Chapter 6

Stereoelectronic substituent effects determine the conformational preferences of mannuronic acid based iminosugar cations

6.1 Introduction

Stereoelectronic effects of substituents on a cyclic compound have a profound effect on its three-dimensional structure. Where substituents on a cyclic compound generally have a preference for an (pseudo)-equatorial position for steric reasons, the electronic spatial preferences depend on different forces such as charge-charge and dipole-dipole interactions.[1] The conformation and reactivity of carbohydrates is determined to a large

extent by the nature and orientation of the substituents. This influence becomes apparent in glycosylation reactions, where the amount, nature and orientation of the hydroxyl groups, protected with electron withdrawing esters or more electron neutral ether groups, determine the overall reactivity.[2-4] It has long been known that in glycosylations axial substituents are less deactivating or "disarming" than their equatorially positioned equivalents.[5-9] Similarly, the basicity of iminosugars (or "azasugars"), carbohydrates of which the endocyclic oxygen is replaced by an amine, is influenced by the orientation of the ring substituents and azasugars bearing more axially positioned hydroxyl groups are more basic than their stereoisomers bearing equatorially positioned substituents.[10-13] These effects can be explained by the interaction of the electronegative oxygen substituents with the positive charge present on the azasugar ring in a protonated state and the (partial) positive charge of oxocarbenium ion (-like) intermediates in glycosylation reactions. Two explanations can be forwarded to account for the more favorable interaction of axially positioned oxygen substituents with the positive charge in the carbohydrate and azasugar rings. Firstly, the interaction of the dipole moment associated with the ring substituent and the positive charge is less unfavorable if the substituent is positioned axially.[10,12-13] Secondly, properly positioned oxygen substituents can donate electron density into the electron-depleted carbocations.[14-16]

Insightful studies of the Woerpel laboratory, involving a series of addition reactions onto mono-substituted five and six-membered oxocarbenium ions, have shown that electronegative substituents at the C3 position in furanosides and at the C3 and C4 position in pyranosides, prefer to adopt a pseudoaxial orientation in an oxocarbenium ion, thereby determining which envelope (the ^{3}E or E_3) or half chair (the $^{3}H_4$ or $^{4}H_3$) is energetically more favorable.[1,14-20] In Chapter 2 a free energy surface (FES) scanning method was introduced to determine the energies of all possible conformers of fully substituted furanosyl oxocarbenium ions. The calculations described in this work corroborated the axial preference for the furanosyl ring C3 substituent, determined experimentally by the Woerpel laboratory, and also provided detailed insight into the overall effect of the full decoration of the furanosyl rings.

In glycuronic acids, pyranosides having a C5 carboxylic acid appendage, the electron withdrawing carboxylic acid functionality also has a profound effect on the reactivity of the pyranoside, making glycuronic acids generally less reactive than their "non-oxidized" counterparts.[21-22] In studies towards the glycosylation properties of mannuronic acids it has been noted that mannuronic acid derived glycosyl donors display an unusual high reactivity.[23-25] In addition, glycosylation reactions of mannuronic acid donors proceed with a striking stereoselectivity to provide the 1,2-*cis*-linked products.[22-24,26-28] To account for these results it has been postulated that the $^{3}H_4$ oxocarbenium ion plays a decisive role. In

this oxocarbenium ion conformer the C3 and C4 oxygen substituents take up their preferred axial orientation, where the C2 oxygen atom is placed equatorially to allow for a hyperconjugative stabilization of the neighboring oxocarbenium ion. Importantly, the C5 carboxylic acid is placed in an axial position in this half chair, an orientation that is energetically significantly favored over the alternative equatorial position.[25,27] Thus, all ring substituents can adopt an energetically most favorable orientation in the $^{3}H_{4}$ half chair oxocarbenium ion, making this species relatively favorable. It has also been observed that various mannuronic acid donors are inclined to undergo a conformational flip towards the "axially rich" $^{1}C_{4}$ chair and this unusual conformational behavior has been rationalized by linking their structural preference to that of the mannuronic acid oxocarbenium ion.[28]

Because of the parallel between glycosyl donor reactivity, conformational behavior and azasugar basicity the properties of mannuronic acid based azasugars are studied in this chapter. Bols and co-workers have previously described a great variety of azasugars and related their basicity to the amount of axially oriented substituents.[12-13,29] They also noted that in specific cases azasugar rings flip their conformation to position the substituents such that their electron withdrawing effect is minimized.[12] Here the conformational behavior of a set of mannuronic acid based azasugars is described as studied by NMR spectroscopy and through DFT calculations (Figure 6.1). It is shown that the conformational flexibility displayed by (fully protected) mannuronic acid glycosyl donors (in apolar solvents) extends to mannuronic acid azasugars in very polar solvents (H_2O).

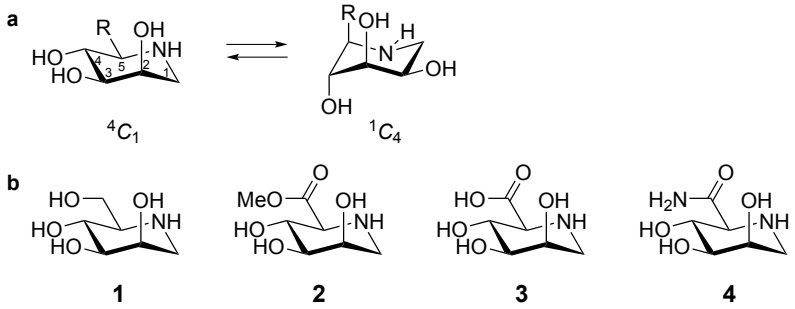
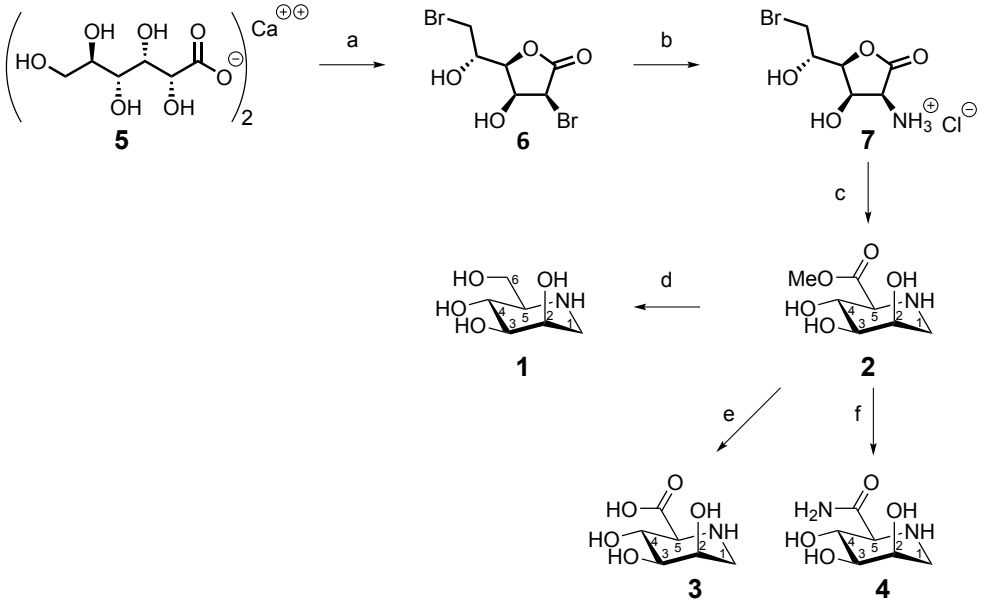


Figure 6.1 a) The $^{4}C_{1}$ and $^{1}C_{4}$ chair conformations. b) The investigated iminosugars, DMJ (**1**), DMJ methyl ester (**2**), DMJ acid (**3**), DMJ amide (**4**).

6.2 Results and discussion

The four azasugars investigated here are depicted in Figure 6.1 and they include the mannuronic acid ester azasugar **2**, the free acid **3** and amide **4** and the "reduced" counterpart, 1-deoxymannojirimicin **1** (DMJ) for comparison. The synthesis of DMJ **1** and its C5 analogs was achieved according to the route devised by Wrodnigg and co-workers.[30] As depicted in Scheme 6.1, methyl 2,6-dideoxy-2,6-imino-D-mannonate (**2**) was obtained in four steps from the commercially available calcium D-gluconate monohydrate (**5**).[31] Acid **5** was treated with HBr in acetic acid to form 3,5-di-*O*-acetyl-2,6-dideoxy-2,6-dibromo-D-manno-1,4-lactone after a series of acid catalyzed transformations (*i.e.* substitution of the C2 and C6 hydroxyl groups, intramolecular ring closure and acetylation of the remaining hydroxyl groups). Next the acetyl groups at O3 and O5 were removed in an acid catalyzed transesterification with methanol to provide the pure dibromolactone **6** after crystallization from chloroform/water in 26% yield over the two steps. Regioselective displacement of the C2-bromide with an azide occurred with retention of configuration, which Bock et al.[32] proposed to originate from epimerization of the C2-bromide to the higher reactive glucose configuration before introduction of the azide. This reaction gave, after palladium catalyzed reduction of the intermediate azide and subsequent crystallization from ethanol, the 2-amino-6-bromo-lactone (**7**) as its hydrochloric acid salt in 55%. Treatment of the salt with triethylamine in methanol led to ring opening and intramolecular bromide displacement by the C2 amine to give methyl ester **2**. Purification of this compound from the triethylammonium and sodium salts formed in the reaction proved difficult, because of the high polarity of the compound as well as the lability of the methyl ester towards hydrolysis. Attempts to crystallize the compound were to no avail. Therefore, all hydroxyl groups in **2** were capped with trimethylsilyl groups[33] to allow for the purification of the compound by chromatography. After desilylation, the pure methyl ester (**2**) was obtained as its hydrochloric acid salt. DMJ (**1**) was synthesized from **2** by a sodium borohydride mediated reduction and was obtained in 29% yield after column chromatography. D-Mannonic acid **3** and amide **4** were obtained from **2** through saponification with sodium hydroxide or aminolysis with methanolic ammonia respectively.

Scheme 6.1 Synthesis of DMJ and its C5 analogs for this study.



Reagents and conditions: (a) i) HBr, AcOH; ii) MeOH, 26% over 2 steps; (b) i) NaN_3, acetone; ii) H_2, Pd/C, HCl (aq.), MeOH, 55% over 2 steps; (c) Et_3N, MeOH, quant.; (d) $NaBH_4$, EtOH, 29%; (e) NaOH, H_2O, quant.; (f) NH_3, MeOH, quant.

The conformational behavior at different pH* (the pH measured in D_2O) values of the set of azasugars was investigated by NMR spectroscopy.[34] In Figure 6.2, the ^{1}H NMR spectra of DMJ (**1**) in D_2O at pH* 1-12 are shown. From pH* 1 to pH* 6.5 no changes are observed in either chemical shifts or coupling constants. The coupling constants are indicative of a "normal" $^4C_1$ chair conformation for the azasugar ring. Going from pH* 6.5 to pH* 12 a significant shift in chemical shift is observed for all ring protons, with the direct neighbors of the amino group experiencing the largest shift. No changes occur in the coupling constants of the ring protons, indicating that no major conformation change takes place.

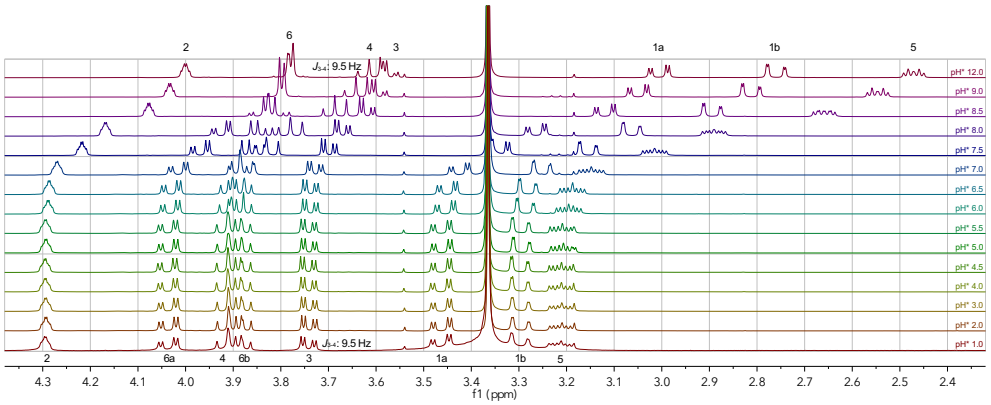


Figure 6.2 ^{1}H NMR spectra for DMJ (**1**) under different pH*; spectra are referenced to residual methanol, intensities are aligned to H2.

In Figure 6.3, the ^{1}H NMR spectra of methyl ester **2** at different pH* values are displayed. Because hydrolysis of the methyl ester was observed above pH* 8, no spectra were recorded above this pH*. Large chemical shift changes are seen with increasing pH*. Especially H5 undergoes a large chemical shift change and shifts from δ = 4.04 at pH* 2 to 3.22 at pH* 8. Also a change in coupling constants is observed for the ring protons. For example, the $J_{3,4}$ changes from 9.4 Hz at basic pH* to 7.5 Hz at acidic pH*, indicative of a change in conformation of the azasugar ring. At high pH* the azasugar adopts a single conformation, where both the $^{1}C_{4}$ and $^{4}C_{1}$ conformers are present at low pH* (*vide infra*).

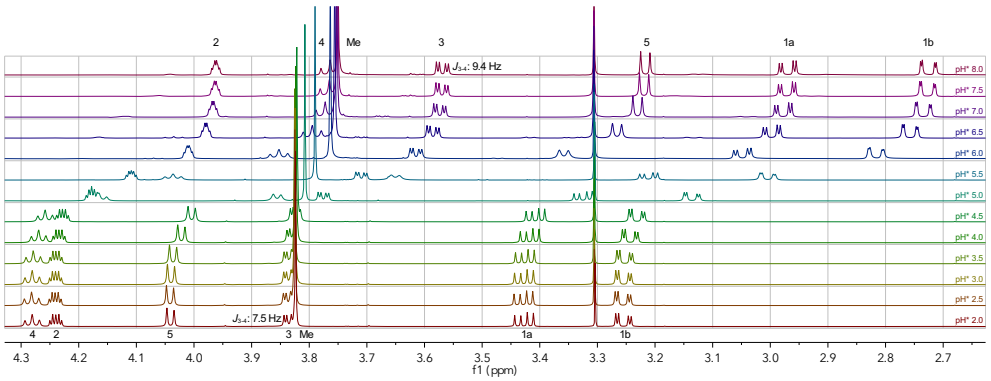


Figure 6.3 ^{1}H NMR spectra for 2,6-dideoxy-2,6-imino-mannonic acid methyl ester (**2**) under different pH*; spectra are referenced to residual methanol, intensities are aligned to the methyl ester.

Mannonic acid **3** can occur in three different charged states: the fully protonated state, the neutral zwitterionic state and the negatively charged state. In Figure 6.4, the ^{1}H NMR spectra of **3** are shown from pH* 1 to pH* 12. Again large chemical shift changes are observed (especially for H5 shifting from 3.9 to 2.9 ppm). Also a small change in coupling constants is apparent. The $J_{3,4}$ changes from 9.8 at high pH* to 8.8 Hz at neutral pH* to 8.3

Hz at acidic pH*. In line with the conformational behavior of methyl ester **2**, mannonic acid **3** can change its conformation in a pH-dependent manner.

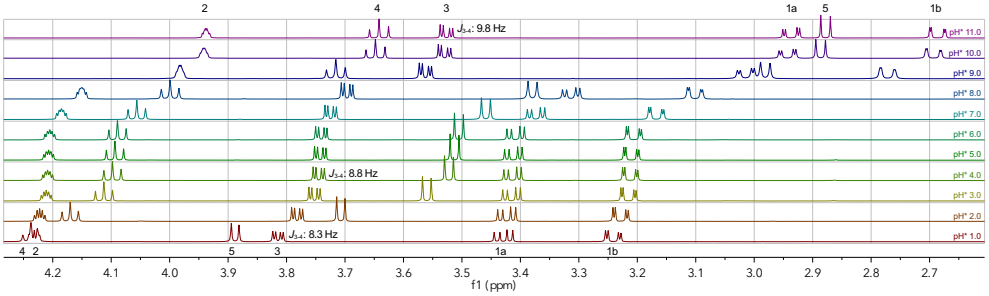


Figure 6.4 ^{1}H NMR spectra for 2,6-dideoxy-2,6-imino-mannonic acid (**3**) under different pH*; spectra are referenced to water, intensities are aligned to H4.

Figure 6.5 displays the collection of ^{1}H NMR spectra for amide **4** at different pH* values. Smaller changes are observed for the chemical shift change of H5 and there is no significant change of the coupling constants, indicating minimal conformation changes going from high to low pH*.

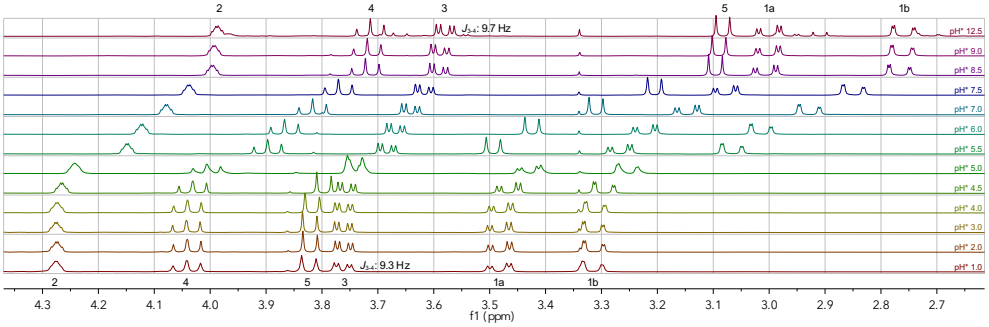


Figure 6.5 ^{1}H NMR spectra for 2,6-dideoxy-2,6-imino-mannonic amide (**4**) under different pH*; spectra are referenced to water, intensities are aligned to H2.

Although it is clear from the ^{1}H NMR spectra depicted above that both the methyl ester **2** and the acid **3** can undergo a pH-dependent conformational change, it is not possible to accurately determine the ratio of $^{1}C_{4}$ and $^{4}C_{1}$ conformers present because the interconversion between the two chair forms is too fast at ambient temperature, leading to coalescence of the resonances of both conformers. Therefore DFT calculations were used to determine the coupling constants of the two conformers of both the protonated and deprotonated azasugars. To this end a set of starting conformers was generated and the structures were optimized with Gaussian 03[35], by employing the B3LYP density functional and the 6-31G* basis set. The solvation in these optimizations was accounted for using the polarizable continuum model (PCM) function for the solvent used (H_2O or

methanol). The structures of relevant optimized chair conformers are depicted in Figure 6.6. The energies associated with the structures were determined by single point calculation employing the 6-311++G** basis set. The ^{1}H NMR coupling constants were generated from the optimized structures using the Gauge-Independent Atomic Orbital (GIAO) NMR method with the 6-311+G(2d,p) basis set, by employing the SpinSpin and Mixed options. The energies of the different conformers were used to determine the distribution of the two present in solution at room temperature. Table 6.1 shows the measured coupling constants ($J_{3,4}$) for the four azasugars at low and high pH*, the calculated $J_{3,4}$ values for the $^{1}C_{4}$ and $^{4}C_{1}$ conformers, the ratio of the two conformers, established from the measured average coupling constants, the difference in free energy established by the DFT calculations as well as the theoretical ratio of the two conformers, based on the difference in calculated free energy.

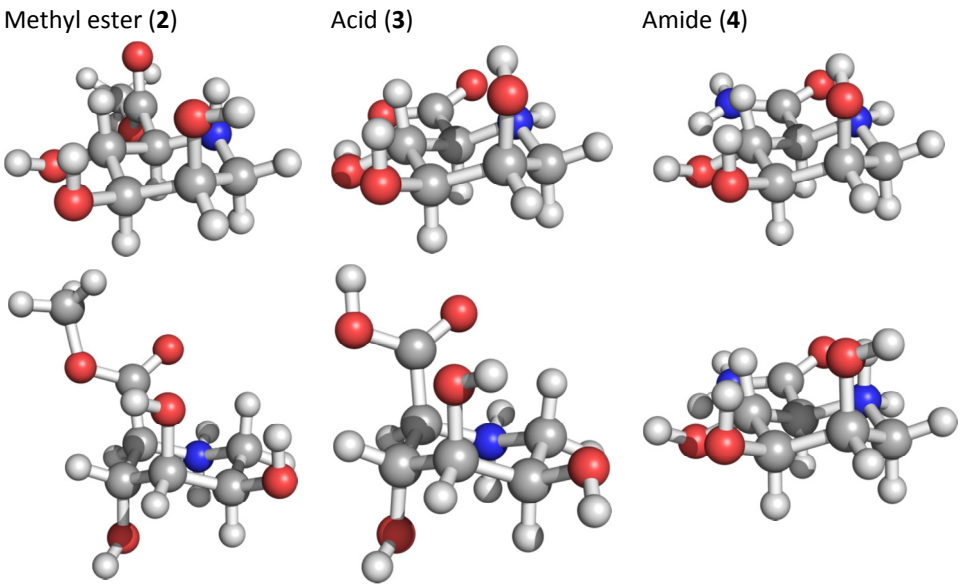
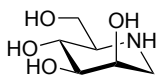
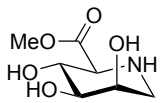
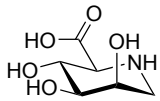
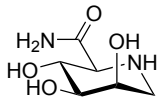
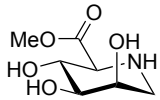


Figure 6.6 Calculated non-protonated (top) and protonated (bottom) optimized structures.

Table 6.1 Measured and calculated ratios in D_2O and MeOD.

Compound		pH* / TFA	$J_{3\text{-}4}$ Obs.	$J_{3\text{-}4}$ calc. $^4C_1$	$^1C_4$	Ratio by NMR $^4C_1$:$^1C_4$	ΔE [$^1C_4$-$^4C_1$] (kcal·mol^{-1})	Ratio theory $^4C_1$:$^1C_4$	pK_a
In D_2O:									
1		9	9.5	9.0	3.3	100:0	4.0	100:0	7.4
		2	9.5	9.4	4.3	100:0	2.3	98:2	
2		8	9.4	9.4	3.9	99:1	2.4	98:2	5.3
		2	7.5	9.5	4.9	56:44	0.3	64:36	
3		11	9.8	9.3	3.1	100:0	3.2	100:0	7.5
		5	8.8	9.1	3.8	94:6	3.7	100:0	
		1	8.3	9.5	4.7	75:25	0.1	54:46	
4		9	9.7	9.0	2.8	100:0	3.4	100:0	5.8
		2	9.3	9.1	4.7	100:0	1.7	95:5	
In MeOD:									
2		0 µl	9.2	9.3	4.2	99:1	2.4	98:2	
		25 µl	4.8	9.3	4.7	1:99	-0.1	45:55	

As can be seen from Table 6.1, there is good agreement between the calculated and measured coupling constants at high pH*. With the two extreme values for $J_{3,4}$ the ratio of the $^1C_4$ and $^4C_1$ conformers was established and it is clear that DMJ takes up a single $^4C_1$ conformation at both low and high pH values. The calculated difference in energy between the possible $^1C_4$ and $^4C_1$ conformers was calculated to be 4.0 kcal mol^{-1} for the unprotonated amine, and 2.3 kcal mol^{-1} for the protonated amine. Although the difference in energy between the two conformers is significantly smaller for the protonated amine, it is so large that the $^4C_1$ conformer is almost exclusively present at both high and low pH values. For the methyl ester **2** the situation is different. For the unprotonated amine, present at high pH values, the difference in energy between the two chair conformers is 2.4 kcal mol^{-1} in favor of the $^4C_1$ chair. In the protonated form however, the energy difference is minimal (0.3 kcal mol^{-1}), explaining the conformational mixture. With the calculated values of the coupling constants for both conformers (9.5 Hz and 4.9 Hz) and the measured average coupling constant (7.5 Hz) the ratio of the two conformers was established to be 56:44. This ratio is well in line with the ratio determined from the energy difference determined by the DFT calculations (0.3 kcal mol^{-1}), being 64:36.[36]

In a similar vein the ratio of the two chair conformers of the acid (**3**) was determined at three different pH values. As can be seen in Table 6.1, there is reasonable agreement between the theoretical calculations and the measured ratios. At high pH, the anionic azasugar **3** is present as a single conformer. At pH 5 the measured average coupling constant indicates a 94:6 mixture of conformers, where theory predicts a single conformer. At low pH two conformers are observed in a 75:25 $^4C_1$: $^1C_4$ ratio, where the theoretical ratio is 54:46.[36] Finally, for the amide **4**, at both high and low pH the $^4C_1$ chair is almost exclusively present.

The pK_a values for the four compounds were determined by titration, and these data are also tabularized in Table 6.1. For DMJ a pK_a value of 7.4 was measured, which is in line with the pK_a previously established for this compound (7.5).[12] The pK_a's of methyl ester **2**, amino acid **3** and amide **4** were determined to be 5.3, 7.5 and 5.8, respectively. The drop in pK_a value for the ester and the amide is a clear manifestation of the electron withdrawing effect of the carboxylic acid ester and amide functionalities. In acid **3** the electron withdrawing effect of the carboxylate is minimized because of its negative charge.

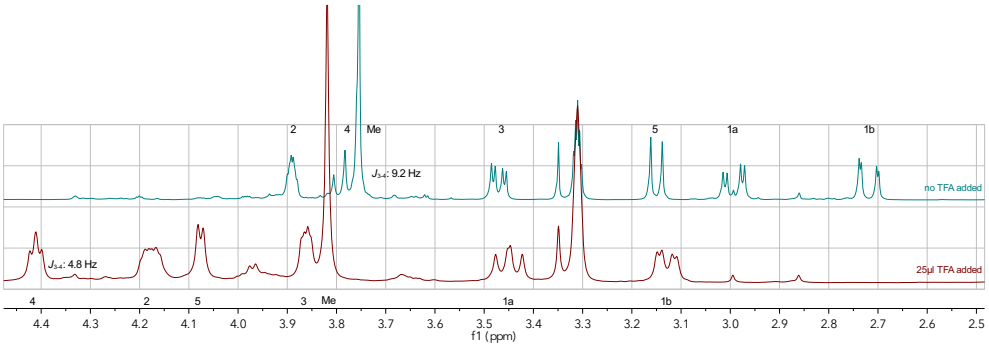


Figure 6.7 DMJ Methyl ester (**2**) in MeOD with non-protonated (no TFA added, top) and protonated (25 µl TFA added, bottom)

Finally the azasugar showing the largest conformational change, that is methyl ester **2**, in a less polar solvent (MeOD) was investigated. In Figure 6.7 the spectra of the non-protonated and protonated azasugar are depicted. In this medium the $J_{3,4}$ coupling constant changes from 9.2 Hz to 4.8 Hz upon protonation, indicating a much stronger conformational flip than the one observed in D_2O. Also for this solvent the structures of the two chair conformers were optimized, and the associated energies and coupling constants calculated as described above. Using the obtained values (depicted in Table 6.1) for the coupling constants of the protonated and deprotonated species, it is concluded that the non-protonated azasugar almost exclusively resides in the $^4C_1$ conformation where the protonated species is found in the opposite $^1C_4$ conformation. Although the

theoretical free energy difference between the two chairs predicts the $^{1}C_4$ chair to be most favorable, the difference between the two chair forms is smaller than what is concluded from the NMR measurements ($^{4}C_1$: $^{1}C_4$ calculated = 45:55; $^{4}C_1$: $^{1}C_4$ measured = 1:99).[36]

The NMR results together with the DFT calculations show that DMJ analogues having a methyl ester or carboxylic acid at C5 (as in **2** and **3**, respectively) change their conformation from the $^{4}C_1$ chair to the opposite $^{1}C_4$ chair upon protonation. This conformational change is seen even in a highly polar medium such as water and is significantly enhanced in a more apolar solvent (MeOD). The nature of the substituent at the C5 of the DMJ analogues is of major importance, because DMJ (**1**) and the C5 amide DMJ (**4**) do not display a change in conformational preference. The difference between the ester and amide is notable, because both functional groups, the C5 ester and C5 amide respectively, have a similar effect on the basicity of the azasugars. The electron withdrawing effect of both groups leads to a significant drop in the pK_a values for **2** and **3**, with the strongest electron withdrawing functionality -the ester- having the strongest inductive effect. The conformational flip of ester **2** and acid **3** can be accounted for by taking into account that electron withdrawing groups prefer to occupy an axial position on a positively charged carbohydrate ring to minimize their destabilizing effect. The fact that amide **4** does not change its conformation to accommodate this intrinsic preference may be due to internal hydrogen bonds that can be formed between the amide -NH_2 and the C4-OH which provides an extra stabilizing factor in the $^{4}C_1$ amide (See DMJ amide pictures in Figure 6.6).[37]

6.3 Conclusion

Mannuronic acid based azasugars can change their conformation upon protonation of the endocyclic amine from a “normal” $^{4}C_1$ chair to the inverted $^{1}C_4$ chair conformation. The molecules thereby position their substituents such that they are optimally positioned to accommodate the positive charge. Although the conformational behavior of any other glycuronic acid based azasugars, having different substituent configurations has not yet been studied in detail, it is likely that the spatial preferences of the substituents in the mannuronic acid azasugar work in concert to affect the ring flip. This behavior is in line with the conformational effects observed for fully protected mannuronic acid glycosyl donors and therefore the results described here provide an extra indication that the (partial) positive charge at the anomeric center of these donors is responsible for the observed unusual ring flip. The flexibility of the mannuronic acid azasugars may be exploited in the inhibition of specific mannosidases, enzymes that cleave mannosyl residues from oligosaccharides. For example, the cleavage of mannosyl residues by

mannosidases belonging to glycosyl hydrolase family 47 (GH47) occurs through a pathway in which the mannosyl substrate follows a $^{3}S_{1} \rightarrow {}^{3}H_{4}{}^{\ddagger} \rightarrow {}^{1}C_{4}$ itinerary.[38-40] To mimic the $^{3}H_{4}$ transition state of this reaction, the flexible mannuronic acid based azasugars may be well suited.

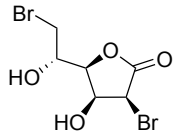
Experimental section

Calculations. All calculations were performed with DFT *ab initio* calculations with the B3LYP model. A set of conformers was optimized by starting from a conformer distribution search option included in the Spartan 04[41] program in gas phase at 6-31G(d) as basis set. All generated geometries were optimized with Gaussian 03[35] at 6-31G*, their zero-point energy corrections calculated, and further optimized with incorporated polarizable continuum model (PCM) to correct for solvation in water or methanol. The energies for each of the structures were calculated by a single point calculation employing the PCM with corresponding solvent and a larger diffuse function containing 6-311++G** basis set.[42] The ^{1}H NMR coupling constants for the structures were generated for the two lowest energy $^{4}C_{1}$ and $^{1}C_{4}$ conformers using the Gauge-Independent Atomic Orbital (GIAO) NMR method with 6-311+G(2d,p) basis set, by employing the SpinSpin and Mixed options.

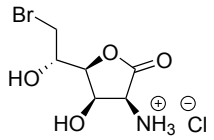
Synthesis

General. All reagents were of commercial grade and used as received unless stated otherwise. Reactions were performed at room temperature unless stated otherwise. Molecular sieves (4Å) were flame dried before use. Flash column chromatography was performed on silica gel (40-63 µm). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV 600, Bruker AV 400 or a Bruker DPX 400 spectrometer in D_2O or CD_3OD. Chemical shifts (δ) are given in ppm relative to the solvent residual signals. Coupling constants (*J*) are given in Hz. All given ^{13}C spectra are proton decoupled. Compound names are given using the standard iminosugar nomenclature numbering, resulting in a different numbering system than in the article.

2,6-dibromo-2,6-dideoxy-D-mannono-1,4-lactone (6). Calcium D-gluconate monohydrate **5** (126 g, 280 mmol) was put under an argon atmosphere before being dissolved in 33% HBr in acetic acid (500 ml, 3.0 mol). The reaction mixture was stirred for 18 hours to form acetylated **6**. MeOH (1 l) was added and the mixture was refluxed for 2 hours. After refluxing the mixture was concentrated to half the volume under reduced pressure before adding another 500 ml of MeOH. The reaction was left to stir overnight after which the mixture was concentrated resulting in a slightly oily residue. This was co-evaporated with 100 ml of MeOH and three times with H_2O. The residue was extracted with diethyl ether (4 x 100 ml), the organic layers were combined, dried with $MgSO_4$, filtered and concentrated under vacuum yielding a yellow oily residue. This was crystallized from $CHCl_3$ / H_2O to yield a white crystalline solid (44 g, 146 mmol, 26% yield). ^{1}H NMR (400 MHz, D_2O): δ 5.20 (1H, d, *J* = 4.5 Hz, C-2), 4.64 (2H, m, C-4, C-3), 4.19 (1H, m, C-5), 3.77 (1H, dd, *J* = 11.4, 2,4 Hz, C-6a), 3.65 (1H, dd, *J* = 11.4, 4.9 Hz, C-6b). ^{13}C NMR (101 MHz, D_2O): δ 174.0 (*C*-1), 81.6 (*C*-4), 69.1 (*C*-3), 66.2 (*C*-5), 47.6 (*C*-2), 36.6 (*C*-6). Melting point: 130 °C. $[\alpha]^{20}_{D}$: +58,6° (c = 1, MeOH)



2-amino-6-bromo-2,6-dideoxy-D-mannono-1,4-lactone hydrochloride (7). 2,6-Dibromo-2,6-dideoxy-D-mannono-1,4-lactone (**6**, 5.0 g , 16.5 mmol) was put under argon and dissolved in dry acetone ($MgSO_4$, 100 ml). Sodium azide (15.0 g, 231 mmol) was added and the suspension was refluxed for 20 hours. The mixture was filtrated and the filtrate concentrated under reduced pressure. The residue was dissolved in H_2O (50 ml) and extracted with diethyl ether (5 x 100 ml), the organic layers were combined, dried over $MgSO_4$, filtered and



concentrated under reduced pressure to give a brown oil which was identified as the 2-azido compound but included some of its diastereoisomer. ^{1}H NMR (400 MHz, D_2O): δ 4.68 (1H, dd, *J* = 4.5, 3.3 Hz, C-3), 4.56 (1H, d, *J* = 4.6 Hz, C-2), 4.46 (1H, dd, *J* = 9.2, 2.7 Hz, C-4), 4.09 (1H, m, C-5), 3.69 (1H, dd, *J* = 11.4, 2.7 Hz, C-6a), 3.56 (1H, dd, *J* = 11.5, 4.9 Hz, C-6b). ^{13}C NMR (101 MHz, D_2O): δ 174.1 (*C*-1), 81.0 (*C*-4), 69.6, 65.7 (*C*-3, *C*-5), 62.3 (*C*-2), 36.6 (*C*-6). The crude compound (16.5 mmol) was put under argon and dissolved in MeOH (100 ml). Palladium on activated carbon (10%, 300 mg, 0.3 mmol) and HCl (37% in H_2O, 10 ml, 121 mmol) were added and the suspension charged with hydrogen atmosphere. The reaction mixture was stirred for 22 hours after which the catalyst was filtered off over a Whatman microfilter. The filtrate was concentrated under reduced pressure and co-evaporated once with HCl (37% in H_2O, 60 ml), thrice with toluene (60 ml) and once with $CHCl_3$ (50 ml). Crystallization from EtOH yielded 2-amino-6-bromo-2,6-dideoxy-D-mannono-1,4-lactone hydrochloride (**7**) as white crystals (2.6 g, 9.2 mmol, 55% over two steps). ^{1}H NMR (400 MHz, D_2O): δ 4.83 (1H, dd, *J* = 4.8, 2.8 Hz, C-3), 4.63 (1H, dd, *J* = 9.2, 2.7 Hz, C-4), 4.59 (1H, d, *J* = 4.9 Hz, C-2), 4.20 (1H, m, C-5), 3.77 (1H, dd, *J* = 11.5, 2.6 Hz, C-6a), 3.65 (1H, dd, *J* = 11.4, 5.0 Hz, C-6b). ^{13}C NMR (101 MHz, D_2O): δ 172.1 (*C*-1), 78.9 (*C*-4), 64.1 (*C*-3), 62.9 (*C*-5), 50.2 (*C*-2), 33.7 (*C*-6). Melting point: 207 °C (decomposed). $[\alpha]^{20}_{D}$: +41.6° (c = 1, MeOH).

1-Deoxymannojirimycin (1). 2-amino-6-bromo-2,6-dideoxy-D-mannono-1,4-lactone hydrochloride (**7**, 501 mg, 1.8 mmol), was three times co-evaporated with dry toluene, put under argon and suspended in dry MeOH (10 ml). The suspension was cooled to 0 °C before addition of distilled triethylamine (1.0 ml, 7.2 mmol) and the resulting clear solution was stirred overnight. The reaction mixture was concentrated under reduced pressure yielding the methyl ester as a white semi-crystalline solid. The residue was put under argon, dissolved in dry EtOH (molsieves, 10 ml) and cooled to 0°C. Sodium borohydride (709 mg, 19 mmol) was added and the suspension was stirred overnight. Dry MeOH (20 ml) was added before the mixture was filtered, concentrated under reduced pressure and co-evaporated with 1M HCl in MeOH (3x, 10 ml). The residue was purified by column chromatography (1:1 EtOAc/EtOH → 100% EtOH) yielding a pure sample of DMJ (**1**) in 29% yield (105 mg, 0.50 mmol). ^{1}H NMR (399 MHz, D_2O): δ 3.99 (1H, dt, *J* = 2.9, 1.6 Hz, C-3), 3.76 (1H, dd, *J* = 12.5, 3.9 Hz, C-7), 3.71 (1H, dd, *J* = 12.5, 5.5 Hz, C-7a), 3.60 (1H, t, *J* = 9.7 Hz, C-5), 3.53 (1H, dd, *J* = 9.6, 3.1 Hz, C-4), 3.03 (1H, dd, *J* = 14.2, 2.8 Hz, C-2a), 2.80 (1H, dd, *J* = 14.2, 1.5 Hz, C-2b), 2.57 (1H, ddd, *J* = 9.7, 4.9, 3.4 Hz, C-6). ^{13}C NMR (101 MHz, MeOD): δ 74.4 (*C*-4), 67.6 (*C*-5), 67.5 (*C*-3), 62.4 (*C*-6), 59.6 (*C*-7), 48.9 (*C*-2). $[\alpha]^{20}_{D}$: -14.0° (c = 0.5, MeOH).

Methyl 2,6-dideoxy-2,6-imino-D-mannonate hydrochloride (2). 2-amino-6-bromo-2,6-dideoxy-D-mannono-1,4-lactone hydrochloride (**7**, 0.60 g, 2.2 mmol), was co-evaporated thrice with dry toluene, put under argon and suspended in dry MeOH (12 ml). The suspension was cooled to 0 °C before addition of distilled triethylamine (1.2 ml, 8.7 mmol) and the resulting clear solution was stirred overnight. The reaction mixture was concentrated under reduced pressure before being taken up in acetonitrile (15 ml) charged with 1,1,1,3,3,3-hexamethyldisilazane (2.5 ml, 12 mmol) and copper sulphate pentahydrate (cat.). After 1 hour, the reaction mixture was concentrated and a fraction of 234 mg (0.57 mmol) was purified by column chromatography (1-2.5% 1,4-dioxane/DCM) to give 162 mg (0.40 mmol) of the per-TMSylated compound. The protected product was put under argon, dissolved in MeOH (8 ml) and acetyl chloride added to generate HCl *in situ*. The mixture was stirred for 0.5 hour, after which the compound was concentrated and coevaporated with MeOH yielding the title compound (98 mg, 0.40 mmol, 70% over 2 steps). ^{1}H NMR (400 MHz, MeOD) δ 4.40 (dd, *J* = 5.3, 4.8 Hz, 1H, C-5), 4.17 (ddd, *J* = 9.5, 4.1, 2.8 Hz, 1H, C-3), 4.09 (d, *J* = 4.4 Hz, 1H, C-6), 3.86 (dd, *J* = 5.6, 2.7 Hz, 1H, C-4), 3.82 (s, 3H, OC*H*$_3$), 3.44 (dd, *J* = 12.2, 9.6 Hz, 1H, C-2a), 3.13 (dd, *J* = 12.2, 4.2 Hz, 1H, C-2b). ^{13}C NMR (101 MHz, MeOD) δ 168.1 (*C*-7), 71.1 (*C*-4), 69.9 (*C*-5), 64.2 (*C*-3), 58.8 (*C*-6), 53.4 (O*C*H$_3$), 43.5 (*C*-2). $[\alpha]^{20}_{D}$: +31.8 (c = 1, MeOH).

Sodium 2,6-dideoxy-2,6-imino-D-mannonate (3). Methyl 2,6-dideoxy-2,6-imino-D-mannonate hydrochloride (**2**, 24 mg, 0.10 mmol), was dissolved in H_2O (0.5 ml). A sodium hydroxide solution (1M aq., 170 µl, 0.17 mmol) was added and the mixture stirred for 2 hours. The mixture was concentrated under reduced pressure yielding the title compound, pure but with added sodium hydroxide. ^{1}H NMR (400 MHz, D_2O): δ 4.01 (1H, m, C-3), 3.71 (1H, t, *J* = 9.7 Hz, C-5), 3.60 (1H, dd, *J* = 9.6, 3.2 Hz, C-4), 3.01 (1H, dd, *J* = 14.6, 2.7 Hz, C-2a), 2.95 (1H, d, *J* = 9.8 Hz, C-6), 2.75 (1H, dd, *J* = 14.6, 1.6 Hz, C-2b). ^{13}C NMR (101 MHz, D_2O): δ 178.4 (*C*-7), 74.1 (*C*-4), 70.6 (*C*-5), 69.1 (*C*-3), 65.2 (*C*-6), 47.9 (*C*-2). $[\alpha]^{20}_D$: -7.2 (c = 1, MeOH).

2,6-dideoxy-2,6-imino-D-mannonic amide (4). 2-amino-6-bromo-2,6-dideoxy-D-mannono-1,4-lactone hydrochloride (**7**, 500 mg, 1.8 mmol), was co-evaporated thrice with dry toluene, put under argon and suspended in dry MeOH (10 ml). The suspension was cooled to 0 °C before addition of distilled triethylamine (1.0 ml, 7.2 mmol) and the resulting clear solution was stirred overnight. The reaction mixture was concentrated under reduced pressure yielding the methyl ester as a white semi-crystalline solid. The residue was dissolved in 6M ammonia in MeOH (10 ml, 60 mmol) and was stirred overnight. The reaction mixture was concentrated under reduced pressure yielding 2,6-dideoxy-2,6-imino-D-mannonic amide (**4**) in quantitative yield. An analytical sample was made by crystallisation from pure MeOH (133 mg, 0.76 mmol, 42%). ^{1}H NMR (399 MHz, D_2O): δ 3.97 (1H, m, C-3), 3.70 (1H, t, *J* = 9.7 Hz, C-5), 3.57 (1H, dd, *J* = 9.6, 3.1 Hz, C-4), 3.07 (1H, d, *J* = 9.8 Hz, C-6), 2.99 (1H, dd, *J* = 14.6, 2.7 Hz, C-2a), 2.75 (1H, dd, *J* = 14.6, 1.6 Hz, C-2b). ^{13}C NMR (101 MHz, DMSO): δ 173.5 (*C*-7), 74.9 (*C*-4), 70.0 (*C*-5), 68.7 (*C*-3), 63.3 (*C*-6), 49.1 (*C*-2). $[\alpha]^{20}_D$: -31,6° (c = 0.5, H_2O). HR-MS: [M+H$^+$] calculated for $C_6H_{12}O_4N_2$: 177.08698; found: 177.08683.

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[37] It has previously been observed that mannuronic acid ester thiodonors take up a $^1C_4$ conformation when fully protected, while they reside in a $^4C_1$ conformation when the C4-OH is free. This contrasting conformational behavior can also be accounted for by a stabilizing hydrogen bond between the C5-carboxylic acid ester and the C4-OH in the $^4C_1$ chair conformation.

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