

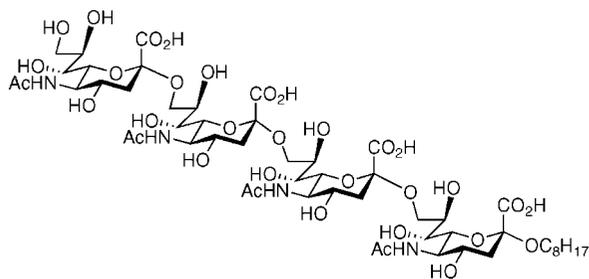
Stereoselective Synthesis of $\alpha(2,9)$ Di- to Tetrasialic Acids, Using a 5,4-*N,O*-Carbonyl Protected Thiosialoside

Hiroshi Tanaka,* Yuji Nishiura, and Takashi Takahashi*

Department of Applied Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, 2-12-1 Ookayama, Meguro, Tokyo 152-8552, Japan

thiroshi@apc.titech.ac.jp; ttak@apc.titech.ac.jp

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An efficient stereoselective synthesis of $\alpha(2,9)$ tetra- to disialic acids **1–3**, using the 5,4-*N,O*-carbonyl protected thiosialoside **4**, is described. The cyclic protecting group was effective for α -sialylation without the need for acetonitrile as the solvent. The donor **4** enabled the formation of a tetramer in excellent yield and selectivity. Deprotection of the cyclic protecting groups of the protected di- to tetrasialic acids proceeded smoothly to give the fully deprotected $\alpha(2,9)$ tetra- to disialic acids **1–3**.

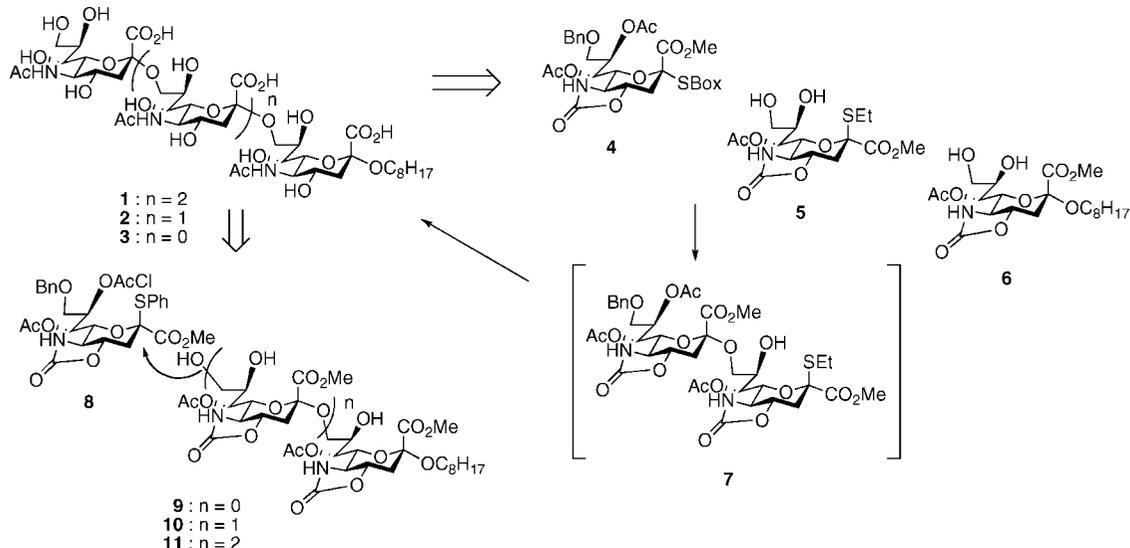
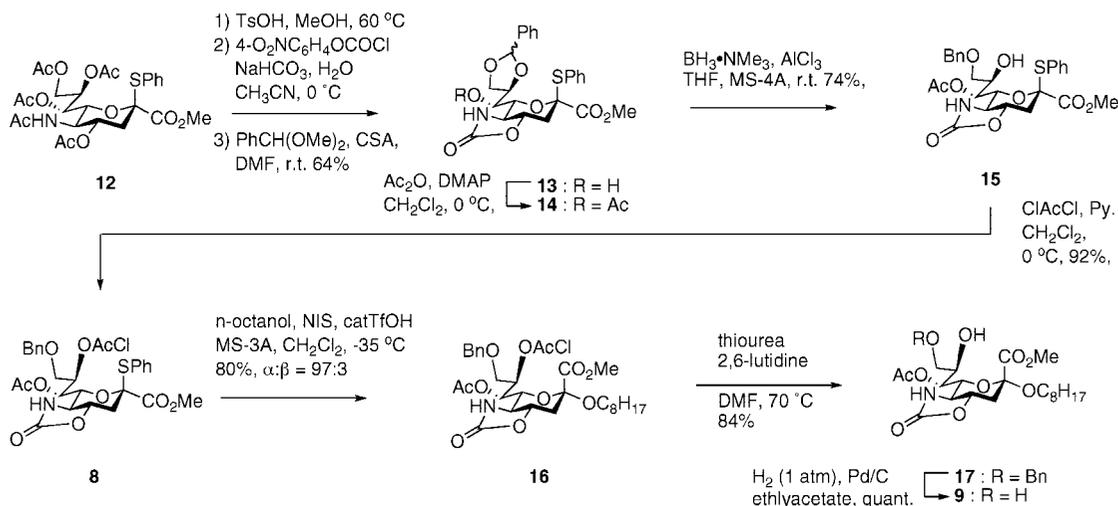
Sialic acids are a family of the most complex monosaccharide units in naturally occurring oligosaccharides, and are frequently located at the nonreducing ends of oligosaccharides.¹ Recent progress in glycobiology suggests that $\alpha(2,8)$ and $\alpha(2,9)$ di/oligosialic and polysialic acids may play important roles in biological events that occur on the cell surface.² The $\alpha(2,9)$ disialic acid unit is attached to lactosaminoglycan in human teratocarcinoma cells (PA1).³ The $\alpha(2,9)$ polysialic acids have been reported to be present in C-1300 mouse neuroblastoma cells (NB41A3).⁴ In addition, a glycoprotein carrying $\alpha(2,9)$ polysialic acids has been identified in sea urchin sperm flagella.⁵ A straightforward chemical synthesis of these oligosaccharides

would be highly desirable, in that it would facilitate our understanding of their biological roles.

The synthesis of α -linked sialic acid derivatives represents one of the most difficult and challenging processes in the chemical synthesis of oligosaccharides.⁶ The carboxyl group at the C1 position reduces the reactivity of the anomeric position toward glycosidation. The lack of a participating auxiliary adjacent to the anomeric center makes it difficult to form thermodynamically and kinetically glycosylation-disfavored α -sialosides and promotes β -elimination with the production of a glycal. Acetonitrile and propionitrile are frequently used as effective solvents in direct α -sialylations because these solvents block the β face of the intermediate oxonium cation, thus promoting α -selective sialylation.⁷ The nitrile-promoted α -sialylation is more efficient when secondary alcohols are used in the glycosylation compared to primary alcohols. Several reports have appeared regarding the synthesis of $\alpha(2,9)$ disialic acid based on nitrile-promoted α -sialylation.⁸ The 8,9 diols were found to be effective acceptors for $\alpha(2,9)$ sialylation reactions.^{8b} The conversion of the acetamide group at the C5 position of the sialyl donor to *N,N*-diacetyl, azido, *N*-TFA, *N*-Troc, *N*-Fmoc, *N*-trichloroacetyl, and *N*-phthalimide groups has also recently been reported to be effective for improving the reactivity of the sialyl donor toward glycosidation.^{9,10} These donors undergo α -sialylation in nitrile solvents. Wong and co-workers reported on the synthesis of protected $\alpha(2,9)$ tetrasialic acids using 5-azido sialyl phosphates.^{10b} Lin and co-workers successfully prepared a protected $\alpha(2,9)$ pentasialic acid based on an iterative glycosidation strategy using an *N*-TFA protected sialyl donor.^{10f} However, although dimerization proceeded in excellent yield and selectivity, the formation of tetra- and trimers as donors resulted in a significant reduction in α -selectivity. We recently reported on a new and effective method for α -sialylation using the 5,4-*N,O*-carbonyl protected sialyl donor.¹¹ The donors undergo α -sialylation and a nitrile solvent is not required in

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SCHEME 1. Strategies for Synthesis of the $\alpha(2,9)$ Di- to Tetrasialic Acids **3**, **2**, and **1**SCHEME 2. Preparation of the Sialyl Donor and Acceptor **8** and **9**

the reaction.^{12,13} The 5,4-*N,O*-carbonyl protection permitted α -sialylation without use of nitrile solvents, but the mechanism for this unique α -sialylation was not examined in detail. The method described herein is particularly useful in glycosylation reactions involving the use of a primary alcohol in terms of both yield and stereoselectivity, compared to nitrile-promoted α -sialylation. Because of the above findings, we initiated a study of the synthesis of $\alpha(2,9)$ oligosialic acids using 5,4-*N,O*-carbonyl protected sialyl donors.

Our initial approach to this synthesis was a one-pot glycosylation involving the chemoselective sialylation of thioglycoside **5** with the *S*-benzoxazolyl (*S*-Box) sialyl donor **4** and the subsequent use of disialic acid **7** as a glycosyl donor (Scheme 1).¹⁴ This approach was effective in that manipulation of the protecting group in the oligosaccharide synthesis could be minimized. However, although the formation of disaccharide **7**

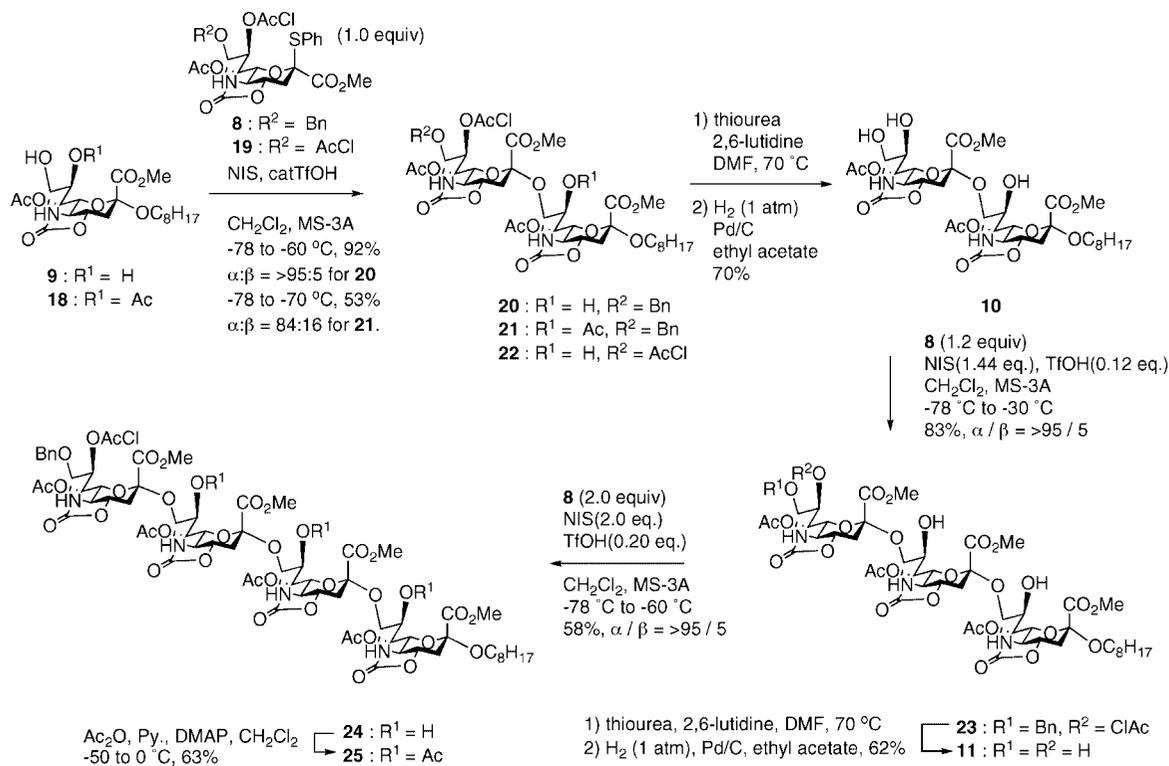
from donor **4** and acceptor **5** proceeded in excellent yield and with α -selectivity, disaccharide donor **7** underwent glycosylation of the sialyl acceptor **6** with reduced α -selectivity. These results suggest that glycosylation with di/oligosialic acid units would be difficult to carry out in an α stereoselective manner. The second approach to the synthesis of $\alpha(2,9)$ oligosialic acids involves glycosylation of oligosialic acids **9**, which contain an 8,9 diol at the nonreducing end of the sialic acid with the monosialic acid unit **8**. The 8,9 diols **9**–**11** would be effective as acceptors in $\alpha(2,9)$ sialylation reactions, because the C8 hydroxyl group does not adversely interfere with glycosylation at the C-9 position.^{8b} The 8-*O*-chloroacetyl and 9-*O*-benzyl β -thiosialoside **8** were used as donors because the protecting groups at the C8 and C9 hydroxyl groups can be removed chemoselectively.

Preparation of the building blocks **8** and **9** is shown in Scheme 2. The known tetraacetyl thiosialoside **12** was used as the starting material. The tetraacetates and the acetamide were removed under acidic conditions. 5,4-*N,O*-Carbonyl protection, followed by regioselective acetalization with benzyl aldehyde at the C8 and C9 positions afforded benzylidene acetal **13** in 64% yield as a diastereomixture (1:1). Acetylation of the

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SCHEME 3. Synthesis of the Protected $\alpha(2,9)$ Di- to Tetrasialic Acids **10**, **23**, and **24**

hydroxyl group at the C7 position, followed by the regioselective reductive opening of the acetal provided the benzyl ether **15** in 74% yield. The remaining hydroxyl groups at the C8 position were protected by reaction with chloroacetic acid to yield the donor **8** in 92% yield. Treatment of donor **8** and *n*-octanol with NIS and a catalytic amount of TfOH in CH₂Cl₂ provided the α -sialoside **16** in 80% yield with $\alpha:\beta = 97:3$. The chloroacetyl group was removed by treatment with thiourea to give alcohol **17** in 84% yield. Hydrogenolysis of the benzyl ether **17** at the C9 position provided diol **9** in quantitative yield.

The synthesis of tetra- to di- $\alpha(2,9)$ sialic acids **1–3** was examined. Treatment of α -thiosialoside **8** and 1.0 equiv of diol **9** with NIS and a catalytic amount of TfOH in the presence of MS-3A in CH₂Cl₂ stereoselectively provided α -sialoside **20** in 92% yield with $\alpha:\beta = >95:5$. On the other hand, sialylation of the alcohol acceptor **18** under the same reaction conditions resulted in a significantly reduced yield of α -disialic acid **21** and α -selectivity (53% yield with $\alpha:\beta = 83:17$). These results indicate that 8,9 diol would be an excellent choice for use in α -sialylation preparations with use of the 5,4-*N,O*-carbonyl protected sialyl donor **8**. In addition, the 8,9 dichloroacetyl donor **19** was adapted for use in $\alpha(2,9)$ sialylation. The coupling product **22** requires only one additional step for the synthesis of the next 8,9 diol acceptor.¹⁵ However, the glycosylation of acceptor **9** with donor **19** resulted in the formation of side-reaction products that were difficult to separate from the coupling product **22**. The preparation of the disialoside acceptor **10** was achieved as follows: (1) removal of the chloroacetyl

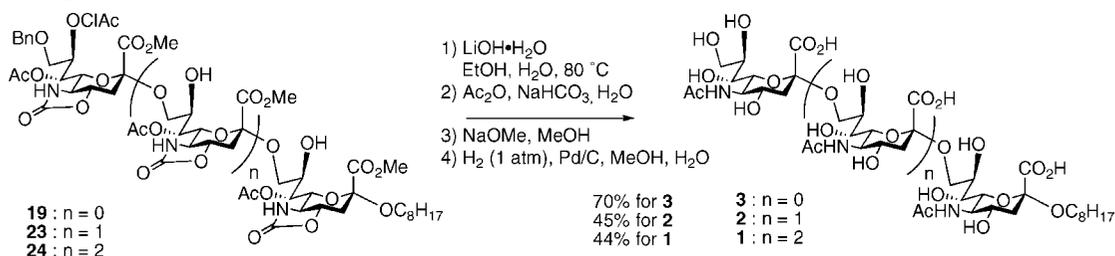
group with thiourea and (2) hydrogenolysis of the benzyl ether at the C9 position. Sialylation of the disialoside **10** at the 9 position was achieved by using a slight excess (1.2 equiv) of donor **8** to provide the protected $\alpha(2,9)$ trisialic acid **23** in 83% yield with $\alpha:\beta = >95:5$. Deprotection of the chloroacetyl group and the benzyl ether afforded the tetraol acceptor **11** in 62% yield. Tetrasialic acid saccharide formation from **11** with 2.0 equiv of donor **8** under the same reaction conditions provided tetrasaccharide **24** in 58% yield with $\alpha:\beta = >95:5$. The coupling constants ³J_{C1-H3ax} (7.3, 6.5, 5.0, and 4.8 Hz) for **24** indicated that the configuration of all of the glycosidic linkages was α .¹⁶ The regioselectivity for each glycosylation step was confirmed by ¹H NMR analysis of the acetylated product **25** (Scheme 3).

Deprotection of the protected di- to tetrasialic acids **19**, **23**, and **24** is shown in Scheme 4. The protected tetrasialic acids **20**, **23**, and **24** were exposed to basic conditions, to remove the 5,4-*N,O*-carbonyl protecting group as well as ester groups, followed by acetylation of the resulting amines. The partially generated *O*-acetyl groups were then removed by hydrolysis under basic conditions. Finally, removal of the benzyl ether at the C9 hydroxyl group was achieved by hydrogenolysis by using a palladium catalyst to afford the fully deprotected di- to tetrasialosides **3**, **2**, and **1** in 87%, 89%, and 69% overall yields, respectively.

In conclusion, we describe an efficient method for the stereoselective synthesis of $\alpha(2,9)$ di- to tetrasialic acids **3**, **2**, and **1** via the use of a simple glycosylation and deprotection procedure. The 5,4-*N,O*-carbonyl protection of donor **8** was effective for the α sialylation of the primary alcohol and acetonitrile was not required for the reaction to proceed. The 8,9 diols **9**, **10**, and **11** possessing a 5,4-*N,O*-carbonyl protecting

(15) The 8,9-benzilidene thiosialoside **14** represents an alternative candidate donor, which can be converted to 8,9 acceptors in a single operation. However, the use of a mixture of stereoisomers derived from the benzilidene acetal makes it difficult to analyze the structure of the coupling product. In addition, the selective synthesis of the acetal derivative as a single diastereomer or separation of these isomers was difficult. Therefore, thiosialoside **14** was not used as a donor in the synthesis of these complex oligosaccharides.

(16) ³J_{C1-H3ax} of α -sialosides is >4 Hz: Haverkamp, J.; Spoomaker, T.; Dorland, L.; Vliegthart, J. F. G.; Schauer, R. *J. Am. Chem. Soc.* **1979**, *101*, 4851–485.

SCHEME 4. Deprotection of the $\alpha(2,9)$ Di- to Tetrasialic Acids **19**, **23**, and **24**

group acted as an effective acceptor for the synthesis of di- to tetra- $\alpha(2,9)$ -sialic acids **3**, **2**, and **1**.

Experimental Section

General Procedure for Glycosylation with the 5,4-*N,O*-Carbonyl Protected Thiosialoside. A mixture of donor **8** (40.7 mg, 88.2 μmol , 1.00 equiv), acceptor **9** (53.7 mg, 88.2 μmol , 1.00 equiv), and pulverized activated MS-3A (44.1 mg, 0.50 g/mmol) in anhydrous CH_2Cl_2 (900 μL , 10.0 mL/mmol) was stirred at room temperature for 30 min under argon to remove traces of water. The reaction mixture was then cooled to -78°C . *N*-Iodosuccinimide (23.8 mg, 102 μmol , 1.20 equiv) and a catalytic amount of trifluoromethanesulfonic acid (0.80 μL , 8.82 μmol , 0.10 equiv) were added to the reaction mixture at -78°C . The reaction temperature was gradually increased to -60°C . After completion of the reaction, as evidenced by TLC, the reaction mixture was neutralized with triethylamine and filtered through a pad of Celite. The filtrate was poured into a mixture of saturated aq NaHCO_3 and saturated aq $\text{Na}_2\text{S}_2\text{O}_3$ with cooling. The aqueous layer was extracted with two portions of ethyl acetate. The combined extracts were washed with saturated aq NaHCO_3 , saturated aq $\text{Na}_2\text{S}_2\text{O}_3$, and brine, dried over MgSO_4 , filtered, and evaporated in vacuo. The residue was chromatographed on silica gel with 98:2 chloroform–methanol as the eluent and further purified by gel permeation chromatography (GPC) to give disaccharide **20** (77.4 mg, 80.7 μmol , 92%, $\alpha/\beta = >95/5$). The α/β ratio was determined by ^1H NMR analysis. $[\alpha]_D^{21} -6.33$ (c 0.43, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 0.88 (t, 3H,

$J = 6.8$ Hz), 1.28 (m, 10H), 1.54 (br dt, 2H), 2.02 (s, 3H), 2.08 (dd, 1H, $J = 12.1, 13.0$ Hz), 2.15 (s, 3H), 2.92 (dd, 1H, $J = 3.9, 12.1$ Hz), 3.01 (dd, 1H), 3.02 (dd, 1H, $J = 9.6, 12.6$ Hz), 3.08 (dd, 1H, $J = 9.7, 10.6$ Hz), 3.24 (dt, 1H, $J = 6.8, 8.7$ Hz), 3.40 (dd, 1H, $J = 2.4, 9.7$ Hz), 3.52–3.59 (m, 2H), 3.70 (m, 1H), 3.74 (m, 1H), 3.81 (s, 3H), 3.83 (m, 1H), 3.85 (s, 3H), 3.92 (m, 1H), 3.93 (m, 1H), 4.07 (dd, 1H, $J = 2.4, 9.7$ Hz), 4.16 (m, 1H), 4.18 (d, 1H, $J = 15.5$ Hz), 4.23 (dd, 1H, $J = 1.4, 9.7$ Hz), 4.38 (d, 1H, $J = 12.1$ Hz), 4.39 (d, 1H, $J = 15.5$ Hz), 4.61 (d, 1H, $J = 12.1$ Hz), 4.98 (dd, 1H, $J = 2.4, 9.2$ Hz), 5.33 (dd, 1H, $J = 1.4, 10.1$ Hz), 5.39 (br s, 1H), 5.41 (m, 1H), 5.46 (br s, 1H), 7.25–7.38 (m, 5H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 20.7, 20.9, 22.7, 25.9, 29.2, 29.3, 29.5, 31.8, 37.1, 37.4, 41.5, 53.2, 53.6, 57.8, 57.9, 65.3, 65.5, 67.3, 67.3, 68.6, 69.3, 71.0, 73.5, 74.0, 74.3, 76.6, 99.9, 100.2, 128.1, 128.1, 128.6, 137.1, 159.2, 159.3, 166.7, 168.7, 169.5, 171.2, 171.4; IR (KBr) 772, 1023, 1376, 1742, 2856, 2924, 3399, 3527 (cm^{-1}); HRMS (ESI-TOF) calcd for $\text{C}_{43}\text{H}_{59}\text{N}_2\text{O}_{20}\text{ClNa}$ $[\text{M} + \text{Na}]^+$ 981.3242, found 981.3242.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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