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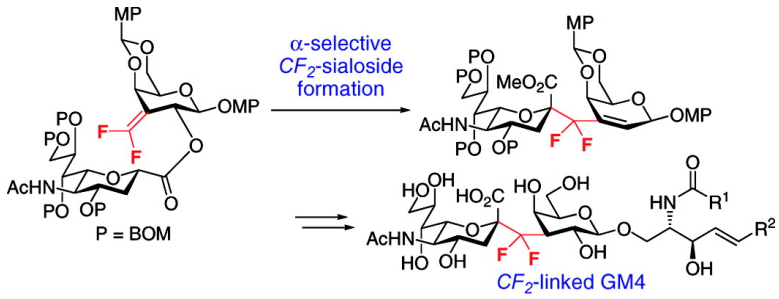

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Stereocontrolled and Convergent Entry to CF_2-Sialosides: Synthesis of CF_2-Linked Ganglioside GM4

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Gangliosides (sialic acid-containing glycosphingolipids) are known to be involved in intra- and intercellular cell signaling.[1,2] For example, GM3 binds to epidermal growth factor receptor (EGFR) and inhibits EGFR-dependent cell proliferation.[3] Among the four known human sialidases (neuraminidase),[4] NEU2, NEU3, and NEU4 but not NEU1 cleave the glycosidic linkage of sialic acid in gangliosides.[4a,5] Plasma membrane-associated sialidase (NEU3) seems to play a critical role in cell survival because it is upregulated in human cancer cells and tissues.[6] However, the physiological roles of gangliosides are still not fully clarified, partly because rapid metabolism of gangliosides makes biological research difficult. For example, sialidases hydrolyze GM3 and GM4 (**1**) to lactosylceramide and galactosylceramide,[7] which show different biological activities.[8] Therefore, chemically and biologically stable ganglioside analogues would be very useful as probes for research to elucidate the roles of these gangliosides in normal and cancer cells. α-Difluorophosphonate derivatives are excellent nonhydrolyzable phosphate ester mimics, and the difluoromethylene group is now recognized as bioisosteric to an oxygen atom.[9] Therefore, we envisioned the synthesis of difluoromethylene-linked (CF_2-linked) α(2,3)sialylgalactose (Figure 1) as a core structure of sialidase-resistant ganglioside mimics,[10] because the α(2,3)sialylgalactose structure is a key structure of not only GM3 and GM4, but also most other gangliosides.

Various *C*-glycoside compounds, in which the oxygen atom of the glycosidic linkage is replaced by a carbon atom, have already been synthesized as hydrolytically stable glycoside mimics,[11] but efficient and stereoselective synthesis of *C*-sialoside is difficult, because the anomeric position of the sialoside (*C*2′) is a tetrasubstituted carbon center.[12] To our knowledge, only two methodologies for the stereoselective synthesis of the *C*-linked α(2,3)sialylgalactose have been developed,[13] and synthesis of CF_2-linked α(2,3)sialylgalactose has not been reported. Linhardt and co-workers have reported a convergent and stereoselective synthesis of *CH(OH)*-linked α(2,3)sialylgalactose derivatives based on the SmI_2-mediated coupling reaction[13a,14] of the sialylsulfone derivative with the aldehyde prepared from galactose. First, we examined transformation of their *CH(OH)*-linked α(2,3)sialylgalactose to CF_2-linked α(2,3)sialylgalactose. However, this was unsuccessful, probably due to the steric hindrance around the *C*-glycoside carbon linkage.[15] Therefore, for the synthesis of the CF_2-linked α(2,3)-sialylgalactose unit, a new strategy was required. Herein we report a convergent and highly stereoselective method for the construction of the CF_2-linked α(2,3)sialylgalactose unit, and the conversion of this unit to the CF_2-linked analogue of ganglioside GM4 (**2**).[16]

As shown in Figure 2, we planned to construct the key C2′–CF_2 bond via Ireland–Claisen rearrangement of the ester **III**.[17] We anticipated that rearrangement would occur from the α-face of the anomeric center (*C*2′) through the chairlike transition state **II** after formation of the (*Z*)-silyl enolate. The resulting product **I** could be converted to the desired CF_2-α(2,3)sialylgalactose unit **3** in a stereocontrolled manner.

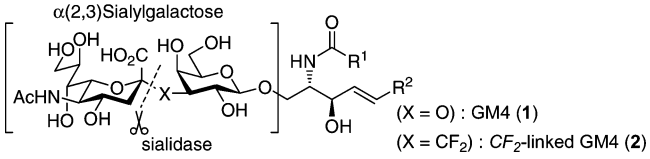

Figure 1. Structure of GM4 (**1**) and CF_2-linked GM4 (**2**).

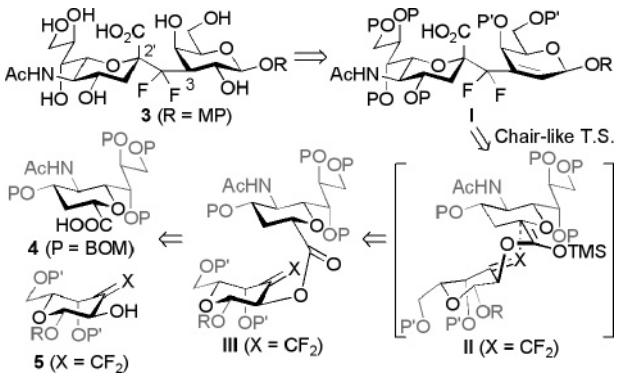

Figure 2. Strategy for the stereocontrolled synthesis of CF_2-sialoside.

The precursor for the Ireland–Claisen rearrangement **9** was prepared by condensation of **4**[18] with **8**, which was prepared from **6**[15] as shown in Scheme 1. Ireland–Claisen rearrangement of **9** proceeded smoothly even at ambient temperature on treatment with LHMDS and TMSCl in THF. After treatment with TMS–diazomethane, methyl ester **10** possessing a α-CF_2-sialoside linkage was obtained as a single isomer in 86% yield. The strong HMBC correlation between α3′-H and 1′-C indicated that the C2′ tetrasubstituted carbon center has the desired α-stereochemistry.

To achieve stereocontrolled introduction of a C2 hydroxyl group into the galactose unit, **10** was converted to the conformationally fixed lactone **11**. After removal of *p*-methoxybenzylidene acetal, protection of the 6-OH group with TBS, and saponification of the methyl ester, the desired lactone **11** was obtained in good yield by treatment with carbodiimide (Scheme 2). Although attempts at hydroboration or epoxidation of **11** were unsuccessful, we were pleased to find that dihydroxylation of **11** using a stoichiometric amount of OsO_4 proceeded in a completely stereoselective manner to afford the desired diol **12**. Regio- and stereoselective reduction of the C3-hydroxyl group was successfully achieved by radical reduction of the cyclic thiocarbonate. Namely, treatment of **12** with thiophosgene, followed by AIBN and Bu_3SnH, gave **13** as a single isomer. Hydrogenolysis of the four BOM groups, together with removal of the TBS group, afforded the key 4-methoxyphenyl CF_2-linked α(2,3)sialylgalactose lactone unit **14**. The stereochemical assignment of the newly formed chiral carbon centers (C2′, C2, and C3) was confirmed by X-ray crystallographic analysis of **14**.

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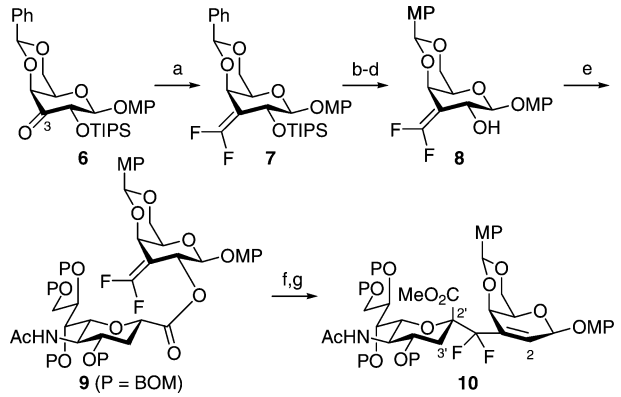
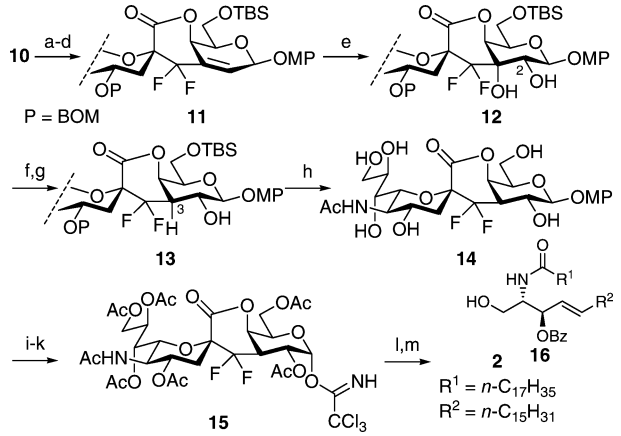
Scheme 1 [a]



[a] Reaction conditions: (a) CF_2Br_2, HMPT, THF, rt, 4 h, 76% (see ref 19); (b) conc. HCl, MeOH−CH_2Cl_2 (4:1), rt, 92%; (c) (4-OMe)Ph-$CH(OMe)_2$, TsOH, CH_3CN, rt; (d) TBAF, THF, rt, 74% (two steps); (e) EDC·HCl, DMAP, **4**, CH_2Cl_2, rt, 72%; (f) LHMDS, TMSCl, THF, −78 °C then rt; (g) $TMSCHN_2$, Et_2O−MeOH (1:1), 86% (two steps).

Scheme 2 [a]



[a] Reaction conditions: (a) 80% AcOH aq., rt, 80%; (b) TBSCl, Et_3N, DMAP, CH_2Cl_2, rt, 89%; (c) 2 M KOH aq., THF, 60 °C; (d) EDC·HCl, DMAP, CH_2Cl_2, rt, 77% (two steps); (e) OsO_4 (1.7 equiv), pyridine, rt, then sat. $NaHSO_3$ aq., rt, 90%; (f) thiophosgene, DMAP, CH_2Cl_2, rt; (g) Bu_3SnH, AIBN, toluene, 100 °C, 84% (two steps); (h) $Pd(OH)_2$/C, H_2, MeOH, rt, 99%; (i) Ac_2O, pyridine, rt, 94%; (j) $Ce(NH_4)_2(NO_3)_6$, CH_3CN−H_2O (4:1), rt; (k) Cl_3CCN, DBU, CH_2Cl_2, rt, 75% (two steps); (l) **16**, TfOH, CH_2Cl_2, 0 °C, 33% (based on recovery); (m) NaOMe, MeOH, rt, then H_2O, rt, 60%.

The overall yield of this CF_2-linked sialoside unit from **6** was 13% (15 steps).

To demonstrate the potential of this novel CF_2-linked sialylgalactose unit **14** as an intermediate for the synthesis of ganglioside analogues, conversion of **14** to CF_2-linked GM4 (**2**) was performed. After acetylation of all hydroxyl groups, the MP group at the anomeric position was converted to trichloroacetoimidate to give a glycosyl donor **15**. Glycosylation of **15** with the ceramide derivative **16**[20] was conducted in the presence of TfOH in CH_2Cl_2. Finally, synthesis of **2** was completed by methanolysis and hydrolysis. Although the yield of glycosidation with ceramide needs to be improved, to our knowledge, this is the first synthesis of a ganglioside analogue containing a CF_2-sialoside linkage.

CF_2-linked GM4 (**2**) showed moderate inhibition of NEU2 (IC_{50} = 754 μM) and NEU4 (IC_{50} = 930 μM).[15] A preliminary study indicated that **2** also showed remarkable inhibition for human lymphocyte proliferation.[15,21] These results suggest that CF_2-sialoside can indeed act as a mimic of *O*-sialoside.

In conclusion, the CF_2-α(2,3)sialylgalactose unit was synthesized in a completely stereoselective manner, and this nonhydrolyzable sialylgalactose unit was confirmed to be suitable as a glycosyl donor for the synthesis of a GM4 analogue. This new strategy based on Ireland−Claisen rearrangement should be applicable not only for the synthesis of CF_2-sialylgalactose but also for the construction of various other (un)substituted *C*-sialoside units, which are expected to be useful for the synthesis of novel ganglioside-mimicking molecules. Synthesis of larger gangliosides, such as a GM3, and biological experiments using CF_2-linked ganglioside analogues are currently under way.

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Supporting Information Available: Experimental procedure, characterization of new compounds, biological evaluations, ^1H NMR and ^{13}C NMR spectra, and CIF file for compound **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

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