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Computational studies of sialyllactones: methods and uses

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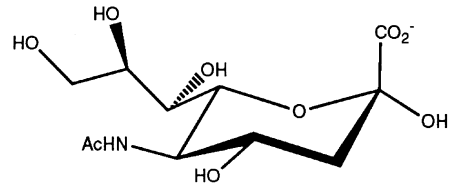
***N*-Acetylneuraminic acid (1) is a common sugar in many biological recognition processes. Neuraminidase enzymes recognize and cleave terminal sialic acids from cell surfaces. Viral entry into host cells requires neuraminidase activity, thus inhibition of neuraminidase is a useful strategy for development of drugs for viral infections. A recent crystal structure for influenza viral neuraminidase with sialic acid bound shows that the sialic acid is in a boat conformation [*Prot Struct Funct Genet* 14: 327 (1992)]. Our studies seek to determine if structural pre-organization can be achieved through the use of sialyllactones. Determination of whether siallylactones are pre-organized in a binding conformation requires conformational analysis. Our inability to find a systematic study comparing the results obtained by various computational methods for carbohydrate modeling led us to compare two different conformational analysis techniques, four different force fields, and three different solvent models. The computational models were compared based on their ability to reproduce experimental coupling constants for sialic acid, sialyl-1,4-lactone, and sialyl-1,7-lactone derivatives. This study has shown that the MM3 forcefield using the implicit solvent model for water implemented in Macromodel best reproduces the experimental coupling constants. The low-energy conformations generated by this combination of computational methods are pre-organized toward conformations which fit well into the active site of neuraminidase.**

***Keywords*: Sialyllactones, neuraminidase, computational models, coupling constants**

Introduction

Sialic acid, (**1**, *N*-acetylneuraminic acid)‖ is important in many biological recognition processes including lymphocyte homing [1], tumor metastasis [2], and pathogenic bacterial infections [3]. The recognition and cleavage of sialic acid from cell-surface carbohydrates is also an important step in viral infection by the influenza virus [4]. The crystal structure of a sialic acid/neuraminidase complex has been determined, Figure 1 [5], and shows that the bound sialic acid is the less stable alpha anomer and that it is bound to neuraminidase in a boat conformation (Figure 2). Previous studies developing neuraminidase inhibitors, focused on the use of unsaturated sialic acid analogs as scaffolds. For example, von Itzstein recently showed compound **2** is a potent neuraminidase inhibitor [6]. This study explores whether sialyllactones or their derivatives could be used as a molecular scaffold in the design of high-affinity neuraminidase inhibitors.

The design of carbohydrate-derived neuraminidase inhibitors would be facilitated by the ability to accurately model the conformations of such compounds. Many methods have been used to model carbohydrates [7–20], but a systematic comparison of multiple methods has proven hard to find. This research compares the ability of two conformational search methods, four molecular mechanics forcefields and three different solvent models to reproduce experimental coupling constants for *N*-acetylneuraminic acid (**1**) and its 1,4- (**3**) and 1,7-lactones (**4**). Molecular mechanics has been sufficiently parameterized for the types of functional groups present in most



1

Scheme 1.

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‖Sialic acids are a class of carbohydrates, the most common of which is *N*-acetylneuraminic acid, and the name sialic acid is often used in place of *N*-acetylneuraminic acid.

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Figure 1. Crystal structure of neuraminidase with sialic acid bound.

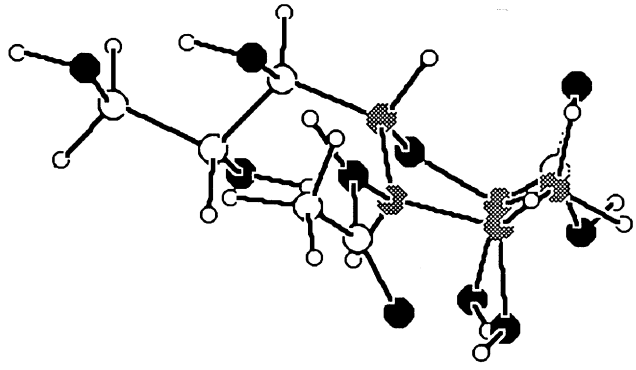
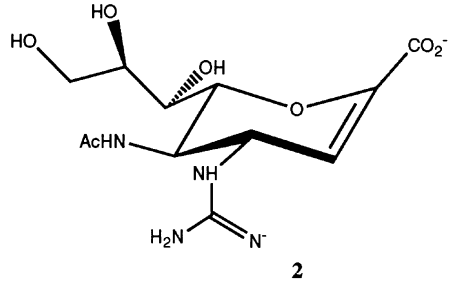


Figure 2. Conformation of bound sialic acid.



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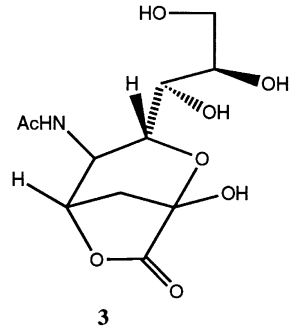
Scheme 2.

carbohydrates so that a higher level of theory, such as semi-empirical or ab initio, is not necessary and would only consume a great deal more time than necessary.



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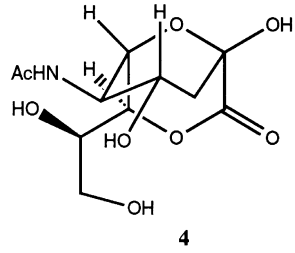
Scheme 3.



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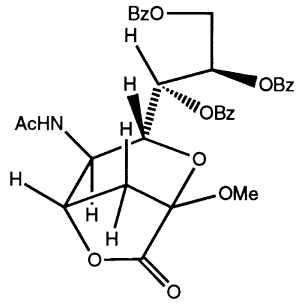
Scheme 4.

Experimental

Conformational search and minimization

Initial structures of sialic acid (**1**) and its 1,4- (**3**) and 1,7-lactone (**6**) were built using MacMimic, version 3.0 beta 1 [21]. The initial structure for each compound was minimized using the MM2 forcefield [22], with no solvent model, before being used as a starting point for the conformational searches. Conformational searches in Macromodel [23] were performed using the MM2 forcefield and no solvent model during minimizations of structures. A total of 10 000 structures were generated during the conformational search, and structures within the lowest 12 kcal (50 kJ) were saved. Conformational searches performed in this manner were assumed to be relatively comprehensive as most of the structures within 1 kcal of the global minimum were found more than five times each. Conformational searches in WIZARD III [24] were performed using the genetic search with a 60% crossover rate and a 5% mutation rate. The population factor and generation factor were set to 15 and 0.6, respectively. WIZARD III utilizes the DREIDING force field [25] for energy calculations but does no structural minimizations. Thus the structures generated by WIZARD III were minimized using the MM2 forcefield with no solvent model implemented in Macromodel [23]. Searches performed in this manner were assumed to be relatively comprehensive if an additional search yielded no new conformations within 1 kcal of the global minimum. The structures generated during the conformational searches were re-minimized using the MM2 [22],



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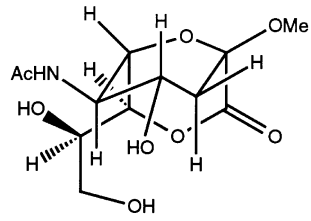
Scheme 5.



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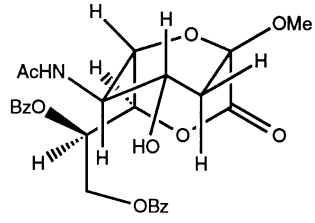
Scheme 6.



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Scheme 7.

MM3 [26], AMBER [27] and OPLS [28] forcefields in combination with no solvent model, and the continuum solvent models for water and chloroform.

Forcefields

The forcefields used in this study are the MM2 [22], MM3 [26], AMBER [27] and OPLS [28] forcefields implemented in Macromodel 4.5 [23]. MM2 and MM3 were developed by the Allinger group to reproduce conformational energy differences in small molecules and have been extensively parameterized for the functional groups present in sialic acid and the sialyllactones. AMBER was developed initially by the Kollman group for modeling proteins in explicit solvent simulations. OPLS was developed by the Jorgenson group for explicit solvent simulations. These forcefields are appropriate for comparison in this study even though a continuum solvent model was used because the evaluation of the GB/SA model was performed with the OPLS forcefield [30] and the OPLS: GB/SA results compared well with free energy perturbation results. Additionally, Macromodel 4.5 contains adjusted acetal parameters for the AMBER and OPLS forcefields which reproduce the gas phase axial-equitorial difference and solvent stabilization of the equitorial form reported by Wiberg [30].

Solvent models

The continuum treatment of solvents used in this study is the GB/SA model [29] implemented in Macromodel [23]. The bulk dielectric used in the GB/SA model for water calculations was 78.3 Da and 4.81 Da for chloroform. Use of a bulk dielectric to represent local environment would introduce inaccuracies which are accounted for in the GB/SA model by assuming neutral neighboring atoms displace the bulk dielectric.

Calculation of coupling constants

A program developed for this project was used to calculate the three-bond coupling constants based on the quantitative electronegativity correction to the Karplus equation developed by Haasnoot *et al.* [31]. This program accepts as input a multi-structure file containing conformations of a single compound. The coupling constant program is linked with the Babel library which currently allows the program to read 47 different file formats [32]. The program also can use a file of either fractions or percentages that each conformation would be expected to contribute to the population if weighted average coupling constants are of interest. Alternatively, the program accepts a temperature and energy unit conversion and calculates the weights for each conformation based on the Boltzmann distribution. For this study, the fraction for each conformation was calculated by the coupling constant program based on the Boltzmann distribution: partition = exp(-delta E/RT) at 297 K. The program utilizes the method of Haasnoot *et al.* [31] to calculate all H–C–C–H coupling constants, the method of Tvaroska [33] to calculate all C–C–C–H and C–O–C–H coupling constants, and the method of Bystrov [34] to calculate all H–N–C–H coupling constants for a single conformation. These are multiplied by the fraction provided for that conformation to generate a contribution to the overall coupling constant by that conformation. The contributions from each conformation to the overall coupling constant are summed over all conformations to determine the calculated coupling constants. These coupling constants were then compared to the experimentally determined coupling constants.

Table 1. Results for sialic acid conformational search by the Monte Carlo method.

Forcefield	*Solvent*	*3a/4* (Hz)	*3e/4* (Hz)	*4/5* (Hz)	*5/6* (Hz)	*6/7* (Hz)	*7/8* (Hz)	*8/9* (Hz)	*8/9′* (Hz)	*avg. abs. error*
Exp. [9]	D_2O	11.4	4.9	10.3	10.3	1.0	8.9	2.7	6.5	
MM2	None	10.3	5.0	8.9	10.2	8.8	9.1	4.5	9.8	2.0
MM3	None	7.8	4.6	7.6	10.3	9.1	9.4	4.6	10.9	2.7
AMBER	None	10.9	5.2	9.2	9.7	7.9	8.6	4.5	10.3	1.9
OPLS	None	11.6	3.1	9.3	4.4	0.1	6.9	7.9	10.0	2.6
MM2	Water	8.0	5.8	8.3	9.9	2.6	4.7	2.9	6.4	1.6
MM3	Water	10.4	5.1	9.2	10.3	3.1	4.1	2.3	4.8	1.4
AMBER	Water	11.3	4.4	9.4	8.6	2.3	3.7	5.2	3.1	2.0
OPLS	Water	11.6	3.7	8.2	2.7	5.8	6.6	7.9	9.1	3.2
MM2	$CHCl_3$	10.9	5.1	9.6	10.3	9.4	9.3	3.3	10.5	1.9
MM3	$CHCl_3$	10.8	5.2	9.5	10.3	8.8	9.3	3.2	10.9	1.9
AMBER	$CHCl_3$	11.6	3.5	8.3	2.8	0.1	2.1	5.2	0.4	3.4
OPLS	$CHCl_3$	11.6	3.1	9.4	4.5	0.1	6.9	7.9	10.0	2.5

Table 2. Results for sialic acid conformational search by the WIZARD method.

Forcefield	*Solvent*	*3a/4* (Hz)	*3e/4* (Hz)	*4/5* (Hz)	*5/6* (Hz)	*6/7* (Hz)	*7/8* (Hz)	*8/9* (Hz)	*8/9′* (Hz)	*avg. abs. error*
Exp. [9]	D_2O	11.4	4.9	10.3	10.3	1.0	8.9	2.7	6.5	
MM2	None	11.0	4.9	9.2	10.4	− 0.1	2.0	2.2	0.9	2.0
MM3	None	10.8	5.1	9.4	10.3	9.1	9.4	4.1	11.3	2.1
AMBER	None	11.3	4.6	9.3	10.4	8.5	2.5	4.8	0.3	3.0
OPLS	None	11.5	3.9	9.9	10.4	9.6	7.3	7.7	3.9	2.4
MM2	Water	11.1	4.8	9.5	10.4	− 0.1	1.9	2.3	0.7	2.0
MM3	Water	10.9	4.9	9.5	10.3	0.6	9.4	3.1	0.4	1.1
AMBER	Water	11.4	4.4	9.6	10.4	0.0	3.0	3.2	0.4	1.9
OPLS	Water	11.6	3.3	9.0	4.1	9.8	6.4	8.2	9.5	3.6
MM2	$CHCl_3$	11.0	4.9	9.3	10.4	0.6	9.4	3.5	0.2	1.2
MM3	$CHCl_3$	10.8	5.0	9.5	10.3	8.8	9.3	3.2	10.9	1.8
AMBER	$CHCl_3$	11.4	4.4	9.6	10.4	0.0	3.0	3.2	0.4	1.9
OPLS	$CHCl_3$	11.1	4.9	9.4	10.3	− 0.1	7.5	8.6	7.5	1.3

Table 3. Results for sialic acid 1,4-lactone conformational search by the Monte Carlo method.

Forcefield	*Solvent*	*3a/4 (Hz)*	*3e/4 (Hz)*	*4/5 (Hz)*	*5/6 (Hz)*	*6/7 (Hz)*	*7/8 (Hz)*	*8/9 (Hz)*	*8/9′ (Hz)*	*avg. abs. error*
Exp.	$CHCl_3$	5.5	0.0	0.0	10.2	0.0	7.6	2.5	5.8	
MM2	None	6.0	0.8	1.7	7.2	3.7	3.4	3.7	5.4	2.6
MM3	None	5.5	0.8	0.9	10.1	9.3	9.1	4.9	8.7	2.2
AMBER	None	5.6	0.8	3.1	1.2	9.6	7.1	4.8	6.3	3.2
OPLS	None	5.8	0.7	2.9	1.6	10.0	6.9	7.7	3.8	3.8
MM2	Water	6.2	0.8	1.4	7.9	4.2	2.7	3.3	8.0	2.2
MM3	Water	5.4	0.9	0.9	10.2	6.1	5.8	4.0	7.3	1.6
AMBER	Water	5.6	0.7	3.1	1.2	6.6	3.2	4.3	8.6	3.6
OPLS	Water	5.7	0.8	2.6	2.3	10.0	7.3	8.5	7.8	3.7
MM2	$CHCl_3$	6.0	0.8	1.7	7.1	2.9	3.2	3.4	7.0	1.9
MM3	$CHCl_3$	5.5	0.9	0.9	10.1	8.8	8.8	3.9	8.3	2.0
AMBER	$CHCl_3$	5.5	0.8	3.2	1.1	7.9	3.1	4.8	3.2	3.8
OPLS	$CHCl_3$	5.8	0.7	2.7	2.1	10.0	7.0	8.1	6.1	3.5

Table 4. Results for sialic acid 1,4-lactone conformational search by the WIZARD method.

Forcefield	*Solvent*	*3a/4* (Hz)	*3e/4* (Hz)	*4/5* (Hz)	*5/6* (Hz)	*6/7* (Hz)	*7/8* (Hz)	*8/9* (Hz)	*8/9′* (Hz)	*avg. abs. error*
Exp.	$CHCl_3$	5.5	0.0	0.0	10.2	0.0	7.6	2.5	5.8	
MM2	None	6.0	0.8	1.1	9.1	1.4	2.9	3.9	2.3	1.8
MM3	None	5.1	1.0	1.2	10.4	5.6	9.4	10.4	6.6	2.3
AMBER	None	5.4	0.7	3.2	1.1	8.7	3.1	5.2	1.2	4.2
OPLS	None	5.8	0.7	3.0	1.4	10.0	6.9	7.7	3.8	3.9
MM2	Water	6.2	0.7	1.2	8.5	0.7	2.5	3.5	8.3	1.7
MM3	Water	5.3	0.9	1.0	10.3	1.7	6.9	4.4	5.7	0.8
AMBER	Water	5.6	0.7	3.6	0.7	9.3	2.7	4.7	0.2	4.5
OPLS	Water	5.8	0.7	2.8	1.5	10.0	7.4	8.5	6.4	3.7
MM2	$CHCl_3$	6.0	0.8	1.2	8.6	1.3	2.8	3.7	4.6	1.5
MM3	$CHCl_3$	5.5	0.9	0.9	10.2	7.9	8.2	2.8	1.9	1.8
AMBER	$CHCl_3$	5.4	0.7	3.3	0.9	9.3	2.8	5.0	0.3	4.5
OPLS	$CHCl_3$	5.8	0.7	3.0	1.4	10.0	7.1	8.0	4.5	3.8

Table 5. Results for sialic acid 1,7-lactone conformational search by the Monte Carlo method.

Forcefield	*Solvent*	*3a/4* (Hz)	*3e/4* (Hz)	*4/5* (Hz)	*5/6* (Hz)	*6/7* (Hz)	*7/8* (Hz)	*8/9* (Hz)	*8/9′* (Hz)	*avg. abs. error*
Exp.	DMSO	0.0	0.0	0.0	0.0	0.0	5.9	3.5	5.6	
MM2	None	9.6	6.6	9.7	7.1	0.2	2.9	4.3	6.0	4.7
MM3	None	9.2	7.1	9.9	7.3	0.8	6.8	4.1	7.8	4.7
AMBER	None	9.9	6.5	8.9	4.1	0.0	5.2	4.7	6.9	4.1
OPLS	None	10.8	5.5	9.8	5.5	0.1	3.2	7.9	8.6	5.2
MM2	Water	9.5	6.7	9.7	7.2	0.1	3.3	3.1	8.5	4.9
MM3	Water	9.0	7.3	9.9	7.4	0.0	7.8	3.2	8.4	4.8
AMBER	Water	10.3	6.1	9.4	4.6	0.0	2.4	4.7	5.2	4.4
OPLS	Water	10.7	5.7	9.3	4.4	0.2	2.9	8.1	8.5	5.1
MM2	$CHCl_3$	9.5	6.7	9.7	7.1	0.1	3.6	3.6	7.8	4.7
MM3	$CHCl_3$	9.3	7.0	9.9	7.3	0.0	6.8	3.5	8.0	4.6
AMBER	$CHCl_3$	10.5	5.9	9.5	4.8	0.0	2.5	5.0	0.3	4.8
OPLS	$CHCl_3$	10.8	5.6	9.7	5.4	0.1	3.0	8.0	8.4	5.2

Table 6. Results for sialic acid 1,7-lactone conformational search by the WIZARD method.

Forcefield	*Solvent*	*3a/4* (Hz)	*3e/4* (Hz)	*4/5* (Hz)	*5/6* (Hz)	*6/7* (Hz)	*7/8* (Hz)	*8/9* (Hz)	*8/9′* (Hz)	*avg.abs. error*
Exp.	DMSO	0.0	0.0	0.0	0.0	0.0	5.9	3.5	5.6	
MM2	None	3.1	2.9	2.2	0.9	0.3	1.4	2.7	9.9	2.4
MM3	None	3.1	2.9	2.1	0.9	0.2	9.4	3.6	11.1	2.3
AMBER	None	9.3	7.2	8.9	4.3	0.0	1.6	5.5	0.5	5.1
OPLS	None	2.0	4.2	2.1	1.7	0.6	4.1	7.4	10.9	2.7
MM2	Water	3.1	2.9	2.2	0.9	0.2	1.5	2.4	9.5	2.3
MM3	Water	3.0	3.0	1.9	0.9	0.3	1.1	2.0	9.6	2.4
AMBER	Water	2.2	3.7	1.9	1.3	0.1	3.7	3.5	0.3	2.1
OPLS	Water	2.0	4.1	1.9	1.5	0.7	3.0	8.1	9.4	2.7
MM2	$CHCl_3$	3.1	2.9	2.3	0.9	0.2	1.4	2.6	9.8	2.4
MM3	$CHCl_3$	3.1	2.9	2.1	0.9	0.1	9.5	3.2	0.4	2.3
AMBER	$CHCl_3$	9.6	6.9	8.9	4.1	0.0	1.8	5.6	0.6	5.1
OPLS	$CHCl_3$	2.0	4.2	2.0	1.7	0.6	4.2	7.5	10.9	2.7

Table 7. Summary of most successful computational methods.

Molecule	*Monte Carlo*		*WIZARD*	
	Method	*Avg. Abs. Err.*	*Method*	*Avg. Abs. Err.*
Sialic Acid	MM3/water	1.4	MM3/water	1.1
Sialyl-1,4-lactone	MM3/water	1.6	MM3/none	2.3
Sialyl-1,7-lactone	AMBER/none	4.1	AMBER/water	2.1

Results

The conformations of sialic acid (**1**) were determined and minimized by several different methods and utilized to calculate weighted average coupling constants. The results are summarized in Tables 1 and 2. The average absolute errors from the modeling of sialic acid indicate that conformational search using the genetic algorithm implemented in WIZARD followed by minimization with the MM3 forcefield and the water dielectric (average absolute error = 1.1) best reproduces the experimental determination of the sialic acid coupling constants in D_2O. The MM3 forcefield and water dielectric also best reproduced the experimental coupling constants when the Monte Carlo conformational search was used (average absolute error = 1.4).

The conformations of the 1,4-lactone of sialic acid (**3**) generated by several methods were utilized to calculate weighted average coupling constants. These results are summarized in Tables 3 and 4. The experimental values shown are for the per-benzoylated methyl glycoside (**5**) in chloroform. These results show that WIZARD conformational search followed by minimization with the MM3 forcefield utilizing the water dielectric best reproduces the experimentally determined coupling constants in chloroform (average absolute error = 0.8). The best results obtained with the other forcefields were in combination with the chloroform model, as would be expected for experimental results in chloroform. The MM3 forcefield with water best reproduced the experimental coupling constant when the Monte Carlo conformational search was used (average absolute error = 1.6). Results obtained using the chloroform solvent model were similar. Work is continuing on the synthesis of the unprotected 1,4-lactone.

The conformations of the 1,7-lactone methyl glycoside (**6**) of sialic acid generated by several methods were utilized to calculate weighted average coupling constants. These results are summarized in Tables 5 and 6. The experimental values shown are for the 8,9-dibenzoylated methyl glycoside (**7**). These results show that in the Monte Carlo conformational search, the AMBER forcefield utilizing no solvent model best reproduces the experimentally determined coupling constants in dimethyl sulfoxide (average absolute error = 4.1). The AMBER forcefield utilizing the water dielectric best reproduces the experimentally determined coupling constants after the genetic algorithm conformational search (average absolute error = 2.1). Work is currently under way to perform the Monte Carlo conformational search on the 8,9-dibenzoylated methyl glycoside (**7**). The large protecting groups can have an effect on the relative energies of conformations which would account for the relatively poor agreement between the calculated coupling constants and the experimental values. Preliminary results indicate that the average absolute errors are in the 2–3 Hz range for the coupling constants calculated from the dibenzoylated lactones (**7**) compared to being in the 4–5 Hz range for those calculated from the methyl glycoside (**6**). These preliminary results indicate that the MM2 forcefield with the chloroform dielectric best reproduces the experimental coupling constants (average absolute deviation = 2.02 for the Monte Carlo conformation search method and 2.44 for the genetic algorithm search method)

In general, MM2 and MM3 more successfully reproduced the experimental coupling constants than did AMBER and OPLS. This may simply reflect the different objectives used during forcefield design. Inclusion of AMBER parameters developed for saccharides by Homans [14] and Glennon [11] would likely improve results obtained by the AMBER forcefield.

Table 7 summarizes the computational methods which were most successful at reproducing the experimental coupling constants. These results indicate that the MM3 forcefield is an appropriate forcefield for calculations involving these carbohydrates although it is not the only method which provided reasonable results.

The global minimum for both the 1,4- (**3**) and the 1,7-sialyllactone (**6**) mimics the conformation of sialic acid bound in the neuraminidase active site. Root mean square fits on the heavy atom positions are approximately 0.5 Å, indicating that sialyllactones and their derivatives could be used as scaffolds in the design of neuraminidase 1-inhibitors.

Conclusions

This study has shown that the MM3 forcefield implemented in Macromodel 4.5 in combination with the dielectric for water consistently reproduces experimental coupling constants for the carbohydrates studied. The MM3 forcefield reproduced the coupling constants for sialic acid (**1**) with an

average absolute error of 1.1 Hz and 1.4 Hz when used to minimize the results of conformational searches by the genetic algorithm and Monte Carlo, respectively. The coupling constants for sialyl-1,4-lactone (**3**) were reproduced by this combination of computational parameters with an average absolute error of 0.8 Hz and 1.6 Hz when used to minimize the results of conformational searches by the genetic algorithm and Monte Carlo, respectively. The genetic algorithm search combined with MM2 using the water dielectric as well as MM3 with the gas-phase dielectric produced similar results for the sialyl-1,4-lactone (**3**) with average absolute errors of 2.6 and 2.3, respectively. The coupling constants for sialyl-1,7-lactone (**6**) were reproduced with an average absolute error of 2.1 Hz and 4.1 Hz when used to minimize the results of conformational searches by the genetic algorithm and Monte Carlo, respectively. These errors were obtained using AMBER with no solvent model for the results of the Monte Carlo search and AMBER with no solvent model for the results of the genetic algorithm search. The genetic search resulted in similar coupling constants using the MM2 and MM3 forcefields with all three solvent options. Preliminary results indicate that the average absolute errors for the coupling constants obtained from the Monte Carlo search can be lowered when performing the conformational search on the dibenzoylated 1,7-lactone (**7**). These preliminary results indicate that MM2 and MM3 reproduce the experimental coupling constants more closely than AMBER.

WIZARD reproduces the experimental coupling constants more closely than Monte Carlo. WIZARD also generates fewer conformations. These missing conformations may be real conformations that were overlooked during the conformational search, or they may be other saddle points, such as maxima, or points with very small gradients rather than minima on the conformational energy surface. Current minimizers used in molecular mechanics tend to get 'stuck' at points on the energy surface with zero gradients (such as maxima and minima) and any conformations starting at energy maxima would not drop down to low energy conformations. This would result in the incorporation of coupling constants which may not actually contribute to the experimentally observed coupling constants.

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