Sialylation

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1,5-Lactamized Sialyl Acceptors for Various Disialoside Syntheses: Novel Method for the Synthesis of Glycan Portions of Hp-s6 and HLG-2 Gangliosides**

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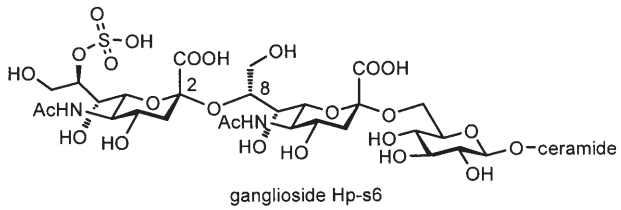
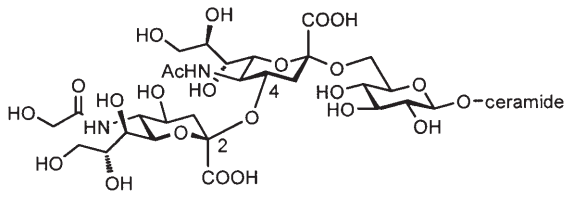
The ongoing studies on oligosaccharide synthesis have resulted in the development of precise synthetic methods by which a large portion of the complex natural oligosaccharides can be duplicated.[1] Although the synthesis of the sequence Neu5Acα(2→8)Neu5Ac (α(2→8)disialic acid; Neu5Ac = *N*-acetylneuraminic acid) has been a major difficulty, the emergence of several exquisite methods[2] that employ indirect coupling by using a C3-functionalized *N*-acetyl sialyl donor and direct coupling by using an *N*-trifluoroacetyl (TFAc)-protected sialic acid donor with the help of the nitrile solvent effect have paved the way for the successful synthesis of α(2→8)disialic acid containing oligosaccharides, such as those with GD3[2c] and GQ1b[3] glycan portions. However, it is obvious that the synthesis of new congeners of disialic acid, such as 8-*O*-sulfo-Neu5Acα(2→8)Neu5Ac in ganglioside Hp-s6[4] and Neu5Gcα(2→4)Neu5Ac in ganglioside HLG-2[5] (Scheme 1), is still difficult because of the diverse modifications possible at the functionality level. On the basis of the predicted biological functions of the disialic acid congener containing oligosaccharides relevant to functions such as neural network formation and fertilization, the establishment of an expedient synthetic method that includes the entire disialic acid family seems essential not only for the progress of glycochemistry but also for studying in detail the molecular basis underlying the biological functions of these compounds. In this study, we report a novel synthetic method for the synthesis of disialic acid congener containing glycans that uses highly reactive lactamized sialyl acceptors and an *N*-2,2,2-trichloroethoxycarbonyl (Troc)-protected sialyl donor.





Scheme 1. Structures of novel disialyl gangliosides Hp-s6 and HLG-2.

Recently, we reported an *N*-Troc-protected sialyl donor (*N*-Troc donor **1**) that shows elevated reactivity and a high degree of accessibility for various sialic acid congeners such as *N*-glycolylneuraminic acid (Neu5Gc), 8-*O*-sulfo-Neu5Ac, and 1,5-lactam-Neu.[6] Initially, we anticipated that use of the *N*-Troc donor would enable the design of HLG-2 and Hp-s6 glycan sequences in an expedient manner. However, as depicted in Scheme 2, the results of the condensations with 4-OH and 8-OH sialyl acceptors, **2** and **3**, respectively, did not meet expectations with regard to yields and stereoselectivity. Even in the case of **2**, which showed the relatively higher reactivity, α-disialyl glycoside was obtained in less than 5%. We hypothesized that the poor results were mainly due to unfavorable hydrogen bonding with the amide moiety at C5, as proposed previously by Tsvetkov and Schmidt.[7] This hypothesis was the basis of the idea that the conformational transformation from the $^2C_5$ chair form to the fixed boat form with the 1,5-lactam bridge would result in increased reactivity of both the C4- and C8-hydroxy groups.[8]

To form the 1,5-lactam bridge in the sialoside, the previously reported *N*-TFAc-sialic acid derivative **6**[9] was used as the key precursor (Scheme 3). After the coupling reaction of **6** and tribenzylated glucosyl acceptor **7**, the resulting sialyl-α(2→6)Glc disaccharide, **8**, was subjected to 1,5-lactamization. First, we attempted a carbodiimide-mediated intramolecular amide formation after the complete deacylation and saponification of **8**, but this reaction yielded a complex mixture. The optimum yield was obtained when **8** was treated with methanolic sodium methoxide in the presence of Drierite under reflux to provide the 1,5-lactam-sialyl glucoside **9** in 85% yield; through regioselective benzoylation of the C9-hydroxy group of **9** with benzoyl chloride and pyridine, under kinetic control, triol acceptor **10** was produced. For the synthesis of the 8-hydroxy-1,5-lacta-

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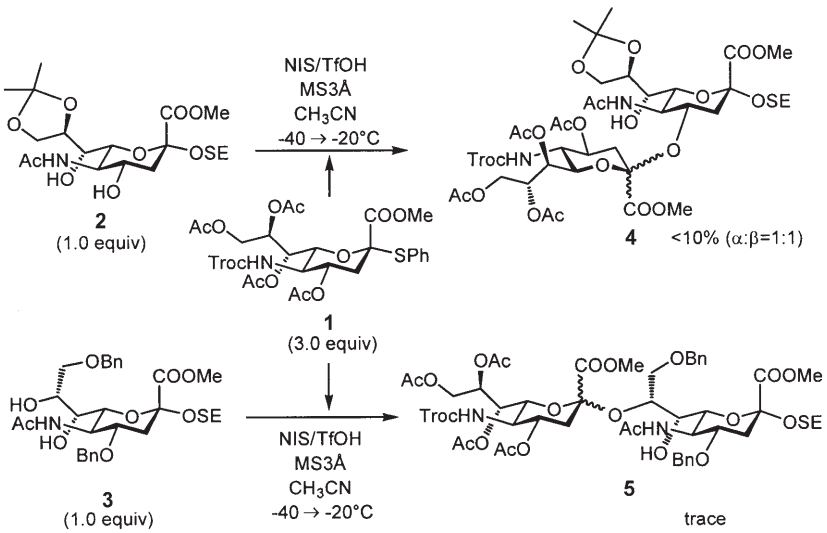
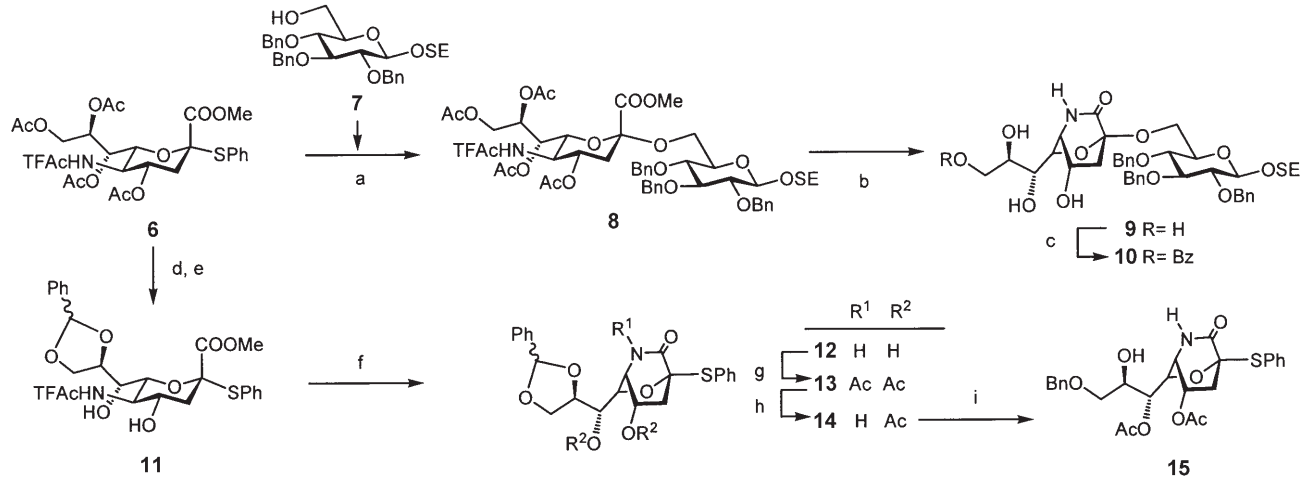


Scheme 2. Unsuccessful sialylation to 4-OH and 8-OH sialyl acceptors. Bn = benzyl, MS = molecular sieves, NIS = *N*-iodosuccinimide, SE = 2-(trimethylsilyl)ethyl, TfOH = trifluoromethanesulfonic acid.

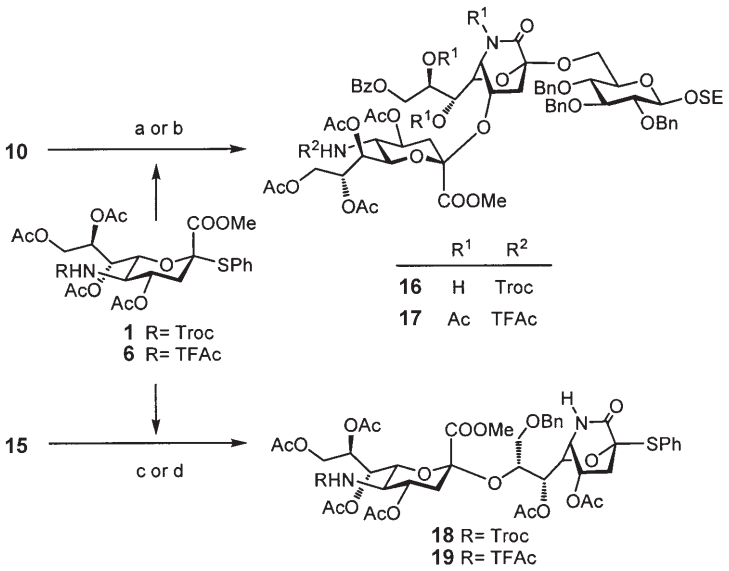
mized sialyl acceptor, 8,9-*O*-benzylidenation was required prior to the lactamization because 8,9-*O*-acetalization of the lactamized derivative was unsuccessful. Thus, compound **6** was de-*O*-acetylated and this was followed by the conventional 8,9-*O*-bezylidenation with benzaldehyde dimethyl acetal and camphorsulfonic acid to produce **11**, which was then subjected to the one-pot 1,5-lactam formation mentioned earlier to yield bicyclo-sialoside **12** in 89% yield. Next, **12** was completely acetylated with Ac_2O in the presence of pyridine and the product was successively de-*N*-acetylated with hydrazinium acetate in a chemoselective manner to produce compound **14**. Finally, reductive ring opening of the benzylidene acetal moiety, influenced by $BH_3{\cdot}NMe_3$ and $AlCl_3$ in THF,[10] produced the 8-OH lactam acceptor **15**.

Next, we carried out the glycosylation of the lactam acceptors **10** and **15** with *N*-Troc- and *N*-TFAc-sialyl donors to evaluate their properties as glycosyl acceptors (Scheme 4). First, the triol acceptor **10** was treated with *N*-Troc donor **1** in the presence of NIS, TfOH, and a molecular sieve in EtCN[11] at −40 °C to provide the Neuα(2→4)Neuα-(2→6)Glc sequence **17**, along with the corresponding β isomer. The anomeric configuration of the new ketosidic linkage was determined on the basis of previous reports[12] by measuring the long-range $^3J_{C1,H3ax}$ coupling constants. For compound **17** this coupling constant was 5.4 Hz, whereas for the β isomer it was less than 1.0 Hz, a fact indicating that the amomeric configuration of **17** was α. Similarly, the coupling reaction with the *N*-TFAc donor **6** and the complete acetylation that followed yielded the corresponding Neuα(2→4)Neuα(2→6)Glc sequence **17** in 41% yield, along with the β isomer (10%) and the Neu(2→8){[Neu(2→4)]Neuα}(2→6)Glc sequences as an anomeric mixture (8%).

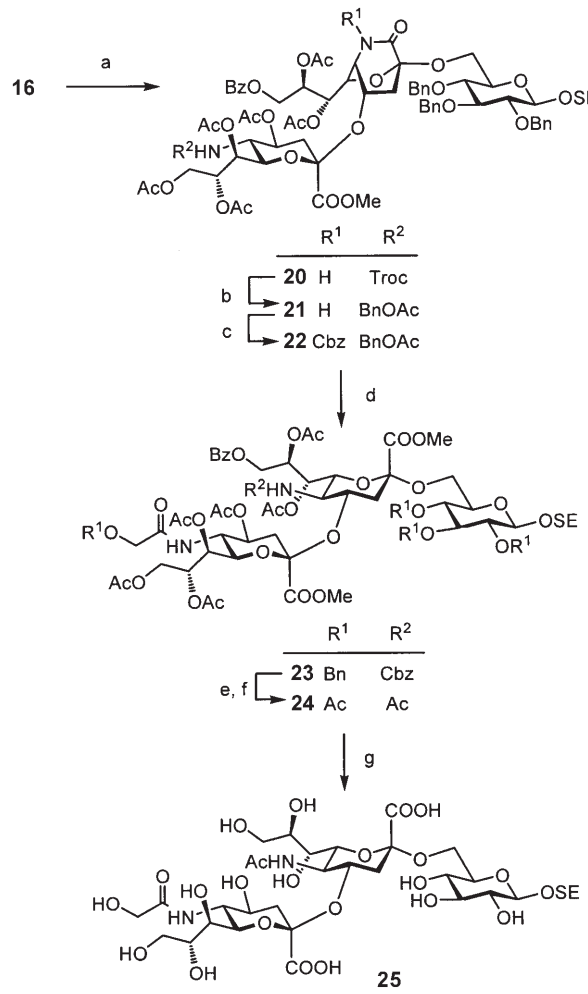
Next, we attempted to fashion the purest form of Neuα(2→8)Neu sequence (Scheme 4). As initially expected, the glycosylation reactions of the lactam acceptor **15** with *N*-Troc and *N*-TFAc donors (**1** and **6**) yielded the corresponding Neuα(2→8)Neu sequences. Thus, *N*-Troc donor **1** and *N*-TFAc donor **6** were incorporated, in the presence of NIS, TfOH, and a molecular sieve in EtCN, at −80 °C to yield α(2→8)disialosides **18** and **19** in 49 and 71% yield, respectively; no corresponding β form was generated in either event. To the best of our knowledge, the yield of addition to the C8-hydroxy group of sialic acid (71%) during the sialylation process was the highest value obtained by direct coupling methods[2] In keeping with the results of the previous experiments, the anomeric configuration of the new linkages was determined to be α from $^3J_{C1,H3ax}$ coupling constants that



Scheme 3. a) **7**, NIS, TfOH, CH_3CN/CH_2Cl_2, MS (3 Å), −30 °C, 5 min, 74%; b) NaOMe, MeOH, Drierite, reflux, 44 h, 85%; c) BzCl, py/CH_2Cl_2, −40 °C, 90 min, 79%; d) NaOMe, MeOH, room temperature, 29 h; e) $PhC(OMe)_2$, CSA, DMF, 40 °C, 2 h, 88% (2 steps); f) NaOMe, MeOH, Drierite, reflux, 5 d, 89%; g) Ac_2O, py, DMAP, room temperature, 3 h; h) $NH_2NH_2{\cdot}AcOH$, THF, room temperature, 80 min, 94% (2 steps); i) $BH_3{\cdot}NMe_3$, $AlCl_3$, THF, MS (4 Å), 0 °C→RT, 6 h, 74%. Bz = benzoyl, CSA = (±)-10-camphorsulfonic acid, DMF = *N*,*N*-dimethylformamide, DMAP = 4-dimethylaminopyridine, py = pyridine, THF = tetrahydrofuran.



Scheme 4. a) **1** (2.0 equiv), NIS (3.0 equiv), TfOH (0.3 equiv), EtCN, MS (3 Å), −40 °C, 6 h, 84% (α/β 66:18); b) 1. **6** (2.0 equiv), NIS, TfOH, EtCN, MS (3 Å), −40 °C, 6 h; 2. Ac_2O, py, DMAP, 40 °C, 17 h, 51%; c) **1** (3.0 equiv), NIS, TfOH, EtCN, MS (3 Å), −80 °C, 5 h, 49% (α only); d) **6** (3.0 equiv), NIS, TfOH, EtCN, MS (3 Å), −80 °C, 3 h, 71% (α only).



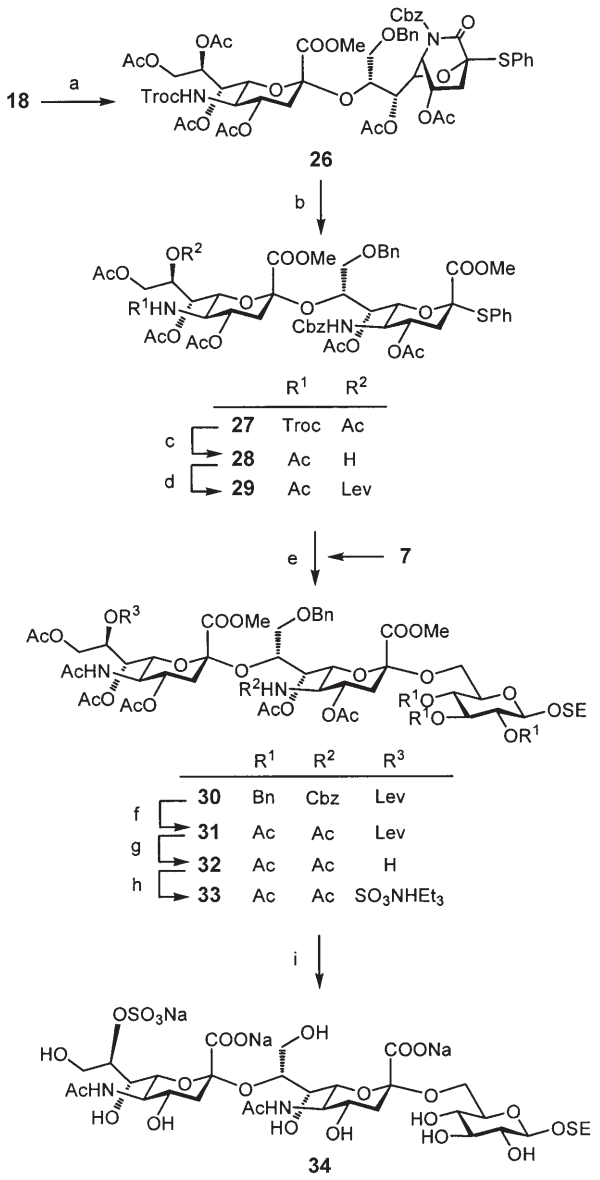
Scheme 5. a) Ac_2O, py, room temperature, 10 h, 89%; b) 1. Zn, AcOH, room temperature, 2 h; 2. BnOAcCl, THF, room temperature, 1 h, 74% (2 steps); c) Cbz_2O, DMAP, py, 40 °C, 26 h, 95%; d) 1. Et_3N, H_2O/CH_3CN, room temperature, 2 d; 2. MeI, K_2CO_3, DMF, room temperature, 30 min, 74% (2 steps); e) 1. H_2, 10% $Pd(OH)_2$/C, NH_3, EtOH, room temperature, 2 h; 2. AcCl, room temperature, 1 h, 68% (2 steps); f) 1. H_2, 10% $Pd(OH)_2$/C, EtOH; 2. Ac_2O, py, room temperature, 54% (2 steps). Cbz = benzyloxycarbonyl.

ranged from 6.7 to 6.9 Hz. Furthermore, the phenylsulfenyl group at the bridgehead anomeric center of acceptor **15** remained unaffected during the coupling reactions. This result confirmed our initial hypothesis, based on Bredt's rule, suggesting the basis of a novel method for the complete deactivation of a sialyl donor.

On the basis of the results obtained with regard to the performance of 1,5-lactamized sialic acid acceptors **10** and **15** in the sialylation reactions, we focused on the synthesis of the glycan portions of HLG-2 and Hp-s6 gangliosides of **16** and **18**, respectively, in order to demonstrate the practical efficacy of the synergic strategy for synthesizing variant disialosides from the 1,5-lactam-sialyl acceptor and *N*-Troc-sialyl donor.

In the initial stages of the synthesis of the HLG-2 glycan portion (Scheme 5), the trisaccharide **16** was *O*-acetylated to provide **20**, to which the *N*-glycolyl moiety was introduced by our reported method,[6] thereby providing **21** in a relatively high yield (66% from **16**). Next, we attempted to recover the $^2C_5$ conformation of the inner sialic acid unit. The following reaction sequences supplied HLG-2 glycan frame **23** in a high yield: *N*-benzyloxycarbonylation, basic hydrolysis, and ensuing methylation of the carboxy group. Debenzylation and acetylation to replaced the Cbz group of **23** by the acetyl group and full deprotection of the product **24** yielded the HLG-2 glycan structure **25**.

In the case of the Hp-s6 glycan frame, the "locked-up" phenylsulfenyl group at the bridgehead carbon atom of **18** was converted into an active state in the initial stages (Scheme 6). To be precise, the reaction sequences mentioned earlier yielded $^2C_5$ conformer **27** in 62% overall yield. For the purpose of 8-*O*-sulfonylation in the final stages of the synthesis, **27** was further transformed into the 8-hydroxy derivative **28** by our regioselective acetyl-transfer method,[6] and the C8 hydroxy group was capped with a levulinoyl group to produce high yields (89%) of the suitably protected disialic acid donor **29** in two steps. Compound **29** was then treated with glucosyl acceptor **7**, influenced by the NIS/TfOH activator system in EtCN at −80→−60 °C, to provide Neuα-(2→8)Neuα(2→6)Glc sequence **30** in 66% yield, predominantly in the α configuration. Next, replacement of the Cbz and benzyl groups of trisaccharide **30** by the acetyl group, followed by chemoselective deblocking of the levulinoyl group with hydrazinium acetate[13] and sulfonylation with SO_3·pyridine resulted in the formation of a completely protected Hp-s6 glycan frame, **33**.[14] The ^{1}H NMR signal for the C8 proton of the outer sialic acid appeared in compound **33** at lower magnetic field ($\delta = 4.92$ ppm) than in compound **32** ($\delta = 4.22$ ppm), and the heteronuclear multiple-bond coherence (HMBC) spectrum of compound **33** contained cross-coupling signals between carbonyl carbon atoms of acetyl groups at C7 and C9, and H7 ($\delta = 5.40$ ppm) and H9



Scheme 6. a) CbzOSu, DMAP, py, room temperature, 42 h, 79%; b) 1. Et_3N, H_2O/CH_3CN, 40°C, 45 h; 2. MeI, K_2CO_3, DMF, room temperature, 3 h, 79% (2 steps); c) Zn, AcOH, THF, room temperature, 28 h, 94%; d) LevOH, DCC, DMAP, CH_2Cl_2, room temperature, 2 h, 95%; e) **7**, NIS, TfOH, EtCN, MS (3 Å), −80→−60°C, 4 d, 66%; f) 1. H_2, 10% $Pd(OH)_2$/C, NH_3, EtOH, room temperature, 1 h; 2. Ac_2O, py, room temperature, 30 min; 3. H_2, 10% $Pd(OH)_2$/C, EtOH, 40°C, 3 h; 4. Ac_2O, py, room temperature, 12 h, 86% (4 steps); g) NH_2NH_2·AcOH, EtOH, room temperature, 6 h, 90%; h) SO_3·py, py, room temperature, 7 h, 65%. Lev = levulinoyl = 4-oxopentanoyl, Su = succinimidyl, DCC = *N*,*N*′-dicyclohexyl carbodiimide.

($\delta = 4.19$ ppm). Thereby, the installation of the sulfonyl group on the C8-hydroxy group was determined.

In conclusion, we have discovered that 1,5-lactam bridging in sialic acid endows high reactivity to the C4- and C8-hydroxy groups, thereby leading to the supply of α(2→4)- and α(2→8)disialic acid sequences in high yields. Furthermore, the practical efficacy of the synergic synthetic approach toward diverse disialic acid containing oligosaccharides, based on the *N*-Troc donor and the lactamized acceptors as the main units, has been demonstrated by the novel method for the synthesis of the HLG-2 and Hp-s6 glycan chains. On the basis of these results, we are now investigating the synthesis of α(2→8)-linked oligosialic acids.

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