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Fluorous Oligosaccharide Synthesis Using a Novel Fluorous Protective Group

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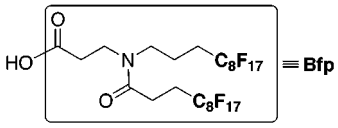
ABSTRACT



The Bfp (bisfluorous chain type propanoyl) group, a novel fluorous protecting reagent, was able to be prepared easily. The Bfp group was readily introduced to carbohydrate, was removed in high yield, and was recyclable after cleavage. Use of the Bfp group made it possible to synthesize a tetrasaccharide by minimal column chromatography purification. Each synthetic intermediate was able to be easily purified by using only simple fluorous-organic solvent extraction and was monitored by NMR, mass spectroscopy, and TLC.

The oligosaccharides on cell surfaces play important roles in biological processes, such as cell−cell interaction, cell adhesion, and immunogenic recognition.[1] The synthesis of oligosaccharides is very difficult, in contrast to peptides and nucleotides, which are easily prepared by a solid phase synthesis using an automatic synthesizer. The solid phase synthesis of oligosaccharides has also been actively studied.[2] Recently, the synthesis of oligosaccharides using a peptide synthesizer have been reported;[3] however, the development of a practical automatic oligosaccharide synthesizer has not yet been accomplished. In addition, the solid phase method suffers from some serious disadvantages, such as reduced reactivity and the inability to monitor the reaction by NMR, mass spectroscopy, and TLC. Recently, fluorous chemistry has been developed for use in several fields such as combinatorial chemistry, parallel synthesis, and catalytic chemistry.[4] A highly fluorinated compound is readily separated from nonfluorinated compounds by a simple fluorous-organic phase separation. A highly fluorinated compound is also soluble in common organic solvents and can be measured by NMR and mass spectroscopy as a single compound. Therefore, fluorous synthesis has become an excellent strategic alternative to solid phase synthesis. Highly fluorinated acetal, silyl, and benzyl protective groups for a hydroxyl function have already been reported.[5,6] Acetal protective groups cannot be used to synthesize oligosaccharides due to their lability to acid. Curran and co-workers reported the fluorous disaccharide synthesis using the fluorous benzyl protective group by a glycal method.[6] Unfortunately, their glycosylation method using a fluorous glycosyl donor gave only 2-deoxy disaccharides. In addition, the yield for the reaction step to introduce the perfluorinated benzyl group to the hydroxyl function was not satisfactory.

We would like to report here the development of a novel fluorous acyl protective group and its application to fluorous oligosaccharide synthesis. Our concept of fluorous oligosac-

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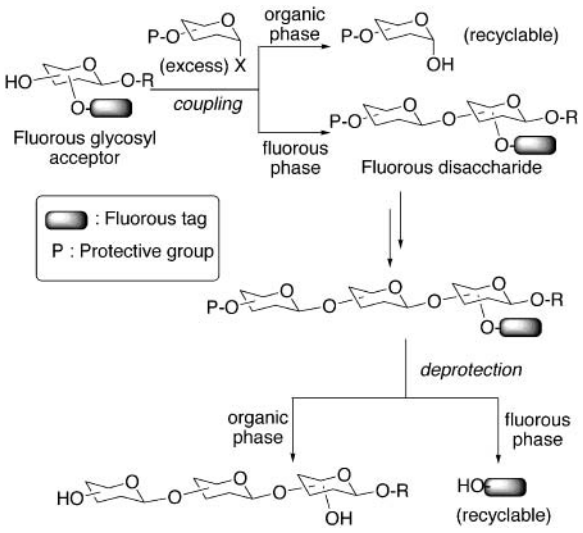
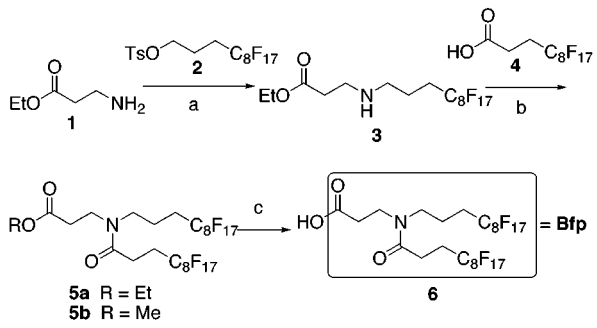


Figure 1. Concept of fluorous oil gosaccharide synthesis.

charide synthesis is shown in Figure 1. We adopted the introduction of a fluorous tag to the glycosyl acceptor but not to the glycosyl donor in order to efficiently synthesize the longer chain oligosaccharides. The glycosyl acceptor containing the fluorous tag couples with the glycosyl donor to afford the fluorous disaccharide. After the partition of the reaction mixture with fluorous and normal organic solvents, the fluorous disaccharide and the excess amount of the glycosyl donor are extracted by the fluorous phase and organic phase, respectively. After selective deprotection, repeating this procedure gives the fluorous oligosaccharide, which is able to be purified only by liquid−liquid extraction without column chromatography. Finally, the fluorous tag is removed to give the desired oligosaccharide extracted with an organic solvent. The fluorous tag is extracted by a fluorous solvent and is recyclable.

We designed and synthesized compound **6** as a novel fluorous acyl protecting reagent which is shown in Scheme 1. The reaction of the β-alanine ethyl ester (**1**) with fluorous

Scheme 1[a]

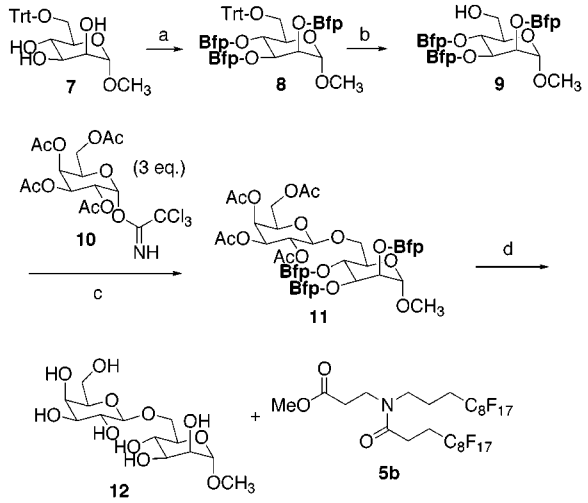


[a] Reagents and conditions: (a) K_2CO_3, MeCN, reflux, 17 h, 83%; (b) PyBOP, Et_3N, CH_2Cl_2, rt, 3 h, 93%; (c) 1 M NaOH, dioxane, 70 °C, 4 h, 98%.

tosylate **2**[7] provided the monoalkylating product **3** in 83% yield. Compound **3** was coupled with perfluorooctylpropionic acid (**4**[8]) to afford compound **5a** in 93% yield. The treatment of **5a** with aqueous sodium hydroxide gave the desired fluorous carboxylic acid **6**[9] in 98% yield. We thought that the two fluorous chains of **6** enhance the efficiency of the liquid−liquid extraction. Some methylene spacer might effectively block the strong electron-withdrawing effect of the long perfluoroalkyl chain without a decrease in the reactivity of the carboxylic group.

Among the many useful methods for glycosylation, we selected Schmidt's imidate method as the most popular synthetic method to make a natural oligosaccharide.[10] We first attempted to synthesize the disaccharide **12** as shown in Scheme 2. The Bfp (bisfluorous chain type propanoyl)

Scheme 2[a]



[a] Reagents and conditions: (a) **6**, DCC, DMAP, CH_2Cl_2, rt, 2 h, 87%; (b) CSA, MeOH−$CHCl_3$, rt, 19 h, 88%; (c) TMS-OTf, molecular sieves (AW-300), Et_2O, 0 °C, 1 h, 69%; (d) NaOMe, Et_2O−MeOH, rt, 1 h, 93%.

group was introduced to the three hydroxyl functions of the mannose derivative **7** using dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) to give **8** in 87% yield. The triphenylmethyl (Trt) group of **8** was removed by treatment of the camphorsulfonic acid (CSA) in MeOH−ether to afford the flourous glycosyl acceptor **9** in 88% yield after purification by FC72[11]−toluene extraction. The reason-

(7) The tosylate **2** was prepared from perfluorooctylpropanol (DAIKIN, Tokyo) according to a literature procedure. Pozzi, G.; Cavazzini, M.; Quici, S.; Fontana, S. *Tetrahedron Lett.* **1997**, *38*, 7605.

(8) Compound **4** was prepared from perfluorooctylpropanol by Jones oxidation.

(9) Compound **6**: white powder, ^{1}H NMR (400 MHz, $CDCl_3$−CD_3OD = 5: 3): δ = 1.92 (m, 2H), 2.15 (m, 2H), 2.65 (m, 6H), 3.49 (m, 2H), 3.66 (m, 2H). MALDI-TOF-MS: calcd for $C_{25}H_{16}F_{34}NO_3$ (M + H^+) 1024.1, found 1022.6; calcd for $C_{25}H_{15}F_{34}NO_3Na$ (M + Na^+) 1046.1, found 1044.6.

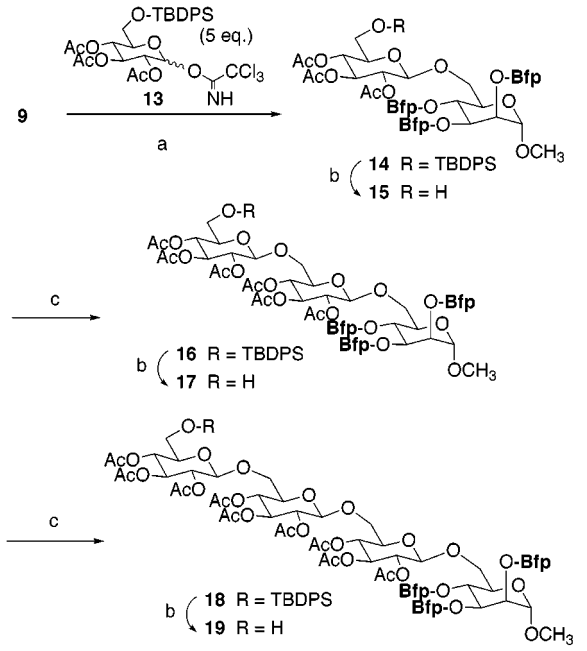
(10) Schmidt, R. R. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212. Schmidt, R. R. *Pure Appl. Chem.* **1989**, *61*, 1257.

(11) FC72 is a commercially available fluorocarbon solvent (3M, Tokyo), which consists of perfluorohexane (C_6F_{14}) isomers.

ably pure fluorous disaccharide **11**[12] was obtained in 69% yield by the reaction of **9** with 3 equiv of the galactose derivative **10** in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-OTf) in ether, followed by FC72−toluene extraction after a normal workup. The galactose derivative **10** used in excess was obtained as the 1-hydroxyl form of **10** from the toluene extract. Furthermore, we confirmed the deprotection of the Bfp group in the usual manner using NaOMe. After FC72−MeOH extraction, the disaccharide **12** was obtained in 93% yield from the MeOH layer, and the methyl ester **5b** was obtained in 92% yield from the FC72 layer. Treatment of the methyl ester **5b** with aqueous NaOH gave **6** that was able to be reused as the fluorous protecting reagent.

To clarify the partition efficiency of the oligosaccharide having the Bfp group as a fluorous tag, we synthesized the longer chain oligosaccharide as shown in Scheme 3. The

Scheme 3[a]



[a] Reagents and conditions: (a) TMS-OTf, molecular sieves (AW-300), Et_2O, 0 °C, 1 h; (b) HF−Py, THF, rt, 24 h; (c) 13 (8−20 equiv), TMS-OTf, molecular sieves (AW-300), Et_2O, 0 °C, 1 h.

reaction of the fluorous glycosyl acceptor **9** with 5 equiv of the glucose derivative **13** in the presence of TMS-OTf in ether, followed by FC72−toluene extraction, afforded the disaccharide **14**[13] in 75% yield from the FC72 layer. The glucose derivative **13** used in excess was obtained as the 1-hydroxyl form of **13** from the toluene extract. The *tert*-butyldiphenylsilyl (TBDPS) group of **14** was removed by treatment with HF−pyridine in THF to give the pure fluorous glycosyl acceptor **15**[13] that was extracted with FC72 by partition between FC72, water, and toluene. The disaccharide **15** coupled with the glycosyl donor **13** under similar Schmidt's conditions to afford the crude trisaccharide **16**,[13] which was extracted with FC72 by being partitioned between FC72 and toluene. The analysis of the crude trisaccharide **16** by ^{1}H NMR spectroscopy and TLC showed that it contained the starting glycosyl acceptor **15** (22%). For characterization of **16**, the crude trisaccharide **16** was purified by silica gel chromatography to provide pure **16**[14] in 50% yield.[15] Treatment of pure **16** with HF−pyridine in THF gave pure **17**,[13] which was extracted with FC72 by being partitioned between FC72, water, and toluene. The coupling of the trisaccharide **17** with **13** by a similar glycosylation provided the tetrasaccharide **18**, which was extracted with toluene by being partitioned between FC72 and toluene. The unreacted **17** was easily separated from **18** only by partition between FC72 and toluene. The toluene extract containing **18** and the 1-hydroxyl form of **13** was treated with HF−pyridine in THF to afford the tetrasaccharide **19**,[13] which was extracted with FC72 by being partitioned between FC72 and toluene. The yield of **19** was 10% (two steps) from **17** and was dependent on the glycosylation step.[15] Although the tetrasaccharide **18** was not extracted with FC72 at all, the deprotected tetrasaccharide **19** was easily extracted with FC72 by three Bfp groups. To effectively extract longer oligosaccharides, the development of other types of fluorous protective groups and the use of fluorous silica gel[16] are now in progress.

In conclusion, the use of the Bfp group as a fluorous protective group made it possible to synthesize a natural oligosaccharide by minimal column chromatography purification. Each synthetic intermediate was able to be easily purified by simple FC72−organic solvent extraction and monitored as a single compound by NMR, mass spectroscopy, and TLC in contrast to the solid phase synthesis. The fluorous protecting reagent **6** (Bfp-OH) was able to be easily prepared on a large scale. The Bfp group was readily introduced to the carbohydrate hydroxyl functions, was removed in high yield by the usual procedure, and was recyclable after cleavage. With only three Bfp groups was it possible to extract the derivative of the tetrasaccharide with the FC72 phase. Further application to the synthesis of a bioactive carbohydrate and glycoconjugate is now in progress.

(12) For characterization of **11**, the reasonably pure **11** (90% purity) was purified by silica gel chromatography to provide pure **11**. Compound **11**: colorless oil, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.88 (m, 6H), 1.97 (s, 3H), 2.04 (s, 3H), 2.06 (s, 3H), 2.10 (m, 6H), 2.13 (s, 3H), 2.56 (m, 18H), 3.41 (s, 3H), 3.44 (m, 6H), 3.64 (m, 7H), 3.92 (m, 3H), 4.09 (m, 1H), 4.20 (m, 1H), 4.45 (m, 1H), 4.74 (m, 1H), 5.05 (m, 1H), 5.22 (m, 4H), 5.41 (m, 1H). MALDI-TOF-MS: calcd for $C_{96}H_{71}F_{102}N_3O_{21}Na$ ($M + Na^+$) 3562.2, found 3561.1; calcd for $C_{96}H_{71}F_{102}N_3O_{21}K$ ($M + K^+$) 3578.4, found 3577.2.

(13) Compounds **14**, **15**, **16**, **17**, and **19** were not detected by TLC from the toluene layer after three extractions with FC72. These results show that these compounds were quantitatively extracted with FC72.

(14) Compound **16**: colorless oil, ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.04 (s, 9H), 1.88 (s, 3H), 1.90 (m, 6H), 1.93 (s, 3H), 1.98 (s, 6H), 2.05 (s, 3H), 2.06 (s, 3H), 2.08 (m, 6H), 2.56 (m, 13H), 2.72 (m, 5H), 3.39 (s, 3H), 3.43 (m, 7H), 3.54 (m, 8H), 3.72 (m, 3H), 3.92 (m, 2H), 4.07 (m, 1H), 4.50 (m, 2H), 4.74 (brs, 1H), 4.87−5.31 (m, 9H), 7.40 (m, 6H), 7.65 (m, 4H). MALDI-TOF-MS: calcd for $C_{122}H_{103}F_{102}N_3O_{28}SiNa$ ($M + Na^+$) 4046.5, found 4045.1.

(15) Compounds **16** and **18** were obtained without complete optimization of glycosidation conditions.

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

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