

An Efficient Convergent Synthesis of GP1c Ganglioside Epitope

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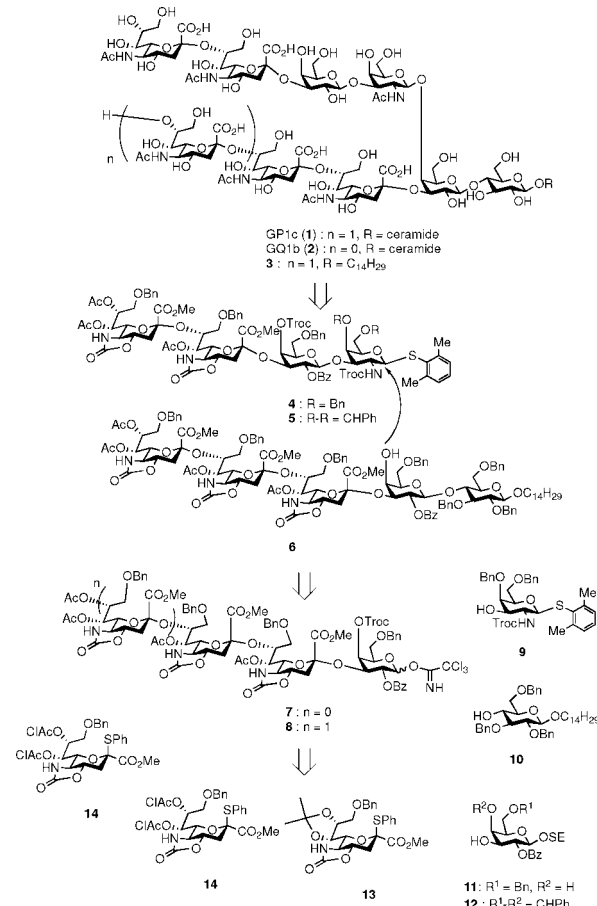
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Gangliosides are a family of glycolipids, which possess sialic acids that play important roles in biological events on cell surfaces.¹ GP1c glycolipid (**1**) is one of the most complex c-series gangliosides; it is composed of a tetrasaccharide (Gal β (1,3)GalNAc β (1,4)Gal β (1,4)Glc) that bears α (2,8) di- and trisialic acid units.² Glycolipids that vary in the number of sialic acids are known as the a-, b-, and c-series of gangliosides.³ Chemical synthesis of these di/oligo-sialo-containing glycolipids and their derivatives would facilitate identification of their biological roles. However, synthesis of both the α (2,8) oligosialic acid unit and the compact and rigid 3,4 dibranched galactoside unit is challenging. Therefore, considerable efforts have been directed toward the synthesis of these complex oligosaccharides.⁴ Kiso and co-workers reported an efficient synthesis of the GQ1b ganglioside (**2**) that has two α (2,8) disialic acid units. In this method, the disialic acid units were prepared by hydrolysis of colominic acid.^{4c} Herein, we report an efficient convergent synthesis of the GP1c ganglioside epitope **3**.

We planned the synthesis of the GP1c epitope **3** bearing a C14 alkyl chain instead of ceramide for establishing the methodology for the synthesis of sugar parts of a-, b-, and c-series of gangliosides. Scheme 1 shows the convergent strategy for the synthesis of the GP1c epitope **3**. The nonasaccharide **3** was prepared by coupling the pentasaccharide **6** and the tetrasaccharide donor **4** at the C4 axial hydroxyl group of the latter. The pentasaccharide **6** was prepared from the α (2,8) trisialylgalactosyl imidate **5** and the glucoside **10**. The tetrasaccharide donor **4** was prepared by chemoselective glycosylation of the 2,6-dimethylphenylthiogalactoside **9** with the disialyl galactosyl imidate **7**. The 2,6-dimethylphenylthio leaving group is effective in chemoselective glycosylation without aglycon transfer of thioglycoside **9**.⁶ The tri- and disialylgalactosides **8** and **7** were prepared from galactoside **11** and the sialyl donors **13** and **14**, which possess a 5*N*,4*O*-carbonyl protecting group, using a simple glycosylation and deprotection procedure.⁷ The sialyl donor **13** protected with an isopropylidene at the C7 and C8 hydroxyl groups was used for α (2,3) sialylation. The C7 and C8 di-*O*-chloroacetyl sialyl donor **14** was used for α (2,8) sialylation to prepare disialylgalactosides **7**. Further α (2,8) sialylation of the resulting disialylgalactosides provided the trisialylgalactosides **5**.

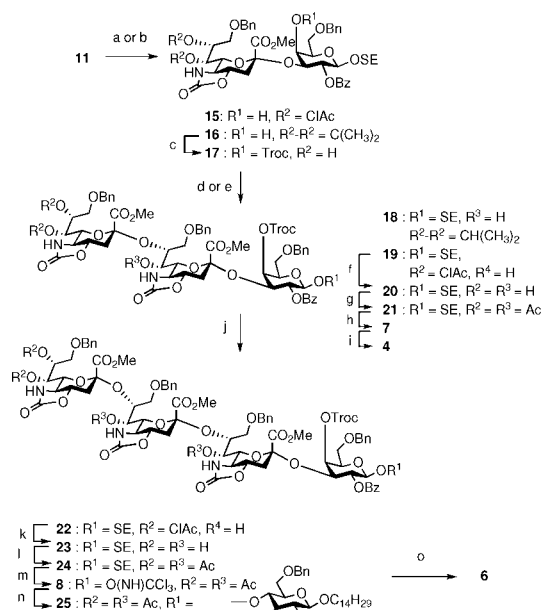
The syntheses of the di- and trisialyl building blocks **4** and **6** are shown in Scheme 2. Treatment of both 3,4-dihydroxyl galactoside **11** and 1.0 equiv of the sialyl donor **13**, which is protected with an isopropylidene at the C7 and C8 hydroxyl groups, with NIS/cat. TfOH in CH₂Cl₂ at -78 °C provided the α (2,3)-sialyl galactoside **16** in 90% yield with complete α -selectivity. Use of the di-*O*-chloroacetyl donor **14** reduced both the yield and α -selectivity of the coupling product **15**. Use of the 4,6-*O*-benzylidene galactoside **12** as an acceptor for α (2,3) sialylation also reduced the yield and selectivity.⁸ Protection of the resulting C4 hydroxyl group of **16** with a Troc group, followed by removal of the acetal, provided the diol **17**. Treatment of the diol **17** and

Scheme 1



the di-*O*-chloroacetyl-protected sialyl donor **14** (2.0 equiv) with NIS/cat. TfOH in CH₂Cl₂/CH₃CN = 3:2 at -78 °C provided the α (2,8) disialoside **19** in 91% yield with complete α -selectivity. The use of pure CH₂Cl₂ as solvent for α (2,8) sialylation of **17** reduced the coupling yield of **19** but did not affect the α -selectivity. The sialyl donor **13** was not effective in the α (2,8) sialylation, and use of **13** resulted in a reduction in α -selectivity (77%, α : β = 54:46). Deprotection of the chloroacetyl groups on **19** afforded the triol acceptor **20** in 87% yield. Trisialyl formation from **20** using 3.0 equiv of donor **14** under the same reaction conditions provided the trisialyl galactoside **22** in 72% yield (α : β > 90:10). Trisialoside **22** had an α -anomeric configuration, as determined based on the ³J_{C1-H3ax} coupling constants (³J_{C1-H3ax} = 4.9, 4.9, and 6.1 Hz). The imidates **7** and **8** were prepared in a stepwise procedure, as follows: (i) removal of the chloroacetyl groups from **19** and **22**; (ii) acetylation of the remaining hydroxyl group; (iii) removal of the 2-trimethylsilyl ethyl group at the anomeric position; and, (iv) treatment of the hemiacetal with trichloroacetonitrile to provide the

Scheme 2

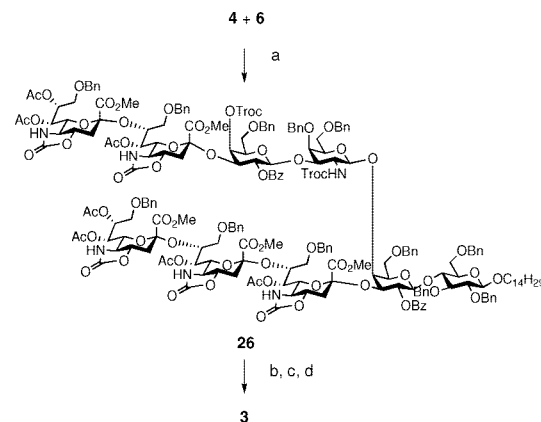


Reagents and conditions: (a) **13** (1.0 equiv), NIS, cat. TfOH, CH_2Cl_2 , MS3A, $-78^\circ C$, 90%, α only; (b) **14** (1.0 equiv), NIS, cat. TfOH, CH_2Cl_2 , MS3A, $-78^\circ C$, 82%, $\alpha/\beta = 87:13$; (c) (i) TrocCl, Py, CH_2Cl_2 , 96%; (ii) CSA, MeOH, rt, 92%; (d) **14** (2.0 equiv), NIS, cat. TfOH, $CH_2Cl_2/CH_3CN = 3:2$, MS3A, $-78^\circ C$, 91%, $\alpha/\beta = >95:5$; (e) **13** (2.0 equiv), NIS, cat. TfOH, $CH_2Cl_2/CH_3CN = 3:2$, MS3A, $-78^\circ C$, 77%, $\alpha/\beta = 54:46$; (f) thiourea, 2,6-lutidine, DMF, $60^\circ C$, 87%; (g) Ac_2O , Py, CH_2Cl_2 , cat. DMAP, $-50^\circ C$, 92%; (h) (i) TFA, CH_2Cl_2 ; (ii) CCl_3CN , CS_2CO_3 , CH_2Cl_2 , $0^\circ C$, 93% from **21**, $\alpha/\beta = 68:32$; (i) **9**, TMSOTf, CH_2Cl_2 , MS4A, -78 to $-45^\circ C$, 95%; (j) **14** (3.0 equiv), NIS, cat. TfOH, $CH_2Cl_2/CH_3CN = 3:2$, MS3A, $-78^\circ C$, 72%, $\alpha/\beta = >90:10$; (k) thiourea, 2,6-lutidine, DMF, $60^\circ C$, 75%; (l) A_2O , Py, cat. DMAP, CH_2Cl_2 , $-30^\circ C$, 87%; (m) (i) TFA, CH_2Cl_2 , $0^\circ C$, 90% from **24**, $\alpha/\beta = 84:16$; (n) **10**, TMSOTf, CH_2Cl_2 , MS4A, -65 to $-40^\circ C$, 93%; (o) Zn dust, AcOH, THF, $0^\circ C$, 94%.

corresponding imidates **7** and **8**. The chloroacetyl esters at the C7 and C8 positions underwent partial hydrolysis during imidate formation with trichloroacetonitrile. Treatment of the trisialyl galactosyl donor **8** and glucoside **10** with a catalytic amount of TMSOTf provided the pentasaccharide **25** in 93% yield. The minor isomer generated in the trisialylation can be removed in the purification of acetylated product **24**. Selective deprotection of the Troc group afforded acceptor **6** in 94% yield. In addition, preparation of the donor **4** was achieved by chemoselective glycosidation of **7** at the secondary alcohol with 1.2 equiv of thioglycoside **9** in the presence of a catalytic amount of TMSOTf, providing the tetrasaccharide **4** in 95% yield.

Synthesis of the GP1c epitope **3** from **4** and **6** is illustrated in Scheme 3. Treatment of both the pentasaccharide acceptor **6** and 2.0 equiv of the tetrasaccharide donor **4** with NIS and a catalytic amount of TfOH at $0^\circ C$ provided the protected nonasaccharide **26** in 64% yield. In contrast, use of the corresponding 4,6-benzyliden acetal-protected tetrasaccharide **5** resulted in a complex reaction mixture. These results indicate that 4,6-di-*O*-benzyl protection on the galactoside is important for the synthesis of the compact and rigid branched structure from the sialyl galactoside. Deprotection of the protected nonasaccharide **26** was achieved using a three-

Scheme 3



Reagents and conditions: (a) **4** (2.0 equiv), NIS, cat. TfOH, MS4A, $0^\circ C$, 64%; (b) LiOH·H₂O, Dioxane, H₂O, $80^\circ C$; (c) Ac_2O , NaHCO₃, H₂O then LiOH, H₂O; (d) Pd(OH)₂, H₂, MeOH, H₂O, 53% from **26**.

step procedure: (i) hydrolysis of acyl protecting groups; (ii) acetylation of the C5 amino groups; and (iii) hydrogenolysis of the resulting benzyl ethers to provide the fully deprotected nonasaccharide **3** in 53% yield based on **26**.

In conclusion, we describe an efficient convergent synthesis of the GP1c epitope **3**. The reactivity of the saccharide structure was tuned by careful selection of a protecting group to match the requisite glycosidation and glycosylation. Various glycosyl acceptors and donors with different oligosialoglycans can be prepared by use of two 5*N*,4*O*-carbonyl-protected thiosialosides. The method described herein is amenable to the synthesis of a-, b-, and c-series ganglioside epitopes.

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Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- Details were described in the Supporting Information.

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