Fluorous Oligosaccharide Synthesis

Oligosaccharide Synthesis on a Fluorous Support**

*Tsuyoshi Miura, Kohtaro Goto, Daisuke Hosaka, and Toshiyuki Inazu**

A fluorous solvent such as perfluorohexane is insoluble in most organic solvents and in water, and a mixture of these three solvent types will form three layers. A highly fluorinated (fluorous) compound is soluble in fluorous solvents, and is readily separated from nonfluorinated compounds through simple fluorous–organic solvent partitioning. Since Horváth and Rabái used these properties to introduce the concept of the fluorous biphasic system in 1994,[1] fluorous chemistry has mainly developed in the field of catalysis.[2] Curran and co-workers have also investigated fluorous synthesis as a strategic alternative to solid-phase synthesis for use in several fields such as combinatorial chemistry and parallel synthesis.[3] Recently, they also reported fluorous-mixture synthesis using a fluorous silica gel.[4] Several fluorous protecting groups, which are essential for the performance of fluorous synthesis, have been reported. However, their application is limited to the synthesis of small molecules because the fluorine content in each fluorous protecting group is low.[5] Herein we describe the development of a fluorous support with a high fluorine content and its application in the synthesis of oligosaccharides.

The oligosaccharides on cell surfaces play important roles in biological processes such as cell–cell interaction, cell adhesion, and immunogenic recognition.[6] However, the synthesis of an oligosaccharide is not easy. Although the solid-phase synthesis of oligosaccharides has been actively studied,[7] the usual solid-phase method suffers from some serious disadvantages, such as the difficulty of large-scale synthesis, reduced reactivity, and the inability to monitor the reaction by TLC, NMR spectroscopic analysis, or mass spectrometry. Solid-phase oligosaccharide synthesis on a soluble polyethylene glycol (PEG)-based support has been reported.[8] Although this improved solid-phase method overcomes some of the disadvantages of traditional solid-phase methods, reactions that use the soluble PEG-based support can not be monitored by TLC and the intermediates can not be purified by silica-gel column chromatography. Curran and co-workers have reported a 2-deoxydisaccharide synthesis using a fluorous benzyl protecting group.[5h] Recently, we

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reported a method for fluorous oligosaccharide synthesis involving the novel fluorous protecting reagent **1** (Bfp-OH), which contains two fluorous chains.[9] Fluorous oligosaccharide synthesis avoids the disadvantages of solid-phase oligosaccharide synthesis. The introduction of the fluorous protecting group Bfp at three or four hydroxy groups on the glycosyl acceptors allowed us to rapidly synthesize a simple tetrasaccharide[9] as well as the Gb3 oligosaccahride that is bound to verotoxins.[10]

Herein we report the development of a novel fluorous support as an alternative to a solid support and its application in oligosaccharide synthesis. Our concept of oligosaccharide synthesis using a fluorous support is shown in Figure 1. Introduction of the fluorous support only at the anomeric hydroxy functionality of the glycosyl acceptor enables the efficient synthesis of free, long-chain oligosaccharides. The glycosyl acceptor bound to the fluorous support couples with the glycosyl donor to afford the fluorous disaccharide. The reaction mixture is partitioned between fluorous and normal organic solvents, such that the fluorous disaccharide and the excess glycosyl donor are extracted into the fluorous phase and the organic phase, respectively. After selective deprotection, repetition of the above procedure produces the fluorous oligosaccharide, which can be purified by liquid–liquid extraction, without the need for column chromatography. Finally, the fluorous support is removed to give the desired oligosaccharide, which can be extracted from the mixture with a common organic solvent. The fluorous support can be extracted with a fluorous solvent and is recyclable. Oligosaccharide synthesis using a fluorous support is similar to the solid-phase synthesis procedure shown in Figure 2. Liquid–liquid extraction is used for the purification steps in fluorous oligosaccharide synthesis, whereas filtration is used in solid-phase synthesis. Fluorous oligosaccharide synthesis is thus an excellent strategic alternative to solid-phase synthesis.

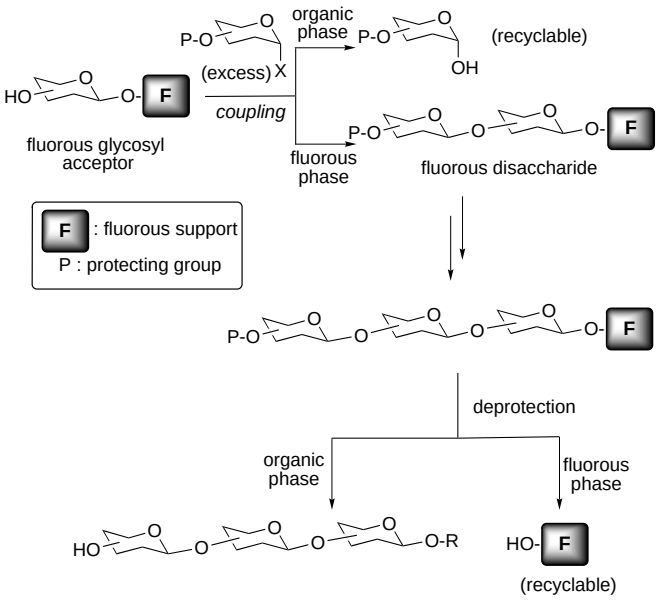


Figure 1. Concept of oligosaccharide synthesis using a fluorous support.

A fluorous support with more fluorous chains than previously used would enhance the performance of the procedure described above. Therefore, we designed and synthesized compound **8**, which contains six fluorous chains, as a novel fluorous support (Scheme 1). We expected the six fluorous chains of **8** to enhance the efficiency of extraction into the fluorous solvent significantly. The reaction of Bfp-OH (**1**)[9] with the fluorous amine **2**[9] provided compound **3** in 96% yield. Treatment of **3** with aqueous NaOH gave the fluorous carboxylic acid **4** with three fluorous chains in 99% yield. The two primary amino functions of diethylenetriamine (**5**) were protected with triphenylmethyl groups, and the resulting intermediate was coupled with methyl hydrogen glutarate to afford compound **6**. Treatment of **6** with hydrochloric acid gave the hydrochloric acid salt **7**. Compound **7** was coupled with **4** (2 equiv), followed by treatment with aqueous NaOH to give the fluorous support **8** (M_W = 3290) with six fluorous chains in good yield. We named the

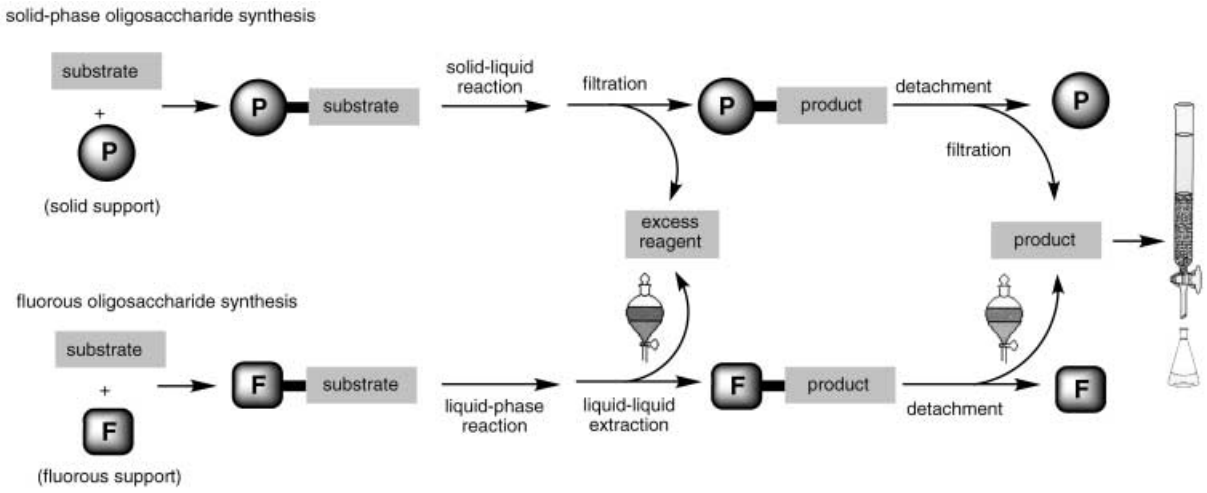
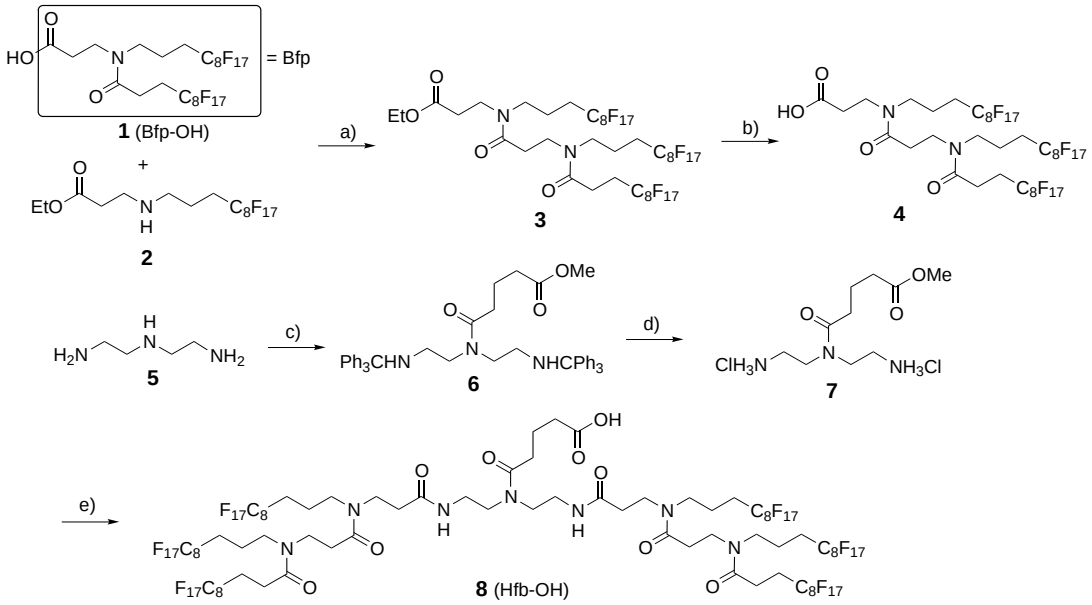


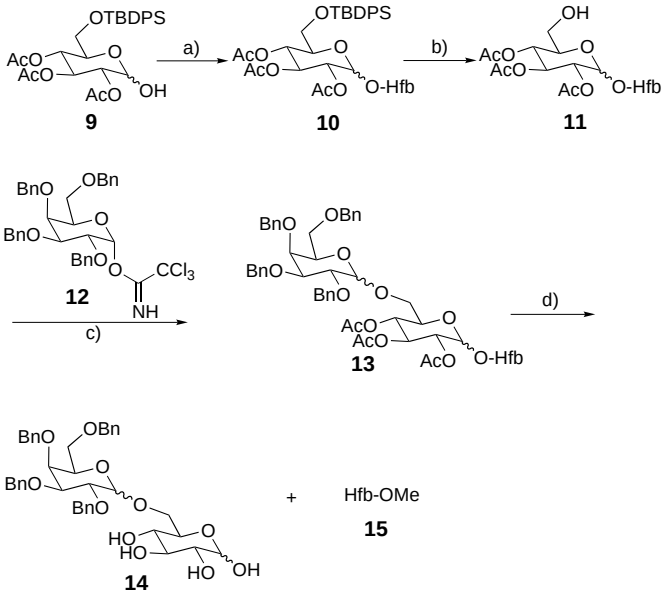
Figure 2. Comparison of fluorous oligosaccharide synthesis with solid-phase oligosaccharide synthesis.



Scheme 1. Reagents and conditions: a) PyBOP, Et_3N, CH_2Cl_2, room temperature, 20 h, 96%; b) NaOH (1 M), dioxane, 50°C, 4 h, 99%; c) 1) Ph_3CCl, Et_2NH, *i*PrOH, room temperature, 21 h, 95%; 2) monomethyl glutarate, PyBOP, Et_3N, CH_2Cl_2, room temperature, 4 h, 87%; d) HCl, dioxane, 60°C, 16 h, 98%; e) 1) **4**, PyBOP, Et_3N, CH_2Cl_2, room temperature, 24 h, 81%; 2) NaOH (1 M), dioxane, 50°C, 24 h, 98%. PyBOP = benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate.

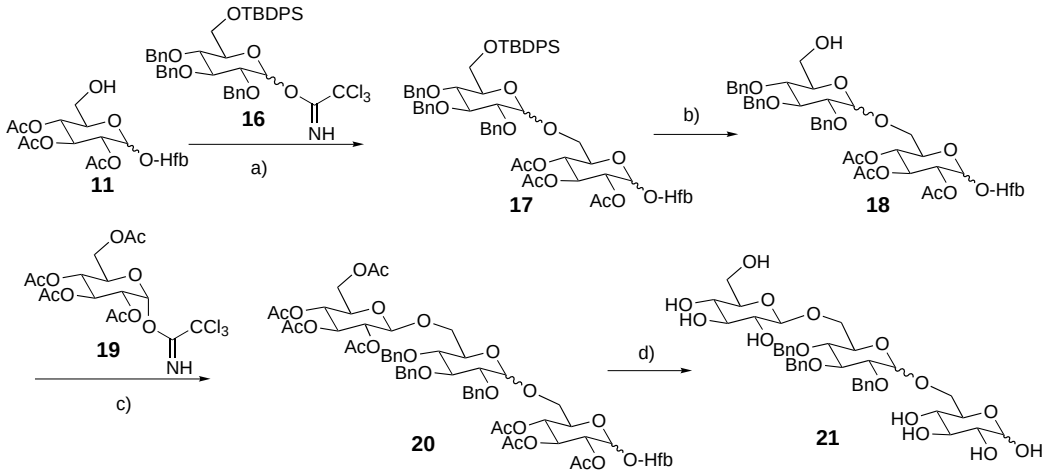
acyl moiety of **8** the Hfb (hexakisfluorous chain type butanoyl) group.

We synthesized the disaccharide **14** as shown in Scheme 2. The fluorous support Hfb was attached to the anomeric hydroxy group of the glucose derivative **9** by using PyBOP and DMAP to give the fluorous compound **10**.[11] The TBDPS group of **10** was removed by treatment with HF–pyridine in THF to afford the fluorous glycosyl acceptor **11**.[11] The fluorous disaccharide **13**[11,12] was obtained by the reaction of **11** with the glycosyl donor **12** (9 equiv)[13] in the presence of TMSOTf in $Et_2O/EtOC_4F_9$.[14] The fluorous intermediates **10, 11**, and **13** were each extracted with the fluorous solvent FC-72[15] by partitioning the product mixtures between FC-72 and an organic solvent. No further purification such as silica gel column chromatography was carried out. The Hfb group of **13** was removed by treatment with NaOMe in $MeOH/EtOC_4F_9$ to afford crude **14**, which was extracted with MeOH by partitioning the mixture between FC-72 and MeOH. The methyl ester of Hfb (Hfb-OMe, **15**) was obtained from the FC-72 layer. Compound **15** was treated with aqueous NaOH to give **8**, which was reused. Finally, the pure disaccharide **14**[16] was obtained from a single silica-gel column-chromatographic purification step in 67% overall yield from **9** (four steps).



Scheme 2. Reagents and conditions: a) **8**, PyBOP, DMAP, CH_2Cl_2, room temperature, 20 h; b) HF–pyridine, THF, room temperature, 20 h; c) TMSOTf, molecular sieves (4 Å), $EtOC_4F_9$, Et_2O, 0°C, 20 min; d) NaOMe, $EtOC_4F_9$, MeOH, room temperature, 16 h, then silica-gel chromatography, 67% from **9**. Bn = benzyl, DMAP = 4-dimethylaminopyridine, TBDPS = *tert*-butyldiphenylsilyl, TMSOTf = trimethylsilyl trifluoromethanesulfonate.

Next, we synthesized the trisaccharide **21** as shown in Scheme 3. The fluorous glycosyl acceptor **11** was coupled with the glycosyl donor **16** (9 equiv)[17] to afford the fluorous disaccharide **17**.[11,18] The TBDPS group of **17** was removed by treatment with HF–pyridine in THF to give the fluorous compound **18**.[11] The reaction of the fluorous glycosyl acceptor **18** with the glycosyl donor **19** (20 equiv)[13] under similar glycosidation conditions afforded the fluorous trisaccharide **20**.[11] The intermediates **17, 18**, and **20** were isolated in analogy with **10, 11**, and **13**. The pure trisaccharide **21**[19] was obtained in the same way as **14** in 42% overall yield from **9** (six steps).



Scheme 3. Reagents and conditions: a) TMSOTf, molecular sieves (4 Å), $EtOC_4F_9$, Et_2O, 0 °C, 20 min; b) HF–pyridine, THF, room temperature, 22 h; c) TMSOTf, molecular sieves (4 Å), $EtOC_4F_9$, Et_2O, 0 °C, 2 min; d) NaOMe, $EtOC_4F_9$, MeOH, room temperature, 14 h, then silica-gel chromatography, 42% from **9**.

In conclusion, although the introduction of several Bfp groups into the glycosyl acceptor was required for fluorous oligosaccharide synthesis,[9] the attachment of the fluorous support Hfb through just the anomeric hydroxy functionality of the glycosyl acceptor allowed efficient oligosaccharide synthesis because the fluorine content in the Hfb group is very high. The fluorous support reagent **8** (Hfb-OH) could be prepared on a large scale. The Hfb support could be removed in high yield under standard dilute basic conditions for the deprotection of acetyl or benzoyl groups, and was recyclable after cleavage. The use of the fluorous support Hfb made it possible to synthesize an oligosaccharide rapidly, without the need for purification of the fluorous intermediates by column chromatography. Each fluorous synthetic intermediate could be obtained in a straightforward manner by simple fluorous–organic solvent extraction, and the reactions could be monitored by TLC, NMR spectroscopic analysis, and mass spectrometry, in contrast to solid-phase reactions. Thus, the reaction conditions for each synthetic step could be optimized rapidly. Although the fluorous intermediates could also be subjected to silica-gel column chromatography if necessary, only the final compounds were purified by chromatography. This fluorous oligosaccharide synthesis should be applicable to large-scale synthesis as it is performed in the liquid phase. Thus, oligosaccharide synthesis on the fluorous support Hfb is an excellent strategic alternative to solid-phase oligosaccharide synthesis. The optimization of the glycosidation conditions and further applications to the synthesis of several bioactive carbohydrates and glycoconjugates are now in progress.

Experimental Section

10: DMAP (243 mg, 1.98 mmol) and PyBOP (1.03 g, 1.98 mmol) were added to a solution of **9** (1.00 g, 1.84 mmol) and Hfb-OH (**8**, 2.17 g, 0.660 mmol) in dry CH_2Cl_2 (110 mL). The mixture was stirred for 20 h at room temperature, then MeOH (110 mL) was added and the CH_2Cl_2 was evaporated. The reaction mixture was then extracted with FC-72 (3 × 110 mL) and the combined FC-72 layers were concentrated. Crude **10** (2.45 g) was used in the next step without further purification.

11: HF–pyr (4.45 ml, 159 mmol) was added to a solution of crude **10** (2.45 g) in THF (38 mL). The resulting mixture was stirred for 20 h at room temperature, and then added to saturated aqueous $NaHCO_3$ (150 ml). Toluene (150 mL) was added to the reaction mixture, which was then extracted with FC-72 (3 × 150 mL). The FC-72 layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated. Crude **11** (2.30 g) was used in the next step without further purification.

13: Powdered molecular sieves (4 Å, 1.4 g) were added to a solution of crude **11** (395 mg) and **12** (680 mg, 0.992 mmol) in a mixture of dry Et_2O (6.0 mL) and $EtOC_4F_9$ (3.2 mL) under an argon atmosphere. The resulting mixture was stirred for 2 h at room temperature, then TMSOTf (140 μL, 0.773 mol) was added at 0 °C. The mixture was stirred for a further 20 min at 0 °C, then triethylamine (0.5 mL) was added and the reaction mixture was filtered. The filtrate was added to saturated $NaHCO_3$, and the mixture was extracted three times with EtOAc. The EtOAc layers were washed with brine, dried over anhydrous Na_2SO_4, and evaporated. The residue was partitioned between MeOH and FC-72. The FC-72 layer was concentrated to give crude **13** (372 mg), which was used in the next step without further purification.

14: A solution of NaOMe in MeOH (28%, 10 μL) was added to a solution of crude **13** (349 mg) in a mixture of $EtOC_4F_9$ (8 mL) and MeOH (16 mL). The reaction mixture was stirred for 16 h at room temperature, then amberlite IR-120 (H^+ form) was added, and the mixture was neutralized. The mixture was then filtered, and the filtrate was concentrated. The residue was partitioned between MeOH and FC-72. The MeOH layer was concentrated to give crude **14**. The FC-72 layer was evaporated to afford pure **15**. The crude **14** was purified by column chromatography on silica gel to give pure **14** (50 mg, 67% over four steps) as a white powder.

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[11] Product mixtures containing the fluorous compounds **10, 13**, and **20** were partitioned between FC-72 and MeOH. Those containing the fluorous compounds **11** and **18** were partitioned between FC-72, toluene, and water. The product mixture containing the fluorous compound **17** was partitioned between FC-72 and acetonitrile. None of the fluorous compounds were detected by TLC of the organic layer after three extractions with FC-72, which shows that these compounds were quantitatively extracted with FC-72.

[12] The anomer ratio of compound **13** (at the newly formed anomeric position) was determined to be $\alpha/\beta = 50{:}50$ by NMR spectroscopic analysis.

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[14] $EtOC_4F_9$ is a commercially available fluorocarbon solvent (3M, Tokyo) called Novec HFE-7200.

[15] FC-72 is a commercially available fluorocarbon solvent (3M, Tokyo) called Fluorinert FC-72, which consists of perfluorohexane (C_6F_{14}) isomers.

[16] Compound **14** was identified by comparison of spectroscopic data of the debenzylated products (melibiose and allolactose) with those of authentic samples: K. Bock, C. Pedersen, H. Pedersen, *Adv. Carbohydr. Chem. Biochem.* **1983**, *42*, 193; K. Ajisaka, H. Fujimoto, H. Nishida, *Carbohydr. Res.* **1988**, *180*, 35.

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