ORGANIC LETTERS

2007
Vol. 9, No. 12
2285−2288

Oligosaccharide Synthesis in Microreactors

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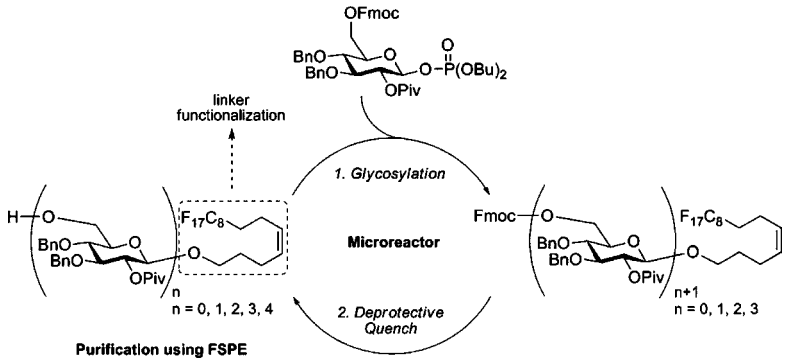
Received March 5, 2007

ABSTRACT



Described is the combination of microreactors and fluorous phase chemistry to assemble oligosaccharides. The synthesis of a β-(1→6) linked D-glucopyranoside homotetramer serves to illustrate this approach. Glycosylations employing a Fmoc-protected glucosyl phosphate building block were performed in a silicon-based micro-structured device to optimize reaction conditions and for reaction scale-up. A perfluorinated linker at the reducing end of the oligosaccharides allowed for purification by fluorous solid-phase extraction (FSPE) and further functionalization.

Microreactors are receiving increasing interest for conducting chemical transfomations.[1] The small dimensions of micro-structured reactors allow for high heat- and mass-transfer rates, efficient mixing by laminar diffusion, and exact control of reaction parameters. Microreactors lend themselves particularly well for the use of unconventional reaction conditions such as high coupling temperatures.[2] Less consumption of material compared to traditional processes, rapid screening of reaction conditions and numbering-up, as well as scale-out processes circumvent traditional challenges for synthetic chemists.[1] The silicon-glass microreactor[2a] used here was chosen for its excellent thermal conductivity and stability to a broad range of organic solvents and reagents. The internal volume of 78.3 μL renders it suitable for reaction screening and larger scale production; several successful applications have already been reported.[2] In the field of oligosaccharide synthesis, microreactors had only been used to prepare disaccharides.[2a,c,3]

Here, we demonstrate the synthesis of a protected β-(1→6) linked D-glucopyranoside homotetramer in a microfluidic device by iterative glycosylations using the Fmoc-protected glycosyl phosphate **1**[4] and perfluorinated linker **2**[5] (Scheme

(1) For reviews, see: (a) Ehrfeld, W.; Hessel, V.; Löwe, H. *Microreactors*: *New Technology for Modern Chemistry*; Wiley-VCH: Weinheim, Germany, 2000. (b) Jensen, K. F. *Chem. Eng. Sci.* **2001**, *56*, 293−303. (c) Haswell, S. J.; Middleton, R. J.; O'Sullivan, B.; Skelton, V.; Watts, P.; Styring, P. *Chem. Commun.* **2001**, *5*, 391−398. (d) Jähnisch, K.; Hessel, V.; Löwe, H.; Baerns, M. *Angew. Chem., Int. Ed.* **2004**, *43*, 406−446. (e) Geyer, K.; Codée, J. D. C.; Seeberger, P. H. *Chem. Eur. J.* **2006**, *12*, 8434−8442. (f) Brivio, M.; Verboom, W.; Reinhoudt, D. N. *Lab. Chip* **2006**, *6*, 329−344. (g) Mason, B. P.; Price, K. E.; Steinbacher, J. L.; Bogdan, A. R.; McQuade, D. T. *Chem. Rev.* published online Mar. 21, http://dx.doi.org/10.1021/cr050944c, and the references cited therein. See also the following articles: (h) Kawaguchi, T.; Miyata, H.; Ataka, K.; Mae, K.; Yoshida, J. *Angew. Chem., Int. Ed.* **2005**, *44*, 2413−2416. (i) Iwasaki, T.; Kawano, N.; Yoshida, J. *Org. Process Res. Dev.* **2006**, *10*, 1126−1131. (j) Iwasaki, T.; Nagaki, A.; Yoshida, J. *Chem. Commun.* In press; and references cited therein.

(2) (a) Ratner, D. M.; Murphy, E. R.; Jhunjhunwala, M. D.; Snyder, A.; Jensen, K. F.; Seeberger, P. H. *Chem. Commun.* **2005**, *5*, 578−580. (b) Flögel, O.; Codée, J. D. C.; Seebach, D.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2006**, *45*, 7000−7003. (c) Geyer, K.; Seeberger, P. H. *Helv. Chim. Acta* **2007**, *90*, *395−403*.

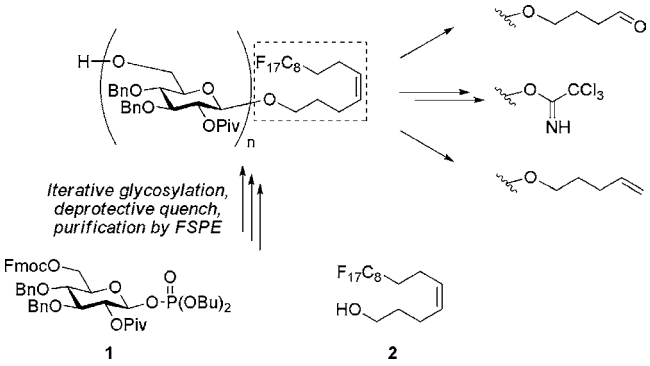
(3) Fukase, K.; Takashina, M.; Hori, Y.; Tanaka, D.; Tanaka, K.; Kusumoto, S. *Synlett* **2005**, *15*, 2342−2346.

(4) Carrel, F.; Seeberger, P. H. *J. Carbohydr. Chem.* **2007**, in press.

(5) The design of linker **2** was inspired by the octenediol linker reported in: Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1811−1814.

10.1021/ol0705503 CCC: $37.00
Published on Web 05/10/2007

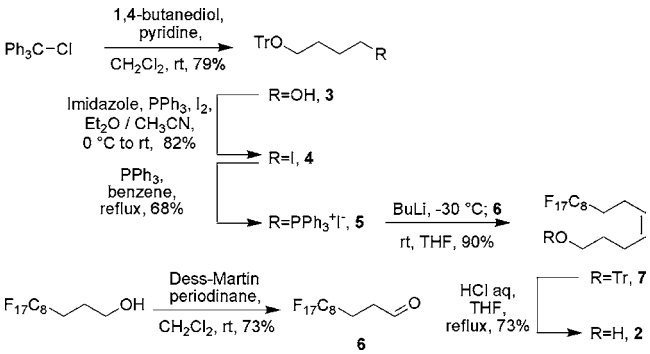
Scheme 1. Synthetic Strategy and Linker Functionalization (n = 1, 2, 3, 4)



1). The new linker system allowed for the use of fluorous solid-phase extraction (FSPE) as an efficient purification method.[6] After completion of the synthesis, the fluorous tag can be transformed into aldehydes, glycosyl trichloroacetimidates, or *n*-pentenyl glycosides (Scheme 1).[7] Alternatively, noncovalent attachment to perfluorinated glass-slides is possible.[8]

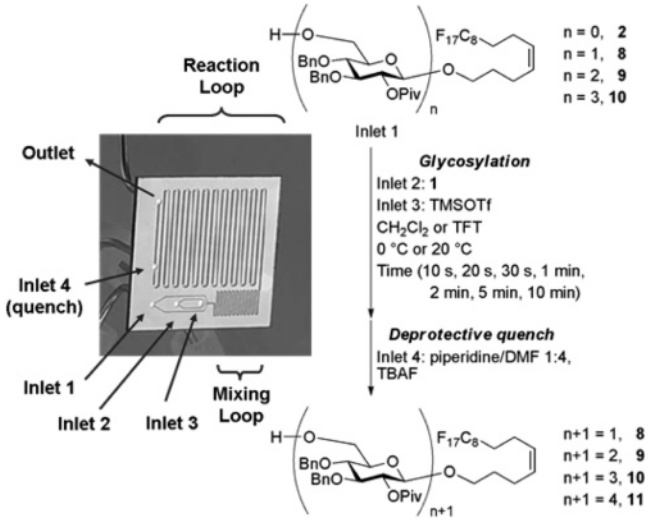
The synthesis of fluorinated linker **2** commenced with monotritylation of 1,4-butanediol to afford **3**. Hydroxyl−halogen exchange furnished **4**.[9] Subsequent treatment of alkyl iodide **4** with PPh_3 in refluxing benzene yielded the desired phosphonium salt **5**. Oxidation of the commercially available 3-(perfluorooctyl)propan-1-ol with Dess−Martin periodinane afforded aldehyde **6**. Wittig olefination of **5** and **6** delivered **7**, exclusively as the *Z*-isomer. Final detritylation under aqueous acidic conditions yielded the fluorinated acceptor **2** (Scheme 2).

Scheme 2. Synthesis of the Fluorous Linker



To rapidly investigate the iterative glycosylations, the silicon-glass microreactor was employed to examine the coupling reactions involving the fluorinated acceptor (**2**, **8**, **9**, or **10**), glycosyl phosphate **1**, and the activator (TMSOTf). A mixture of DMF and piperidine, containing methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside as internal reference for LC/MS analysis, was injected to quench the reaction and to cleave the Fmoc group (Scheme 3).

Scheme 3. Optimization of Glycosylation Reactions with Use of a Silicon-Based Microreactor



Each glycosylation reaction was screened at seven different reaction times, as summarized in Table 1. Since the fluorinated acceptor **2** was poorly soluble in CH_2Cl_2 at 0 °C, the first glycosylation was carried out in trifluorotoluene (TFT) (entries 1 and 2).[10] For the first glycosylation,

Table 1. Screening of Glycosylation Conditions[a]

entry	nucleophile (μmol)	**1** (equiv)	TMSOTf (equiv)	solvent	temp (°C)	reaction times (s)
1	**2** (1.25)	2	2	TFT	0	10, 20, 30, 60, 120, 300
2	**2** (1.25)	2	2	TFT	20	10, 20, 30, 60, 120, 300
3	**8** (1.25)	2	2	CH_2Cl_2	20	10, 20, 30, 60, 120, 300, 600
4	**9** (1.25)	2	2	CH_2Cl_2	20	10, 20, 30, 60, 120, 300, 600
5	**9** (0.83)	3	3	CH_2Cl_2	20	10, 20, 30, 60, 120, 300, 600
6	**10** (0.83)	3	3	CH_2Cl_2	20	10, 20, 30, 60, 120, 300, 600

[a] Experiments were performed in triplicate, except for entry 6.

(6) For reviews on F-tag chemistry, see: (a) Zhang, W. *Tetrahedron* **2003**, *59*, 4475−4489. (b) Miura, T. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 351−358 and references cited therein. For F-tag oligosaccharide synthesis, see: (c) Miura, T.; Satoh, A.; Goto, K.; Murakami, Y.; Imai, N.; Inazu, T. *Tetrahedron: Asymmetry* **2005**, *16*, 3−6. (d) Manzoni, L.; Castelli, R. *Org. Lett.* **2006**, *8*, 955−957. (e) Mizuno, M.; Goto, K.; Miura, T.; Inazu, T. *QSAR Comb. Sci.* **2006**, *25*, 742−752 and references cited therein.

(7) Buskas, T.; Söderberg, E.; Konradsson, P.; Fraser-Reid, B. *J. Org. Chem.* **2000**, *65*, 958−963.

(8) (a) Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162−13163. (b) Mamidyala, S. K.; Ko, K. S.; Jaipuri, F. A.; Park, G.; Pohl, N. L. *J. Fluorine Chem.* **2006**, *127*, 571−579.

a clear conversion optimum was found at 20 °C and 30 s reaction time (Figure 1). For batch syntheses, reaction times are typically longer and reaction temperatures are lower.[11]

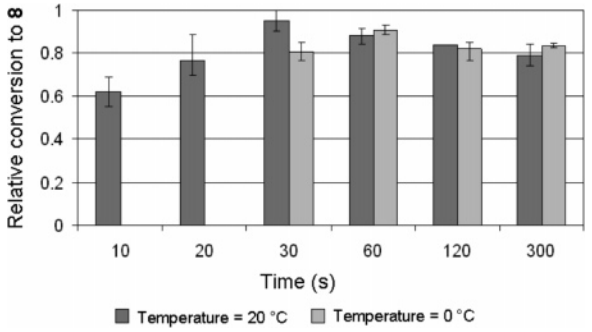
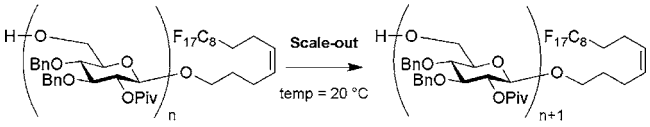


Figure 1. Optimization of the first glycosylation.

After FSPE,[12] the monoglycoside **8** was contaminated with the C(6)-*O*-TMS derivative that was desilylated by treatment with silica gel.[13] Complete conversion to disaccharide **9** required a reaction time of 20 s at 20 °C (entry 3).[13] The same reaction temperature was applied to the third glycosylation to form trisaccharide **10** (entry 4).[13] In all cases, unreacted starting material **9** was observed. Increasing amounts of glycosyl phosphate **1** (3 equiv) drove the glycosylation to completion at an optimal reaction time of 60 s (entry 5). For the final glycosylation step, formation of the desired tetrasaccharide **11** was investigated by employing again 3 equiv of glycosyl phosphate **1** (entry 6).[13] Complete conversion to tetrasaccharide **11** required 60 s of reaction time.

The optimized reaction conditions for each glycosylation were scaled-out to furnish larger quantities of the desired oligosaccharides (Scheme 4). The different oligosaccharides

Scheme 4. Oligosaccharide Synthesis with Use of the Optimized Reaction Conditions

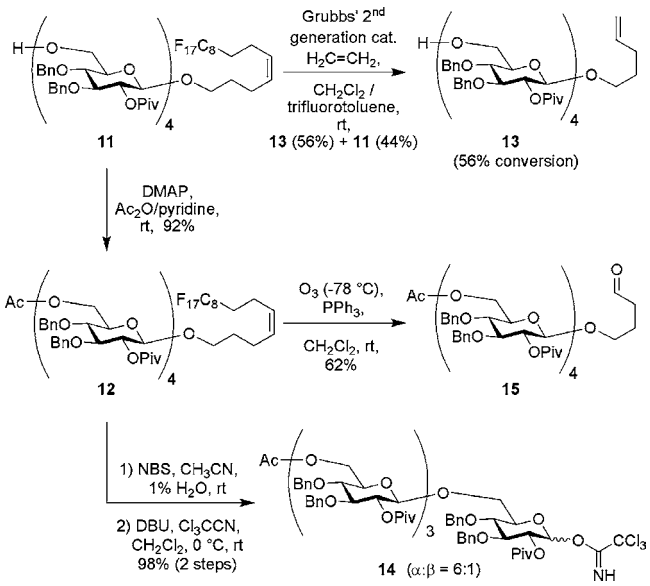


nucleophile	scale (mmol)	solvent	**1** (equiv)	TMSOTf (equiv)	time [s]	TBAF (equiv)	product	yield
n = 0, **2**	0.214	TFT	2	2	30	1.5	**8**	99%
n = 1, **8**	0.176	CH_2Cl_2	2	2	20	1.5	**9**	97%
n = 2, **9**	0.102	CH_2Cl_2	3	3	60	2	**10**	90%
n = 3, **10**	0.073	CH_2Cl_2	3	3	60	2	**11**	95%

were obtained after FSPE in excellent yield and purity (**8**, 99%; **9**, 97%; **10**, 90%; and **11**, 95%) and were directly used for the next step. The first glycosylation produces 11.3 mmol of product per day.

Following oligosaccharide assembly, the perfluorinated linker of tetrasaccharides **11** and **12** was transformed into different functional groups (Scheme 5). Olefin cross-

Scheme 5. Functionalization of the Linker



metathesis of **11** and ethylene with use of Grubbs' second generation catalyst afforded the desired *n*-pentenyl glycoside **13** (56%), while starting material **11** remained (44%). The acetylated derivative **12** was hydrolyzed under Fraser-Reid's conditions[14] to yield the lactol that was further transformed into glycosyl trichloroacetimidate **14** (ratio α:β = 6:1, 98% yield for 2 steps). Ozonolytic cleavage of the double bond of **12** afforded the desired aldehyde **15** in 62% yield.

In summary, we report the synthesis of a β-(1→6) linked D-glucopyranoside homotetramer in a silicon-glass microreactor using iterative glycosylations. Each glycosylation was optimized and scaled-out in the microfluidic device to obtain the desired oligosaccharides in excellent yield. Notably, the continuous-flow microreactor allowed for glycosylations by using β-glycosyl phosphates at ambient temperature. A perfluorinated linker was incorporated and allowed for the successful purification of the oligosaccharides by FSPE and additionally served as an *n*-pentenyl-type linker for further functionalization.

(9) Nicolaou, K. C.; Nikovic, S.; Sarabia, F.; Vourloumis, D.; He, Y.; Vallberg, H.; Finlay, M. R. V.; Yang, Z. *J. Am. Chem. Soc.* **1997**, *119*, 7974−7991.

(10) At 0 °C in TFT, clogging of the microchannels occurred at 10 s and 20 s reaction time at the quench inlet due to increased back-pressure. This problem was not encountered at lower flow rates. Therefore, no data points are given in Graph 1 for these two reaction times.

(11) Generally, β-glycosylphosphates are activated at temperatures in a range of −78 to −40 °C and typical reaction times range from 5 to 30 min.

(12) A "Siliabond Tridecafluoro, Silicycle" self-packed column was used for FSPE.

(13) Only for the first two glycosylations were the observed C(6)-*O*-TMS derivatives transformed into the desired glycosides **8** and **9** respectively by treatment with silica gel. For large-scale syntheses, formation of the undesired side-products was circumvented by addition of TBAF to the quenching solution.

(14) Motoo, D. R.; Date, V.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1988**, *110*, 2662−2663.

Acknowledgment. We thank the ETH, the Swiss National Science Foundation (SNF grant 200020-109555), and The Netherlands Organization for Scientific Research (N.W.O.) (for J.D.C.C.) for financial support. We gratefully acknowledge Prof. K. F. Jensen and Dr. E. R. Murphy (MIT, USA) for providing the silicon-glass microreactor and Silicycle (Quebec, Canada) for the fluorinated silica gel used for FSPE.

Supporting Information Available: Detailed experimental procedures and compound characterization data, including NMR spectra for all described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

OL0705503