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Concentration Dependence of Glycosylation Outcome: A Clue to Reproducibility and Understanding the Reasons Behind

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Dedicated to the memory of the late Professor Leon V. Backinowsky

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Changes in the concentration of reagents (0.009–0.2 M) have been shown to dramatically effect the yield and stereoselectivity of glycosylation with a sialic acid based glycosyl donor in a complex nonlinear manner that correlates with changes in the structures of the supramers of the reagents. The yield of disaccharide gradually increases with concentration and levels off at concentrations of glycosyl donor higher than 69 mM. The ratio of anomers is very high at some concentrations (α/β ≈ 20:1), moderate (α/β ≈ 8:1) or very low (α/β ≈ 4:1) at others. The formation of mixed supramers of glycosyl donor and glycosyl acceptor at concentrations exceeding 69 mM was detected by polarimetry and laser light scattering.

Introduction

Sialic acid containing glycoconjugates are involved in a wide range of biological phenomena ranging from cell–cell adhesion and mobility to oncogenesis and recognition by viruses and bacteria.[1] Therefore the synthesis and the biomedical investigation of sialic acid containing glycoconjugates, oligosaccharides, and their analogues is a very important area of research aimed at understanding their biological roles and determining their therapeutic importance. For this reason, tremendous effort has been made to develop efficient methods for the synthesis of sialo-oligosaccharides.[2,3] Sialic acids are attached to other carbohydrates by means of a glycosylation reaction called sialylation.[2a] Although substantial progress has recently been achieved in the synthesis of sialo-oligosaccharides,[3] poor predictability and reproducibility of yield and stereoselectivity are still typical of the sialylation reaction. The problem becomes even more complicated when both *N*-acetyl- and *N*-glycolyl-substituted sialo-oligosaccharides are required. Although it is possible to synthesize each type of oligosaccharide separately,[4] the use of a sialyl donor with suitable temporary protection at N(5) is generally considered more reasonable because in this case the number of nontrivial sialylation steps is minimized.[2d,2e] One of the practical approaches to libraries of sialo-oligosaccharides, which comprise the (α-2→3)-intersaccharidic linkage, with almost any N substituent from the single precursor involves the use of a sialyl-(α-2→3)-galactose building block with a removable protecting group at the N(5) of the sialic acid residue.[5]

In our own work, during the development of a preparative synthesis of the sialyl-galactose building block **6**[6] (Scheme 1) we faced a problem of reproducibility of yield and stereoselectivity of sialylation (**1b** + **5** → **6**). Especially annoying results were obtained when we tried to change the concentrations of the reagents. Clearly, this reaction required optimization, which could be accomplished in a number of ways.[7] We are trying to develop rational approaches to the design and optimization of glycosylation experiments. These approaches are based on the recently introduced supramer concept,[8] which emphasizes the importance of supramolecular aggregation in the reaction mixture leading to the formation of supramers[9] (*supra*molecular iso*mers*), which are differently arranged supramolecular assemblies of the same molecular entities. According to this concept, molecular structures of reactants and reaction conditions (solvent, temperature, concentration, presence of "nonreacting" compounds, etc.) would determine the aggregation type and the spatial arrangement ("structure") of the supramers formed in each particular case. Modification of the protecting groups or other nonreacting functional groups in the molecule could modulate

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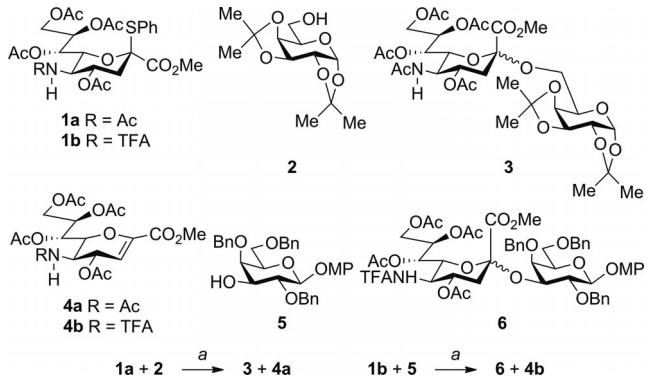
the aggregation type and the structure of the supramers formed from the same molecular scaffold. Supramers with different structures or compositions are expected to react differently. The accessibility of the reaction center in the supramers present would determine the apparent (macroscopic) reactivity and the outcome of a reaction [product yield and reaction (stereo/regio-)selectivity]. The supramer approach has been shown to be useful to explain, predict, and discover a series of unexpected phenomena[8,10] and led to the development of a novel sialyl donor, *N*,*N*-diacetylsialyl chloride,[11] with improved glycosylating properties.



Scheme 1. Structures of sialic acid glycosyl donors **1a**,**b** [R = Ac (**a**), TFA (**b**)], glycosyl acceptors **2** and **5**, disaccharides **3** and **6**, and glycals **4a**,**b**. Reagents and conditions: *a*) NIS-TfOH, MeCN, –40 °C.

At present, little is known about supramers and their structures. Nevertheless, by studying solutions of a typical sialyl donor **1a** by IR spectroscopy and polarimetry it was possible to detect changes in the structures of hydrogen-bonded supramers of sialyl donor **1a** upon a change in the concentration.[8b] This supramer rearrangement occurs in a very narrow concentration range and is detectable by polarimetry and IR spectroscopy (Figure 1, *a*). Not surprisingly, the yield of disaccharide **3**, formed from this glycosyl donor **1a** by the reaction **1a** + **2** → **3**, shown in Scheme 1, also experiences discontinuity in the same concentration range (Figure 1, *b*) in which supramers with different structures and reactivities are formed.[8b]

There are two important messages from Figure 1. The first is that polarimetry and IR spectroscopy are very sensitive[12,13] to supramolecular aggregation and the second is that critical points on the plots of optical rotation or the intensity of a relevant band (e.g., NH vibrations) in the IR spectrum against concentration can give us information concerning changes in the structures of supramers and hence changes in their reactivity and outcome of glycosylation. Thus, by studying the concentration dependence of the optical rotation or the intensity of bands in the IR spectra of solutions of glycosyl donor in the reaction solvent, one may find ranges of concentration in which anomalies in chemical reactivity could be expected.

Thus, the following scenario for the optimization of glycosylation can be proposed, which is based on the supramer approach. Before embarking on any glycosylation, a study

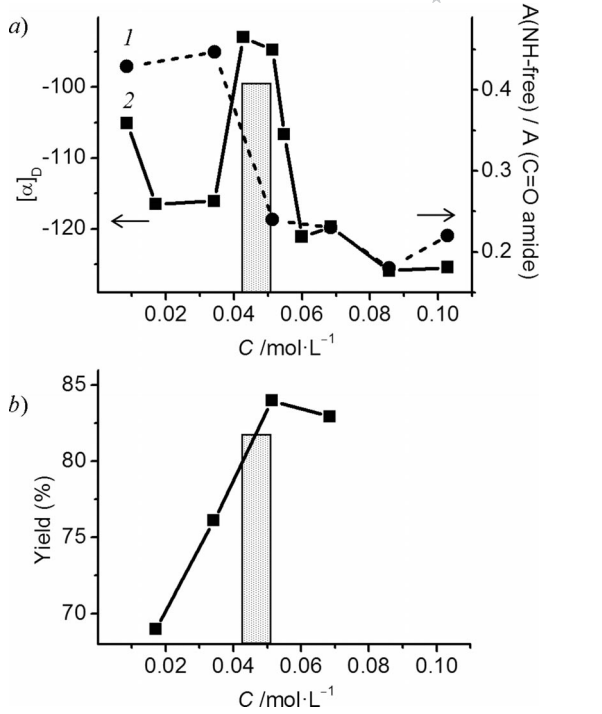


Figure 1. Concentration-induced rearrangement of supramers in a solution of glycosyl donor **1a** in MeCN (*a*), and the yield of disaccharide **3** (MeCN, NIS–TfOH, –40 °C, 3 h) at different concentrations of **1a** (*b*): *1* (circles, dashed line): Amount of "free" NH groups in a solution of glycosyl donor **1a** in MeCN (ca. 25 °C) calculated as the ratio of the absorptions of the NH band at 3365 cm^{-1} and the amide C=O band at 1676 cm^{-1} [*A*(NH free)/*A*(C=O amide)], the intensity of the latter band being an "internal standard"; *2* (squares, solid line): specific optical rotation ($[\alpha]_D$) of a solution of **1a** in MeCN (averaged over a range of 23–26 °C). The rectangle denotes the concentration range of the supramer rearrangement. Adapted from ref.[8b].

of solutions of the glycosyl donor by polarimetry, IR spectroscopy, or other appropriate physical methods should be attempted. Analysis of the corresponding concentration plots may reveal discontinuities. The corresponding critical points would serve as guidelines for choosing concentrations for glycosylation. When performing chemical experiments, it is important to include critical concentrations and at least one intermediate different critical concentrations or between them.

Herein we report the use of the supramer approach for the optimization of the troublesome sialylation reaction **1b** + **5** → **6** (Scheme 1). This proof-of-principle study has led to the discovery of completely unprecedented results, which are described below.

Results and Discussion

The first task was to find the critical concentrations in solutions of glycosyl donor **1b**[15] in MeCN, which was the reaction solvent. However, unlike in the previous study of

the glycosyl donor **1a** with the AcNH group at C(5) (see Figure 1, *a*),[8b] an attempt to use IR spectroscopy to monitor changes in hydrogen-bond-mediated aggregation in solutions of glycosyl donor **1b** with the TFANH group at C(5) was unsuccessful. Although changes in the relative intensities of "free" and "bound" NH vibrations and in the positions and intensities of carbonyl vibration bands were clearly visible upon changing the concentration of glycosyl donor **1b**, their quantitative assessment was found difficult due to substantial overlap of the NH and solvent bands.

Then we measured the optical rotations of a series of solutions of the glycosyl donor **1b** in MeCN. Two maxima and one minimum are observed in the plot of specific rotation against concentration of glycosyl donor (Figure 2, *b*). To simplify further comparison, these concentrations (50, 69, and 103 mM) are marked with thick arrows, which are also present in the following figures. These arrows indicate the critical concentrations of the glycosyl donor solution, as detected by polarimetry. It is important to emphasize that the shape of the optical rotation plot clearly indicates the formation of supramolecular aggregates in solution and their interconversion upon changes in concentration.

After studying the properties of solutions of glycosyl donor **1b** we performed a series of chemical experiments at critical points and at some intermediate concentrations (Figure 2, *a* and Table 1). We found that by changing the concentrations of reagents one can dramatically modulate the yield and stereoselectivity of sialylation (Figure 2, *a*). The concentration dependence of the yield in this case (dashed line, Figure 2, *a*, *1*) is strikingly similar to that previously obtained[8b] for the glycosyl donor **1a** with the AcNH group at C(5) (Figure 1, *b*). The yield gradually increases with the concentration of the glycosyl donor **1b** with the TFANH group at C(5) and levels off after the critical concentration of 69 mM. The concentration dependence of the stereoselectivity (solid line, Figure 2, *a*, *2*) is even more complex. Note that discontinuities of this line correspond well to the critical concentrations detected by polarimetry (see Figure 2, *b*), which confirms the validity of our approach. In the particular case of a sialylation reaction (**1b** + **5** → **6**), the ratio of anomers of the glycosylation product **6** can be very high at some concentrations (α/β ≈ 20:1), moderate (α/β ≈ 8:1), or very low (α/β ≈ 4:1) at others, in all cases the equatorial glycoside **6**[16] dominating due to the "nitrile effect"[17] (see Figure 2, *a* and Table 1).[18]

However, more careful examination of this plot suggests that at high concentrations the situation is more complex than we initially assumed. The second maximum (at 103 mM) in the optical rotation plot (Figure 2, *b*), unlike the first one (at 50 mM), corresponds to a poorly resolved discontinuity in the stereoselectivity plot (Figure 2, *a*) rather than to a pronounced maximum. For this reason, we decided to include the second component of the reaction, that is, the glycosyl acceptor **5**,[19] in our supramer model. This idea is quite natural because there are a number of examples reported in the literature in which the nature of the glycosyl acceptor influences the stereoselectivity of glycosylation.[20,21]

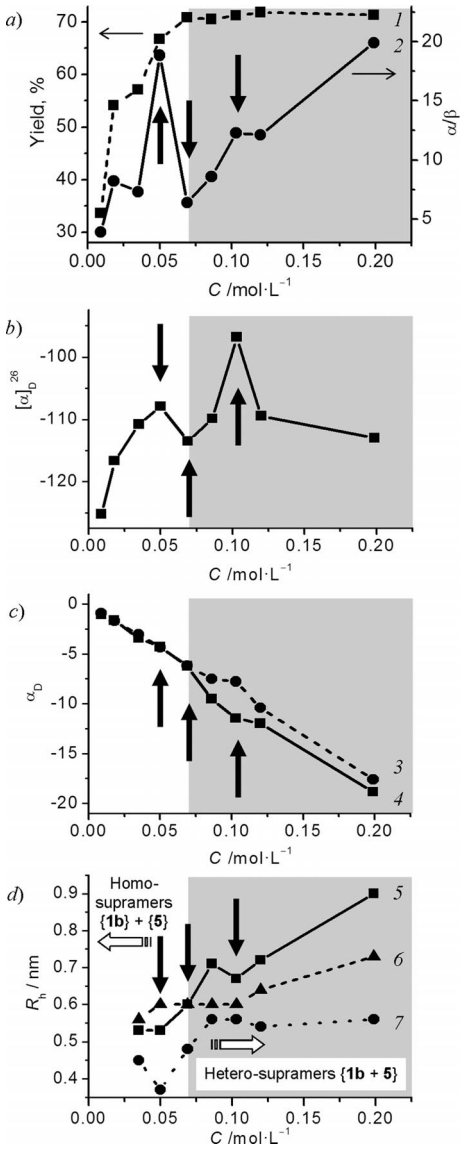


Figure 2. Outcome of sialylation (*a*) and its correlation with optical rotation (*b*,*c*) and DLS data (*d*) for solutions at different concentrations: (*a*) *1* (squares, dashed line): Yield of disaccharide **6**; *2* (circles, solid line): anomeric ratio (α/β) of **6**. (*b*) Specific optical rotation ($[\alpha]_D^{28}$) of sialyl donor **1b** in MeCN. (*c*) Optical rotation (α_D^{28}) in MeCN; *3* (circles, dashed line): the sum of rotations of **1b** and **5** measured separately; *4* (squares, solid line): optical rotation of 1:1 mixtures of **1b** and **5**. (*d*) Comparison of the average hydrodynamic radii (R_h) of light-scattering particles in MeCN: *5* (squares, solid line): 1:1 mixtures of **1b** and **5**; *6* (triangles, dashed line): solution of glycosyl acceptor **5**; *7* (circles, dotted line): solution of glycosyl donor **1b**. Vertical thick arrows indicate critical concentrations (50, 69, and 103 mM). Shaded area marks the concentration range in which mixed supramers {**1b** + **5**} exist.

We studied the aggregation in 1:1 mixtures of glycosyl donor **1b** and glycosyl acceptor **5** in MeCN by polarimetry (Figure 2, *c*). The solid line (Figure 2, *c*, *4*) represents the observed optical rotation of this mixture in MeCN. The dashed line (Figure 2, *c*, *3*) corresponds to the calculated sum of the optical rotation values of glycosyl donor **1b** and glycosyl acceptor **5** measured separately. It can clearly be

Table 1. Conditions and products of glycosylation.[a]

Entry	Conc. [mM]	Reaction time	Yield of **6** [%][b]	Yield of α isomer of **6** [%][c]	Anomeric ratio for **6** (α/β)[d]
1	9	138 h	33.6	26.8	3.9:1[e]
2	18	4 h	54.1	44.9	8.2:1
3	35	2.25 h	57.1	51.0	7.3:1
4	50	1 h	66.7	60.6	18.8:1
5	69	45 min	70.8	64.5	6.4:1
6	86	1 h	70.5	64.3	8.6:1
7	103	40 min	71.2	64.7	12.3:1
8	120	45 min	71.8	66.2	12.1:1
9	199	40 min	71.3	66.0	19.9:1
10[f]	50	1 h	28.1	12.2	1:1.3[e]

[a] Reagents and conditions: 1 equiv. **1b**, 1 equiv. **5**, MeCN, NIS–TfOH, 3 Å molecular sieves, –40 °C. The reaction was quenched after complete consumption of the glycosyl donor (TLC control). [b] The disaccharide fraction was isolated by gel chromatography on Bio-Beads S-X3 (toluene) and then the isomers were separated by silica gel column chromatography (the sum of the isolated yields of the α and β isomers of **6** is given). [c] As for [b] (the isolated yield of the α isomer of **6** is given). [d] Determined from the ^{1}H NMR spectroscopic data for the disaccharide fraction isolated by gel chromatography on Bio-Beads S-X3 (toluene). [e] Approximate value obtained by weighing the purified α and β isomers of **6**. [f] The reaction was performed in CH_2Cl_2.

seen that at the critical concentration of 69 mM, these two lines become separated. This means that at higher concentrations these two compounds are no longer independent and do interact. We believe that this deviation from additivity at high concentrations is related to the formation of mixed supramers (heterosupramers) {**1b** + **5**} comprising molecules of both reagents.

To confirm independently the existence of supramers in our system we used dynamic light scattering (DLS).[22] Typical correlation functions and size distributions of light-scattering particles in solutions of the glycosyl donor **1b**, glycosyl acceptor **5**, and a 1:1 mixture **1b** and **5** in MeCN at one of the concentrations studied (69 mM) are shown in Figure 3.[24] The plot of hydrodynamic radii of light-scattering particles in these solutions against concentration of solute (Figure 2, *d*) also indicates the formation of mixed supramers {**1b** + **5**} of sialyl donor **1b** and glycosyl acceptor **5** at concentrations exceeding 69 mM. It can clearly be seen in this plot that in the high concentration range, marked with a shaded background, the hydrodynamic radius of the light-scattering particles in the mixture of glycosyl donor **1b** and glycosyl acceptor **5** (solid line, Figure 2, *d*, *5*) is larger than the hydrodynamic radii of particles in solutions of the individual components (dashed and dotted lines, Figure 2, *d*, *6* and *7*). Thus, the existence of mixed supramers {**1b** + **5**} at high concentrations may be considered established.

It is important to stress that in the high concentration range (at concentrations exceeding 69 mM), when mixed supramers {**1b** + **5**} exist (Figure 2, *c* and *d*), the yield of disaccharide **6** does not depend on concentration although it clearly does at lower concentrations, when homosupramers {**1b**} exist (Figure 2, *a*, *1*). Because all the reactions were quenched after the complete consumption of the glycosyl donor, the decrease in disaccharide yield reflects the increase in the yields of products of the side-reactions that usually accompany the sialylation reaction, the major one being glycal **4b**[25] formed by elimination. In other words, a change in the supramer structure of glycosyl donor **1b** induced by a change in concentration resulted in a change in the chemoselectivity of the reaction, that is, the chemical properties of **1b** have changed. This is not surprising from the supramer point of view. As mentioned earlier, changes in the structures of the supramers can modulate the accessibility of the reaction center for the nucleophile and hence the selectivity of the reaction, leading to either the substitution (S_N1) product (disaccharide **6**) or the elimination (E1) product (glycal **4b**). Apparently, unimolecular elimination begins to dominate when substitution (glycosylation) is disfavored.

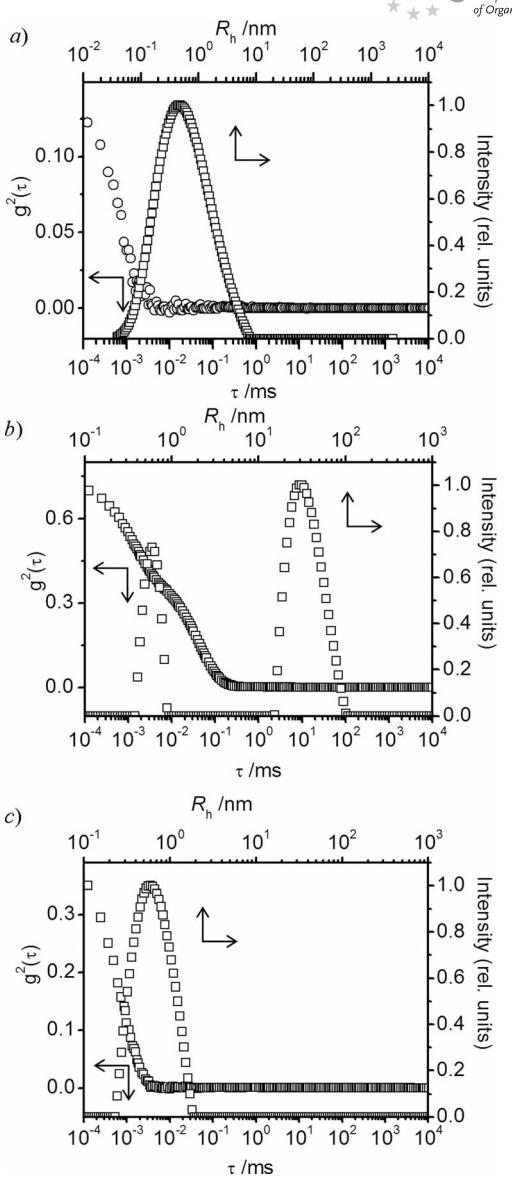
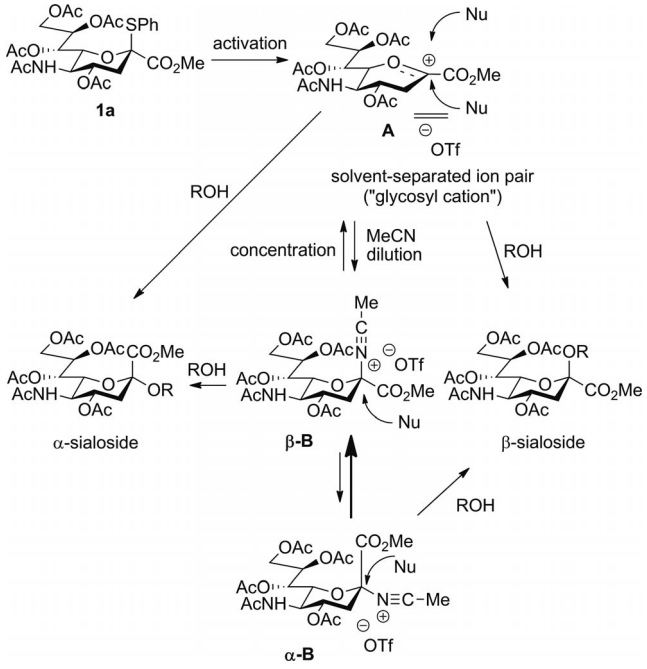


Figure 3. Correlation functions and intensity-weighted size distributions in solution of the glycosyl donor **1b** (*a*), glycosyl acceptor **5** (*b*), and their 1:1 mixture (*c*) in MeCN at 69 mM concentration.

Although the influence of concentration on the stereoselectivity of the glycosylation is very complex in this particular case (Figure 2, *a*, *2*), it can be rationally discussed by using the supramer approach. One can even say that without knowing the critical concentrations, which were determined by the supramer approach, we would not be able to reveal this complex picture. This complex concentration dependence of the stereoselectivity of sialylation is very important from both practical and mechanistic points of view. From a practical point of view, during the optimization, without knowing the critical concentrations it is very easy to miss good conditions, under which the ratio of anomers is very high (α/β ≈ 20:1). The theoretical implications are even more important. It is commonly believed that sialylation with thioglycoside glycosyl donors is a multistep process that proceeds via the formation of an oxacarbenium ion (**A**, Scheme 2),[2,26,27] which may be stabilized in MeCN by the solvent molecules to form the so-called "nitrilium intermediate" **B**.[2,17] Because α-selective sialylation can be achieved only under kinetically controlled conditions (as the β anomer is more thermodynamically stable due to the anomeric effect),[2] the stereoselectivity of sialylation essentially reflects the different preferences for the attack of a nucleophile from different diastereotopic faces of a glycosyl donor (or, more correctly, species generated from it upon activation). Current views on the origin of the stereoselectivity of sialylation in MeCN (and other glycosylation reactions with glycosyl donors without stereocontrolling participating substituents) predict that the proportion of equatorial glycoside[16] should be higher in dilute solutions, which is attributed to increased solvent participation upon dilution.[28] However, the practical validation of such a prediction is accompanied by unexpected difficulties. Indeed, it is virtually impossible to perform glycosylation at every feasible concentration. We found that the results of the experimental verification depend on the set of concentrations used, as can be seen from Figure 2 (*a*) and Table 1. Depending on the choice of concentrations one can obtain results that either support this view (entries 4 and 5 or 4 and 6, or 4, 7, and 8 in Table 1) or contradict it[29] (entries 1, 3, 6, 7, and 9). It is even possible to "prove" that stereoselectivity almost does not depend on concentration[30] by the appropriate selection of experimental concentrations (entries 2, 3, and 6). It looks like almost any theory and view can find experimental support. We wish to stress that this paradoxical situation can emerge only if one does not consider the existence of critical concentrations, which indicate changes in supramer structure, and their relevance to the outcome of glycosylation. Because previous studies[28,30] of the influence of concentration on the outcome of glycosylation reactions did not pay any attention to the choice of experimental set of concentrations, it is only natural that the results obtained earlier often seem to be confusing.

The supramer approach recognizes all the established factors that could influence the stereoselectivity of sialylation at the molecular level.[2] In fact, our results confirm the existence of the stereocontrolling "nitrile effect".[17,18] In addition, we believe that selective screening of one of the



Scheme 2. Simplified, generally accepted mechanism of stereocontrol during sialylation (thioglycoside **1a** shown as an example) in MeCN, which implies an increase in α stereoselectivity upon dilution.

faces of the anomeric center in a sialic acid supramer by the neighboring molecules that comprise the supramer would considerably influence the stereoselectivity of glycosylation. It is not unexpected that dramatic changes in supramer structure upon changes in concentration, as evidenced by the polarimetry and DLS data presented herein, are accompanied by significant and correlated changes in the stereoselectivity of sialylation (Figure 2). Interestingly, the supramer approach is better correlated with the recently introduced[31] "conformer and counterion distribution hypothesis" (which emphasizes the conformational preferences of a glycosyl donor as the major factor influencing the stereoselectivity of glycosylation) rather than with the commonly accepted "solvent-coordination hypothesis" (which rationalizes, e.g., the "nitrile effect"[17] by assuming preferential coordination of acetonitrile to the reactive cation on the β side of the anomeric carbon in sialic acid derivatives[16]). It is important that the latter explanation (see Scheme 2) does not leave any room for the effects of concentration and the presence of glycosyl acceptor, which were revealed in this study. Our optical rotation data (Figure 2, *b* and *c*) suggest dramatic changes in the molecular conformations of both reagents (**1b** and **5**) upon changes in concentration.[12] These changes might modify the barriers of the pathways leading to different conformers of the true reacting species (glycosyl cation-like intermediates), hence their populations in the reaction mixtures and the overall stereoselectivity of glycosylation.

One has to comment on the apparent discrepancy of the discovery in this study of the complex concentration dependence of stereoselectivity of the sialylation of glycosyl ac-

ceptor **5** with sialyl donor **1b**, the α/β ratio ranging from 4:1 to 20:1 (see Scheme 1 and Figure 2, *a*, *2*), with our own previous results[8b] in which the stereoselectivity of sialylation of the glycosyl acceptor **2** with sialyl donor **1a** (see Scheme 1) at the end of the reaction was almost independent of concentration (α/β ≈ 7:1–10:1 after 3 h of reaction). Later, we showed that considerable changes in the stereoselectivity of sialylation may occur during the course of the NIH–TfOH-promoted glycosylation (in some cases the α/β ratio approached 27:1 after 15 min of reaction) and related them to changes in the supramer structure of the glycosyl donor induced by changes in the concentration of the succinimide formed during the reaction.[8c] In our opinion, the main difference in these two cases lies in the noticeable difference in reactivity between the glycosyl donor **1a** with an AcNH group at C(5) and glycosyl donor **1b** with a TFANH group at C(5), the latter being much more reactive. This leads to shorter reaction times with **1b** (40–60 min is enough for complete consumption of the starting thioglycoside **1b** in the high concentration range; see Table 1).[32] The high stereoselectivities achieved in this study suggest that during this period of time no significant rearrangement of supramers, induced by succinimide, takes place. This suggests that a minimum time of around 30 min is required for supramer rearrangement induced by succinimide, which is in agreement with our previous results.[8c]

Caution should be exercised when transferring our findings to other systems. This proof-of-principle study represents the situation in the particular system of glycosyl donor **1b** and glycosyl acceptor **5** in MeCN. The results obtained for other combinations of glycosyl donor and glycosyl acceptor (or in other solvents) may be different; the supramer approach even predicts that they should be different because supramers with different structures, and hence chemical properties, can be formed in these cases. More systems should be studied and analyzed by using the supramer approach before any substantiated generalizations on the influence of concentration (and other factors including the molecular structures of reagents) on the outcome of glycosylation can be made.

This necessarily pessimistic view is supported by the generally accepted fact that currently it is almost impossible to predict the outcome of a specific glycosylation.[33] In our opinion, at the present level of development, only the supramer approach allows rational discussion of the influence of the nature of the glycosyl acceptor on the yield and stereoselectivity of glycosylation.[2h,20,21b,27] The commonly accepted concept of double diastereoselection,[21] although helpful in some cases, cannot treat adequately the sometimes pronounced influence of a change in protecting group pattern (especially at positions remote from the reaction center, for example, in aglycon of an oligosaccharide glycosyl acceptor[20c]) on the same molecular scaffold.[20] This is especially true for glycosylations involving large oligosaccharide glycosyl donors and acceptors. Even though a model reaction with monosaccharide derivatives may proceed smoothly, reaction of the corresponding oligosaccharide derivatives, with formally the same monosaccharide residues (with identical protecting group pattern) involved in the actual chemical reaction, may (or may not) be problematic or even impossible. This situation is expected from the supramer point of view. Larger oligosaccharides necessarily have additional sites capable of intermolecular interactions[35] and the supramers formed from such oligosaccharides (unlike much smaller monosaccharides) may be arranged in such a way that the reaction center (anomeric center in the case of glycosylation) is partially shielded (leading to modified selectivity) or completely blocked (giving no reaction at all) by the neighboring molecules comprising the supramer. The supramer approach might be useful in interpreting other examples (non-carbohydrate) in which a model reaction in fact does not model anything at all or when difficult and unexpected reactivities of functional groups are observed.[36]

The results obtained in this study suggest that at different concentrations, glycosyl donors form different supramers, which apparently have very distinct chemical properties. In other words, we can now claim that the same chemical compound can react differently depending on the type of supramer formed. One can even say that the chemical properties are a feature of a supramer rather than that of an isolated molecule.[37] This unusual notion may have important implications and consequences, which are yet to be completely understood, in other areas of chemistry.

Conclusions

The results obtained in this study can be summarized in the following way. A change in the concentration of reagents was found to influence the outcome of glycosylation in a complex way. Depending on the concentration, molecules of reagents form fundamentally different supramers, which can be distinguished by physical methods and differ in their chemical properties. A better understanding of the real reasons that determine the stereoselectivity of glycosylation can be obtained by consideration of the nature and supramolecular structures of all the components of the reaction mixture rather than by analysis of the molecular structure of the glycosyl donor alone. The commonly accepted view of the origin of the stereoselectivity of sialylation in MeCN, which relies on solvent participation, is not supported by the results obtained herein. The results obtained in this study allow a fresh look at the problems of reproducibility of glycosylation.

Experimental Section

General: The reactions were performed with commercial reagents. Solvents for reactions were distilled and purified before use according to standard procedures. HPLC far-UV-grade acetonitrile (99.9%, water content <0.02%, Acros Organics, Cat. No. 268260010) was used for optical rotation and DLS measurements. 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 $CDCl_3$ solutions were recorded with a

Bruker AVANCE-600 spectrometer. ^{1}H and ^{13}C NMR chemical shifts are given relative to residual $CHCl_3$ (δ = 7.27 and 77.0 ppm). Signals were assigned through 2D NMR (COSY, HSQC, HMBC) and DEPT-135 experiments. Anomeric configurations of sialic acid derivatives were determined by measurement of the $^3J_{C\text{-}1,3ax\text{-}H}$ coupling constants[38] using the J-HMBC experiment. HRMS (electrospray ionization, ESI) spectra were recorded with a Bruker micrOTOF II mass spectrometer for 2×10^{-5} M solutions in MeCN. IR spectra of amide **1b** and 1:1 mixtures of **1b** and alcohol **5** in MeCN (concentration range 9–199 mM) were obtained with a Bruker IFS 25 FTIR spectrometer. Optical rotations were measured for filtered (0.45 μm) solutions of amide **1b** and 1:1 mixtures of **1b** and alcohol **5** in MeCN (concentration range 9–199 mM; exact concentrations correspond to the concentrations listed in Table 1) with a PU-07 automatic polarimeter (Russia) at 28 °C in a jacketed cell (10 cm length). The temperature was maintained with an accuracy of ±0.2 °C. Each measurement at a particular concentration was repeated 10 times on one day and then repeated again on another day (10 times), and then averaged and plotted against concentration (see Figure 2, *b*, Figure 2, *c*). The standard deviations were calculated by using the Student distribution (95% probability) and did not exceed 1% for either observed (α_D) or specific ($[\alpha]_D$) rotation values. DLS experiments were performed on filtered (0.45 μm) solutions of amide **1b**, alcohol **5**, and 1:1 mixtures of **1b** and **5** in MeCN (concentration range 9–199 mM; exact concentrations correspond to the concentrations listed in Table 1) at 24 °C with an ALV Correlation Goniometer System 5000/6010 (Langen, Germany). To obtain intensity correlation functions [$g^2(\tau)$], data were averaged over 20 independent measurements (the total collection time was 40 min for each point on the graph in Figure 2, *d*) and then processed by using the CONTIN algorithm to calculate contributions to the scattered intensity from particles of each observable size (the so-called "intensity-weighted size distribution",[23] see Figure 3) and hydrodynamic radii (R_h) of light-scattering particles (see Figure 2, *d*), which were calculated at the maxima of intensity-weighted size distributions.

Typical Glycosylation Procedure: A mixture of thioglycoside **1b**[15] (132.0 mg, 0.206 mmol, 1 equiv.) and alcohol **5**[19] (115.2 mg, 0.206 mmol, 1 equiv.) was dried in vacuo for 2 h and then anhydrous MeCN (3 mL, distilled from P_2O_5, stored over 3 Å molecular sieves) was added under argon. Freshly activated (220 °C, 6 h, in vacuo) powdered 3 Å molecular sieves (300 mg, Fluka; 100 mg per 1 mL of solvent) were added to the resulting solution and the reaction flask was flushed with argon. The suspension was stirred under argon at room temp. for 1 h and then cooled to –40 °C (liquid N_2/MeCN bath). Solid NIS (70.4 mg, 0.312 mmol, 1.5 equiv. per 1 equiv. donor) was added followed by TfOH. Only the minimum amount of TfOH (2–5 μL) required to generate persistent color was added. The reaction mixture was stirred under argon at –40 °C until complete consumption of the starting thioglycoside **1b** (TLC monitoring; the time is specified in Table 1), then diluted with $CHCl_3$ (20 mL), and filtered through a pad of Celite. 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 plug of cotton wool, 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-X3 (200–400 mesh, Bio-Rad) using toluene as the eluent and a differential refractometer (Knauer) as the detector. The first eluted fraction contained disaccharide **6**, which was analyzed by NMR spectroscopy to obtain the anomeric ratios α/β [see Table 1 and Figure 2, *a*, *2*; to determine the ratio of anomers of disaccharide **6** the integral intensities of signals of α-3eq-H (δ = 2.59 ppm) and β-3eq-H (δ = 2.77 ppm) of the Neu5Ac residue were used]. Later eluted fractions contained Neu5TFA glycal **4b**[25] and finally unreacted alcohol **5**. A base-line separation of all mentioned components was repeatedly achieved. The disaccharide fraction was purified by chromatography on a silica gel 60 column to give pure α- and β-linked isomers of disaccharide **6** (for the yields, see Table 1 and Figure 2, *a*, *1*; all yields were calculated with respect to glycosyl donor **1b**). TLC data: R_f = 0.40 (**1b**), 0.20 (**4b**), 0.63 (**5**), 0.36 (α-**6**), 0.60 (β-**6**) (benzene/acetone, 9:1 v/v).

Disaccharide α-6: $[\alpha]_D^{27}$ = –14.7 (*c* = 2.0, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.44–7.41 (m, 2 H, Ph), 7.36–7.25 (m, 13 H, Ph), 7.05–7.01 (m, 2 H, $MeOC_6H_4$), 6.82–6.79 (m, 2 H, $MeOC_6H_4$), 6.58–6.52 (m, 1 H, NH), 5.46 (ddd, $J_{8,7}$ = 7.7, $J_{8,9}$ = 5.6, $J_{8,9'}$ = 2.4 Hz, 1 H, 8-H Neu), 5.29 (dd, $J_{7,6}$ = 2.0, $J_{7,8}$ = 7.7 Hz, 1 H, 7-H Neu), 5.06 (ddd, $J_{4,3ax}$ = 12.1, $J_{4,3eq}$ = 4.8, $J_{4,5}$ = 10.4 Hz, 1 H, 4-H Neu), 4.97 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-H Gal), 4.95 (d, *J* = 11.8 Hz, 1 H, PhC*H*), 4.90 (d, *J* = 11.6 Hz, 1 H, PhC*H*), 4.84 (d, *J* = 11.8 Hz, 1 H, PhC*H*), 4.54 (d, *J* = 11.6 Hz, 1 H, PhC*H*), 4.51 (d, *J* = 11.6 Hz, 1 H, PhC*H*), 4.45 (d, *J* = 11.6 Hz, 1 H, PhC*H*), 4.42 (dd, $J_{9',8}$ = 2.4, $J_{9a,9b}$ = 12.6 Hz, 1 H, 9b-H Neu), 4.25 (dd, $J_{3,2}$ = 9.9, $J_{3,4}$ = 2.9 Hz, 1 H, 3-H Gal), 4.08 (dd, $J_{6,5}$ = 10.7, $J_{6,7}$ = 2.0 Hz, 1 H, 6-H Neu), 4.02–3.95 (m, 1 H, 5-H Neu), 3.99 (dd, $J_{9,8}$ = 5.6, $J_{9a,9b}$ = 12.6 Hz, 1 H, 9a-H Neu), 3.95 (dd, $J_{2,1}$ = 7.6, $J_{2,3}$ = 9.9 Hz, 1 H, 2-H Gal), 3.78 (s, 3 H, $CH_3OC_6H_4$), 3.77–3.72 (m, 2 H, 6b-H Gal, 5-H Gal), 3.75 (s, 3 H, CO_2Me), 3.71 (d, $J_{4,3}$ = 2.9 Hz, 1 H, 4-H Gal), 3.70–3.65 (m, 1 H, 6a-H Gal), 2.59 (dd, $J_{3eq,3ax}$ = 13.2, $J_{3eq,4}$ = 4.8 Hz, 1 H, 3eq-H Neu), 2.13 (s, 3 H, Ac), 2.04 (dd, $J_{3ax,3eq}$ = 13.2, $J_{3ax,4}$ = 12.1 Hz, 1 H, 3ax-H Neu), 2.00 (s, 3 H, Ac), 1.97 (s, 3 H, Ac), 1.94 (s, 3 H, Ac) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.76, 170.44, 170.04, 169.78 (4 Me*C*O), 168.02 (C-1 Neu; $^3J_{C\text{-}1,3ax\text{-}H}$ = 6.9 Hz, *J*-HMBC data), 157.54 (q, *J* = 38.0 Hz, CF_3CO), 155.12, 151.70 (C-1, C-4 $MeOC_6H_4$), 138.97, 138.90, 138.09 (3 C_{quat} Ph), 128.31, 128.08, 128.06, 127.94, 127.68, 127.59, 127.35, 127.26 (Ph), 118.40 [C-2(6) or C-3(5) $MeOC_6H_4$], 115.40 (q, *J* = 288.0 Hz, CF_3CO), 114.43 [C-3(5) or C-2(6) $MeOC_6H_4$], 102.91 (C-1 Gal), 98.59 (C-2 Neu), 77.45 (C-2 Gal), 76.32 (C-3 Gal), 76.08 (C-4 Gal), 74.91 (2 PhCH_2O), 73.48 (PhCH_2O), 73.34 (C-5 Gal), 71.64 (C-6 Neu), 69.16 (C-8 Neu), 68.63 (C-6 Gal), 68.50 (C-4 Neu), 67.25 (C-7 Neu), 61.99 (C-9 Neu), 55.63 ($CH_3OC_6H_4$), 52.91 (CO_2CH_3), 50.15 (C-5 Neu), 36.62 (C-3 Neu), 21.09, 20.61, 20.49, 20.37 (4 CH_3CO) ppm. HRMS (ESI): calcd. for $C_{54}H_{60}F_3NNaO_{19}$ $[M + Na]^+$ 1106.3604; found 1106.3636.

Disaccharide β-6: $[\alpha]_D^{24}$ = –13.5 (*c* = 2.0, CH_2Cl_2). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.51–7.24 (m, 15 H, 3 Ph), 7.04–7.01 (m, 2 H, $MeOC_6H_4$), 6.82–6.78 (m, 2 H, $MeOC_6H_4$), 5.22–5.18 (m, 2 H, 8-H Neu, NH), 5.15 (dd, $J_{7,6}$ = 2.4, $J_{7,8}$ = 2.4 Hz, 1 H, 7-H Neu), 5.05 (dd, $J_{9b,8}$ = 2.3, $J_{9a,9b}$ = 12.2 Hz, 1 H, 9b-H Neu), 5.00 (ddd, $J_{4,3ax}$ = 11.6, $J_{4,3eq}$ = 4.9, $J_{4,5}$ = 10.2 Hz, 1 H, 4-H Neu), 4.98 (d, *J* = 11.3 Hz, 1 H, PhC*H*), 4.95 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1-H Gal), 4.90 (d, *J* = 13.0 Hz, 1 H, PhC*H*), 4.84–4.81 (m, 2 H, 2 PhC*H*), 4.61–4.55 (m, 2 H, PhCH_2), 4.25 (dd, $J_{3,2}$ = 9.9, $J_{3,4}$ = 2.0 Hz, 1 H, 3-H Gal), 4.06 (dd, $J_{2,3}$ = 9.9, $J_{2,1}$ = 7.5 Hz, 1 H, 2-H Gal), 4.03–3.91 (m, 5 H, 9a-H Neu, 4-H Gal, 5-H Neu, 6-H Neu, 5-H Gal), 3.82 (dd, $J_{6a,6b}$ = 9.6, $J_{6b,5}$ = 6.3 Hz, 1 H, 6b-H Gal), 3.79–3.75 (m, 1 H, 6a-H Gal), 3.78 (s, 3 H, $CH_3OC_6H_4$), 3.41 (s, 3 H, CO_2Me), 2.77 (dd, $J_{3eq,3ax}$ = 13.7, $J_{3eq,4}$ = 4.9 Hz, 1 H, 3eq-H Neu), 2.08 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.01 (s, 6 H, 2 Ac), 1.87 (dd, $J_{3ax,3eq}$ = 13.7, $J_{3ax,4}$ = 11.6 Hz, 1 H, 3ax-H Neu) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 170.64, 170.52, 170.11, 169.71 (4 Me*C*O), 166.53 (C-1 Neu; $^3J_{C\text{-}1,3ax\text{-}H}$ = 2.5 Hz, *J*-HMBC data), 157.53 (q, *J* = 38.1 Hz, CF_3CO), 155.14, 151.46 (C-1, C-4 $MeOC_6H_4$), 139.27, 138.28, 137.95 (3 C_{quat} Ph), 128.73, 128.59, 128.33, 128.21, 127.81, 127.77, 127.68, 127.40, 126.78 (Ph), 118.09 [C-2(6) or C-3(5) Me-

OC_6H_4], 115.24 (q, J = 287.0 Hz, CF_3CO), 114.48 [C-3(5) or C-2(6) $MeOC_6H_4$], 102.82 (C-1 Gal), 99.51 (C-2 Neu), 77.61 (C-4 Gal), 77.23 (C-2 Gal), 76.16 (C-3 Gal), 75.05 ($PhCH_2O$), 73.98 (C-6 Neu), 73.87 ($PhCH_2O$), 73.51 ($PhCH_2O$), 72.57 (C-8 Neu), 71.47 (C-5 Gal), 68.88 (C-6 Gal), 68.67 (C-7 Neu), 67.98 (C-4 Neu), 62.38 (C-9 Neu), 55.61 ($CH_3OC_6H_4$), 52.54 (CO_2CH_3), 49.53 (C-5 Neu), 37.25 (C-3 Neu), 20.88, 20.72, 20.51, 20.49 (4 CH_3CO) ppm. HR MS (ESI): calcd. for $C_{54}H_{60}F_3NNaO_{19}$ $[M + Na]^+$ 1106.3604; found 1106.3589.

Supporting Information (see footnote on the first page of this article): Copies of ^1H and ^{13}C NMR spectra for disaccharides α-**6** and β-**6**.

Acknowledgments

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under examination. In fact, DLS measures the relaxation time of a time autocorrelation function $g^2(\tau)$, or the correlation time, τ_0, which is assumed to be inversely proportional to the translational diffusion coefficient, D, and proportional to the hydrodynamic radius R_h ($\tau_0 \sim R_h \sim 1/D$). Note that the changes in supramer structure may not be always accompanied by change in supramer size. In such a case, DLS may not be able to provide information on supramers.

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[24] Note the bimodal size distribution in solutions of glycosyl acceptor **5** (Figure 3, b), the slower mode (millisecond range of correlation time) corresponds to very large supramers with R_h of 30–60 nm. The slower mode disappears in mixtures of glycosyl donor **1b** and glycosyl acceptor **5**. For this reason, all further discussion is limited to the faster mode (microsecond range of correlation time).

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[34] B. Davis, *J. Chem. Soc. Perkin Trans. 1* **2000**, 2137–2160.

[35] The supramer concept considers remote functional or protecting groups as the sites capable of intermolecular interactions, which may determine the structure of the supramer formed in each particular case and hence its reactivity. For example, a significant and unexpected influence of a remote functional group in aglycon on the reactivity of the hydroxy group of a trisaccharide glycosyl acceptor in glycosylation during the chemical synthesis of HNK-1 pentasaccharide (ref.[20c]) can easily be rationalized by the supramer approach. Owing to the presence of an extra hydrogen-bonding acceptor site (azide), which allows the formation of more tightly packed supramers with a reaction center buried within the supramer core, 2-azidoethyl glycoside was much less reactive than the corresponding allyl glycoside of an otherwise identical trisaccharide (glycosylation was five times slower and gave a lower yield of the pentasaccharide). Similarly, consideration of the possibility of an additional hydrogen bond formed by an extra amide group in a spacer allowed a rational explanation of the dramatic dependence of hydrolytic stability of *closo*-carborane–lactose neoglycoconjugates on the nature of the spacer (ref.[8d]).

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