn at the 50% probability level

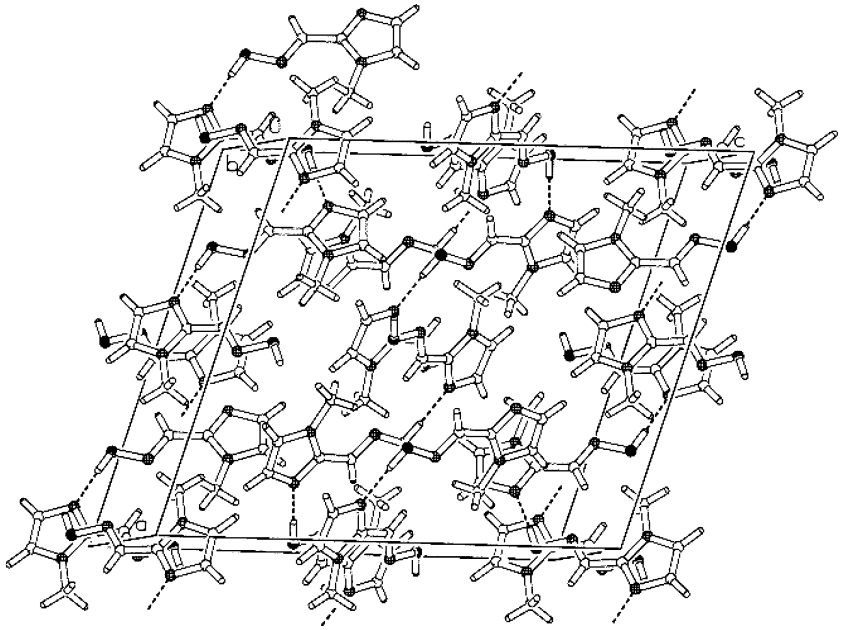


Fig. 4 Hydrogen bonding in **4**

Calculations showed that *Z* isomer is more stable than is *E* isomer (1.8 kcal/mol). The tendency of quinuclidinium oximes to form *Z* isomers was also noticed during the crystallization process of the starting oxime since only *Z* isomer could be obtained under different crystallization conditions and its structure unequivocally determined by X-ray crystallography. Furthermore, calculations showed that *Z* isomer as compared to *E* has slightly better correlation with experimental ^{13}C NMR chemical shifts: $R^2 = 0.987$, $F = 695$ (Fig. 6) vs. $R^2 = 0.986$, $F = 631$. In all the correlations C–5, C=O, and CH_3 shifts are omitted because of significant difference in chemical surroundings from the real structures.

Other two *N*-glucoconjugates (**6** and **7**) have more complex experimental NMR spectra because all of the signals are splitted. Table 6 shows the calculated ^{13}C NMR chemical shifts of the most stable *E* and *Z* isomers, and appropriate experimental ^{13}C NMR values of the *N*-glucoconjugate **6** with pyridinium oxime as heterocyclic part.

Although calculated *E* and *Z* isomer show different ^{13}C NMR chemical shifts, it is not possible to conclude which of the two signals (*exp* or *exp′*) belong to which isomer, because all the possible correlations are good ($R^2 > 0.97$). The best correlation is that of *Z* isomer with exp′ chemical shifts ($R^2 = 0.983$, $F = 451$), so we can suppose that exp′ originate from *Z*, and exp from *E* isomer ($R^2 = 0.973$, $F = 284$).

Table 3 Hydrogen bonding in **4**

D–H···A	*d*(D–H)	*d*(H···A)	*d*(D···A)	<(DHA)
O11–H11···N21[a]	0.97(3)	1.75(3)	2.718(2)	177(2)
O21–H22···N11	0.92(3)	1.81(3)	2.723(3)	177(3)

[a]Symmetry code: $1/2 + x, -1/2 + y, z$.

Calculations showed that *E* isomer is more stable than is *Z* isomer (3.2 kcal/mol).

Table 7 shows the calculated ^{13}C NMR chemical shifts of the most stable *E* and *Z* isomers and appropriate experimental ^{13}C NMR values of the *N*-glucoconjugate **7** with imidazolium oxime as heterocyclic part.

This case is similar to the previous one; oxime isomerization causes the signal splitting, but it is not possible to connect

Table 4 Interatomic distances (Å) and angles (°) in **4**

	Mol I	Mol II
Interatomic distances (Å)		
O11–N13/O21–N23	1.385(2)	1.394(2)
N11–C11/N21–C21	1.323(2)	1.326(2)
N11–C12/N21–C22	1.365(3)	1.360(3)
N12–C11/N22–C21	1.357(2)	1.353(2)
N12–C13/N22–C23	1.365(3)	1.355(3)
N12–C14/N22–C24	1.461(2)	1.458(2)
N13–C15/N23–C25	1.267(2)	1.267(2)
C11–C15/C21–C25	1.441(3)	1.440(3)
C13–C12/C23–C22	1.345(3)	1.353(3)
Interatomic angles (°)		
O11–N13–C15/O21–N23–C25	111.33(15)	111.31(16)
N11–C11–N12/N21–C21–N22	111.29(17)	110.95(17)
N11–C11–C15/N21–C21–C25	120.82(17)	121.65(17)
N11–C12–C13/N21–C22–C23	110.6(2)	110.1(2)
N12–C11–C15/N22–C21–C25	127.89(17)	127.38(16)
N12–C13–C12/N22–C23–C22	106.4(2)	106.7(2)
N13–C15–C11/N23–C25–C21	123.50(18)	123.32(18)
C11–N11–C12/C21–N21–C22	105.08(18)	105.37(17)
C11–N12–C13/C21–N22–C23	106.62(17)	106.94(17)
C11–N12–C14/C21–N22–C24	127.92(17)	127.95(17)
C13–N12–C14/C23–N22–C24	125.44(18)	125.10(18)

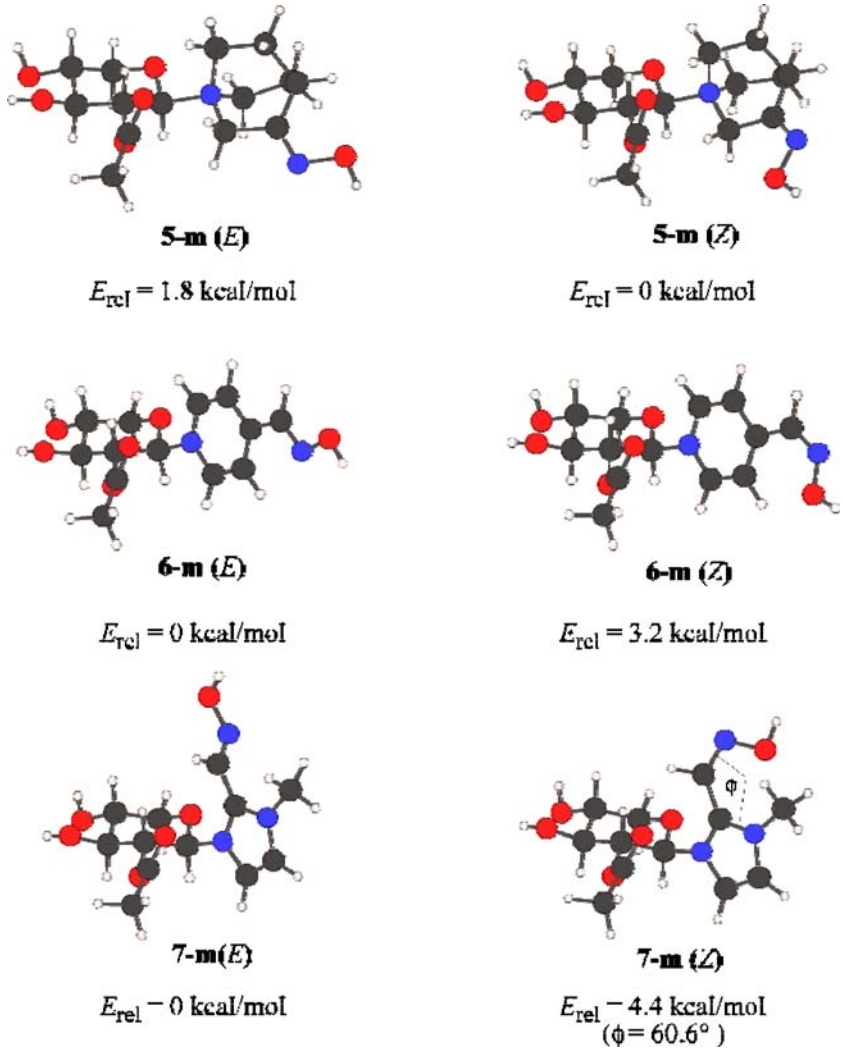


Fig. 5 Optimized model structures of examined *N*-glucoconjugates at the B3LYP/6-31G(d) level of theory (m: model)

calculated and experimental data with certainty because all of the possible correlations are good ($R^2 > 0.97$). Since the best correlation is that of *Z* isomer with exp′ chemical shifts ($R^2 = 0.998$, $F = 661$), we can suppose that exp′ originate from *Z*, and exp from *E* isomer ($R^2 = 0.976$, $F = 279$). Calculations showed that *E* isomer is more stable than *Z* isomer (4.4 kcal/mol).

Therefore, the conclusion can be made that the difference in energies between *E* and *Z* isomers indicates that *Z* isomer formed in the case of the quiniclidinum conjugate **5** while *E* isomers predominate in the case of pyridinium and imidazolium conjugates **6** and **7**.

Conclusion

In this paper, we present the synthesis of *β*-*N*-glucoconjugates with heterocyclic oximes. Some of them formed in two isomeric forms (*E* and *Z*) due to the restricted rotation around the oxime's double bond. This was proved by comparison of NMR spectra with the calculated spectra and by X-ray structural analysis of heterocyclic oxime reagents.

Experimental

General remarks

Column chromatography was performed on Silica Gel (Merck) and TLC on Kieselgel G (Merck) with solvent A, benzene:EtOAc (10:1) or solvent B, $CHCl_3$:MeOH (5:1). The detection was effected by charring with H_2SO_4 or J_2.^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV600 spectrometer at room temperature. Chemical shifts are given in ppm downfield from TMS as internal standard. Optical rotations were measured in MeOH at ∼25°C using the Optical Activity LTD AA-10 Automatic Polarimeter. Melting points were determined in open capillary using a Büchi

Table 5 Calculated and experimental ^{13}C NMR chemical shifts of 5-m (*E* and *Z*)

	E_{calc}	Z_{calc}	Experimental
q C-3	150.86	150.08	148.27
C-1	105.54	105.86	91.96
C-3	83.59	83.59	67.38
C-4	78.77	78.87	66.40
C-2	78.16	79.05	74.37
q C-2	67.76	66.86	60.58
q C-7	65.56	66.26	55.87
q C-6	58.61	60.06	54.05
q C-4	33.59	38.25	26.73
q C-8	34.43	35.27	23.02
q C-5	32.45	34.14	22.97

B-540 melting point apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer FTIR 1725 X spectrometer. Elemental analyses were performed with a Perkin-Elmer PE 2400 Series II CHNS/O Analyser.

Single crystals for X-ray analysis of **2** and **4** were obtained by recrystallization of the compounds from isopropanol-diisopropil ether.

N-(tetra-*O*-pivaloyl-β-D-glucopyranosyl)-3-hydroxyiminoquinuclidinium bromide (**5**)

Tetra-*O*-pivaloyl-α-D-glucopyranosyl bromide (**1**, 250 mg, 0.43 mmol) was added to the solution of 3-hydroxyiminoquinuclidine (**2**, 60 mg, 0.43 mmol) in sulfolane (5 mL). The mixture was stirred under nitrogen, at 50°C for 24 h under nitrogen. The course of the reaction was monitored by TLC (solvent B). The reaction mixture was then chromatographed on a column of silica gel, first with $CHCl_3$ to elute sulfolane (R_F 0.8), followed by solvent B to obtain compound **5** (45 mg, 14%) as yellow crystals: m.p. 124–128°C; $[\alpha]_D^{25} + 14$ (c = 1.0 MeOH); R_F 0.3. IR (KBr):$\tilde{\nu}$ = 3434, 3323, 2918, 1737, 1653, 1479 cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.11 (s, 9H, Piv), 1.15 (s, 9H, Piv), 1.22 (s, 9H, Piv), 1.24 (s, 9H, Piv), 2.18–2.21 (m, 4H, *q* (H-5 and H-8)), 2.99–3.04 (m, 1H, *q* H-4), 3.57–3.84 (m, 2H, H-6), 3.96–4.04 (m, 1H, *q* H-6), 4.12 (dd, *J* = 4.33; *J* = 13.07, 1H, *q* H-2), 4.25–4.31 (m, 2H, *q* (H-6 and H-7)), 4.36–4.38 (m, 1H, *q* H-2), 4.52–4.54 (m, 1H, H-5), 4.69–4.72 (m, 1H, *q* H-7), 5.21 (app t, *J* = 9.96; *J* = 9.63, 1H, H-4), 5.46 (app t, *J* = 8.83; *J* = 8.52, 1H, H-2), 5.56 (app t, *J* = 8.52; *J* = 8.76, 1H, H-3), 6.61 (d, *J* = 9.07, 1H, H-1), 10.71 (s, 1H, NOH) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 22.97 (*q* C-5), 23.02 (*q* C-8), 26.73 (*q* C-4), 26.83 ($(\underline{C}H_3)_3CCO$), 27.02 ($(\underline{C}H_3)_3CCO$), 27.07 ($(\underline{C}H_3)_3CCO$), 27.24 ($(\underline{C}H_3)_3CCO$), 38.63 ($(CH_3)_3\underline{C}CO$), 38.65 ($(CH_3)_3\underline{C}CO$), 38.83 ($(CH_3)_3\underline{C}CO$), 39.12 ($(CH_3)_3\underline{C}CO$), 51.19 (C-6), 54.05 (*q* C-6), 55.87 (*q* C-7), 60.58 (*q* C-2), 66.40 (C-4), 67.38 (C-3), 74.37 (C-2), 74.92 (C-5), 91.96 (C-1), 148.27 (*q* C-3), 176.23 (C=O), 176.30 (C=O), 177.15 (C=O), 177.76 (C=O) ppm. ESMS: *m*/*z* = 639.38 (100%) [M$^+$]. $C_{33}H_{55}BrN_2O_{10}$ (719.70): calculated C 55.07, H 7.70, N 3.89; found C 55.00, H 7.57, N 3.86.

Table 6 Calculated and experimental ^{13}C NMR chemical shifts of 6-m (*E* and *Z*)

	E_{calc}	Z_{calc}	exp	exp′
pyr C-4	156.42	147.11	152.78	151.72
CH=NO	147.30	143.73	144.46	144.27
pyr C-2	145.58	145.40	144.05	143.21
pyr C-6	142.48	144.43	142.27	142.66
pyr C-3	130.39	131.84	141.86	141.34
pyr C-5	124.04	130.69	124.22	124.58
C-1	105.25	105.69	91.30	94.29
C-2	83.83	83.99	72.90	72.78
C-3	81.95	81.96	71.52	71.65
C-4	79.63	79.60	66.52	66.36

Table 7 Calculated and experimental ^{13}C NMR chemical shifts of 7-m (*E* and *Z*)

	E_{calc}	Z_{calc}	exp	exp′
im C-2	143.50	143.01	153.63	139.59
CH=NO	139.77	134.15	131.52	128.34
im C-5	125.31	127.12	124.40	124.10
im C-4	127.49	125.81	119.77	119.27
C-1	100.70	100.48	84.64	84.18
C-2	82.09	80.18	71.92	71.73
C-3	82.29	82.06	70.65	70.17
C-4	79.84	79.88	66.52	66.68
CH_3N	50.04	46.05	38.07	36.99

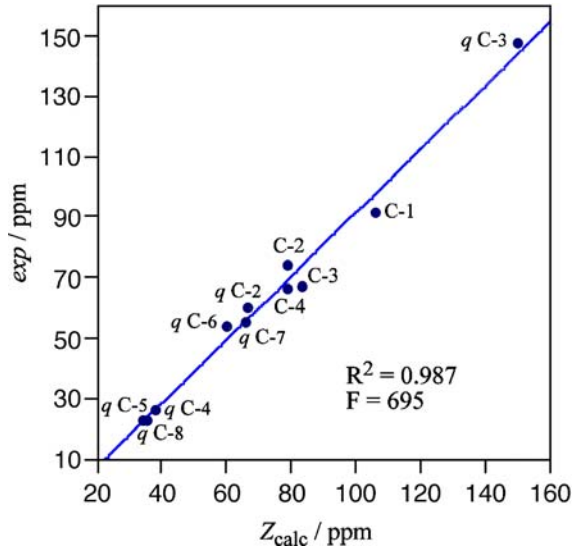


Fig. 6 Correlation of Z_{calc} vs. exp ^{13}C NMR chemical shifts of **5**

N-(Tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)-4-hydroxyiminomethylpyridinum Bromide (**6**)

Tetra-*O*-pivaloyl-*α*-D-glucopyranosyl bromide (**1**, 100 mg, 0.17 mmol) was added to the solution of 4-hydroxyiminomethylpyridine (**3**, 21 mg, 0.17 mmol) in sulfolane (2 mL). The mixture was stirred at 50°C for 24 h. The course of the reaction was monitored by TLC (solvent B). The reaction mixture was then chromatographed on a column of silica gel, first with $CHCl_3$ to elute sulfolane (R_F 0.8), followed by solvent B to obtain compound **6** as mixture of *E*/*Z* isomers in ratio 1:3, (34 mg, 28%) as yellow crystals: m.p. 119–124°C; $[\alpha]_D^{25}$ + 16 ($c = 1.0$ MeOH); R_F 0.3. IR(KBr):$\tilde{\nu}$ = 3465, 3395, 2950, 2850, 1747, 1643, 1480 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.00$ (s, 9H, Piv), 1.11 (s, 9H, Piv), 1.17 (s, 9H, Piv), 1.18 (s, 9H, Piv), 1.19 (s, 9H, Piv), 1.24 (s, 9H, Piv), 1.25 (s, 9H, Piv), 1.26 (s, 9H, Piv), 3.87–4.11 (m, 2H, H-6), 4.23–4.32 (m, 2H, H-5, and H-5′), 4.41–4.60 (m, 2H, H-6), 5.19–5.38 (m, 2H, H-3, and H-3′), 5.42–5.47 (m, 2H, H-4, and H-4′), 5.56–5.85 (m, 2H, H-2, and H-2′), 6.10 (d, $J = 8.53$, 1H, H-1), 7.00 (d, $J = 9.17$, 1H, H-1′), 8.24 (s, 1H, CH=NO′), 8.42 (s, 1H, CH = NO), 8.94 (d, $J = 6.55$, 2H, *pyr*(H-3 and H-5)), 8.98 (d, $J = 6.66$, 2H, p*yr*(H-3′ and H-5′), 9.17 (d, $J = 6.66$, 2H, *pyr*(H-2 and H-6)), 9.40 (d, $J = 6.51$, 2H, *pyr*(H-2′ and H-6′)), 12.17 (s, 1H, NOH′), 12.36 (s, 1H, NOH) ppm.^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 26.86$ (($\underline{C}H_3)_3CCO$), 26.88 (($\underline{C}H_3)_3CCO$), 26.99 (2 × ($\underline{C}H_3)_3CCO$), 27.04 (($\underline{C}H_3)_3CCO$), 27.06 (2 × ($\underline{C}H_3)_3CCO$), 27.08 (($\underline{C}H_3)_3$ CCO), 38.60 ($(CH_3)_3\underline{C}CO$), 38.69 (2 × $(CH_3)_3\underline{C}CO$), 38.74 ($(CH_3)_3\underline{C}CO$), 38.80 (2 × $(CH_3)_3\underline{C}CO$), 38.82 (2 × $(CH_3)_3\underline{C}CO$), 60.73 (C-6), 61.20 (C-6′), 66.36 (C-4′), 66.52 (C-4), 71.52 (C-3′), 71.65 (C-3), 72.78 (C-2′), 72.90 (C-2), 75.69 (C-5), 75.70 (C-5′), 91.30 (C-1), 94.29 (C-1′), 91.30 (C-1), 124.22 (*pyr* C-5), 124.58 (*pyr* C-5′), 141.34 (*pyr* C-3′), 141.86 (*pyr* C-3), 142.27 (*pyr* C-6), 142.66 (*pyr* C-6′), 143.21 (*pyr* C-2′), 144.05 (*pyr* C-2), 144.27 (CH=NO′), 146.46 (CH=NO), 151.72 (*pyr* C-4′), 152.78 (*pyr* C-4), 176.12 (C=O), 176.25 (2 × C=O), 176.30 (C=O), 176.93 (C=O), 176.70 (C=O), 177.79 (C=O), 177.94 (C=O) ppm. ESMS: $m/z = 621.33$ (100%) [M^+]. $C_{32}H_{49}BrN_2O_{10}$ (701.64): calculated C 54.78, H 7.04, N 3.99; found C 54.78, H 7.08, N 3.97.

N-(Tetra-*O*-pivaloyl-*β*-D-glucopyranosyl)-2-hydroxyiminomethyl-3-methylimidazolium bromide (**7**)

Tetra-*O*-pivaloyl-*α*-D-glucopyranosyl bromide (**1**, 200 mg, 0.35 mmol) was added to the solution of *N*-methyl-2-hydroxyiminomethylimidazole (**4**, 43 mg, 0.35 mmol) in sulfolane (4 mL). The mixture was stirred at 50°C for 24 h. The course of the reaction was monitored by TLC (solvent B). The reaction mixture was then chromatographed on a column of silica gel, first with $CHCl_3$ to elute sulfolane (R_F 0.8), followed by solvent B to obtain compound **7** as mixture of *E*/*Z* isomers in ratio 1:3, (29 mg, 12%) as yellow crystals: m.p. 110–114°C; $[\alpha]_D^{25}$ +22 ($c = 0.51$ MeOH); R_F 0.2. IR(KBr):$\tilde{\nu}$ = 3415, 3187, 2980, 2850, 1750, 1539, 1462 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): $\delta = 1.05$ (s, 9H, Piv), 1.07 (s, 9H, Piv), 1.11 (s, 9H, Piv), 1.12 (s, 9H, Piv), 1.15 (s, 9H, Piv), 1.16 (s, 9H, Piv), 1.22 (s, 9H, Piv), 1.24 (s, 9H, Piv), 4.10 (s, 3H, CH_3N'), 4.17 (s, 3H, CH_3N), 4.20–3.34 (m, 6H, H-5, H-5′, H-6, and H-6′), 5.31–5.34 (m, 3H, H-3′, H-4, and H-4′), 5.44–5.47 (m, 1H, H-3), 5.53–5.69 (m, 2H, H-2 and H-2′), 6.12 (d, $J = 9.13$, 1H, H-1), 6.56 (d, $J = 9.39$, 1H, H-1′), 7.13 (s, 1H, CH=NO), 7.28 (s, 1H, CH=NO′), 7.48 (d, $J = 2.03$, 1H, *im* H-4′), 7.60 (d, $J = 2.01$, 1H, *im* H-4), 7.78 (d, $J = 1.91$, 1H, *im* H-5), 7.94 (d, $J = 2.02$, 1H, *im* H-5′), 10.95 (s, 1H, NOH), 10.97 (s, 1H, NOH′) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): $\delta = 26.90$ (($\underline{C}H_3)_3CCO$), 26.93 (($\underline{C}H_3)_3CCO$), 27.01 (($\underline{C}H_3)_3CCO$), 27.10 (($\underline{C}H_3)_3CCO$), 27.12 (($\underline{C}H_3)_3CCO$), 27.16 (2 × ($\underline{C}H_3)_3CCO$), 27.22 (($\underline{C}H_3)_3CCO$), 36.99 (CH_3N'), 38.07 (CH_3N), 38.79 (2 × $(CH_3)_3\underline{C}CO$), 38.85 (2 × $(CH_3)_3\underline{C}CO$), 38.90 (2 × $(CH_3)_3\underline{C}CO$), 38.94 (2 × $(CH_3)_3\underline{C}CO$), 60.86 (C-6), 60.95 (C-6′), 66.52 (C-4), 66.68 (C-4′), 70.17 (C-3′), 70.65 (C-3), 71.73 (C-2′), 71.92 (C-2), 75.45 (C-5′), 75.75 (C-5), 84.18 (C-1′), 84.64 (C-1), 119.27 (*im* C-4′), 119.77 (*im* C-4), 124.10 (*im* C-5′), 124.40 (*im* C-5′), 128.34 (CH=NO′), 131.52 (CH=NO), 139.59 (*im* C-2′), 153.63 (*im* C-2), 176.15 (C=O), 176.36 (C=O), 176.710 (2 × C=O), 177.24 (2 × C=O), 177.34 (C=O), 177.77 (C=O) ppm. ESMS: $m/z = 624.35$ (100%) [M^+]. $C_{31}H_{50}BrN_3O_{10}$ (704.65): calculated C 52.84, H 7.15, N 5.96; found C 53.09, H 7.21, N 5.90.

X-ray crystallography

The X-ray diffraction data for compounds **2** and **4** were collected on the Oxford Diffraction Xcalibur3 CCD diffractometer with monochromated Mo K_α radiation ($\lambda = 0.71073$ Å). The measured intensity data were corrected for Lorentz and polarisation effects. The molecular and crystal structures were solved by direct methods implemented in the program SIR97 [31] and refined on F^2 with anisotropic displacement parameters for all nonhydrogen atoms (SHELXL97) [32]. Crystal data and data for the structure solutions and refinements are collected in Table 8. Positions of the hydrogen atoms in **2** were calculated geometrically and they were refined using the riding model. Hydrogen atoms in **4** were found in difference Fourier map and were refined isotropically. Those on the methyl groups had poor geometry and were placed in calculated position and refined using the riding model.

Table 8 Crystallographic data for **2** and **4**

Crystal data	Compound **2**	Compound **4**
Formula	$C_7H_{12}N_2O$	$C_5H_7N_3O$
M_r	140.18	125.14
Crystal system and habit	Orthorhombic, prism	Monoclinic, prism
Space group	*P*nma	*C*2/*c*
a (Å)	11.7494(15)	15.532(3)
b (Å)	9.4830(13)	9.733(2)
c (Å)	6.5317(9)	17.760(4)
α (°)	90	90
β (°)	90	108.23(2)
γ (°)	90	90
Volume (Å^3)	727.76(17)	2550.0(10)
Z	4	16
Density (calculated, g cm^{-3})	1.279	1.304
μ(Mo Kα) (mm^{-1})	0.088	0.096
F(000)	304	1056
2θ range for data collection (°)	9.34–58.00	8.72–56.00
h, k, l range	−16 to 16, −12 to 12, −8 to 8	−20 to 20, −10 to 12, −23 to 23
Scan type	ω scan	ω scan
No. of measured reflections	7777	11,816
No. of independent reflections	1011	3049
No. of refined parameters	55	195
No. of observed reflections, $[I \geq 2\sigma(I)]$	901	1838
g_1, g_2 in w^a	0.0590, 0.2206	0.0732, 0.0000
R^b, wR^c $[I \geq 2\sigma(I)]$	0.0649, 0.1517	0.0536, 0.1293
R, wR (all data)	0.0756, 0.1580	0.0942, 0.1538
Goodness-of-fit on F^2, S^d	1.244	1.047
Max., min. electron density (e Å^{-3})	0.285 − 0.202	0.155–0.127

$^a w = 1/\left[\sigma^2\left(F_o^2\right) + (g_1 P)^2 + g_2 P\right]$, where $P = \left(F_o^2 + 2F_c^2\right)/3$.

$^b R = \sum \left||F_o| - |F_c|\right| / \sum |F_o|$.

$^c wR = \left[\sum \left(F_o^2 - F_c^2\right)^2 / \sum w\left(F_o^2\right)^2\right]^{1/2}$.

$^d S = \sum \left[w\left(F_o^2 - F_c^2\right)^2 / N_{obs} - N_{param}\right]^{1/2}$.

Supplementary material

Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC-258876 (**2**) and -258877 (**4**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-(0)1223/336-033; or e-mail: deposit@ccdc.cam.ac.uk.

Acknowledgments We thank the Ministry of Science, Education and Sport of Croatia for support of this work (0119610, 0119612, and 0119632). We thank Mrs. Mirjana Nehyba for technical assistance.

References

1. Ljevaković Đ, Tomić S, Tomašić J (1988) Carbohydr Res 182:197–205
2. LjevakovićĐ, Tomić S, Tomašić J (1992) Carbohydr Res 230:107–115
3. Petrović V, Tomić S, Matanović M (2002) Carbohydr Res 337:863–867
4. Tomić S, Petrović V, Matanović M (2003) Carbohydr Res 338:491–494
5. Ljevaković Đ, Treščec A, Tomić S, Tomašić J (1991) Carbohydr Res 210:191–198
6. Petrović V, Tomić S, Ljevaković Đ, Tomašić J (1997) Carbohydr Res 302:13–18
7. Kunz H, Harreus A (1982) Liebigs Ann Chem 41–48
8. Simeon-Rudolf V, Reiner E, Škrinjarić-Špoljar M, Radić B, Lucić A, Primožič I, Tomić S (1998) Arch Toxicol 72:289–295
9. Reiner E, Škrinjarić-Špoljar M, Dunaj S, Simeon-Rudolf V, Primožič I, Tomić S (1999) Chem Biol Int 119/120:173–181
10. Primožič I, Odžak R, Tomić S, Simeon-Rudolf V, Reiner E (2004) J Med Chem Def 2, http://www.jmedchemdef.org/Issue_0201/Reiner_0704.html
11. Heldman E, Ashani Y, Raveh L, Rachaman S (1986) Carbohydr Res 151:337–347
12. Iversen P, Lund H (1966) Acta Chem Scand 20:2649–2657
13. Fournari P, de Cointet P, Laviron E (1968) Bull Soc Chim Fr 6:2438–2446
14. Sternbach LH, Kaiser S (1952) J Am Chem Soc 74:2215–2218
15. Martinez-Ripoll M, Lorenz HP (1976) Acta Cryst B 32:2322–2325
16. Martinez-Ripoll M, Lorenz HP (1976) Acta Cryst B 32:2325–2328
17. Schouten A, Kantres JA, Kroon J (1994) Acta Cryst C50:128–129
18. Vicković I, Pavlić L, Mrvoš-Sermek D, Mesić M (1995) Z Kristallogr 210:282–286
19. Deschamps JR, George C, Flippen-Anderson JL, Musallam HA (1997) J Chem Crystallogr 27:541–548
20. Bühl M, Schleyer PVR (1992) J Am Chem Soc 114:477–491
21. Gaussian 03, Revision C.02. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery Jr, JA, Vreven T, Kudin KN, Burant JC, Millam, JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Bakken V, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, and Pople JA, Gaussian, Inc., Wallingford CT, 2004
22. Foresman JB, Frisch Æ (1993) Exploring chemistry with electronic structure methods: a guide to using Gaussian. Gaussian, Inc., Pittsburg, PA
23. Hehre WJ, Radom L, Schleyer PVR, Pople JA (1986) Ab initio molecular orbital theory. Wiley, New York

24. Jensen F (1999) Introduction to computational chemistry. Wiley, Canada
25. Young DC (2001) Computational chemistry: a practical guide for applying techniques to real-world problems. Wiley, New York
26. Koch W, Holthausen MC (2002) A chemist's guide to density functional theory. Wiley-VCH, Weinheim, Germany
27. Wolinski K, Hinton JF, Pulay P (1990) J Am Chem Soc 112:8251–8260
28. Barfield M, Fagerness P (1997) J Am Chem Soc 119:8699–8711
29. Forsyth DA, Sebag AB (1997) J Am Chem Soc 119:9483–9494
30. GaussView, Version 3.09. Dennington II, R, Keith T, Millam J, Eppinnett K, Hovell WL, and Gilliand R. Semichem, Inc., Shawnee Mission, KS, 2003
31. Altomare A, Cascarano G, Giacovazzo C, Guagliardi A, Moliterni AGG, Burla MC, Polidori G, Camalli M, Spagna R (1997): SIR 97 A package for crystal structure solution by direct methods and refinement, Roma, Italy
32. Sheldrick GM (1997) SHELXL97: Program for the Refinement of Crystal Structures, Universität Göttingen, Germany
33. Chowdhary MS, Hough L, Richardson AC (1984) J Chem Soc Perkin Trans 1:419–427
34. Garcia RC, Hough L, Richardson AC (1990) Carbohydr Res 200:307–317