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Stereo- and regioselective synthesis of spacer armed α2-6 sialooligosaccharides

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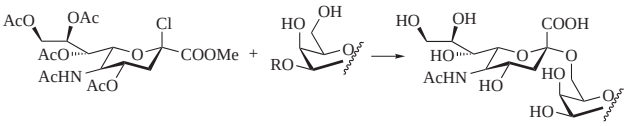
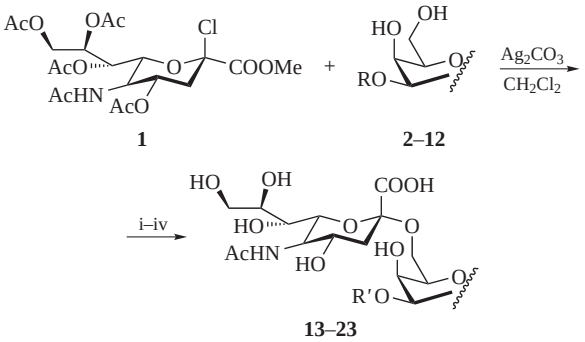
A simple protocol for the preparation of α2-6 sialooligosaccharides including SiaTn, SiaTF, 6′SL and 6′SLN in moderate yields involves room temperature glycosylation of 4,6-diol acceptors with routine sialic donor and one step isolation.



α2-6 Sialylglycans are known as receptors for siglecs and targets for human influenza viruses. Innate response to sialylglycans is a new area of glycobiologists interest.[1,2] Great progress in development of glycoarrays[3,4] requires maximal variety of sialylglycans as the array ligands. As a consequence, reliable routine protocols for their synthesis readily reproducible by chemists without previous experience in the carbohydrate area are in demand. Previously, we described a method using the conditions of the Koenigs–Knorr reaction[5] with chloride of acetylated *N*-acetylneuraminic acid methyl ester **1** as a glycosyl donor and similar more sophisticated donors, for example, acetylated chlorides of 9-deoxy-9-NAcNeu5Ac, Neu5Gc, Neu5Acα2-8Neu5Ac and Neu5Acα2-8Neu5Acα2-8Neu5Ac.[6] The significant advantages of this method include simple preparation of glycosyl donors and room temperature sialylation providing good yield and α-stereospecificity, which are at least the same or higher than reported[7–11] when more complicated sialyl donors and/or lower temperature (–40 to –70 °C) were used. Herein, we report on our experience in glycosylation with donor **1**, circumscribe available glycosyl acceptors, and describe a simplified general protocol for the isolation of target sialylglycans in form of aminoalkyl glycosides (Table 1).

Sialylation of acceptors **2**–**12**† with neuraminic acid ester chloride **1**[18] was performed in the presence of silver carbonate[19] according to general procedure.‡ When obtaining SiaTn (**14**), 6′SLN (**16**), Sia6′Le^c (**18**) and Sia6′LN3′LN (**23**), in contrast to other sialylglycans, products of glycosylation were isolated using chromatography on silica gel followed by removal of the protecting groups. When regeneration of unreacted glycosyl acceptor is not required, all components of the reaction mixture



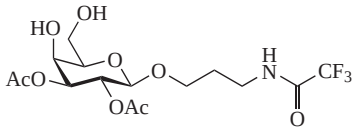
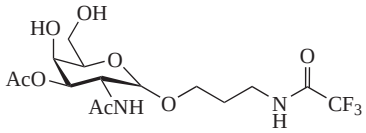
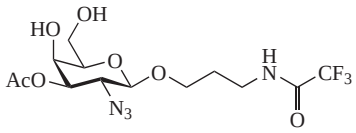
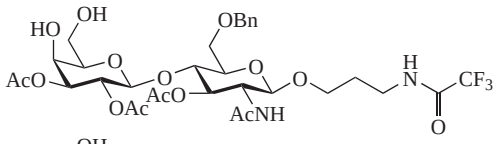
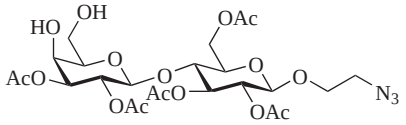
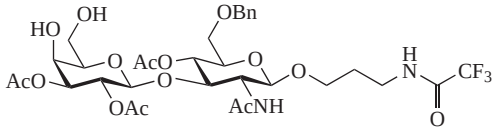
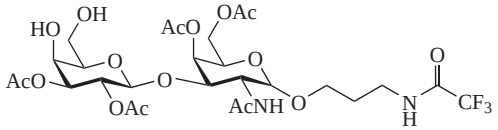
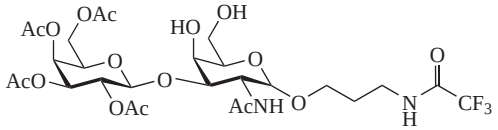
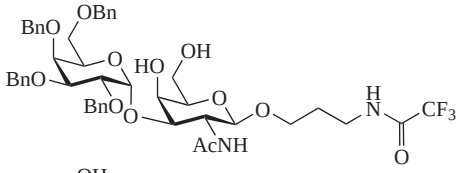
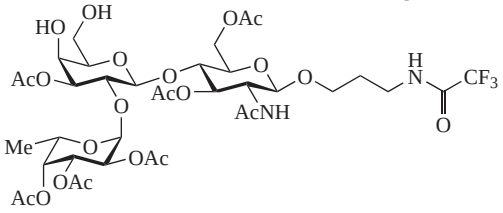
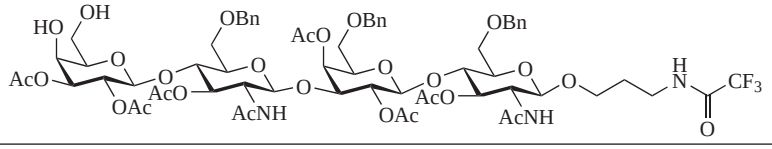
Scheme 1 *Reagents and conditions*: i, 10% Pd/C, H_2, MeOH; ii. 0.1 M MeONa/MeOH, 30 min, room temperature, then 0.1 M aq. NaOH, 16 h; iii, 10% Pd/C, H_2, MeOH/H_2O (1:1) for **6**; iv, Dowex H^+: elution with 1 M aq. pyridine.

† Syntheses of acceptors **2**–**12** were described earlier.[12–17]

‡ A solution of neuraminic acid ester chloride **1**[18] (0.6 mmol) in dry CH_2Cl_2 (3 ml) was added to the mixture of an acceptor (0.2 mmol), Ag_2CO_3[19] (1.8 mmol), freshly activated molecular sieves 4 Å (1 g) and dry CH_2Cl_2 (7 ml) and the formed suspension was vigorously stirred in the dark at room temperature in tightly closed flask for 7 days. Then the reaction mixture was filtered, the solids were washed with $CHCl_3$–MeOH (1:1, 5×20 ml), and the combined filtrates were concentrated *in vacuo*. The residue was subjected to chromatography on silica gel or deprotected without the chromatography step.

In case of the presence of benzyl and/or azide groups in acceptor, hydrogenolysis step was additionally performed. The residue was dissolved in MeOH (20 ml), 10% Pd/C (600 mg) was added, and the mixture was stirred under H_2 (1 atm) at ambient temperature for 16 h (in case of 2-deoxy-2-azido sugars, Ac_2O was added to the reaction mixture). The catalyst was filtered off, washed with MeOH (3×10 ml), and the combined filtrates were concentrated to dryness with co-evaporation of toluene. The residue was dissolved in dry MeOH (6 ml) and 2 M MeONa/MeOH (0.3 ml) was added. The mixture was kept for 30 min at room temperature and evaporated followed by addition of 6 ml H_2O [in case of azide group in spacer, instead of basic treatment hydrogenation was carried out in H_2O–MeOH (1:1)]. After 10–15 h (room temperature) the volatiles were evaporated, the residue was dissolved in 2 ml of water, and the solution was applied on Dowex 50×4–400 (H^+) ion-exchange resin column (1.5×6 cm). The resin was washed sequentially with water (50 ml), 1 M aq. pyridine (50 ml) and 1 M aq. NH_3 (50 ml). Elution with H_2O gave the glycal (an acid); the product (an amino acid) was retained on the column and was completely eluted with 1 M aqueous pyridine; the non-reacted glycosyl acceptor (an amine) was eluted with 1 M aq. NH_3. The pyridine fraction was evaporated and subjected to ion-exchange chromatography on DEAE SephadexA-25 (AcO^- form; elution with 0.01 M aq. pyridine–AcOH, pH 6.5). The pure anomers were obtained by low-pressure chromatography of the protected sialosides on silica gel. Alternatively, unpurified material was deprotected, and the individual α-anomer was separated by HPLC chromatography on reversed-phased C18 silica gel (Phenomenex Luna, 21.2×250 mm, 5 μm, pore size 100 Å) by elution with water (10.0 ml min^{-1}, 30 °C).

Table 1 Sialylation of 4,6-diols.

	Glycosyl acceptor	Final product (trivial name)	Yield[a] α (%) (α:β)[b]
2		Neu5Acα2-6Galβ-O(CH$_2$)$_3$NH$_2$ **13**	64 (45:1)
3		Neu5Acα2-6GalNAcα-O(CH$_2$)$_3$NH$_2$ **14** (SiaTn)	65[c] (25:1)
4		Neu5Acα2-6GalNAcβ-O(CH$_2$)$_3$NH$_2$ **15**	58 (19:1)
5		Neu5Acα2-6Galβ1-4GlcNAcβ-O(CH$_2$)$_3$NH$_2$ **16** (6′SLN)	60[c] (34:1)
6		Neu5Acα2-6Galβ1-4Glcβ-O(CH$_2$)$_2$NH$_2$ **17** (6′SL)	69 (35:1)
7		Neu5Acα2-6Galβ1-3GlcNAcβ-O(CH$_2$)$_3$NH$_2$ **18**	54[d,21]
8		Neu5Acα2-6Galβ1-3GalNAcα-O(CH$_2$)$_3$NH$_2$ **19**	45 (8:1)
9		Neu5Acα2-6(Galβ1-3)GalNAcα-O(CH$_2$)$_3$NH$_2$ **20** (SiaTF)	25 (2:1)
10		Neu5Acα2-6(Galα1-3)GalNAcα-O(CH$_2$)$_3$NH$_2$ **21**	28 (1.6:1)
11		Neu5Acα2-6(Fucα1-2)Galβ1-4GlcNAcβ-O(CH$_2$)$_3$NH$_2$ **22**	34 (23:1)
12		Neu5Acα2-6Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ-O(CH$_2$)$_3$NH$_2$ **23**	55[d]

[a]Yields of the target products are calculated based on the glycosyl acceptor. [b]α:β ratios are given for isolated products. [c]For gram scale. [d]β-anomer was not detected.

are deprotected followed by isolation of the required sialylglycan using simple cation-exchange chromatography. This procedure was used for obtaining compounds **13**, **15**, **17**, and **19**–**22**.

The 2-6-sialylation appeared to be well reproducible[20] and scalable. Gram amounts of SiaTn (**14**) and 6′SLN (**16**) derivatives were obtained without dropping the yield; in case of 4,6-diol acceptors without bulky substituent at 2- and 3-positions the yields were 45–69% along with good stereoselectivity.

The structure of all synthesized compounds was confirmed by high resolution ^{1}H NMR spectroscopy and mass spectrometry

data. Spectra of derivatives **14**, **16**, **18** coincided with those previously reported.[21–23] Chemical shifts of H-3_{eq} at 2.6–2.7 ppm for α-anomers and 2.2–2.4 ppm for β-anomers are consistent with the literature data.[7] The structure of pentasaccharide **23** was also corroborated by completely assigned ^{1}H NMR spectrum of its peracetylated derivative **23a**.

Evaluation of obtained results enables us to formulate the following rules in selection of glycosyl acceptor for donor **1**.

i) Derivatives with single 6-positioned OH group are poor acceptors for **1** under the selected conditions.

ii) The high α-specificity and acceptable yields (> 55%) have been achieved with 4,6-diols of monosaccharides like Galβ, GalNAcα, GalNAcβ, as well as with oligosaccharides containing terminal 4,6-$(OH)_2$-Gal diol motif.§

iii) The presence of the carbohydrate substituent at O^3 or O^2 of 4,6-$(OH)_2$-Gal moiety does not hinder glycosylation, however, the conversion and stereospecificity in these cases are lower.

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Online Supplementary Materials

Supplementary data associated with this article (^{1}H NMR and MALDI-TOF mass spectra of the synthesized key compounds) can be found in the online version at doi:10.1016/j.mencom.2016.09.004.

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§ This type of diols is easily accessible by deprotection of the corresponding 4,6-benzylidene derivatives.

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