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Rapid Oligosaccharide Synthesis Using a Fluorous Protective Group

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The Bfp-OH, a novel fluorous protecting reagent, was able to be easily prepared. The Bfp group was readily introduced to a carbohydrate, removed in high yield, and recyclable after cleavage. The use of the Bfp group made it possible to synthesize a pentasaccharide by minimal column chromatography purification. Each synthetic intermediate was able to be easily purified only by simple fluorous−organic solvent extraction and monitored by TLC, NMR, and MS.

Introduction

The oligosaccharides on cell surfaces play important roles in biological processes such as cell−cell interactions, cell adhesion, and immunogenic recognition.[1] However, the synthesis of oligosaccharides is not easy, in contrast to peptides and nucleotides, which are easily prepared by a solid-phase synthesis using a commercially available automatic synthesizer. Although the solid-phase synthesis of oligosaccharides has also been actively studied,[2] the usual solid-phase method suffers from some serious disadvantages, such as reduced reactivity, the difficulty of large-scale synthesis, and the inability to monitor the reaction by TLC, NMR spectroscopic analysis, or mass spectrometry.

A fluorous solvent such as perfluorohexane is insoluble in most organic solvents and water, and three layers are formed. A highly fluorinated (fluorous) compound exhibits a high solubility for fluorous solvents and is readily separated from nonfluorinated compounds by the simple fluorous−organic solvent partition. Since Horváth and Rabái used these properties to introduce the concept of the fluorous biphasic system in 1994,[3] fluorous chemistry has been developed for use in several fields such as combinatorial chemistry, parallel synthesis, and catalytic chemistry.[4,5] Curran and co-workers elaborated the fluorous synthesis (fluorous-tag method) as a strategic alternative to solid-phase synthesis.[5] Recently, they have also reported a fluorous mixture synthesis using a fluorous silica gel.[6] The fluorous protecting groups are essential for the fluorous synthesis performance. Several fluorous oxygen protecting groups such as acetal, silyl, and benzyl groups have already been reported.[7] Other fluorous protecting groups for amino and carboxyl functions have also been reported.[8] Curran and co-workers reported the fluorous disaccharide synthesis using the fluorous benzyl protective group by a glycal method.[7e] Unfortunately, their glycosylation method using a fluorous glycosyl donor gave only the 2-deoxy disaccharides. In addition, the yield for the reaction step to introduce the fluorous benzyl group to the hydroxyl function was

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(1) (a) Varki A. *Glycobiology* **1993**, *3*, 97. (b) Dwek, R. A. *Chem. Rev.* **1996**, *96*, 683. (c) Blithe, D. L. *Trends Glycosci. Glycotech.* **1993**, *5*, 81.

(2) (a) Manabe, S.; Ito, Y. *J. Am. Chem. Soc.* **2002**, *124*, 12638. (b) Ando, H.; Manabe, S.; Nakahara, Y.; Ito, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3848. (c) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, *291*, 1523. (d) Eichler, E.; Yan, F.; Sealy, J.; Whitfield, D. M. *Tetrahedron* **2001**, *57*, 6679. (e) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2510. (f) Douglas, S. P.; Whitfield, D. M.; Krepinsky, J. J. *J. Am. Chem. Soc.* **1995**, *117*, 2116; and references therein.

(3) Horváth, I. T.; Rábai, J. *Science* **1994**, *266*, 72.

(4) (a) Tzschucke, C. C.; Markert, C.; Bannwarth, W.; Roller, S.; Hebel, A.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 3964. (b) Nishikido, J.; Kamishima, M.; Matsuzawa, H.; Mikami, K. *Tetrahedron* **2002**, *58*, 8345. (c) Rocaboy, C.; Gladysz, J. A. *Org. Lett.* **2002**, *4*, 1993. (d) Nakamura, Y.; Takeuchi, S.; Okumura, K.; Ohga, Y. *Tetrahedron* **2001**, *57*, 5565. (e) Barrett, A. G. M.; Braddock, D. C.; Catterick, D.; Chadwick, D.; Henschke, J. P.; McKinnell, R. M. *Synlett* **2000**, 847. (f) Horváth, I. T. *Acc. Chem. Res.* **1998**, *31*, 641 and references therein.

(5) (a) Zhang, Q.; Luo, Z.; Curran, D. P. *J. Org. Chem.* **2000**, *65*, 8866. (b) Curran, D. P. *Pure Appl. Chem.* **2000**, *72*, 1649. (c) Curran, D. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 1174 and references therein.

(6) (a) Zhang, W.; Luo, Z.; Chen, C. H.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 10443. (b) Curran, D. P.; Furukawa, T. *Org. Lett.* **2002**, *4*, 2233. (c) Zhang, Q.; Rivkin, A.; Curran, D. P. *J. Am. Chem. Soc.* **2002**, *124*, 5774. (d) Curran, D. P. *Synlett* **2001**, 1488. (e) Luo, Z.; Zhang, Q.; Oderaotoshi, Y.; Curran, D. P. *Science* **2001**, *291*, 1766.

(7) (a) Wipf, P.; Reeves, J. T.; Balachandran, R.; Giuliano, K. A.; Hamel, E.; Day, B. W. *J. Am. Chem. Soc.* **2000**, *122*, 9391. (b) Röver, S.; Wipf, P. *Tetrahedron Lett.* **1999**, *40*, 5667. (c) Wipf, P.; Reeves, J. T. *Tetrahedron Lett.* **1999**, *40*, 5139. (d) Wipf, P.; Reeves, J. T. *Tetrahedron Lett.* **1999**, *40*, 4649. (e) Curran, D. P.; Ferritto, R.; Hua, Y. *Tetrahedron Lett.* **1998**, *39*, 4937. (f) Studer, A.; Curran, D. P. *Tetrahedron* **1997**, *53*, 6681.

(8) (a) Curran, D. P.; Amatore, M.; Guthrie, D.; Campbell, M.; Go, E.; Luo, Z. *J. Org. Chem.* **2003**, *68*, 4643. (b) Schwinn, D.; Bannwarth, W. *Helv. Chim. Acta* **2002**, *85*, 255. (c) Filippov, D. V.; Zoelen, D. J.; Oldfield, S. P.; Marel, G. A.; Overkleeft, H. S.; Drijfhout, J. W.; Boom, J. H. *Tetrahedron Lett.*, **2002**, *43*, 7809. (d) Pardo, J.; Cobas, A.; Guitlán, E.; Castedo, L. *Org. Lett.* **2001**, *3*, 3711. (e) Luo, Z.; Williams, J.; Read, R. W.; Curran, D. P. *J. Org. Chem.* **2001**, *66*, 4261.

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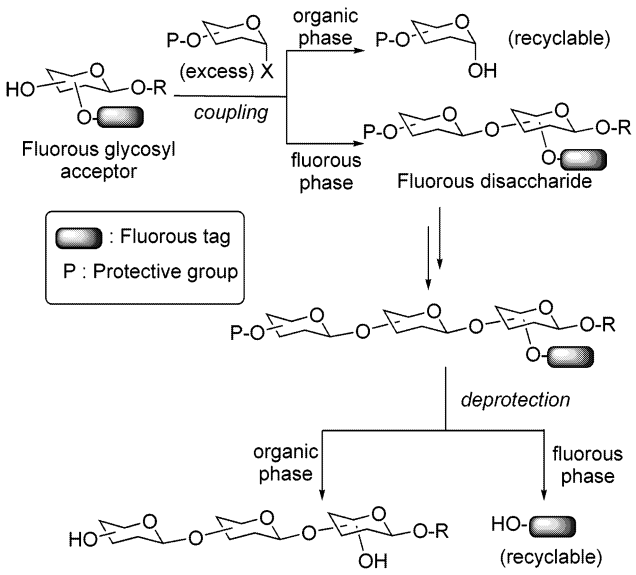


FIGURE 1. Concept of fluorous oligosaccharide synthesis.

not satisfactory. Recently, we reported a method for the fluorous oligosaccharide synthesis involving the novel fluorous acyl protective group and the fluorous support in a preliminary communication.[9] We would like to report the full details of the development of a novel fluorous acyl protective group Bfp and its application to the rapid synthesis of oligosaccharides.

Our concept of fluorous oligosaccharide 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.

Results and Discussion

We designed and synthesized compound **6**, which contains two fluorous chains, as a novel fluorous acyl protecting reagent (Scheme 1). The reaction of the *β*-alanine ethyl ester (**1**) with a fluorous tosylate **2**[10] provided the monoalkylating product **3** in 83% yield. Compound **3** was coupled with perfluorooctylpropionic acid (**4**[11]) to afford compound **5a** in 93% yield. Treatment of **5a** with aqueous sodium hydroxide gave the desired fluorous carboxylic acid **6** (M_W = 1023) in 98% yield. We thought that the two fluorous chains of **6** enhance the efficiency of the liquid−liquid extraction. Some methylene spacers might effectively block the strong electron-withdrawing effect of the long perfluoroalkyl chain without a decrease in the reactivity of the carboxylic group. We named the acyl moiety of **6**, the Bfp (bisfluorous chain type propanoyl) group.

Among the many useful methods for glycosylation, we selected Schmidt's popular imidate method for oligosaccharide synthesis.[12] We first attempted to synthesize the disaccharide **12** as shown in Scheme 2. The Bfp group was easily introduced to the three hydroxyl functions of the mannose derivative **7**[13] using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) to give the fluorous compound **8**. The triphenylmethyl (Trt) group of **8** was removed by treatment with camphorsulfonic acid (CSA) in MeOH−ether to afford the flourous glycosyl acceptor **9**. The fluorous disaccharide **11** was obtained by the reaction of **9** with the excess glycosyl donor **10** in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-OTf) in ether/$EtOC_4F_9$.[14] The glycosylation yield was much improved using ether/$EtOC_4F_9$ as the reaction solvent system.[9a] The fluorous intermediates **8**, **9**, and **11** 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 Bfp group of **11** was easily removed by treatment with NaOMe in MeOH/ether to afford the crude **12**, which was extracted with MeOH by partitioning the mixture between FC-72 and MeOH. The methyl ester of Bfp (Bfp-OMe, **5b**) was recovered from the FC-72 layer in 81% yield. Compound **5b** was treated with aqueous sodium hydroxide to give Bfp-OH (**6**), which was reused. Finally, the pure disaccharide **12** was obtained from a single silica gel column chromatographic purification step in 59% overall yield from **7** (four steps).

Next, we synthesized the longer chain oligosaccharide as shown in Scheme 3. The fluorous glycosyl acceptor **9** was coupled with the glycosyl donor **13**[16] to afford the fluorous disaccharide **14**. The TBDPS group of **14** was removed by treatment with HF−pyridine in THF to give the fluorous compound **15**. The reaction of the glycosyl acceptor **15** with the glycosyl donor **13** under similar glycosylation conditions afforded the fluorous trisaccharide **16**. The repeat of similar deprotection and glycosylation gave the fluorous tetrasaccharide **19**. The fluorous intermediates **14**−**19** were each extracted with the fluorous solvent FC-72 by partitioning the product mixtures between FC-72 and an organic solvent (MeOH or MeCN). No further purification such as silica gel column chro-

(9) (a) Miura, T.; Hirose, Y.; Ohmae, M.; Inazu, T. *Org. Lett.* **2001**, *3*, 3947. (b) Miura, T.; Inazu, T. *Tetrahedron Lett.* **2003**, *44*, 1819. (c) Miura, T.; Goto, K.; Hosaka, D.; Inazu, T. *Angew. Chem., Int. Ed.* **2003**, *42*, 2047.

(10) Campo, F. D.; Lastécouères, D.; Vincent, J.-M.; Verlhac, J.-B. *J. Org. Chem.* **1999**, 64, 4969.

(11) Hungerhoff, B.; Sonnenschein, H.; Theil, F. *J. Org. Chem.* **2002**, *67*, 1781.

(12) Schmidt, R. R.; Michel, J.; Roos, M. *Liebigs Ann. Chem.* **1984**, 1343.

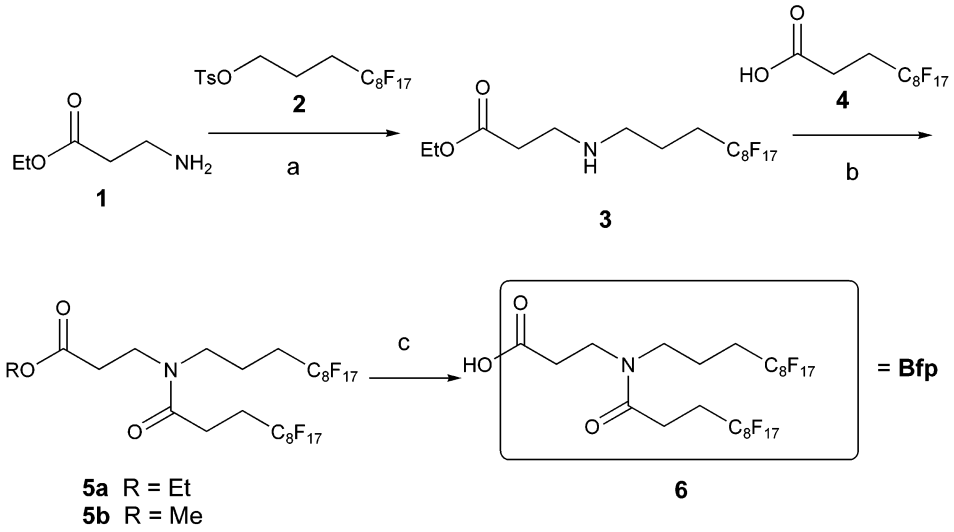
(13) Tennant-Eyles, R. J.; Davis, B. G.; Fairbanks, A. J. *Tetrahedron: Asymmetry* **2000**, *11*, 231.

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

(15) FC-72 is a commercially available fluorocarbon solvent (3M, Tokyo), which consists of perfluorohexane (C_6F_{14}) isomers and is called Fluorinert FC-72. The boiling point of FC-72 is 76 °C.

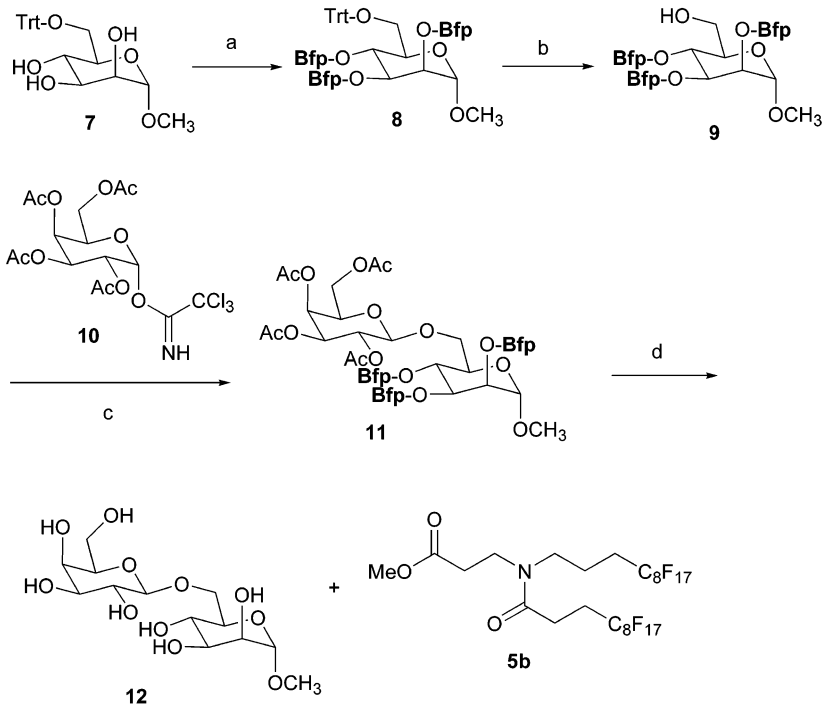
(16) Nicolaou, K. C.; Pfefferkorn, J. A.; Roecker, A. J.; Cao, G.-Q.; Barluenga, S.; Mitchell, H. J. *J. Am. Chem. Soc.* **2000**, *122*, 9939.

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%.

SCHEME 2 [a]

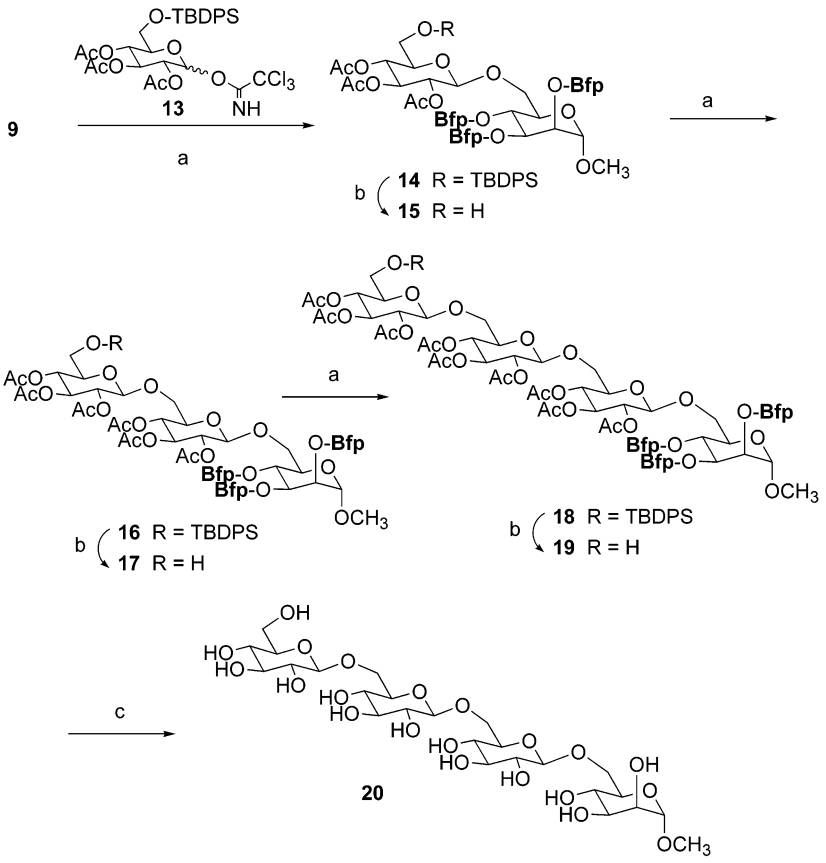


[a] Reagents and conditions: (a) **6**, DCC, DMAP, CH_2Cl_2, rt, 20 h; (b) CSA, MeOH−$CHCl_3$, rt, 3 h; (c) TMS-OTf, molecular sieves 4A, Et_2O, $EtOC_4F_9$, 0 °C, 30 min; (d) NaOMe, Et_2O−MeOH, rt, 22 h, then silica gel chromatography, 59% from **7**.

matography was carried out. The pure fluorous tetrasaccharide **19** was obtained from a single silica gel column chromatographic purification step in 11% overall yield from **7** (eight steps). Although the yields for the glycosylation step to synthesize the trisaccharide **16** (50%) and the tetrasaccharide **18** (10%) were not satisfactory in the preliminary communication,[9a] the glycosylation yields were much improved using ether/$EtOC_4F_9$ as the reaction solvent system (up to 70−85% from 10 to 75% yield in each glycosylation step). The Bfp groups of **19** were removed by treatment with NaOMe in MeOH/ether to afford the tetrasaccharide **20**, which was extracted with MeOH by partitioning the mixture between FC-72 and MeOH.

We also measured the liquid−liquid partition coefficients for the fluorous oligosaccharides (**9**, **14**, **16**, and **18**) between FC-72 and two organic solvents (methanol and toluene) as shown in Table 1. The fluorous compounds show higher partition coefficients for separation by FC-72−ethanol extraction than FC-72−toluene extraction. Although the fluorine content of the fluorous compound **18** is low (44.9%), its partition coefficient is high in contrast to other known fluorous compounds.[5a]

SCHEME 3 [a]



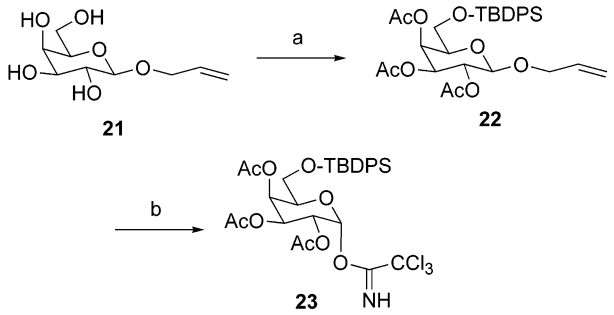
[a] Reagents and conditions: (a) TMS-OTf, **13**, molecular sieves 4A, $EtOC_4F_9$, 0 °C; (b) HF-Py, THF, rt; (c) NaOMe, Et_2O, MeOH, rt, then silica gel chromatography, 11% from **7**.

TABLE 1. Partition Coefficients of Fluorous Oligosaccharide in 50/50 (v/v) of FC-72/Organic Solvents ($P = C_{\text{fluorous phase}}/C_{\text{organic}}$ phase)

compd	F content (wt %)	FC-72/methanol	FC-72/toluene
9	60.4	>100	>100
14	51.9	85	9.4
16	48.1	60	3.9
18	44.9	21	2.2

Next, we synthesized the galactose β-(1−6) pentamer **36**, which is known as a component part of arabinogalactan-proteins,[17] as an all-free oligosaccharide (Scheme 5). To synthesize the galactose β-(1−6) pentamer **36**, we used compound **23** as the glycosyl donor and compound **26** as the glycosyl acceptor, which had the anomer hydroxyl function protected by a benzyl group. The glycosyl donor **23** was prepared as shown in Scheme 4. The introduction of the TBDPS group to the primary hydroxyl function of **21**[18] followed by treatment with acetic anhydride afforded compound **22** in 93%onwedtion yield. The treatment of compound **22** with $Pd(PPh_3)_4$ in acetic acid, followed by the reaction with trichloroacetonitrile gave the glycosyl donor **23** in 70% yield. The galactose β-(1−6) pentamer **36** was synthesized using compound **23** as a glycosyl donor as shown in Scheme 5. The Bfp group was introduced to the three hydroxyl

SCHEME 4 [a]



[a] Reagents and conditions: (a) TBDPS-Cl, Py, rt, 18 h, then Ac_2O, rt, 3 h; (b) (i) $Pd(PPh_3)_4$, AcOH, 80 °C, 1 h; (ii) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 1 h.

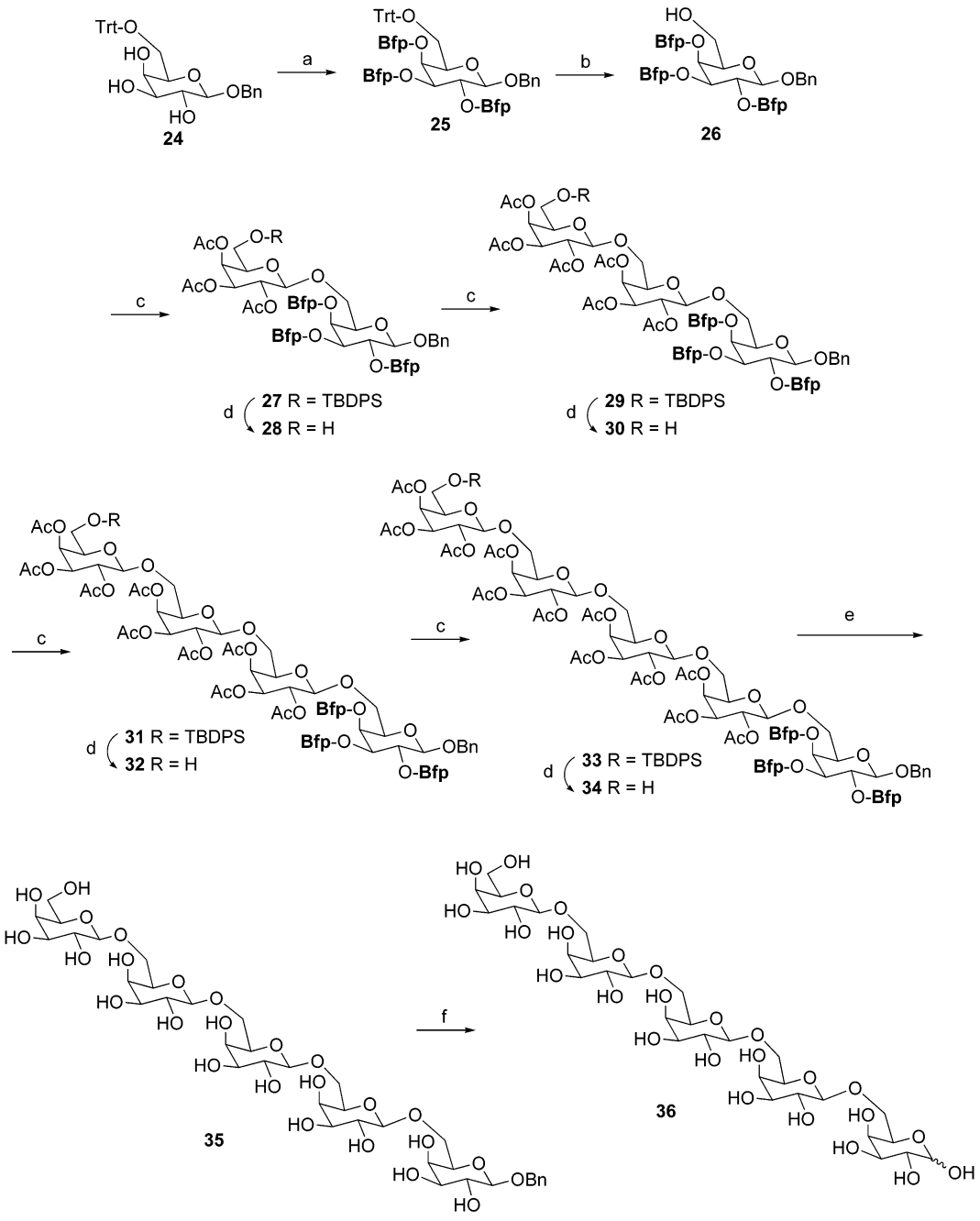
functions of the galactose derivative **24**[19] using DCC and DMAP to give the fluorous compound **25**. The triphenylmethyl (Trt) group of **25** was removed by treatment with camphorsulfonic acid (CSA) in MeOH−$CHCl_3$ to afford the flourous glycosyl acceptor **26**. Compound **26** was unstable because the Bfp group (4-position) of compound **26** easily migrated to the primary hydroxyl function (6-position). Therefore, it was difficult to isolate compound **26** by silica gel column chromatographic purification. The fluorous glycosyl acceptor **26** was coupled with the glycosyl donor **23** to afford the fluorous disaccharide **27**.

(17) Kuroyama, H.; Tsutsui, N.; Hashimoto, Y.; Tsumuraya, Y. *Carbohydr. Res.* **2001**, *333*, 27.

(18) Lee, R. T.; Lee, Y. C. *Carbohydr. Res.* **1974**, *37*, 193.

(19) Miyai, K.; Jeanloz, R. W. *Carbohydr. Res.* **1972**, *21*, 45.

SCHEME 5 [a]



[a] Reagents and conditions: (a) **6**, DCC, DMAP, CH_2Cl_2, rt, 2 h; (b) CSA, LiCl, $CHCl_3$, MeOH, rt, 2h; (c) **23** TMS-OTf, molecular sieves 4A, $EtOC_4F_9$, Et_2O, 0 °C, (d) HF-Py, THF, rt; (e) NaOMe, Et_2O, MeOH, rt, 3 h; (f) H_2, Pd/C, EtOH, H_2O, rt, 18 h.

The TBDPS group of **27** was removed by treatment with HF-pyridine in THF to give the fluorous compound **28**. The reaction of the glycosyl acceptor **28** with the glycosyl donor **23** under similar glycosylation conditions afforded the fluorous trisaccharide **29**. The repeat of similar deprotection and glycosylation gave the fluorous pentasaccharide **33**. The intermediates **25**−**33** were each extracted with FC-72 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 pure fluorous pentasaccharide **33** was obtained from a single silica gel column chromatographic purification step in 29% overall yield from **24** (nine steps). After treatment of **33** with HF-pyridine, the Bfp group of **34** was removed by treatment with NaOMe in MeOH/ether to afford the crude **35**, which was extracted with MeOH by partitioning the mixture between FC-72 and MeOH. The crude product **35** was isolated by ODS silica gel column chromatographic purification in 92% yield from **33** (two steps). The benzyl group of **35** was removed by hydrogenation in the presence of Pd/C to afford the galactose β-(1−6) pentamer **36**. Compounds **28**, **30**, **32**, and **34** were also unstable because the acetyl group easily migrated to the primary hydroxyl function. It is worth noting that the unstable compounds (**26**, **28**, **30**, **32**, and **34**) were able to be

quickly purified by partitioning the product mixtures between FC-72 and an organic solvent without decomposition.

Conclusion

The use of Bfp group as a fluorous protective group made it possible to rapidly synthesize a natural oligosaccharide by minimal column chromatography purification. The fluorous protecting reagent **6** (Bfp-OH) could be easily prepared on a large scale. The Bfp group was readily introduced to the carbohydrate hydroxyl functions, removed in high yield by the usual procedure, and was recyclable after cleavage. Only three Bfp groups made it possible to extract the derivative of the pentasaccharide with the FC-72 phase.[20] Each fluorous synthetic intermediate could be obtained in a straightforward manner by simple FC-72-organic solvent extraction. The reaction conditions for each synthetic step could be rapidly optimized because the reactions could be monitored as a single compound by TLC and mass spectrometry, in contrast to the usual solid-phase reactions. The fluorous intermediates could also be measured by NMR spectroscopic analysis. The peaks in the NMR of the fluorous compounds containing the Bfp group are somewhat broad due to the influences of the amide linkages and the fluorous groups.[7e] 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 using the fluorous protecting group Bfp is an attractive strategic alternative to solid-phase oligosaccharide synthesis, and removed some of the disadvantages of the usual solid-phase method. Further application to the synthesis of bioactive carbohydrates and glycoconjugates is now in progress.

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Supporting Information Available: Experimental procedure and ^{1}H NMR and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(20) The fluorous monosaccharide derivative, which was introduced only one Bfp group into the anomeric hydroxyl function of 2,3,4,6-tetra-*O*-benzyl-D-galactose, cannot be extracted with FC-72 by partitioning between FC-72 and organic solvent.

(21) Eisenbraun, E. J. *Organic Syntheses*; Wiley: New York, 1973; Collect. Vol. V, p 310.