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Synthesis and Elimination of C-3-Labeled Thiosialosides

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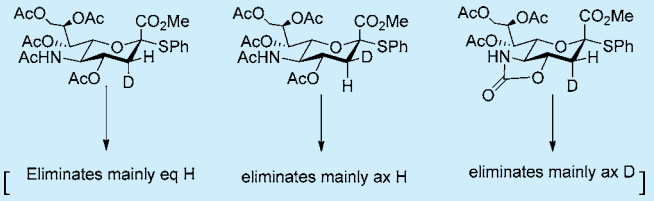
Supporting Information

ABSTRACT: The synthesis of C-3-labeled phenylthio sialic acid derivatives and an investigation of stereoselectivity in elimination reactions for the synthesis of 2,3-dehydro derivatives (glycals) is described. The experimental results are consistent with the existence of a conformational change and may be indicative of the intermediacy of an all-axial oxacarbenium ion.



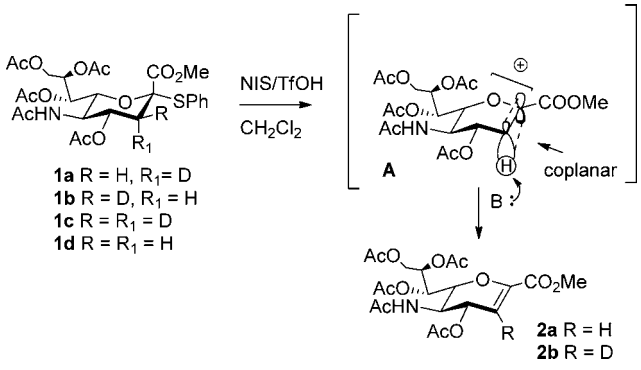
N-Acetylneuraminic acid (Neu5Ac) is the most common member of the sialic acid family, which is found at the terminal position of glycoconjugate chains.[1,2] Its involvement in numerous biological phenomena, ranging from cell−cell communication to pathogen recognition and oncogenesis, has been a driving force in the search of efficient synthetic methodologies to obtain oligosaccharides and glycoconjugates containing α-sialic acid residues. In the field of sialylation reactions, a dramatic improvement in the stereoselectivity toward the natural α-glycosidic linkage has been recorded in the past decades.[3−5] Another major research effort is the synthesis of 2,3-dehydro derivatives of sialic acids and evaluation thereof as neuraminidase inhibitors and substrates. In particular, a fast growing field has been the search for neuraminidase influenza inhibitors.[6−8]

Regardless of the synthetic target, it is clear that the development of efficient methodologies in glycochemistry, specifically in sialic acid chemistry, depends on extensive knowledge of the mechanism of substitution reactions, to form glycosidic bonds, or elimination reactions for the synthesis of glycals. Complete understanding of a reaction mechanism is in some cases a utopian task, as several mechanisms might compete and the prevalence of one over another might be decided by small alterations in reaction features.[9,10] On the other hand, for some systems, it is possible to narrow down the mechanism to one specific pathway and, in that case, gain a much better control of it.

As part of a research program dedicated to investigating reaction mechanisms in sialic acid chemistry, herein we report a study on the stereoselectivity of elimination of C-3-labeled thiophenyl sialosides. For this purpose, labeled compounds **1a** and **1b**, bearing deuterium at the C-3 axial and C-3 equatorial positions, respectively, were synthesized and their reactivity/selectivity was studied in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in dichloromethane as solvent (Scheme 1).

We anticipated that knowing whether the axial or equatorial hydrogen at C-3 was eliminated would give some useful insights into the course of the elimination reaction, its outcome, and mechanism. This, in turn, might allow for the development of improved methodologies. To begin our investigation, we assumed that the major elimination pathway would be via an E1 mechanism, where the oxacarbenium ion, formed upon the departure of the leaving group, would eliminate mainly (or only) the pseudo-axial H-3 due to its coplanarity in respect to the empty orbital of the anomeric carbon (**A**, Scheme 1). It should be noted that, through the text, 3-axial and 3-equatorial are referring to the substituent orientation in the $^2C_5$ chair conformation, standard for most sialic acids, whereas pseudo-axial/equatorial refer to the orientation in the oxacarbenium intermediates.

Scheme 1. Elimination Reactions of Labeled and Non-labeled Sialosides 1a−d



To evaluate the viability of this assumption, sialoside **1a** bearing an axial deuterium at the C-3 position was obtained following the protocol described by Friebolin and Schmidt, i.e., treatment of sialic acid **3** in alkaline solution with deuterium oxide (Scheme 2).[11] Under these reaction conditions, the monosubstitution occurs exclusively in the axial position.

The labeling reaction can be monitored by ^{1}H NMR following the disappearance of the signal corresponding to the

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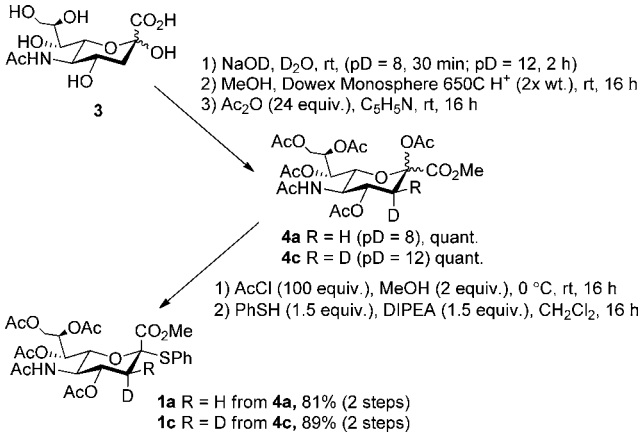
Scheme 2. Synthesis of D-3ax Sialoside 1a



H-3ax in **3** (Figure 1a–c). Concomitant conversion of the H-3eq signal from a double doublet to a doublet is indicative of monodeuteration at the C-3 axial position. In the case of overdeuteration, which leads to the formation of **1c**, complete disappearance of H-3 signals is observed. In order to stop the reaction at the monodeuteration level, the pD value was found to be of critical importance. Thus, after thorough fine-tuning of the reaction conditions, we concluded that pD = 8 allows for a nearly quantitative monodeuteration. It should be noted that the reaction at pD = 12 predominantly yields the dideuterated product, as previously reported.[11]

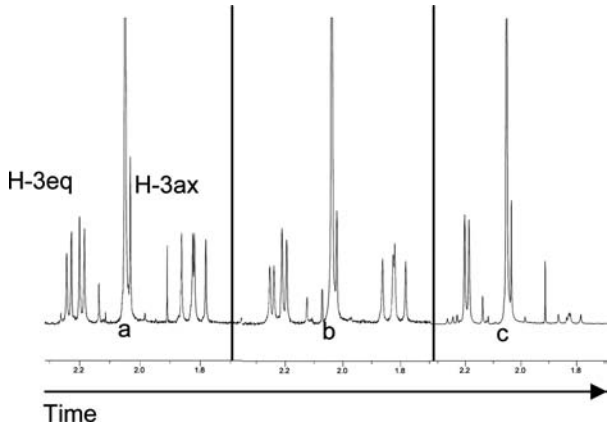
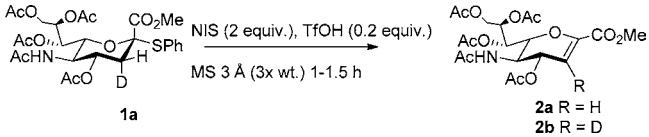


Figure 1. Monitoring labeling process by ^{1}H NMR in D_2O (400 MHz, *a* = 0 min; *b* = 60 min; *c* = 120 min).

Upon labeling, esterification of the reaction mixture in an acidic solution of MeOH, followed by acetylation, gave either the mono- or dideuterated products **4a** or **4c**, respectively. Conversion of **4a** in the corresponding chloride by treatment with acetyl chloride in methanol, followed by the reaction with thiophenol in the presence of diisopropylethylamine (DIPEA), gave thioglycoside **1a** in 81% yield over two steps (Scheme 2). Having obtained the labeled thiosialoside **1a**, we turned our attention to studying its properties in the elimination reaction in the presence of NIS/TfOH as the promoter system in dichloromethane. The ratio between H-3 glycal **2a** and D-3 glycal **2b**, the two possible elimination products, was determined by ^{1}H NMR analysis of the crude reaction mixture. As summarized in Table 1, D-3 glycal **2b**, resulting from the elimination of the H-3eq in **1a**, was found

Table 1. Elimination of D-3ax Sialoside 1a

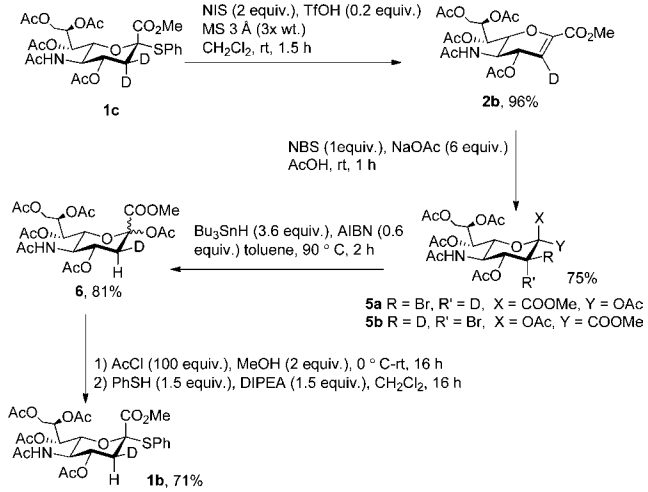


entry	solvent/temp (°C)	ratio **2a**:**2b**	yield (%)	major elimination
1	CH_2Cl_2/rt	1:5	98	H-3eq
2	CH_2Cl_2/0	1:6	87	H-3eq
3	CH_2Cl_2/−40	1:6	84	H-3eq

to be the major product of the reaction performed at room temperature (**2a**:**2b** = 1:5, entry 1). Somewhat surprisingly, this ratio was practically unaffected when the elimination reaction was performed at a lower temperature of 0 or −40 °C (entries 2 and 3).

The preference of **1a** to eliminate the pseudo-equatorial H-3 can be rationalized by the isotopic effect created by the stronger C–D bond at C-3 rather than an intrinsic tendency of this system to eliminate in any case from this position.[12] To confirm this possibility, we proceeded to the synthesis and elimination of thiosialoside **1b** having inverted configuration at C-3 (D-3eq, H-3ax) with respect to that of **1a**. The direct introduction of deuterium in the equatorial position at C-3 of sialic acid **3** did not seem feasible. Hence, we chose to originate **1b** from the dideuterated intermediate **1c**. As depicted in Scheme 3, elimination of **1c** in the presence of

Scheme 3. Synthesis of D-3eq Sialoside 1b



NIS/TfOH yielded glycal **2b** in 96% yield. Treatment of **2b** with *N*-bromosuccinimide (NBS), in the presence of sodium acetate and acetic acid, gave two 3-brominated diastereomers **5a** and **5b**, which could be separated by silica gel chromatography. Reduction of either **5a** or **5b** with tributyltin hydride occurred with high stereoselectivity, and in both cases, the hydride was delivered in the axial position almost exclusively, probably due to steric hindrance. Therefore, both intermediates **5a** and **5b** could be used as precursors for the synthesis of D-3eq thiosialoside **6**. Conversion of **6** into the corresponding chloride, followed by the reaction with thiophenol in the presence of DIPEA, afforded the desired D-3eq thiosialoside **1b** in 71% yield (Scheme 3).

With the D-3eq thiosialoside **1b** in hand, we turned our attention to studying its elimination reaction in the presence

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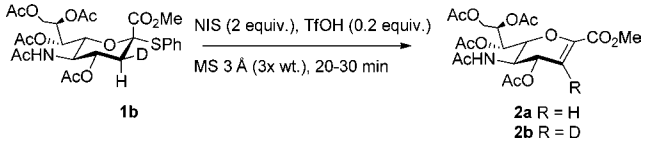
of NIS/TfOH. As listed in Table 2, elimination of **1b** at room temperature gave a mixture of H-3 glycal **2a** and D-3 glycal **2b** in a high yield (entry 1). The observed ratio of **2a**:**2b** = 1:2 was indicative of the preferential elimination of the H-3ax in **1b**. The effect of the temperature, although still minimal, was in this case more pronounced at −40 °C, with a ratio of **2a**:**2b** = 1:4 being obtained (entry 3). These results prove that elimination from the pseudo-axial position can occur, and the overall stereoselectivity observed in the elimination of **1a** and **1b** is mainly controlled by the relative position of hydrogen vs deuterium.

Table 2. Elimination of D-3eq Sialoside 1b

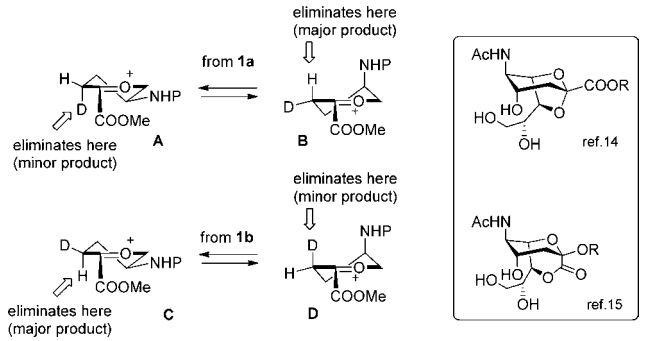


entry	solvent/temp (°C)	ratio **2a**:**2b**	yield (%)	major elimination
1	CH_2Cl_2/rt	1:2	93	H-3ax
2	CH_2Cl_2/0	1:2	63	H-3ax
3	CH_2Cl_2/−40	1:4	71	H-3ax

One explanation of the cumulative experimental data presented in Tables 1 and 2 invokes the existence of an equilibrium between an all-equatorial and all-axial conformations of the oxacarbenium ion, the key intermediate en route via the E1 pathway.

In this context, a conformational equilibrium of the oxacarbenium ion, and its contribution to controlling the overall efficiency and stereoselectivity of glycosylation reactions with model compounds and hexoses, has been acknowledged by many groups.[13] Oxacarbenium ions and conformations thereof within the sialic acid series are much less studied. However, the ease of the synthesis of 2,7-dehydro[14] and 1,7-lactone[15] derivatives (Scheme 4) is

Scheme 4. Conformational Equilibrium at the Oxacarbenium Ion Level and Known Sialosides with $^5C_2$ Conformation

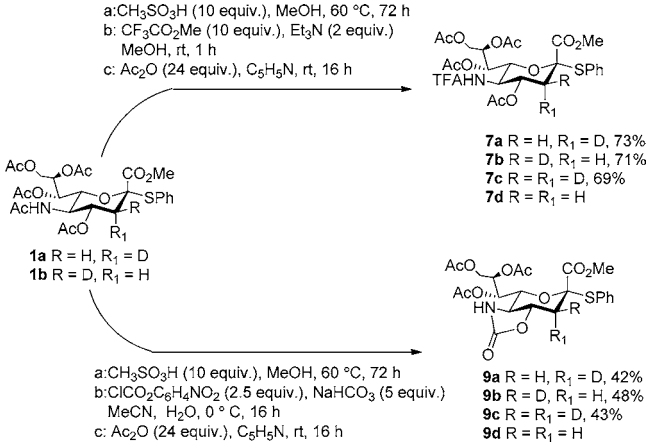


certainly indicative of the existence of all-axial conformations for sialic acid intermediates and substrates. In view of this equilibrium, our data might be the result of isotopic influenced pseudoaxial-eliminations from the two conformations.

In fact, regardless of the orientation of H-3 in the starting thioglycoside, the system would adjust itself to provide a suitable conformation to facilitate the elimination of more weakly bonded H-3 rather than D-3. Thus, starting from thiosialoside **1a**, two conformations of the oxacarbenium intermediate **A** and **B** can be envisaged with the major elimination product arising from **B** (Scheme 4). On the other hand, starting from thiosialoside **1b**, two conformations of the oxacarbenium intermediate **C** and **D** can be envisaged, with the main elimination occurring in this case from the all-equatorial conformation **C** (Scheme 4).

To support this general concept and elucidate a possible influence of the all-axial conformation, we decided to obtain and study two new series of sialosides modified at C-5, trifluoroacetamido sialosides **7a**,**b**, and oxazolidinones **9a**,**b** (Scheme 5).

Scheme 5. Synthesis of Sialosides 7a,b and 9a,b



Structural modifications at C-5 have found a broad application in sialic acid chemistry due to their profound effect on the reactivity, selectivity, and yields.[4] For our testing, sialosides **7a** and **7b** bearing a strongly electron-withdrawing 5-trifluoroacetamido group should provide additional stabilization of the all-axial conformation while disfavoring the all-equatorial counterpart.[16] On the other hand, 4,5-oxazolidinone-substituted sialosides **9a** and **9b** having trans-fused rings should limit the conformational freedom of the pyranose ring. As a result of these induced structural modifications and conformational preferences, we expected different results from those acquired with standard 5-acetamido sialosides **1a** and **1b**, which would imply a profound effect of conformation on stereoselectivity.

The syntheses of D-3ax sialosides **7a** and **9a** and their D-3eq counterparts **7b** and **9b** were accomplished from the respective labeled precursors **1a** and **1b** following standard manipulations as depicted in Scheme 5.[17−19] As a control standpoint, we also obtained and tested 3-D,D and 3-H,H sialosides **7c**, **9c** and **7d**, **9d**, respectively. Sialosides **7c** and **7d** gave the corresponding glycals **8a**,**b** within minutes and in moderate/high yield (89% for **7c** and 97% for **7d**). Conversely, elimination of the sialosides **9c** and **9d** afforded the corresponding glycals 3-H **10a** and 3-D **10b** within minutes in high yield (73% for elimination of **9c** and 74% for elimination of **9d**).

Elimination reactions of D-3ax **7a** gave results similar to those of the corresponding N-acetyl donor **1a** (**8a**:**8b** = 1:6, entry 1, Table 3). On the other hand, when D-3eq sialoside **7b** was tested, we obtained a mixture of glycals closer to a 1:1 ratio (**8a**:**8b** = 1:1.4, entry 2, Table 3). The difference in the ratio observed between **7b** and **1b** (**2a**:**2b** = 1:2), although

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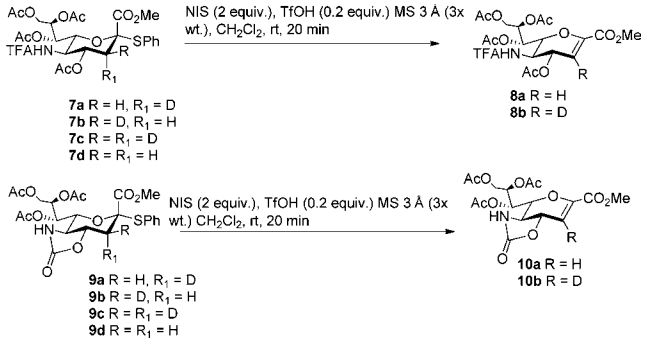
not very high, might be related to the effect of the C-5 modification on the conformational equilibrium of the oxacarbenium ion.

Encouraged by these results, we proceeded with testing the elimination reactions of **9a** and **9b** having, at C-5, oxazolidinone groups and D-3ax and H-3ax, respectively. Elimination of thiosialoside **9a** gave a mixture of glycals **10a**,**b** with a ratio of 2:1, showing a preference of this system to cleave the stronger pseudo-axial C−D bond rather than the weaker pseudo-equatorial C−H bond. Conversely, elimination of **9b**, with deuterium in the equatorial position, gave mainly elimination of the pseudo-axial H-3 (**10a**:**10b** = 1:7). The opposite stereoselectivity observed for the elimination of **9a** vs **1a** suggests that a conformational change of the oxacarbenium ion intermediate might be a strong factor governing the stereoselectivity of elimination reactions.

Table 3. Elimination of C-5-Modified Thiosialosides



entry	sialoside	products	ratio	yield (%)	major elimination
1	**7a**	**8a**:**8b**	1:6	69	H-3eq
2	**7b**	**8a**:**8b**	1:1.4	87	H-3ax
3	**9a**	**10a**:**10b**	2:1	58	D-3ax
4	**9b**	**10a**:**10b**	1:7	62	H-3ax

In conclusion, novel C-3 deuterium-labeled sialosides **1**−**9** were synthesized, including C-5 modified sialosides with oxazolidinone and trifluoroacetyl groups. Elimination reactions of each of these sialosides suggested the presence of a conformational equilibrium which is strongly influenced by the isotopic effect, conformational constraints, and electronegativity of the C-5 substituent.[20] Although the ratio of the glycals obtained did not provide a quantitative measurement of the conformational equilibrium (Curtin−Hammett principle),[21] it did confirm, in our opinion, the presence and the relevance of an all-axial oxacarbenium ion intermediate, which should be taken into account when analyzing results of elimination and sialylation reactions.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ DEDICATION

This paper is dedicated to the late Dr. Masangu Shabangi and to the late Dr. Kevin Johnson (Southern Illinois University Edwardsville).

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