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Simple stereoselective synthesis of α2-6 sialooligosaccharides

Galina Pazynina, Alexander Tuzikov, Alexander Chinarev, Polina Obukhova and Nicolai Bovin*

Shemyakin & Ovchinnikov Institute of Bioorganic Chemistry, 16/10 Miklukho-Maklaya str., Moscow 117997, Russian Federation

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Abstract—The sialylation approach reported by A. Khorlin et. al 30 years ago has been modified into a highly practical procedure for stereoselective α2-6 sialylation of galactopyranose 4,6-diols.

Glycoprotein and glycolipid carbohydrate chains terminated by sialic acids play an exceptional role in cell recognition and cross-talk, cell-pathogen interactions, immune response and in other cellular events. For example, they might act as 'self' ligands that interact with siglecs on mieloid cells, thereby preventing inappropriate self-reactivity.[1] An approach to the systematic understanding of structure–function relationships of sialooligosaccharides requires efficient synthetic methods for regio- and stereoselective sialylation. However, the presence of an electron-withdrawing carboxy group as well as the lack of a co-participating substituent adjacent to the anomeric centre of the sialic acid, often results in low stereoselectivity of sialylation (especially when a primary hydroxyl group is used as the glycosyl acceptor),[2] and in a high rate of concurrent 2,3-elimination in the sialyl donor.

The first synthesis of oligosaccharides containing the α-*N*-acetylneuraminic (Neu5Ac) residue was described by Khorlin et al. about three decades ago. In the cited work, chloride **1** was used as the glycosyl donor, silver carbonate was chosen as the promoter-catalyst; the yields of α2-6-sialodisaccharides reached 18%.[3] Later, the search for effective sialylation methods was focused on the modification of the promoter-catalyst, the glycosyl donor, and the reaction conditions. So, the donor **1** was used with silver silicate, silver salicylate and silver carbonate/triflate; thioglycoside donors **2** in combination with various electrophilic promoters were also studied.[4] An additional group (X = Cl, Br or SeR, see **2**, Fig. 1) was introduced at C-3 for co-participation in glycosylation, which gave an improvement of the yield and selectivity, but such groups had to be removed after the reaction.[5] Extra acylation of the 5-*N*-acetyl group or its substitution by N_3 (see **3**, Fig. 1) increased the efficiency of glycosylation.[2,6,7] A significant rise in α-stereoselectivity was observed for donor **4** with the acetoacetoxy groups at C-4 and C-9.[7] Although these studies improved the yield and/or α-stereoselectivity of sialylation, these advantages were nullified by the multistep synthesis of the complicated Neu5Ac-donors and by the need for elimination of additional substituents.

We reinvestigated the synthesis of α2-6-sialooligosaccharides under the classical Koenigs–Knorr conditions used by Khorlin et al., e.g. Ag_2CO_3-promoted glycosylation with the readily prepared donor **1**. In contrast to the cited work, where acceptors with one free hydroxyl group at C-6 were used, we glycosylated less hindered 4,6-diols, namely, the mono- and disaccharide acceptors **5**–**9** with two free hydroxyl groups (Scheme 1). Monosaccharide **10** with three unprotected hydroxyls was also sialylated (Scheme 2).[†] A typical procedure for

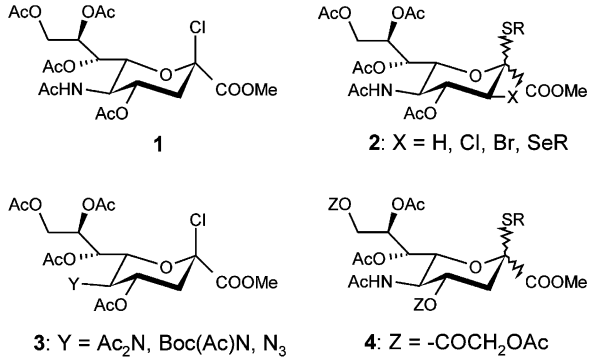

Figure 1. Sialyl donors.[2–7]

Keywords: oligosaccharides; sialic acids; sialylation.

* Corresponding author. Tel.: +7-095-330-7138; fax: +7-095-330-5592; e-mail: bovin@carb.siobc.ras.ru

† Syntheses of acceptors **5**–**10** were described earlier;[7,11,12] synthesis of the glycosyl chloride **1** was performed according to the simple one-pot procedure.[13]

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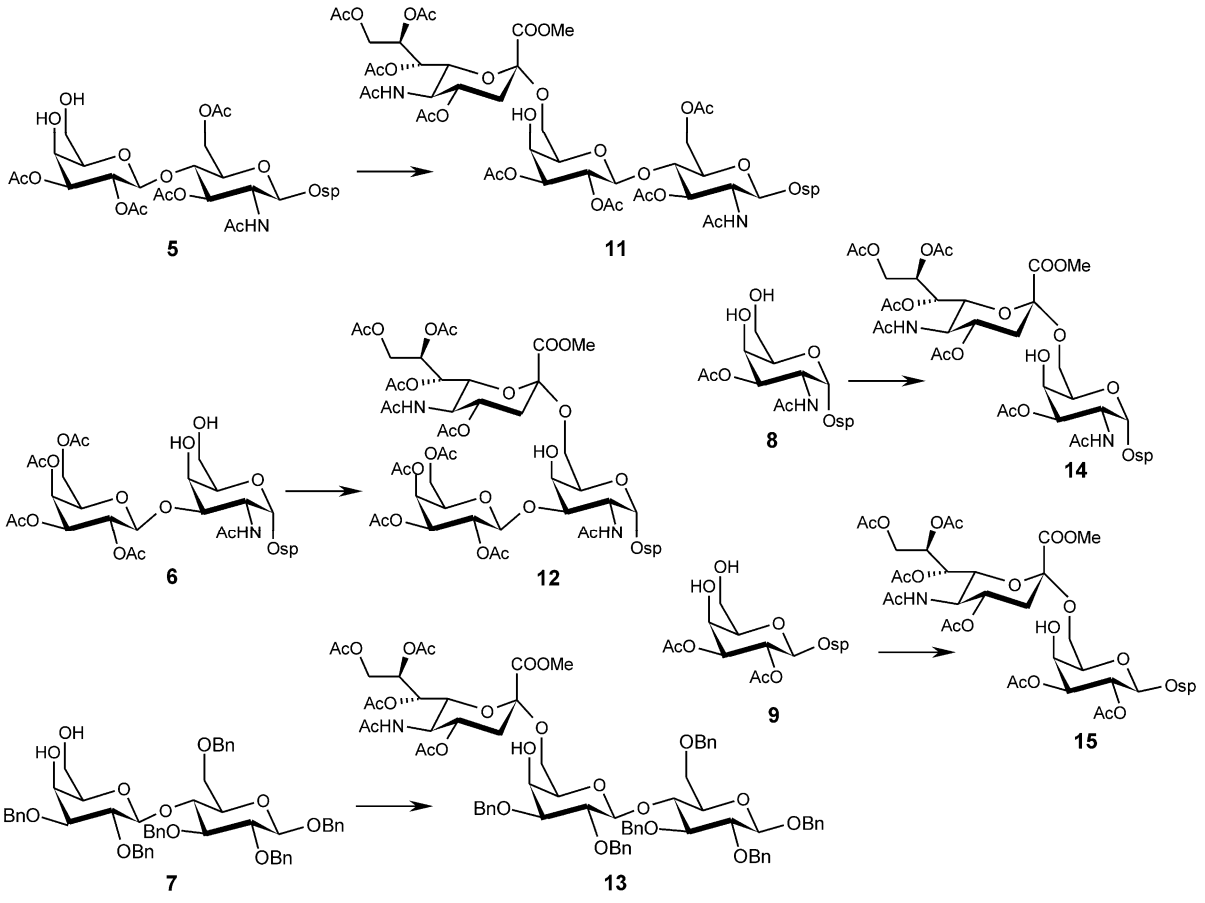
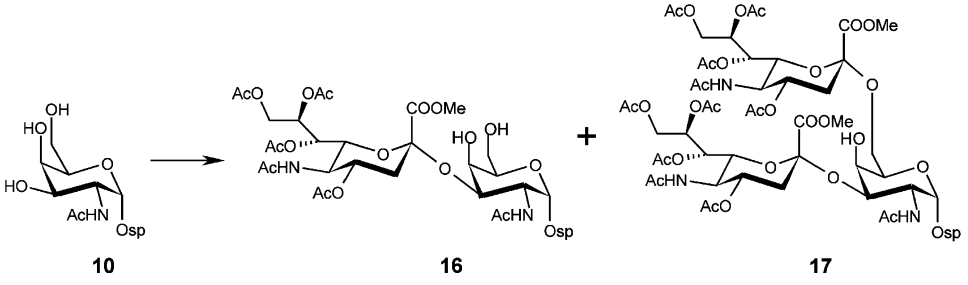


Scheme 1. Sialylation of mono- and disaccharide diols with **1** under the Koenigs–Knorr conditions, sp = $(CH_2)_3NHCOCF_3$.



Scheme 2. Unusual sialylation of triol **10** with **1** under the Koenigs–Knorr conditions, sp = $(CH_2)_3NHCOCF_3$.

sialylation was as follows. To a mixture of acceptor **5**–**10** (0.1 mmol), Ag_2CO_3 (0.7 mmol), and molecular sieves 4 Å (1 g) in CH_2Cl_2 or $ClCH_2CH_2Cl$ (5 mL) was added a solution of the glycosyl chloride **1** (0.3 mmol) in the same solvent (5 mL) (for details see Table 1). The reaction mixture was stirred at room temperature with

Table 1. Sialylation of saccharides under the Koenigs–Knorr conditions (Schemes 1–2)

Glycosyl acceptor	Product	Solvent	Time (days)	Yield of the α-anomer (%)[a]	α/β anomer ratio	Recovery of the acceptor (%)
5	**11**	CH_2Cl_2	3	95	β in trace	10
6	**12**	CH_2Cl_2	7	84	6/1	50
7	**13**	CH_2Cl_2	7	90	β in trace	25
8	**14**	$ClCH_2CH_2Cl$	5	88	25/1	20
9	**15**	CH_2Cl_2	3	82	β in trace	10
10	**16**	$ClCH_2CH_2Cl$	7	48	β in trace	10
	17			41		

[a] Yields of α-sialooligosaccarides **11**–**17** are given based on the glycosyl acceptor reacted.

TLC monitoring, then filtered, the solids were washed with $CHCl_3$/MeOH 10/1, and the combined filtrate was concentrated in vacuo. The residue was subjected to chromatography on silica gel. The ^1H NMR spectra of the sialooligosaccharides **11**, **12**, **14** obtained are identical to those described earlier.[8–10] The structures of **13**, **15**, **16** and **17** were confirmed by ^1H NMR; the spectra are available as supplementary material.

Glycosylation of acceptors **5**–**9** with an excess of donor **1** in the presence of silver carbonate in CH_2Cl_2 or $ClCH_2CH_2Cl$ gave rise to α2-6 sialooligosaccharides in 85–95% yields. A significant prevalence of the α-anomer was observed; in some cases the β-anomer was detected in only trace amounts. The results obtained are summarised in Table 1.

The stereoselectivity of sialylation and the degree of acceptor conversion depend on the nature of the solvent (CH_2Cl_2 or $ClCH_2CH_2Cl$), which should be chosen so as to provide acceptor solubility. The combination of silver carbonate with silver triflate as a co-promoter accelerates the sialylation, but, at the same time, increases the yields of the β-anomer and the glycal.

The results of sialylation of triol **10** appeared to be rather unexpected (Scheme 2). Earlier, upon glycosylation of this compound by the glycosyl bromides of Gal, GlcNAc, and Galβ1-4GlcNAc,[11] the conventional reactivity of the galactopyranose hydroxyl groups was observed, i.e. the substitution occurred at the primary 6-OH group. However, glycosylation with donor **1** resulted in two products, 3-*O*-sialylated **16** and 3,6-di-*O*-sialylated **17**, while none of the expected 6-*O*-sialylated product was isolated. The observed elevated reactivity of the 3-OH group, might be explained by participation of the acetamido group at C-2 of the galactose moiety. Attempts to sialylate the galactose derivatives with a OAc or OH group at C-2 instead of NHAc did not give rise to the 3-*O*-sialoside. The six saccharides tested as glycosyl acceptors, either with one hydroxyl group at C-3, or with two OH groups at C-2 and C-3, or C-3 and C-4, or with three at C-2, C-3 and C-4 of the galactose yielded no 3-*O*-sialylated product. Thus, except in one case, the silver carbonate-promoted sialylation of saccharides with the donor **1** occurs at the primary 6-OH group.

A comparison of the results presented here with the data on α2-6 sialylation[2–10] with more complex glycosyl donors demonstrates that, being more convenient, Koenigs–Knorr glycosylation is also rather effective with respect to the yield and stereoselectivity. Another advantage is that Ag_2CO_3-promoted sialylation does not destroy the acetamido group in the glycosyl acceptor as documented for glycosylation with Neu5Ac thioglycosides.[12] As to generality of this method, it should be mentioned that the synthesis of an oligosaccharide containing *N*-glycolylneuraminic acid has been successfully performed.‡ The only drawback of the heterogeneous reaction described is that in some cases a part of a glycosyl acceptor remains unreacted; however, the unreacted substance is recovered quantitatively.

Being highly practical, the approach presented gives hope that the synthesis of 6-sialooligosaccharides indispensable for glycobiological investigations, will become routine.

Acknowledgements

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References

1. Crocker, P.; Varki, A. *Immunology* **2001**, *203*, 137–145.
2. Yu, C.-S.; Niikura, K.; Lin, C.-C.; Wong, C.-H. *Angew. Chem., Int. Ed.* **2001**, *40*, 2900–2903.
3. Khorlin, A. Ya.; Privalova, I. M.; Bystrova, I. B. *Carbohydr. Res.* **1971**, *19*, 272–275.
4. Hasegawa, A.; Nagahama, T.; Ohki, H.; Hotta, K.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1991**, *10*, 493–498.
5. Nakahara, Y.; Iijima, H.; Ogawa, T. In *Synthetic Oligosaccharides*; Kovac, P., Ed.; ACS: Washington, DC, 1994; Vol. 560, pp. 2833–2840.
6. Demchenko, A. V.; Boons, G.-J. *Chem. Eur. J.* **1999**, *5*, 1278–1283.
7. Simeoni, L. A.; Byramova, N. E.; Bovin, N. V. *Russ. J. Bioorg. Chem.* **2000**, *26*, 206–214.
8. Sherman, A. A.; Yudina, O. N.; Shashkov, A. S.; Men'shov, V. M.; Nifant'ev, N. E. *Carbohydr. Res.* **2001**, *330*, 445–458.
9. Simeoni, L. A.; Byramova, N. E.; Bovin, N. V. *Russ. J. Bioorg. Chem.* **1999**, *25*, 55–61.
10. Simeoni, L. A.; Byramova, N. E.; Bovin, N. V. *Russ. J. Bioorg. Chem.* **1997**, *23*, 683–691.
11. Pazynina, G. V.; Bovin, N. V. *Mendeleev Commun.* **2000**, *4*, 132–133.
12. Nifant'ev, N. E.; Tsvetkov, Y. E.; Shashkov, A. S.; Kononov, L. O.; Menshov, V. M.; Tuzikov, A. B.; Bovin, N. V. *J. Carbohydr. Chem.* **1996**, *15*, 939–953.
13. Byramova, N. E.; Tuzikov, A. B.; Bovin, N. V. *Carbohydrate Res.* **1992**, *237*, 161–175.

‡ Glycosylation of **8** with *N*-glycolyl analog of chloride **1** gave rise to 76% yield of α-disaccharide, α/β = 6:1.