JOC*Note*

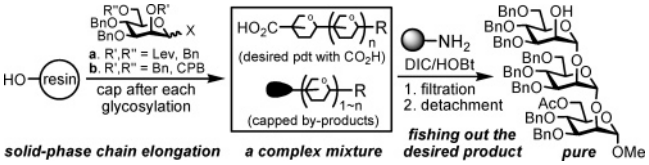
Cap and Capture−Release Techniques Applied to Solid-Phase Synthesis of Oligosaccharides

Jian Wu and Zhongwu Guo*

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, Michigan 48202

zwguo@chem.wayne.edu

Received February 7, 2006



This paper reports a new strategy for oligosaccharide synthesis by combining solid-phase methods with cap and capture−release separation techniques, using the *p*-(5-(ethoxycarbonyl)pentyloxy)benzyl group (CPB) as a tag for the capture of desired oligosaccharides. After a complex carbohydrate mixture was obtained by solid-phase synthesis, the desired oligosaccharide containing a free carboxyl group derived from CPB was attached to an amino resin. The loaded resin was readily separated from side products by filtration and finally treated with acid to release the pure oligosaccharide product.

Among the three major classes of biooligomers, which include oligosaccharides, oligonucleotides, and oligopeptides, the structure of oligosaccharides is by far the most complex and diverse. As a result, the chemical synthesis of oligosaccharides posts an important challenge in organic chemistry,[1] despite the significant advancement in carbohydrate chemistry in the past three decades.[2−5] For instance, although automated solid-phase synthesis of oligonucleotides and oligopeptides using synthesizers has become routine, the attempt to automate solid-phase oligosaccharide synthesis has just started.[6] Solid-phase carbohydrate synthesis has shown great promise,[7−9] as it can significantly simplify the oligosaccharide assembly by eliminating the time-consuming process of intermediate purification. However, it is exactly this feature that often complicates the final product purification.

Compared to the coupling reactions used in peptide and nucleotide synthesis, glycosylation reactions established for carbohydrate synthesis are much less effective,[10] which is even more so for the two-phase reactions in solid-phase synthesis. Consequently, after a few steps of glycosylations, a significant amount of side products with deficient carbohydrate sequences will accumulate on the resin, whereas only a low yield of the desired oligosaccharide will be formed. After the products are released from the resin, the desired oligosaccharide has to be isolated from a large quantity of impurities, which can be especially challenging. Meanwhile, the removal of an array of side products that are different by just one sugar unit is another notable difficulty.[11] Therefore, an efficient method to purify the desired final product is one of the key issues that needs to be addressed to achieve practical, automated solid-phase carbohydrate synthesis.

In recent years, many new and interesting strategies have been developed for product isolation and purification in organic synthesis,[12,13] among which the capture−release technique using various unique "capture" reagents has been extensively investigated in combinatorial, solution-phase, and solid-phase synthesis of peptides, carbohydrates, and other molecules.[14,15] The basic principle of this strategy is to label the desired product with a tag during the synthetic process. After the synthesis is finished, the tagged structures are captured by materials, such as resin or column, which can specifically bind or react with the tag to facilitate the separation of the desired products. Insoluble polymers are commonly used as capture materials, as they enable separation by filtration.

The polymer-based capture−release technique has brought up many brilliant designs for carbohydrate synthesis.[15] For example, in soluble polymer-supported carbohydrate synthesis, Ito and co-workers[10,16,17] used the chloroacetyl (ClAc) group as a temporary protection of hydroxyl groups and as the tag for solid-phase capture−release as well. After each glycosylation reaction, the product was treated with a capture resin having free thio groups. Although the unreacted glycosyl acceptor was inert to capture resin, the elongated sugar chains, which contained ClAc, would link to the resin via the thio groups. The loaded resin could be easily separated from the side products by filtration. Thereafter, the oligosaccharide on the capture resin was released and then subjected to the next cycle of polymer-supported reaction and capture−release purification. A useful feature of this approach is that each glycosylation product was purified. Another elegant approach was developed by Fukase and co-workers[18,19] using 3-chloro-4-azidobenzyl (ClAzb) as a temporary protecting group and the tag of incoming sugar units. After the elongation was completed and the carbohydrates were detached from the solid-phase support, the desired oligo-

(1) Paulsen, H. *Chem. Soc. Rev.* **1984**, 15.
(2) Boons, G. J. *Contemp. Org. Synth.* **1996**, *3*, 173.
(3) Koeller, K. M.; Wong, C.-H. *Chem. Rev.* **2000**, *100*, 4465.
(4) Demchenko, A. V. *Synlett* **2003**, 1225.
(5) Boons, G.-J. *Tetrahedron* **1996**, *54*, 1095.
(6) Plante, O. J.; Palmacci, E. R.; Seeberger, P. H. *Science* **2001**, 1523.
(7) Seeberger, P. H.; Danishefsky, S. J. *Acc. Chem. Res.* **1998**, *31*, 685.
(8) Seeberger, P. H.; Haase, W.-C. *Chem. Rev.* **2000**, *100*, 4349.
(9) Sears, P.; Wong, C. H. *Science* **2001**, *291*, 2344.
(10) Ando, H.; Manabe, S.; Nakahara, Y.; Ito, Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 4725.
(11) Palmacci, E. R.; Hewitt, M. C.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2001**, *40*, 4433.
(12) Curran, D. P. *Angew. Chem., Int. Ed.* **1998**, *37*, 1174.
(13) Tzschucke, C. C.; Markert, C.; Bannwarth, W.; Roller, S.; Hebel, A.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *41*, 3964.
(14) Kirschning, A.; Monenschein, H.; Wittenberg, R. *Chem.−Eur. J.* **2000**, *6*, 4445.
(15) Ito, Y.; Manabe, S. *Chem.−Eur. J.* **2002**, *8*, 3076.
(16) Hanashima, S.; Manabe, S.; Ito, Y. *Synlett* **2003**, 979.
(17) Hanashima, S.; Manabe, S.; Ito, Y. *Angew. Chem., Int. Ed.* **2005**, *44*, 4218.
(18) Egusa, K.; Kusumoto, S.; Fukase, K. *Synlett* **2001**, 777−780.
(19) Egusa, K.; Kusumoto, S.; Fukase, K. *Eur. J. Org. Chem.* **2003**, 3435.

10.1021/jo060255g CCC: $33.50
Published on Web 07/29/2006

saccharide was captured by a resin that could selectively react with the azido group. One potential problem that may affect the efficiency of the capture−release purification is that the unreacted glycosyl acceptors may participate in subsequent chain elongation.

Another useful and closely related technique in organic synthesis is the employment of a scavenger resin to remove byproducts or the excessive reagents.[20−22] Seeberger and co-workers[11] have made creative use of this technique by combining it with automated carbohydrate synthesis and the cap technique. After each glycosylation, the unreacted glycosyl acceptor was capped by the 2-azido-2-methylpropionyl (AMP) group. AMP also served as a tag to facilitate the scavenge technique. Once the chain elongation was complete and the products were detached from the solid-phase support, side products were removed by a resin via its selective reaction with AMP and filtration. The use of the cap technique reduced the complexity of the resultant product mixture.

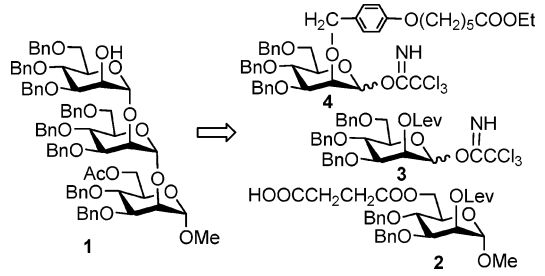
In the present paper, we described a new strategy for carbohydrate synthesis that combines solid-phase synthesis with cap and capture−release techniques. We expect that it will significantly facilitate the final product purification and improve carbohydrate synthesis efficiency.

Our strategy is outlined as follows. After each glycosylation, we use a cap to block the remaining hydroxyl groups, which can prevent the deficient structures from further elongation. Next, the temporary protecting group of the installed sugar unit will be removed to expose a free hydroxyl group and subject to the next cycle of glycosylation, capping, and deprotection. The same protocol will be repeated to introduce other monosaccharide units. The last glycosyl donor is designed to bear a unique functionality. Thus, the final synthetic target, and only the desired one, will contain this unique group to facilitate its capture by a resin. After the side products, which remain in solution, are removed through filtration, the desired oligosaccharide will be released from the capture resin. Theoretically, the product thus obtained should be homogeneous.

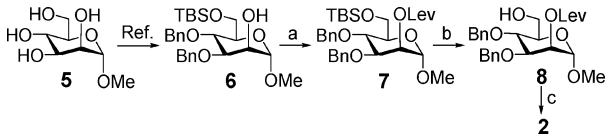
This new strategy will benefit the final product purification in several aspects. First, the cap technique will minimize the number and complexity of side products formed on the resin. Second, no matter how complex or big the desired oligosaccharide is, only the last glycosyl donor needs a special design. This can simplify the selection of protection tactics and the synthesis of building blocks. Third, the capture resin will catch or react with the desired product only, because in the mixture only it contains the tag. Therefore, even if the coupling reaction is less than perfect, only the recovery rate of the desired product will be compromised and not its purity.

For the new strategy to work well, we need to address several issues. First, the cap reaction must be efficient and easily achievable, and the cap must be stable to the reactions involved in carbohydrate chain elongation. In this respect, the acetyl group seems to be an ideal choice. Second, the reaction conditions used to remove the temporary protecting group must be compatible with the cap as well as the linker between the solid support and the sugar chain. We chose the levulinoyl group (Lev) for this use, as it can be easily removed by reaction with

SCHEME 1



SCHEME 2[a]



[a] Reagents and conditions: (a) LevOH, DIPC, DMAP, CH_2Cl_2, 0 °C to room temperature, 4 h, 95%; (b) TBAF, HOAc, THF, o/n, 95%; (c) succinic anhydride, DMAP, pyridine, o/n, quantitative.

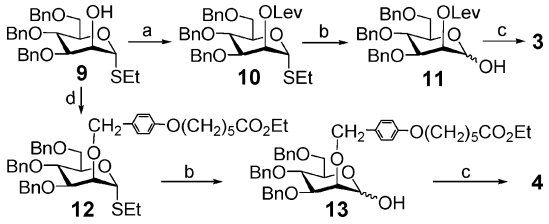
hydrazine at room temperature.[23] Third, the reaction used to couple the desired oligosaccharide and the capture resin has to be simple and effective. To fulfill this condition, we designed the *p*-(5-carboxypentyloxy)benzyl (CPB) group as a tag of the terminal sugar unit. The carboxyl group of CPB can react with amino resins effectively enabling the product capture. Meanwhile, CPB should be easily removable under mild oxidative or moderate acidic conditions to realize the release of attached oligosaccharides later on.

We chose an α-linked trimannoside **1** as our synthetic target to test the new strategy (Scheme 1). This trisaccharide appears in many natural glycoconjugates[24,25] and is therefore the focus of a number of synthetic studies.[11] Following the discussion above, **1** was disconnected into three logical building blocks **2**−**4**. As **2** contained a free carboxylic group that enabled its attachment to the solid-phase support, its 2-OH was temporarily protected by a Lev group. Glycosyl donor **3** was the typical building block used to elongate carbohydrate chains for the new strategy, and **4** was the terminal sugar unit having the unique tag. For glycosylations, we planned to use the Schmidt method[26] because the reagents and byproducts of this method can be easily washed off after the reaction is finished.

Compound **2** was prepared according to a route shown in Scheme 2 with methyl D-mannoside (**5**) as the starting material. The conversion of **5** to **6** followed a reported procedure.[27] The introduction of Lev to **6** was realized by reaction with levulinic acid and *N*,*N*′-diisopropylcarbodiimide (DIC), to afford an excellent yield (95%) of the desired ester **7**. This condition was also used to install Lev in the synthesis of **3**. Thereafter, the *tert*-butyldimethylsilyl (TBS) group was selectively removed with tetrabutylammonium fluoride (TBAF), which was followed by reaction with succinic anhydride to afford **2**. The free carboxyl group in **2** enabled its coupling to the polymer support for solid-phase synthesis.

(20) Booth, R. J.; Hodges, J. C. *Acc. Chem. Res.* **1999**, *32*, 18
(21) MacCoss, R. N.; Brennan, P. E.; Ley, S. V. *Org. Biomol. Chem.* **2003**, *1*, 2029.
(22) Ito, Y.; Kanie, O.; Ogawa, T. *Angew. Chem., Int. Ed.* **1996**, *35*, 2510.
(23) Van Boom, J. H.; Burgers, P. M. J. *Recl. Trav. Chim. Pays-Bas.* **1978**, *93*, 73.
(24) Ferguson, M. A. J.; Williams, A. F. *Annu. Rev. Biochem.* **1988**, *57*, 285.
(25) Kornfeld, R.; Kornfeld, S. *Annu. Rev. Biochem.* **1985**, *54*, 631.
(26) Schmidt, R. R.; Kinzy, W. *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21.
(27) Gurjar, M. K.; Talukdar, A. *Tetrahedron* **2004**, *60*, 3267.

SCHEME 3[a]



[a] Reagents and conditions: (a) LevOH, DIPC, DMAP, CH_2Cl_2, 0 °C to room temperature, 4 h, ≥95%; (b) NIS, CH_3CN/H_2O (9:1), 5 min, >99%; (c) NaH, CCl_3CN, CH_2Cl_2, 0 °C, 20 min, ≥90%; (d) NaH, ethyl 6-(4-bromomethylphenyloxyl)hexanoate, DMF, −5 °C, 1 h, 90%.

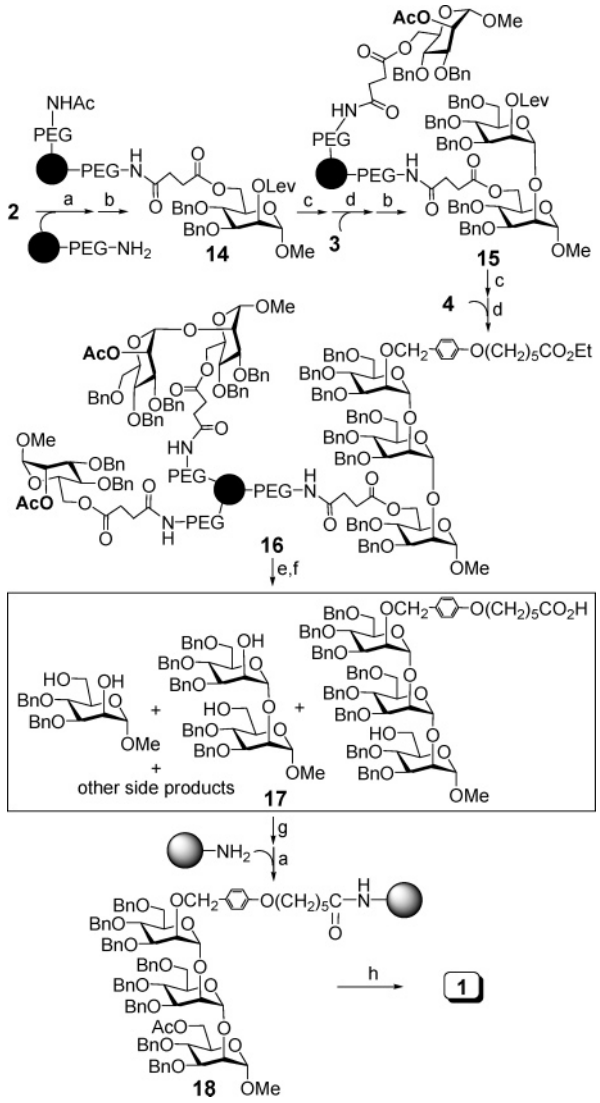
Compounds **3** and **4** were prepared from a common intermediate **9** (Scheme 3).[28] After the introduction of a Lev group to the 2-OH of **9** and oxidative hydrolysis at the anomeric position of **10**, the hemiacetal product **11** was transformed into the Schmidt donor **3** in an excellent yield. In the preparation of **4**, prior to the oxidative hydrolysis and subsequent imidation of the anomeric hydroxyl group, the 2-OH of **9** was protected by CPB first. All transformations shown in Scheme 3 were very efficient.

For solid-phase oligosaccharide assembly (Scheme 4), we used commercially available poly(ethylene glycol) (PEG)-grafted polystyrene with an amino headgroup (0.77 mmol/g) as the solid-phase support. The coupling of **2** to this resin was realized with DIC/HOBt as the condensation reagent. The reaction was effective, judging from the reaction yield (81%) calculated on the basis of the weight gain following the coupling reaction. The remaining free amino groups were capped by treating the resin with acetic anhydrate in pyridine (1:2). Ultimately, the carbohydrate loading of resin **14** was ca. 0.46 mmol/g of dry resin. Thereafter, the carbohydrate chain was elongated by repeated deprotection, glycosylation, and capping, using **3** and **4** as glycosyl donors. The deprotection to remove the Lev group was achieved with hydrazine.[23] To ensure complete deprotection, the resin was treated with hydrazine for a prolonged time (overnight). For Schmidt glycosylation, trimethylsilyl triflate (TMSOTf) was used as the promoter, and the reactions were performed at −20 °C using excessive donors (3 equiv). This reaction did not affect the protecting groups or the linkers. We found that the glycosylation using **3** gave a 52% yield calculated on the basis of the weight gain of the resin. Similar results were obtained with **4**, and the reaction yield was 50%. During carbohydrate chain elongation, the cap of unreacted hydroxyl groups was realized by treating the resin with acetic anhydride in pyridine (1:2).

After two cycles of chain elongation, the carbohydrate chains were released from the polymer support by NaOMe in methanol/dichloromethane, which also removed the acetyl caps. To make sure that the carboxyl group of CPB was deprotected, the product was further treated with NaOH. These operations eventually resulted in a complex mixture (**17**) as proved by its NMR spectrum (Figure 1A). The free hydroxyl groups in **17** were then blocked by acetyl groups, followed by treatment with the fishing resin. The coupling between the fishing resin and CPB was achieved with DIC/HOBt. After washing with solvents, the loaded resin was treated with 10% trifluoroacetic acid (TFA) in dichloromethane. The resin was filtered off and washed with dichloromethane. The filtrate and the washing were

(28) Paulsen, H.; Heume, M. *Carbohydr. Res.* **1990**, *200*, 127.

SCHEME 4[a]



[a] Reagents and conditions: (a) DIC, HOBt, DMF/CH_2Cl_2 (1:1), room temperature, 24 h; (b) Ac_2O/pyridine (1:2), room temperature, 6 h; (c) hydrazine monohydrate, Py/HOAc (3:2), room temperature, o/n; (d) TMSOTf (0.1 equiv), CH_2Cl_2, −20 °C, 2 h; (e) NaOMe, CH_2Cl_2/MeOH (9:1), room temperature, 6 h; (f) NaOH, THF/MeOH (5:1), room temperature, o/n; (g) Ac_2O/pyridine (1:10); (h) TFA/CH_2Cl_2 (1:9), room temperature, 2 h.

combined, rinsed, dried, and condensed to give **1** that was positively identified by NMR and MS spectroscopy. The NMR spectrum of **1** (Figure 1B) suggested its homogeneity. To prove the purity, it was further subjected to silica gel column chromatography. The NMR spectrum (Figure 1C) of chromatography-purified **1** was essentially identical to that of the product released from the fishing resin.

We have proposed and tested in this paper a new strategy for carbohydrate synthesis, which combines solid-phase synthesis with the cap and polymer-based capture−release techniques for reducing side-product formation and facilitating final product purification, respectively. The basic concept is that during solid-phase elongation of the carbohydrate chains acetic anhydride was employed to block free hydroxyl groups of unreacted glycosyl acceptors after each glycosylation, so that the side products with deficient sequences would not grow further. Meanwhile, the last glycosyl donor used was modified

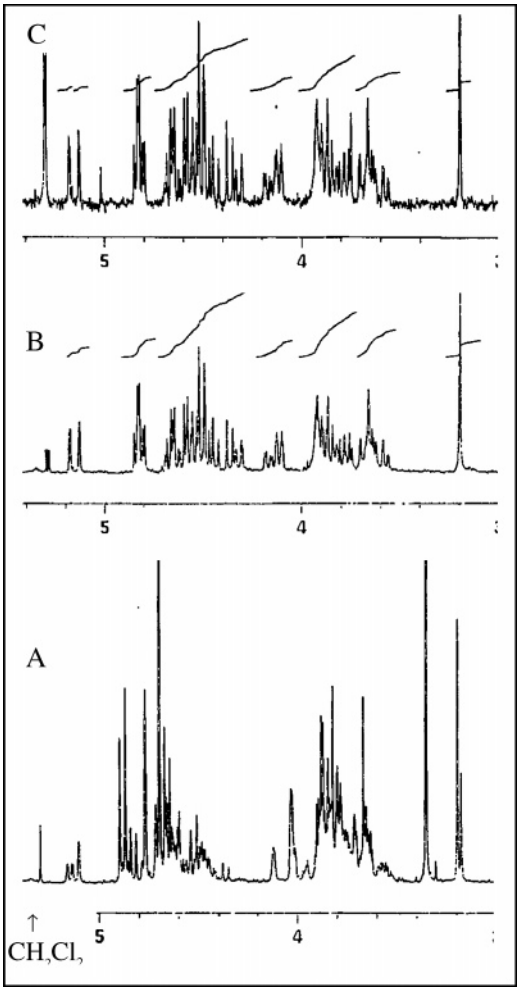


FIGURE 1. ^{1}H NMR spectra of (A) **17**; (B) **1** released from the fishing resin; and (C) **1** after further purification by silica gel column chromatography.

with CPB that contained a carboxyl group. After all products were detached from the solid-phase support, the CPB-containing carbohydrates were able to couple to a second resin, i.e., the capture resin, that contained free amino groups. Although a complex carbohydrate mixture was derived from solid-phase synthesis, only the desired oligosaccharide had CPB and could be captured by this resin. The loaded capture resin was conveniently isolated by filtration and then treated with TFA to release the captured oligosaccharide. Our results showed that the oligosaccharide released from the capture resin was pure and that no further purification was necessary. Because solid-phase carbohydrate synthesis almost always gives complex mixtures, the isolation of the desired oligosaccharide from a large quantity of similar impurities is extremely challenging. The synthetic strategy described herein can significantly simplify and accelerate the final product purification process and result in pure oligosaccharides. Thus, the new strategy should be especially useful for promoting the use of a solid-phase strategy in oligosaccharide synthesis. It is worth noting that our designs are very simple with regard to the synthesis of the tagged sugar unit and the cap and capture−release separation techniques, which makes this strategy practical. The results have unequivocally demonstrated the effectiveness of all designs and have thus provided a proof of principle. We predict that this strategy should be applicable to more complex structures. In fact, we envision that the more complex the synthetic targets are, the more advantageous the new strategy can be.

Experimental Section

Detachment of Carbohydrates from the Solid-Phase (Preparation of 17). Dry resin **16** (215 mg) was mixed with CH_2Cl_2 (2.7 mL), and the resultant suspension was shaken under argon for 10 min. A solution of MeONa (0.46 mmol) in MeOH (0.3 mL) was added, and the mixture was shaken under an N_2 atmosphere for 6 h. TLC analysis confirmed the formation of a carbohydrate mixture in solution. The resin was filtered off and rinsed with CH_2Cl_2/MeOH (9:1, 3 × 5 mL). This detachment procedure was repeated to examine if the cleavage was complete. Then, cation-exchange resin (HP^+ form) was added to the combined filtrate and washed to neutralize the solution. After the ion-exchange resin was filtered off, the filtrate was concentrated to yield a mixture (70 mg). It was dissolved in a 5:1 mixture of THF and MeOH (5 mL), and an aqueous NaOH solution (4 M, 0.1 mmol) was added dropwise at room temperature. After stirring overnight, the reaction mixture was diluted with CH_2Cl_2 and washed with H_2O. The organic layer was dried (Na_2SO_4) and concentrated in a vacuum to offer **17** as a mixture.

Fishing Out the Desired Oligosaccharide from 17 (Preparation of 18). After a mixture of **17**, Ac_2O (0.2 mL), and pyridine (2 mL) was stirred at room temperature for 6 h, it was diluted with CH_2Cl_2, followed by washing with 1 M HCl aq. solution and H_2O. The organic layer was dried over Na_2SO_4 and concentrated in a vacuum. The residue, HOBt (32 mg, 0.24 mmol), and DIC (0.037 mL, 0.24 mmol) were dissolved in dry CH_2Cl_2 (0.5 mL). The resultant solution was added to NovaGel-NH_2 (60 mg, 0.048 mmol) swollen in DMF (0.5 mL) under an argon atmosphere dropwise at room temperature. After the mixture was shaken for 24 h, the solid material was filtered off, washed with H_2O (2 × 15 mL), DMF (2 × 5 mL), THF (2 × 10 mL), and CH_2Cl_2 (2 × 10 mL), and finally dried under vacuum to give **18** (72 mg).

Release of the Desired Oligosaccharide 1 from the Fishing Resin. The resin **18** obtained above (72 mg) was mixed with a solution of 10% TFA in CH_2Cl_2 (1 mL), and the resultant suspension was shaken under argon at room temperature for 2 h. Thereof, TLC analysis confirmed the release of a single carbohydrate product. The resin was then filtered off and rinsed with CH_2Cl_2. This procedure was repeated once to confirm the complete release of carbohydrates from the resin. The combined filtrate and washing were washed with $NaHCO_3$ and H_2O. The organic layer was dried over Na_2SO_4 and concentrated in a vacuum to afford the desired product **1** (10 mg). R_f = 0.34 (toluene/EtOAc = 10:1). $[\alpha]_D^{20}$ +26.5° (*c* 0.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz): 7.40−7.14 (m, 40 H), 5.18 (bs, 1 H), 5.13 (bs, 1 H), 4.86-4.78 (m, 4 H), 4.69-4.41 (m, 12 H), 4.36 (d, J = 12.4 Hz, 1 H), 4.32 (bd, J = 10.0 Hz, 1 H), 4.17 (dd, J = 11.6, 4.0 Hz, 1 H), 4.11 (m, 2 H), 3.99-3.74 (m, 9 H), 3.71-3.61 (m, 4 H), 3.57 (m, 1 H), 3.20 (s, 3 H), 2.39 (d, 1 H), 1.86 (s, 3 H). ^{13}C NMR (125 MHz, $CDCl_3$) 171.3, 138.8, 138.6, 138.6, 138.5, 138.4, 138.4, 138.2, 133.2, 129.9, 128.7, 128.6, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 128.0, 127.9, 127.8, 127.8, 127.7, 127.6, 123.8, 116.1, 101.2, 101.0, 99.8, 80.2, 79.5, 75.6, 75.3, 75.0, 74.53, 74.2, 73.6, 73.4, 72.4, 72.4, 72.3, 71.8, 71.7, 69.7, 69.7, 69.4, 69.2, 68.8, 64.2, 63.5, 55.0, 32.2, 31.8, 29.9, 29.9, 29.6, 21.0. FAB MS (*m*/*e*): calcd for $C_{77}H_{84}NaO_{17}$ (M + Na^+), 1303.6; found 1304.0.

Acknowledgment. We thank the NSF for the financial support of this work through a research grant, CHE-0554777.

Supporting Information Available: Experimental details and selected NMR spectra of intermediates and the final product. This material is available free of charge via the Internet at http://pubs.acs.org.

JO060255G