CONFORMATIONAL STUDIES OF THE FURANOSIDES, IMPORTANT COMPONENTS OF BACTERIAL GLYCANS

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Many of biological compounds, e.g. polysaccharides and oligosaccharides, which are important components of number of microorganisms, fungi and plants, contain furanose ring in their structure (Fig. 1). Importantly, oligosaccharides involving furanosyl constituents are present in various microorganisms whereas these are absent in the mammals glycans. This fact suggests that the enzymes involved in the metabolism of such sugars in bacteria, fungi and protozoa would constitute a good target for the design of new drugs.

Despite of their ubiquity in biological structures, furanosides have received much less attention than pyranosides as regards the conformational analysis. This is because different conformations of five-membered rings have quite similar energies, whereas six-membered rings are normally present in a single low-energy chair conformation. Therefore, conformations of pyranosides in solution are easily identified by NMR techniques, which is not the case with furanosides. These are equilibrating rapidly on the NMR scale and averaging of coupling constants occurs. However, when a tetrahydrofuran ring is conformationally restricted by a rigid second skeleton, it is possible to predict its conformation [2-4].

To study conformations of a furanose ring we synthesized three series of the conformationally restricted furanosides. The first one consists of D-glucofuranosidurono-6,3-lactones (compounds **1-12**). The second one consists of 2,3-*O*-isopropylidene-D-ribofuranosides (compounds **13-17**). The third one consist of 1,2-*O*-isopropylidene-D-ribofuranoses (compounds **18-23**).

Fig. 1 Arabinofuranoside-based hexasaccharide in *mycobacteria* [1].
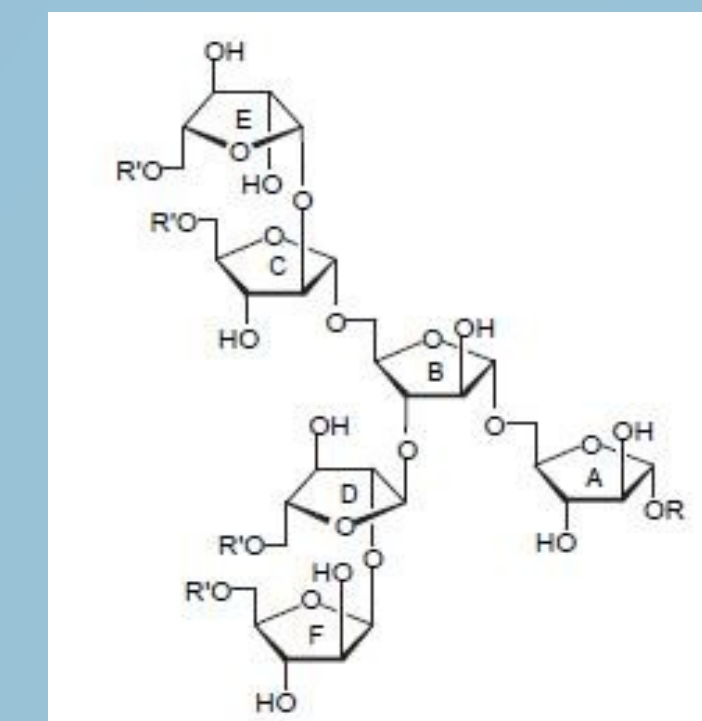
LAM: R=arabinomannan; R'=H
AG: R=arabinogalactan; R'=mycolic acids

Scheme 1 Synthesis of alkyl β-D-glucofuranosidurono-6,3-lactones (**1-6**).

conformation $^1T_2$
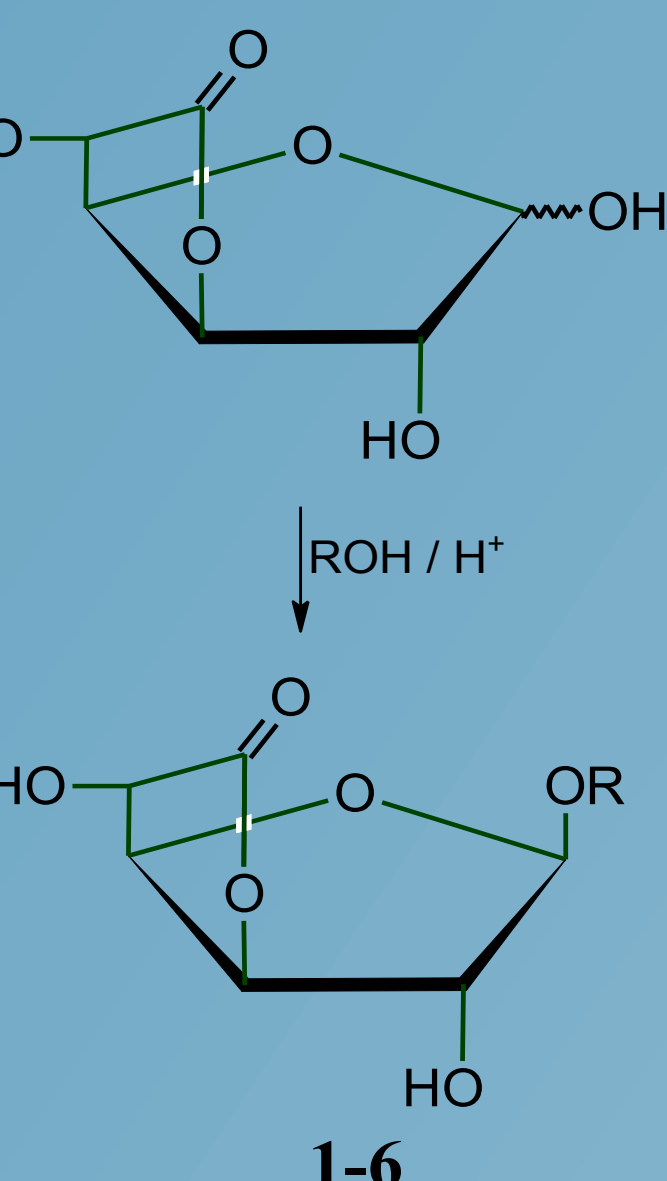
Table 1
Chemical shifts (ppm) and ^{1}H-^{1}H coupling constants (Hz) in the ^{1}H NMR spectra (CDCl$_3$) of β-D-glucofura-nosidurono-6,3-lactones (**1-6**).

no.	R=	H-1	H-2	H-3	H-4	H-5	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
1	CH$_2$CH$_3$	5,12 (s)	4,45 (bs)	4,87 (d)	5,02 (dd)	4,37 (t)	-	-	5,2	6,8
2	CH$_2$CH$_2$CH$_3$	5,11 (s)	4,44 (s)	4,88 (d)	5,02 (dd)	4,38 (bs)	-	-	5,2	6,8
3	CH(CH$_3$)$_2$	5,22 (s)	4,41 (d)	4,87 (d)	5,00 (dd)	4,36 (dd)	-	-	4,8	7,2
4	CH$_2$CH$_2$CH$_2$CH$_3$	5,10 (s)	4,43 (d)	4,88 (d)	5,02 (dd)	4,38 (bt)	-	-	4,8	6,8
5	C(CH$_3$)$_3$	5,34 (s)	4,29 (d)	4,89 (d)	4,59 (dd)	4,36 (dd)	-	-	5,2	7,2
6	CH$_2$Ph	5,17 (s)	4,44 (s)	4,82 (d)	4,97 (dd)	4,33 (d)	-	-	4,8	6,4
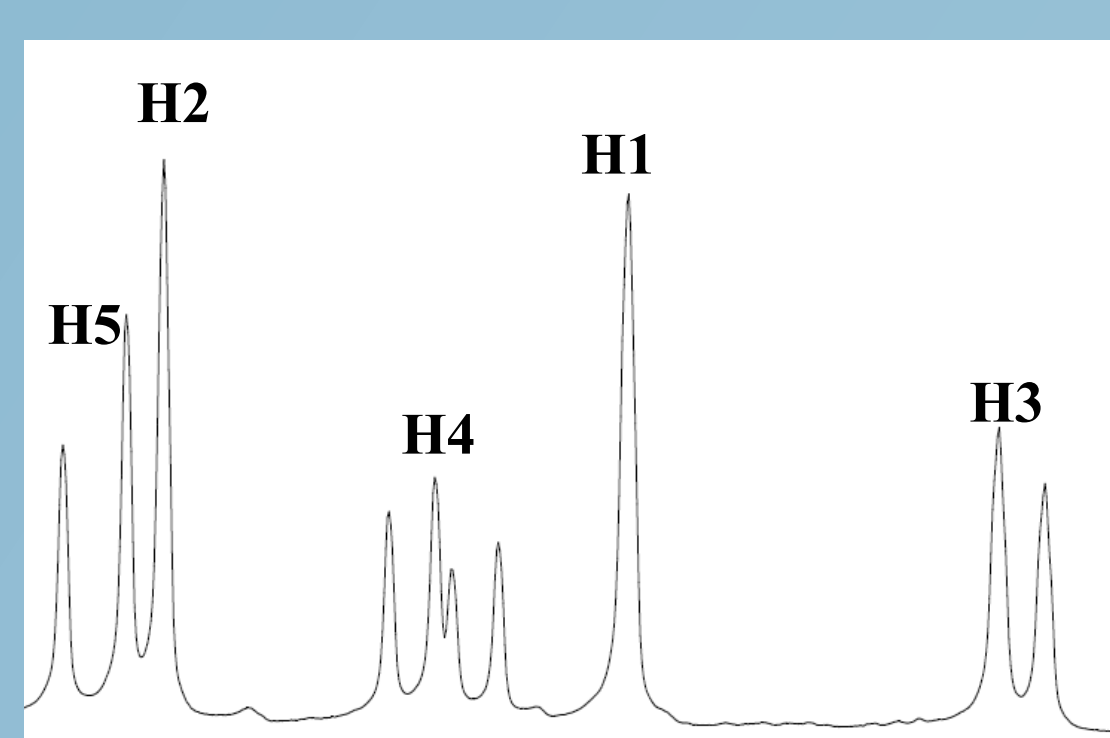
Characteristic ^{1}H NMR spectra of furanosides **7-11** consist of two singlets (H1 and H2), two doublets (H3 and H5) and doublet of doublets (H4) (Fig. 2). Really important is that there is lack of coupling between H1 and H2 as well as H2 and H3 protons, which is characteristic for the $^1T_2$-like conformation [5]. The ^{1}H NMR spectra of furanosides **1-6** are slightly more complicated because of the coupling with the hydroxyl protons. However, these are also characteristic by the lack of coupling between H1 and H2 as well as H2 and H3 protons, indicating the $^1T_2$ conformation.

Fig. 2 Characteristic ^{1}H NMR spectra of furanosides **7-11**.
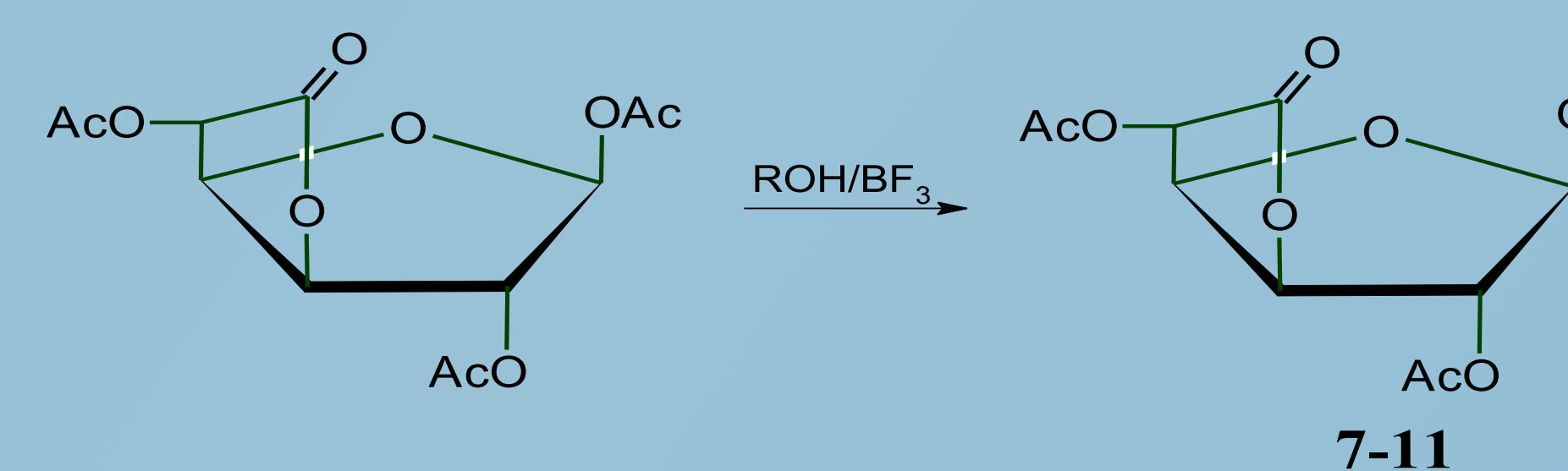
Scheme 2 Synthesis of alkyl 2,5-di-*O*-acetyl-β-D-glucofuranosidurono-6,3-lactones (**7-11**).

Table 2
Chemical shifts (ppm) and ^{1}H-^{1}H coupling constants (Hz) in the ^{1}H NMR spectra (CDCl$_3$) of 2,5-di-β-*O*-acetyl-D-glucofuranosidurono-6,3-lactones (**7-11**).
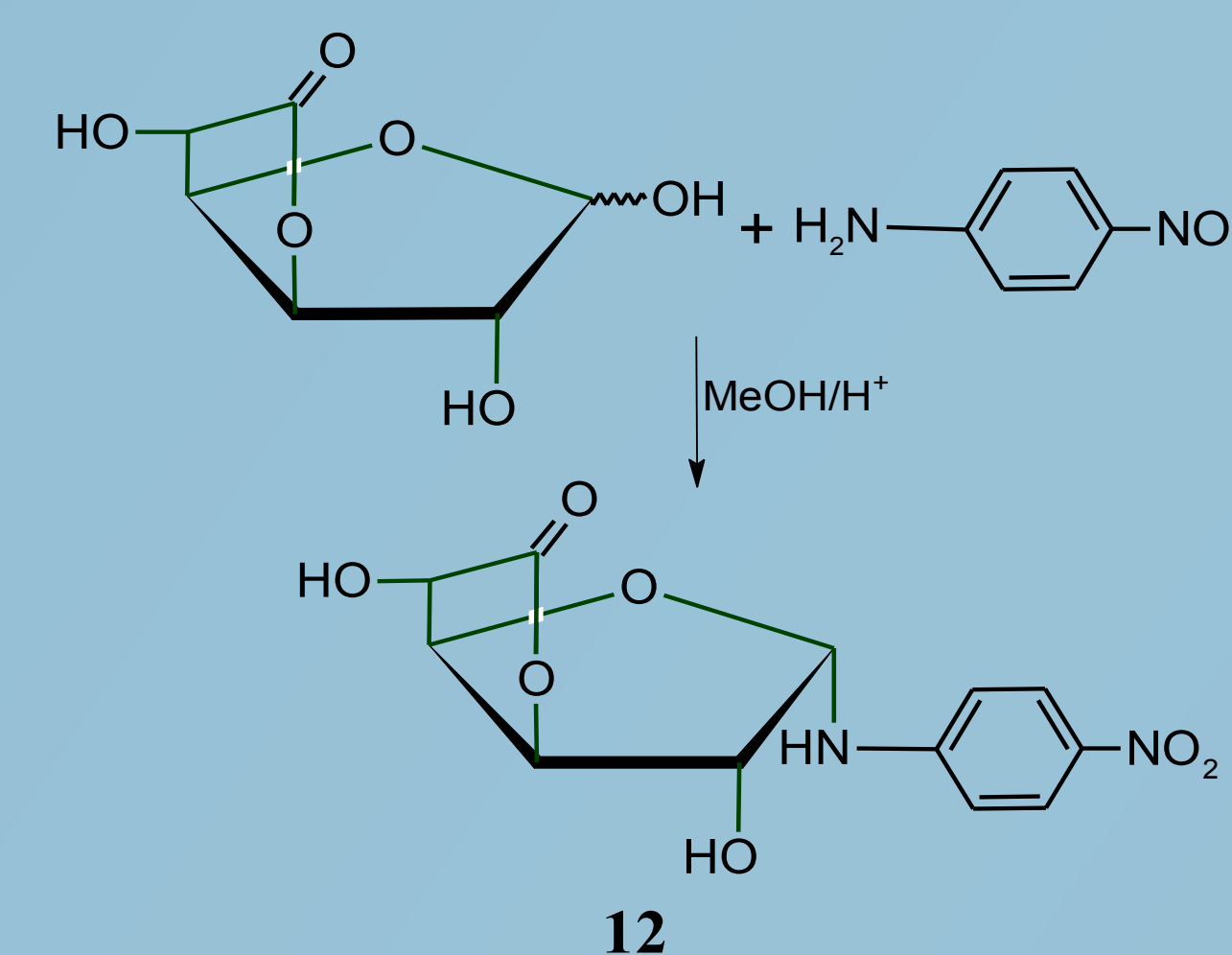
no.	R=	H-1	H-2	H-3	H-4	H-5	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
7	CH$_2$CH$_3$	5,08 (s)	5,21 (s)	4,97 (d)	5,13 (dd)	5,23 (d)	-	-	5,2	6,8
8	CH$_2$CH$_2$CH$_3$	5,07 (s)	5,21 (s)	4,96 (d)	5,13 (dd)	5,23 (d)	-	-	5,2	7,2
9	CH(CH$_3$)$_2$	5,16 (s)	5,20 (s)	4,96 (d)	5,11 (dd)	5,23 (d)	-	-	5,2	6,8
10	CH$_2$CH$_2$CH$_2$CH$_3$	5,07 (s)	5,21 (s)	4,96 (d)	5,13 (dd)	5,23 (d)	-	-	5,2	7,2
11	CH$_2$Ph	5,16 (s)	5,29 (s)	5,00 (d)	5,18 (dd)	5,30 (d)	-	-	4,8	7,2

Table 3
Crystal data and structure refinement for **12**.

Empirical formula	C$_{12}$H$_{12}$N$_2$O$_7$, CH$_4$O
Formula weight	328.28
Temperature (K)	295
Wavelength (Å)	0.71073
Crystal system	orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 5.5455(8) Å b = 7.8178(8) Å c = 32.251(4) Å
V (Å^3)	1398.2(3)
Z	4
D_{calcd} (Mg m^{-3})	1.560
Absorption coefficient (mm^{-1})	0.131
$F(000)$	688
Θ Range for data collection (°)	3.63 − 25.05
Limiting indices	-6 ≤ h ≤ 6 -9≤ k ≤ 9 -38 ≤ l ≤ 26
Reflections collected/unique	1486 / 917 [R_{int} = 0.0985]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1486/0/209
Completeness 2Θ = 24.99° (%)	99.7
Final R indices [I>2σ(I)]	R$_1$ = 0.0584 wR$_2$ = 0.1318
R indices (all data)	R$_1$ = 0.1064 wR$_2$ = 0.1549
Goodness-of-fit on F^2	1.123
Largest diff. peak and hole (e Å^{-3})	0.202 and -0.270

Scheme 3 Synthesis of *N*-(α-D-glucofura-nurono-6,3-lactonyl)-*p*-nitroaniline (**12**).
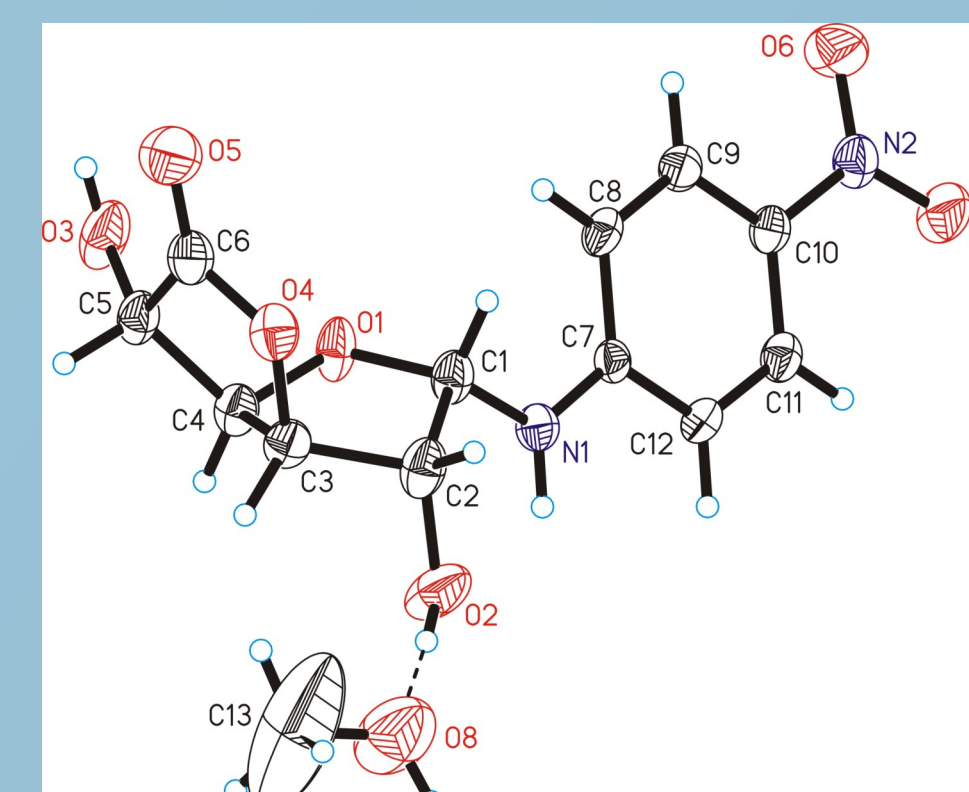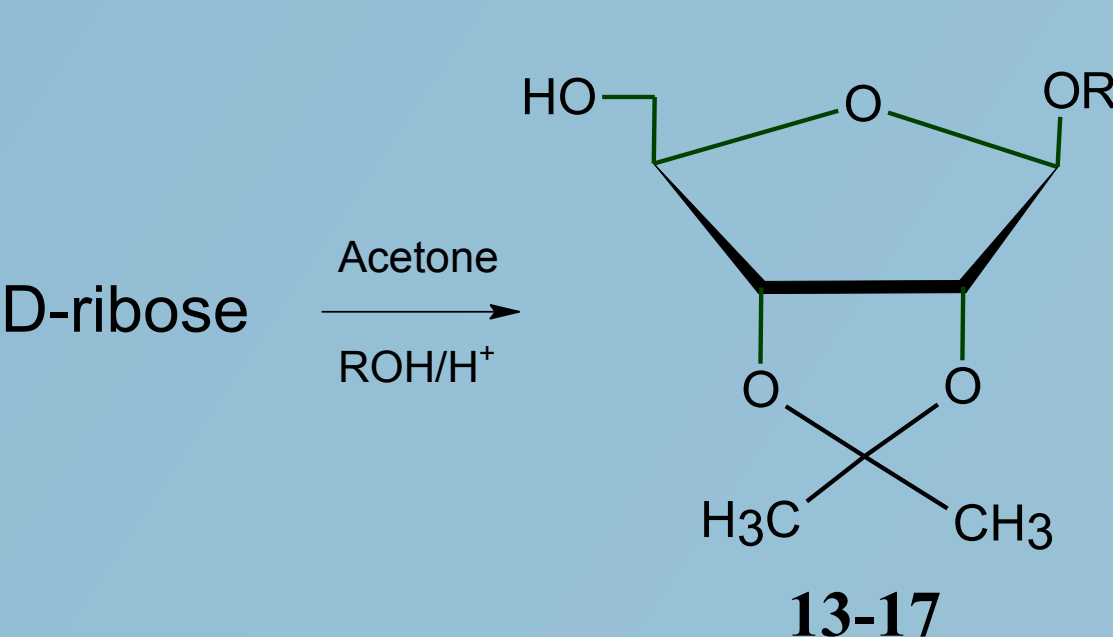
conformation ^3E

Fig. 3 Structure of **12** showing 25% probability displacements for ellipsoids. Compound **12** crystallines with one molecule of methanol. The O—H···O hydrogen bond, represented by a dashed line, is created between MeOH and 2-OH.

Table 4
Chemical shifts (ppm) and ^{1}H-^{1}H coupling constants (Hz) in the ^{1}H NMR spectra (DMSO) of *N*-(α-D-glucofuranurono-6,3-lactonyl)-*p*-nitroaniline (**12**).

H-1	H-2	H-3	H-4	H-5	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
5,44 (d)	5,31 (d)	4,89 (d)	4,65 (dd)	4,48 (t)	4,0	-	3,6	4,8

The ^{1}H NMR spectra of *N*-(α-D-glucofuranouron-6,3-lactonyl)-*p*-nitroaniline (**12**) show, that the change of an aglycone and an anomeric carbon atom configuration results in a coupling between the H1 and H2 protons, but there is still lack of coupling between the H2 and H3 protons (Table 4). In the crystal lattice **12** adopts the ^3E conformation (Fig. 3). It is possible that the same conformation is adopted by **12** in solution.

D-ribose → (Acetone, ROH/H$^+$) → **13-17**

Scheme 4 Synthesis of alkyl 2,3-*O*-isopropylidene-D-ribofuranosides (**13-17**).

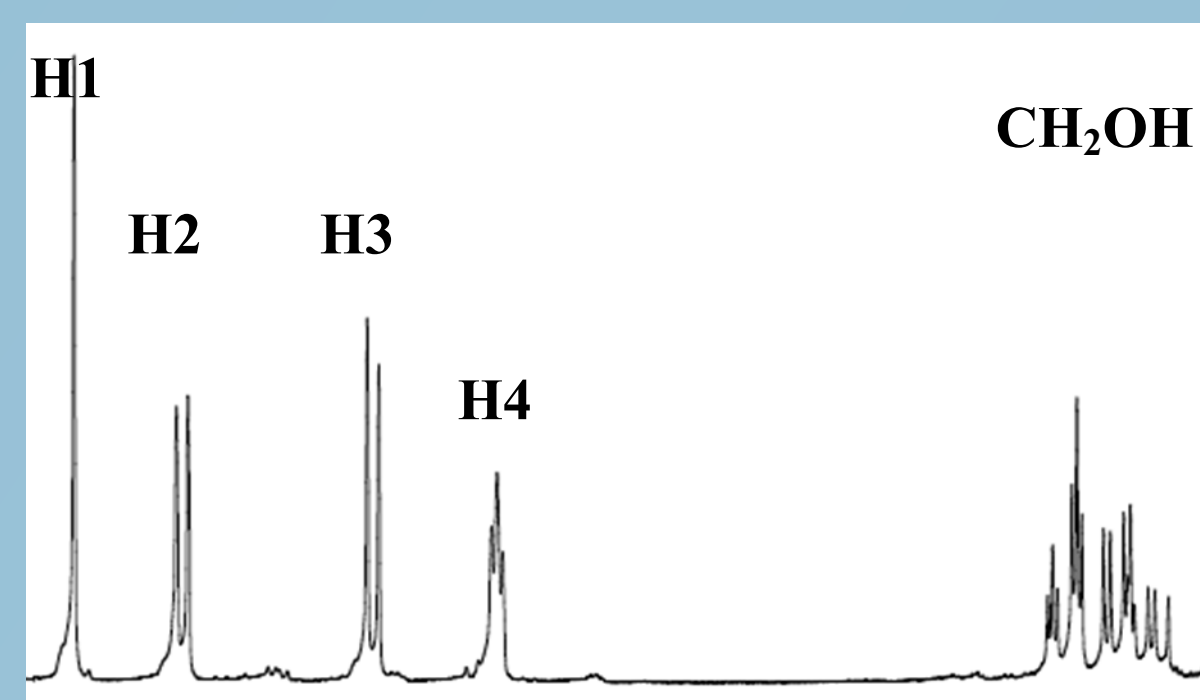**Fig. 4** Characteristic ^{1}H NMR spectra of furanosides **13-17**.

Table 5
Chemical shifts (ppm) and ^{1}H-^{1}H coupling constants (Hz) in the ^{1}H NMR spectra (CDCl$_3$) of 2,3-*O*-isopropylidene-D-ribofuranosides (**13-17**).

no.	R=	H-1	H-2	H-3	H-4	CH$_2$OH	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
13	CH$_3$	4,97 (s)	4,83 (d)	4,58 (d)	4,42 (t)	3,65 (m)	-	6,4	-	2,8
14	CH$_2$CH$_3$	5,07 (s)	4,84 (d)	4,59 (d)	4,41 (t)	3,68 (m)	-	6,4	-	2,8
15	CH$_2$CH$_2$CH$_3$	5,06 (s)	4,84 (d)	4,60 (d)	4,41 (t)	3,68 (m)	-	6,0	-	2,8
16	CH(CH$_3$)$_2$	5,17 (s)	4,85 (d)	4,56 (d)	4,40 (t)	3,67 (m)	-	6,0	-	2,4
17	CH$_2$CH$_2$CH$_2$CH$_3$	5,05 (s)	4,83 (d)	4,59 (d)	4,40 (t)	3,73 (m)	-	6,4	-	2,4

The ^{1}H NMR spectra of 2,3-*O*-isopropylidene-D-ribofuranosides (**13-17**) are also typical and consist of one singlet (H1), two doublets (H2 and H3), one triplet (H4) and one multiplet (CH$_2$OH) (Table 5). In this case, there is lack of coupling between H1 and H2 as well as H3 and H4 protons (Fig. 4).

conformation ?

Table 6
Chemical shifts (ppm) and ^{1}H-^{1}H coupling constants (Hz) in the ^{1}H NMR spectra (CDCl$_3$) of compounds **18-23**.

no.	H-1	H-2	H-3	H-4	H-5	H-5'	H-6	H-6'	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$
18	5,88 (d)	4,46 (d)	4,14 (d)	4,15 (m)	3,77 (m)	3,77 (m)			3,6	-	2,8	6,0
19	5,93 (d)	4,51 (d)	5,24 (d)	4,48 (t)	4,24 (m)	4,24 (m)			3,6	-	3,2	6,8
20	5,86 (d)	4,47 (d)	4,19 (d)	4,01 (dd)	3,88 (m)		3,58 (dd)	3,75 (dd)	3,6	-	2,8	6,4
21	5,91 (d)	4,75 (d)	5,34 (d)	4,12 (dd)	5,21 (m)		4,26 (dd)	4,56 (dd)	3,6	-	2,8	6,8
22	5,93 (d)	4,52 (d)	4,32 (m)	4,06 (dd)	4,32 (m)		4,16 (dd)	3,97 (dd)	3,6	-	2,8	6,7
23	5,87 (d)	5,25 (d)	5,25 (d)	4,21 (m)	4,21 (m)		4,08 (dd)	4,01 (dd)	3,6	-	2,0	b.d.

The ^{1}H NMR spectra of compunds **18-23** (Fig. 5) are also typical and consist of tree doublets (H1, H2, H3), one doublet of doublets (H4), multiplet (H5) and two doublets of soublets (H6, H6') (Table 6). In this case, there is lack of coupling between H2 and H3.
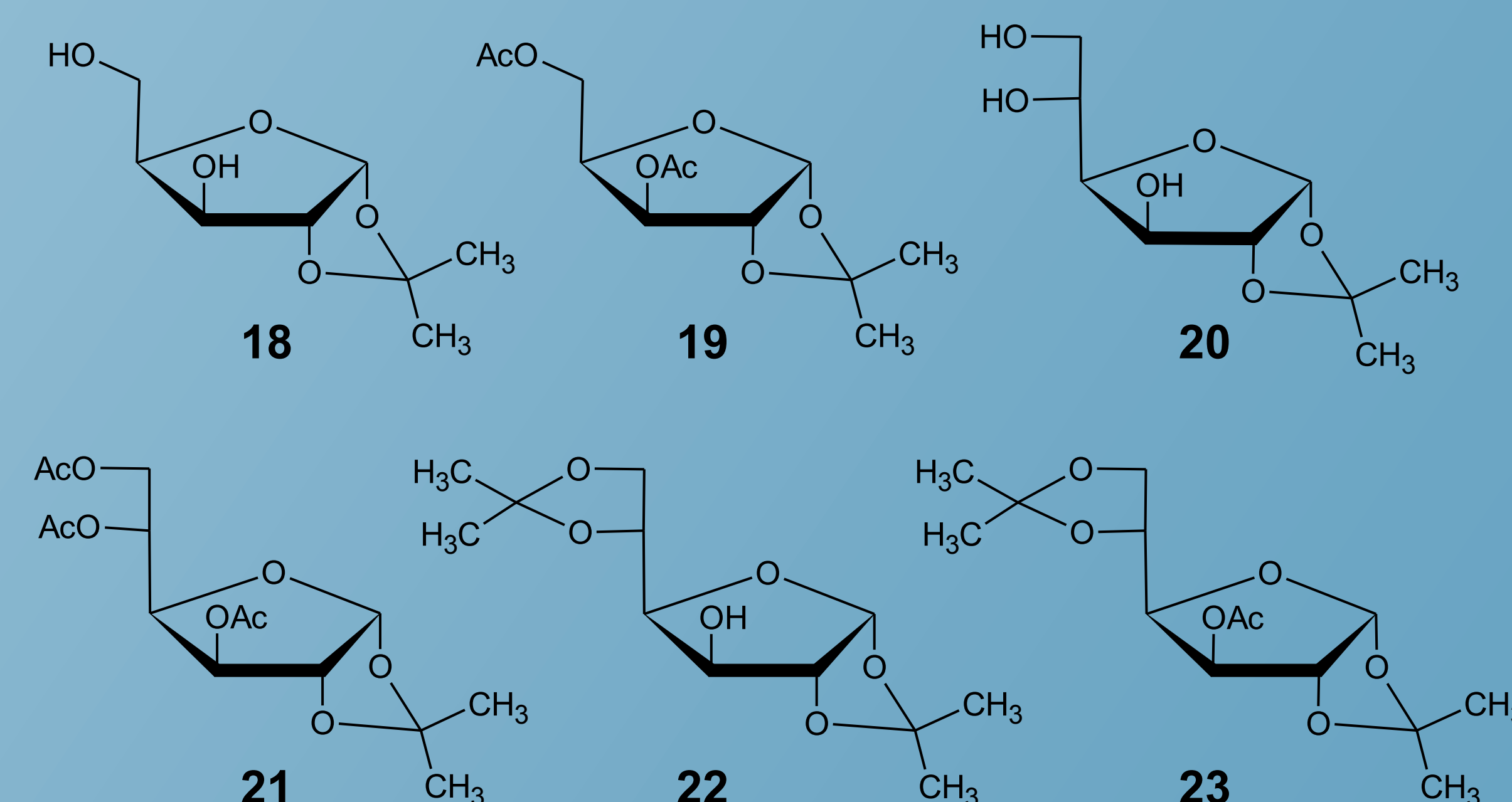
conformation ?

Fig. 5 1,2-*O*-isopropylidene-D-ribofuranoses **18-23**.

References:
1. P. J. Brennan, H. Nikaido, *Annu. Rev. Biochem.* 64: 29, 1995.
2. J. B. Houseknecht, T. L. Lowary. *J. Org. Chem.* 67: 4150-4164, 2002.
3. U. Ellervik, G. Magnusson. *J. Am. Chem. Soc.* 116: 2340-2347, 1994.
4. P. Bouř, I. Raich, J. Kaminský, R. Hrabal, J. Čejka, V. Sychrovský. *J. Phys. Chem.* 108: 6365-6372, 2004.
5. B. Liberek, D. Tuwalska, I. do Santos-Zounon, A. Konitz, A. Sikorski, Z. Smiatacz, *Carbohydr. Res.* 341: 2275-2285, 2006.