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On-Resin Real-Time Reaction Monitoring of Solid-Phase Oligosaccharide Synthesis

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The roles of glycoconjugates in various aspects of biological events have been well defined in recent years.[1] To gain facile access to a synthetic oligosaccharide in significant amount, solid-phase synthesis has received much attention in the past decade.[2] However, solid-phase reaction has an obvious drawback due to the inherent difficulties in monitoring the reaction profile. As a result, a significant time and trial-and-error effort are often required to establish a practical route and optimize each reaction condition. For monitoring solid-phase peptide synthesis, the Kaiser test (ninhydrin test) that detects the residual amino group is widely used.[3] To attain the similar situation in solid-phase oligosaccharide synthesis, the detection of the hydroxy group is required, which is much more difficult. Although spectroscopic means such as high-resolution magic angle spinning NMR,[4] gated decoupling NMR using a ^{13}C enriched acetyl group,[5] and the ^{19}F NMR technique[6] have proved to be powerful, these methods are not suitable for real-time monitoring. We wish to report herein a practical method for the real-time monitoring of solid-phase oligosaccharide synthesis, which enables side-by-side detection of glycoside bond formation and acceptor consumption. It relies solely upon on-resin color tests and is readily performed in any organic chemistry laboratory.

Recently, we developed the real-time monitoring method for solution-phase polymer-supported carbohydrate synthesis, which employs MALDI-TOF MS and a color test for the chain elongation and selective deprotection, respectively.[7]

For the latter purpose, the chloroacetyl (CAc) group was used as the “temporary” hydroxy protection, removal of which can be achieved in a highly chemoselective manner. Its presence can be detected with high precision by a red color generated with (*p*-nitrobenzyl)pyridine (PNBP) under basic conditions.[8] This procedure was expected to be suitable for on-resin detection; the color density increases as the CAc having a sugar unit accumulates and fades away after deprotection of the CAc group. On the other hand, to observe the disappearance of the hydroxy group, modified Taddei’s method was to be adopted, which employs cyanuric chloride−Disperse Red conjugate **2**. It readily reacts with nucleophiles such as hydroxy or amino groups to give a persistent red color on the resin (Figure 1).[9]

Our experiment commenced with the preparation of resin-supported acceptor, which was performed as depicted in Scheme 1. The 3-position of **3** was selectively protected as TBS ether, and subsequent benzoylation gave **4**.[10] Reductive ring opening of the benzylidene group was accompanied by desilylation, and resultant diol was converted to **5**. After desilylation, the acid-withstanding linker **8**[11] was introduced to give **6**. The latter was immobilized onto piperazine modified TentaGel **9** in the presence of 1,3-diisopropylcarbodiimide to give amido linked **7**.

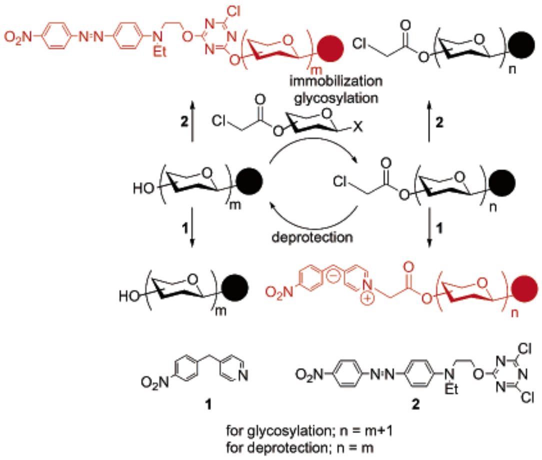
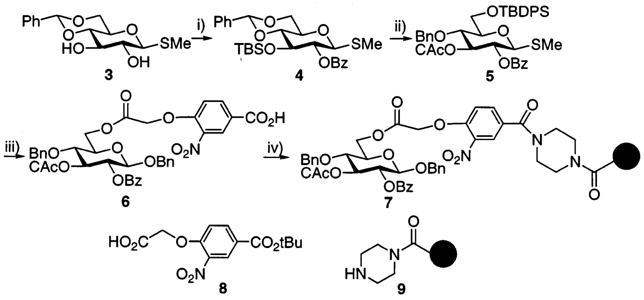


Figure 1. The (*p*-nitrobenzyl)pyridine method and Disperse Red−cyanuric chloride conjugate method monitoring cycle in solid-phase oligosaccharide synthesis.

Scheme 1 [a]



[a] Reagents and conditions: (i) (a) TBSCl, imidazole, DMF, quant., (b) benzoyl chloride, DMAP, pyridine, 75%; (ii) (a) $BH_3{\cdot}NMe_3$, $AlCl_3$, MS 4A, CH_2Cl_2, (b) TBDPSCl, Et_3N, DMAP, CH_2Cl_2 (95%, two steps), (c) chloroacetic anhydride, pyridine, CH_2Cl_2, 92%; (iii) (a) BnOH, NIS, TESOTf, 97%, (b) aqueous HF, CH_3CN, 85%, (c) **8**, PPh_3, DEAD, CH_2Cl_2, 99%, (d) TFA, CH_2Cl_2, quant.; (iv) **9**, 1,3-diisopropylcarbodiimide, CH_2Cl_2.

At this stage, successful immobilization was clearly seen by the color test with PNBP **1**, while a positive response to Disperse Red **2** indicated the presence of the residual amino group (Figure 2). After capping with Ac_2O, we monitored the deprotection reaction of CAc in a real-time manner. Thus, **7** was reacted with 10 equiv of HDTC (hydrazinedithiocarbonate)[12] to yield **10** in 5 min as indicated by the PNBP color test.

Subsequently, acceptor-immobilized resin **10** was submitted to the glycosylation reaction using **11** as a donor. Completion of the reaction was ascertained by the Disperse Red (negative) as well as PNBP (strongly positive) test. The quality of disaccharide **12** was confirmed after cleavage from resin by NaOMe to afford **14** in

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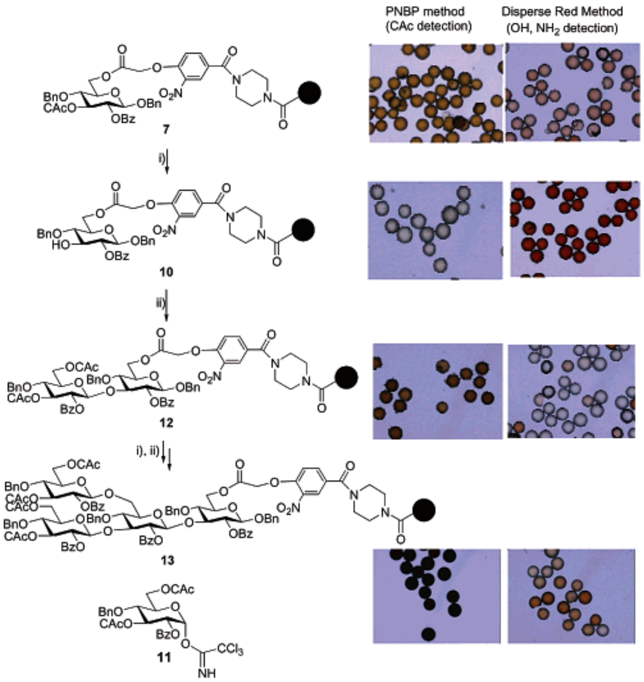


Figure 2. Reaction monitoring by PNBP and Disperse Red method. (i) (a) Ac$_2$O, *i*-Pr$_2$NEt, CH$_2$Cl$_2$, (b) HDTC, DMF; (ii) **11**, BF$_3$·OEt$_2$, CH$_2$Cl$_2$.

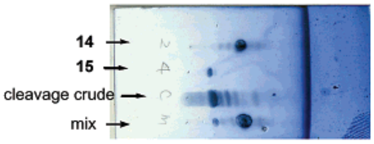
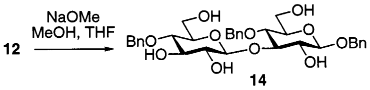
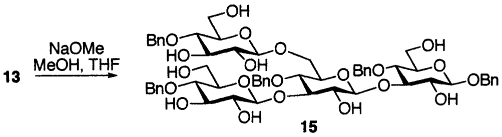


Figure 3. TLC profile of the crude mixture after cleavage of **13** (CHCl$_3$:MeOH 8:1).

Scheme 2



Scheme 3



45% yield (Scheme 2) (for TLC profile, see the Supporting Information).

Resin-bound disaccharide **12** was subjected to the second round of dechloroacetylation-glycosylation, and the tetrasaccharide, which corresponds to the repeating unit of the immuno-active oligosaccharide schizophyllan,[13] was constructed successfully again with on-resin real-time monitoring. After cleavage, the tetrasaccharide **15** was obtained in a reasonably pure form (Scheme 3, Figure 3). After chromatographic purification, **15** was isolated in 30% overall yield based on the initiated loading of **9** (Scheme 3, Figure 3).

In summary, a practical method for the real-time reaction monitoring on-beads for oligosaccharide construction was established. The deprotection of CAc was monitored by the PNBP method, whereas the glycosylation was monitored by the cyanuric chloride−Disperse Red conjugate method. It is operationally simple and quick and does not require any special equipment. Because PNBP and Disperse Red color tests are complementary to each other, the combination allows one to gain a clear indication of the progress of glycosylation, even if complete conversion is not obtained. Several examples of fluorescence or dye based screening methods of reaction conditions are known;[14] however, to our knowledge, this is the first example for the multistep transformation monitored on resin, except peptide synthesis. Further application of this methodology will be reported in due course.

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Supporting Information Available: Detailed experimental procedures for the synthesis of all compounds, general procedure, and deprotection and glycosylation reactions on solid-phase, ^{1}H and ^{13}C NMR spectra (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) *Essentials of Glycobiology*; Varki, A., Cummings, R., Esko, J., Freeze, H., Hart, G., Marth, J., Eds.; Cold Spring Harbor Laboratory Press: Plainview, NY, 1999.
(2) (a) Ito, Y.; Manabe, S. *Curr. Opin. Chem. Biol.* **1998**, *2*, 701−708. (b) Seeberger, P. H.; Hasse, W.-C. *Chem. Rev.* **2000**, *100*, 4349−4394.
(3) Kaiser, E.; Colescott, R L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595−598.
(4) Seeberger, P. H.; Beebe, X.; Sukenick, G. D.; Pochapsky, S.; Danishefsky, S. J. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 491−493.
(5) (a) Kanemitsu, T.; Kanie, O.; Wong, C.-H. *Angew. Chem., Int. Ed.* **1998**, *37*, 3415−3418. (b) Kanemitsu, T.; Wong, C.-H.; Kanie, O. *J. Am. Chem. Soc.* **2002**, *124*, 3591−3599.
(6) Mogemark, M.; Elofsson, M.; Kihlberg, J. *Org. Lett.* **2001**, *3*, 1463−1466.
(7) Ando, H.; Manabe, S.; Nakahara, Y.; Ito, Y. *J. Am. Chem. Soc.* **2001**, *123*, 3848−3849.
(8) Kuisle, O.; Lolo, M.; Quiñoá, E.; Riguera, R. *Tetrahedron* **1999**, *55*, 14807−14812.
(9) (a) Attardi, M. E.; Falchi, A.; Taddei, M. *Tetrahedron Lett.* **2000**, *41*, 7395−7399. (b) Attardi, M.; Taddei, M. *Tetrahedron Lett.* **2001**, *42*, 2927.
(10) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; DeRoose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449−450.
(11) (a) Manabe, S.; Nakahara, Y.; Ito, Y. *Synlett* **2000**, 1241−1244. (b) Manabe, S.; Ito, Y. *Chem. Pharm. Bull.* **2001**, *49*, 1241−1242. (c) Wu, X.; Grathwohl, M.; Schmidt, R. R. *Org. Lett.* **2001**, *3*, 747−750.
(12) van Boechel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983**, *24*, 3775−3778.
(13) (a) Tabata, K.; Ito, W.; Kojima, T.; Kawabata, S.; Misaki, A. *Carbohydr. Res.* **1981**, *89*, 121. (b) Nishimura, K.; Nishimura, S.; Nishi, N.; Saiki, I.; Tokura, S.; Azuma, I. *Vaccine* **1984**, *2*, 93−99. (c) Chihara, G. *Cancer Res.* **1986**, 85−96. (d) Schizophyllan was demonstrated to form a triple helix with single-stranded RNA: Sakurai, K.; Shinkai, S. *J. Am. Chem. Soc.* **2000**, *122*, 4520−4521. (e) For a recent example of solution-phase synthesis of immuno-active β-glycan, see: Yang, G.; Kong, F. *Synlett* **2000**, 1423−1426.
(14) (a) Weingarten, M. D.; Sekanina, K.; Still, W. C. *J. Am. Chem. Soc.* **1998**, *120*, 9112−9113. (b) Shaughnessy, K. H.; Kim, P.; Hartwig, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 2123−2132. (c) Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4306−4307. (d) Korbel, G. A.; Lalic, G.; Shair, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 361−362.

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