Enhanced Sialylating Activity of O-Chloroacetylated 2-Thioethyl Sialosides

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Dedicated to Professor Richard R. Schmidt on the occasion of his 70th birthday

Abstract: It has been shown that *O*-chloroacetyl protecting groups enhance significantly sialylating activity of 2-thioethyl sialosides in model reactions of α(2-8)-sialylation. Ethyl 4,7,8,9-tetra-*O*-chloroacetyl-3,5-dideoxy-2-thio-5-trifluoroacetamido-D-glycero-α-D-galacto-non-2-ulopyranoside that combines electron-withdrawing *O*-chloroacetyl and *N*-trifluoroacetyl protecting groups displayed the best reactivity and enabled the most efficient synthesis of the Neu5Acα(2-8)Neu5Ac dimer. The GD_3 tetrasaccharide was synthesized in stepwise manner using this sialyl donor in the key (2-8)-coupling, albeit the yield was noticeably lower than in the model sialylation of monosaccharide acceptors.

Key words: chloroacetyl protecting group, 2-thioethyl sialoside, glycosylation, reactivity, GD_3 oligosaccharide

The disaccharide sequence Neu5Acα(2-8)Neu5Ac is a constituent of a number of gangliosides such as GD_3, GT_{1a}, GQ_{1b}, and others, and plays a key role in their biological activities.[1] The simplest representative of this group, the ganglioside GD_3 (**1**), was shown to be the human melanoma associated antigen (Figure 1).[2]

Neu5Ac-α-(2-8)-Neu5Ac-α-(2-3)-Gal-β-(1-4)-Glc-β-O-Ceramid

1

Figure 1

Chemical preparation of the Neu5Acα(2-8)Neu5Ac dimer is strongly complicated by the low reactivity of the 8-OH group of neuraminic acid and remains so far one of the main problems of the oligosaccharide synthesis. The most of successful syntheses were based on application of modified sialyl donors bearing an additional function at C-3 which can control the stereochemistry of substitution at the anomeric center and prevent 2,3-elimination.[3] Multistep synthetic procedures are required for preparation of these donors and an additional step is needed for removal of the auxiliary from the sialylation product.[4] An alternative strategy[5] consisting in the use of the Neu5Acα(2-8)Neu5Ac dimer obtained by mild acid hydrolysis of colominic acid[6] circumvents the problems of the chemical synthesis of the α(2-8)-linkage but is limited by low availability of the starting material. Recently, a promising approach was reported[7] that employed N-trifluoroacetylated derivatives of neuraminic acid as both sialyl donor and acceptor. The improved glycosyl donor properties of di-*N*-acetyl[8] and *N*-trifluoroacetyl (*N*-TFA) derivatives of neuraminic acid were explained by minimization of side reactions at the *N*-acyl moieties due to reducing their nucleophilicity.[7,9]

We supposed that electron-withdrawing N-protecting groups are able also to increase the positive charge on C-2 of all cationic species (such as sialyl or sialyl nitrilium[10] cations) involved in sialylation and increase thereby their reactivity towards nucleophiles. If this is the case, electron-withdrawing O-protecting group would produce the same effect on the reactivity of sialyl donors. Here we describe the effect of *O*-chloroacetyl protecting groups in 2-thioethyl α-sialosides on their efficiency as sialyl donors in α(2-8)-sialylation. *O*-Chloroacetyl groups can be easily introduced, are stable under sialylation conditions, and can be removed selectively, if necessary, in the presence of other acyl protections.

To facilitate NMR analysis of compounds, α-thioethyl sialoside **3** but not a more readily available α,β-mixture[3] was chosen as a starting material for the preparation of *O*-chloroacetylated sialyl donors. α-Thiosialosides can be obtained more easily in anomerically pure form than the corresponding β-anomers. Compound **3** was synthesized from chloride **2**[11] according to the modified published procedure[12] followed by deacetylation in 79% yield (Scheme 1). Since fully acetylated derivatives of Neu5Ac are conventional sialyl donors, tetraacetate **4a** was chosen as a reference point. Its chloroacetylated analog **4b** was prepared from **3** by reaction with chloroacetic anhydride in the presence of $NaHCO_3$.[13] *O*-Acetyl and *O*-chloroacetyl *N*-TFA thioethyl sialoside **7a** and **7b**[14] were synthesized from **3** by acidic de-N-acetylation[15] followed by N-trifluoroacetylation of the amino group in **5** and O-acylation of **6** formed with Ac_2O or $(ClCH_2CO)_2O$.

7,8-Diol **14** was used as a sialyl acceptor in model sialylation reactions. It could be selectively sialylated at 8-OH because of its much higher reactivity as compared to that of 7-OH.[4c,16] Diol **14** was prepared as follows (Scheme 2). The starting α-benzyl sialoside **8** was synthesized from chloride **2** and benzyl alcohol according to the described procedure[17] followed by de-O-acetylation. Treatment of **8** with 2,2-dimethoxypropane and CSA in acetone afforded 8,9-isopropylidene derivative **9** which was selectively benzoylated at O-4 with benzoyl chloride to give monobenzoate **10**. Removal of the isopropylidene group followed by treatment of triol **11** with α,α-dimethoxytoluene in the presence of CSA yielded a mixture of 7,9- and

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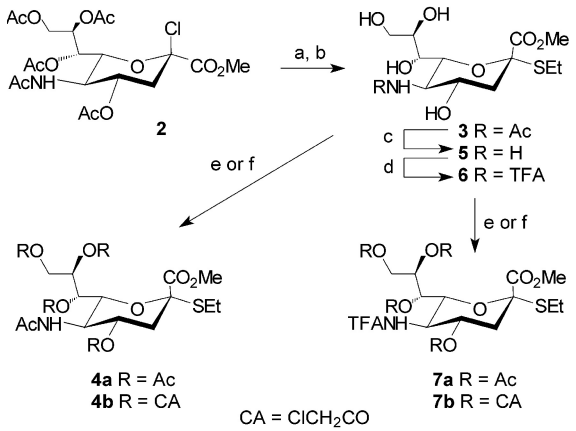
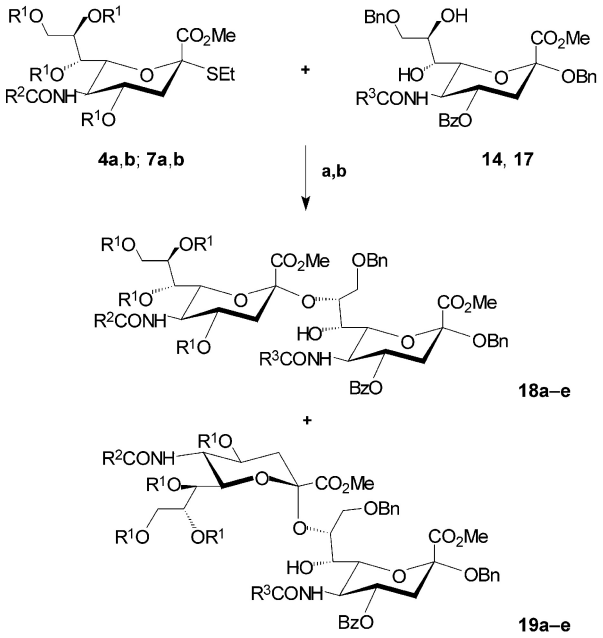


Scheme 1 *Reagents and conditions*: a) EtSNa, MeCN, r.t.; b) MeONa, MeOH, r.t., 79% over two steps; c) MsOH, MeOH, reflux; d) Et_3N, CF_3CO_2Et, MeOH, r.t., 84% over two steps; e) Ac_2O, pyridine, r.t., 96%; f) $(ClCH_2CO)_2O$, $NaHCO_3$, DMF, r.t., 84% for **4b**, 93% for **7b**.

8,9-benzylidene derivatives **12** and **13** in a ratio of about 1:1. This mixture was subjected, without separation of the isomers, to reductive acetal ring-opening with $H_3B{\cdot}NMe_3$–$AlCl_3$ in the presence of water[18] to give 7,8-diol **14** in high yield.

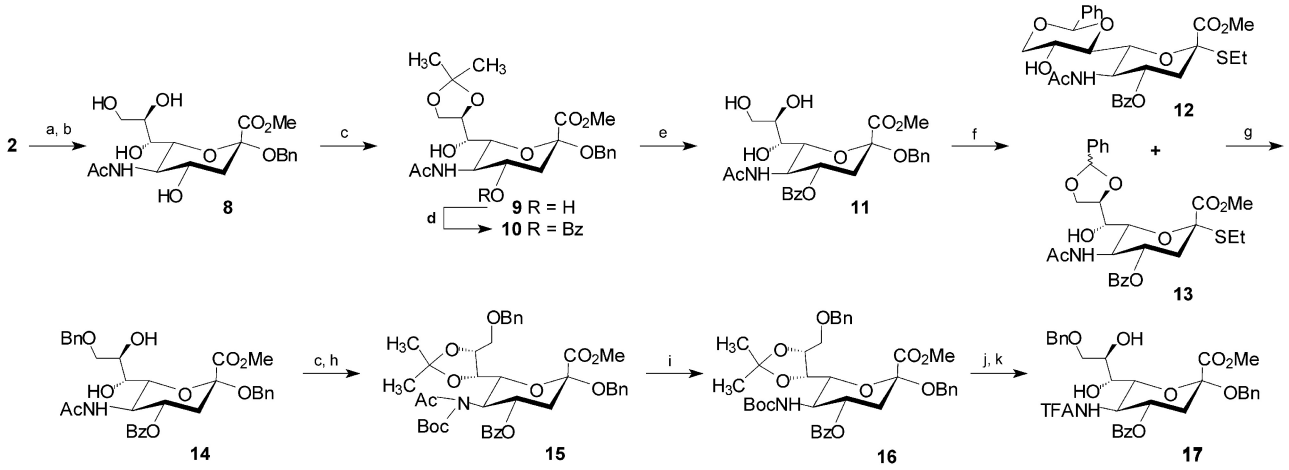
For comparison of the sialyl acceptor properties of **14** and its *N*-TFA counterpart (cf. ref.[7]), diol **17** was also prepared from **14** using the procedure of de-N-acylation of secondary amides via intermediate *N*-Boc-imide formation[19] (Scheme 2). Diol **14** was converted into 7,8-*O*-isopropylidene derivative which was treated, without isolation, with Boc_2O in the presence of DMAP to give imide **15**. Selective removal of the *N*-acetyl group in **15** with hydrazine hydrate afforded Boc-protected amine **16**. Simultaneous cleavage of acid-labile isopropylidene and Boc groups followed by N-trifluoroacetylation yielded *N*-TFA



Scheme 3 *Reagents and conditions*: a) NIS, TfOH, MS 3 Å, MeCN, –40 °C; b) $(H_2N)_2CS$, MeOH–HOAc (3:1), 7–10 min, r.t.

derivative **17**. The transformation **14** → **17** could be accomplished without chromatographic purification of the intermediate compounds and afforded diol **17** in 85–90% overall yield.

Then NIS/TfOH-promoted sialylation of acceptors **14** and **17** with two equivalents of thioglycosides **4a**,**b** and **7a**,**b** was examined[20] (Scheme 3). As we reported earlier,[21] glycosylation of substrates containing acetamido groups with ethyl thioglycosides can be accompanied by the transfer of SEt residue onto these groups resulting in the formation of EtS(Ac)N-derivatives. This side reaction strongly complicated analysis and separation of reaction mixtures and



Scheme 2 *Reagents and conditions*: a) BnOH, Ag_2CO_3, $AgClO_4$, MS 4 Å, CH_2Cl_2, r.t.; b) MeONa, MeOH, r.t., 90% over two steps; c) $Me_2C(OMe)_2$, CSA, acetone, r.t., 94% for **9**; d) BzCl, pyridine–CH_2Cl_2, 0 °C, 88%; e) 80% aq HOAc, 40° C, 94%; f) $PhCH(OMe)_2$, CSA, MeCN, r.t.; g) $H_3B{\cdot}NMe_3$, $AlCl_3$, H_2O, THF, r.t., 81% over two steps; h) Boc_2O, DMAP, THF, reflux, 97% over two steps; i) $N_2H_4{\cdot}H_2O$, DMF, r.t., 95%; j) 90% aq CF_3COOH, CH_2Cl_2, r.t.; k) $CF_3COOC_6F_5$, Et_3N, CH_2Cl_2, r.t., 91% over two steps.

decreased the yields of target products. It was found that a brief treatment with thiourea in a mixture of MeOH–HOAc[22] caused complete conversion of EtS(Ac)N moiety into the parent acetamide, whereas chloroacetyl groups remained intact under these conditions. To provide a reliable comparison of results of the model reactions, sialylation reaction mixtures were subjected to the above procedure. The disaccharide products were separated from unreacted sialyl acceptors and monosaccharide by-products by gel permeation chromatography. Pure anomers **18** and **19** were isolated by HPLC. The results are summarized in Table 1.

As expected, acetylated donor **4a** gave low yield of the corresponding α(2-8)-linked product **18a** (entry 1). Replacement of *O*-acetyl groups by chloroacetyl ones (donor **4b**, entry 2) increased two-fold the yield of **18**. Essentially the same effect gave rise *N*-TFA group (donor **7a**, entry 3). Combination of *O*-chloroacetyl and *N*-TFA groups (donor **7b**, entry 4) resulted in further increase of the yield of **18**; this yield is comparable with those obtained on sialylation of 3-OH in many galactose derivatives.[3] *N*-TFA sialyl acceptor **17** (entry 5) displayed higher reactivity[7] than the *N*-acetyl counterpart **14** and allowed preparation of α-dimer **18e** in excellent yield of 55%. This is the highest yield of α(2-8)-disaccharide achieved on the use of a non-modified at C-3 sialyl donor. Boons et al. prepared a similar α(2-8)-dimer in the same yield of 55% but with three equivalents of sialyl donor.[7] It is noteworthy that yields of the corresponding β-anomers **19** did not change much with protecting groups variation, and hence, α-stereoselectivity also increased upon accumulation of electron-withdrawing groups in the sialyl donor molecule. The increase of α-stereoselectivity may be attributed to the stabilization of β-configurated sialyl nitrilium intermediates (cf. ref.[23]) due to the *–I* effect of protecting groups.

Structure of disaccharides **18** and **19** was proved by ^{1}H NMR spectroscopy data.[24] Configurations of newly formed sialoside bonds were deduced from HMBC spectra (optimized for a coupling constant of 8 Hz). A strong correlation between C-1′ and H-3′$_{ax}$ indicated *trans*-diaxial orientation of the methoxycarbonyl group and the proton H-3$_{ax}$ and, hence, α-configuration of **18**. The lack of C-1′–H-3′$_{ax}$ correlation in HMBC spectra of **19** proved their β-configuration. The presence of signals for 7-OH in ^{1}H NMR spectra of both **18** and **19** showed that they were (2-8)-linked disaccharides.

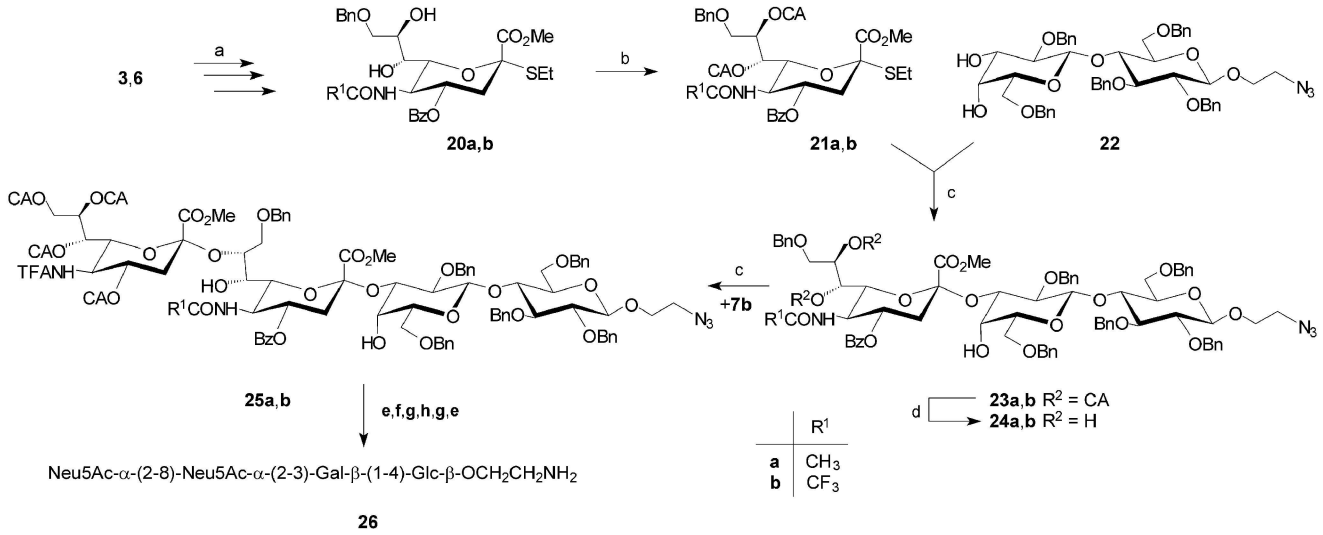
The known empirical rules for the assignment of the anomeric configuration of tetraacetylated Neu5Ac residue[3] were found to be also applicable to *O*-chloroacetyl and *N*-TFA sialosides. Although signals for H-4 of the glycosylating residue in *N*-TFA α-disaccharides **18c**–**e** appeared at δ = 5.0–5.1 (i.e. shifted by ca. 0.2 ppm downfield as compared to signals for H-4 in *N*-acetyl derivatives **18a**,**b**), this region did not overlap that of corresponding β-anomers **19c**–**e** (δ > 5.40 ppm). The difference in Δδ{H9′–H9} values of the glycosylating Neu moiety for α-anomers **18** (0.28–0.37 ppm) and β-anomers **19** (0.45–0.63 ppm) was not as pronounced as in 'conventional' sialosides, but nonetheless allowed reliable configuration assignment within each anomeric pair. Coupling constant values $J_{7,8}$ of the non-reducing Neu residue were also characteristic for its anomeric configuration being 8.0–8.6 Hz for α-anomers **18** and 2.8–4.6 Hz for β-anomers **19**.

We have found some other regularities in ^{1}H NMR spectra of **18** and **19** which may be useful for the assignment of the anomeric configuration in (2-8)-dimers of neuraminic acid. Thus, irrespective of the protecting groups pattern, the signals for H-6 in the glycosylating Neu residue appeared at δ = 3.95–4.15 for α-anomers **18** and at δ = 4.55–4.89 for β ones **19**. The signals for H-8 in the glycosylated Neu residue located in the region of δ = 4.40–4.58 for α-disaccharides **18** and in the region of δ = 4.02–4.07 for β-isomers **19**.

Recently, Schmidt and co-workers[25] described an efficient synthesis of the ganglioside GD_3 (**1**) that was based on the consecutive introduction of two Neu5Ac residues into an oligosaccharide chain. A sialyl donor with 3-SPh participating auxiliary was used in the second sialylation. We applied sialyl donor **7b**, which had displayed the best reactivity in the model (2-8)-sialylations, to a similar preparation of 2-aminoethyl glycoside of the GD_3 tetrasaccharide. To minimize manipulations with protecting groups in the stage of GM_3 trisaccharides, selectively protected sialyl donors were employed in the first coupling step. Their *N*-acetyl and *N*-TFA precursors **20a**,**b** were obtained in good overall yields from tetrols **3** and **6** using the synthetic pathway described above for the transformation **8** → **14** (Scheme 4). Chloroacetylation of **20a**,**b**

Table 1 Effect of O- and N-Protecting Groups on Efficiency of (2-8)-Sialylation

Entry	Donor	Acceptor	Products	R^1	R^2	R^3	Yield of **18** (%)	Yield of **19** (%)
1	**4a**	**14**	**18a**, **19a**	CH_3CO	CH_3	CH_3	8	7
2	**4b**	**14**	**18b**, **19b**	$ClCH_2CO$	CH_3	CH_3	18	9
3	**7a**	**14**	**18c**, **19c**	CH_3CO	CF_3	CH_3	20	7
4	**7b**	**14**	**18d**, **19d**	$ClCH_2CO$	CF_3	CH_3	37	8
5	**7b**	**17**	**18e**, **19e**	$ClCH_2CO$	CF_3	CF_3	55	7



Scheme 4 *Reagents and conditions*: a) see steps c–g in Scheme 1, 69% overall for **20a**, 60% for **20b**; b) $ClCH_2COCl$, pyridine–CH_2Cl_2, 0 °C, 92% for both **21a**,**b**; c) NIS, TfOH, MS 3 Å, MeCN, –40 °C, 62% for **23a**, 72% for **23b**; 15% for **25a**, 17% for **25b**; d) $(H_2N)_2CS$, 2,4,6-collidine, MeOH, reflux, 97% for **24a**, 93% for **24b**; e) 1 M aq NaOH, MeOH, r.t.; f) Ac_2O, MeOH, r.t.; g) H_2, $Pd(OH)_2$/C, MeOH, r.t.; h) CF_3CO_2Et, MeOH, r.t.

afforded sialyl donors **21a**,**b**[26] in which chloroacetates are activating and also selectively removable temporary protecting groups. NIS/TfOH-promoted sialylation of lactoside **22**[27] with small excess (1.2 equiv) of **21a**,**b** gave protected GM_3 trisaccharides **23a**,**b**[28] in yields of 62% and 72%, respectively. Only minute amounts of the corresponding β-anomers (6% for **23a** and 3% for **23b**) were isolated in both sialylations. Removal of chloroacetyl groups from **23a**,**b** with thiourea resulted in triols **24a**,**b** almost quantitatively. Thus, the use of the 7,8-di-*O*-chloroacetyl-thiosialosides **21a**,**b** provided highly efficient and stereoselective sialylation of lactoside **22** and subsequent one-step, high-yielding deprotection of the sialylation products **23** into new sialyl acceptors **24**.

However, sialylation of *N*-acetyl and *N*-TFA acceptors **24a**,**b** with two equivalents of thiosialoside **7b** was not as efficient as could be expected from the results of model disaccharide syntheses and gave GD_3 tetrasaccharide derivatives **25a**,**b**[29] in modest and practically equal yields of 15% and 17%, respectively. TLC examination of tetrasaccharide fractions obtained after gel permeation chromatography[20] revealed only traces of products that might be identified as the corresponding β-anomers. About 70% of the acceptor was recovered in both cases. We suppose that such a considerable decrease of efficiency of (2-8)-sialylation compared to model experiments, and the lack of the difference in reactivity of the *N*-acetyl and *N*-TFA acceptors, may be caused by additional steric hindrances around the 8-OH group that are created by the bulky benzylated disaccharide aglycon in the acceptors **24**.

Anomeric configuration of the terminal neuraminic acid residue in **25a**,**b** was proved by the data of HMBC spectra (vide supra) and the position of sialylation was confirmed by the presence of signals for 7-OH of internal neuraminic acid in ^{1}H NMR spectra of both tetrasaccharides. Chemical shifts for H-4 (δ = 5.05 and 5.07 ppm) and H-6 (δ = 4.12 and 4.08 ppm), and the values of $J_{7,8}$ (8.8 and 8.2 Hz) and $\Delta\delta$ {H9–H9′} (0.38 and 0.23) confirmed also the α-configuration of the terminal neuraminic acid residues in **25a**,**b**.

Transformation of **25a**,**b** into 2-aminoethyl glycoside of GD_3 **26** was accomplished in 6 steps involving alkaline hydrolysis of all ester and *N*-TFA groups followed by N-acetylation; reduction of the spacer azide group by hydrogenation and subsequent temporary protection of arising amine by an *N*-TFA group; hydrogenolysis of benzyl ethers; and, finally, alkaline hydrolysis with liberation of the spacer amino group in 79% overall yield. ^{1}H NMR and ^{13}C NMR data of **26**[30] were almost identical to those recently published for the GD_3 3-azidopropyl glycoside.[31]

In conclusion, we have found that *O*-chloroacetylation increase considerably the reactivity of 2-thioethyl sialosides in sialylation reactions. 2-Thiosialoside **7b**, which combines *O*-chloroacetyl and *N*-TFA protecting groups, showed the best sialylating activity and provided the most efficient synthesis of the α(2-8)-linked dimer of neuraminic acid.

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References

(1) Lloyd, K. O.; Furukawa, K. *Glycoconjugate J.* **1998**, *15*, 627.

(2) Nudelman, E.; Hakomori, S.; Kannagi, R.; Levery, S.; Yeh, M.-Y.; Hellström, K. E.; Hellström, I. *J. Biol. Chem.* **1982**, *257*, 12752.

(3) Boons, G.-J.; Demchenko, A. V. *Chem. Rev.* **2000**, *100*, 4539.

(4) (a) Ito, Y.; Ogawa, T. *Tetrahedron* **1990**, *46*, 89. (b) Ercegovic, T.; Magnusson, G. *J. Org. Chem.* **1995**, *60*, 3378. (c) Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schmidt, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 5434.

(5) Ishida, H.; Ohta, Y.; Tsukada, Y.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1993**, *246*, 75.

(6) Roy, R.; Pon, R. A. *Glycoconjugate J.* **1990**, *7*, 3.

(7) De Meo, C.; Demchenko, A. V.; Boons, G.-J. *J. Org. Chem.* **2001**, *65*, 5490.

(8) Demchenko, A. V.; Boons, G.-J. *Chem.–Eur. J.* **1999**, *5*, 1278.

(9) De Meo, C.; Demchenko, A. V.; Boons, G.-J. *Austr. J. Chem.* **2002**, *55*, 131.

(10) Schmidt, R. R. *ACS Symp. Ser.* **1994**, *560*, 276.

(11) Kuhn, R.; Lutz, P.; MacDonald, D. L. *Chem. Ber.* **1966**, *99*, 611.

(12) Lubineau, A.; Le Gallic, J. *J. Carbohydr. Chem.* **1991**, *10*, 263.

(13) Chittenden, G. J. F.; Regeling, H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 44.

(14) Analytical data for **7b**: mp 145–146 °C (EtOAc–hexane); $[\alpha]_D$ +24 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 1.21 (t, 3 H, *J* = 7.5 Hz, SCH_2CH_3), 2.03 (t, 1 H, $J_{3ax,3eq}$ = 12.9 Hz, $J_{3ax,4}$ = 12.3 Hz, H-3*ax*), 2.55 (dq, 1 H, J_{gem} = 12.3 Hz, SCH_2CH_3), 2.75 (dq, 1 H, SCH_2CH_3), 2.85 (dd, 1 H, $J_{3eq,4}$ = 4.8 Hz, H-3*eq*), 3.83 (s, 3 H, CH_3O), 3.99, 4.02 (2 d, 2 H, J_{gem} = 15.0 Hz, $ClCH_2$), 4.07 (m, 4 H, H-5, H-6, $ClCH_2$), 4.17, 4.20 (2 d, 2 H, J_{gem} = 14.6 Hz, $ClCH_2$), 4.20, 4.32 (2 d, 2 H, J_{gem} = 15.2 Hz, $ClCH_2$), 4.25 (dd, 1 H, $J_{9,9'}$ = 12.7 Hz, $J_{9,8}$ = 5.3 Hz, H-9), 4.58 (dd, 1 H, $J_{9',8}$ = 2.5 Hz, H-9′), 5.08 (m, 1 H, $J_{4,5}$ = 10.0 Hz, H-4), 5.38 (d, 1 H, $J_{7,8}$ = 8.0 Hz, H-7), 5.50 (m, 1 H, H-8), 6.91 (d, 1 H, $J_{NH,5}$ = 9.9 Hz, NH).

(15) Sugata, T.; Kan, Y.; Nagaregava, Y.; Miyamoto, T.; Higuchi, R. *J. Carbohydr. Chem.* **1997**, *16*, 917.

(16) (a) Ercegovic, T.; Magnusson, G. *J. Org. Chem.* **1996**, *61*, 179. (b) Castro-Palomino, J. C.; Tsvetkov, Y. E.; Schneider, R.; Schmidt, R. R. *Tetrahedron Lett.* **1997**, *38*, 6837.

(17) Hasegawa, A.; Ito, Y.; Ishida, H.; Kiso, M. *J. Carbohydr. Chem.* **1989**, *8*, 125.

(18) Sherman, A. A.; Mironov, Y. V.; Yudina, O. N.; Nifantiev, N. E. *Carbohydr. Res.* **2003**, *338*, 697.

(19) Grehn, L.; Gunnarson, K.; Ragnarsson, U. *J. Chem. Soc., Chem. Commun.* **1985**, 1317.

(20) **General Procedure for Model Sialylation.** A mixture of sialyl donor (0.33 mmol), sialyl acceptor (0.165 mmol) and powdered molecular sieves 3 Å (1.5 g) in MeCN (4 mL) was stirred at r.t. for 2 h, then cooled to –40 °C. Then, NIS (149 mg, 0.66 mmol) and TfOH (6 μL, 0.07 mmol) were added and the resulting mixture was stirred at –40 °C for 1.5 h. A drop of pyridine was added, the mixture was diluted with $CHCl_3$ and filtered through Celite. The solids were washed with $CHCl_3$, combined filtrates were washed with 1 M $Na_2S_2O_3$ solution, H_2O, and concentrated. The residue was dissolved in a mixture of MeOH (3 mL) and HOAc (1 mL) and thiourea (20 mg) was added. After being stirred for 7–8 min, the mixture was diluted with $CHCl_3$, thoroughly washed with H_2O, and the solvent was evaporated. The residue was subjected to gel permeation chromatography on a column with Bio Beads S-X3 (Bio-Rad) in toluene. Disaccharide fractions were pooled and concentrated to give a mixture of **18** and **19**. Pure anomers were isolated by preparative HPLC (LiChrosorb Si-60 column, K-2401 RI detector, both from Knauer) using toluene–acetone mixtures as eluents.

(21) (a) 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. (b) Nifant'ev, N. E.; Sherman, A. A.; Yudina, O. N.; Cheshev, P. E.; Tsvetkov, Y. E.; Khatuntseva, E. A.; Kornilov, A. V.; Shashkov, A. S. *Pure Appl. Chem.* **2004**, *76*, 1705.

(22) Kessler, W.; Iselin, B. *Helv. Chim. Acta* **1966**, *49*, 1330.

(23) Yu, C.-S.; Niikura, K.; Lin, C.-C.; Wong, C.-H. *Angew. Chem. Int. Ed.* **2001**, *40*, 2900.

(24) Selected ^1H NMR assignments for anomeric (2-8)-disialosides **18** and **19**.
Compound **18a**: δ = 4.86 (H-4′), 3.95 (H-6′), $J_{7',8'}$ = 8.3 Hz, 4.52 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.28.
Compound **19a**: δ = 5.31 (H-4′), 4.55 (H-6′), $J_{7',8'}$ = 4.3 Hz, 4.06 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.51.
Compound **18b**: δ = 4.88 (H-4′), 3.97 (H-6′), $J_{7',8'}$ = 8.7 Hz, 4.56 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.37.
Compound **19b**: δ = 5.37 (H-4′), 4.72 (H-6′), $J_{7,8'}$ = 2.8 Hz, 4.07 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.60.
Compound **18c**: δ = 5.03 (H-4′), 4.11 (H-6′), $J_{7',8'}$ = 8.0 Hz, 4.53 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.25.
Compound **19c**: δ = 5.42 (H-4′), 4.76 (H-6′), $J_{7',8'}$ = 4.6 Hz, 4.03 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.45.
Compound **18d**: δ = 5.06 (H-4′), 4.15 (H-6′), $J_{7',8'}$ = 8.6 Hz, 4.58 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.36.
Compound **19d**: δ = 5.47 (H-4′), 4.89 (H-6′), $J_{7',8'}$ = 2.8 Hz, 4.02 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.63.
Compound **18e**: δ = 5.09 (H-4′), 4.13 (H-6′), $J_{7',8'}$ = 8.3 Hz, 4.40 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.32.
Compound **19e**: δ = 5.47 (H-4′), 4.82 (H-6′), $J_{7',8'}$ = 2.8 Hz, 4.03 (H-8), $\Delta\delta\{H\text{-}9_a'\text{–}H\text{-}9_b'\}$ = 0.60.

(25) Castro-Palomino, J. C.; Simon, B.; Speer, O.; Leist, M.; Schmidt, R. R. *Chem.–Eur. J.* **2001**, *7*, 2178.

(26) Analytical data for **21a**: $[\alpha]_D$ +39 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 1.22 (t, 3 H, *J* = 7.5 Hz, SCH_2CH_3), 1.78 (s 3 H, CH_3CO), 2.11 (t, 1 H, $J_{3ax,3eq}$ = 12.7 Hz, $J_{3ax,4}$ = 12.2 Hz, H-3*ax*), 2.58 (dq, 1 H, J_{gem} = 12.3 Hz, SCH_2CH_3), 2.80 (dq, 1 H, SCH_2CH_3), 2.94 (dd, 1 H, $J_{3eq,4}$ = 4.6 Hz, H-3*eq*), 3.56 (dd, 1 H, $J_{9,9'}$ = 11.0 Hz, $J_{9,8}$ = 4.6 Hz, H-9), 3.77 (dd, 1 H, $J_{9',8}$ = 3.5 Hz, H-9′), 3.86 (s, 3 H, CH_3O), 3.96 (dd, 1 H, $J_{6,7}$ = 1.4 Hz, H-6), 4.06, 4.23 (2 d, 2 H, J_{gem} = 14.7 Hz, $ClCH_2$), 4.22, 4.38 (2 d, 2 H, J_{gem} = 15.0 Hz, $ClCH_2$), 4.29 (q, 1 H, $J_{5,6}$ = 10.7 Hz, H-5), 4.49, 4.53 (2 d, 2 H, J_{gem} = 11.8 Hz, $PhCH_2$), 5.07 (m, 1 H, $J_{4,5}$ = 11.0 Hz, H-4), 5.41 (d, 1 H, $J_{NH,5}$ = 9.9 Hz, NH), 5.49 (m, 1 H, H-8), 5.55 (dd, 1 H, $J_{7,8}$ = 8.8 Hz, H-7), 7.17–7.98 (m, 10 H, arom.).

(27) Cheshev, P. E.; Khatuntseva, E. A.; Tsvetkov, Y. E.; Shashkov, A. S.; Nifantiev, N. E. *Russ. J. Bioorg. Chem.* **2004**, *30*, 60.

(28) Analytical data for **23a**: $[\alpha]_D$ +11 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 1.78 (s, 3 H, CH_3CO), 2.14 (t, 1 H, $J_{3ax,3eq}$ = 12.6 Hz, $J_{3ax,4}$ = 11.4 Hz, H-3*ax* NeuAc), 2.73 (dd, 1 H, $J_{3eq,4}$ = 4.5 Hz, H-3*eq* NeuAc), 3.73, 4.02 (2 d, 2 H, J_{gem} = 14.9 Hz, $ClCH_2$), 3.81 (s, 3 H, CH_3O), 4.15, 4.29 (2 d, 2 H, J_{gem} = 15.0 Hz, $ClCH_2$), 4.40 (d, 1 H, $J_{1,2}$ = 8.0 Hz, H-1 Glc), 4.65 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1 Gal), 5.04 (m, 1 H, $J_{4,5}$ = 10.3 Hz, H-4 NeuAc), 5.35 (d, 1 H, $J_{NH,5}$ = 10.1 Hz, NH), 5.51 (dd, 1 H, $J_{7,6}$ = 1.7 Hz, $J_{7,8}$ = 9.0 Hz, H-7 NeuAc), 5.57 (m, 1 H, H-8 NeuAc).

(29) Analytical data for **25a**: $[\alpha]_D$ –15 (*c* 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 1.92 (s, 3 H, CH_3CO), 2.18 (t, 1 H, $J_{3ax,3eq}$ = 13.2 Hz, $J_{3ax,4}$ = 11.6 Hz, H-3*ax* NeuTFA), 2.21 (t, 1 H, $J_{3ax,3eq}$ = 13.1 Hz, $J_{3ax,4}$ = 11.2 Hz, H-3*ax* NeuAc), 2.61 (dd, 1 H, $J_{3eq,4}$ = 4.8 Hz, H-3*eq* NeuAc), 2.77 (br d, 1 H, 4-OH Gal), 2.82 (dd, 1 H, $J_{3eq,4}$ = 4.7 Hz, H-3*eq* NeuTFA), 3.79 (s, 3 H, CH_3O), 3.85 (s, 5 H, CH_3O, $ClCH_2$), 4.05 (s, 2 H, $ClCH_2$), 4.12 (dd, 1 H, $J_{6,5}$ = 10.5 Hz, $J_{6,7}$ = 1.6 Hz, H-6 NeuTFA), 4.13, 4.16 (2 d, 2 H, J_{gem} = 15.0 Hz, $ClCH_2$), 4.17, 4.28 (2 d, 2 H, J_{gem} = 15.1 Hz, $ClCH_2$), 4.19 (dd, 1 H, $J_{9,9'}$ = 12.4 Hz, $J_{9,8}$ = 6.0 Hz, H-9 NeuTFA), 4.28 (m, 1 H, H-8 NeuAc), 4.40 (dd, 1 H, $J_{1,2}$ = 7.8 Hz, H-1 Glc), 4.45 (dd, 1 H, $J_{1,2}$ = 8.2 Hz, H-1 Gal), 4.55 (dd, 1 H, $J_{9',8}$ = 2.6 Hz, H-9′ NeuTFA), 5.05 (ddd, 1 H, $J_{4,5}$ = 10.4 Hz, H-4 NeuTFA), 5.27 (dd, 1H, $J_{7,8}$ = 8.8 Hz, H-7 NeuTFA), 5.32 (ddd, 1 H, $J_{4,5}$ = 10.5 Hz, H-4 NeuAc), 5.52 (m, 1 H, H-8 NeuTFA), 6.00 (d, 1 H, $J_{NH,5}$ = 8.7 Hz, NH NeuAc), 6.40 (d, 1 H, $J_{NH,5}$ = 9.4 Hz, NH NeuTFA).

(30) Analytical data for **26**: $[\alpha]_D$ +5 (*c* 0.5, H_2O). ^1H NMR (500 MHz, D_2O, relative to internal acetone, δ_H = 2.225); terminal Neu5Ac: δ = 2.03 (CH_3CO), 2.78 (dd, $J_{3eq,4}$ = 4.1 Hz, $J_{3eq,3ax}$ = 12.2 Hz, H-3*eq*), 1.74 (t, H-3*ax*), 3.67 (H-4), 3.83 (H-5), 3.61 (H-6), 3.58 (H-7), 3.88 (H-8), 3.65 (H-9), 3.87 (H-9′); internal Neu5Ac: δ = 2.06 (CH_3CO), 2.68 (dd, $J_{3eq,4}$ = 3.9 Hz, $J_{3eq,3ax}$ = 12.0 Hz, H-3*eq*), 1.74 (t, H-3*ax*), 3.61 (H-4), 3.82 (H-5), 3.71 (H-6), 3.86 (H-7), 4.11 (H-8), 3.75 (H-9), 4.17 (dd, $J_{9',8}$ = 2.8 Hz, $J_{9',9}$ = 12.1 Hz, H-9′); Gal: δ = 4.52 (d, $J_{1,2}$ = 7.6 Hz, H-1), 3.57 (dd, $J_{2,3}$ = 9.7 Hz, H-2), 4.08 (dd, $J_{3,4}$ = 2.3 Hz, H-3), 3.96 (d, H-4), 3.72 (H-5), 3.73 (H-6), 3.75 (H-6′); Glc: δ = 4.54 (d, $J_{1,2}$ = 8.2 Hz, H-1), 3.38 (t, $J_{2,3}$ = 8.5 Hz, H-2), 3.66 (H-3), 3.68 (H-4), 3.62 (H-5), 3.86 (H-6), 4.00 (dd, $J_{6',6}$ = 12.4 Hz, $J_{6',5}$ = 1.8 Hz, H-6′), 3.27 (t, *J* = 4.9 Hz, $OCH_2CH_2NH_2$), 3.88, 4.12 ($OCH_2CH_2NH_2$). ^{13}C NMR (125 MHz, D_2O, relative to internal acetone, δ_C = 31.45); terminal Neu5Ac: δ = 176.2 (C-1), 41.7 (C-3), 69.7 (C-4), 53.0 (C-5), 74.0 (C-6), 69.4 (C-7), 72.2 (C-8), 63.9 (C-9), 23.3 (CH_3CO), 174.4 (CH_3CO); internal Neu5Ac: δ = 176.2 (C-1), 41.0 (C-3), 69.0 (C-4), 53.5 (C-5), 75.1 (C-6), 70.5 (C-7), 79.3 (C-8), 62.7 (C-9), 23.5 (CH_3CO), 174.4 (CH_3CO); Gal: δ = 104.0 (C-1), 70.5 (C-2), 76.7 (C-3), 68.7 (C-4), 76.5 (C-5), 62.3 (C-6); Glc: δ = 103.2 (C-1), 73.9 (C-2), 75.3 (C-3), 79.2 (C-4), 76.0 (C-5), 61.1 (C-6); 67.0 ($OCH_2CH_2NH_2$), 40.7 ($OCH_2CH_2NH_2$).

(31) Zou, W.; Borelli, S.; Gilbert, M.; Liu, T.; Pon, R. A.; Jennings, H. J. *J. Biol. Chem.* **2004**, *279*, 25390.