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N-Trifluoroacetyl Sialyl Phosphite Donors for the Synthesis of α(2 → 9) Oligosialic Acids

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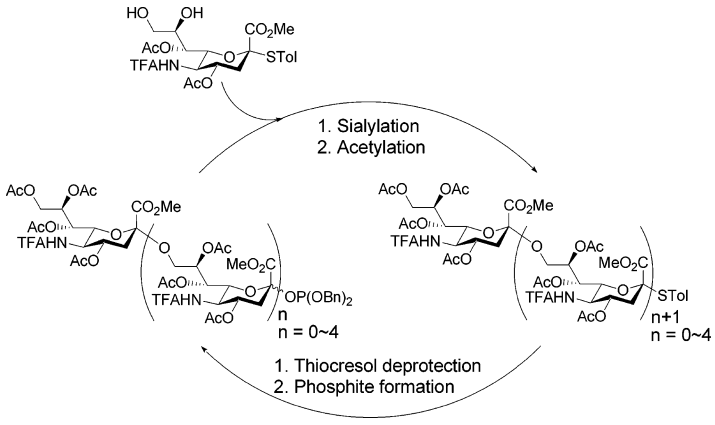
ABSTRACT



A new method for the synthesis of α(2 → 9) oligosialic acids is developed using phosphite sialyl donors that are protected with a C-5 *N*-trifluoroacetyl (NHTFA) substituent. Compared with conventional donors, these donors gave a higher degree of α-anomeric selectivity during glycosidic bond formation and better yields during iterative sialylation in the synthesis of oligosialic acids.

Sialic acids are nine-carbon carboxylated saccharides and are often found at the nonreducing terminus of oligosaccharide chains that are part of glycolipids or glycoproteins in avian and mammalian tissues. Sialic acids are involved in a number of biological processes, such as cell−cell interaction, cell differentiation and proliferation, tumor metastasis, malignant alteration, pathongen-host recognition, toxin-receptor interaction, and neural network development.[1] Among the more than 40 members of the sialic acid family, the monomeric *N*-acetyl neuraminic acid (Neu5Ac) is a prominent one because of its wide occurrence in bioconjugates.[2] Linear polysialic acids consist of contiguous Neu5Ac's linked by α2-8, α2-9, and alternating α2-8 and α2-9 linkages; these chains are found on the surface of bacteria (e.g., *Escherichia coli* K1 and *Neisseria meningitides* groups B and C), where they function as virulence factors. Their presence on the bacterial surface make them a good target for bactericidal antibodies and potential antigens for antibacterial vaccine development.[3a]

The polysaccharides used to prepare vaccines are generally isolated from their natural sources. The natural isolates, however, may be heterogeneous and/or contaminated with other antigenic components. Thus, it is of great interest to develop synthetic polysaccharide vaccines having greater

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immunogenicity.[3b] However, the chemical synthesis of polysialic acids presents a formidable challenge in that the sialylation reaction often proceeds with low yield, low α-stereoselectivity, and undesired 2,3-elimination due to an electron-withdrawing group at the anomeric center, the lack of a participating auxiliary substituent adjacent to the anomeric center, and a sterically hindered tertiary anomeric center.[4] Fortunately, recent progress in the development of sialic acid donors for better α-selectivity has been achieved with moderate success by modifying the amino protecting group at the C-5 position[5−8] and inserting an auxiliary group at the C-1[9] or C-3[10] position while phosphite,[11] sulfide,[12] xanthanate[13] or hydroxyl group[14] was used as a leaving group. However, for oligosialic acids, the preparation of suitable donors and acceptors with differentially protected hydroxyl functions is a laborious task, although effective strategies have been developed in recent years.[4] In view of the aforementioned points, we investigated whether a homo-oligosialic acid could be synthesized by iterative glycosylation using a minimum of reaction types; we also measured the extent to which synthesis could be carried out with acceptable α-stereocontrol. Here, we report our synthesis of homooligosialic acids having α2-9 intersialyl linkages.

Before embarking on the synthesis of homooligosialic acids, we took cognizance of literature precedence to design appropriate sialyl donor−acceptor combinations for the proposed synthesis. Over the past several years, it has been well recognized that replacement of the *N*-acetyl functional group at C-5 with *N*,*N*-diacetyl (NAc_2),[5] *N*-trifluoroacetyl (NTFA),[6] *N*-2,2,2-trichloroethoxycarbonyl (NTroc),[7] or an azido group[8] in the sialyl donor results in higher yields and, in some cases, better α-selectivity during sialylation. These groups are also considered to inhibit hydrogen bond formation between NH at C-5 and OH at C-8/C-9. Thus, the nucleophilicity of the C-9 OH group can be enhanced, which would be advantageous if α2−9 sialylation is desired.[4c,15] Although several leaving groups at the anomeric center can enhance the α-selectivity of sialyl donors,[11−14] the sulfide- and phosphite-based donors are most commonly used,[11,12] with the sulfide-based donors having the comparative advantage of being very stable. Conversely, phosphite-based donors can be activated by a catalytic amount of promoter (usually TMSOTf) and usually lead to predominant formation of the α-product during glycosylation.[11c] On the basis of the above precedence, TFA and Troc were chosen as protecting groups at the *N*-5 of sialic acid, phosphite was used as the leaving group during sialylation, and thiocresol served as the protecting group of the anomeric center.

As shown in Figure 1, three phosphite donors with NHAc, NHTroc, and NHTFA at the C-5 position (**1**, **2**, and **3**, respectively) were tested with regard to their α-selectivities in sialylation with sulfide acceptors **4**[16] and **5**. The results

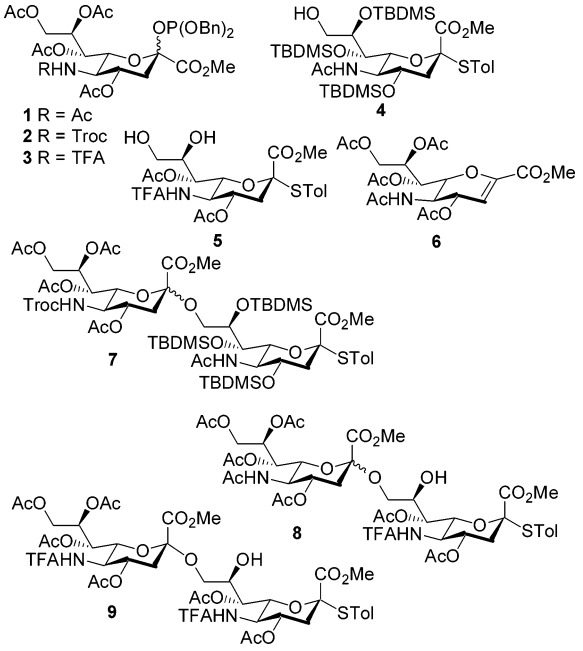

Figure 1. Acceptors, donors, and products of sialylation.

of sialylations were shown in Table 1 The reaction between bulky acceptor **4** and **1** yielded the elimination product only

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Table 1. Sialylation of C-5 *N*-Protected Sialic Acid Derivatives[a]

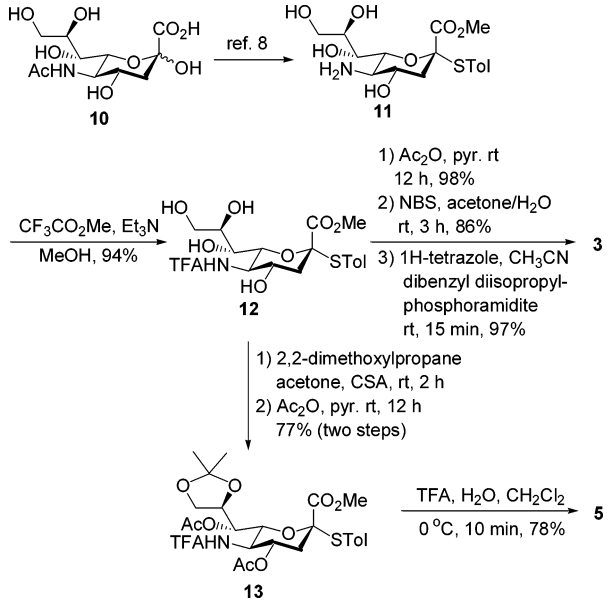
entry	donor	acceptor	product	yield (%)	α/β
1	1	4	6	83	
2	2	4	7	33	1.5/1
3	1	5	8	55	4.7/1
4	3	5	9	77	α

[a] Tol = *p*-methyl phenyl.

(entry 1), whereas the reaction between **2** and **4** produced the desired dimer with 33% yield. Although the α-selectivity was not good (α/β = 1.5), donor **2** had better reactivity for the bulky acceptor (entry 2). When the less sterically hindered acceptor **5** was sialylated with **1**, as expected, only the primary hydroxyl group reacted with the donor, and both the reaction yield (55%) and the α-selectivity increased (entry 3). Thus, the donor with higher activity, **3**, was reacted with **5** to produce the α2-9 sialic acid dimer **9** with 77% yield (entry 4). These model studies demonstrated that the TFA protecting group at the C-5 position of sialic acid is a good choice for both sialyl donor and acceptor.

Synthesis of donor **3** and acceptor **5** are outlined in Scheme 1. The synthesis of the key intermediate **12** was achieved

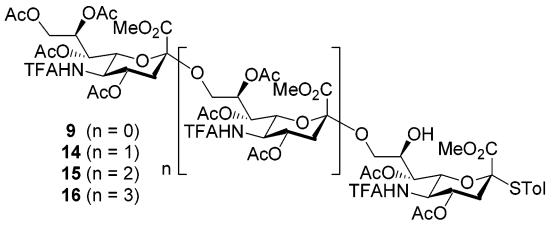
Scheme 1. Synthesis of Donor **3** and Acceptor **5**



by acylation of **11**,[6c] which was obtained from neuraminic acid **10**, as previously reported.[5,8] Peracetylation of **12** was followed by deprotection of thiocresol[17] to give a hydroxyl compound that was transformed to phosphite[11b] to produce **3**. Selective acetonation of **12** was followed by acetylation and then acidic deprotection of acetonide to yield **5**.

For extending the sialic acid chain, we adopted a strategy in which the chain was elongated from the nonreducing end toward the reducing end. Thus, the free hydroxyl group at C-8 of the reducing end sialyl unit of **9** was capped with an acetyl group (95% yield), and subsequently the sulfide group was converted to a hydroxyl group (84% yield) by treating with *N*-bromosuccinimide (NBS)[17] in aqueous acetone. Treatment of the resulting intermediate with dibenzyl *N*,*N*-diisopropylphosphoramidite produced the disialyl phosphite donor (97% yield).[11b] The second generation of sialylation was performed using the phosphite dimer with acceptor **5** to give the trimer, **14**. Iterating the sialylation, acetylation, thiocresol deprotection, and phosphite formation produced the higher-order sialosides. The execution and results of this strategy are summarized in Table 2. The α-selectivity of

Table 2. Synthesis of α2-9 Oligosialic Acids[a]



entry		glycosylation (α/β)	acetylation	thiocresol deprotection	phosphite formation
1	**9**	77% (α)	95%	84%	82%
2	**14**	70% (4/1)	94%	88%	80%
3	**15**	74% (1.6/1)	96%	88%	85%
4	**16**	45% (1/1.3)	95%		

[a] Tol = *p*-methyl phenyl.

sialylation decreased with increasing phosphite donor size. Eventually, the β isomer became the major product in the synthesis of pentasialic acid (β/α = 1.3) using our newly developed method. Notably, the α/β mixture of **14**, **15**, and **16** could not be separated without acetylation of the free hydroxyl group. The new formed anomeric configuration of **9** was determined by the long-range $J_{C-1,H-3ax}$ coupling constant.[8a,18] By selective proton decoupled ^{13}C NMR experiments, the coupling pattern of C-1 of the α anomer gave a doublet C-1 signal and the coupling constant was 5.6 Hz. The configurations of nonreducing end sialic acids of **14**, **15**, and **16** were determined by empirical rules[8a,b,19] and the

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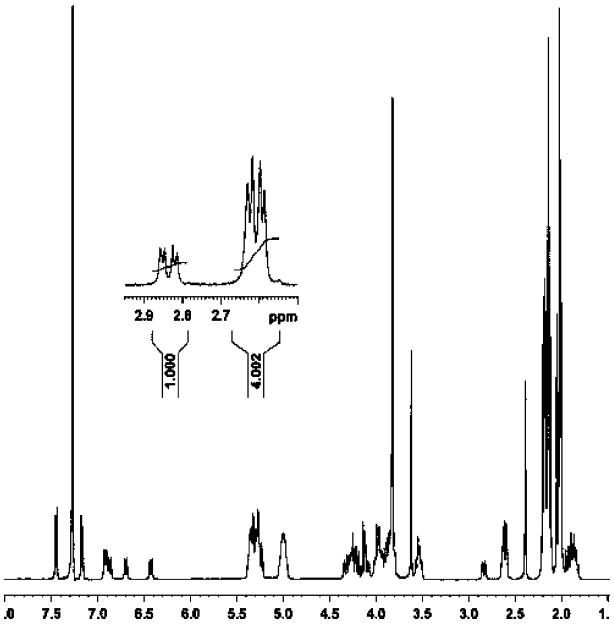

Figure 2. Proton NMR spectrum of α2-9 pentasialic acid **16**.

chemical shifts of H-3eq of nonreducing end sialic acids. The chemical shifts of H-3eq of α anomers were more downfield than those of β anomers. The proton NMR spectrum of the acetylated α2-9 pentasialic acid derivative, **16**, is shown in Figure 2. Note that the chemical shifts of H-3eq protons of the nonreducing end saccharides are at the same position and that of the reducing end sugar shows downfield shift.

Several important features of this work should be emphasized. New phosphite-based sialyl donors have demonstrated their utility for efficient construction of di-, tri-, tetra-, and pentasialosides by iterative sialylation. The α-selectivity displayed by the donors, especially during the formation of di- and trisialosides, has very few parallels compared with the reported sialyl donors.[5,6c,8c] The yields obtained in the sialylation reactions are comparable or superior to those previously reported for disialosides and trisialosides.[8c] Finally, to the best of our knowledge, this is the first chemical synthesis of pentasialic acid through glycosidic bond formation.

In conclusion, we have demonstrated the utility of new phosphite-based donors for efficient and highly α-selective synthesis of oligosialic acids (up to pentasialoside) using iterative sialylation. Although the α-selectivity decreased with increasing size of the donor, pure pentasialic acid was obtained on the 10 mg scale. We believe that these phosphite donors will have applications in the development of polysialic acid based vaccines.

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Supporting Information Available: Detailed synthetic procedures and spectroscopic characterization of compounds **3**, **5**, **7**−**9**, and **12**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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