Tag-Reporter Strategy for Facile Oligosaccharide Synthesis on Polymer Support

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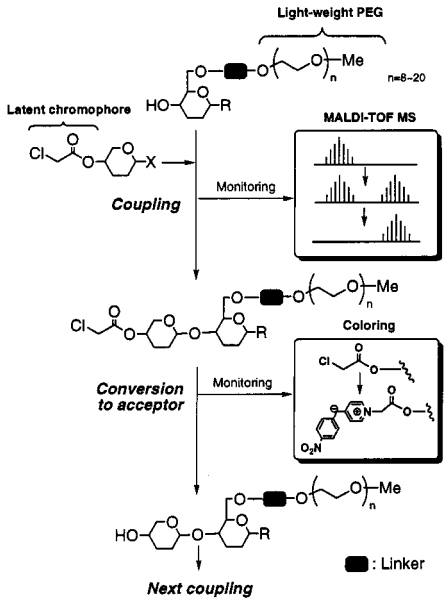
Development of a general method for the rapid assembly of oligosaccharides based on polymer support technology has been the subject of intense effort.[1] Considering the structural diversity of glycoconjugate derived oligosaccharides, the advantages of polymer support synthesis are obvious, in terms of speeding up as well as potential for extension to combinatorial and automated synthesis. However, to reach this goal, several fundamental problems have yet to be overcome, the most serious of which are (1) the reduced reactivity of substrates bound to a polymer support, (2) the difficulty of monitoring such reactions, and (3) limitations on the ability to scale up reactions. Taking account of these shortcomings, it would be most advantageous to merge polymer support technology with solution-phase chemistry, so that the major drawbacks inherent to solid-phase reactions could be kept to a minimum.[2] Herein, we report a novel technology for monitorable, high-yielding soluble polymer support oligosaccharide synthesis[3] based on the "tag-reporter" strategy. This exploits low-molecular weight poly(ethylene)glycol (PEG)[4] as a polymer support tag in combination with temporary chloroacetyl (CAc) protection, which also serves as a reporter group (Scheme 1). With this combination, the chain elongation process (glycosylation) and chemoselective deprotection can be monitored by matrix-associated laser desorption/ionization mass spectrometory (MALDI-TOF MS)[5] and a coloring reaction, respectively.

Scheme 1. Tag-Reporter Strategy



Starting with PEG-tagged acceptor, each glycosylation reaction is monitored by MALDI-TOF MS, because PEG-bound materials are easily distinguished from others by their mountain-like shape which derives from statistical distribution of the PEG chain length consisting of 8−20 ethylene glycol units. Retrieval of the coupled product can be achieved simply by direct chromatography of the reaction mixture on silica gel. With ethyl acetate (AcOEt), the PEG-tagged component stays at the origin, while nonsupported sugars move rapidly through the column. After AcOEt washing, PEG-bound products can be readily eluted with AcOEt−MeOH. The CAc group was adopted as a "latent chromophore", relying upon its reactivity with *p*-nitrobenzylpyridine (PNBP) and piperidine to form a strongly colored internal salt.[6]

As a demonstration of the power of our strategy, the assembly of tetrasaccharide **17** was conducted. Preparation of polymer-bound sugar primer **12** began with straightforward synthesis of nitro-containing linker **3**[7] from commercially available **1** via **2**, which was subsequently bound to glucosamine derivative **4** in a manner similar to that for conventional benzylidene formation (Scheme 2). Subsequent manipulation via **6**, **7**, **8**, and **9** gave **10**, which was subsequently deblocked by removal of the ^{t}Bu group to liberate the carboxylic acid, which was used for coupling with PEG (av MW 550) to afford **11**. As we hoped, the dechloroacetylation of **11** was easily monitored by the coloring test which was carried out by successive treatment with PNBP and piperidine. Quantification of the color density was carried out on TLC plates which were processed using a scanner and the NIH Image[8]

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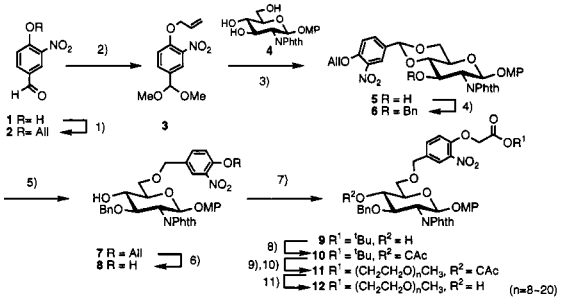
Scheme 2. Synthesis of PEG-Tagged Sugar[a]



[a] Reagents and yields: 1) allyl bromide, K_2CO_3/CH_3CN, 60 °C, quant.; 2) $CH(OCH_3)_3$, CSA/Drierite, CH_3CN, 40 °C; 3) **4**, CSA/Drierite, CH_3CN, 55 °C, 95%; 4) BnBr, NaH/DMF, 93%; 5) 4.0 M HCl in dioxane, $NaBH_3CN$/THF, quant.; 6) $Pd(PPh_3)_4$, Et_3SiH, AcOH/toluene, 92%; 7) $BrCH_2CO_2{}^tBu$, K_2CO_3/CH_3CN, 60 °C, 92%; 8) $(ClCH_2CO)_2O$, Pyr/CH_2Cl_2, 0 °C, 97%; 9) TFA/CH_2Cl_2; 10) PEG monomethyl ether, DCC, DMAP/CH_2Cl_2, 89% (over 2 steps); 11) DMAP, 50% aq Pyr (pH 8−9), 95%.

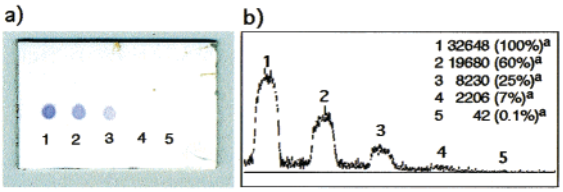
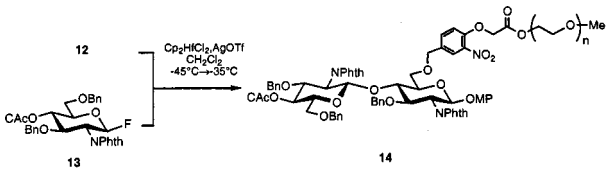


Figure 1. Monitoring of dechloroacetylation of **11** by coloring with PNBP and piperidine. (a) TLC profile (lane 1: 0 h, lane 2: 0.5 h, lane 3: 3.0 h, lane 4: 3.5 h, lane 5: 6 h), (b) plot profile and density values analyzed by NIH Image program (numbers are corresponding to theirs in (a)). [a]Density percentage to that of lane 1.

Scheme 3. Coupling Reaction on Polymer Support



program. As shown in Figure 1, dechloroacetylation was accompanied by almost complete disappearance of color. The resultant **12** was determined to be quantitatively deprotected by ^{1}H NMR, which revealed the complete disappearance of the low-field H-4 signal that originally appeared at δ 5.25.

The coupling reaction was performed using fluoride **13** under Suzuki conditions[9] and was monitored by MALDI-TOF MS (Scheme 3, Figure 2). Complete consumption of **12** as well as near quantitative formation of the disaccharide was observed as a shift of the broad peak. The PEG-bound fractions were separated using a silica gel column, and formation of disaccharide **14** was confirmed by ^{1}H NMR. The coupling yield was estimated to be greater than 98% judging from the relative intensity of the H-1 signals of the disaccharide (δ 5.41 and 5.26) and the unreacted monosaccharide (δ 5.63). This nearly quantitative coupling was reproducible on gram scales.

Thus obtained disaccharide **14** was subjected to two dechloroacetylation (89−96%)/coupling (96−97%) cycles, monitored by color and MALDI-TOF MS, to afford tetrasaccharide **16** (Scheme 4). After the CAc group was removed, liberation of the tetrasaccharide from the polymer support was performed under previously reported conditions for reductive cyclorelease by the action of $Sn(SPh)_2$, PhSH, and Et_3N in benzene.[7] The resulting hydroxamic acid **17a** was treated with CSA in MeOH at 45 °C[7] to give **17b** (74% from **16**; overall yield from **14**, 59%).

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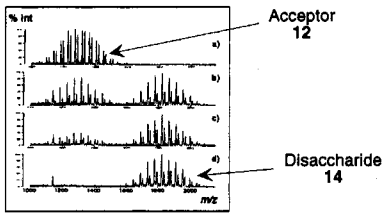
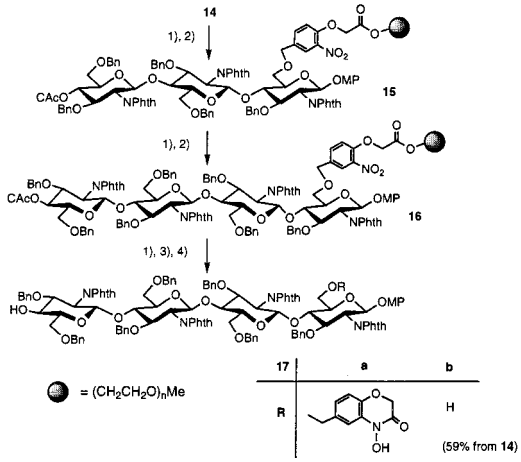


Figure 2. MALDI-TOF MS spectra showing the course of coupling reaction of **12** and **13**. (a) 0 min; (b) 1 min; (c) 3 min; (d) 20 min. Monosaccharide (**12** + Na)$^+$ and (**12** + K)$^+$ are appearing as a mountaneous shape on left side (m/z = 1104 to 1648) and disaccharide (**14** + Na)$^+$ and (**14** + K)$^+$ are appearing on right side (m/z = 1652 to 2064).

Scheme 4. Tetrasaccharide Assembly on Polymer Support and Its Release[a]



In summary, we have developed a new method for monitoring polymer-supported glycan chain assembly. The process is rapid, efficient, and high-yielding, and in addition, the course of each reaction can be monitored in real-time and to very sensitive degree. Research to further refine this technology is ongoing as part of work on developing a general method for automated oligosaccharide synthesis.

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Supporting Information Available: Experimental details, ^{1}H NMR spectra of compounds **2**, **3**, **5**, **6**, **7**, **8**, **9**, **10**, **11**, and **17b**, ^{1}H- and ^{13}C NMR (DEPT) of **12**, **14**, **15**, and **16**, and MALDI-TOF MS profiles of coupling reactions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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