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Intermolecular Hydrogen-Bonding Pattern of a Glycosyl Donor: The Key to Understanding the Outcome of Sialylation

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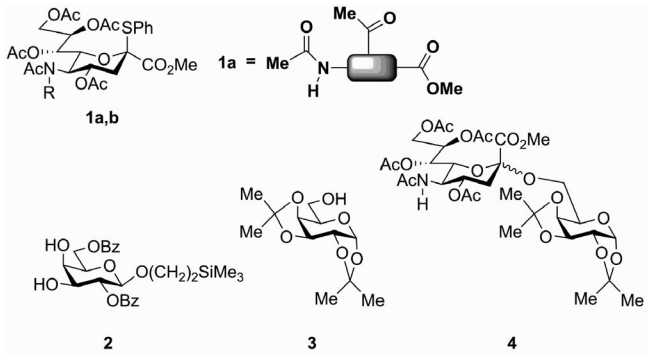
Changes in the hydrogen-bonding pattern in solutions of glycosyl donor induced by the addition of external amides/imides or by concentration changes influence the yield and stereoselectivity of sialylation.

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 to develop efficient methods for the synthesis of sialooligosaccharides.[2a,2b] Although substantial progress has recently been achieved in this booming area,[2d,2e,2f] it is still almost impossible to predict the outcome of a particular sialylation reaction. In many cases, seemingly minor changes in the structures of the reactants or reaction conditions dramatically influence the yield and stereoselectivity of glycosylation.[2] There has been a long-standing need for an approach to rationalize the outcome of a particular glycosylation reaction, which can be confusing. We are developing a novel concept that emphasizes the importance of supramolecular aggregation in the reaction mixture. This aggregation may be caused by intermolecular hydrogen bonding (inter alia). Molecular structures of reactants and reaction conditions would determine the aggregation type and the structure of the aggregates. Accessibility of the reaction center in the formed supramolecular aggregates would determine their reactivity, product yield, and stereoselectivity of glycosylation. In this communication we provide evidence that modulation of the intermolecular hydrogen-bonding pattern in solutions of glycosyl donors may result in dramatic changes in the outcome of sialylation.

Results and Discussion

Recently, synergistic activation of sialic acid *N*-acetylthioglycoside **1a** in the presence of *N*,*N*-diacetylthioglycoside **1b** in the reaction with **2** has been discovered (Scheme 1).[3] Glycosylation of **2** with a 1:1 mixture of **1a** and **1b** was complete within 5 min, whereas it took 3 h for **1a** alone to be consumed. We hypothesized that the synergism is related to changes in the hydrogen-bonding network in solutions of **1a** upon the addition of **1b**. The addition of **1b**, which is a hydrogen-bond acceptor competing with **1a** for hydrogen-bond donors (NH groups of **1a** molecules), would lead to "depolymerization" of hydrogen-bonded "oligomers" $[\mathbf{1a}]_n$,[4a] whose reactivity is lowered in comparison to that of the unbound molecules of **1a**,[4b] and to an increase in the apparent reactivity of **1a**. The postulated change in the hydrogen-bonding pattern in solutions of **1a** upon the addition of **1b** was corroborated by variable-temperature IR spectroscopy.[3] Furthermore, if our hypothesis is correct, then other amides may influence the intermolecular hydrogen-bonding pattern of glycosyl donors and, hence, the outcome of sialylation.



Scheme 1. Structures of sialic acid glycosyl donors **1a**,**b** [R = H (a), Ac (b)], glycosyl acceptors **2** and **3**, and disaccharide **4**.

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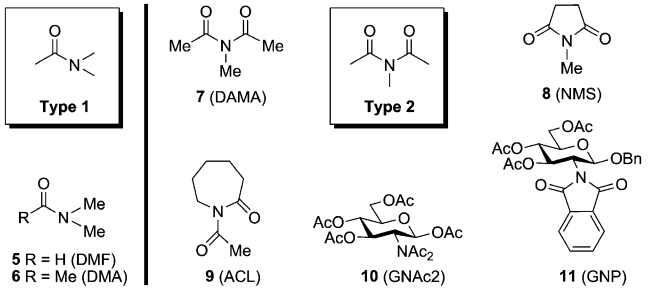
Two types of amides with structures resembling that of **1b** and capable of influencing the hydrogen-bonding network in the reaction mixture may be proposed (Scheme 2).[5] For testing the effect of amides **5**–**11** we used a model reaction between glycosyl donor **1a**[6] and glycosyl acceptor **3**[7] promoted by NIS–TfOH[8a] (NIS = *N*-iodosuccinimide) or DMTST[8b] [DMTST = dimethyl(methylthio)sulfonium tetrafluoromethanesulfonate] in MeCN or CH_2Cl_2.



Scheme 2. Two types of amides with structures resembling that of **1b**.[5]

When NIS–TfOH in MeCN was used as the promoter, the effect of the type of amide on the yield of disaccharide **4**[9] was clearly visible (Figure 1).[10] The addition of Type 2 amides (**7**–**11**) did not result in a decreased yield of disaccharide **4** (*f*–*k*) relative to that obtained with the reaction without additives (*a*). On the contrary, an excess amount of Type 1 amides (**5**,**6**) led to a decrease in the yield of disaccharide **4** (*b*–*e*). This previously unknown dose-dependent influence of the addition of Type 1 amides on the outcome

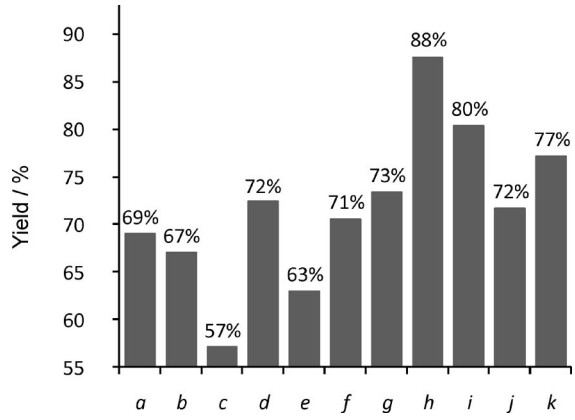


Figure 1. Yield of disaccharide **4** (%) in MeCN, NIS–TfOH, –40 °C, 3 h. Additives (equiv.): *a*) none, *b*) **5** (1), *c*) **5** (3), *d*) **6** (1), *e*) **6** (3), *f*) **9** (1), *g*) **9** (3), *h*) **1b** (1), *i*) **7** (1), *j*) **7** (3), *k*) **10** (3). Concentration of **1a**: 10 mg mL^{-1}.

of sialylation warranted further investigation. We studied the effect of the addition of amides **5** and **6** on the hydrogen-bonding pattern of glycosyl donor **1a** in MeCN by using IR spectroscopy. When 1 equiv. of amide **5** or **6** was added to the solution of **1a** (Figure 2, *a*) the ester carbonyl band was shifted, which is indicative of a weakening of the amide–ester hydrogen bond. Interestingly, the amide carbonyl did not experience any shift, which implies that the hydrogen bonding between the two amide groups was not affected. The situation changed dramatically when 3 equiv. of amide **5** or **6** was added (Figure 2, *b*). The ester carbonyl band experienced a substantial shift, which indicates a fur-

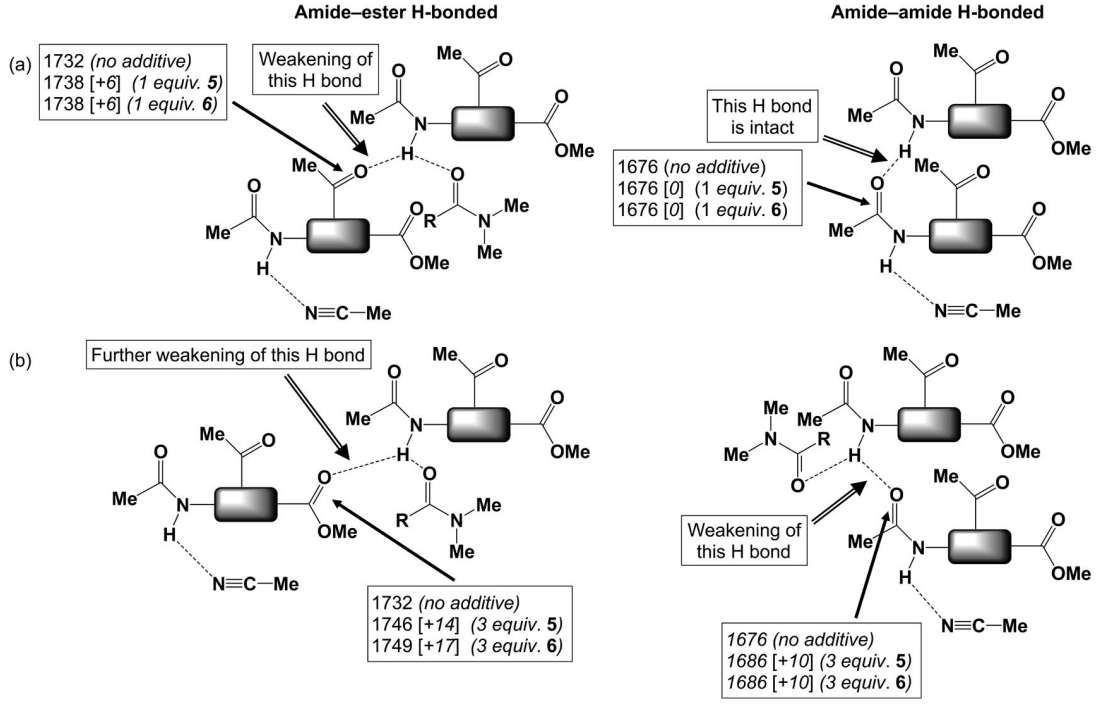


Figure 2. Effects of the amount of Type 1 amides added on the H-bonding pattern of **1a** in MeCN (10 mg mL^{-1}): *a*) 1 equiv., *b*) 3 equiv. Frequencies of amide C=O vibrations [*change*] (ν / cm^{-1}) are shown. Dimers are shown for clarity.

ther weakening of the amide–ester hydrogen bond. More importantly, amide carbonyl band was also shifted. This implies the weakening of the amide–amide hydrogen bond. It is very important that these changes in the hydrogen-bonding pattern upon the addition of variable amounts of Type 1 amides are correlated with the different outcomes of the sialylation reactions (Figures 1 and 2).

The highest yields of disaccharide **4** were obtained when the glycosylation was performed in the presence of Type 2 amides **1b**, **7**, and **10** (Figure 1, *h*, *i*, *k*). In particular, 1 equiv. of **7** is almost as activating as the addition of **1b** (80 vs. 88%). The common feature of the molecules of compounds **1b**, **7**, and **10** is the presence of an *N*,*N*-diacetylamino moiety (NAc_2), which can act as an efficient hydrogen-bond acceptor. Not surprisingly, their influences on the outcome of the model sialylation reaction are similar apparently due to their ability to incorporate into the hydrogen-bonding network and to form similarly organized supramolecular structures.

The most popular method used to activate sialic acid thioglycosides (NIS–TfOH), which we used, has an intrinsic limitation for the study of the effect of added amides because of the basicity of the latter. For this reason, increased amounts of TfOH are required[10] to promote the reaction when external amides are present in the reaction mixture. Although the results reported in Figure 1 are based on variations of three parameters (nature and amount of amide additive and amount of TfOH, which is proportional to the amount of the former), one can compare experiments and find that there is no correlation (and therefore no relationship) between the amount of TfOH added and the outcome of the glycosylations. Almost the *same* yields (71–73%) were obtained in the experiments with *different* amounts of TfOH used to promote the reaction in the presence of different or identical amide additives (see *d*, *f*, *g*, and *j* in Figure 1). These yields are very close to that (69%) obtained in the reaction *without* additives (*a*). In contrast, the differences in yield range from 57 (*c*) to 88% (*h*). This implies that the variations in the yields of disaccharide **4** are *not* related to the amount of TfOH used.

In order to completely exclude any possibility of influence of the amount of TfOH on the hydrogen-bonding pattern of glycosyl donor **1a**, we performed a series of experiments in the *absence* of any acid by using DMTST in MeCN rather than the NIS–TfOH combination as the promoter (Figure 3).[10] In this case, a more complex picture was observed indicating that not all Type 2 amides are the same in their influence on the outcome of sialylation. In our opinion, this is not surprising, as external amides are able to incorporate into hydrogen-bonding networks and form supramolecular structures organized differently depending on the spatial orientation of their carbonyl groups (Figure 2). The yield of disaccharide **4** varied in these experiments from 19 (*b*) to 47% (*f*), depending on the *nature* of the added amide, which was the single varied parameter here. Addition of compound **9**, which is a Type 2 amide, to the reaction mixture resulted in a dramatic decrease in the yield of the disaccharide [from 46 in (*a*) to 20% in (*d*); i.e., more than *twice*] similar to that observed upon the addition of Type 1 amides (*b*, *c*). This is a very important observation that rules out the possibility that the influence of Type 1 amides on the yield of disaccharide may be related to their potential ability to form anomeric imidates, which would react through an entirely different glycosylation pathway.

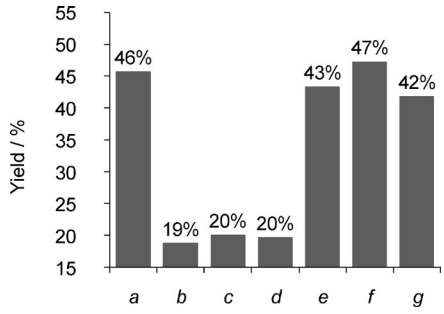


Figure 3. Yield of disaccharide **4** (%) in MeCN, DMTST, ≈ 25 °C, 18 h. Additives (equiv.): *a*) none, *b*) **5** (3), *c*) **6** (2.5), *d*) **9** (3), *e*) **7** (3), *f*) **11** (3), *g*) **8** (3). Concentration of **1a**: 10 mg mL^{-1}.

The influence of the addition of amides on the stereoselectivity of sialylation is even more intriguing. In MeCN, glycosylations promoted by NIS–TfOH consistently gave α-configured disaccharide **4** as the major product (α/β, 4:1–10:1), whereas those promoted by DMTST were less stereoselective (α/β, ca. 1:1 to 2:1).[10]

Usually, sialylation in CH_2Cl_2 leads mainly to the β isomer, and this is commonly explained by the absence of stereocontrolling nitrile effect.[2a] However, the addition of amide **10** dramatically increased the α/β ratio of the anomers of **4** even in CH_2Cl_2 (Figure 4).[10] This highly unusual *reversal* of stereoselectivity by the addition of an external nonreacting compound can be easily rationalized within the framework of our concept, as the accessibility of the α and β faces of **1a** can vary considerably in differently organized supramolecular aggregates formed in the presence of additives. In this case, because NIS–TfOH was used as a promoter system it is important to emphasize that two of the three experiments (*b*, *c*) presented in Figure 4 were performed in the presence of the *same* amount of TfOH (the only parameter varied here was the *nature* of the amide additive), and comparison of these results leads to the same conclusions as the comparison of all three experiments shown in Figure 4.

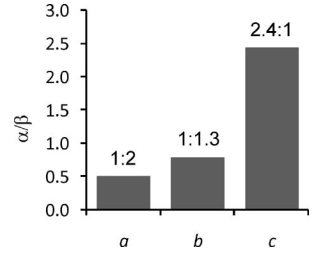


Figure 4. Stereoselectivity of sialylation in CH_2Cl_2 (α/β ratio for disaccharide **4**), NIS–TfOH, –40 °C, 3 h. Additives (equiv.): *a*) none, *b*) **7** (3), *c*) **10** (3). Concentration of **1a**: 10 mg mL^{-1}.

The final proof for the validity of our concept came from the study of the IR spectral pattern of glycosyl donor **1a** in various solvents.[10] Frequency range[11] and concentration dependence of the relative intensities of the "free" and "bound" NH vibrations suggest *inter*molecular hydrogen bonding of **1a** in *all* solvents studied.[12] For example, Figure 5 shows how the amount of "free" NH groups in solution of glycosyl donor **1a** in MeCN changes with the concentration of the solute (*a*). A phase transition apparently related to the rearrangement of the hydrogen-bonding network seems to occur. Incidentally, in the same concentration range, the optical rotation also experiences discontinuity (*b*), which confirms the phase transition.[10,13] More importantly, the yield of disaccharide also changes abruptly in the concentration range of phase transition (*c*).[10,14] All plots in Figure 5 experience discontinuity in the same narrow concentration range, which implies that there is a relationship between them. We may conclude that the rearrangement of the intermolecular hydrogen-bonding network influences the yield of disaccharide.

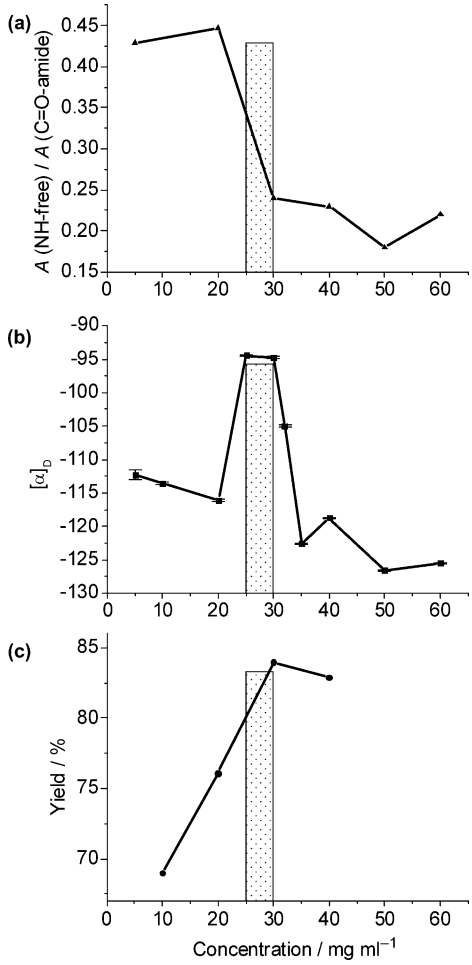


Figure 5. Correlation of various properties at different concentrations of glycosyl donor **1a**: (*a*) amount of "free" NH groups in solution of glycosyl donor **1a** in MeCN (≈25 °C) calculated as the ratio of absorptions of the NH band at 3365 cm^{-1} and the amide C=O band at 1676 cm^{-1} [*A*(NH-free)/*A* (C=O amide)]; the intensity of the latter band is an "internal standard", (*b*) specific rotation of a solution of glycosyl donor **1a** in MeCN (averaged over 23–26 °C range), (*c*) yield of disaccharide **4** (%) (MeCN, NIS–TfOH, –40 °C, 3 h). Filled rectangle designates concentration range of phase transition.

Conclusions

Higher yields of disaccharide **4** are obtained in those cases when a better suppression of the side processes that usually accompany sialylation reactions occurs. It is astonishing how the addition of external amides/imides can modulate the selectivity of the reaction leading to the substitution (S_N1) product (disaccharide) versus the elimination (E1) product (glycal). Apparently, unimolecular elimination begins to dominate when substitution (glycosylation) is disfavored. This can happen if the approach of a nucleophile to the anomeric center in the intermediate glycosyl-cation-like intermediate is hindered. Changes in the concentration or addition of external amides/imides result in rearrangement of the intermolecular hydrogen-bonding network and formation of supramolecular structures organized differently (see above). This change in the structure of the supramolecular aggregates can modulate the accessibility of the reaction center for the nucleophile and hence the selectivity of the reaction and the yield of disaccharide.

Our concept may provide insight into seemingly incomprehensible results. For example, there is inconsistency in the relative reactivity series of various sialyl donors with modified substituents at N(5) determined with separate[15a,15b] (*N*-TFA > *N*,*N*-diacetyl) or simultaneous[15c] (*N*,*N*-diacetyl > *N*-TFA) presence of glycosyl donors in the reaction mixture. Our results strongly suggest that estimation of the relative reactivity of sialyl donors under competitive conditions[3,15c] may be incorrect.

In summary, we demonstrated that changes in the hydrogen-bonding network may influence the yield of disaccharide and stereoselectivity of sialylation. Intermolecular hydrogen bonding should be considered as an important factor that is currently almost ignored during analysis of the outcome of glycosylation. The suggested supramolecular approach for modulation of the reactivity of the amide-containing substances may find wider application in other areas of chemistry.

Supporting Information (see footnote on the first page of this article): Experimental procedures, optical rotation data for all temperatures, and Table with all yields and α/β ratios of disaccharide **4**.

Acknowledgments

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[10] See the Supporting Information.

[11] Frequencies of "free" and "bound" NH vibrations of **1a** (concentration 10 mg mL^{-1}), respectively: 3428 and 3371 (CCl_4), 3422 and 3371 (CH_2Cl_2), 3365 and 3224 cm^{-1} (MeCN). These pairs of values should be compared with the IR frequencies of "non-hydrogen-bonded" and "hydrogen-bonded" NH vibrations for noncarbohydrate amides: 3435–3455 and 3310–3370 cm^{-1} ($CDCl_3$) [E. S. Stevens, N. Sugawara, G. M. Bonora, C. Toniolo, *J. Am. Chem. Soc.* **1980**, *102*, 7048–7050]; 3450–3460 and 3300–3350 cm^{-1} (CH_2Cl_2), 3394–3406 and 3300–3320 cm^{-1} (MeCN) [S. H. Gellman, G. P. Dado, G.-B. Liang, B. R. Adams, *J. Am. Chem. Soc.* **1991**, *113*, 1164–1173].

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