Multi-step Synthesis of a Protected Monosaccharide Unit by Iterative Reactions in Microreactors and Fluorous Liquid-phase Extractions

Hiroko Kawakami, Kohtaro Goto, and Mamoru Mizuno*
Laboratory of Glyco-organic Chemistry, The Noguchi Institute, 1-8-1 Kaga, Itabashi-ku, Tokyo 173-0003

(Received June 9, 2009; CL-090548; E-mail: mmizuno@noguchi.or.jp)

We describe a process that simultaneously achieves microreaction synthesis and fluorous liquid-phase extraction in a single system. Use of this method in multi-step synthesis of a protected monosaccharide unit yielded the desired product. Moreover, the total synthesis time, including an only single column chromatography as the last step, was significantly shortened compared with general synthetic methods.

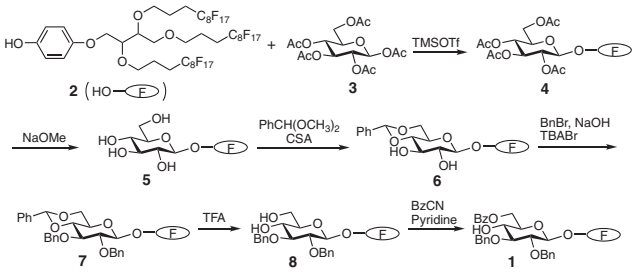
In recent years there has been increasing focus on microreaction synthesis methodologies in organic synthesis.[1] The use of microreactors allows a high frequency of collision among molecules because of a short molecular diffusion length, faster heat transfer, and increased mixing as a result of the increased surface area to volume ratio. It also facilitates scaling-up of the reaction volume by numbering-up procedures.

On the other hand, regioselectivity is a demanding task in carbohydrate chemistry, because sugars contain several chiral centers substituted with hydroxy groups. Protecting-group strategy is therefore of substantial importance in carbohydrate chemistry. Usually, synthesis of a protected saccharide unit requires time-consuming processes, such as multiple reactions involving workup and purification. A few studies have described the application of microreactors to carbohydrate chemistry syntheses;[2] in these studies microreaction synthesis provided several advantages, such as improved reactivity and efficiency.

Use of a microreactor increases reaction efficiencies but still requires time for the workup and purification processes. Reported studies of microreaction synthesis have used various purification strategies, including packed columns of solid-supported reagents,[3] liquid–liquid phase separation using a thin porous fluoropolymer membrane,[4] and a fluorous solid-extraction method.[5]

To expedite the purification processes, we selected a simple phase extraction method. Fluorous liquid-phase extraction is a method for refining by liquid–liquid extraction by using fluorous fluids that are immiscible with water and many organic solvents.[6] Hence, we expected a synergetic effect from the microreaction synthesis and the fluorous liquid-phase extraction technique. In this article, we report multi-step synthesis of the protected monosaccharide unit **1**[7] for use as a glycosyl acceptor (Scheme 1) using the combination of a microreactor and fluorous chemistry as a single system.

The pathway for the synthesis of the monosaccharide unit **1** is shown in Scheme 1. The fluorous tag **2**[7] must be incorporated at the reducing end of glucose to facilitate purification by fluorous liquid-phase extraction. The reaction was performed in the following sequence. Starting from the introduction of the fluorous tag, first, the fluorous tag **2** was coupled with glucose pentaacetate **3** in the presence of TMSOTf to give fluorous glucose **4**. Second, after removal of the acetyl groups from **4** by treatment with NaOMe, the glucose derivative **5** was functionalized by using benzaldehyde dimethyl-acetal to provide 4,6-benzylidene acetal **6**. Third, benzylation of C-2 and C-3 hydroxy groups of **6** using benzyl bromide yielded dibenzylate **7**. Fourth, treatment of **7** with trifluoroacetic acid (TFA) opened the benzylidene acetal ring to give 4,6-diol **8**. Finally, the C-6 hydroxy group of **8** was protected as the benzoyl ester using benzoyl cyanide to give the desired product **1**.



Scheme 1. Synthesis of monosaccharide unit **1**.

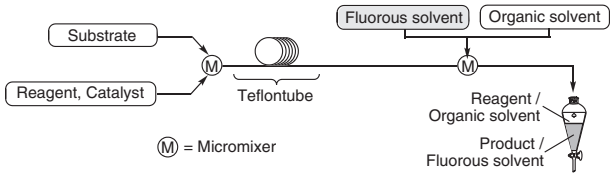


Figure 1. Microreaction system for synthesis of monosaccharide unit **1**.

The typical method for each reaction of this sequence is illustrated in Figure 1. Using syringe pumps, substrate possessing a fluorous tag was introduced into one inlet of a Y-shaped micromixer[8] (inner diameter = 0.5 mm) and the reagent was introduced into the other inlet. The reaction mixture was passed through an additional Teflon tube reactor ($\Phi = 0.5$ mm, $L = 5$ or 10 m), which was connected to the end of the micromixer. The reaction mixture, a fluorous solvent ($MeOC_4F_9$–FC72), and an organic solvent (aq CH_3CN or aq MeOH) were introduced into the next mixer in the system for quenching and separation.[7] The eluate was poured into a separating funnel. The fluorous layer was evaporated under reduced pressure, and the dried product was used as a precursor for synthesis in the next reaction, without further purification.

First, we optimized the conditions for each microreaction. All reactions were performed at room temperature, and yields were obtained after purification by silica-gel column chromatography.

The introduction of a sugar moiety to the fluorous tag **2** was tested. A solution of the fluorous tag **2** as a glycosyl acceptor and a glycosyl donor **3** in CH_2Cl_2–$MeOC_4F_9$ (1:1), and a solution of TMSOTf (1.0 equiv) in CH_2Cl_2–$MeOC_4F_9$ (1:1) were introduced to the micromixer. When the glycosylation donor **3** was used at 5.0 equiv at a flow rate of 166 μL min^{-1} for 6 min in an additional tube ($L = 10$ m), the reaction was complete, and

the glucose derivative **4** was obtained in 80% (based on **2**) or 16% (based on **3**) yield (See Supporting Information, Table S1, Entry 5).[9] The reaction with a higher concentration of acceptor **2** (60 mM) resulted in a lower conversion rate. If a shorter residence time or a smaller amount of glycosyl donor **2** was used, the reaction was incomplete.

Second, deprotection of acetyl groups was performed according to the Zemplén procedure.[10] A solution of tetraacetate **4** (30 mM) in MeOH–$MeOC_4F_9$ (1:1) and a solution of varying concentrations of NaOMe (0.2, 0.5, and 1.0 equiv) in MeOH–$MeOC_4F_9$ (1:1) were introduced into the micromixer. Acetyl groups were easily cleaved in 1.0 equiv of NaOMe at a flow rate of 250 μL min^{-1} for 4 min in an additional tube ($L = 10$ m), giving a 94% yield of **5** (Table S2, Entry 5).[9] If a smaller amount of NaOMe (0.2 and 0.5 equiv) was used, the deprotection reaction was incomplete.

Third, introduction of a benzylidene group was tested. 4,6-Benzylidene acetal **6** was synthesized. The glucose derivative **5** in MeCN–$MeOC_4F_9$ (1:1) and various amounts of benzaldehyde dimethylacetal and camphorsulfonic acid (1.0 equiv) in MeCN–$MeOC_4F_9$ (1:1) were mixed in the micromixer. When 5.0 equiv of **5** was used at the rate of 250 μL min^{-1} for 2 min in an additional tube ($L = 5$ m), acetal **6** was obtained in 85% yield (Table S3, Entry 4).[9] If a smaller amount of $PhCH(OCH_3)_2$ and a shortened residence time were used, the protection reaction was incomplete.

Fourth, benzylation was tested. Benzylation of C-2 and C-3 hydroxy groups of **6** was performed with benzyl bromide, aqueous NaOH, and tetrabutylammonium bromide (TBABr) as the phase transfer catalyst. A solution of **6** (10 mM) and TBABr (10 equiv) dissolved in *N*,*N*-dimethylformamide (DMF)–$MeOC_4F_9$ (4:1) and 20 equiv of NaOH dissolved in DMF–H_2O (3:2) was introduced into the micromixer. The reaction mixture was then passed through an additional tube ($L = 5$ m) at the rate of 250 μL min^{-1} for 120 s and was mixed with BnBr (20 equiv) in DMF in the second micromixer. The solution was then passed through a second additional tube ($L = 5$ m) at the rate of 250 μL min^{-1} for 80 s. The desired product **7** was obtained int 86% yield (Table S4, Entry 4).[9] Under the same conditions using tetrahydrofuran or DMF–$MeOC_4F_9$–H_2O (8:5:2), the reactions were considered incomplete because the reaction mixtures were not homogeneous solutions.

Fifth, removal of benzylidene acetal of **7** under acidic conditions was tested. The optimum condition for the reaction, a solution of **7** (6 mM) dissolved in $MeOC_4F_9$ and 10 equiv of TFA dissolved in $MeOC_4F_9$, was introduced into the micromixer. The mixture was passed through an additional tube ($L = 5$ m) at the rate of 250 μL min^{-1} for 2 min (Table S5, Entry 6),[9] and **8** was obtained in 94% yield. The reaction with a more highly concentrated substrate, lower concentration of TFA, or shorter residence time was incomplete.

Finally, selective benzoylation[11] of the C-6 hydroxy group of substrate **8** was tested. A solution of 10 mM of **8** containing 10% pyridine in $MeOC_4F_9$ and benzoyl cyanide (10 equiv) in $MeOC_4F_9$ was mixed in the micromixer and passed through an additional tube ($L = 10$ m) at the rate of 125 μL min^{-1} for 8 min, to give the benzoate **1** in 76% yield (Table S6).[9,11] No 4,6-dibenzoate was observed. The reaction with a less concentrated substrate tended to form by-products.

Furthermore, using these optimized conditions in the microreactor, the multi-step synthesis of monosaccharide unit **1** was examined with single silica-gel column chromatography purification as the final step. Using 0.15 mmol (240 mg) of fluorous tag **2** as the starting material, the reaction and separation time required was 6 h (time for each step: 30, 20, 30, 60, 100, and 120 min, respectively), and the desired product **1** was obtained in 55% (based on **2**) or 11% (based on **3**) overall yield. The total synthesis time, including the final column chromatography, was only about 9 h.

In summary, we achieved a multi-step synthesis of the protected carbohydrate unit **1** by using the combination of a microreactor and fluorous chemistry in a single system. Moreover, the multi-step synthesis, including single column chromatography as the last step, was achieved within a day. In regard to the optimization conditions at each step, it is not necessarily the case that the yields of microreactions are higher than those of the corresponding batch reactions; moreover, the relative amounts of reagents used in microreactions are greater than those used in the corresponding batch reactions. Nevertheless, these results show that the use of a microreactor enables the reaction time to be shortened.

References and Notes

1 a) K. F. Jensen, *Chem. Eng. Sci.* **2001**, *56*, 293. b) P. D. I. Fletcher, S. J. Haswell, E. Pombo-Villar, B. H. Warrington, P. Watts, S. Y. F. Wong, X. Zhang, *Tetrahedron* **2002**, *58*, 4735. c) K. Jähnisch, V. Hessel, H. Löwe, M. Baerns, *Angew. Chem., Int. Ed.* **2004**, *43*, 406. d) P. Watts, S. J. Haswell, *Chem. Soc. Rev.* **2005**, *34*, 235. e) A. J. deMello, *Nature* **2006**, *442*, 394. f) T. Fukuyama, M. T. Rahman, M. Sato, I. Ryu, *Synlett* **2008**, 151.

2 a) D. M. Ratner, E. R. Murphy, M. Jhunjhunwala, D. A. Snyder, K. F. Jensen, P. H. Seeberger, *Chem. Commun.* **2005**, 578. b) K. Geyer, J. D. C. Codée, P. H. Seeberger, *Chem.—Eur. J.* **2006**, *12*, 8434. c) K. Geyer, P. H. Seeberger, *Helv. Chim. Acta* **2007**, *90*, 395. d) S. Tanaka, T. Goi, K. Tanaka, K. Fukase, *J. Carbohydr. Chem.* **2007**, *26*, 369. e) K. Tanaka, K. Fukase, *Synlett* **2007**, 164.

3 a) M. Baumann, I. R. Baxendale, S. V. Ley, C. D. Smith, G. K. Tranmer, *Org. Lett.* **2006**, *8*, 5231. b) I. R. Baxendale, J. Deeley, C. M. Griffiths-Jones, S. V. Ley, S. Saaby, G. K. Tranmer, *Chem. Commun.* **2006**, 2566.

4 a) H. R. Sahoo, J. G. Kralj, K. F. Jensen, *Angew. Chem., Int. Ed.* **2007**, *46*, 5704. b) J. G. Kralj, H. R. Sahoo, K. F. Jensen, *Lab Chip* **2007**, *7*, 256.

5 a) O. Flögel, J. D. C. Codée, D. Seebach, P. H. Seeberger, *Angew. Chem., Int. Ed.* **2006**, *45*, 7000. b) F. R. Carrel, K. Geyer, J. D. C. Codée, P. H. Seeberger, *Org. Lett.* **2007**, *9*, 2285.

6 For fluorous-organic partition, see: *Handbook of Fluorous Chemistry*, ed. by J. A. Gladysz, D. P. Curran, I. T. Horváth, Wiley-VCH, Weinheim, **2004**.

7 K. Goto, M. Mizuno, *Tetrahedron Lett.* **2007**, *48*, 5605.

8 Y-shaped connector P-512, http://www.idex-hs.com/.

9 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

10 G. Zemplén, E. Pacsu, *Ber. Dtsch. Chem. Ges.* **1929**, *62*, 1613.

11 S. A. Abbas, A. H. Haines, *Carbohyder. Res.* **1975**, *39*, 358.

Published on the web (Advance View) August 22, 2009; doi:10.1246/cl.2009.906