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Cap-and-Tag Solid Phase Oligosaccharide Synthesis

Frédéric R. Carrel and Peter H. Seeberger*

Laboratory of Organic Chemistry, Swiss Federal Institute of Technology (ETH) Zürich, Wolfgang-Pauli-Strasse 10, 8093 Zürich, Switzerland

seeberger@org.chem.ethz.ch

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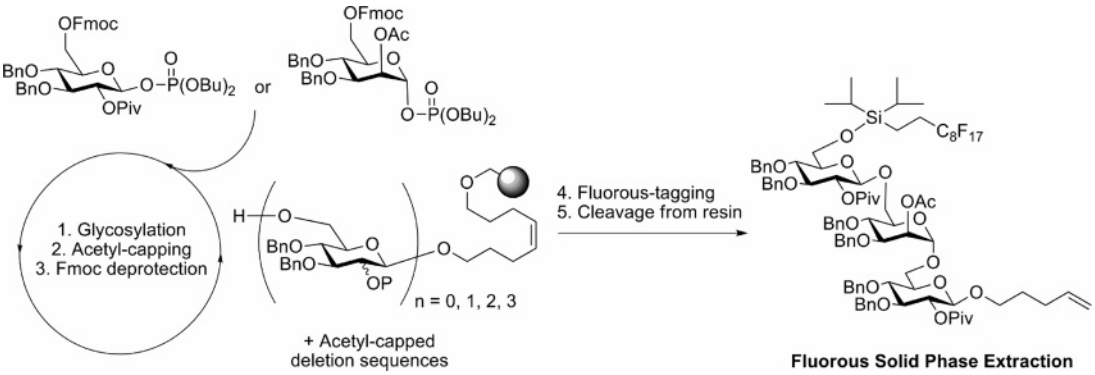

A new "cap-and-tag" strategy is applied to solid phase oligosaccharide synthesis. Acetyl-capping and fluorous-tagging allowed for the facile separation of the desired F-tagged oligosaccharide from the acetyl-capped deletion sequences using fluorous solid phase extraction. To illustrate this approach, a protected Glc-β-(1→6)-Man-α-(1→6)-Glc-β-1→pentenyl trisaccharide was synthesized.

Introduction

Solid phase assembly is commonly employed for the synthesis of complex biomolecules.[1] Due to its heterogeneous character, solid phase synthesis allows for reactions to be driven to completion by addition of excess reagents. Purification is achieved by simple filtration, in contrast to solution phase synthesis where time-consuming chromatographic steps are generally required after each transformation. However, solid phase oligosaccharide synthesis[2] (SPOS) does not always result in quantitative glycosylations and may produce deletion sequences as byproducts (Figure 1A).

These deletion sequences are often difficult to separate from the desired oligosaccharide, especially when the deletion sequence lacks only one sugar unit. In an attempt to tackle this problem, a cap/tag method, where the fluorous cap also acted as tag, was introduced (Figure 1B).[3] The tagged deletion sequences were separated from the desired oligosaccharide by fluorous solid phase extraction (FSPE).[4] Despite the attractiveness of the concept, the need to use the expensive fluorous tag[2a,5]

(1) *Innovation and Perspectives in Solid Phase Synthesis & Combinatorial Libraries*; Epton, R., Ed.; Mayflower Worldwide Limited: Birmingham, 1998.

(2) For books and reviews on solid phase oligosaccharide synthesis, see: (a) *Solid Support Oligosaccharide Synthesis and Combinatorial Carbohydrate Libraries*; Seeberger, P. H., Ed.; Wiley-Interscience: New York, 2001. (b) Seeberger, P. H.; Haase, W. C. *Chem. Rev.* **2000**, *100*, 4349−4393 and references cited therein. See also the articles: (c) Jonke, S.; Liu, K. G.; Schmidt, R. R. *Chem. Eur. J.* **2006**, *12*, 1274−1290. (d) Timmer, M. S. M.; Codee, J. D. C.; Overkleeft, H. S.; van Boom, J. H.; van der Marel, G. A. *Synlett* **2004**, *12*, 2155−2158. (e) Wu, X.; Schmidt, R. R. *Eur. J. Org. Chem.* **2004**, *12*, 2826−2832. (f) Mogemark, M.; Elofsson, M.; Kihlberg, J. *J. Org. Chem.* **2003**, *68*, 7281−7288.

(3) Palmacci, E. R.; Hewitt, M. C.; Seeberger, P. H. *Angew. Chem., Int. Ed.* **2001**, *40*, 4433−4436.

(4) For a review on FSPE, see: (a) Zhang, W.; Curran, D. P. *Tetrahedron* **2006**, *62*, 11837−11865 and references cited therein. See also the articles: (b) Carrel, F. R.; Geyer, K.; Codée, J. D. C.; Seeberger, P. H. *Org. Lett.* **2007**, *9*, 2285−2288. (c) Zhang, W.; Lu, Y. *J. Comb. Chem.* **2006**, *8*, 890−896. (d) Matsugi, M.; Yamanaka, K.; Inomata, I.; Takekoshi, N.; Hasegawa, M.; Curran, D. P. *QSAR Comb. Sci.* **2006**, *25*, 713−715. (e) Hu, G.; Lee, J. S. H.; Li, D. *J. Colloid Interface Sci.* **2006**, *301*, 697−702. (f) Ko, K. S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162−13163. (g) Mamidyala, S. K.; Ko, K. S.; Jaipuri, F. A.; Park, G.; Pohl, N. L. *J. Fluorine Chem.* **2006**, *127*, 571−579.

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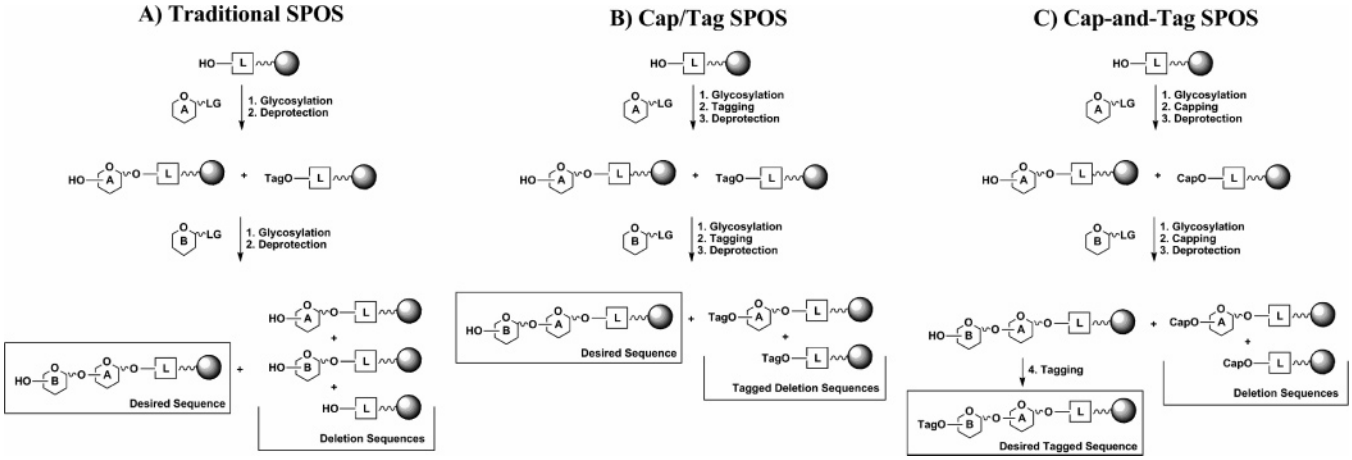


FIGURE 1. Capping and tagging approaches for solid phase oligosaccharide synthesis.

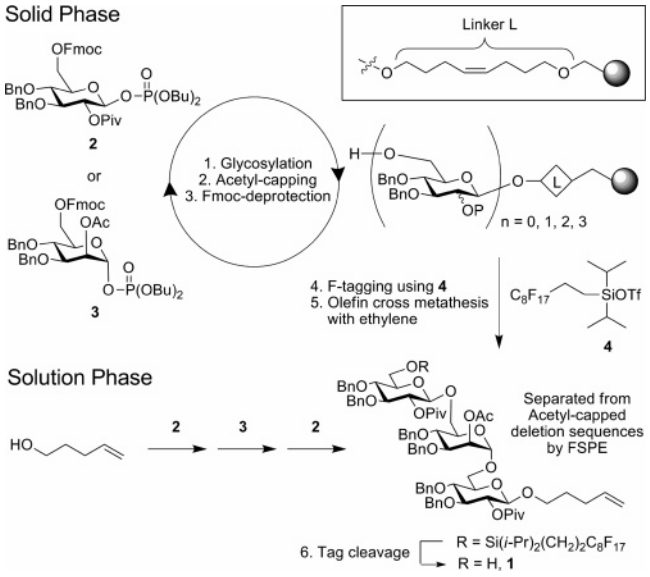
in excess following each coupling limits the utility of this approach.

Here, we report a new approach to cap-and-tag solid phase oligosaccharide synthesis (Figure 1C) by adapting a method developed originally for solid phase peptide synthesis.[6] The deletion sequences are capped by acetylation, and the desired oligosaccharide is marked with a fluorous tag. Following cleavage from the resin, the desired F-tagged oligosaccharide is separated from the acetyl-capped deletion sequences by FSPE. The methodology is illustrated by the solid phase synthesis of a model trisaccharide. Quantitative analysis confirmed the high efficiency of the purification by FSPE.

Results and Discussion

The protected Glc-β-(1→6)-Man-α-(1→6)-Glc-β-1→pentenyl trisaccharide **1** was selected as a model to develop cap-and-tag solid phase oligosaccharide synthesis. The *n*-pentenyl aglycone allowed us to compare both solution and solid phase approaches. Glucose and mannose building blocks **2** and **3**[7] containing a C(2)-participating group were selected to control the stereochemistry at the anomeric center. The C(6)-Fmoc group served to quantify the glycosylation steps by spectroscopic measurement of the piperidine-dibenzofulvene adduct released during

SCHEME 1. Acetyl-Cap and Fluorous-Tag Solid Phase Oligosaccharide Synthesis



Fmoc cleavage.[8] Acetylation was achieved using acetic anhydride/pyridine, and the C_8F_{17}-tag was introduced using silyl triflate **4**.[3] Cleavage of the oligosaccharide from the resin was achieved by olefin cross metathesis with ethylene (Scheme 1).[3,9]

Functionalized Merrifield resin was prepared by treatment of 8-(4,4′-dimethoxytrityloxy)oct-4(*Z*)-en-1-ol[9] with potassium hydride in the presence of 18-crown-6 to generate the alkoxide, which was trapped with chloromethylpolystyrene resin (Scheme 2). The DMT protecting group was cleaved under acidic conditions, and the loading was determined by UV quantification at 503 nm.[10]

Fluorous-tag **4** was synthesized starting from commercially available perfluorooctylethan-2-ol (Scheme 3). Hydroxyl-halogen exchange furnished iodide **5**.[11] Treatment of **5** with

(5) For reviews on fluorous tags, see: (a) Zhang, W. *Chem. Rev.* **2004**, *104*, 2531−2556 and references cited therein. (b) Zhang, W. *Tetrahedron* **2003**, *104*, 4475−4489 and references cited therein. (c) Miura, T. *Trends Glycosci. Glycotechnol.* **2003**, *15*, 351−358 and references cited therein. See also the articles: (d) Malkov, A. V.; Figlus, M.; Stoncius, S.; Kocovsky, P. *J. Org. Chem.* **2007**, *72*, 1315−1325. (e) Mizonu, M.; Matsumoto, H.; Goto, K.; Hamasaki, K. *Tetrahedron Lett.* **2006**, *47*, 8831−8835. (f) Curran, D. P.; Zhang, Q.; Richard, C.; Lu, H.; Gudipati, V.; Wilcox, C. S. *J. Am. Chem. Soc.* **2006**, *128*, 9561−9573. (g) Zu, L.; Li, H.; Wang, J.; Yu, X.; Wang, W. *Tetrahedron Lett.* **2006**, *47*, 5131−5134. (h) Fustero, S.; Garcia, S. A.; Chiva, G.; Sanz-Cervera, J. F.; del Pozo, C.; Acena, J. L. *J. Org. Chem.* **2006**, *8*, 955−957. (i) Goto, K.; Miura, T.; Mizuno, M. *Tetrahedron Lett.* **2005**, *46*, 8293−8297. (j) Kasuya, M. C. Z.; Ito, A.; Cusi, R.; Sato, T.; Hatanaka, K. *Chem. Lett.* **2005**, *34*, 856−857. (k) Manku, S.; Curran, D. P. *J. Org. Chem.* **2005**, *70*, 4470−4473. (l) McAllister, L. A.; McCormick, R. A.; Brand, S.; Procter, D. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 452−455. (m) Goto, K.; Miura, T.; Mizuno, M.; Takaki, H.; Imai, N.; Murakami, Y.; Inazu, T. *Synlett* **2004**, *12*, 2221−2223. (n) Dandapani, S.; Jeske, M.; Curran, D. P. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 12008−12012.

(6) Filippov, D. V.; van Zoelen, D. J.; Oldfield, S. P., van der Marel, G. A.; Overkleeft, H. S.; Drijfhout, J. W.; van Boom, J. H. *Tetrahedron Lett.* **2002**, *43*, 7809−7812.

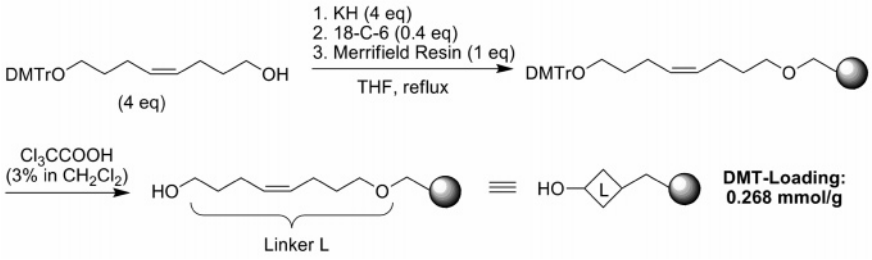
(7) Carrel, F. R.; Seeberger, P. H. *J. Carbohydr. Chem.* **2007**, *26*, 125−139.

(8) Quantification of the piperidine-dibenzofulvene adduct was achieved by measurement of the absorbance at 301 nm in DMF/piperidine (4:1) (ϵ = 7800 M^{-1} cm^{-1}): Niggemann, J.; Michaelis, K.; Frank, R.; Zander, N.; Höfle G. *J. Chem. Soc., Perkin Trans. 1* **2002**, 2490−2503.

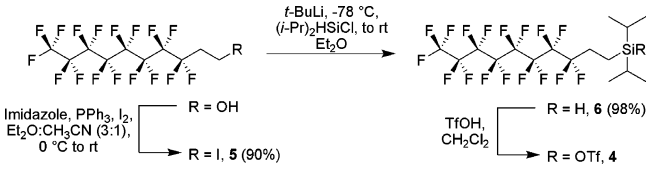
(9) Andrade, R. B.; Plante, O. J.; Melean, L. G.; Seeberger, P. H. *Org. Lett.* **1999**, *1*, 1811−1814.

(10) Pon, R. T. In *Methods in Molecular Biology: 20. Protocols for Oligonucleotides and Analogs*; Agrawal, S., Ed.; Humana Press: Totowa, 1993, pp 467−468.

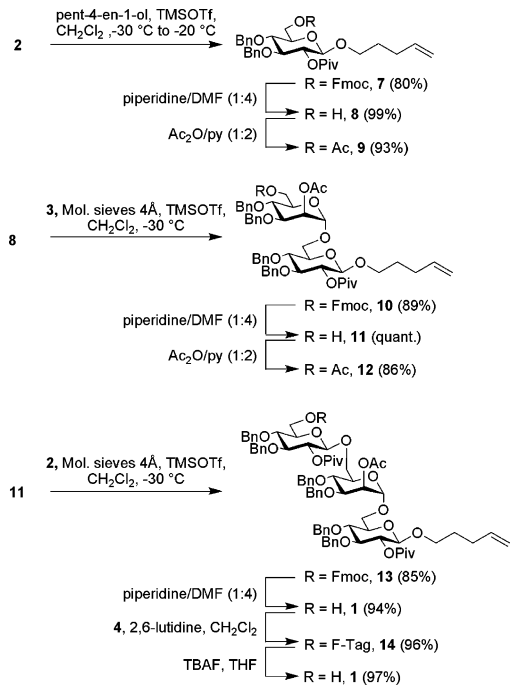
SCHEME 2. Functionalization of Merrifield Resin



SCHEME 3. Synthesis of Fluorous-Tag 4



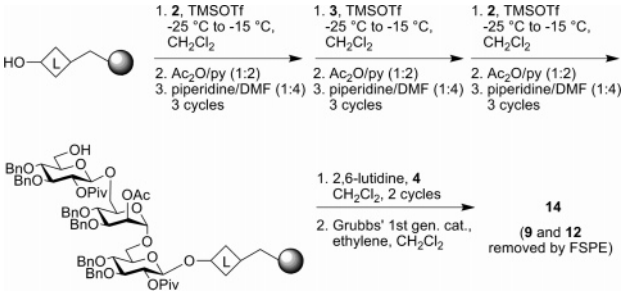
SCHEME 4. Solution Phase Synthesis of 1



t-butyllithium, followed by addition of chlorodiisopropylsilane, afforded the corresponding fluorinated silane **6**.[12] Since triflate **4** cannot be stored, it was freshly prepared before use by treatment of **6** with TfOH.

Trisaccharide **1** was initially synthesized in solution phase (Scheme 4). Glycosylation of pent-4-en-1-ol using **2** furnished monosaccharide **7** (80%). The Fmoc group of **7** was cleaved using piperidine/DMF to afford **8** (99%). Acetylation of **8** generated the first deletion sequence **9** in 93% yield. Union of **8** and **3** afforded disaccharide **10** (89%). After Fmoc cleavage furnished **11** in quantitative yield, acetylation afforded the second deletion sequence **12** (86%). Glycosylation of **11** using building block **2** yielded 85% of trisaccharide **13**. Finally, Fmoc removal from **13** furnished trisaccharide **1** in 95% yield (overall yield starting from **2**: 56% over six steps). Fluorous-tag chemistry was also investigated in solution phase. Placement of F-Tag **4** on **13** afforded **14** (96%). Cleavage of the F-tag of **14** by addition of TBAF furnished trisaccharide **1** in 97% yield.

SCHEME 5. Cap-and-Tag Solid Phase Oligosaccharide Synthesis of 14



Compatibility of the acetyl-capping step with an Fmoc-based SPOS was investigated using monosaccharides **7** and **9**. The Fmoc group of **7** proved to be stable to the Ac_2O/pyridine-capping conditions over 20 h. Similarly, the acetyl group of **9** was stable under prolonged Fmoc deprotection conditions (20 h).

The cap-and-tag solid phase assembly of trisaccharide **14** is summarized in Scheme 5. In order to produce deletion sequences, each glycosylation was carried out once, using 4 equiv of glycosyl phosphate **2** or **3** with equimolar amounts of the activator TMSOTf. Acetyl capping required 1 h, while Fmoc cleavage was repeated three times for 10 min. After constructing the trisaccharide, the resin was F-tagged.[3] Finally, olefin cross-metathesis using Grubbs' catalyst and ethylene[3,9] afforded the desired F-tagged trisaccharide **14**.

Even under these conditions, no significant quantities of deletion sequences **9** and **12** were observed by LC-MS (Figure 2A). To favor the formation of **9** and **12**, the amount of glycosyl phosphates **2** and **3** added to the resin were reduced. Using 2.5 equiv of building blocks, **9** and **12** were only formed in traces (Figure 2B). Interestingly, when employing 1.5 equiv, monosaccharide **9** was formed as the major deletion sequence, whereas only traces of disaccharide **12** were observed (Figure 2C). This finding can be rationalized by considering that the relative amount of building block increases as the unreacted nucleophile is capped. Thereby, the second and third glycosylations are driven to completion.

Fluorous solid phase extraction was employed to purify the products of all solid phase syntheses. Conventional FSPE[14] of the synthesis using 4 equiv of building block (Figure 2A) afforded **14** in 73% yield. The same yield was obtained after

(11) 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.

(12) Repeating the synthetic procedure described in reference 3 did not allow for the isolation of **6**. The procedure was therefore slightly modified and is described in the Experimental Section.

(13) Absence of 0.1% TFA in the eluent resulted in a slight change in the retention time of **14** (entry A versus B and C).

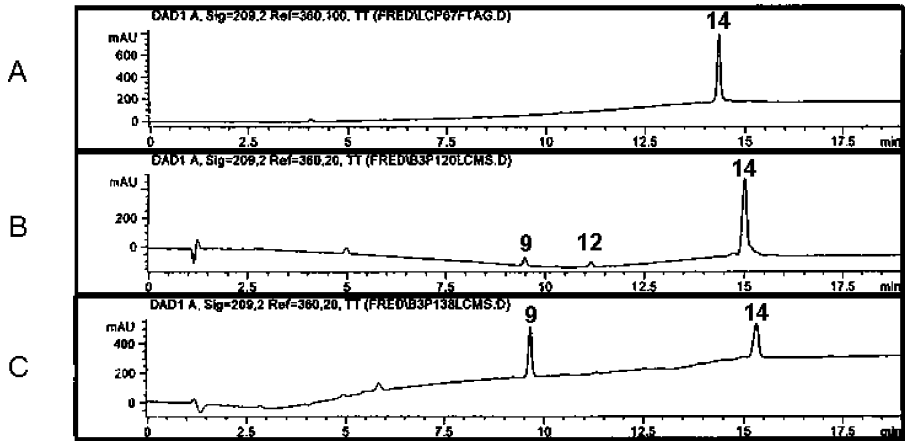


FIGURE 2. Cap-and-tag synthesis of **14** using 4 equiv (A),[13] 2.5 equiv (B), and 1.5 equiv (C) of glycosyl phosphate building blocks **2** or **3**.

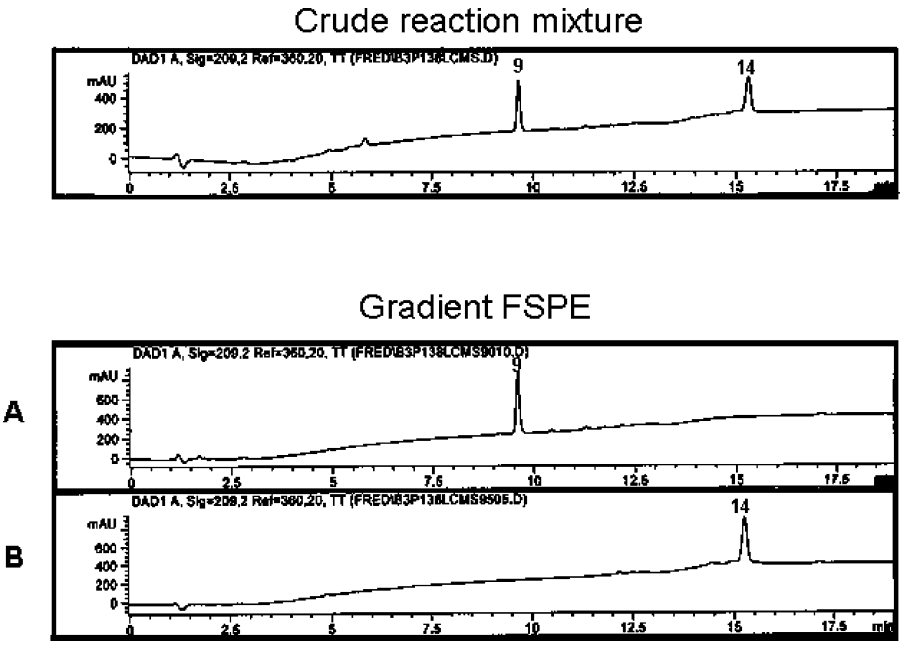


FIGURE 3. Gradient FSPE of the crude reaction mixture for the trisaccharide assembly using 1.5 equiv of building block: (A) fraction 90/10; (B) fraction 95/5 (MeOH/H_2O).

gradient FSPE[15] of the synthesis employing 2 equiv (Figure 2B). Gradient FSPE was also required for the purification of the synthesis using 1.5 equiv (Figure 2C). Trisaccharide **14**, the desired product, was eluted using 95/5 (MeOH/H_2O), whereas the deletion sequence **9** was collected in the 90/10 (MeOH/H_2O) fraction (Figure 3).[16]

The efficiency of FSPE purification could not be determined with these experiments, since the amount of each deletion sequence was not well defined. To mimic the contamination of the oligosaccharide product with well-defined amounts of deletion sequences, we created such deletion sequences by adding a capped glycosyl phosphate to the coupling mixture. Each coupling step was performed twice using 4 equiv of a mixture of **2** and **15**[7] (4:1). After three glycosylations, a 51.2/12.8/16/20 ratio of **16**/**17**/**18**/**19** should be obtained. Quantification of the piperidine-dibenzofulvene adduct[8] revealed that the efficiency of each glycosylation was approximatively 80% ($Fmoc_1$ = 12.7 μmol, $Fmoc_2$ = 10.1 μmol, $Fmoc_3$ = 7.9 μmol; back-calculation of initial amount: $Fmoc_0$ = 15.9 μmol). After the three glycosylations, one-fifth of the resin was cleaved without F-tagging to provide the reference LC-MS chromatogram. The remaining resin was F-tagged twice prior to cleavage (Scheme 6).

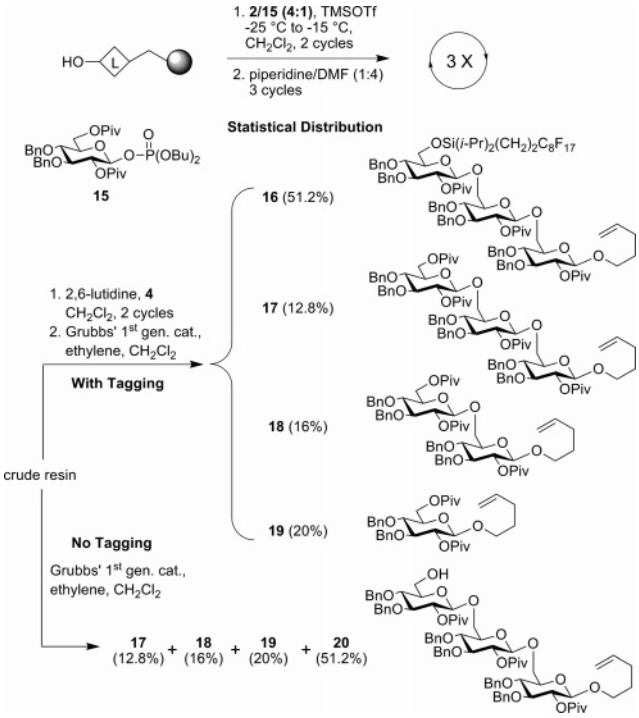
Comparison of the LC-MS chromatograms without (Figure 4A) and with F-tagging (Figure 4B) showed a significant shift of the F-tagged trisaccharide **16** due to the hydrophobic fluorous chain. This observation aided the HPLC separation of the mixture of **16**/**17**/**18**/**19**, although **16** was recovered in only 41% yield. Conventional FSPE[14] failed to separate the **16**/**17**/**18**/**19**

(14) Conventional FSPE was performed according to the procedure described in reference 4a: The crude product was loaded on the column using DMF:H_2O (9:1). Non-fluorous compounds were eluted using MeOH/H_2O (80/20), whereas fluorinated compounds were recovered using pure MeOH.

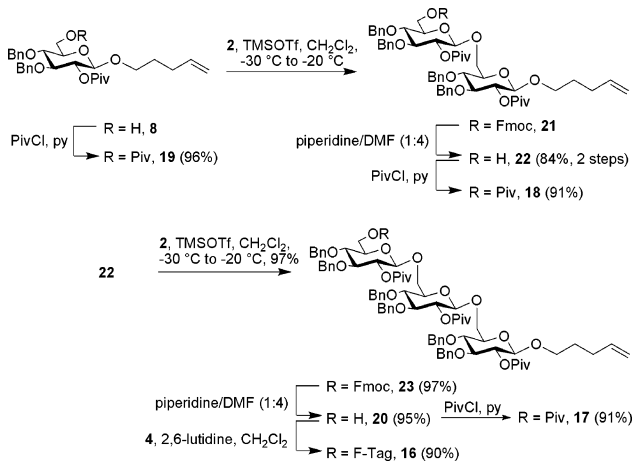
(15) Gradient FSPE was performed using a gradient of MeOH/H_2O (from 80% to 100%, in steps of 5%). Each FSPE fraction was checked by TLC and LC-MS.

(16) The LC-MS deletion sequences **9** and **12** have the same retention time as those synthesized in solution.

SCHEME 6. Creation of Defined Amounts of Deletion Sequences



SCHEME 7. Solution Phase Synthesis of the Oligoglucoses 16−23



mixture. Gradient FSPE[15] yielded the deletion sequences **18** and **19** that were collected together (Figure 4C), while **17** was eluted later (Figure 4D). The F-tagged oligosaccharide **16** was eluted with 95/5 MeOH/H_2O (Figure 4E) and isolated in 49% yield. The efficiency of recovery and purification using FSPE was calculated to be 94%.

To rigorously prove the identity of the various deletion sequences **17**, **18**, and **19** and the trisaccharides **16** and **20**, these compounds were prepared in solution phase (Scheme 7). Monosaccharide **8** was protected as the pivaloyl derivative **19** in 96% yield. Glycosylation of **8** using glycosyl phosphate **2** afforded disaccharide **21** before Fmoc-cleavage furnished **22** (84% yield for two steps). Pivaloylation of **22** gave the second deletion sequence **18** (91%). Final glycosylation of **22** using **2** afforded the trisaccharide **23** in 97% yield prior to Fmoc removal to yield 95% of **20**. Finally, **20** was either F-tagged to give **16** (90%) or pivaloylated to afford the deletion sequence **17** (91%). The retention time of compounds **16**, **17**, **18**, **19**, and **20** were identical to those observed in the previous LC-MS chromatograms.

Conclusions

Here, we report a cap-and-tag approach to solid phase oligosaccharide synthesis. Acetyl-capping and fluorous-tagging allowed for facile separation of deletion sequences from the desired F-tagged oligosaccharide by gradient FSPE. A model study using intentionally created deletion sequences demonstrated the efficient recovery of the F-tagged product using the method.

Experimental Section

General Methods. All chemicals were reagent grade and used as supplied unless mentioned otherwise. All reactions were performed in flame-dried glassware under an argon atmosphere. Dichloromethane (CH_2Cl_2), diethylether (Et_2O), tetrahydrofuran (THF), and toluene were purified by a J. C. Meyer solvent dispensing system (two packed columns of neutral alumina). Solvents for chromatography and workup procedures were distilled from commercially available technical grade solvents. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F_{254} plates (0.25 mm). Compounds were visualized by UV and/or by dipping the plates in a cerium sulfate−ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Fluka silica gel 60 (40−63 μm). The ^{1}H NMR spectra (300 MHz) are expressed in ppm relative to $CHCl_3$ (7.26 ppm) as internal reference; the coupling constants are reported in Hz. The same is valid for ^{13}C NMR spectra (75 MHz, internal reference $CDCl_3$: 77.0 ppm). For ^{19}F NMR (282 MHz) spectra, CCl_3F (δ = 0 ppm) was used as the internal reference. Optical rotations $[\alpha]_D$ were recorded in $CHCl_3$ using a sodium lamp (λ = 589 nm) at room temperature with a 10 cm/1 mL cell. The solvent and the concentration are specified, e.g., c = 1 = 10 mg/mL. IR spectra were recorded in $CHCl_3$ and are expressed in cm^{-1}. LC-MS spectra were recorded on a Agilent 1100 LC MSD high-performance liquid chromatograph with a Waters Symmetry C18 column (3.9 × 150 mm, 5 μm), using a defined gradient of water/isopropanol (80/20 + 0.1% TFA: solvent A) and acetonitrile/isopropanol (80/20 + 0.1% TFA: solvent B) (flow rate 1 mL/min): 0−1 min: 60/40 (A/B); 1−11 min: 60/40 to 0/100 (A/B); 11−20 min: 0/100 (A/B). The spectra were detected at 208, 234, 254, and 280 nm. Preparative HPLC was performed on Waters HPLC apparatus with a Waters SunFire Prep C8 column (10 × 150 mm, 5 μm), using a gradient of water/isopropanol (80/20 + 0.1% TFA: solvent A) and acetonitrile/isopropanol (80/20 + 0.1% TFA: solvent B) (flow rate 5 mL min^{-1}): 0−10 min: 100/0 (A/B); 10−50 min: 100/0 to 40/60 (A/B); 50−90 min: 40/60 to 0/100 (A/B); 90−100 min: 0/100 (A/B). The spectra were detected at wavelengths of 208 and 254 nm. High-resolution mass spectroscopy (HRMS) was performed on an IonSpect Ultima; 2,5-Dihydroxybenzoic acid (DHB) was used as matrix.

Synthesis of Functionalized Merrifield Resin: To a solution of KH (prewashed with pentane and dried, 44 mg, 1.097 mmol) in THF (2 mL) was added, at room temperature, a solution of 8-(4,4′-dimethoxytrityloxy)oct-4(*Z*)-en-1-ol (490 mg, 1.097 mmol)[9] in THF (2 mL), followed by crown ether 18-C-6 (29 mg, 0.27 mmol). After 15 min, the alkoxide solution was transferred onto the Merrifield resin (Polymer Laboratories, PL-CMS Resin, 0.38 mmol/g, 75−150 μm, 721 mg, 0.27 mmol) swollen in THF (6 mL). After 19 h at room temperature, the resin was heated at 70 °C for 3 h. MeOH (1 mL) was added, and the solution was filtered off. The resin was washed with THF (3 × 10 mL), CH_2Cl_2 (3 × 10 mL), CH_2Cl_2/MeOH (9:1, 3 × 10 mL), CH_2Cl_2 (3 × 10 mL), THF (3 × 10

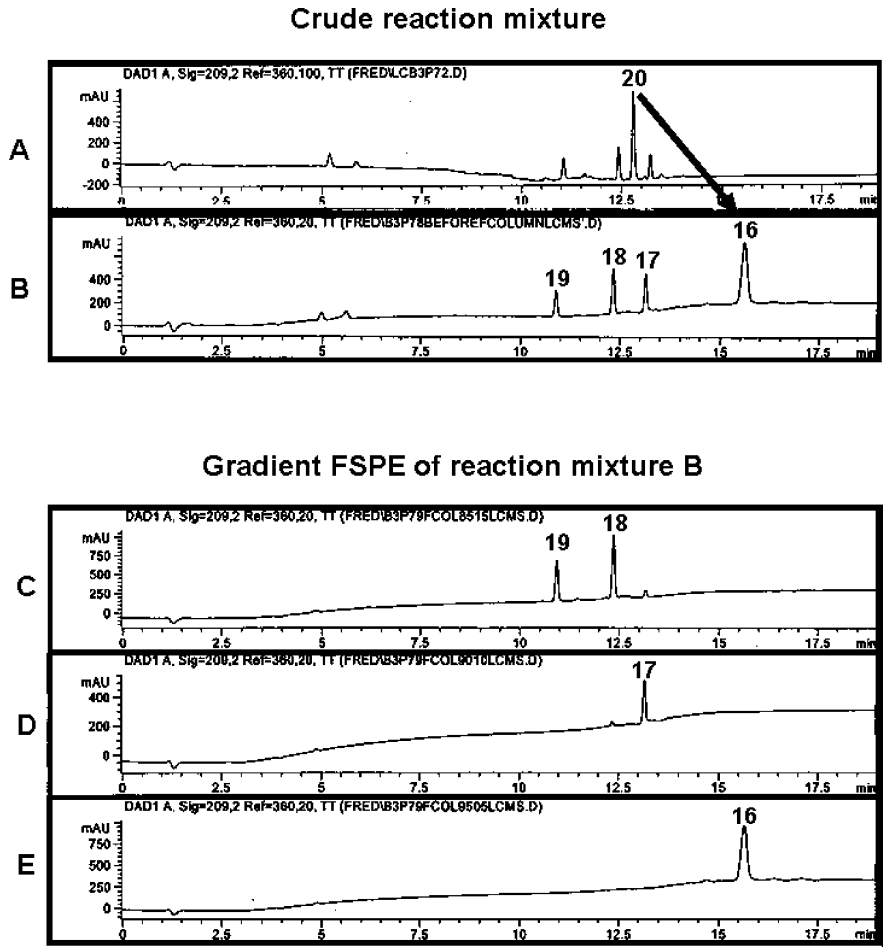


FIGURE 4. Crude reaction mixture of (A) without F-tagging and (B) with F-tagging. Gradient FSPE: (C) fraction 85/15, (D) fraction 90/10, (E) fraction 95/5 (MeOH/H_2O).

mL), CH_2Cl_2/MeOH (9:1, 3 × 10 mL), and CH_2Cl_2 (3 × 10 mL) and dried under high vaccuum.

DMT Quantification:[10] Two aliquots of the resin (A: 7.6 mg; B: 7.3 mg) were treated with Cl_3CCOOH (3% w/w in CH_2Cl_2, 10 mL). The resulting red solutions were diluted 50 times, and absorbance was measured at 503 nm (A_A = 0.283, A_B = 0.268), which corresponds to an average DMT_{ON} loading = 0.253 mmol/g (loading = (A·Vol·dil)/(76·mass)). The remaining resin (693 mg) was deprotected using Cl_3CCOOH (3% w/w in CH_2Cl_2, 4 × 20 mL). The resin was washed with CH_2Cl_2 (5 × 20 mL), CH_2Cl_2 (+1% Et_3N, 5 × 20 mL), 5× {CH_2Cl_2/MeOH (9:1, 20 mL), then MeOH (20 mL)}, and CH_2Cl_2 (5 × 20 mL) and was dried under high vacuum to afford the octenediol-functionalized Merrifield resin (655 mg, DMT_{OFF} loading = 0.268 mmol/g).

General Procedures. Solution Phase Glycosylation: Acceptor (pent-4-en-1-ol, **8**, **11**, **22**) and donor (**2** or **3**) were dissolved in CH_2Cl_2. Molecular sieves (4 Å) were added when indicated and stirred at room temperature for 30 min. The solution was cooled to −30 °C, and TMSOTf (1.0−1.1 equiv) was added. The solution was kept between −30 °C and −20 °C for 15 to 30 min. Pyridine was added at −30 °C to quench the reaction. The solvents were evaporated, residual pyridine was coevaporated with toluene, and the residue was purified by flash column chromatography on silica gel to afford the Fmoc-protected saccharide.

Glycosylation on Solid Phase: A solution of the donor (**2**, **3**, or mixture **2**:**15** (4:1)) in CH_2Cl_2 (1.5 mL) was added to the dried resin, and the solution was cooled to −25 °C. Equimolar amounts of TMSOTf were added, and the solution was shaken between −25 °C and −15 °C for 30 min. The solution was filtered off, and the resin was washed with CH_2Cl_2 (5 × 2 mL), CH_2Cl_2/MeOH (9:1, 5 × 2 mL), THF (5 × 2 mL), and CH_2Cl_2 (10 × 2 mL). The resin was dried under high vacuum.

Capping by Acetylation: The resin was swollen in pyridine (2 mL) and Ac_2O (1 mL) and shaken for 1 h at room temperature. The solution was filtered off. The resin was washed with CH_2Cl_2 (5 × 2 mL), 5 times {THF (+1% CH_3COOH (v/v), 2 mL), then CH_2Cl_2 (2 mL)}, THF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL), 5× {CH_2Cl_2/MeOH (9:1, 2 mL), then MeOH (2 mL), then CH_2Cl_2 (2 mL)}, and CH_2Cl_2 (10 × 2 mL). The resin was dried under high vacuum.

Fmoc Deprotection and Quantification: The resin was swollen in DMF/piperidine (4:1, 10 mL) and shaken for 10 min at room temperature. The solution was filtered off. Fmoc quantification could be measured by 10-fold dilution of the filtrate in DMF/piperidine (4:1) and by measurement of the absorbance at 301 nm (ϵ = 7800 M^{-1} cm^{-1}).[8] After three deprotection cycles, the resin was washed with DMF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL), 5× {THF (+1% CH_3COOH (v/v), 2 mL), then CH_2Cl_2 (2 mL)}, THF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL), 5× {CH_2Cl_2/MeOH (9:1, 2 mL), then MeOH (2 mL), then CH_2Cl_2 (2 mL)}, and CH_2Cl_2 (10 × 2 mL). The resin was dried under high vacuum.

F-Tagging: The resin was swollen in CH_2Cl_2 (1 mL), and 2,6-lutidine (8 equiv) was added at room temperature. Fresly prepared **4** (5 equiv) in CH_2Cl_2 (0.5 mL) was added, and the solution was shaken for 1 h. The solution was filtered off, and a second F-tagging cycle was performed. The solution was filtered off, and the resin was washed with CH_2Cl_2 (5 × 2 mL), 5× {THF (+1% CH_3COOH (v/v), 2 mL), then CH_2Cl_2 (2 mL)}, THF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL), 5× {CH_2Cl_2/MeOH (9:1, 2 mL), then MeOH (2 mL), then CH_2Cl_2 (2 mL)}, and CH_2Cl_2 (10 × 2 mL).

Cleavage from Solid Support: The resin was swollen in CH_2Cl_2 (1.5 mL), and Grubbs' first-generation catalyst (20% mol) was added. The solution was purged five times with ethylene, and the pink solution was stirred under atmospheric pressure of ethylene overnight. The black solution was filtered, and the resin was washed three times with CH_2Cl_2 (2 mL). The combined filtrates were evaporated to afford a black oil.

Gradient FSPE: The residue was dissolved in the minimum amount of DMF/H_2O (9:1) and loaded on top of selfed-packed 3 g Tridecafluoro SiliaBond (Silicycle, Quebec, Canada) column. The column was eluted under pressure with gradient MeOH/H_2O solvent extraction systems: 80/20 (20 mL), 85/5 (20 mL), 90/10 (20 mL), 95/5 (20 mL), and 100/0 (20 mL). The solvents were evaporated, and each residue was analyzed by TLC and/or LC-MS.

Pent-4-enyl 3,4-Di-*O*-benzyl-2-*O*-pivaloyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-*β*-D-glucopyranoside (1). Method A, Fmoc Deprotection: A solution of **13** (114 mg, 74 μmol) in piperidine/DMF (0.6 mL/2.4 mL) was stirred at room temperature for 30 min. The solvents were evaporated, and residual piperidine was coevaporated with toluene (2 × 10 mL). The residue was purified by silica gel flash column chromatography (gradient eluent EtOAc/cyclohexane (1:8) to (1:2)) to afford **1** (92 mg, 94%) as a colorless oil.

Method B, Cleavage of F-Tag: To a solution of **14** (47 mg, 25 μmol) in THF (1 mL) was added TBAF (1 M in THF, 50 μL, 50 μmol) at room temperature. After 15 min at room temperature, the solution was diluted with CH_2Cl_2 (10 mL) and treated with a saturated aqueous solution of $NaHCO_3$ (3 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried over $MgSO_4$, and the solvents were evaporated. The residue was purified by silica gel flash column chromatography (gradient eluent EtOAc/cyclohexane (1:5) to (1:2)) to afford **1** (32 mg, 97%) as a colorless oil. Ret. time (40−100) = 12.5 min; R_f (EtOAc:hexane (1:2)) = 0.39. ^{1}H NMR (300 MHz, $CDCl_3$): 7.36−7.17 (m, 30H), 5.76 (dddd, J = 17.1, 10.3, 6.5, 6.5 Hz, 1H), 5.40 (dd, J = 3.4, 1.9 Hz, 1H), 5.10 (dd, J = 8.7, 7.5 Hz, 1H), 5.06 (dd, J = 9.3, 8.1 Hz, 1H), 5.02−4.90 (m, 2H), 4.89 (d, J = 11.5 Hz, 1H), 4.85 (d, J = 1.6 Hz, 1H), 4.82−4.68 (m, 7H), 4.62 (d, J = 10.9 Hz, 1H), 4.54 (d, J = 10.9 Hz, 1H), 4.52 (d, J = 11.5 Hz, 2H), 4.42 (d, J = 7.5 Hz, 1H), 4.38 (d, J = 8.1 Hz, 1H), 3.94−3.36 (m, 17H), 2.34 (dd, J = 7.2, 6.5 Hz, 1H), 2.14 (s, 3H), 2.08−1.98 (m, 2H,), 1.66−1.54 (m, 2H), 1.21 (s, 9H), 1.17 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): 176.6, 176.4, 170.1, 138.5, 138.1, 138.0, 137.8, 128.4, 128.3, 128.2, 128.0, 127.9, 127.6, 127.6, 127.5, 127.4, 127.2, 114.7, 101.5, 101.0, 97.8, 83.5, 82.9, 77.7, 75.5, 74.9, 74.6, 74.2, 73.2, 73.1, 71.4, 70.9, 69.1, 68.9, 68.2, 66.1, 62.0, 38.9, 38.8, 30.2, 28.9, 27.3, 27.3, 21.2. $[\alpha]_D$ ($CHCl_3$, c = 1): 2.9°. IR ($CHCl_3$): 3466, 3066, 3008, 2876, 1737, 1604, 1497, 1479, 1454, 1397, 1365, 1277, 1088, 912. HRMS (MALDI) calcd for $C_{77}H_{94}O_{19}Na$, 1345.6282; found, 1345.6300.

Diisopropyl-(1H,1H,2H,2H-perfluorodecyl)silyl Triflate (4). To a solution of **6** (200 mg, 352 μmol) in CH_2Cl_2 (2 mL) was added triflic acid (28 μL, 320 μmol) at room temperature. The solution was stirred for 60 min, and the volume of the solution was readjusted to 2 mL. (NB: Due to the low stability of **4** upon storage, this stock solution was freshly prepared prior to use.)

1-Iodo-1H,1H,2H,2H-perfluorodecane (5). To a solution of perfluorooctylethanol (13.92 g, 30 mmol) in Et_2O (75 mL) and acetonitrile (25 mL) was added successively imidazole (6.127 g, 90 mmol) and triphenylphosphine (11.8 g, 45 mmol). The solution was cooled to 0 °C, and iodine (11.42 g, 45 mmol) was added portion wise over 15 min. The solution was kept for a further 15 min at 0 °C and was then allowed to warm to room temperature overnight. After 19 h, Et_2O (200 mL) was added to the red solution and filtered over a pad of Celite (height = 3 cm). The Celite was washed with Et_2O (4 × 50 mL). The combined organic layer was washed with a saturated aqueous solution of $Na_2S_2O_3$ (until destruction of residual iodine, ∼50 mL) and with brine (50 mL) and was dried over $MgSO_4$. The solvents were evaporated, and the residue was purified by silica gel flash column chromatography (product absorbed on silica gel, eluent hexane, R_f (hexane) = 0.76, $KMnO_4$ stain) to afford **5** (15.38 g, 89%) as a white solid. The NMR data and the melting point are in agreement with literature[17] and are the same as the commercial product from Fluorous Technologies Inc. (Pittsburgh, U.S.A., http://fluorous.com/).

Diisopropyl-(1H,1H,2H,2H-perfluorodecyl)silane (6).[12] To a stirred solution of *t*-BuLi (1.7 M in pentane, 19.1 mL, 32.5 mmol) in Et_2O (55 mL) at −78 °C was added a solution of **5** (9.326 g, 16.24 mmol) in Et_2O (75 mL) over 15 min. The turbid yellow solution was stirred at −78 °C for 1 h. Chlorodiisopropylsilane (2.45 mL, 13.54 mmol) was added, and the solution was allowed to warm to room temperature. After 2 h, the gray solution was slowly treated with a saturated aqueous solution of NH_4Cl (50 mL) and water (until clear aqueous phase). The aqueous phase was extracted with CH_2Cl_2 (3 × 150 mL). The combined organic layers were washed with brine (50 mL, the chlorinated phase was above the aqueous phase), dried over $MgSO_4$, and the solvents were evaporated. The residue was distilled under reduced pressure to give **6** (7.314 g, 96%, R_f (hexane) = 0.89, $KMnO_4$ stain) as a colorless liquid. The NMR data were the same as the commercial product from Fluorous Technologies Inc. (Pittsburgh, USA, http://fluorous.com/).

Pent-4-enyl 3,4-Di-*O*-benzyl-6-*O*-(fluorenylmethoxycarbonyl)-2-*O*-pivaloyl-*β*-D-glucopyranoside (7). General procedure for glycosylation in solution using pent-4-en-1-ol (96 mg, 1.11 mmol), **2** (379 mg, 0.44 mmol), and TMSOTf (96 μL, 0.53 mmol) in CH_2Cl_2 (4.4 mL) between −30 °C and −20 °C for 30 min. The reaction was quenched with pyridine (100 μL). Silica gel flash column chromatography (gradient eluent EtOAc/cyclohexane (1:9) to (1:4)) afforded **7** (260 mg, 80%) as a colorless oil. R_f (EtOAc:cyclohexane (1:4)) = 0.41. ^{1}H NMR (300 MHz, $CDCl_3$): 7.77 (d, J = 7.5 Hz, 2H), 7.65−7.60 (m, 2H), 7.44−7.24 (m, 14H), 5.78 (dddd, J = 17.1, 10.3, 6.5, 6.5 Hz, 1H), 5.10 (dd, J = 9.1, 8.2 Hz, 1H), 5.04−4.92 (m, 2H), 4.84 (d, J = 11.0 Hz, 1H), 4.59 (d, J = 11.0 Hz, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.73 (d, J = 11.2 Hz, 1H), 4.47 (dd, J = 11.5, 1.9 Hz, 1H), 4.45−4.23 (m, 5H), 3.86 (ddd, J = 9.3, 6.3, 6.3 Hz, 1H), 3.75 (dd, J = 9.1, 8.5 Hz, 1H), 3.68 (dd, J = 9.3, 8.5 Hz, 1H), 3.61 (ddd, J = 9.3, 4.9, 1.9 Hz, 1H), 3.46 (ddd, J = 9.3, 6.3, 6.3 Hz, 1H), 2.12−2.02 (m, 2H), 1.70−1.60 (m, 2H), 1.21 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): 176.6, 154.9, 143.3, 143.2, 141.2, 141.2, 137.9, 137.8, 137.5, 128.4, 128.3, 128.0, 127.9, 127.6, 127.4, 127.1, 125.2, 125.1, 119.9, 114.7, 101.0, 83.2, 77.3, 75.0, 75.0, 72.9, 69.9, 69.0, 66.4, 46.6, 38.7, 29.9, 28.7, 27.1. $[\alpha]_D$ ($CHCl_3$, c = 1): −4.7°. IR ($CHCl_3$): 3068, 3032, 2960, 2872, 1740, 1640, 1602, 1497, 1478, 1452, 1397, 1363, 1263, 1137, 1036, 1011, 972, 916. HRMS (MALDI) calcd for $C_{45}H_{50}O_9Na$, 757.3347; found, 757.3335.

Pent-4-enyl 3,4-Di-*O*-benzyl-6-*O*-{(1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluorodecyl)diisopropylsilyl}-2-*O*-pivaloyl-*β*-D-glucopyranosyl-(1→6)-2-*O*-acetyl-3,4-di-*O*-benzyl-α-D-mannopyranosyl-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-*β*-D-glucopyranoside (14). Method A, Solid Phase Oligosaccharide Synthesis Using 4 equiv of Donor 2 (69 mg, 80 μmol) or 3 (65 mg, 80 μmol) and TMSOTf (15 μL, 80 μmol): Octenediol-functionalized resin (DMT loading: 0.267 mmol/g, 20 μmol, 75 mg) was loaded in a solid phase vessel. A first glycosylation using donor **2** was performed, followed by acetyl-capping and Fmoc deprotection. The second glycosylation was performed using donor **3**, followed by acetyl-capping and Fmoc deprotection. The third glycosylation using **2**, followed by acetyl-capping and Fmoc deprotection, afforded the trisaccharide-functionalized resin (100 mg). Part of the resin (80%, 80 mg, 16 μmol) was F-tagged. The resulting resin was cleaved for 17 h using Grubbs' first-generation catalyst (2.6 mg, 3.2 μmol), and the LC-MS was recorded. Conventional F-SPE[14] afforded **14** (22 mg, 73%) as a colorless oil.

(17) Feiring, A. E. *J. Org. Chem.* **1985**, *50*, 3269−3274.

Method B, F-Tagging in Solution Phase: To a solution of **6** (119 mg, 0.212 mmol) in CH_2Cl_2 (1.5 mL) was added triflic acid (17 μL, 0.194 mmol) at room temperature. Thirty-five minutes later, a solution of **1** (93 mg, 0.070 mmol) and 2,6-lutidine (41 μL, 0.352 mmol) in CH_2Cl_2 (0.5 mL) was added at room temperature, and the solution was stirred for 15 min. The solution was diluted with CH_2Cl_2 (10 mL) and treated with a saturated aqueous solution of $NaHCO_3$ (3 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 3 mL). The combined organic layers were dried over $MgSO_4$, and the solvents were evaporated. The residue was purified by silica gel flash column chromatography (gradient eluent EtOAc/cyclohexane (1:8) to (1:5)) to afford **14** (127 mg, 96%) as a colorless oil.

Ret. Time (40−100) = 15.2 min. R_f (EtOAc/cyclohexane (1:7)) = 0.23. ^{1}H NMR (300 MHz, $CDCl_3$): 7.36−7.16 (m, 30H), 5.78 (dddd, J = 17.0, 10.4, 6.6, 6.6 Hz, 1H), 5.41 (dd, J = 3.3, 1.9 Hz, 1H), 5.13 (dd, J = 9.1, 7.9 Hz, 1H), 5.13 (dd, J = 9.3, 8.0 Hz, 1H), 5.04−4.92 (m, 2H), 4.90 (d, J = 11.0 Hz, 1H), 4.89 (d, J = 1.9 Hz, 1H), 4.84−4.68 (m, 7H), 4.63 (d, J = 11.0 Hz, 1H), 4.84−4.68 (m, 4H), 4.38 (d, J = 7.9 Hz, 1H), 4.07 (bd, $J \approx$ 11 Hz, 1H), 3.96−3.32 (m, 16H), 2.23−2.02 (m, 7H), 1.68−1.56 (m, 2H), 1.22 (s, 9H), 1.19 (s, 9H), 1.07−0.98 (m, 14H), 0.92−0.82 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 176.6, 176.4, 170.2, 138.5, 138.0, 138.0, 137.9, 137.8, 128.3, 128.3, 128.2, 128.1, 127.9, 127.8, 127.6, 127.4, 127.4, 127.3, 127.2, 114.6, 101.0, 100.6, 97.6, 83.4, 83.2, 77.6, 77.2, 76.1, 74.9, 74.8, 74.6, 74.3, 74.1, 73.0, 72.8, 71.2, 70.7, 68.6, 68.1, 67.2, 65.5, 62.4, 38.7, 38.6, 30.0, 28.7, 27.2, 27.1, 25.4 (t, J = 24 Hz), 20.9, 17.4, 17.3, 17.3, 12.1, −0.2. ^{19}F NMR (282 MHz, $CDCl_3$): −80.6 (t, J = 10 Hz, 3F), −115.9 to −116.2 (m, 2F), −121.4 to −121.9 (m, 6F), −122.5 (bs, 2F), −122.9 (bs, 2F), −125.9 (bs, 2F). $[\alpha]_D$ ($CHCl_3$, c = 1): −1.2°. IR ($CHCl_3$): 3066, 3008, 2939, 2869, 1737, 1497, 1479, 1455, 12997, 1364, 1278, 1248, 1146, 1092, 909, 886. HRMS (MALDI) calcd for $C_{93}H_{111}F_{17}O_{19}SiNa$, 1905.7110; found, 1905.715.

Pent-4-enyl 3,4-Di-*O*-benzyl-6-*O*-{(1,1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8-heptadecafluorodecyl)diisopropylsilyl}-2-*O*-pivaloyl-*β*-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-*β*-D-glucopyranosyl-(1→6)-3,4-di-*O*-benzyl-2-*O*-pivaloyl-*β*-D-glucopyranoside (16). Method A, Solid Phase Oligosaccharide Synthesis: A (4:1) stock-mixture of **2** (457 mg, 0.532 mmol) and **15** (96 mg, 0.133 mmol) was prepared. Octenediol-functionalized resin (DMT loading: 0.267 mmol/g, 25 μmol, 93.4 mg) was loaded in a flame-dried solid phase reaction vessel. Two glycosylation cycles were performed using the 2/15 stock mixture (83 mg, 0.1 mmol) in CH_2Cl_2 (1.5 mL) and TMSOTf (18 μL, 0.1 mmol). The Fmoc group was deprotected using piperidine/DMF and quantified ($Fmoc_1$ = 12.7 μmol). Initial Fmoc loading of the resin was calculated (0.170 mmol/g). The second and third glucose units were incorporated using the same conditions, and the Fmoc release was quantified ($Fmoc_2$ = 10.1 μmol, $Fmoc_3$ = 7.8 μmol). The trisaccharide-functionalized resin was dried under high vacuum (123.3 mg). An aliquot of the resin (20% w/w) was cleaved without F-tagging (Figure 4A). Another aliquot of the resin (40% w/w) was F-tagged twice and then cleaved. Preparative HPLC (Waters SunFire Prep C8) afforded **16** (2.6 mg, 42% (to Fmoc loading)). The rest of resin (40% w/w) was F-tagged twice and then cleaved. Gradient FSPE afforded **16** (5.9 mg, 49% (to Fmoc loading)). The efficiency of recovery by FSPE was calculated to be 94%.

Method B, Solution Phase Synthesis: To a stirred solution of **6** (55 mg, 98 μmol) in CH_2Cl_2 (1 mL) was added triflic acid (7.7 μL, 88 μmol) at room temperature. Forty-five minutes later, a solution of **20** (44 mg, 32 μmol) and 2,6-lutidine (19 μL, 163 μmol) in CH_2Cl_2 (1 mL) was added at room temperature. After 15 min, the reaction mixture was diluted with CH_2Cl_2 (5 mL) and treated with a saturated aqueous solution of $NaHCO_3$ (2 mL). The aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were dried over $MgSO_4$, and the solvents were evaporated. The residue was purified by silica gel flash column chromatography (gradient eluent EtOAc/cyclohexane (1:9) to (1:7)) to afford **16** (56 mg, 90%) as a colorless oil.

Ret. Time (40−100) = 15.9 min. R_f (EtOAc/cyclohexane (1:7) = 0.30. ^{1}H NMR (300 MHz, $CDCl_3$): 7.35−7.19 (m, 30H), 5.80 (dddd, J = 17.1, 10.3, 6.5, 6.5 Hz, 1H), 5.08−4.94 (m, 5H), 4.82−4.54 (m, 13H), 4.51 (d, J = 8.1 Hz, 1H), 4.35 (d, J = 8.1 Hz, 1H), 4.06−3.39 (m, 16H), 3.24 (dbt, J = 9.7 Hz, 1H), 2.24−2.00 (m, 4H), 1.72−1.60 (m, 2H), 1.21 (s, 9H), 1.20 (s, 9H), 1.19 (s, 9H), 1.10−0.94 (m, 14H), 0.94−0.82 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): 176.6, 176.5, 138.1, 138.0, 138.0, 137.9, 137.8, 137.8, 128.4, 128.3, 128.2, 127.8, 127.8, 127.7, 127.6, 127.5, 127.3, 127.2, 127.1, 114.8, 101.2, 100.8, 100.6, 83.1, 78.1, 77.1, 75.9, 75.5, 74.8, 74.8, 72.9, 72.8, 72.6, 68.8, 67.8, 67.2, 62.6, 38.7, 38.7, 38.7, 30.0, 28.7, 27.2, 27.1, 25.3 (t, J = 23 Hz), 17.4, 17.3, 12.3, −0.2. ^{19}F NMR (282 MHz, $CDCl_3$): −80.6 (t, J = 10 Hz, 3F), −115.6 to −115.9 (m, 2F), −121.1 to −121.7 (m, 6F), −122.3 (bs, 2F), −122.5 (bs, 2F), −125.6 (bs, 2F). $[\alpha]_D$ ($CHCl_3$, c = 1): −11.0°. IR ($CHCl_3$): 3068, 3032, 2963, 2869, 1737, 1603, 1497, 1479, 1455, 1397, 1362, 1278, 1144, 1089, 915, 886. HRMS (MALDI) calcd for $C_{96}H_{117}F_{17}O_{19}SiNa$, 1947.7579; found, 1947.7525.

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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.

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