DOI: 10.1002/ejoc.200801017

Stereoselectivity of Glycosylation May Change During the Reaction Course: Highly α-Stereoselective Sialylation Achieved by Supramer Approach

Leonid O. Kononov,*[a] Nelly N. Malysheva,[a] and Anna V. Orlova[a]

Keywords: Glycosylation / Stereoselectivity / Sialic acids / Hydrogen bonds / Amides

The stereoselectivity of NIS/TfOH-promoted glycosylation with *O*-acetylated *N*-acetylneuraminic acid phenyl thioglycoside was found to be dependent on the reaction time. Highly α-stereoselective sialylation (27:1 anomer ratio) was achieved by decreasing the reaction time (from 3 h to 15 min) and by adding a non-reacting compound (1 equiv.) capable of modifying the structure of hydrogen-bonded supramolecular aggregates (supramers) of the glycosyl donor.

Introduction

Sialic acid containing glycoconjugates are involved in a wide range of cell-surface recognition phenomena in living systems.[1] For this reason, tremendous efforts have been made in order to develop efficient methods for the synthesis of sialo-oligosaccharides.[2] Although substantial progress has recently been achieved in this booming area,[2c,2e–2g] poor predictability and reproducibility of results are still typical of the sialylation reaction. Especially notorious in this respect is the stereoselectivity of sialylation, which can sometimes vary considerably even upon repetitive glycosylations performed by the same person, not to mention the results for seemingly identical sialylations reported by different research groups. There has been a long-standing need for an approach to rationalize the outcome of a particular glycosylation experiment, which can be confusing.

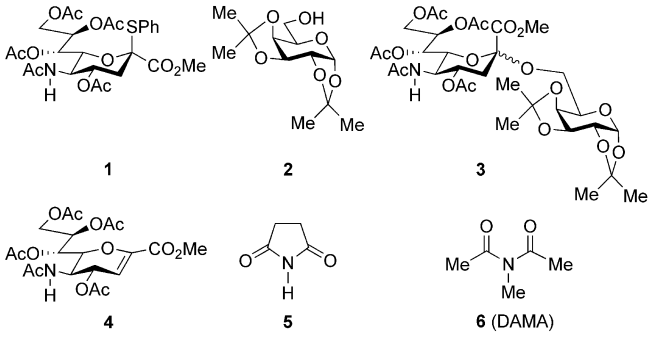
We have recently suggested[3] a novel concept (the "supramer approach") that emphasizes the importance of supramolecular aggregation in solution leading to the formation of supramers,[4] which are *supra*molecular iso*mers*, i.e. compounds of the same composition but differing in properties. Molecular structures of reactants and reaction conditions would determine the aggregation type and the structure of supramers. Accessibility of the reaction center in the supramers formed would influence their reactivity, product yield and stereoselectivity of glycosylation. Formation of supramers in the reaction mixture should be considered as an important factor which is currently almost ignored during the analysis of the outcome of glycosylation and other reactions in carbohydrate chemistry. Our concept may provide insight into seemingly incomprehensible results. Among them are such different observations as a novel phenomenon of synergism in sialylation,[3a] significant influence of a remote functional group in the aglycon on the reactivity during the chemical synthesis of HNK-1 pentasaccharide[5] or unprecedented dependence of the hydrolytic stability of *closo*-carborane–lactose neoglycoconjugates on the nature of a spacer,[6] to mention a few. All these and other seemingly unrelated "strange" facts can easily be rationalized if one considers that the real reacting species are differently organized supramers. Thinking along this line we were able to demonstrate that changes in the hydrogen-bond-mediated aggregation in solutions of the glycosyl donor induced by the addition of external non-reacting amides/imides or by concentration changes may dramatically influence the outcome of sialylation.[3b]

In this communication we discuss the issues of stereoselectivity of sialylation by the supramer approach. This analysis has led to the discovery of completely unprecedented and unexpected results, which are described below.

[a] N. K. Kochetkov Laboratory of Carbohydrate Chemistry,
N. D. Zelinsky Institute of Organic Chemistry,
Leninsky prospect 47, 119991 Moscow, Russian Federation
Fax: +7-499-137-6148
E-mail: kononov@ioc.ac.ru
leonid.kononov@gmail.com

Results and Discussion

In our previous publication[3b] we showed that changes in the concentration or addition of external amides/imides may result in variations of the *yield* of sialylation product from 57% to 88%, when the sialylation of glycosyl acceptor **2**[7] with glycosyl donor **1**[8] was promoted by *N*-iodoscuccinimide NIS) and catalytic amounts of TfOH (NIS/TfOH)[9a] in MeCN (Scheme 1). To our surprise no substantial changes in *stereoselectivity* were observed in these glycosylation reactions. Moderate stereoselectivities were achieved: α/β ratios were mostly within the 7:1–10:1 range, and only in two of 14 cases were the α/β ratios somewhat lower (4:1–5:1).[3b]



Scheme 1. Structures of sialic acid glycosyl donor **1**, glycosyl acceptor **2**, disaccharide **3**, glycal **4** and imides **5** and **6**.

This clear *insensitivity* of the stereoselectivity to the presence of additives and their nature is intriguing since it apparently contradicts our concept, which has been fairly successful in explaining the variety of experimental results so far.[3,5,6] According to the supramer concept, the influence of the addition of amides/imides[3b] on the outcome of sialylation (including stereoselectivity) is related to the rearrangement of the intermolecular hydrogen-bonding network and the formation of supramers of the glycosyl donor, organized differently as compared to the situation without any additives (see above). This change in the structure of the supramers can modulate the accessibility of the different faces of the glycosyl donor in the supramers formed and hence the stereoselectivity of glycosylation. In order to resolve this apparent inconsistency we have to analyze the problem in more detail.

Until this study we were considering[3a,3b] only a *static* situation, which corresponded to the structure of the supramers *before* the beginning of the reaction. Of course, this is only the first approximation. When the glycosylation commences, the concentration of the glycosyl donor **1** starts to decrease. This change in concentration of amide **1** would lead to changes in the hydrogen-bonding pattern and hence the structures of hydrogen-bonded supramers as it was demonstrated earlier.[3b] However, at the same time the concentration of the glycosylation product **3** is gradually increasing. For similar reasons, the change in the concentration of product **3**, which is an amide too, would modulate the structure of hydrogen-bonded supramers of glycosyl donor **1**. The situation appears to be rather complex. Fortunately, as can easily be seen from the comparison of the molecular structures of compounds **1** and **3**, the spatial arrangements of hydrogen-bond acceptor and donor sites (especially those of the amide group) in their molecules are almost identical. This suggests that the intermolecular hydrogen bonding patterns of each of these two molecules are similar. More important is the conclusion that the decrease in concentration of **1** is almost completely *compensated* by the increase in concentration of **3**, and therefore no substantial changes in the hydrogen-bonding pattern would occur.[10] For this reason, no change in the structure of hydrogen-bonded supramers of glycosyl donor **1** induced by the changes in concentrations of the glycosyl donor and the products should be expected during the course of the reaction.

However, more careful consideration of the composition of the reaction mixture reveals another possible hydrogen-bonding partner, succinimide **5**, which is gradually formed during the NIS/TfOH-promoted glycosylations,[11] the amount of **5** being proportional to the amount of glycosyl acceptor **2** reacted. The spatial arrangement of hydrogen-bond acceptor and donor sites in the molecule of succinimide **5**, which comprise two acceptor sites (C=O groups) and, more importantly, one *donor* site (NH group), differs considerably from those of amides **1**, **3**, **4** or amides/imides [e.g., DAMA (**6**)] used[3b] for modulation of the hydrogen-bond network earlier. Since the NH proton of imide **5** is much more acidic than those of amides **1** or **3**, the former compound is a much stronger hydrogen-bond donor capable of competing effectively with amides **1** and **3** for hydrogen-bond acceptors present in the reaction mixture. Therefore, the increase in concentration of succinimide **5** would induce completely different and substantial changes in the structure of hydrogen-bonded supramers of glycosyl donor **1**. For this reason, at the *end* of the reaction, regardless of the presence of other amides/imides[3b] lacking the NH group, the structure of hydrogen-bonded supramers of the glycosyl donor would be mainly determined by this *additional* strong hydrogen-bond donor (**5**). Hence the stereoselectivity of glycosylation would be *similar* in all these cases as indeed was earlier observed[3b] (see above). This also means that one may expect considerable changes in the stereoselectivity of silalylation to occur *during* the course of the reaction since the influence of succinimide **5** would increase with its concentration.

To verify this hypothesis, we performed the standard NIS/TfOH-promoted sialylation of glycosyl acceptor **2** with glycosyl donor **1**, but quenched the reaction after 15 min rather than after 3 h as in our previous experiments,[3b] after which all glycosyl donor was completely consumed. The results were impressive. Although the yield of the glycosylation product **3** somewhat decreased (62% after 15 min vs. 69% after 3 h) the amount of the α-anomer *doubled* (α/β = 13:1 after 15 min vs. 7:1 after 3 h) (Table 1, Entries 1 and 4). This higher α-stereoselectivity at the beginning of the glycosylation, which deteriorated at the end of the reaction, is completely unprecedented to the best of our knowledge.

Although the observation of the change in stereoselectivity during the course of sialylation in MeCN (promoted by NIS/TfOH) was predicted by the supramer concept, one may argue that there could be other reasons for this phenomenon. The first possibility that immediately comes to mind is anomerization of the primary α-configured glycosylation product **α-3** by the TfOH present in the reaction mixture, which could lead to the increase in the proportion of the thermodynamically more stable β-anomer of **3** during the course of the reaction. No reports of such anomerization has been reported so far, and the NIS/TfOH promoter system is currently considered to be fairly safe in this respect.[11] The reactions between various sialic acid thiogly-

Table 1. Conditions and products of glycosylation.

Entry	Promoter	Reaction time	Additive (equiv.)	Yield (%) of **3**[a]	Anomeric ratio for **3** (α/β)[b]	Anomeric ratio (α/β) range
1	NIS/TfOH[c]	15 min	none	62	13:1	
2	NIS/TfOH[c]	15 min	DAMA (1)	61	27:1	13:1–27:1
3	NIS/TfOH[c]	15 min	DAMA (3)	57	18:1	
4	NIS/TfOH[c]	3 h	none	69	7:1	
5	NIS/TfOH[c]	3 h	DAMA (1)	80	8:1	7:1–8:1
6	NIS/TfOH[c]	3 h	DAMA (3)	72	7:1	
7	DMTST[d]	15 min	none	37	ca. 2:1[e]	ca. 2:1
8	DMTST[d]	18 h	none	46	ca. 2:1[f]	ca. 2:1

[a] Yield of disaccharide fraction isolated by gel chromatography on BioBeads S×3. [b] NMR spectroscopic data. [c] 1 equiv. of **1**, 1 equiv. of **2**, NIS/TfOH, MS (3 Å), –40 °C. [d] 1 equiv. of **1**, 1 equiv. of **2**, DMTST, MS (3 Å), ca. 25 °C. [e] α/β = 1.7:1. [f] α/β = 1.7:1.

cosides and alcohols promoted by NIS/TfOH were performed by various research groups. In many cases the reaction mixtures were left for much longer periods of time than 3 h used in our work without any decrease in stereoselectivity. For example, thioglycoside **1** was used for sialylation of a Le^x trisaccharide acceptor to give exclusively the α-configured glycosylation product in 58% yield after overnight treatment with NIS/TfOH at –35 °C.[12] For this reason, there are no grounds to suppose that the reaction used in our study is an exception in this respect. In addition, when the sample of disaccharide **3**, obtained after 15 min of reaction (Table 1, Entry 1), was subjected to the reaction conditions [NIS/TfOH/MS (3 Å)/MeCN/–40 °C] for 3 h it was recovered quantitatively and was identical to the original sample before this treatment. No change in anomeric ratio (α/β) of the disaccharide obtained, as determined by ^{1}H NMR spectroscopy and specific rotation ($[\alpha]_D$), was observed (data not shown). This excludes the possibility of anomerization completely.

Another possibility might be the putative difference in kinetics of the formation of α- and β-isomers of disaccharide **3**, i.e. the major portion of α-isomer is mainly formed at the beginning of the reaction, whereas the β-isomer is mainly formed at the end of the glycosylation. Essentially, the rate of formation of the β-isomer should substantially increase during the course of the reaction. The reason for such an increase could only be the change in reaction conditions. We may conclude that the effect of the reaction time on the stereoselectivity of the sialylation in MeCN could be related to the changes in reaction conditions during the course of the sialylation, which are apparently caused by the changes in concentrations of reagents and products of the reaction. This notion immediately leads us back to the relevance of the change in the structure of hydrogen-bonded supramers of the glycosyl donor, induced by the change of concentration of succinimide **5** as discussed above.

Succinimide **5** is only formed in glycosylation reactions promoted by NIS/TfOH.[9a,11] However, glycosylation reactions promoted by DMTST[9b,11] do not produce NH-containing by-products capable of influencing the hydrogen-bonding pattern in supramers of glycosyl donor **1**. Therefore, if the use of DMTST as the promoter would exclude a dramatic change in the stereoselectivity during the course of the reaction, then we may safely conclude that it is succinimide **5** that is mainly responsible for supramer structure changes during the sialylation. This was found to be the case. Sialylation of glycosyl acceptor **2** with glycosyl donor **1**, promoted by DMTST,[9b] gave samples of disaccharide **3** with *identical* anomeric ratios both after 15 min and 18 h of reaction (Table 1, Entries 7 and 8).

One may also argue that succinimide **5** might react with the "glycosyl cation", thus forming another reactive glycosyl intermediate, and the glycosylation reaction would proceed along a different pathway, thus changing the stereoselectivity. Indeed, in some cases reactions of thioglycosides (not sialic acid derivatives) promoted by NIS (in the presence of TfOH,[13a–13d] TMSOTf,[13e] TESOTf[13f] or without any acids[13g]) were reported to result in the formation of *N*-glycosylsuccinimides[13] rather than the expected glycosylation products. In all cases these compounds were formed either in the absence of a glycosyl acceptor or when a glycosyl acceptor with low nucleophilicity was used. It is important that *N*-glycosylsuccinimides were not able to act as glycosyl donors under the reaction conditions[13g] and therefore cannot be considered as reactive intermediates in the glycosylation reactions.

The achieved success in increasing the stereoselectivity of the sialylation by shortening the reaction time is somewhat spoiled by the decrease in the disaccharide yield due to incomplete consumption of the glycosyl acceptor. Since addition of *N*,*N*-diacetylmethylamine (**6**, DAMA) to the reaction mixture was found to be highly favorable for the increase in the yield of glycosylation product **3** when the glycosyl donor was allowed to react for 3 h (Table 1, Entries 4–6),[3b] we studied the effect of decreasing the reaction time on the outcome of sialylation performed in the presence of variable amounts of DAMA. Unfortunately, after 15 min of reaction, no increase in the product yield was observed (Table 1, Entries 2, 3) in comparison with the reaction without additives (Table 1, Entry 1). Nevertheless, we were delighted to find that the stereoselectivity of the silaylation increased even *further* in these experiments. Especially striking is the result obtained after 15 min of reaction in the presence of equimolecular amount of DAMA (Table 1, Entry 2), in which almost only one isomer of disaccharide **3** was formed (α/β = 27:1). This dramatic increase in the

stereoselectivity of the sialylation induced by the addition of a non-reacting compound is in full accord with the supramer concept, which predicts changes in the accessibility of the α- and β-faces of the glycosyl donor in differently organized supramers formed by the glycosyl donor in the presence of compounds, such as DAMA, capable of modifying the hydrogen-bonding network.[3b]

Additives have been previously employed in glycosylation reactions to modulate the stereoselectivity, and some of them are believed to act by forming reactive glycosyl intermediates upon reaction with "glycosyl cation", such as is the case with tetramethylurea (TMU),[14] hexamethylphosphortriamide (HMPA),[14a] *N*,*N*-dimethylformamide (DMF)[14b,14c] and, in the sialic acid field, with diaryl sulfoxides.[15] The existence of such intermediates was demonstrated by NMR spectroscopy in the case of DMF[14b,14c] and diphenyl sulfoxide.[15c]

Participation of Ph_2SO in the stabilization of the "glycosyl cation" resulting in the formation of a glycosyloxysulfonium cationic intermediate[15] does not necessarily lead to an increase in α-stereoselectivity. In this respect it is very instructive to compare the stereoselectivities of sialylations of the same glycosyl acceptor [methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**7**)] in CH_2Cl_2 with similar glycosyloxysulfonium species (as claimed by the authors) generated from *N*-acetyl- and *N*,*N*-diacetylsialic acid derivatives. Whereas the former gave mainly β-sialoside (α/β = 1:3),[15b] the latter gave almost equimolecular mixture of both isomers (α/β = 1.2:1).[15c] This clearly demonstrates that the situation is very complex, and the influence of the 5-*N*-substituent in the molecule of a sialic acid glycosyl donor is substantial (see refs.[3a,3b] for a discussion of the possible reasons of different behavior of *N*-acetyl- and *N*,*N*-diacetylsialic acid derivatives in view of the supramer concept). It is very important that the sialylation of the same acceptor **7** with glycosyloxysulfonium species bearing an *N*,*N*-dimethylglycolamide C1 auxiliary, introduced as a tool for anomeric stereocontrol in the synthesis of α-sialyl glycosides,[15a] resulted in a completely *unselective* glycosylation (α/β = 1:1).[15b] Moreover, sialylation of alcohol **2**, used in our study, with this C1-glycolamide/C2-hemiketal sialyl donor under the conditions of a dehydrative sialylation protocol, resulted in poor stereoselectivity (α/β = 4:1)[15b] even though the participation of a putative glycosyl imidate cationic intermediate was presumably involved. It is worth mentioning that in the same article[15b] the dehydrative sialylation involving formation of glycosyloxysulfoinium species in EtCN, which was expected to exert a "nitrile effect", was demonstrated, by using a different example, to be completely non-selective (α/β = 1:1). This means that the exceptionally high α-selectivity observed in the presence of DAMA in our case cannot be entirely attributed to the participation of putative imidate cationic intermediates presumably derived from DAMA and a "glycosyl cation". Such intermediates, if any, are expected to be more easily formed in the presence of excess of DAMA (as was indeed observed in the presence of excess Ph_2SO[15c]). However, in our case, although addition of 1 equiv. of DAMA did increase the stereoselectivity greatly (Table 1, Entry 2), the excess of DAMA resulted in a decrease rather than a further increase in stereoselectivity of the sialylation (Table 1, Entry 3). Variable amounts of amide additives have recently been shown by IR spectroscopy to modify the hydrogen-bonding network in solutions of glycosyl donor **1** in different ways.[3b] These changes in the hydrogen-bonding pattern were correlated with changes in the outcomes of sialylation reactions performed in the presence of different amounts of additives, thus supporting the supramer approach.[3b] It is evident that factors other than the formation of imidate cationic intermediates seem to be more essential for the stereochemical outcome of the sialylation, and we believe that supramolecular aggregation in the reaction mixture is one of them.

A comparison of the results of the "regular" sialylation (Table 1, Entry 4) and the "optimized" one (Table 1, Entry 2) clearly suggests that, by using exceptionally simple means, we were able to develop a highly α-stereoselective sialylation (gain of anomeric ratio α/β from 7:1 to 27:1) accompanied by a small decrease in the yield of the glycosylation product (drop from 69% to 61%). This apparent decrease in the efficiency of the sialylation can be tolerated considering the enormous increase in stereoselectivity achieved. However, since the reaction in the presence of DAMA (1 equiv.) performed for 3 h (Table 1, Entry 5) gives the product in 80% yield, one can expect that recovery of the unreacted acceptor and repetition of the glycosylation for another 15 min would give the glycosylation product in at least 70% overall yield.

Conclusions

By using the supramer approach we performed a series of experiments, which demonstrated for the first time that the stereoselectivity of glycosylation may change during the reaction period, and developed a highly α-stereoselective sialylation achieved by exceptionally simple means: by the addition of 1 equiv. of a non-reacting compound (DAMA) and by the decrease in reaction time from 3 h to 15 min.

A similar approach could be used for optimizing other NIS/TfOH-promoted glycosylations, in which the formation of hydrogen-bonded supramers could be expected. Addition of compounds capable of modifying the structure of supramers of the glycosyl donor (or/and acceptor) to the reaction mixture and varying the reaction time may be a useful alternative to the conventional optimization of the molecular structure of the glycosyl donor, which is by no means a trivial task.

This is yet another example of productivity of the supramer concept, which has only recently started to be developed.[3,6] Just one simple assumption that the real reacting species are supramers rather than isolated molecules has already led to a rational explanation of a series of unusual observations and the generation of new results which are completely unpredictable from the traditional point of view.

Experimental Section

General: The reactions were performed by using commercial reagents. Solvents were distilled and purified before the use according to standard procedures. TLC was carried out on Silica Gel 60 F_{254} plates (Merck), spots were visualized under UV light and by heating the plates after immersion in a 1:10 (v/v) mixture of 85% aqueous H_3PO_4 and 95% EtOH. NMR spectra of solutions in $CDCl_3$ were recorded with a Bruker AVANCE-600 instrument. The ^1H chemical shifts are given relative to the signal of the residual $CHCl_3$ (δ = 7.27 ppm), the ^{13}C chemical shifts were measured relative to the signal of $CDCl_3$ (δ = 77.0 ppm). The optical rotation ($[\alpha]_D$) was measured for solutions of compound **1a** in $CHCl_3$ with a JASCO DIP-360 polarimeter at 25 °C.

Typical Glycosylation Procedure (Activation with NIS/TfOH): A mixture of thioglycoside **1** (58.3 mg, 0.1 mmol, 1 equiv.) and alcohol **2** (26 mg, 0.1 mmol, 1 equiv.) was dried in vacuo for 2 h, then anhydrous MeCN [6 mL, distilled from P_2O_5, stored over molecular sieves (3 Å)] was added under argon followed by DAMA (**6**; 1 or 3 equiv.; if appropriate). Freshly activated (220 °C, 6 h, in vacuo) powdered molecular sieves (3 Å; 300 mg, Fluka) were added to the resulting solution, and the reaction flask was flushed with argon. The suspension was stirred under argon at room temperature for 1 h, then cooled to –40 °C (liquid N_2/MeCN bath). Solid NIS (34 mg, 0.15 mmol, 1.5 equiv.) followed by TfOH was added. Only the minimum amount of TfOH required to generate a persistent color was added. The actual amount of TfOH varied with the amount of DAMA (**6**) added (7.5 μL, 0.08 mmol, 0.8 equiv. in the absence of DAMA; 15 μL, 0.17 mmol, 1.7 equiv. in the presence of 1 equiv. of DAMA; 25 μL, 0.28 mmol, 2.8 equiv. in the presence of 3 equiv. of DAMA). The reaction mixture was stirred under argon at –40 °C for the time specified in Table 1 (15 min or 3 h), then diluted with $CHCl_3$ (20 mL) and filtered through a Celite pad. The solids were thoroughly washed with $CHCl_3$ (100 mL), and the filtrate was successively washed with 20% aqueous $Na_2S_2O_3$ (2 × 50 mL) and water (2 × 50 mL), filtered through a cotton wool plug and concentrated. The residue was dissolved in toluene (2 mL) and separated by gel chromatography on a column (50 × 2.5 cm) with Bio-Beads S×3 (200–400 mesh, Bio-Rad) using toluene as the eluent and a differential refractometer (Knauer) as the detector. The first eluted fraction contained disaccharide **3**, which was analyzed by NMR spectroscopy. Later eluted fractions contained Neu5Ac glycal **4** and finally unreacted alcohol **2**. A base-line separation of all mentioned components was repeatedly achieved. All yields were calculated with respect to the glycosyl acceptor **2** taken (Table 1). TLC data: R_f (benzene/acetone, 3:2) = 0.45 (**1**), 0.62 (**2**), 0.40 (**3**).

Typical Glycosylation Procedure (Activation with DMST): A mixture of thioglycoside **1** (58.3 mg, 0.1 mmol, 1 equiv.) and alcohol **2** (26 mg, 0.1 mmol, 1 equiv.) was dried in vacuo for 2 h, then anhydrous MeCN [6 mL, distilled from P_2O_5, stored over molecular sieves (3 Å)] was added under argon. Freshly activated (220 °C, 6 h, in vacuo) powdered molecular sieves (3 Å; 300 mg, Fluka) were added to the resulting solution, and the reaction flask was flushed with argon. The suspension was stirred under argon at room temperature for 1 h, then solid DMTST [206.4 mg as a 1:1 mixture with molecular sieves (3 Å), 0.4 mmol, 4 equiv.] was added. The reaction mixture was stirred under argon at room temperature (ca. 25 °C) for the time specified in Table 1 (15 min or 18 h), then diluted with $CHCl_3$ (20 mL) and filtered through a Celite pad. The solids were thoroughly washed with $CHCl_3$ (100 mL), and the filtrate was successively washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and water (2 × 50 mL), filtered through a cotton wool plug and concentrated. The residue was dissolved in toluene (2 mL) and separated by gel chromatography on a column (50 × 2.5 cm) with Bio-Beads S×3 (200–400 mesh, Bio-Rad) using toluene as the eluent and a differential refractometer (Knauer) as the detector as described above. The yields are presented in Table 1. The NMR spectroscopic data for the disaccharide fraction are identical to those reported in ref.[16] For the determination of the anomer ratio of disaccharide **3** integral intensities of the signals of α-H-3′eq (δ = 2.61, dd, J = 12.8, 4.7 Hz) and β-H-3′eq (δ = 2.48, dd, J = 12.9, 4.8 Hz) of the Neu5Ac residue were used.

Acknowledgments

This work was supported by the Russian Foundation for Basic Research (projects No. 05-03-32579 and 08-03-00839) and the Council on Grants at the President of the Russian Federation (project MK-3244.2008.3). We are grateful to V. I. Bragin for recording NMR spectra with a Bruker Avance 600 spectrometer.

[1] a) R. Schauer, "Biochemistry of Sialic Acid Diversity", in *Carbohydrates in Chemistry and Biology* (Eds.: B. Ernst, G. W. Hart, P. Sinaÿ), Wiley-VCH, Weinheim, **2000**, part II (Biology of Saccharides), vol. 3 (Biosynthesis and Degradation of Glycoconjugates), pp. 227–244; b) T. Angata, A. Varki, *Chem. Rev.* **2002**, *102*, 439–469.

[2] a) G.-J. Boons, A. V. Demchenko, *Chem. Rev.* **2000**, *100*, 4539–4565; b) D. K. Ress, R. J. Linhardt, *Curr. Org. Synth.* **2004**, *1*, 31–46; c) C. De Meo, U. Priyadarshani, *Carbohydr. Res.* **2008**, *343*, 1540–1552; d) K. Ikeda, M. Aizawa, K. Sato, M. Sato, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2618–2620; e) M. D. Farris, C. De Meo, *Tetrahedron Lett.* **2007**, *48*, 1225–1227, and references cited therein; f) D. Crich, W. Li, *J. Org. Chem.* **2007**, *72*, 2387–2391, and references cited therein; g) S. Tanaka, T. Goi, K. Tanaka, K. Fukase, *J. Carbohydr. Chem.* **2007**, *26*, 369–394, and references therein.

[3] a) L. O. Kononov, N. N. Malysheva, E. G. Kononova, O. G. Garkusha, *Russ. Chem. Bull.* **2006**, *55*, 1311–1313; b) L. O. Kononov, N. N. Malysheva, E. G. Kononova, A. V. Orlova, *Eur. J. Org. Chem.* **2008**, 3251–3255; c) L. O. Kononov, "A Novel Concept of Supramer-Mediated Reactivity of Carbohydrates", *XXIV International Carbohydrate Symposium*, Oslo, 27 July – 1 August **2008**, Book of Abstracts, A-P134.

[4] This term has recently been coined to describe stereoisomerism at the supramolecular level (in the crystal): M. Czugler, N. Bathori, *Cryst. Eng. Commun.* **2004**, *6*, 494–503.

[5] A. V. Kornilov, A. A. Sherman, L. O. Kononov, A. S. Shashkov, N. E. Nifant'ev, *Carbohydr. Res.* **2000**, *329*, 717–730.

[6] A. V. Orlova, L. O. Kononov, B. G. Kimel, I. B. Sivaev, V. I. Bregadze, *Appl. Organomet. Chem.* **2006**, *20*, 416–420.

[7] P. P. Singh, M. M. Gharia, F. Dasgupta, H. C. Srivastava, *Tetrahedron Lett.* **1977**, *18*, 439–440.

[8] A. Marra, P. Sinaÿ, *Carbohydr. Res.* **1989**, *187*, 35–42.

[9] a) A. Hasegawa, T. Nagahama, H. Ohki, K. Hotta, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1991**, *10*, 493–498; b) O. Kanie, M. Kiso, A. Hasegawa, *J. Carbohydr. Chem.* **1988**, *7*, 501–506.

[10] One has also to consider the formation of glycal **4** during the sialylation, which is similar to **3** with respect to hydrogen-bond formation. Since the combined amounts of glycal **4** and disaccharide **3** are equal to the amount of the starting thiosialoside **1**, the overall changes in the hydrogen-bond pattern induced by changes in the concentrations of glycosyl donor **1**, disaccharide **3** and glycal **4** are negligible.

[11] A. V. Demchenko (Ed.), *Handbook of Chemical Glycosylation: Advances in Stereoselectivity and Therapeutic Relevance*, Wiley-VCH, Weinheim, **2008**, pp. 261–379.

[12] A. Hasegawa, K. Fushimi, H. Ishida, M. Kiso, *J. Carbohydr. Chem.* **1993**, *12*, 1203–1216.

[13] a) Z. Zhang, I. R. Ollmann, X.-S. Ye, R. Wischnat, T. Baasov, C.-H. Wong, *J. Am. Chem. Soc.* **1999**, *121*, 734–753; b) H. Tsukamoto, Y. Kondo, *Tetrahedron Lett.* **2003**, *44*, 5247–5249; c) L. Liao, F.-I. Auzanneau, *J. Org. Chem.* **2005**, *70*, 6265–6273; d) J. Xue, P. Liu, Y. Pan, Z. Guo, *J. Org. Chem.* **2008**, *73*, 157–161; e) G. Gu, Y. Du, H. Hu, C. Jin, *Carbohydr. Res.* **2003**, *338*, 1603–1607; f) E. J. Grayson, S. J. Ward, A. L. Hall, P. M. Rendle, D. P. Gamblin, A. S. Batsanov, B. G. Davis, *J. Org. Chem.* **2005**, *70*, 9740–9754; g) C. Krog-Jensen, S. Oscarson, *J. Org. Chem.* **1996**, *61*, 1234–1238; h) M. Á. Rodríguez, O. Boutureira, X. Arnés, M. I. Matheu, Y. Díaz, S. Castillón, *J. Org. Chem.* **2005**, *70*, 10297–10310.

[14] a) J. Bogusiak, W. Szeja, *Carbohydr. Res.* **2001**, *330*, 141–144; b) Y. Nishida, Y. Shingu, H. Dohi, K. Kobayashi, *Org. Lett.* **2003**, *5*, 2377–2380; c) Y. Shingu, A. Miyachi, Y. Miura, K. Kobayashi, Y. Nishida, *Carbohydr. Res.* **2005**, *340*, 2236–2244.

[15] a) J. M. Haberman, D. Y. Gin, *Org. Lett.* **2001**, *3*, 1665–1668; b) J. M. Haberman, D. Y. Gin, *Org. Lett.* **2003**, *5*, 2539–2541; c) D. Crich, W. Li, *Org. Lett.* **2006**, *8*, 959–962.

[16] E. Kirchner, F. Thiem, R. Dernick, J. Heukeshoven, J. Thiem, *J. Carbohydr. Chem.* **1988**, *7*, 453–486.

Received: October 16, 2008
Published Online: December 29, 2008

