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Synthesis of S-Linked α(2→9) Octasialic Acid via Exclusive α S-Glycosidic Bond Formation

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Linear polysialic acids consist of contiguous *N*-acetylneuraminic acid molecules linked by α(2→8), α(2→9), or alternating α(2→8) and α(2→9) linkages. These chains have been identified as pathogenic determinants in capsular antigens of *Neisseria meningitides*, *Escherichia coli*, and *Pasteurella hemolytica*.[1] To study the structure−activity relationship of polysaccharides,[2] in general, the chemically pure oligosaccharides were synthesized and used as biochemical probes. However, because polysaccharides are susceptible to degradation by glycosyl hydrolases in vivo, hydrolysis-resistant carbohydrate mimics have been proposed for application in various ways to study glycobiology.[3] Many investigations have shown that substitution of the glycosidic oxygen atom with a sulfur atom can result in resistance of the glycosidic linkage to either chemical or enzymatic hydrolysis[4] with retention of polysaccharide bioactivity. Recently, both Schmidt and co-workers[5] and Bundle and co-workers[6] synthesized distinct sialic acid-containing tumor antigens in which the nonreducing end of sialic acid was linked via a S-glycosidic bond. Because of their resistance to hydrolysis, S-linked sialic acid-containing antigens showed prolonged stability in vivo, resulting in enhanced immunogenicities compared with antigens containing an O-linked sialic acid. To investigate the S-linked polysaccharide as a potential carbohydrate-based vaccine antigen, we initiated the synthesis of S-linked oligosialic acid.

The synthesis of thiooligosaccharides has been achieved by a variety of methods,[7] the majority of which use either a nonanomeric unprotected thiol or an anomeric thiol protected with moderately stable groups (e.g., thioacetates, thiocyanates, or S-alkyl thiouronium salts)[8] as the donor (nucleophile). However, undesired elimination, anomerization of the anomeric center, and complicated isolation of the product[9] have impeded the practical application of current methods to the synthesis of S-linked oligosaccharides, especially oligosialic acid. To achieve the synthesis of S-linked α(2→9) oligosialic acid, we preassembled the S-glycosidic bond in the α orientation at the anomeric position and then performed S_N2 replacement of the anomeric sulfur nucleophile with a C9 leaving group of the sialic acid acceptor (electrophile). The advantage of this "S-alkylation" is that the stereochemistry of the anomeric center is fixed before alkylation, thus yielding only the α anomer. The key features of this S-alkylation are the stereoselective placement of the protected thiol group at the anomeric position, efficient deprotection without changing the anomeric stereochemistry, and inhibition of disulfide bond formation. In addition, the thiol protecting group must be able to tolerate the conditions under which the functional group is transformed during the synthesis of sialic donor/acceptor.

After many attempts[10] to synthesize S-linked oligomers using various protected thiosialic acid compounds (Figure 1), we found that protection via an unsymmetrical *tert*-butyl disulfide satisfied all of the requirements mentioned above. In addition, the use of *tert*-butyl disulfide protection (**6**) alleviated the thiol−thiol exchange

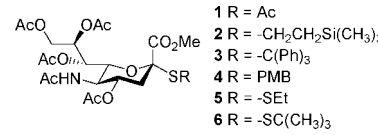
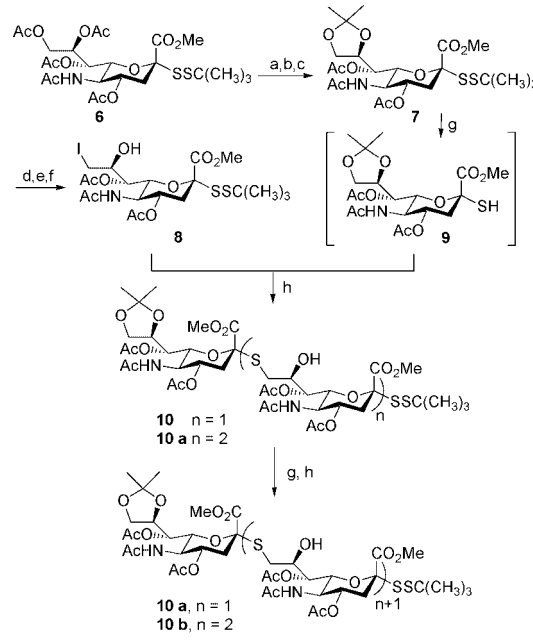


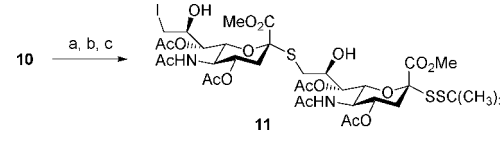
Figure 1. Structures of protected thiosialic acids.

Scheme 1. Synthesis of α(2→9) Tetrathiosialic Acid[a]



[a] Reagents and conditions: (a) NaOMe, MeOH, rt, 85%; (b) 2,2-dimethoxylpropane, acetone, CSA, rt; (c) Ac_2O, pyr, rt, 73% (two steps); (d) TFA, CH_2Cl_2, H_2O, 0 °C; (e) TsCl, pyr, rt; (f) NaI, acetone, reflux, 65% (three steps); (g) sodium 2-mercaptoethanesulfonate, DMF, H_2O, DIPEA, −15 °C to rt; (h) Et_2NH, **8**, DMF, rt; 69% for **10**; 52% for **10a**; 45% for **10b** (two steps).

Scheme 2. Synthesis of the Iodide-Activated Disialoside **11**[a]



[a] Reagents and conditions: (a) TFA, CH_2Cl_2, H_2O, 0 °C; (b) TsCl, pyr, rt; (c) NaI, acetone, reflux, 50% (three steps).

that was observed when the ethyl disulfide-protected compound (**5**)[11] was used in thiol nucleophilic substitution reactions. Moreover, the S-*tert*-butyl disulfide protection was robust in the functional transformation reactions, whereas the disulfide bond was easily cleaved by sodium 2-mercaptoethanesulfonate. To extend the sialic

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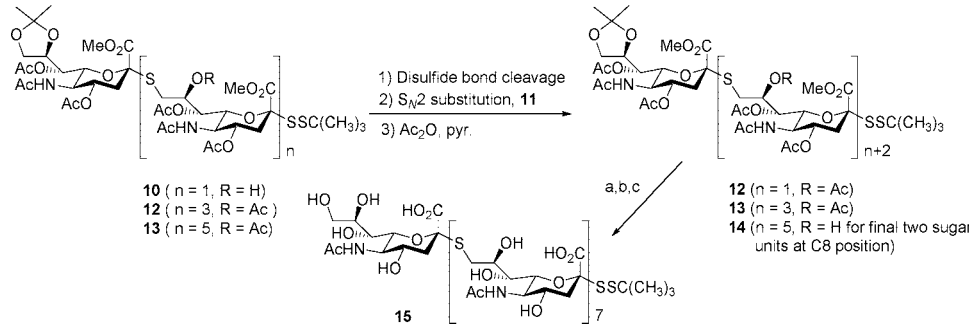
Scheme 3. Synthesis of α(2→9) Thiooligosialic Acids[a]



[a] Reagents and conditions: (a) TFA, CH_2Cl_2, H_2O, 0 °C; (b) NaOMe, MeOH, rt; (c) 1 N NaOH, H_2O, rt, 64% (three steps).

acid chain, we adopted the strategy of elongatating the sugar chain from the nonreducing end toward the reducing end.

As shown in Scheme 1, when **6**[12,13] was used as the precursor, the building block **7** was synthesized by deprotection of the acyl group of **6** followed by acetonidation and peracetylation (total yield 73% for three steps). Deacetonidation of **7** was followed by selective transformation of the primary hydroxyl group to tosylate, which was then converted to the iodide acceptor **8** in the presence of sodium iodide under reflux conditions (65% yield for three steps). The thiol donor **9** was produced by cleavage of the unsymmetrical disulfide bond of **7** with sodium 2-mercaptoethanesulfonate.[14] Without further purification, **9** was reacted with **8** to give the α(2→9) disialoside **10** in 69% yield. The α configuration of **10** was verified by its ^{13}C NMR spectrum ($^3J_{C1-H3ax} = 6.8$ Hz).[15,16] Notably, when **9** was stored at room temperature overnight, the substitution yielded α and β anomers, indicating anomerization at the anomeric position. Finally, we succeeded in synthesizing tri- and tetrathiosialic acids with inverse S-alkylation in yields of 52 and 45%, respectively.

To shorten the overall synthesis, we used the disialoside **10** as a building block to serve as both the donor and acceptor (Scheme 2). Thus, a highly reactive C2 thiol of the disialoside was used as the nucleophile to react with the C9 iodide-activated disialoside. Using this synthetic strategy, we achieved the synthesis of tetra-, hexa-, and octathiooligosialic acids, as shown in Scheme 3. The synthesis of the key building block **11** was achieved using the method for the synthesis of **8** (50% yield for three steps, Scheme 2). We next synthesized S-linked tetra-, hexa-, and octasialic acids by using **11** as the building block, as illustrated in Scheme 3. Iteration of the disulfide bond cleavage, S_N2 substitution, and acetylation steps produced higher-order S-sialosides. The three-step yields for the tetra- and hexasialic acids were 43 and 35%, respectively, and the two-step (without acetylation) yield for the octasialic acid was 35%. The S_N2 reaction yield decreased with increasing length of the sugar chain. Notably, no β anomer product was obtained from any of the substitution reactions.

Deprotection of the S-α(2→9)-octasialoside **14** was then examined. De-O-isopropylidenation of **14** under acidic conditions was followed by deacetylation with sodium methoxide. Finally, removal of the methyl ester protecting group by sodium hydroxide produced the fully deprotected thiooctasialoside **15** (64% overall yield for three steps).

In conclusion, we achieved efficient synthesis of S-linked α(2→9) oligosialosides. The use of *tert*-butyl disulfide protection at the anomeric position allows for the transformation of the functional group and facilitates activation of the thiol nucleophile at the α anomeric position. The use of a disaccharide building block to serve as both the donor and acceptor effectively executes the elongation of the sugar chain with related high total yield. This method potentially could be applied to the synthesis of S-linked sialosides with exclusive α S-glycosidic bond formation.

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Supporting Information Available: Experimental procedures for the syntheses of S-linked α(2→9) oligosialosides, NMR spectral characterization, and complete ref 2. This material is available free of charge via the Internet at http://pubs.acs.org.

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