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Conformational study of synthetic Δ^4-uronate monosaccharides and glycosaminoglycan-derived disaccharides

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Abstract

Sixteen Δ^4-uronate monosaccharides were chemically synthesized. Their carboxy group was protected as a methyl or benzyl ester, the anomeric hydroxyl group as a benzyl glycoside and the 2 and 3 hydroxyl groups were protected with different substitution patterns as both ester and ether derivatives. Disaccharides containing Δ^4-uronates were prepared from heparin using heparin lyases. Their carboxy group was unprotected or protected as a benzyl ester and the two hydroxyls in the uronate moiety were free, as *O*-sulfo derivatives or acylated. The conformation of these unsaturated uronate monosaccharide and disaccharide residues was studied using ^{1}H NMR by examining interproton vicinal coupling constants. The Δ^4-uronate residue adopted either the $^2H_1$ or the $^1H_2$ conformations. The equilibrium between these two conformers was shown to be controlled by substitution pattern. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: Δ^4-uronates; Unsaturated saccharide residue; Synthesis of Δ^4-uronates; Conformational analysis; NMR

1. Introduction

Glycosaminoglycans (GAGs) regulate a number of important biological events through their interaction with diverse proteins [1]. Synthetic oligosaccharide sequences of GAGs, which correspond to GAG-protein binding sites, are the targets of our synthetic program. Polysaccharide lyases break down GAGs into oligosaccharides that contain a non-reducing terminal Δ^4-uronic acid residue [2]. Our laboratory is exploring the use of these oligosaccharides as building blocks for the synthesis of larger GAG oligosaccharides. This approach requires the stereochemically controlled conversion of the non-reducing terminal Δ^4-uronic acid residues to either D-glucopyranosiduronic (GlcA*p*) or L-idopyranosiduronic (L-IdoA*p*) acids. A number of derivatives of Δ^4-uronate have been synthesized [3] that might prove useful as model compounds for developing a general strategy for the regio and

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Abbreviations: GAGs, Glycosaminoglycans; GlcA*p*, Glucopyranosiduronic acid; IdoA*p*, idopyranosiduronic acid; DBU, 1,8-diazobiaylco[5,4,0]undec-7-ene; TBDMSCl, *tert*-butyldimethylsilyl chloride; TLC, thin layer chromatography; CE, capillary electrophoresis; DMF, dimethylformamide; THF, tetrahydrofuran; FABMS, fast atom bombardment mass spectrometry.

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stereoselective conversion of the Δ^4-uronic acid residues of oligosaccharides into D-GlcA*p* or L-IdoA*p*. In addition, Δ^4-uronate disaccharides derived from GAGs have also been synthesized [4,5].

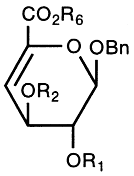
While 2,3-unsaturated pyranosides have been extensively studied, the 4,5-unsaturated pyranoside derivatives, major products of *β*-elimination reactions of uronic acid containing polysaccharides, have received less attention. Over 20 years ago, several 4,5-unsaturated pyranosides were studied by ^{1}H NMR spectroscopy [6]. More recently, Ragazzi et al. [7] reported a compilation of ^{1}H NMR data for unsaturated acid residues of 11 GAG-derived disaccharides and oligosaccharides showing that variation in interproton vicinal coupling constants as a function of configuration, glycosidic linkage and substitution can be unequivocally interpreted in terms of an equilibrium between the $^1H_2$ and the $^2H_1$ conformers (Fig. 1).

Here, the results of ^{1}H NMR studies on chemically modified Δ^4-uronates of a number of monosaccharides and GAG-derived disaccharides are presented. The results of such a conformational analysis should aid in understanding the unusual chemistry and stereoselectivity associated with reactions occurring at such Δ^4-uronates [3].

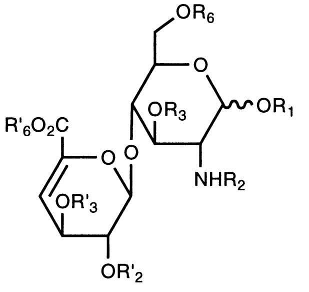
2. Results and discussion

Synthesis of Δ^4-uronates.—The different mono and disaccharides synthesized are presented in Fig. 2. Compounds **1–3**, and **13** were synthesized from the known methyl (benzyl 2,3,4-tri-*O*-acetyl-*β*-D-glucopyranosid)uronate **1a** [8], as shown in Fig. 3. The *β*-elimination of **1a** or **1b**, using

Fig. 1. Half-chair conformations of Δ^4 uronates.



1 $R_1=R_2=H$, $R_6=Me$
2 $R_1=R_2=Ac$, $R_6=Me$
3 $R_1=R_2=Piv$, $R_6=Me$
4 $R_1=R_2=Bz$, $R_6=Me$
5 $R_1=R_2=TBDMS$, $R_6=Me$
6 $R_1=R_2=[(iPr)_2Si]_2O$, $R_6=Me$
7 $R_1=H$, $R_2=TBDMS$, $R_6=Me$
8 $R_1=Piv$, $R_2=H$, $R_6=Me$
9 $R_1=H$, $R_2=Piv$, $R_6=Me$
10 $R_1=H$, $R_2=Bz$, $R_6=Me$
11 $R_1=OP(OEt)_2$, $R_2=Piv$, $R_6=Me$
12 $R_1=Bn$, $R_2=Piv$, $R_6=Me$
13 $R_1=R_2=Bn$, $R_6=Bn$
14 $R_1=Bn$, $R_2=TBDMS$, $R_6=Bn$
15 $R_1=Bn$, $R_2=Bz$, $R_6=Bn$
16 $R_1=Bn$, $R_2=PMB$, $R_6=Bn$



17 $R_1=R_3=R'_3=R'_6=H$, $R_2=R_6=SO_3H$
18 $R_1=R_3=R'_3=Ac$, $R_2=R'_2=R_6=SO_3H$, $R'_6=H$,
19 $R_1=H$, $R_3=R'_3=Ac$, $R_2=R'_2=R_6=SO_3H$, $R'_6=H$
20 $R_1=R'_3=Piv$, $R_2=R'_2=R_6=SO_3H$, $R_3=R'_6=H$
21 $R_1=R_3=R'_3=H$, $R_2=R'_2=R_6=SO_3H$, $R'_6=Bn$
22 $R_1=R_3=R'_2=R'_3=R_6=R'_6=H$, $R_2=Ac$
23 $R_1=R_2=R_3=R_6=R'_2=R'_3=Ac$, $R'_6=H$

Fig. 2. The structure of Δ^4-monosaccharides and Δ^4-disaccharides.

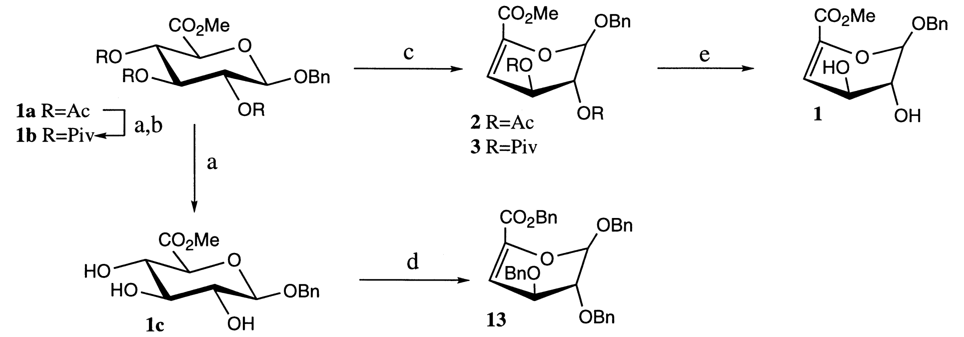


Fig. 3. Synthesis of monosaccharides **1–3** and **13**. (a) NaOMe, MeOH, rt; (b) Piv-Cl, Py, rt, 12 h (65–70 % from **1**); (c) DBU, CH_2Cl_2, rt, 7–30 h (90 %); (d) Ag_2O, BnBr, DMF, rt, 48 h (40 %), (e) NEt_3, MeOH, rt, 12 h (80 %).

1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) [9], gave the corresponding Δ^4-uronates **2** and **3** in high yields (91%). Deacetylation of **2** using triethylamine in methanol led to the free diol **1** in 87% yield. Deacetylation [10] of **1a** followed by a one pot benzylation and β-elimination, using silver oxide, afforded the perbenzylated glycal **13** in 40% yield. All the other monosaccharides were prepared using standard methods from the diol **1**. Compound **4** was obtained in quantitative yield by benzoylation of **1** using benzoyl chloride and pyridine. The 2,3-di-*O*-silyl derivatives **5** and **6** were synthesized by reacting **1** with respectively *tert*-butyldimethylsilyl chloride (TBDMSCl) in presence of imidazole (quant.), or 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane in pyridine (88% yield). Silylation of **1** with 1 equivalent of TBDMSCl in pyridine gave a mixture of the 3-*O*-*tert*-butyldimethylsilyl derivative **7** and 2-*O*-*tert*-butyldimethylsilyl derivative in 82 and 5% yield, respectively. Pivaloylation of **1** using 1 equivalent of pivaloyl chloride gave a mixture of 2-*O*-pivaloyl uronate **8**, 3-*O*-pivaloyl uronate **9**, and 2,3-di-*O*-pivaloyl uronate **3** in a yield of 4, 66 and 14%, respectively. Benzoylation of the 3-*O*-tert-butyldimethylsilyl derivative **7** followed by desilylation using tetrabutylammonium fluoride led to a mixture of 2-*O*-benzoyl derivative, 3-*O*-benzoyl derivative **10**, and 2,3-di-*O*-benzoyl derivative **4** in a yield of 34, 37 and 5%, respectively. Phosphorylation [11] of **9** using triethylphosphite and iodide afforded **11** quantitatively. Direct benzylation of **7** using sodium hydride and benzyl chloride was expected to result in transesterification, giving a mixture of methyl and benzyl esters. To eliminate this potential problem, **7** was first de-esterified using methanolic potassium hydroxide. The corresponding carboxylic acid was then benzylated under standard conditions to give the corresponding benzyl ester **14** in 56% yield, and the perbenzylated compound **13** in 26% yield. Transesterification of **14** using sodium methoxide, followed by desilylation and subsequent pivaloylation led to **12** in 70% yield. Desilylation of **14** followed by benzoylation or acidic benzylation [12] gave respectively **15** and **16** in yields of 90 and 75%, respectively.

The disaccharides **17**, **18**, **20** and **21** had been previously prepared in our laboratory [4,5]. Anomeric deacetylation of **18** using piperidine resulted in a 73% yield of **19**. The desulfated disaccharide **22** was obtained by enzymatic depolymerization of *N*-acetylated, *O*-desulfated heparin [13], and was peracetylated under standard conditions to give **23** in 80% yield.

^{1}H NMR study.—Vicinal coupling constants are useful in determining the conformation of unsaturated substances using ^{1}H NMR. The chemical shifts and coupling constants of the compounds **1–23** are reported in Table 1. For each derivative, the signal for H-4 appears at lowest field (5.9–6.5 ppm) due to deshielding by the double bond, and is either a well resolved doublet, or a doublet of doublets, due to long-range coupling with H-2. The signal for H-1 is a doublet, or a doublet of doublets due to the long-range coupling with H-3. The signals for H-2 and H-3 are a doublet of doublets or a triplet, which is split to multiplet when a long-range coupling is present. In some cases, H-2 appears as a broad singlet, making the precise determination of $J_{2,3}$ difficult. However, these undetermined values can be estimated as being very small (<3.0 Hz). Based on the values of $J_{1,2}$ and $J_{2,3}$, three different groups of monosaccharides can be distinguished.

The first group includes monosaccharides **6**, **7** and **14**. These have high $J_{1,2}$ and $J_{2,3}$ values, varying from 6.3 (**14**) to 8.1 Hz (**6**) and from 5.7 (**14**) to

Table 1
Chemical shifts and coupling constants of **1-23**

Compounds	Chemical shifts (ppm)				Coupling constants (Hz)				
	H1	H2	H3	H4	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{1,3}$	$J_{2,4}$
1	5.3(d)	4.2(m)	3.9(m)	6.3(dd)	2.6	nd	4.6		
2	5.3(dd)	5.2(m)	5.2(dd)	6.2(dd)	2.8	2.1	4.6	< 1	1.2
3	5.3(dd)	5.1(m)	5.2(ddd)	6.2(dd)	3.2	2.5	4.2	< 1	0.6
4	5.6(dd)	5.6(m)	5.6(ddd)	6.4(dd)	2.2	1.7	4.7	1.0	1.2
5	5.2(d)	3.3(dt)	4.4(t)	6.5(dd)	4.3	3.7	4.2		
6	4.9(d)	3.9(t)	4.5(dd)	5.9(d)	8.1	7.3	2.5		
7	4.9(d)	3.7(dt)	4.4(dd)	5.9(d)	7.3	6.6	2.9		
8	5.1(m)	5.5(m)	3.9(m)	6.4(dd)	1.6	nd	4.7	< 1	< 1
9	5.1(d)	4.0(t)	5.3(t)	6.1(d)	5.4	4.6	3.8		
10	5.2(d)	4.2(m)	5.5(t)	6.2(d)	4.9	4.1	4.0		
11	5.5(d)	4.6(ddd)	5.2(dd)	6.2(dd)	2.4	1.3	4.8		1.3
12	5.3(d)	3.8(t)	5.3(t)	6.1(d)	3.4	3.2	4.6		
13	5.1(d)	3.8(t)	4.1(t)	6.2(d)	5.3	5.0	3.7		
14	5.1(d)	3.6(t)	4.4(dd)	6.0(d)	6.3	5.7	3.2		
15	5.3(d)	3.9(t)	5.6(t)	6.3(d)	3.4	3.5	4.4		
16	5.1(d)	3.8(t)	4.1(t)	6.2(d)	5.5	4.1	3.7		
17	5.5(dd)	4.6(m)	4.4(t)	6.0(dd)	3.3	3.4	4.3		
18	5.6(d)	4.7(bs)	5.5(m)	5.9(d)	1.4	nd	5.0		1.0
19	5.5(d)	4.6(m)	5.3(t)	5.9(d)	2.5	2.0	5.0		
20	5.5(d)	4.7(t)	5.4(m)	5.9(d)	2.9	2.6	4.4		1.0
21	5.6(d)	4.6(t)	4.4(m)	6.3(d)	3.0	2.5	4.5		
22	4.9(d)	3.6(dd)	4.1(dd)	5.7(d)	7.2	6.5	3.0		
23	5.3(dd)	4.9(m)	5.2(m)	6.1(d)	1.9	nd	4.8		

7.3 Hz (**6**), respectively. These coupling constants are consistent with axial or quasi-axial H-1, H-2 and H-3 protons. The small $J_{3,4}$ constants for these compounds, 2.5–3.2 Hz, also indicate the quasi-axial orientation of H-3, characteristic for a torsion angle between the allylic proton and the alkene of approximately 90° [14]. These results suggest that **6** solely adopts the $^2H_1$ conformation while **7** and **14** primarily adopt the $^2H_1$ conformation.

The second group includes monosaccharides **1–4**, **8** and **11**. These have low values for $J_{1,2}$ (1.6–3.2 Hz) and $J_{2,3}$ (1.3–2.5 Hz) characteristic of the equatorial-quasi-equatorial orientation of the protons observed in the $^1H_2$ conformation. Moreover, the corresponding values of $J_{3,4}$ of 4.3–4.8 Hz is also characteristic of the quasi-equatorial orientation of H-3. Long-range couplings ($J_{1,3}$ and $J_{2,4}$) in these compounds suggest a planar "W" arrangement, consistent with the $^1H_2$ conformation.

The third group contains **5, 9, 10, 12, 13, 15** and **16**. Their $J_{2,3}$ values are higher than expected between the equatorial and the quasi-equatorial protons H-2 and H-3. These data, together with the absence of long-range coupling indicate that either the $^1H_2$ conformation may be considerably deformed or the equilibria between the $^1H_2$ and the $^2H_1$ may significantly be shifted towards $^2H_1$, as a result of strong interactions between functional groups.

The coupling constants $J_{2,3}$ and $J_{3,4}$ were plotted as a function of the coupling constant $J_{1,2}$ (Fig. 4). Inspection of this figure shows a linear relationship in both plots described by the equations

$$J_{2,3} = b + a \bullet J_{1,2} \text{ and } J_{3,4} = d + c \bullet J_{1,2}$$

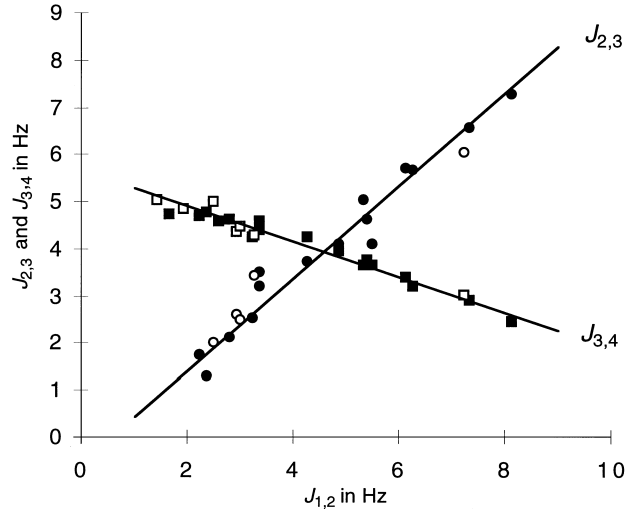


Fig. 4. Plot of $J_{2,3}$ (●) and $J_{3,4}$ (■) in Hz as a function of $J_{1,2}$ for monosaccharides **1–16** and $J_{2,3}$ (○) and $J_{3,4}$ (□) in Hz as a function of $J_{1,2}$ for disaccharides **17–23**.

Table 2
Theoretical coupling constants of **1–23**

Compounds	J values calculated from Haasnoot et al. Eq 15 (Hz)				Extrapolated theoretical J						$^1H_2$ population %
	$^1H_2$		$^2H_1$		$^1H_2$			$^2H_1$			
	$J_{1,2}$	$J_{2,3}$	$J_{1,2}$	$J_{2,3}$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	
1	3.1	6.1	8.0	8.4	2.9	2.4	4.4	8.5	7.7	2.5	100
2	3.1	1.7	8.1	8.6	2.7	2.3	4.5	8.6	7.8	2.5	100
3	2.4	0.8	8.0	8.8	2.0	1.6	4.7	8.5	7.7	2.5	83
4	2.9	1.6	8.0	8.6	2.7	2.2	4.5	8.3	7.6	2.6	100
5	3.3	2.4	7.5	6.3	3.2	2.7	4.3	7.3	6.6	2.9	75
6	3.0	1.3	7.9	7.5	2.6	2.1	4.5	8.0	7.2	2.7	0
7	3.1	1.6	8.0	8.4	2.7	2.3	4.5	8.5	7.7	2.5	20
8	2.9	1.6	8.1	8.4	3.0	2.5	4.4	8.4	7.6	2.6	100
9	3.1	1.8	7.9	8.6	2.8	2.3	4.5	8.5	7.7	2.5	56
10	3.2	1.9	7.9	8.5	2.9	2.4	4.4	8.3	7.5	2.6	65
11	3.1	2.4	7.9	8.1	3.0	2.6	4.4	8.3	7.5	2.6	100
12	3.0	1.9	7.9	8.2	2.9	2.4	4.5	8.3	7.5	2.6	86
13	3.0	2.3	7.9	8.6	2.9	2.5	4.4	8.4	7.6	2.6	53
14	2.9	2.1	8.0	7.6	2.9	2.4	4.5	8.1	7.3	2.7	34
15	3.0	2.1	7.8	7.4	2.9	2.4	4.4	7.9	7.1	2.7	85
16	3.1	2.4	7.9	8.6	3.0	2.5	4.4	8.6	7.8	2.5	63
17	3.2	1.9	8.1	9.0	2.9	2.4	4.4	8.8	8.0	2.4	88
18	3.3	2.0	8.1	9.3	3.0	2.5	4.4	8.9	8.1	2.4	100
19	3.3	2.0	8.1	9.3	3.0	2.5	4.4	8.9	8.1	2.4	100
20	3.2	1.5	8.1	9.3	2.7	2.3	4.5	9.0	8.1	2.4	95
21	3.2	1.9	8.1	8.9	2.9	2.4	4.4	8.7	7.9	2.5	99
22	3.1	1.9	8.0	9.4	2.9	2.4	4.5	8.5	7.7	2.5	22
23	3.1	1.7	8.1	9.3	2.9	2.4	4.4	8.9	8.1	2.4	100

with $a = 0.94 \pm 0.05$; $b = -0.29 \pm 0.26$; $c = -0.34 \pm 0.04$; $d = 5.43 \pm 0.16$. These values are in excellent agreement with those obtained for similar plots for unsaturated disaccharides ($a = 0.98 \pm 0.07$; $b = -0.44 \pm 0.36$; $c = -0.35 \pm 0.05$; $d = 5.67 \pm 0.22$;) previously reported by Ragazzi et al. [7]. Molecular modeling of uronates **1–16** was done using the package SYBYL (version 6.3) from Tripos Inc., St Louis, MO. All energy calculations were performed using parameters from the Tripos force-field. Charges were assigned according to the Gasteiger–Huckel protocol. A distance dependent dielectric constant ($\varepsilon = 1.5$) was used to compensate for the lack of explicit solvation. Molecular mechanics calculations led to two energy minima, corresponding to the half-chairs $^1H_2$ and $^2H_1$. These were taken as the starting points of calculation for all the other uronate derivatives. Ragazzi et al. [7] have also shown that for disaccharides containing similar Δ^4-uronic acid residues, no other minimum ring energy conformation is found. These observations together with the linear relationships described in the equations above, confirm that the coupling constants of these different compounds can be interpreted in terms of an equilibrium between the two conformers, $^1H_2$ and $^2H_1$.

The theoretical J values can be calculated from a Karplus type equation described by Haasnoot et al. [15] containing a correction term for α- and β-electronegative substituents. Using this equation and the calculated dihedral angles H–C–C–H corresponding to the two minimum energy conformers of each uronate, the theoretical coupling constants $J_{1,2}$ and $J_{2,3}$ were calculated (Table 2). These values are in good agreement with the straight line, best fit of the experimental values as shown in Fig. 5. The

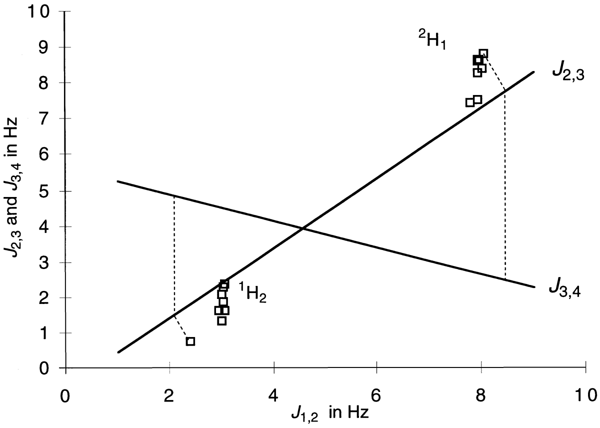


Fig. 5. Plot of the theoretical $J_{1,2}$ and $J_{2,3}$ values (□) calculated from the Haasnoot et al. [15] equation.

Haasnoot et al. [15] equation has not been parameterized for unsaturated moieties and thus, the $J_{3,4}$ values could not be calculated. These $J_{3,4}$ values can, however, be estimated by taking the closest points lying on the experimental lines. These new sets of theoretical values, determined from the Haasnoot et al. [15] equation and from these linear relationships (Table 2) were used for the calculation of the conformer populations, by least-square fitting the experimental coupling constants to the theoretical data. Since the $J_{2,3}$ values for compounds **1** and **8** could not be determined directly from the corresponding ^{1}H NMR spectra, the conformer population for these two uronates were determined from their $J_{2,3}$ values estimated from their $J_{1,2}$ values and the experimental plot $J_{3,4} = f(J_{1,2})$. The quantitative results presented in Table 2 are in good accordance with the previous qualitative conclusions deduced from the ^{1}H NMR study. The conformational equilibria were also determined using $J_{1,2}$ and $J_{2,3}$ alone, without including $J_{3,4}$. The corresponding least-square fitting gave populations very close to the ones previously calculated with a maximum deviation of 3% (except for **22** with 7% deviation).

As expected, Δ^4-uronates **6, 7** and **14**, bearing a bulky electrorepulsive substituent such as a silyl group at the O-3 position, exist mainly in the $^2H_1$ conformation, in which all the substituents adopt the more energetically favored equatorial positions. Thus, the siloxane uronate **6** exists solely as the $^2H_1$ conformer, and the 3-*O*-*tert*-butyldimethylsilyl uronate **7** mainly as the $^2H_1$ conformer (80%). A substituent bulkier than hydrogen at the O-2 position shifts the equilibrium to the $^1H_2$ conformer, as seen for the 2-*O*-benzyl **14** which exists as 67% $^2H_1$ conformer. This is presumably the result of unfavorable steric interactions between the substituents at the O-2 and O-3 positions. The introduction of a second bulky *tert*-butyldimethylsilyl group (compound **5**), clearly amplifies this effect towards the $^1H_2$ conformation, $^1H_2$ becoming the predominant conformer at 75%. It was expected that the predominant $^2H_1$ conformation would be observed for uronates bearing a 3-*O*-benzyl substituent such as **13** and **16**. However, the presence of a second benzyl group at the O-2 position in these two compounds generate both electronic and steric interactions, shifting the equilibrium from $^2H_1$ to $^1H_2$. The population of **13** and **16** in the $^2H_1$ conformer was estimated to be only 47 and 37%, respectively. The 2,3-di-*O*-acetyl and 2,3-di-*O*-benzoyl uronates **2** and **4** exist solely as the $^1H_2$ conformer, the 2,3-di-*O*-pivaloyl derivative **3** and the 2-*O*-benzyl-3-*O*-acyl derivatives **12** and **15**, mainly in the $^1H_2$ conformation (83, 89 and 85%, respectively). This can be explained by an allylic effect [16], which consists in the stabilization of quasi-axial polar allylic substituents. Surprisingly, the 3-*O*-pivaloyl and 3-*O*-benzoyl derivatives **9** and **10** exist in 56 and 65% in the $^1H_2$ conformation, respectively. This observation can be explained by the presence of intramolecular hydrogen bonding between the 2-hydroxyl and the 3-*O*-acyl substituents, clearly shown by molecular modeling of these two compounds.

The chemical shifts and coupling constants obtained for the uronate residues of the disaccharides **17–23** are given in Table 1, and the $J_{2,3}$ and $J_{3,4}$ values are plotted as a function of $J_{1,2}$. (Fig. 4). These experimental values again fit very well with the previously described linear equations, showing that these uronate residues in these disaccharides exist in an equilibrium between the $^1H_2$ and $^2H_1$ conformers. For compounds **18–21** and **23**, Table 1 shows small $J_{1,2}$ (1.4–3.0 Hz) and $J_{2,3}$ (2.0–2.7 Hz) values, suggesting that these disaccharides mainly exist as the $^1H_2$ conformer. The $J_{1,2}$ and $J_{2,3}$ values of 7.2 and 6.5 Hz, respectively for **22** suggest it should primarily adopt the $^2H_1$ conformation. Finally, **17** having intermediate $J_{1,2}$ and $J_{2,3}$ values of 3.3 and 3.4 Hz, should exist as an equilibrium between the $^1H_2$ and $^2H_1$ conformers. The population of each conformer of these disaccharides was calculated as previously described for the monosaccharides (Table 2). As expected, **18, 19** and **23** are exclusively in the $^1H_2$ conformation, and **17, 20** and **21** exist mainly as the $^1H_2$ conformer (88, 95 and 99%, respectively). Disaccharide **22** adopts mainly the $^2H_1$ conformation (77%). For these compounds, the equilibrium is essentially controlled by electrostatic and hydrogen bonding interactions. It should be noted that the calculated conformer populations of compounds **17** and **23** (12 and 77% in the $^1H_2$ conformer) are in accordance with the percentages reported by Ragazzi et al. [7] (8 and 57%, respectively).

3. Experimental

General methods.—^{1}H NMR spectra were recorded at 25 °C, in deuterochloroform on a Varian

Unity 500 MHz spectrometer. Chemical shifts were recorded in ppm (δ) and coupling constants in Hz, relative to tetramethylsilane as internal standard. The ^{1}H NMR spectra were fully assigned by the use of single frequency decoupling. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Thin layer chromatography (TLC) was performed using Merck plates of silica gel 60 with fluorescent indicator. Visualization was effected by spraying plates with Von's reagent [5] followed by heating at 140 °C. Reactions performed on sulfated disaccharides were monitored by capillary electrophoresis (CE) [5]. CE was performed on a Dionex CE system (Sunnyvale, CA) equipped with a variable wavelength detector. All analyses used a fused silica capillary tube (75 μm i.d., 375 μm o.d. and 75 cm long) from Dionex. Operating buffer was 10 mM sodium borate, 50 mM sodium dodecylsulfate adjusted to pH 8.8 with 1 N HCl. Flash chromatography was conducted with silica gel (230–430 mesh, Merck). Dichloromethane (CH_2Cl_2), dimethylformamide (DMF), methanol, pyridine and tetrahydrofuran (THF) were anhydrous solvents available from Aldrich, Milwaukee, WI.

*Methyl (benzyl 4-deoxy-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**1**).—A solution of **2** (5.0 g, 13.7 mmol) in anhydrous methanol (150 mL) containing triethylamine (0.5 mL, 3.6 mmol) was stirred at room temperature under argon for 36 h. The reaction mixture was concentrated under reduced pressure and subjected to flash chromatography (acetone–hexane, v/v 1:4) to give **1** (3.4 g, 87%) as a white amorphous solid. A portion of the pure product was crystallized from ethanol to afford white needles; mp 139–140; $[\alpha_D]^{22} = -106°$ (ca 1, $CHCl_3$): Lit [17]. mp 139–140 (ethanol); $[\alpha_D]^{22} = -94.5°$ (*c* 0.5, CH_3OH); FABMS (+ve) *m/z* 303 $[M+Na^+]^+$; Anal. calcd for $C_{14}H_{16}O_6$ (280.3) C 60.00, H 5.75; Found: C 59.76, H 5.95.

*Methyl (benzyl 2,3-di-*O*-acetyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**2**).—DBU (3.6 mL, 24.1 mmol) was added to a solution of **1a** [6] (8.2 g, 19.3 mmol) in anhydrous CH_2Cl_2 (100 mL) at 0 °C and under argon. After 10 min the ice bath was removed and stirring continued for 7 h at room temperature. The solution was cooled with an ice bath and quenched with 50 mL cold saturated aq. NH_4Cl. The mixture was diluted with 150 mL CH_2Cl_2 and extracted with an additional 150 mL saturated aq. NH_4Cl, then saturated aq. NaCl. The organic layer was dried over anhydrous Na_2SO_4, filtered and evaporated to afford a black oil. Flash chromatography (ethyl acetate–hexane, v/v 1:5) provided **2** (6.4 g, 91%) as a colorless oil. $[\alpha_D]^{22} = -103°$ (*c* 1, $CHCl_3$); FABMS (+ve) *m/z* 387 $[M+Na^+]^+$; Anal. calcd for $C_{18}H_{20}O_8$ (364.4) C 59.34, H 5.53; Found: C 59.14, H 5.58.

*Methyl (benzyl 4-deoxy-2,3-di-*O*-pivaloyl-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**3**).—Compound **1b** (14.5 g, 26.3 mmol) was treated as described for **2**. Flash chromatography (ethyl acetate–hexanes, v/v 1:8) afforded **3** (6.4 g, 91%) as a colorless oil. $[\alpha_D]^{22} = -8.6°$ (*c* 2, $CHCl_3$); FABMS (+ve) *m/z* 471 $[M+Na^+]^+$; Anal. calcd for $C_{24}H_{32}O_8$ (448) C 64.27, H 7.19; Found: C 63.91, H 7.30.

*Methyl (benzyl 2,3-di-*O*-benzoyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**4**).—To a solution of **1** (220 mg, 0.78 mmol) in anhydrous pyridine (8 mL) at 0 °C and under argon was added benzoyl chloride (0.36 mL, 3.14 mmol). After 5 h at room temperature, the reaction mixture was quenched with MeOH (3 mL) and concentrated under reduced pressure. Purification of the resulting oil by flash chromatography (ethyl acetate–hexanes, v/v 1:10) gave **4** (380 mg, quant.) as white needles; $[\alpha_D]^{22} = 25.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{28}H_{24}O_8$ (488.5) C 68.95, H 4.95; Found: C 68.73, H 5.17.

*Methyl (benzyl-2,3-di-*O*-t-butyldimethylsilyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**5**).—To a solution of **1** (1.0 g, 3.57 mmol) in anhydrous DMF (10 mL) at 0 °C and under argon were added imidazole (972 mg, 14.27 mmol) and TBDMSCl (2.15 g, 14.27 mmol). After 12 h at room temperature, the reaction mixture was poured into water (10 mL). The aqueous layer was extracted 2 times with $CHCl_3$ (10 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to afford a pale yellow oil. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:15) provided **5** (1.79 g, quant.) as a colorless oil. $[\alpha_D]^{22} = 29.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{26}H_{44}O_6Si_2$ (508.8) C 61.38, H 8.72, Found: C 61.89, H8.90.

*Methyl (benzyl 4-deoxy-2,3-tetraisopropyldisiloxane-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**6**).—To a solution of **1** (100 mg, 0.36 mmol) in anhydrous pyridine (5 mL) at 0 °C and under argon was added 1,3-dichloro-1,1,3,3-tetraisopropyl-1,3-disiloxane (150 μL, 0.46 mmol). After 12 h at room temperature, the reaction mixture was poured into water (5 mL). The aqueous layer was extracted 2 times with ethyl acetate (5 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:30) afforded **6** (164 mg, 88%) as a colorless oil. $[\alpha_D]^{22} = 18.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{26}H_{42}O_7Si_2$ (522.8) C 59.73, H 8.10; Found: C 59.78, H 8.34.

*Methyl (benzyl-3-*O*-t-butyldimethylsilyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid) uronate* (**7**).—To a solution of **1** (500 mg, 1.78 mmol) in anhydrous pyridine (10 mL) at 0 °C and under argon was added TBDMSCl (269 mg, 1.78 mmol) and the reaction mixture was allowed to warm to room temperature. Every 12 h an additional equivalent of TBDMSCl was added until the disappearance of **1**. The reaction mixture was poured into water (10 mL). The aqueous layer was extracted 2 times with chloroform (10 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:10) gave **7** (575 mg, 82%) and methyl (benzyl 4-deoxy-2-*O*-*tert*-butyldimethylsilyl-*α*-L-threo-hex-4-enopyranosid) uronate (35 mg, 5%) as colorless oils. $[\alpha_D]^{22} = 28.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{20}H_{30}O_6Si$ (394.5) C 60.89, H 7.66; Found: C 61.28, H 7.66.

*Methyl (benzyl 4-deoxy-2-*O*-pivaloyl-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**8**) *and methyl (benzyl 4-deoxy-3-*O*-pivaloyl-α-*L*-*threo*-hex-4-enopyranosid)-uronate* (**9**).—To a solution of **1** (1.0 g, 3.57 mmol) in anhydrous pyridine (8 mL) at 0 °C and under argon was added pivaloyl chloride (0.48 mL, 3.92 mmol). After 12 h at room temperature, the reaction mixture was quenched with water (5 mL) and extracted 2 times with chloroform (10 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:6) yielded the dipivaloyl uronate **3** (221 mg, 14%), the 2-*O*-pivaloyl uronate **8** (48 mg, 4%) and the 3-*O*-pivaloyl uronate **9** (857 mg, 66%) as colorless oils. **8**: $[\alpha_D]^{22} = 49.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{19}H_{24}O_7$ (364.4) C 62.63, H 6.64; Found: C 62.25, H 6.65. **9**: $[\alpha_D]^{22} = -22.0°$ (*c* 1, $CHCl_3$); Anal. calcd for $C_{19}H_{24}O_7$ (364.4) C 62.63, H 6.64; Found: C 62.94, H 6.59.

*Methyl (benzyl 3-*O*-benzoyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**10**).—To a solution of **7** (137 mg, 0.35 mmol) in anhydrous pyridine (10 mL) at 0 °C and under argon was added benzoyl chloride (79 *μ*L, 0.69 mmol). After 2 h at room temperature, the reaction mixture was quenched with MeOH (5 mL) and concentrated under reduced pressure. The residue was dissolved under argon in anhydrous THF (10 mL) and reacted at room temperature with tetrabutylammonium fluoride (0.42 mL, 0.42 mmol). After 2 h at room temperature, the reaction mixture was concentrated under reduced pressure. Purification of the resulting oil by flash chromatography (ethyl acetate–hexane, v/v 1:20) gave the methyl (benzyl 2-*O*-benzoyl-4-deoxy-*α*-L-threo-hex-4-enopyranosid)-uronate (36 mg, 34%), **10** (39 mg, 37%) and the dibenzoyl uronate **4** (7 mg, 5%) as colorless oils. **10**: $[\alpha_D]^{22} = 34.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{21}H_{20}O_7$ (384.4) C 65.62, H 5.24; Found: C 65.56, H 5.63.

*Methyl (benzyl 2-*O*-diethylphosphate-3-*O*-pivaloyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranoside) uronate* (**11**).—Iodide (265 mg, 1.04 mmol) was added to a solution of triethylphosphate (0.2 mL, 1.14 mmol) in anhydrous dichloromethane (5 mL) at 0 °C and under argon. After 5 min, this solution was added to a solution of **9** (345 mg, 0.95 mmol) in anhydrous dichloromethane (10 mL) and pyridine (0.31 mL, 3.8 mmol) cooled at −40 °C. After 30 min at −40 °C, the reaction mixture was allowed to warm at room temperature, diluted with dichloromethane (10 mL), washed with saturated aq. $NaHSO_4$, then with phosphate buffer (pH 7), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The resulting oil was purified by flash chromatography (ethyl acetate–hexane, v/v 1:3) to give **11** (472 mg, quant.) as a colorless oil. $[\alpha_D]^{22} = 8.0°$ (*c* 0.1, $CHCl_3$); Anal. calcd for $C_{23}H_{33}O_{10}P$ (500.5) C 55.20, H 6.65; Found: C55.08, H 6.69.

*Methyl (benzyl 2-*O*-benzyl-4-deoxy-3-*O*-pivaloyl-α-*L*-*threo*-hex-4-enopyranosid) uronate* (**12**).—**14** (73.8 mg, 0.13 mmol) in methanol (5 mL) under argon was transesterified at room temperature with sodium methoxide (0.13 mmol). After 24 h the reaction mixture was neutralized with resin Amberlyst IR 120 (H^+), filtered and concentrated under reduced pressure. The resulting residue was dissolved under argon in anhydrous THF (5 mL) and reacted at room temperature with tetrabutylammonium fluoride (0.16 mL, 0.16 mmol).

After 2 h, the reaction mixture was concentrated under reduced pressure. The resulting residue was dissolved in anhydrous pyridine (5 mL) under argon and acylated by addition of pivaloyl chloride (24 μL, 0.20 mmol). After 12 h at room temperature, the reaction mixture was quenched with water (2 mL) and extracted with chloroform. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:15) afforded **12** (41 mg, 70%) as a colorless oil. Anal. calcd for $C_{26}H_{30}O_7$ (454.5) C 68.71, H 6.65; Found: C 69.12, H 6.99.

*Benzyl (benzyl 2,3-di-*O*-benzyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid) uronate* (**13**).—Sodium methoxide (380 mg, 7 mmol) was added to a vigorously stirred suspension of **1a** (13.2 g, 21.2 mmol) in methanol (100 mL) under argon with ice bath cooling. After 22 h at room temperature, the reaction mixture was cooled to 0 °C, neutralized by adding resin Amberlyst IR 120 (H^+), filtered and concentrated under reduced pressure. Flash chromatography (chloroform–methanol, v/v 13:1) afforded the corresponding methyl (benzyl β-D-glucopyranosid)uronate (8.4 g, 90%) as a white amorphous solid. To a solution of the previous compound (0.18 g, 0.60 mmol) in dry DMF (4 mL) under argon and cooled with an ice-water bath were added benzyl bromide (0.64 mL, 5.4 mmol), Ag_2O (1.4 g, 6.0 mmol) and crushed 4 Å molecular sieves. After 48 h at room temperature, the reaction mixture was diluted with dichloromethane (10 mL), filtered through a pad of celite and concentrated under reduced pressure. Purification by chromatography on silica gel (ethyl acetate–hexane, v/v 1:17) gave **13** (0.13 g, 40%). $[\alpha_D]^{22} = -30°$ (c 1, $CHCl_3$).

*Benzyl (benzyl 2-*O*-benzyl-3-*O*-*tert*-butyldimethylsilyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**14**).—**7** (575 mg, 1.46 mmol) was reacted 2 h at 50 °C with methanolic KOH 0.5 M (4 mL, 1.66 mmol). After disappearance of **7**, the reaction mixture was neutralized with resin Amberlyst IR 120 (H^+), filtered and concentrated under reduced pressure. The residue was dissolved in anhydrous DMF (5 mL) under argon and reacted 30 min with NaH (84 mg, 7.35 mmol). Benzyl chloride (0.51 mL, 4.37 mmol) was added to the reaction mixture. After 48 h at room temperature, the reaction mixture was cooled to 0 °C, quenched with water, and extracted with chloroform (2×5 mL). The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a pale yellow oil. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:30) yielded **14** (458 mg, 56%) and **13** (204 mg, 26%) as colorless oils.

*Benzyl (benzyl 2-*O*-benzyl-3-*O*-benzoyl-4-deoxy-α-*L*-*threo*-hex-4-enopyranosid) uronate* (**15**).—**14** (117 mg, 0.21 mmol) was dissolved under argon in dry THF (5 mL) and desilylated by addition of tetrabutylammonium fluoride (1M in THF, 0.23 mL, 0.23 mmol). After 2 h at room temperature, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in dry pyridine (5 mL) and acylated by addition of benzoyl chloride (47 μl, 0.42 mmol). After 12 h at room temperature, the reaction mixture was quenched with water and extracted with chloroform. The combined organic layers were washed with water, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Purification by flash chromatography (ethyl acetate–hexane, v/v 1:15) afforded **15** (114 mg, 90%). Anal. calcd for $C_{34}H_{30}O_7$ (550.6) C 74.17, H 5.49. Found: C 74.37, H 6.11.

*Benzyl (benzyl 2-*O*-benzyl-4-deoxy-3-*O*-p-methoxybenzyl-α-*L*-*threo*-hex-4-enopyranosid)uronate* (**16**).—To a solution of **14** (90 mg, 0,20 mmol) in anhydrous dichloromethane (5 mL) under argon was added *p*-methoxybenzyltrichloroacetamidate [13] (0.60 mmol) and a catalytic amount of *p*-toluenesulfonic acid. After 20 h at room temperature, the reaction mixture was neutralized with triethylamine and concentrated under reduced pressure. Purification of the resulting oil by flash chromatography (ethyl acetate–hexane, v/v 1:15) afforded **16** (85 mg, 75%) as a colorless oil.

*3-*O*-acetyl-4-deoxy-2-sulfate-α-*L*-*threo*-hex-4-enopyranosyluronic acid-(1→4)-3-*O*-acetyl-2-deoxysulfamido-6-*O*-sulfo-α-*D*-glucopyranose, tetrasodium salt* (**19**).—The tetrasodium salt of disaccharide **18** (90 mg, 0.11 mmol) was suspended with stirring, under argon, in DMF (3 mL) and formamide (200 μL). At 4 °C, piperidine (0.55 mL, 5.5 mmol) was added and the reaction stirred at RT until CE analysis showed the disappearance of starting material. After 17 h, the solution was diluted with 2 mL of water and passed through a column (1×10 cm) of Dowex (H^+) followed by a column (1×10 cm) of Dowex (Na^+). Piperidine was still present in the sample, indicating incomplete salt exchange had occurred. This solution was adjusted at pH 6.8 with dilute HCl, and the volume reduced to 20 mL by evaporation. The concentrated product was decolorized with activated carbon at 40 °C and filtered through a celite pad. The eluent was concentrated under reduced pressure to 6 mL and precipitated with acetone providing a white precipitate that become tan upon standing. The remaining supernatant was evaporated to 2 mL and again precipitated with acetone. The combined precipitates were taken up in water, eluted with water from a 2.5×25 cm Biogel P-2 column and lyophilized. CE analysis showed a purity greater than 95%. Neutralization of the eluent with sodium hydroxide to pH 5.8 afforded, after lyophilization **19** (0.083 mmol, 73%). FABMS (-ve) *m/z* 748, 726, 704, 682 $[M\text{-}5H+4Na]^-$, $[M\text{-}4H+3Na]^-$, $[M\text{-}3H+2Na]^-$, $[M\text{-}2H+Na]^-$, ^1H NMR (500 MHz, D2O), δ 2.09, 2.11 (2s, 6H, 2 OAc), 3.46 (dd, 1H, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 10 Hz, H-2), 4.19 (dd, 1H, $J_{3,4}$ 10 Hz, $J_{4,5}$ 10 Hz, H-4), 4.24 (m, 1H, H-5), 4.31 (dd, 1H, $J_{5,6a}$ 11 Hz, $J_{6a,6b}$ 2.5 Hz, H-6a), 4.34 (dd, $J_{5.6b}$ 4.5 Hz, H-6b), 4.63 (m, 1H, H-2′), 5.13 (dd, 1H, H-3), 5.31 (m, 1H, H-3′), 5.40 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1), 5.54 (d, 1H, $J_{1',2'}$2.5 Hz, H-1′), 5.94 (dd, 1H, $J_{3',4'}$ 5.0 Hz, $J_{2',4'}$ 1 Hz, H-4′).

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