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Efficient Synthesis of an α(2,9) Trisialic Acid by One-Pot Glycosylation and Polymer-Assisted Deprotection

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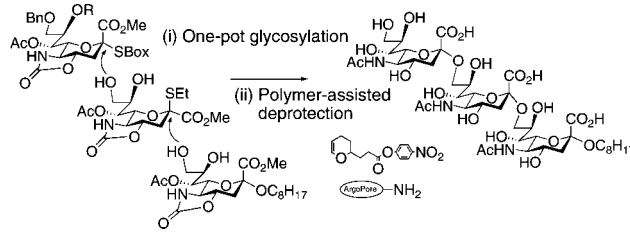
ABSTRACT



An efficient synthesis of α(2,9) trisialic acid has been achieved via one-pot glycosylation and polymer-assisted deprotection. The synthesis involves chemo- and regioselective α-sialylation of ethylthiosialoside with the *S*-benzoxazolyl (*S*-Box) sialyl donor. Use of a prelinker to link an activated ester and a vinyl ether via a carbon chain enables polymer-assisted deprotection of the protected trisialic acids.

Sialic acids, such as Neu5Ac, Neu5Glc, and KDN, are the most complex monosaccharide units in naturally occurring oligosaccharides and are frequently located as monomers at the nonreducing end of oligosaccharides.[1] Recent progress in glycobiology suggests that α(2,8) and α(2,9) di/oligosialic acids may play important roles in the biological events that occur on the cell surface.[2] Chemical synthesis of these oligosaccharides is highly desirable to facilitate discovery of their biological roles. However, α-sialylation is one of the most difficult and challenging processes in the chemical synthesis of oligosaccharides.[3] Recently, modification of the acetamide group at the 5 position of sialyl donors to *N*,*N*-diacetyl,[4a,k] azido,[4b] *N*-TFA,[4c,f] *N*-Troc,[4d,e,h,j,l] *N*-Fmoc,[4e,h] *N*-trichloroacetyl,[4e,h,g] *N*-phthalimide,[4i] and 5*N*,4*O*-carbonyl[4m,n,7] groups was shown to improve the efficiency of α-selective sialylation, providing several effective methods for the synthesis of various α(2,3) and α(2,6) sialosides.[5] However, synthesis of the α(2,8) or α(2,9) oligosialic acids remains a difficult task due to the poor reactivity of sialic acids toward glycosylation. In addition, oligomers of α-sialosides are less stable under acidic conditions than conventional sialic acids and, therefore, require careful treatment during workup and purification of the final products.[6] The synthesis of α(2,9) sialic acids has

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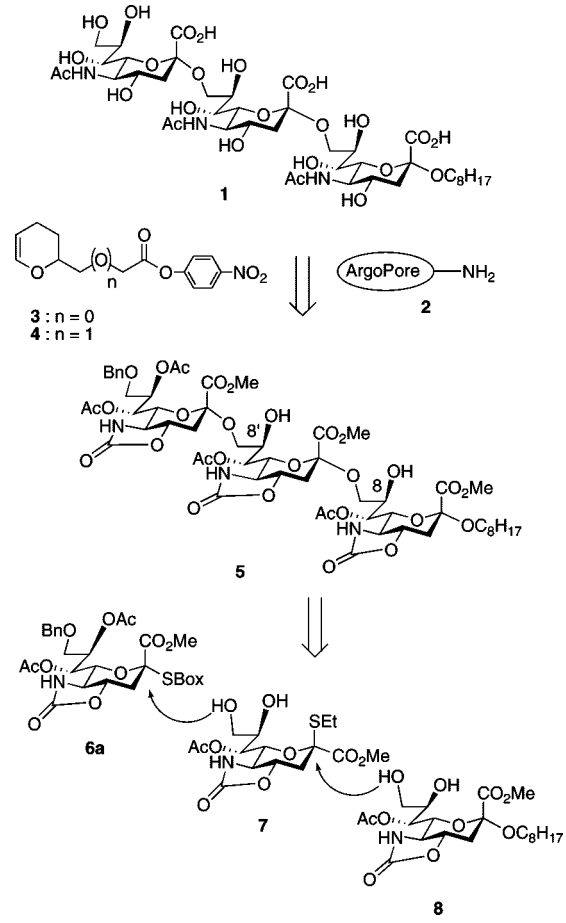
been reported by using 5-azido[4b] and *N*-TFA[4] sialyl phosphites based on a stepwise strategy. Recently, we reported the synthesis of α(2,8) tetrasialic acid using the 5*N*,4*O*-carbonyl-protected thiosialoside.[7] The 5*N*,4*O*-carbonyl protection of sialic acids enables α sialylation without use of acetonitrile. However, these effective methods, which are based on modification of the C5 acetamide group, require conversion of amino groups into the naturally occurring *N*-acetyl derivative (Neu5Ac) or *N*-glycolyl derivative (Neu5Glc) via highly polar amino acid intermediates.

Recently, we developed an efficient method for the synthesis of oligosaccharides that is based one-pot glycosylation and polymer-assisted deprotection.[8−10] The one-pot glycosylation involved sequential chemo- and regioselective glycosylation to provide oligosaccharides from several simple building blocks in one pot.[11] The polymer-assisted deprotection involved deprotection of solid-supported, protected oligosaccharides followed by their release from the solid supports. The prelinker **4**, composed of an activated ester and a vinyl ether linked via an ether bond, was used for quantitative loading of the protected oligosaccharides on to a solid-support. Deprotection was made easier by supporting the substrates on solids.[12] Herein, we report the synthesis of the α(2,9) trisialic acid **1** by one-pot glycosylation and polymer-assisted deprotection using 5*N*,4*O*-carbonyl protected sialyl donors and a new prelinker for polymer-assisted deprotection.

Our strategy for the synthesis of α(2,9) trisialic acid **1**, based on one-pot glycosylation and polymer-assisted deprotection, involves chemo- and regioselective glycosylation of thiosialoside **7** with the *S*-Box sialyl donor **6a** at the 9 position followed by coupling of the resulting disaccharide with α-sialoside **8**[7] at the 9 position (Scheme 1). The 5*N*,4*O*-carbonyl protection of building blocks **6**−**8** is effective for α-sialylation at the 9 position. The S-Box glycosyl donors are adaptable to sialylation and can be selectively activated in the presence of thioglycosides.[13,14] The 8,9 diols **7** and **8** were effective as acceptors for α(2,9) sialylation because the C8 hydroxyl group does not adversely interfere with glycosylation at the C-9 position.[4f] The prelinker **3**, which links an activated ester and a vinyl ether via a carbon chain, was newly designed for polymer-assisted deprotection of the protected trisialoside **5**. The linker **3** undergoes acetalization with the protected trisialic acid **5** at the 8 and/or 8′ positions; amidation with an amino group on ArgoPore thereafter immobilizes the trisaccharide **5** onto a solid support. The ArgoPore resin enables deprotection of solid-supported, protected oligosaccharides via debenzylation through Birch reduction.[10] The ester unit in the carbon linker chain of **3** exhibits enhanced stability under basic conditions. In addition, the reactivity of the vinyl ether of **3** toward acetalization is improved, and the resulting acetal unit is more easily hydrolyzed under acidic conditions compared with linker **4** due to the lack of an electron-withdrawing oxygen unit.

Scheme 1. Strategy for the Synthesis of the α(2,9) Trisialic Acid **1** Based on One-Pot Glycosylation and Polymer-Assisted Deprotection



The chemical properties of the linkers prepared from the prelinkers **3** and **4** were compared. The acylated benzylamines **9** and **10** were used as model compounds. Treatment of **9** at 80 °C for 12 h under basic conditions, which allows for hydrolysis of both the C1 ester and the 5*N*,4*O*-carbonyl protecting group, had no effect on **9**. On the other hand, under similar conditions, hydrolysis of the α-alkoxyacetamide **10** occurred. Next, we exposed **9** and **10** to 1% trifluoromethanesulfonic acid in CH_2Cl_2 in the presence of H_2O

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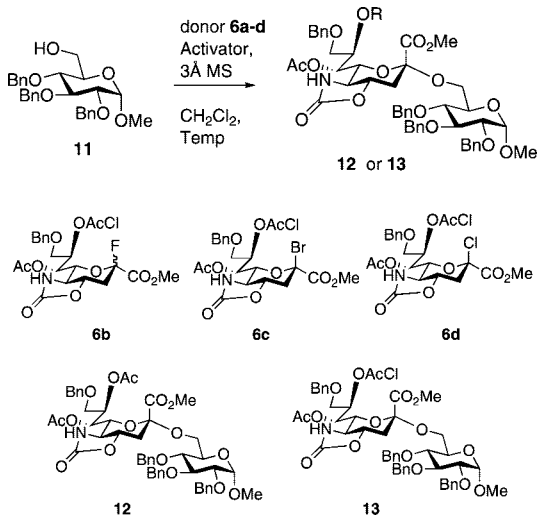
Table 1. Glycosidation of the 5*N*,4*O*-Carbonyl-Protected Sialyl Donors **6a**−**d**, Which Had Different Leaving Groups



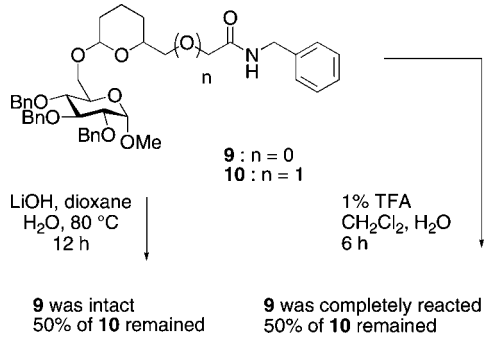
no.	donor	activator	T (°C)	yield of **12** or **13**	α/β ratio[a]
1	**6a**	AgOTf	−60 to −40	92	>95:5
2	**6b**	$ZrCp_2Cl_2$, AgOTf	−30 to rt		
3	**6c**	AgOTf	−60 to −30	51	>95:5
4	**6d**	AgOTf	−60 to −40	87	>95:5

[a] The ratio was determined on the basis of ^{1}H spectra.

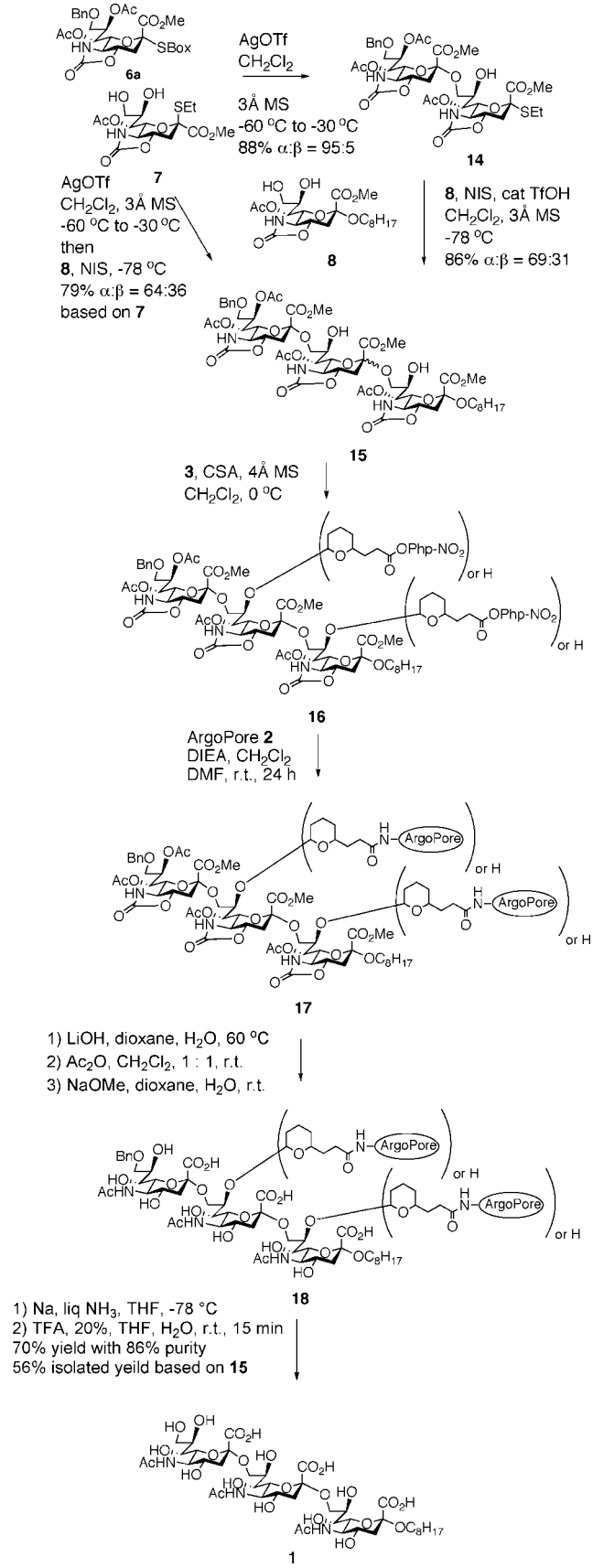
for 6 h, which are the conditions required for release from the resin. HPLC analysis of the reaction mixtures showed that, although 50% of **10** remained intact, **9** had completely disappeared. These results indicated that prelinker **3** enables release of the products under less acidic conditions than prelinker **4**.

We next examined glycosylation of the primary alcohol **11** with the 5*N*,4*O*-carbonyl protected sialyl donors **6a-d**, which had different leaving groups (Table 1). The *S*-benzoxazolyl (*S*-Box) glycosyl donor **6a** underwent α-sialylation with the primary alcohol **11** to provide α-sialoside **12** in excellent yield with α-selectivity. The glycosyl fluoride

Scheme 2. Chemical Properties of the Linkers Prepared from the Prelinkers **3** and **4**



Scheme 3. Synthesis of the α(2,9) Trisialic Acid **1**



6b was not activated by in situ generated $Zr(OTf)_2$.[15] The glycosyl bromide **6c** underwent smooth glycosidation to provide disaccharide **13**. However, purification of the glycosyl bromide **6c** using column chromatography on silica gel was difficult. The glycosyl chloride **6d** exhibited lower reactivity than **6c**. Therefore, we selected the *S*-Box sialyl donor **6a** as the first glycosyl donor for the one-pot glycosylation.

The stepwise synthesis of the protected trisialic acid **5** is shown in Scheme 2. Treatment of β-ethylthiosialoside **7** with 1.2 equiv of the *S*-Box sialoside **6a** in the presence of AgOTf in CH_2Cl_2 at -60 to -30 °C provided the disaccharide **14** in 88% yield with α/β = 95:5. The anomeric ratio was determined based on ^{1}H spectral data NMR ($CDCl_3$, 400 MHz). The α-anomer **α-14** was purified from the mixture by column chromatography on silica gel. Glycosidation of the disialoside **α-14** with the primary alcohol of **8** was successfully achieved by activation of **14** with NIS/cat. TfOH to provide the protected trisialic acid **15** in 86% yield (α/β = 69:31). The anomeric configuration of the major product was determined based on the $^3J_{C1\text{-}H3ax}$ coupling constants ($^3J_{C\text{-}1,H\text{-}3ax}$ = 4.9, 4.9 and 6.1 Hz, $CDCl_3$, 100 MHz) to be α[17].

One-pot glycosylation using **6a**, **7**, and **8** was examined. Chemoselective glycosylation of the thioglycoside **7** with the donor **6a** under the conditions described above provided disaccharide **14**. Subsequent addition of acceptor **8** and NIS to the reaction vessel afforded the protected trisaccharide **15** as two diastereomers in an overall yield of 79% (α/β = 64:36) based on **7**. The protected α(2,9) trisialic acid **α-15** was purified from the mixture of two diastereomers by column chromatography on silica gel. The spectral data (^{1}H and ^{13}C NMR) of the major product were identical with those of the authentic sample prepared by the stepwise synthesis.

Deprotection of the protected trisialic acid was accomplished using the polymer-supported strategy. Treatment of α-sialoside **α-15** with 4 equiv of prelinker **3**, in the presence of CSA at 0 °C for 2 h, provided the protected oligosaccharide **16** as a mixture of mono- and diactivated esters. After removal of the remaining prelinker **3**, the activated esters of **16** were reacted with 3 equiv of the solid-supported amine **2** on ArgoPore resin in DMF−CH_2Cl_2 under basic conditions for 24 h. Compound **16** disappeared from the solution to yield the solid-supported protected oligosaccharides **17**. Hydrolysis of the 5*N*,4*O*-carbonyl protecting group, the methyl ethers, and other ester protecting groups was achieved by treatment with LiOH in dioxane and H_2O at 60 °C for 24 h. Treatment of the resulting amines with Ac_2O, followed by hydrolysis of the partially generated esters provided the solid-supported acetamides **18**. The manipulation for the hydrolysis requires the monitoring of the N-acylation reaction by cleavage of the product. The solid-supported protected oligosaccharides **18** were then subjected to Birch reduction, followed by cleavage from the resin under mildly acidic conditions to provide the fully deprotected α(2,9) trisialic acid in 70% yield with 87% purity based on **15**. The purity was estimated using HPLC with an evaporative light scattering detector (ELSD). Purification of the released product provided the α(2,9) trisialic acid **1** in 56% isolated yield based on **15**. It should be noted that the polymer-assisted deprotection of **15** by using prelinker **4** failed due to release of the sugar from the resin via cleavage of the amide bond during hydrolysis.

In conclusion, we describe the synthesis of the α(2,9) trisialic acid **1** using one-pot glycosylation and polymer-assisted deprotection (Scheme 3). The 5*N*,4*O*-carbonyl-protected S-Box sialyl donor underwent α-selective chemoselective sialylation of β-ethythiosialoside **7**. Subsequent activation of the resulting thioglycoside was achieved by addition of NIS to provide α(2,9) trisialic acid. The prelinker **3**, which links an activated ester and a vinyl ether via a carbon chain, allowed deprotection of solid-supported sialic acids. These methods can be adapted to deprotection and derivatization of various sialo-containing oligosaccharides.

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Supporting Information Available: Experimental procedures and ^{1}H and ^{13C} NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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