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Synthesis of spacer armed Kdn(2→6′) and (2→3′)-lactosamines for immunochemical research

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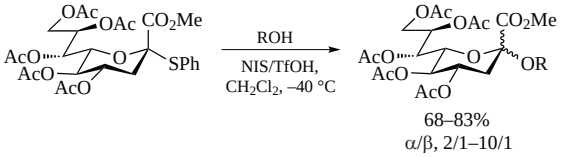
Glycosylation of lactosamine acceptors with Kdn thioglycoside donors in the presence of NIS/TfOH as a promoter affords products with both α- and β-ketosidic linkage (2–6′ or 2–3′) between the Kdn and Gal residues. After deprotection, the synthesized trisaccharides and glycans containing Neu5Ac were printed to a chip and their comparative interaction with human serum antibodies was explored.



ROH = 3′-OH-6-*O*-Bn-*O*-Ac$_4$-LacNAc-O(CH$_2$)$_3$NHC(O)CF$_3$,
6′-OH-*O*-Ac$_5$-LacNAc-O(CH$_2$)$_3$NHC(O)CF$_3$,
HO(CH$_2$)$_3$NHC(O)CF$_3$

Keywords: sialylation, thiosialic donors, sialooligosaccharides, Kdn, glycochip, human serum antibodies.

Within animal cells, sialic acids [*N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc), their two most abundant forms] are normally found at the terminal positions of complex glycans being α2,3- or α2,6- to Gal, or α2,6-linked to GalNAc residues. Additionally, they form polymers with α2,8- or α2,9-linkages between sialic units. Sialylated glycans are involved in a wide range of biological recognition processes.[1]

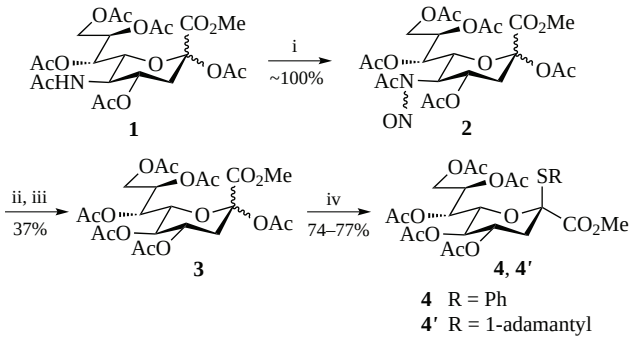
The inherent structural features of sialic acids, namely the hindered anomeric carbon, the electron-withdrawing carboxyl group at the C(2), and the methylene C(3) ring carbon, make chemical sialylation quite challenging. A variety of structural modifications of sialyl donors (*e.g.*, introduction of stereo-directing or co-participating groups, variations in the nature of the leaving group or the nature/position of protecting groups) have been investigated to improve the outcome of sialylation and reviewed in the literature. It is not surprising that chemists focus their efforts mainly on natural α-sialylation; only a few approaches resulted in predominantly β-sialosides are described.[2–4]

Introduction of 2-thiosialic donors (*S*-alkyl/aryl glycosides, xanthates and thioimidates) into synthetic practice was the important milestone; the thioglycosides, due to simple preparation, long shelf life, and high tolerance to most chemical reactions, quickly emerged as versatile building blocks.[5–7] Thus, in recent years, Crich and coworkers reported on the rapid, low-temperature α-selective sialylation using thioglycosides as donors. The optimal activators for 2-thiophenyl donors with most common promoters were sulfenyl triflates ArSOTf prepared *in situ* from arylsulfenyl chlorides (PhSCl or stable commercially available *p*-O$_2$NC$_6$H$_4$SCl) in conjunction with silver triflate; diphenyl sulfoxide in conjunction with triflic anhydride; *N*-iodosuccinimide (NIS) in conjunction with triflic acid. Furthermore, it was found that the more reactive adamantyl thiosialosides could be used in the presence of NIS/TfOH to achieve excellent yield and anomeric selectivity.[8–11] A few direct examples of glycosylation of lactosamine acceptors with 2-thiosialic donors are documented.[12–14]

Recently, in blood of healthy donors, we found antibodies to oligosaccharides terminated by Neu5Ac residue in the β-form not occurring in nature; at the same time, as expected, antibodies to the analogous glycans containing the ubiquitous α-form of Neu5Ac were not detected.[15,16] To explain this, we hypothesized that the discovered antibodies were actually directed to fragments of bacterial polysaccharides, which often contain β-anomers of 2-keto-3-deoxy-D-*glycero*-D-*galacto*-nononic (Kdn), legionaminic (Leg), pseudoaminic (Pse) acids; the latter nonulosonic acids share their nine-carbon backbone with Neu5Ac.[1,17]

In the course of this work, we synthesized spacer armed lactosamines coupled with Kdn residue at 3′- or 6′-positions, and, also, spacer armed Kdn monosaccharide derivatives. Resulting β-sialosides were our main interest, however the α-isomers were also required for investigation of antibodies directed to Neu5Gc glycans.[16] When planning the synthesis, we focused primarily on the results earlier obtained by the Crich's group. Thus, we chose the standard methyl ester of per-*O*-acetylated 2-β-thiopenyl glycoside Kdn **4** as a donor. Since it has no co-participating or stereodirecting auxiliaries, we expected to get the glycosylation products without a strong predominance of any of the anomeric forms. We also used per-*O*-acetylated 2-β-thioadamantyl glycoside Kdn **4′** as the reference with higher reactivity and α-stereoselectivity.

The both thioglycosides were prepared from peracetylated methyl ester Neu5Ac **1** (Kdn is not commercially available on a preparative scale). Oxidative deamination of **1** using the modified Zbiral procedure,[18] that includes nitrosylation with nitrosyl tetrafluoroborate followed by treatment with sodium isoprop-



Scheme 1 *Reagents and conditions*: i, $NO^+BF_4^-$, Py, CH_2Cl_2, –10 °C, 3 h; ii, CF_3CH_2OH, CH_2Cl_2, $NaOPr^i$, –10 °C; 2 min; iii, AcOH, –10 °C, 5 min; iv, RSH (R = Ph or 1-adamantyl), $BF_3 \cdot OEt_2$, CH_2Cl_2, 24 h.

oxide and then acetic acid, gave peracetylated methyl ester Kdn **3** with rather a modest yield (37% *vs.* 51% declared in literature[11]). Conversion of **3** into the desired thioglycosides was achieved by the well-established procedure involving the treatment with the corresponding thiol (PhSH or AdaSH) in the presence of boron trifluoride etherate.[20] The thioglycosides were obtained in excellent yields as separable mixtures of the two anomers; after chromatographic purification, the prevalent β-anomers β-**4** and β-**4′** were used in glycosylation (Scheme 1).

With these donors in hands, we processed glycosylation of the lactosamine acceptors; the results obtained are summarized in Table 1. First of all, we made sure that PhSCl/AgOTf promoting system (successfully used for the preparation of thermodynamically unfavorable equatorial glycosides) did not activate 2-β-thiopenyl glycoside **4** in CH_2Cl_2 at –78 °C (entry 1). This is consistent with the report of the Crich's group that they did not observe activation of the 5-*N*,4-*O*-oxazolidinone-2-β-thiopenyl Neu5Ac glycoside in CH_2Cl_2 at –78 °C.[20] On the other hand, the same donor under the same conditions was capable of glycosylation of a primary galactosyl acceptor when *p*-$O_2NC_6H_4SCl$ was used as the promoter;[10] or the analogous *S*-tolyl donor in conjunction with TolSCl/AgOTf also glycosylated galactosyl acceptors in CH_2Cl_2/MeCN (2 : 1) at –78 °C with excellent yields and α-stereoselectivity.[21] All of this once again demonstrates that even closely related promoters such as sulfenyliums may differ dramatically in their ability to activate donors and that application of the same activation approach to different sialylation processes may lead to failure.

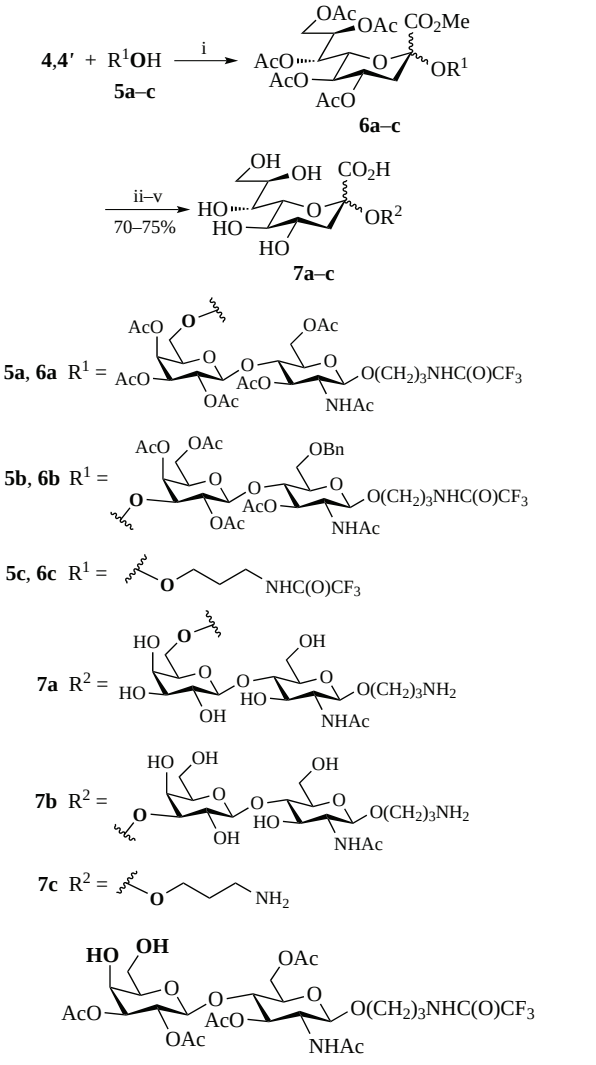
The further experiments were conducted with the milder promoting system, NIS/TfOH, in CH_2Cl_2 at –40 °C. The choice of solvent was determined by the data that sialylation with this promoter, carried out in CH_2Cl_2, gave anomeric products in moderate-to-good yields without pronounced stereoselectivity.[8,11] At the same time, when acetonitrile or its mixtures with CH_2Cl_2 were used as solvents, yield of the α-anomer usually increased due to formation of an axial-oriented nitrilium intermediate.[22,23] Rise in α-stereoselectivity was observed also in certain ethereal solvents (*e.g.*, in 1,2-dimethoxyethane or THF), as well as in their mixtures with CH_2Cl_2.[24,25]

The benchmark sialylation of the lactosamine acceptor **5a** with the single free 6′-OH group using donor **4′** bearing β-adamantylthio leaving group resulted in comparably good yield (72%) and α-stereoselectivity (α/β ~ 7 : 1, see Table 1, entry 2). Replacement of the β-adamantylthio leaving group in the donor to a β-thiophenyl group did not significantly affect the sialylation yield but led to decrease in α-stereoselectivity (α/β ~ 2.5 : 1, entry 3). Sialylation of the less reactive secondary lactosamine acceptor **5b** with 2-β-thiophenyl donor **4** resulted in 57% of the desired (2→3′)-linked product **6b** with only slight prevalence of the α-anomer (α/β ~ 2 : 1, entry 4), *i.e.* no significant difference was observed in outcomes of sialylation between primary and secondary acceptors. For comparison, we also tested lactosamine 4′,6′-diol **5d** as a glycosyl acceptor. Obviously, the synthetic paths both to 3′,4′- or 4′,6′-diol derivatives of lactosamine are shorter than to those with only one free OH-group at C(3) or C(6) in the galactose residue; moreover, the mono-OH acceptors seem to be more sterically hindered. Previously, using the classical Koenigs–Knorr procedure (neuraminic glycosyl chloride as a donor and silver carbonate as a promoter), we successfully sialylated galactopyranosyl diols and triols, obtaining α(2→3)- or α(2→6)-linked disaccharides.[26] Unfortunately, we failed to sialylate diol **5d** with the

Table 1 Sialylation of lactosamine acceptors with Kdn thioglycoside donors.

Entry	Donor	Acceptor	Reagent	Reaction conditions[a]	Product	Yield (%) (α : β ratio)
1	**4**	**5a**	PhCl/AgOTf	–78 °C	NG[b]	–
2	**4′**	**5a**	NIS/TfOH	–40 °C	**6a**	72 (7 : 1)
3	**4**	**5a**	NIS/TfOH	–40 °C	**6a**	70 (2.5 : 1)
4	**4**	**5b**	NIS/TfOH	–40 °C	**6b**	57 (2 : 1)
5	**4**	**5c**	NIS/TfOH	–40 °C	**6c**	83 (10 : 1)
6	**4**	**5d**	NIS/TfOH	–40 °C	NG[b]	–

[a] In CH_2Cl_2/4Å MS. [b] No glycosylation products.



Scheme 2 *Reagents and conditions*: i, NIS/TfOH, CH_2Cl_2, –40 °C (see also Table 1); ii, H_2, Pd/C, MeOH (for **6b**); iii, MeONa, MeOH; iv, NaOH; v, HPLC purification.

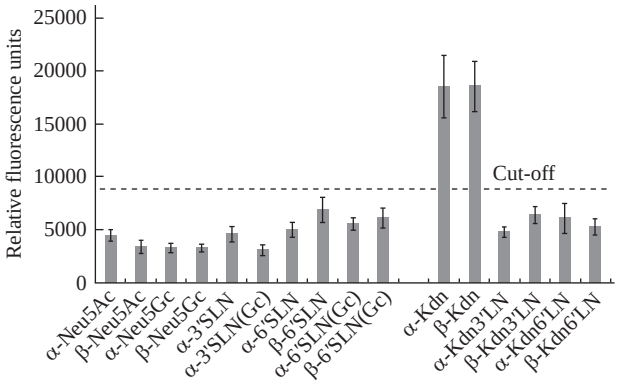


Figure 1 Binding of human serum antibodies with Kdn-glycans on the surface of the glycochip.

2-β-thiophenyl donor **4** when applied conditions used in this study (entry 6); we tend to attribute this fact to the low solubility of **5d** in CH_2Cl_2 at low temperatures. The coupling of 2-β-thiophenyl donor **4** with trifluoroacetamidopropanol **5c** proceeded with a high yield (83%) and a modestly high α-stereselectivity (α/β = 10 : 1, entry 5). Remarkably, the unexpectedly high α-stereoselectivity was observed in the experiments with the primary acceptors **5a** and **5c** (entries 2 and 5) although just standard peracetylated donors **4** and **4′** were used; meanwhile, to increase yield of α-anomer, Crich *et al.* applied the conformationally rigid Kdn donor with the *N*-acetyloxazolidinone ring.[8,11]

Sialooligosaccharides were isolated as individual anomers, after deprotection (see Online Supplementary Materials for details), their anomeric stereochemistry was determined based on chemical shifts and coupling constants for sialic acid H(3) protons;[27,28] the used acceptors were prepared as described in our previous works.[26,29] In the sialylation reaction, only a trace amount of per-*O*-acetylated Kdn glycal is formed.

The synthesized Kdn trisaccharides, α- and β-spacered monosaccharide, as well as about twenty derivatives of Neu5Ac and Neu5Gc were printed as a microchip as described earlier,[30] and using this chip the antibodies of the donor blood serum were examined. In detail, the results will be published separately and can be summarized as follows. Antibodies in any significant amount to Kdn-trisaccharides (as α-, and β-anomers) are not detected in healthy donors, but antibodies to β-anomer of the monosaccharide Kdn are present; this is the evidence in favor of our assumption that the previously observed antibodies to β-derivatives of *N*-acetylneuraminic acid actually appeared in response to Kdn glycoconjugates, bacterial antigens (Figure 1).

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.07.017.

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