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# 8-O-Sialylation of Neuraminic Acid Acceptor Reactivity and Anomeric Stereocontrol[1]

Julio C. Castro-Palomino, Yury E. Tsvetkov, Regine Schneider, and Richard R. Schmidt*

Fakultät Chemie, Universität Konstanz, Fach M 725,

D-78457 Konstanz, Germany

***Abstract***: 2-O-Unprotected and 2-deoxy-2,3-dehydro-neuraminic acid derivatives **2**, **4**, and **10** exhibit enhanced acceptor properties at their 8-hydroxy group in sialylation reactions with phosphite **5** as donor; yet, mainly (**10**) or exclusively (**2**, **4**) β-glycoside bond formation was observed. The 3-phenylthionocarbonate group as stereodirecting auxiliary group in the sialyl donor **11** led with **10** as acceptor to exclusive formation of α(2-8)-linked disaccharide.
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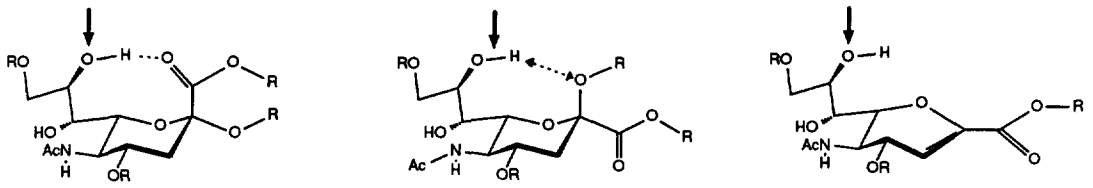
The sequence Neu5Acα(2-8)Neu5Ac is a principal constituent of a number of glycoconjugates including various gangliosides. These compounds were found to play an important role in numerous biological phenomena being, for example, tumor-associated antigens[2,3] or receptors for bacterial toxins and viruses.[3,4]

Successful syntheses of this linkage were based on the use of sialyl donors bearing at C-3 an additional function, namely OH[5] or SPh and SePh,[6] which can control the stereochemistry of substitution at the anomeric center and prevent 2,3-elimination. On the other hand, attempts to prepare the α(2-8)-linkage directly, using α-glycosides as acceptors and conventional sialyl donors, such as thioglycosides[7] or phosphites,[8-10] thus far gave only very low yields[10] and/or β-linkage.[9]

Scheme 1

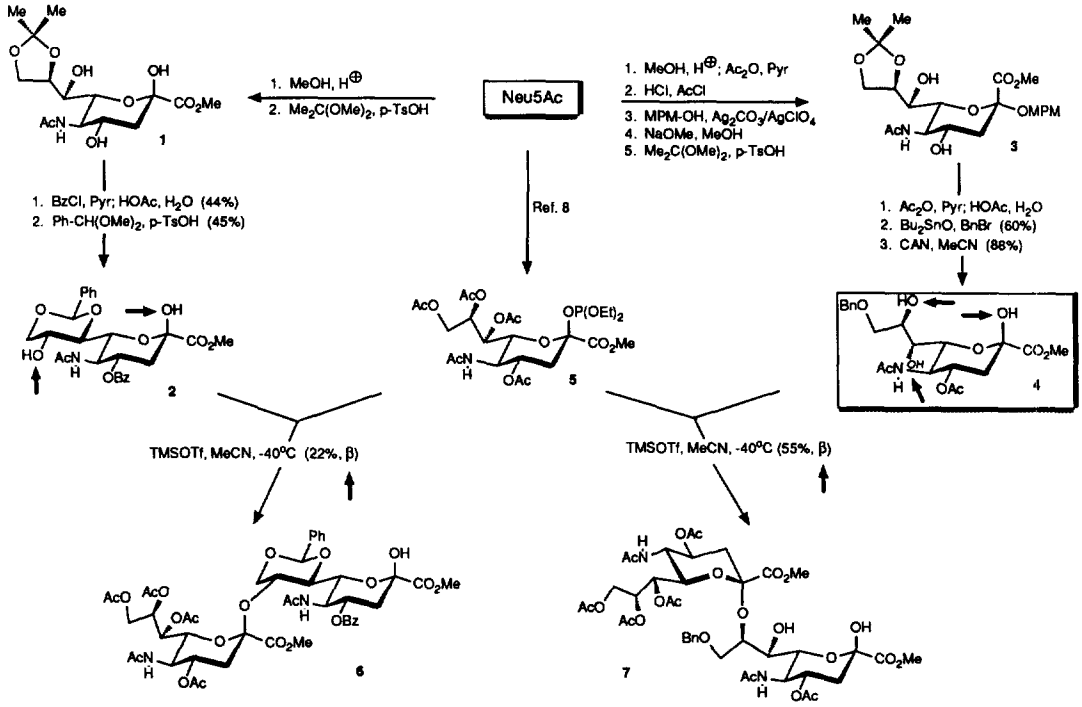


**A** **B** **C**

It was assumed, that the low acceptor reactivity of the 8-OH group in derivatives of Neu5Ac possessing $^2C_5$ conformation is caused by interaction with the 5-acetamido group. Therefore, derivatives of Neu5Ac-1,7-lactone, having a rigid $^5C_2$ conformation, were investigated as sialyl acceptors.[11] Thus, the acceptor reactivities were greatly increased, yet essentially only the undesired β(2-8)-linkage was obtained. Therefore, the study was now focussed to the influence of the functional groups at the anomeric position (OR, $CO_2R$, Scheme 1, **A** and **B**). Obviously, interaction between the carboxylate group and the 8-OH group in the α-anomer **A** (as indicated by an X-ray analysis) could also strongly influence the acceptor properties of the 8-OH group and thus explain their low reactivity.[10,12] Therefore, now the reactivity of the corresponding β-anomers[10] and especially of 2,8-O-unprotected neuraminic acid derivatives (Scheme 1, **B**) was investigated; they adopt the β-configuration and

exhibit low acceptor properties at the anomeric position. Because of steric and stereoelectronic reasons even higher acceptor properties are expected for 2-deoxy-2,3-dehydro-neuraminic acid derivatives **C**.

Scheme 2

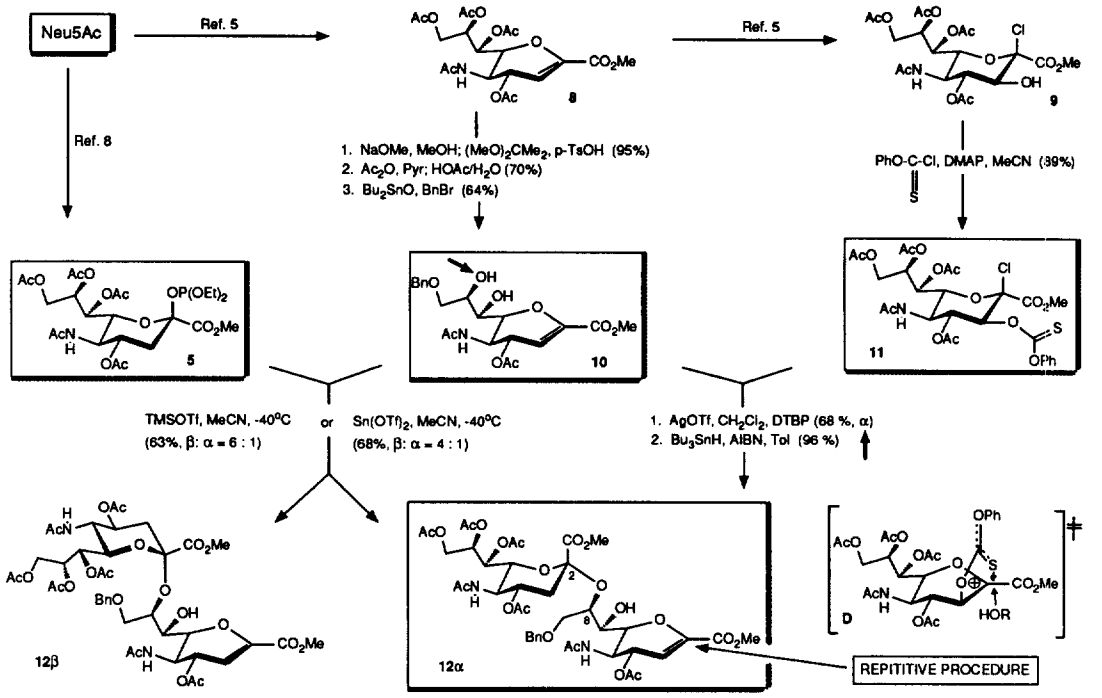



To this aim, Neu5Ac was transformed into 8,9-O-isopropylidene derivative **1** (Scheme 2). Regioselective 4-O-benzoylation and acid catalyzed de-O-isopropylidenation and then direct 7,9-O-benzylidenation with benzaldehyde dimethylacetal in the presence of p-toluenesulfonic acid (p-TsOH) as catalyst afforded the desired acceptor **2**. 9-O-Benzyl-protected acceptor **4** was readily obtained from methoxybenzyl (MPM) neuraminic acid derivative **3** via regioselective 4-O-acetylation and acid catalyzed de-O-isopropylidenation, regioselective 9-O-benzylation with benzyl bromide and dibutyltin oxide, and then oxidative cleavage of the MPP group with ceric ammonium nitrate (CAN) as oxidizing agent. Glycosylation of acceptors **2** and **4** with known sialyl phosphite **5**[8] as glycosyl donor under standard conditions (acetonitrile as solvent at -40°C) gave only β(2-8)-linked disaccharides **6** and **7**,[13] respectively. Yet, as expected, sterically less demanding 9-O-benzyl protected acceptor **4** gave the best results (55%).

This improvement in glycosylation yields with 2-O-unprotected acceptors prompted us to investigate the acceptor properties of 2-deoxy-2,3-dehydro-neuraminic acid derivatives (Scheme 1, **C**). Some literature examples[5] made us hope, that the undesired influence of functional groups at the anomeric position could be entirely overcome and ready access of the α-face of sialyl donors to the 8-OH group would be facilitated. To this aim, known 2,3-anhydro-neuraminic acid derivative **8**[14] (Scheme 3) was transformed into the desired 9-O-benzyl protected acceptor **10** by employing a similar methodology as described for the synthesis of structurally related acceptor **4**. Reaction of **10** with sialyl donor **5** under standard conditions afforded (2-8)-linked

disaccharides **12** in good yield, yet the β-anomer prevailed (**12α**:**12β** = 1:6).[13] Use of $Sn(OTf)_2$ as catalyst (and various other catalysts) led only to a slight change in favor of the α-anomer (**12α**:**12β** = 1:4).

Scheme 3




Obviously, high α-sialylation at the 8-OH group of Neu5Ac derivatives cannot be reached just by increasing the acceptor properties. Presumably, due to steric restraints, stereocontrol by directing groups at the donor moiety is required. Previous investigations with a 3-OH group as auxiliary at the β-side of the donor (for instance, compound **9**[5] in Scheme 3) gave only moderate glycosylation yields and α/β-mixtures.[5] Therefore, 3-phenylthio and 3-phenylselenyl groups were introduced into sialyl donors; they gave the desired high yields and α-selectivities.[6] Yet, preparation of the donor molecules seems to be quite demanding. Therefore, we employed readily available 3-hydroxy substituted halogenose **9**[5] and transformed it with chlorophenylthionocarbonate in the presence of dimethylaminopyridine (DMAP) into thionocarbonate **11**, thus accommodating both, efficient neighboring group participation via a five-membered ring intermediate in the glycosylation step, as evidenced by the presumed glycosylation transition state **D**, and ensuing deoxygenative removal of the auxiliary group by a standard deoxygenation protocol.[15] As shown in Scheme 3, this reaction sequence worked very efficiently: with **10** as acceptor and AgOTf in the presence of 2,6-di-tert-butylpyridine as promoter system exclusively the α(2-8)-linked disaccharide was obtained, which gave upon treatment with $Bu_3SnH$/AIBN in toluene the desired α(2-8)-linked disaccharide **12α** in very good overall yield.

In conclusion, the 8-OH groups of 2-O-unprotected or 2-deoxy-2,3-dehydro-neuraminic acids exhibit strongly increased glycosyl acceptor properties. For α-selective sialylation at the 8-OH group, based on known 3-hydroxylated Neu5Ac derivative **9** sialyl donor **11** is designed, which permits α-stereocontrol via neighboring

group participation and immediate 3-deoxygenation of the product. The usefulness of this donor/acceptor combination for repetitive α(2-8)-linkages (see Scheme 3), as found in oligo- and polysialic acids, is under investigation.[16]

**References and Notes**


1. This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. – J.C.C-P. is grateful for a DAAD stipend. – R.S. is grateful for a stipend within the frame-work of the Landesgraduiertenförderung.
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13. $^1$H NMR data (250 MHz, $CDCl_3$): **6**: $\delta_H$ = 1.83 (dd, 1 H, $J_{3'ax,4'}$ = 11.3, $J_{3'ax,3'eq}$ = 13.0 Hz, 3'ax-H), 2.27 (m, 2 H, 3eq-H, 3'eq-H), 2.46 (dd, 1 H, $J_{3ax,4}$ = 11.3, $J_{3ax,3eq}$ = 12.8 Hz, 3ax-H), 3.57 (m, 1 H, 9a-H), 3.79 (m, 1 H, 8-H), 3.94 (dd, 1 H, $J_{6,7}$ = 1.6, $J_{7,8}$ = 9.2 Hz, 7-H), 4.09 (m, 2 H, 5'-H, 9'a-H), 4.19 (m, 2 H, 9b-H, 6'-H), 4.35 (m, 1 H, 9'b-H), 4.69 (dd, 1 H, $J_{6,7}$ = 1.6, $J_{5,6}$ = 10.1 Hz, 6-H), 4.75 (dd, 1 H, $J_{4,5}$ = 9.9, $J_{5,6}$ = 10.1 Hz, 5-H), 5.30 (m, 1 H, 8'-H), 5.43 (m, 2 H, 7'-H, 4'-H), 5.72 (m, 1 H, 4-H), 6.03 (d, 1 H, $J_{NH,5}$ = 9.1 Hz, NH), 7.04 (d, 1 H, $J_{NH',5}$ = 9.7 Hz, NH'). **7**: $\delta_H$ = 1.80 (dd, 1 H, $J_{3'ax,4'}$ = 11.8, $J_{3'ax,3'eq}$ = 13.2 Hz, 3'ax-H), 2.26 (dd, 1 H, $J_{3ax,4}$ = 10.9, $J_{3ax,3eq}$ = 12.6 Hz, 3ax-H), 2.19 (dd, 1 H, $J_{3eq,4}$ = 5.6, $J_{3eq,3ax}$ = 12.6 Hz, 3eq-H), 2.41 (dd, 1 H, $J_{3'eq,4}$ = 4.7, $J_{3'eq,3'ax}$ = 13.2 Hz, 3'eq-H), 3.60 (m, 1 H, 9a-H), 3.69 (m, 1 H, 9b-H), 3.89 (dd, 1 H, $J_{6,7}$ < 1, $J_{7,8}$ = 9.5 Hz, 7-H), 4.04 (dd, 1 H, $J_{4',5'}$ = 10.2, $J_{5',6'}$ = 10.8 Hz, 5'-H), 4.12 (m, 3 H, 5-H, 9'a-H, 6-H), 4.46 (m, 1 H, 9'b-H), 5.15 (m, 1 H, 8'-H), 5.22 (m, 1 H, 4'-H), 5.33 (dd, 1 H, $J_{6',7'}$ < 1, $J_{7',8'}$ = 7.1 Hz), 5.45 (m, 1 H, 4-H), 6.06 (d, 1 H, $J_{NH,5}$ = 8.2 Hz, NH), 6.77 (d, 1 H, $J_{NH'}$ = 9.9 Hz, NH'). **12α**: $\delta_H$ = 2.44 (dd, $J_{3'ax,3'eq}$ = 12.3, $J_{3'ax,4}$ = 11.4 Hz, 3'ax-H), 2.60 (dd, $J_{3'eq,3'ax}$ = 12.3, $J_{3'eq,4}$ = 4.6 Hz, 3'eq-H), 3.56 (m, 1 H, 9a-H), 3.77 (dd, 1 H, $J_{5',6'}$ = 11.0, $J_{6',7'}$ = 2.3 Hz, 6'-H), 3.84 (m, 1 H, 9b-H), 3.91 (m, 2 H, 5'-H, 9'a-H), 4.31 (m, 2 H, 5-H, 6-H), 4.52 (m, 1 H, 9'b-H), 4.57 (m, 1 H, 8-H), 4.89 (m, 1 H, 4'-H), 5.19 (m, 1 H, 8'-H), 5.30 (dd, 1 H, $J_{6',7'}$ = 2.3, $J_{7',8'}$ = 4.6 Hz, 7'-H), 5.34 (d, $J_{NH',5'}$ = 9.8 Hz, NH'), 5.76 (dd, 1 H, $J_{3,4}$ = 2.5, $J_{4,5}$ = 9.2 Hz, 4-H), 5.92 (d, 1 H, $J_{3,4}$ = 2.5 Hz, 3-H), 6.91 (d, $J_{5,NH}$ = 8.3 Hz, NH). **12β**: $\delta_H$ = 1.70 (dd, $J_{3'ax,3'eq}$ = 13.1, $J_{3'ax,4}$ = 11.8 Hz, 3'ax-H), 2.35 (dd, 1 H, $J_{3'eq,3'ax}$ = 13.1, $J_{3'eq,4}$ = 4.5 Hz), 3.59 (m, 1 H, 9a-H), 3.73 (m, 1 H, 8-H), 3.76 (m, 1 H, 9b-H), 3.94 (dd, $J_{6,7}$ < 1, $J_{7,8}$ = 8.7 Hz, 7-H), 3.97 (dd, $J_{4',5'}$ = 11.1, $J_{5',6'}$ = 10.5 Hz, 5'-H), 4.13 (m, 1 H, 9'a-H), 4.23 (dd, $J_{6,7}$ < 1, $J_{5,6}$ = 11.0 Hz, 6-H), 4.27 (dd, 1 H, $J_{5,6}$ = 11.0, $J_{4,5}$ = 8.7 Hz, 5-H), 4.44 (dd, 1 H, $J_{5',6'}$ = 10.5, $J_{6',7'}$ = 20 Hz, 6'-H), 4.47 (m, 1 H, 9'b-H), 4.94 (m, 1 H, 4'-H), 5.30 (m, 1 H, 8'-H), 5.44 (dd, 1 H, $J_{6',7'}$ = 2.0, $J_{7',8'}$ = 7.2 Hz, 7'-H), 5.62 (dd, 1 H, $J_{3,4}$ = 2.5, $J_{4,5}$ = 8.7 Hz, 4-H), 5.89 (d, 1 H, $J_{3,4}$ = 2.5 Hz, 3-H), 6.18 (d, $J_{5,NH}$ = 7.5 Hz, NH), 6.34 (d, $J_{5',NH'}$ = 10.2 Hz, NH').
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