The first example of synergism in glycosylation. Possible reasons and consequences

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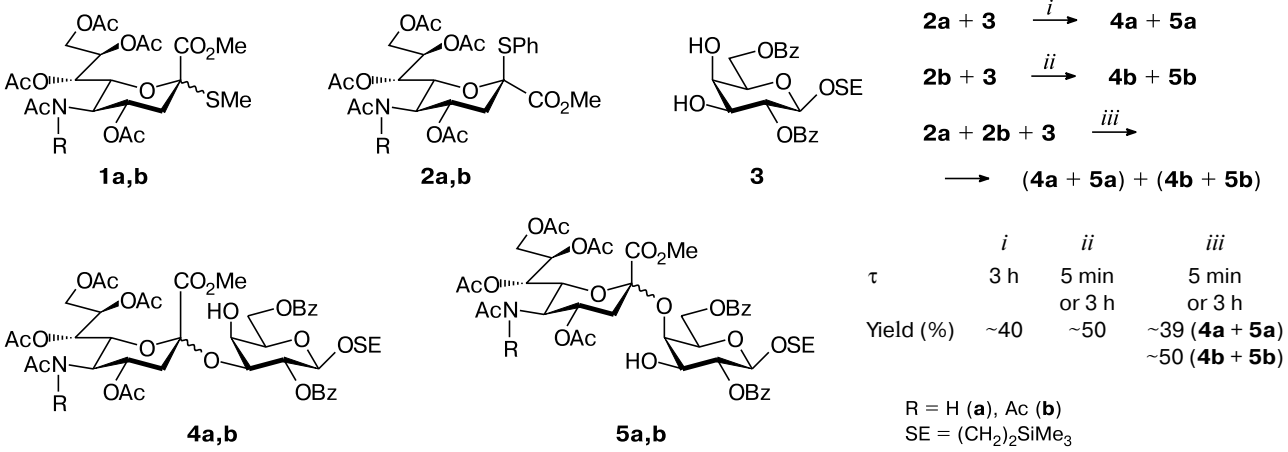
Sialic acid-containing glycoconjugates are involved in a wide range of cell—surface recognition phenomena in living systems.[1] For this reason, tremendous efforts have been made in order to develop efficient methods for the synthesis of sialo-oligosaccharides.[2] Most of the known approaches to the construction of the sialyl glycosidic bond rely on the use of the *N*-acetylneuraminic acid containing one acetyl group at the N(5) atom of the sialic acid residue (for example, **1a**, Scheme 1).[2] Recently, *N*,*N*-diacetyl derivative of sialic acid methyl thioglycoside **1b** has been shown[3] to be a highly reactive and efficient glycosyl donor. Remarkably, the glycosylation reactions with **1b** were finished within 3—5 min while most glycosylations with *N*-monoacetylated thioglycoside **1a** require several hours for completion.[2] Since then, various sialyl donors with modified substituents at C(5) were introduced and shown to have a number of advantages, including enhanced reactivity, in comparison to more traditional 5-acetamido-counterparts.[4—8] However, it should be emphasized that the reasons for different reactivities of these modified glycosyl donors still remain unknown.

Therefore, we decided to study this phenomenon using *N*-acetyl and *N*,*N*-diacetyl derivatives **2a** (see Ref. 9) and **2b** (see Ref. 10) as representative examples. We studied the behavior of these compounds in the glycosylation of galactoside diol **3** (see Ref. 11) under typical sialylation conditions[3] (1.16 equiv. **2** + 1 equiv. **3**, NIS—TfOH, 3Å MS, MeCN, −40 °C). After conventional workup of the reaction mixture,[3] a disaccharide fraction that contained (2-3)-linked disaccharides in addition to the regioisomeric (2-4)-linked disaccharides was isolated by gel chromatography (Bio-Beads Sx3, toluene) (see Scheme 1). The reaction of *N*,*N*-diacetylthioglycoside **2b** was complete after nearly 5 min (according to TLC, no starting thioglycoside was present). During this period, an almost maximum yield (~50%) of *N*,*N*-diacetyldisaccharides **4b**+**5b** was attained and did not change markedly within 3 h of reaction (Fig. 1, *a*). *N*-Monoacetylthioglycoside **2a** was much less reactive. According to TLC analysis, full conversion of **2a** could be realized only after 3 h of reaction resulting in the formation of a mixture of *N*-acetyldisaccharides **4a**+**5a** in ~40% yield. Only a half of

Scheme 1



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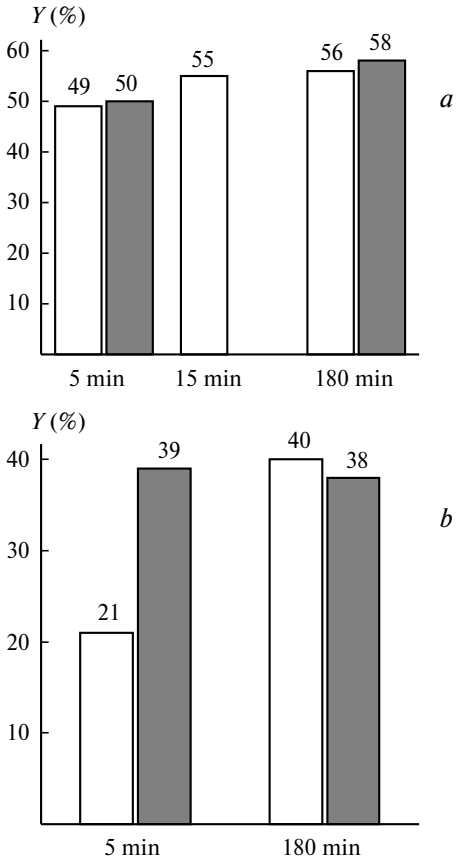


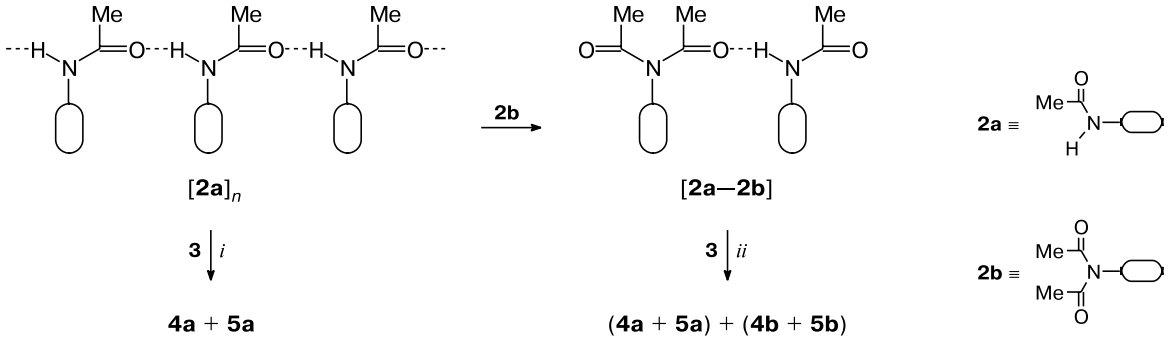
Fig. 1. *a*. Yields (*Y* (%)) of *N*,*N*-diacetyldisaccharides **4b**+**5b** obtained from **2b** (light rectangles) or from a **2a** and **2b** mixture (dark rectangles) after 5, 15, and 180 min of the reaction. *b*. Yields of *N*-acetyldisaccharides **4a**+**5a** prepared from **2a** (light rectangles) or from a **2a** and **2b** mixture (dark rectangles) after 5 and 180 min of the reaction. The average yields of two independent experiments are given.

this amount of *N*-acetyldisaccharides **4a**+**5a** (yield ~20%) was formed during 5 min of the reaction (see Fig. 1, *b*). When diol **3** was glycosylated by an equimolar mixture of **2a** and **2b** ([**2a**] : [**2b**] : [**3**] = 0.58 : 0.58 : 1), *N*-acetylthioglycoside **2a** was consumed within only 5 min and the yield of *N*-acetyldisaccharides **4a**+**5a** (isolated by gel chromatography followed by silica gel chromatography) (~40%) was the same as that obtained in the 3-h reaction (see Fig. 1, *b*).

This apparent activation of thioglycoside **2a** in the presence of thioglycoside **2b** is highly unusual and deserves special comment. It is obvious that no explanation of this phenomenon could be inferred from consideration of intramolecular reasons. The synergism of action of thioglycosides **2a** and **2b** in glycosylation can only be interpreted if one assumes some type of intermolecular interactions between their molecules in solution.

We believe that it is the ability of *N*-acetylthioglycoside **2a** to form hydrogen-bonded aggregates $[\mathbf{2a}]_n$ due to the presence of the NH group in the molecule of amide **2a** (Fig. 2) that causes the reactivity of *N*-acetylthioglycoside **2a** to be lower than that of *N*,*N*-diacetylthioglycoside **2b**. Apparently, these aggregates have lower reactivity than unbound molecules of **2a**. The addition of **2b**, which is an additional hydrogen bond acceptor competing with **2a** for hydrogen bond donors, makes hydrogen bonding between molecules of *N*-acetylthioglycoside **2a** less likely. This may lead to the "depolymerization" of homoaggregates $[\mathbf{2a}]_n$ and the formation of mixed aggregates [**2a**—**2b**], which are more reactive, and, hence, they are converted more rapidly into *N*-acetyldisaccharides **4a**+**5a** (see Fig. 2).

The postulated change in the hydrogen bonding pattern in MeCN solutions of amide **2a** upon addition of *N*,*N*-diacetylthioglycoside **2b** is corroborated by IR-spectroscopy (Nicolet Magna-750 FT IR spectrometer with a low-temperature block). It was shown that upon the addition of compound **2b** to a solution of **2a** in MeCN, the concentration of free NH groups decreases (this followed from the intensity ratio of the bands corresponding to free NH groups (3365 cm^{-1}) and amide C=O groups of **2a**



i. Slowly *ii*. fast

Fig. 2. Destruction of hydrogen-bonded homoaggregates $[\mathbf{2a}]_n$ upon the addition of an equimolar amount of **2b** as a possible reason for the synergistic activation of thioglycoside **2a** in the presence of thioglycoside **2b**.

(1688 cm^{-1}), the latter band was used as the "internal standard"). In the stretching region of the amide C=O groups, the band at 1713 cm^{-1}, present in the spectrum of *N*,*N*-diacetyl derivative **2b** in MeCN, is absent in the spectrum of a mixture of **2a** and **2b**. In the IR spectrum of a mixture of amides **2a** and **2b** in MeCN, the intensity of the second band (1679 cm^{-1}) corresponding to stretching vibrations of the amide C=O group of compound **2b** markedly increases as the temperature decreases from ~25 °C to −30 °C. This indicates that this amide C=O group is involved in intermolecular association, the degree of which is expected to increase at lower temperature.

The suggested reasons for the synergistic action of **2a** and **2b** proposed in this work not only provide a possible explanation of the considerable difference in their reactivity but also provides an insight into the inconsistency in the relative reactivity series of various sialyl donors with modified substituents at N(5) determined with separate[4] (*N*-TFA > *N*,*N*-diacetyl) or simultaneous[5a] (*N*,*N*-diacetyl > *N*-TFA) presence of glycosyl donors. Our results strongly suggest that estimation of relative reactivity of sialyl donors under competitive conditions (several glycosyl donors present in one reaction mixture)[5a] may be incorrect.

However, the most important consequence of our results is that they open a new dimension in the search for possible ways of affecting the reactivity of sialyl donors. It is important to bear in mind that addition of other compounds capable of influencing hydrogen bonding network in the reaction mixture along with more common[2—8,10] change of *N*- and *O*-protective groups in the molecule of a glycosyl donor may also be important for the success. The intermolecular approach to modulation of the reactivity of amide-containing compounds proposed in this communication may find wider application in other areas of chemistry.

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