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Tetrahedron Letters 44 (2003) 1819–1821

TETRAHEDRON LETTERS

Rapid synthesis of oligosaccharide moieties of globotriaosylceramide using fluorous protective group

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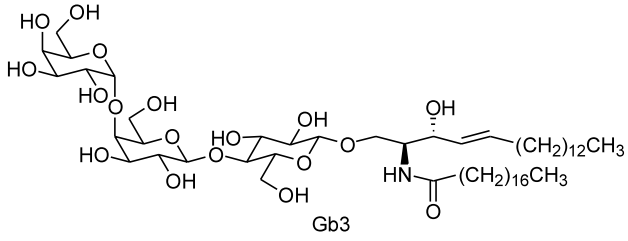
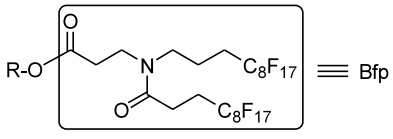
Received 26 November 2002; revised 7 January 2003; accepted 10 January 2003

Abstract—The use of the Bfp (bisfluorous chain type propanoyl) group as a fluorous protective group made it possible to rapidly synthesize galabiose and the Gb3 oligosaccharide derivatives by a simple fluorous–organic extraction purification. The fluorous oligosaccharide synthesis using the Bfp group is an excellent strategic alternative to solid phase oligosaccharide synthesis, and removes some of the disadvantages of the solid phase method.

Globotriaosyl ceramide (Gb3), known as a P^k antigen in the P blood-group system,[1] is a natural ligand of Shiga-like toxins (Stx-I and Stx-II, or verotoxins) produced by *Escherica coli* O-157:H-7.[2] Shiga-like toxins mainly recognize a galactobiosyl α(1–4)-linkage (galabiose moiety) in Gb3, and induce carbohydrate-mediated internalization into the host cell.[2] In order to develop verotoxin inhibitors and detect the verotoxin, galabiose and the Gb3 trisaccharide play an important role as a key molecule.[3] The synthesis of galabiose and the Gb3 trisaccharide have already been accomplished by several groups.[4] However, each synthetic method consumes much time and cost due to the purification procedure such as column chromatography in multi-steps. To provide a rapid synthesis, the solid phase synthesis of oligosaccharides has been actively studied.[5] The solid phase method suffers from some serious disadvantages, such as the difficulty of a large scale synthesis, reduced reactivity, and the inability to monitor the reaction by TLC, NMR, and MS. Recently, fluorous synthesis has been developed for use in several fields by Curran et al.[6] 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 MS as a single compound. Therefore, fluorous synthesis has become an excellent strategic alternative to solid phase synthesis. Recently, we reported the fluorous oligosaccharide synthesis using the Bfp (bisfluorous chain type propanoyl) group as a novel fluorous protective group.[7] Use of the Bfp group made it possible to rapidly synthesize a simple oligosaccharide (Glcβ(1–6)Glc linkage) by purification using fluorous-organic solvent extraction. To demonstrate the usefulness of the Bfp group as a fluorous tag, we attempted to synthesize the oligosaccharide moieties of Gb3 as a more complex bioactive carbohydrate molecule. We would like to report the rapid synthesis of the oligosaccharide moieties of Gb3 using the Bfp group as a fluorous protective group in this communication.



Gb3



1a R = H
1b R = Me

We first synthesized the galabiose derivative **8** as shown in Scheme 1. The Bfp group was introduced to the two hydroxyl functions of the galactose derivative **2** using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) to give **3**.[8] The benzylidene group of **3** was removed by treatment of the camphorsulfonic acid (CSA) in MeOH-$CHCl_3$ to afford **4**.[8] The

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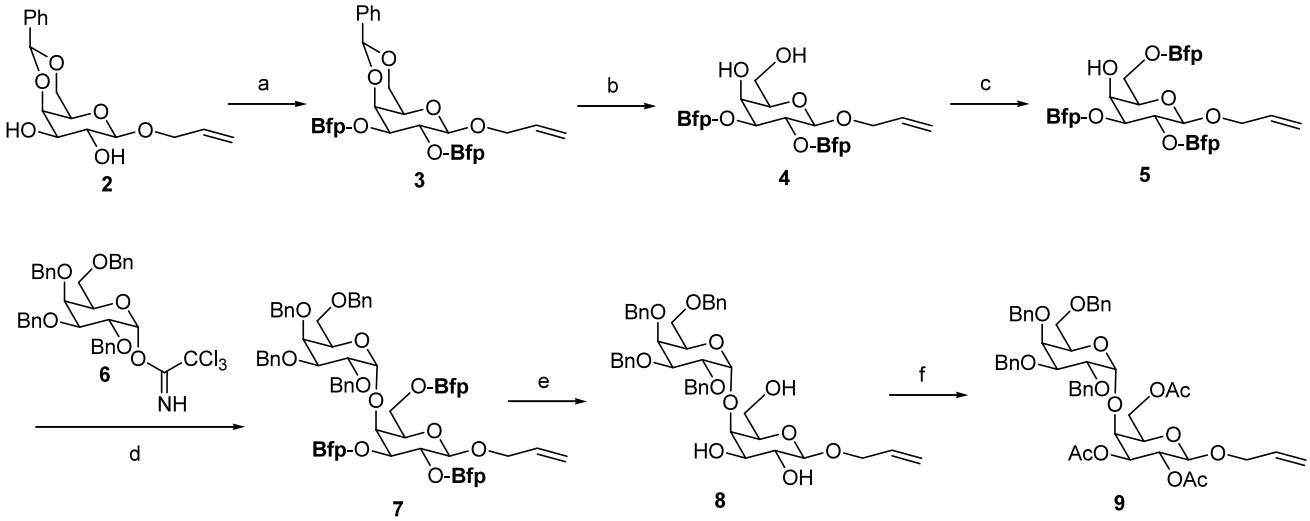
0040-4039/03/$ - see front matter
PII: S0040-4039(03)00091-1

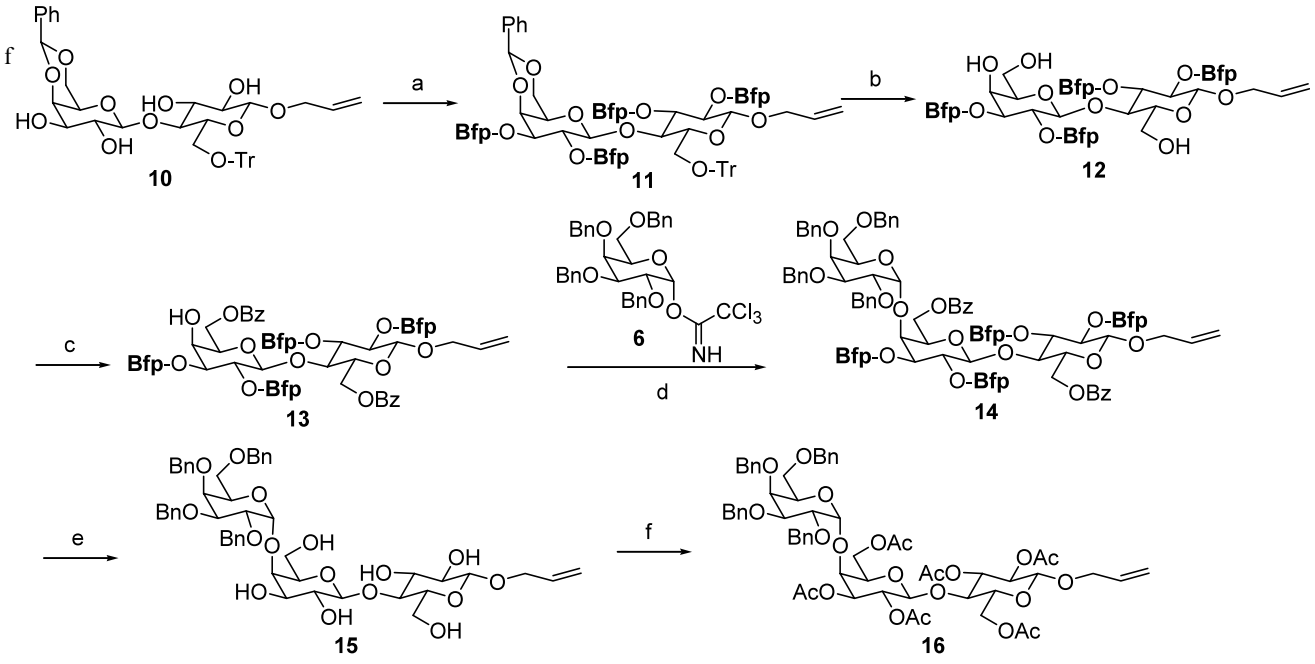


Scheme 1. *Reagents and conditions*: (a) **1a**, DCC, DMAP, CH_2Cl_2, rt, 4 h; (b) CSA, $CHCl_3$–MeOH, rt, 5 h; (c) **1a**, DCC, DMAP, CH_2Cl_2–$EtOC_4F_9$, −5°C, 21 h; (d) TMS–OTf, 4 Å molecular sieves, ether–$EtOC_4F_9$, 0°C, 30 min; (e) NaOMe, Et_2O–MeOH, rt, 1 h, 27% (in 5 steps); (f) Ac_2O, py, rt, 12 h, 95%.

Bfp group was selectively introduced to the primary hydroxyl function of **4** using DCC and DMAP to give the fluorous glycosyl acceptor **5**.[8] The reaction of the fluorous glycosyl acceptor **5** with an excess of the glycosyl donor **6** (6 equiv.) in the presence of TMS-OTf in ether–$EtOC_4F_9$[9] afforded selectively the α-linked fluorous disaccharide **7**.[8] (No β-isomer could be detected.) The fluorous intermediates **3**, **4**, **5**, and **7** were extracted with FC-72[10] by being partitioned between FC-72 and an organic solvent (toluene or methanol), and were purified without silica gel column chromatography. The Bfp group of **7** was removed by treatment with sodium methoxide in MeOH-ether to afford the crude **8**, which was extracted with MeOH by being partitioned between FC-72 and MeOH. The methyl ester **1b**, which was obtained from the FC-72 layer, was treated with aqueous NaOH to give **1a** that was able to be reused as the fluorous protective reagent. Finally, the pure galabiose derivative **8** was obtained by only one silica gel column chromatography purification in the final step for a 27% total yield from **2** (5 steps). The galabiose derivative **8** was introduced to the acetate **9** by the treatment with acetic anhydride in pyridine, and its structure was identified.

Next, we synthesized the Gb3 trisaccharide derivative **15** as shown in Scheme 2. The Bfp group was introduced to the four hydroxyl functions of the lactose derivative **10** using DCC and DMAP to give **11**.[8] The benzylidene group and trityl (Tr) group of **11** were removed by treatment with HCl in AcOEt–$EtOC_4F_9$ to afford **12**.[8] The benzoyl (Bz) group was selectively introduced to the two primary hydroxyl functions of **12** to afford the fluorous glycosyl acceptor **13**.[8] The reaction of the fluorous glycosyl acceptor **13** with an excess of the glycosyl donor **6** (5 equiv.) in the presence of TMS-OTf in ether-$EtOC_4F_9$ afforded selectively the α-linked fluorous trisaccharide **14**.[8] (No β-isomer could be detected.) The fluorous intermediates **11**, **12**, **13**, and **14** were extracted with FC-72 by being partitioned between FC-72 and an organic solvent (toluene or methanol), and were purified without silica gel column chromatography. The Bfp group of **14** was removed by treatment with sodium methoxide in MeOH-ether to afford **15,** which was extracted with MeOH by being partitioned between FC-72 and MeOH. Finally, the pure trisaccharide **15** was obtained by only one silica gel column chromatography purification in the final step for a 34% total yield from **10** (5 steps). The trisaccharide **15** was introduced to **16** by the treatment with acetic anhydride in pyridine, and its structure was identified.

In conclusion, the use of the Bfp group as a fluorous protective group made it possible to rapidly synthesize galabiose and the Gb3 oligosaccharide derivatives by a fluorous-organic extraction purification. The fluorous oligosaccharide synthesis can be applied to large scale synthesis due to the liquid phase synthesis. Because each synthetic intermediate containing the Bfp group was monitored by TLC, NMR, and MS, the reaction conditions in each synthetic step were able to be rapidly optimized. The fluorous intermediates were also able to be purified by silica gel column chromatography in the case which needed purification. After optimization of the reaction conditions in each step, the synthesis in multisteps was accomplished by fluorous-organic partition purification without column chromatography. The only final compounds, which were removed the Bfp group, were purified by column chromatography on silica gel. Therefore, the fluorous oligosaccharide synthesis using the Bfp group is an excellent strategic alternative to solid phase oligosaccharide synthesis, and removes some of the disadvantages of the solid phase method. Further application to the synthesis of a bioactive carbohydrate and glycoconjugate is now in progress.



Scheme 2. *Reagents and conditions*: (a) **1a**, DCC, DMAP, CH_2Cl_2, rt, 3 h; (b) HCl, AcOEt–$EtOC_4F_9$, 0°C, 2 h; (c) BzCl, Et_3N, CH_2Cl_2–$EtOC_4F_9$, −20°C, 7 h; (d) TMS–OTf, 4 Å molecular sieves, ether–$EtOC_4F_9$, 0°C, 20 min; (e) NaOMe, Et_2O–MeOH, rt, 1 h, then silica gel chromatography, 34% from **10** (in 5 steps); (f) Ac_2O, Py, rt, 12 h, 95%.

Acknowledgements

This work was partly supported by a Grant-in-Aid for Scientific Research (C) (No. 13680680), a Grant-in-Aid for Encouragement of Young Scientists (No. 13771349) from the Japan Society for the Promotion of Science, and Takeda Science Foundation. This work was performed through the Noguchi Fluorous Project by our institute.

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8. Fluorous compounds **3**, **7**, **13**, and **14** were partitioned between FC-72 and methanol. Fluorous compounds **4**, **5**, **11**, and **12** were partitioned between FC-72 and toluene. All fluorous compounds were not detected by TLC from the organic layer after three extractions with FC-72. These results show that these compounds were quantitatively extracted with FC-72.
9. $EtOC_4F_9$ is a commercially available fluorocarbon solvent (3M, Tokyo), which is called Novec™ HFE-7200.
10. FC-72 is a commercially available fluorocarbon solvent (3M, Tokyo), which consists of perfluorohexane (C_6F_{14}) isomers and is called Fluorinert™ FC-72.