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Solid-Phase Synthesis of α-Gal Epitopes: On-Resin Analysis of Solid-Phase Oligosaccharide Synthesis with ^{19}F NMR Spectroscopy

Mickael Mogemark,[†] Mikael Elofsson,*[,†] and Jan Kihlberg*[,†,‡]

Organic Chemistry, Department of Chemistry, Umeå University, SE-901 87 Umeå, Sweden, and AstraZeneca R&D Mölndal, SE-431 83 Mölndal, Sweden

mikael.elofsson@chem.umu.se; jan.kihlberg@chem.umu.se

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A route for solid-phase synthesis of the α-Gal epitopes Gal(α1−3)Gal(β1−4)Glc and Gal(α1−3)-Gal(β1−4)GlcNAc is described. These trisaccharide antigens are responsible for hyperacute rejection in xenotransplantation of porcine organs. Optimization of the solid-phase synthesis relied on use of fluorinated protective groups for the carbohydrate building blocks and use of a fluorinated linker. This allowed convenient on-resin analysis of the reactions with gel-phase ^{19}F NMR spectroscopy. Conditions were established which allowed reductive ring-opening of 4,6-*O*-benzylidene acetals to be performed on the solid phase with high regioselectivity to furnish the corresponding 6-*O*-benzyl ethers. It was found that glycosylations could be conveniently carried out by using thioglycosides as donors with *N*-iodosuccinimide and trifluoromethanesulfonic acid as the promoter system. With use of these conditions a challenging α-glycosidic linkage was successfully installed with complete stereoselectivity in the final glycosylation. It was also established that fluorinated benzoates, benzyl ethers, and benzylidene acetals display almost identical chemical properties as their nonfluorinated counterparts, a finding that is essential for future use of fluorinated protective groups in solid-phase oligosaccharide synthesis.

Introduction

Automated procedures for the solid-phase synthesis of oligomers such as peptides and nucleotides have been of major importance in understanding the biological roles of proteins and nucleic acids. However, the functions of carbohydrates found in glycoconjugates such as glycoproteins and glycolipids are less well understood. Increased attention is therefore directed toward generation of protocols for solid-phase synthesis, which will permit preparation of the biologically interesting, and highly diverse,[1] oligosaccharides on an individual basis or in the form of libraries.[2−5] Despite the considerable progress made during recent years,[4−11] the assembly of complex oligosaccharides on solid support still constitutes a substantial challenge.

Difficulties in analyzing the outcome of reactions performed on solid support is a significant limitation when establishing reaction conditions for solid-phase synthesis. To address this issue, gel-phase ^{19}F NMR spectroscopy has been developed as a simple and versatile method for monitoring solid-phase synthesis.[12−27] Recently, we have communicated the use of ^{19}F NMR spectroscopy as an

[†] Umeå University.
[‡] AstraZeneca R&D Mölndal.

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analytical tool in solid-phase glycoconjugate synthesis.[22,27] The method is based on utilization of a fluorinated linker in combination with carbohydrate building blocks carrying fluorinated protective groups. Thus, both quantitative and qualitative information can be obtained in a couple of minutes with a conventional NMR spectrometer. Other approaches to quantify the progress of reactions directly on the solid phase include the use of IR spectroscopy,[28] high-resolution magic-angle-spinning NMR spectroscopy,[29−34] gated decoupling ^{13}C NMR spectroscopy,[35−37] and MALDI-TOF mass spectrometry in combination with a color test.[38,39]

Ideally, steps performed on the solid phase should be selective and high yielding to avoid generation of byproducts, which can complicate purification of the product after cleavage from the solid support. An important requirement for successful oligosaccharide synthesis is the use of carbohydrate building blocks substituted with orthogonal protective groups, which can be removed selectively under mild conditions.[40−43] To be of use in solid-phase oligosaccharide synthesis fluorinated protective groups should show the same overall reactivity, for instance, in glycosylations and during deprotection, as the corresponding nonfluorinated variants. Fluorinated benzoyl groups have previously been found to act as participating groups in oligosaccharide synthesis, just as the ordinary benzoyl group, but the fluorinated variants are removed somewhat more readily.[44] Moreover, the use of fluorinated benzoyl groups, instead of benzoates, allowed problems with β-elimination and epimerization of stereocenters to be circumvented during base-catalyzed deprotection of sensitive glycopeptides.[44]

In an effort to develop improved methodology for solid-phase oligosaccharide synthesis, and to further develop gel-phase ^{19}F NMR spectroscopy as an analytical tool, we have undertaken the synthesis of the α-Gal epitopes Gal(α1−3)Gal(β1−4)GlcNAc (**1**) and Gal(α1−3)Gal(β1−4)Glc (**2**). These saccharides are bound by human anti-Gal antibodies involved in hyperacute rejection of porcine organs in xenotransplantation.[45] Derivatives of α-Gal epitopes have previously been prepared by chemoenzymatic and conventional solution-phase synthesis.[45−57]

TABLE 1. ^{19}F NMR Chemical Shifts for Fluorinated Variants of Protective Groups Attached to Monosaccharides

position of F	protective group δ [ppm]		
	FBz[a]	FBn[b]	FPhCH[c]
ortho	−109.0 to −110.0	−119.0 to −119.4	−121.1
meta	−111.6 to −112.6	−113.5 to −113.8	−113.4
para	−103.9 to −105.5	−114.9 to −115.8	−112.7

[a] *o*-, *m*-, and *p*-*F*-perbenzoates of D-glucose. [b] *o*-, *m*-, and *p*-*F*-benzyl ethers attached to HO-2, -3, -4, and -6 of methyl α-D-glucopyranoside. [c] *o*-, *m*-, and *p*-*F*-4,6-*O*-benzylidene acetals on 4-methylphenyl 1-thio-β-D-galactopyranoside.

Results and Discussion

In parallel with developing an optimized solid-phase synthesis of trisaccharides **1** and **2**, we aimed to investigate the use of fluorinated protective groups and their versatility in monitoring reactions with ^{19}F NMR spectroscopy. First, a set of commercially available fluorinated reagents corresponding to the most commonly used protective groups in oligosaccharide synthesis, i.e., benzoates, benzyl ethers, and benzylidene acetals, were used to protect model gluco- and galactopyranosides (Table 1). NMR spectroscopy revealed that the ^{19}F resonances derived from *o*-, *m*-, and *p*-fluorinated variants of these protective groups were spread over a wide spectral range (−104 to −121 ppm). It should therefore be possible to use saccharide building blocks carrying fluorinated protective groups for monitoring solid-phase oligosaccharide synthesis without encountering problems with chemical shift overlap. In this respect it should be pointed out that it has previously been shown that both line widths and chemical shifts for fluorinated compounds linked to solid supports closely match values found in solution.[14,22]

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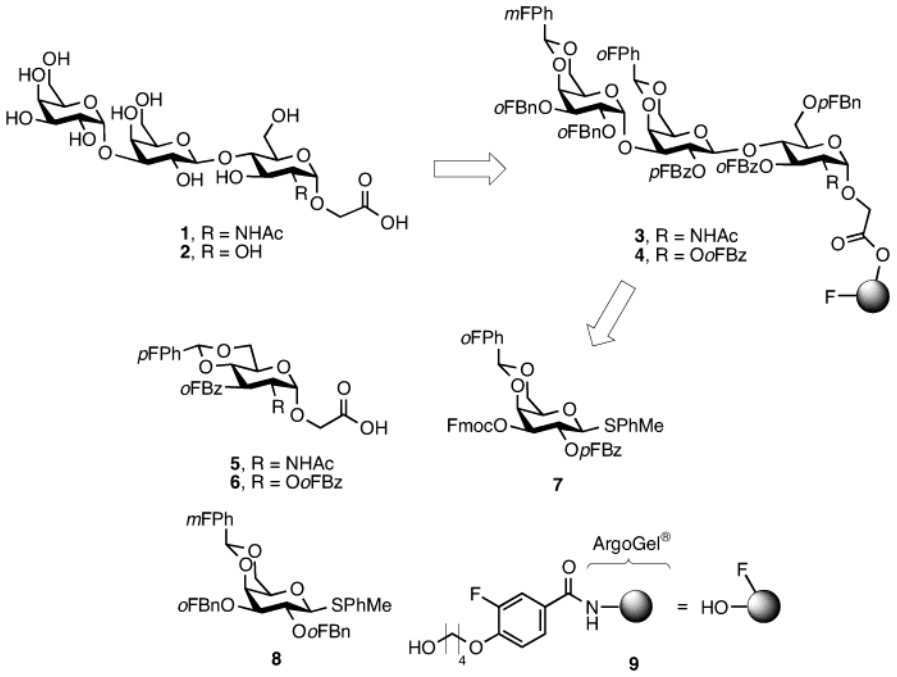
SCHEME 1. Retrosynthetic Analysis of α-Gal Trisaccharides 1 and 2



It was decided to prepare the α-Gal epitopes **1** and **2** from building blocks **5**−**8** (Scheme 1). The building blocks carry different fluorinated protective groups, which serve as markers for monitoring and optimization. In addition, the linker-loaded ArgoGel resin **9**[22] (Scheme 1) was also substituted with a fluorine atom, which serves as an internal standard throughout the synthesis. Resins that have poly(ethylene glycol) chains grafted onto a hydrophobic polystyrene core, e.g. ArgoGel, exhibit better properties for monitoring with MAS NMR spectroscopy than ordinary polystyrene solid supports.[30] Moreover, the ArgoGel resin is compatible with a wide variety of reaction conditions and has physiochemical properties that give it excellent swelling capacities in polar solvents, which is known to increase the diffusion of reagents into the polymer.[58] The choice of temporary protective groups, which can be manipulated selectively in high yields, is critical for the outcome of the synthesis. Among the various hydroxyl protective groups used for carbohydrate synthesis in solution, the 4,6-*O*-benzylidene acetal has proved to be most useful. This protective group is stabile to many reagents used in oligosaccharide synthesis, and has the advantage of protecting two hydroxyl groups at the same time. Moreover, it can be regioselectively reduced to give either of the corresponding 6-*O*-benzyl or 4-*O*-benzyl ethers.[59−61] The *p*-fluorobenzylidene acetal derivatives **5** and **6** (Scheme 1) were prepared to investigate if conditions could be found that allow conversion to the corresponding 4-hydroxy derivatives on the solid phase. The 3-OH position in the key monosaccharide building block **7** was provided with a temporary Fmoc group, which can be removed by treatment with a sterically hindered base. The Fmoc group, which is one of the most commonly used N^{α}-amino protecting groups employed in peptide synthesis,[62] has recently found many applications also in solid-phase oligosaccharide synthesis.[40,63−65] Another important issue in the synthesis of **1** and **2** was the formation of a challenging 1,2-cis α-galactosidic linkage in one of the final steps. The stereochemical outcome of this reaction is difficult to predict due to the lack of neighboring group participation at C-2 of the galactosyl donor. To achieve high α-selectivity, the reactivities of the acceptor and the donor have to be carefully matched. Since 4,6-*O*-benzylidene acetals are known to enhance the α-selectivity,[66,67] we chose to use the benzylidene protected thiogalactoside **8** (Scheme 1), which has nonparticipating fluorinated benzyl groups at O-2 and O-3.

Preparation of Building Blocks 5 and 6. Allyl glycosides **10** and **11** were obtained from glucosamine and glucose, respectively, via Fischer glycosylation followed by protection of the 4- and 6-hydroxyl groups with α,α-dimethoxy-*p*-fluorotoluene under catalysis of *p*-toluenesulfonic acid (50% and 37% yields, respectively, over two steps, Scheme 2). Subsequent acylation of the remaining hydroxyl groups with *o*-fluorobenzoyl chloride provided the fully protected glycosides **12** and **13** (84 and 98% yields). Oxidative cleavage of the double bond of **12** and **13** with the biphasic $RuCl_3$−$NaIO_4$ system[68] furnished building blocks **5** and **6** in 67% and 63% yields, respectively, after purification with reversed phase HPLC. It

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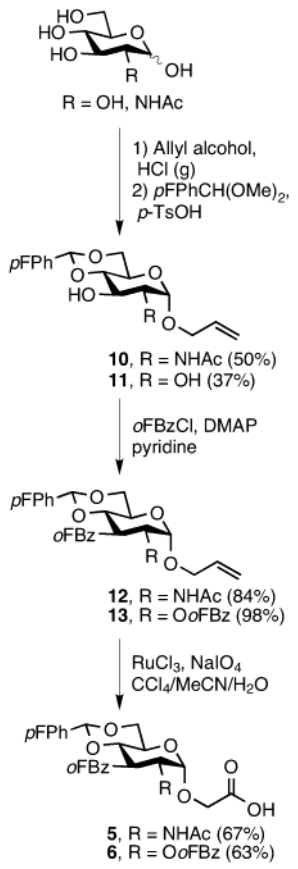
SCHEME 2. Synthesis of Protected Glucosamine and Glucose Building Blocks 5 and 6

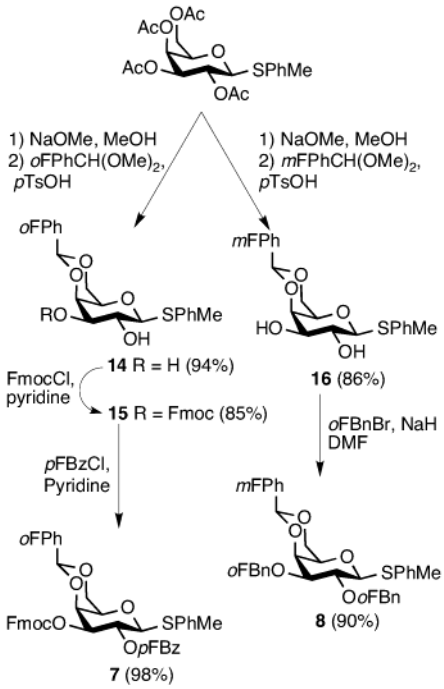


should be emphasized that the benzylidene group was intact throughout the oxidation, and that only trace amounts of aldehyde were formed from the alkene.

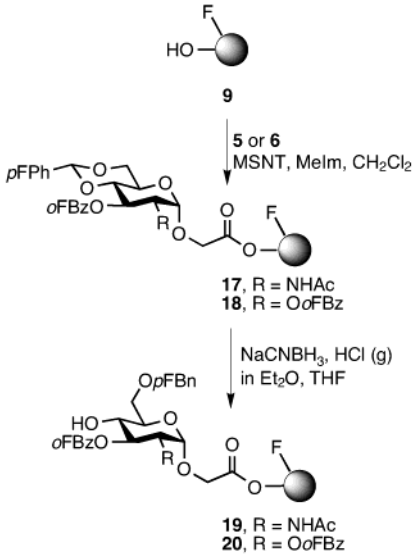
Preparation of Building Blocks 7 and 8. Synthesis of glycosyl donors **7** and **8** started with deacetylation of 4-methylphenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-*β*-D-galactopyranoside[69] followed by trans-acetalization with α,α-dimethoxy-*o*-fluorotoluene and α,α-dimethoxy-*p*-fluorotoluene, respectively, to give benzylidene-protected derivatives **14** and **16** in 94% and 86% yields (Scheme 3). Treatment of **16** with *o*-fluorobenzyl bromide and sodium hydride at 0 °C provided building block **8** in 90% yield. The Fmoc group was introduced at HO-3 of **14** by acylation with 9-fluorenylmethyl chloroformate (FmocCl, 1.2 equiv) in pyridine to give **15** in 85% yield. Subsequent benzoylation of the remaining hydroxyl group with *p*-fluorobenzoyl chloride in pyridine produced building block **7** in 98% yield.

Solid-Phase Synthesis of the α-Gal Epitopes 1 and 2. The route on the solid phase started with immobilization of *N*-acetyl glucosamine derivative **5** and glucoside **6** (3 equiv each) on the linker of the loaded ArgoGel resin **9** (Scheme 4). The ester linkages were formed with 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) as the coupling reagent[70] to afford resins **17** and **18**. The outcome of the reactions was analyzed with ^{19}F NMR

SCHEME 3. Synthesis of Galactosyl Donors 7 and 8



SCHEME 4. Attachment of Building Blocks 5 and 6 to the Solid Support and Regioselective Reduction of Benzylidene Acetals 17 and 18



MSNT = 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole
MeIm = 1-methylimidazole

spectroscopy,[71] and for **17** integration of the ^{19}F resonances from the phenolic linker (−134.5 ppm), the 4,6-*O*-*p*-fluorobenzylidene group (−113.2 ppm), and the *o*-fluorobenzoyl group (−110.5 ppm) revealed the yield to be quantitative.[72] Resin **18** was also formed in quan-

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(71) Gel-Phase ^{19}F NMR spectra were recorded after insertion of a suspension of the resin in a standard NMR tube. $CDCl_3$ was used to swell the resin with $CFCl_3$ (∂_F 0.00 ppm) as internal standard.

(72) A minor byproduct (<10%) was revealed by ^{19}F NMR spectroscopy. The byproduct disappeared in the next, acid-catalyzed step.

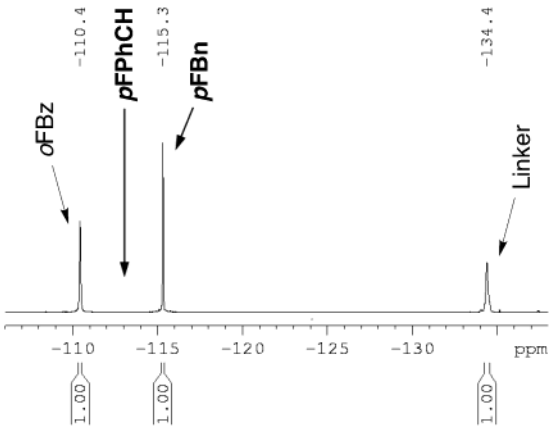
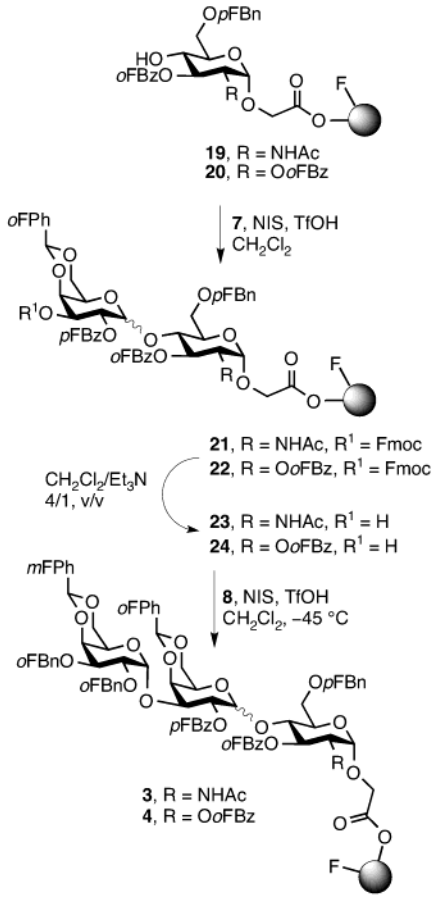


FIGURE 1. ^{19}F NMR spectrum of resin **19**.

titative yield as judged by ^{19}F NMR spectroscopy. Resin-bound glycosides **17** and **18** were subsequently converted into secondary alcohols **19** and **20** via regioselective opening of the 4,6-*O*-*p*-fluorobenzylidene acetal under reductive conditions with use of $NaCNBH_3$ (50 equivalents) in a solution of HCl (g) in diethyl ether. These conditions were established by optimization, using a model system, and it was found that standard solution-phase conditions,[60] i.e., 10 equiv of $NaCNBH_3$, resulted in acidolytic cleavage of the benzylidene acetal. Use of a large excess of $NaCNBH_3$ (>30 equivalents) prevented acidolysis so that only the desired product was formed. Indeed, inspection of the ^{19}F NMR spectrum of the resin-bound product **19** revealed the complete disappearance of the resonance from the 4,6-*O*-*p*-fluorobenzylidene acetal and that a single resonance originating from the *p*-fluorobenzyl group had formed (−115.3 ppm, Figure 1). The *o*-fluorobenzoyl signal shifted slightly upfield, whereas the resonance from the linker remained at the same shift as in starting material **17**. Integration of the fluorine signals indicated that resin **19** was formed in quantitative yield (Figure 1). The reaction proceeded equally well with resin **18** to give resin **20**. These results imply that the reductive opening of benzylidene acetals is a very robust and useful protective group manipulation also on the solid phase, and that it has potential to be adapted for use in automated procedures.

The deprotected hydroxyl group of **19** and **20** was glycosylated with thiogalactoside **7** (4 equiv) in the presence of the promoter system *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH, Scheme 5).[73,74] According to the ^{19}F NMR spectrum of resin **21**, the reaction did not reach completion since resonances from unreacted **19** were still visible, and the yield was estimated to be ∼70% (Figure 2, spectrum A). In addition, all peaks expected from the product were split into doublets, which implied that a second disaccharide product also had been formed. It was assumed that the minor peaks originated from the corresponding α-anomer indicating a 1:2 α/*β* ratio. Repeating the glycosylation under identical conditions completed the conversion of acceptor **19** to afford the resin-bound disaccharide **21**

SCHEME 5. Solid-Phase Synthesis of Trisaccharides 3 and 4 by Attachment of Building Blocks 7 and 8 to Resin-Bound 5 and 6



(Figure 2, spectrum B). Previously, the poor *β*-selectivity in a similar system was proposed to be due to inadequate neighboring group participation of a benzoyl protective group at O-2 of a donor related to **7**.[75] In that study, exchanging the benzoyl group for a pivaloyl group led to a significant increase in the *β*-selectivity. When resin **20** was glycosylated with **7**, using the same conditions and reaction time as for resin **19**, the yield after the first glycosylation could be estimated to be 50% (α/*β*, 1:2). Repeating the glycosylation twice under identical conditions gave the resin-bound disaccharide **22** in quantitative yield with an α/*β* ratio of 1:2. Interestingly, the resin-bound *N*-acetylglucosamine derivative **19** was thus more reactive than the corresponding glucoside **20**. This observation was somewhat surprising since it has been emphasized that HO-4 of *N*-acetylglucosamine derivatives are very poor glycosyl acceptors due to both intra- and intermolecular hydrogen bonding.[76] Since acceptor **19** is bound to a solid support, intermolecular hydrogen bonding is thwarted, which seems to be sufficient to enhance the reactivity.

The Fmoc group of resins **21** and **22** was removed by treatment with Et_3N in dichloromethane to afford resins **23** and **24** in quantitative yield, as judged by ^{19}F NMR spectroscopy. Subsequent α-glycosylation of **23** and **24**

(73) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313−4316.
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(76) Crich, D.; Dudkin, V. *J. Am. Chem. Soc.* **2001**, *123*, 6819−6825.

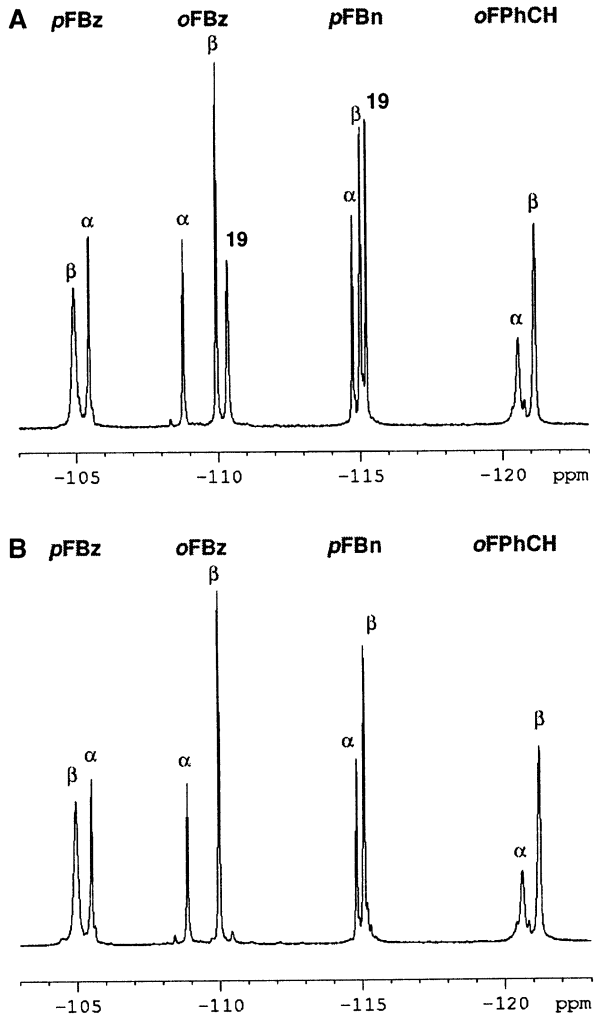
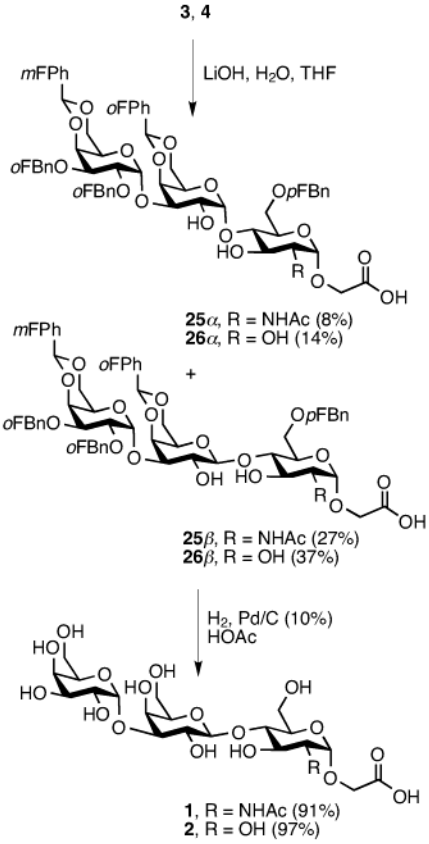


FIGURE 2. ^{19}F NMR spectra of resin **21**: (A) after the first glycosylation of resin **19** (70% yield) and (B) after two glycosylations of resin **19** (quantitative formation of **21**).

with thiogalactoside **8** (6 equiv) was carried out at reduced temperature under activation by NIS and TfOH to give resins **3** and **4**. Inspection of the signals in the ^{19}F NMR spectrum of resin **3** revealed that a single anomer had been formed. Integration indicated an 80% yield of **3**, whereas resin **4** was formed in quantitative yield. Both for **3** and **4** it was established, after cleavage from the solid phase, that the desired α-galactosidic linkage had been formed in the reaction with **8** (cf. below). Thus, the critical α-glycosylations of the galactose 3-OH on resins **23** and **24** with donor **8** proceeded in excellent yields with absolute stereoselectivity. In view of the complexity of α-glycosylations the stereoselectivity obtained is noteworthy. Synthesis of 1,2-cis glycosides constitutes a challenge in solution and successful solid-phase applications are relatively rare.[77] In contrast to solid-phase α-fucosylation that appears to be relatively straightforward,[78−80] α-glucosylations and α-galactosylations often proceed with no or modest stereoselectivity in varying yields.[81−84] In previous studies careful optimization including variation of the solid support, protective groups, glycosyl donor, solvent, and promoter system did, however, provide reliable protocols for successful synthesis of oligosaccharides containing α-glucosidic linkages.[82,83]

SCHEME 6. Cleavage of 3 and 4 from the Solid Phase and Removal of the Remaining Protecting Groups



Cleavage of **3** and **4** from the solid phase under basic conditions with LiOH/H_2O/THF allowed trisaccharides **25α** and **25*β*** to be isolated in 8% and 27% yields, respectively. The corresponding trisaccharides **26α** and **26*β***, which have a glucose moiety at the reducing end, were obtained in 14% and 37% yields, respectively (Scheme 6).[85] The anomeric configurations for the products were confirmed by the $^3J_{1,2}$ coupling constants determined by ^{1}H NMR spectroscopy. Finally, the remaining fluorinated benzylidene acetal and benzyl ethers in trisaccharides **25*β*** and **26*β*** where subjected to hydrogenation. The fluorinated protective groups showed the same reactivity in hydrogenation as the corresponding nonfluorinated variants. Thus, both the fluorinated benzyl ethers and the 4,6-*O*-fluorobenzylidene groups could

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(85) Isolated yields were based on the initial loading capacity of the Amino ArgoGel resin (0.45 mmol/g).

be removed by standard hydrogenation conditions, i.e., by treatment with palladium on activated carbon (10% Pd, w/w) under an atmosphere of hydrogen (1 and 3.7 atm, respectively) to provide the α-Gal epitopes **1** (91%) and **2** (97%).[86] The carboxymethyl functionality at the reducing end of trisaccharides **1** and **2** can be activated and coupled, for instance to an insoluble support, which can be used as filtrate material to exclude circulating α-Gal antibodies from blood. This type of carbohydrate derivative can also be attached to a microchip to furnish microarrays for automated high-throughput screening of carbohydrate binding proteins.[87]

Conclusions

A route for the preparation of α-Gal trisaccharides by solid-phase synthesis has been established. Development of the synthetic route relied on the use of fluorinated benzoates, benzyl ethers, and benzylidene acetals in combination with a fluorinated linker. This allowed the formation of products in each step to be analyzed directly on the resin with gel-phase ^{19}F NMR spectroscopy. Hence, the outcome of each glycosylation, as well as in the protective group transformations, could be conveniently determined. This enabled conditions for reductive opening of benzylidene acetals on the solid phase to be established, and employed in a key step in the synthetic route. In addition, it was demonstrated that thioglycosides, in combination with the promoter system NIS and TfOH, were effective as glycosyl donors for synthesis of both β- and α-glycosides. A challenging α-(1→3)-galactosidic linkage could thus be successfully installed with complete stereoselectivity and in high yield. Importantly, it was confirmed that the fluorinated protective groups had "normal" chemical reactivity, i.e., that they could be employed and manipulated under conditions identical with those used for standard, nonfluorinated protective groups employed in oligosaccharide synthesis.

Experimental Section

Resin 17. MSNT (120 mg, 0.41 mmol) and **5** (206 mg, 0.41 mmol) were dissolved in CH_2Cl_2 (2.5 mL) and added to linker-loaded resin **9**[22] (0.14 mmol). Methyl imidazole (65 μL, 0.81 mmol) was added and after 18 h of agitation at room temperature, the resin was washed with CH_2Cl_2, piperidine/DMF (1/5, v/v), DMF (4 × 3 mL each), and CH_2Cl_2 (6 × 3 mL) to give resin **17** (quant). **17**: ^{19}F NMR ($CDCl_3$) δ −134.5, −113.2, −110.5.

Resin 18. MSNT (61 mg, 0.21 mmol) and compound **6** (130 mg, 0.21 mmol) were dissolved in CH_2Cl_2 (2.5 mL) and added to linker-loaded resin **9**[22] (69 μmol). Methyl imidazole (33 μL, 0.41 mmol) was added and after 16 h of agitation at room temperature, the resin was washed as described for resin **17** to give resin **18** (quant). **18**: ^{19}F NMR ($CDCl_3$) δ −134.4, −113.2, −110.1, −109.9.

Resin 19. Resin **17** (0.14 mmol) and $NaCNBH_3$ (0.51 g, 8.1 mmol) were suspended in THF (3.0 mL) and saturated ethereal HCl was added until the mixture became acidic (pH 2−3, measured by pH paper) and the gas evolution ceased. After 17 h of agitation at room temperature, the resin was washed with THF (5 × 3 mL), sat. $NaHCO_3$ (3 × 3 mL), H_2O (10 × 3 mL), THF, MeOH, THF, DMF, piperidine/DMF (1/5, v/v), DMF, and CH_2Cl_2 (5 × 3 mL each), and then dried under vacuum to give resin **19** (quant). **19**: ^{19}F NMR ($CDCl_3$) δ −134.4, −115.3, −110.4.

Resin 20. Resin **18** (69 μmol) and $NaCNBH_3$ (0.22 g, 3.50 mmol) were suspended in THF (2.0 mL) and saturated ethereal HCl was added until the mixture became acidic (pH 2−3, measured by pH paper) and the gas evolution ceased. After 20 h of agitation at room temperature, the resin was washed as described for resin **19** and then dried under vacuum to give resin **20** (quant). **20**: ^{19}F NMR ($CDCl_3$) δ −134.4, −115.2, −110.0, −109.6.

Resin 21. Triflic acid (1.0 μL, 11.3 μmol) was added to a suspension of resin **19** (52 μmol), NIS (47 mg, 0.21 mmol), and **7** (153 mg, 0.21 mmol) in CH_2Cl_2 (2.00 mL) in the absence of light. After 16 h of agitation at room temperature, the resin was washed with CH_2Cl_2, DMF, THF, and CH_2Cl_2 (5 × 3 mL each) to give a 70% formation of resin **21**. The procedure was repeated to give resin **21** (quant). The β anomer of **21**: ^{19}F NMR ($CDCl_3$) δ −134.6, −121.2, −115.1, −110.0, −104.9. The α anomer of **21**: ^{19}F NMR ($CDCl_3$) δ −134.6, −120.6, −114.9, −108.8, −105.5.

Resin 22. Resin **20** (69 μmol) was reacted with **7** (203 mg, 0.28 mmol), NIS (62 mg, 0.28 mmol), and triflic acid (1.2 μL, 13.8 μmol) in CH_2Cl_2 (2.66 mL) as described for resin **21** to give a 50% formation of resin **22**. The procedure was repeated twice to give resin **22** (quant). The β anomer of **22**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.6, −114.8, −110.3, −109.3, −105.1. The α anomer of **22**: ^{19}F NMR ($CDCl_3$) δ −134.4, −121.4, −114.7, −110.1, −108.9, −105.8.

Resin 23. Resin **21** (52 μmol) was rinsed with Et_3N/CH_2Cl_2 (1/4, v/v) during 10 min, then Et_3N/CH_2Cl_2 (1/4, v/v, 4.0 mL) was added. After 40 min of agitation at room temperature, the resin was washed with CH_2Cl_2, DMF, THF, and CH_2Cl_2 (6 × 3 mL each) and then dried under vacuum to give resin **23** (quant). The β anomer of **23**: ^{19}F NMR ($CDCl_3$) δ −134.4, −121.2, −115.1, −110.2, −105.4. The α anomer of **23**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.5, −114.9, −109.3, −105.9.

Resin 24. Resin **22** (69 μmol) was treated and washed as described for resin **23** to give resin **24** (quant). The β anomer of **24**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.6, −114.8, −110.3, −109.4, −105.5. The α anomer of **24**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.6, −114.8, −109.4, −106.0.

Resin 3. Compound **8** (0.19 g, 0.31 mmol), NIS (70 mg, 0.31 mmol), and resin **23** (52 μmol) were suspended in CH_2Cl_2 (2.0 mL) in the absence of light. The mixture was cooled to −45 °C (CO_2/MeCN) and TfOH (1.0 μL, 11.3 μmol) was added. After 6 h of stirring, the resin was washed with CH_2Cl_2, DMF, piperidine/DMF (1/5, v/v), DMF, and CH_2Cl_2 (5 × 3 mL each), and then dried under vacuum to give a 70% formation of resin **3**. The procedure was repeated once to give resin **3** in 80% yield. The α/β/α anomer of **3**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.7, −119.6, −119.5, −115.1, −113.9, −110.2, −104.8. The α/α/α anomer of **3**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.1, −119.1, −118.9, −115.0, −113.7, −110.0, −105.8.

Resin 4. Resin **24** (69 μmol) was reacted with **8** (0.23 g, 0.37 mmol), NIS (83 mg, 0.37 mmol), and TfOH (1.1 μL, 12.3 μmol) in CH_2Cl_2 (2.0 mL) and washed as described for resin **3** to give resin **4** (quant). The α/β/α anomer of **4**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.9, −119.6, −119.4, −114.8, −113.9, −110.4, −109.4, −104.9. The α/α/α anomer of **4**: ^{19}F NMR ($CDCl_3$) δ −134.4, −120.2, −119.1, −118.9, −114.9, −113.7, −110.1, −108.9, −105.6.

Carboxymethyl 2-Acetamido-6-*O*-(4-fluorobenzyl)-4-*O*-{4,6-*O*-(2-fluorobenzylidene)-3-*O*-[2,3-di-*O*-(2-fluorobenzyl)-4,6-*O*-(2-fluorobenzylidene)-α-D-galactopyranosyl]-⟨α/β-D-galactopyranosyl⟩-2-deoxy-α-D-glucopyranoside (25α/25β). THF (3.0 mL) and aqueous LiOH (3.0 mL, 0.20 M) were added to resin **3** (52 μmol). After 5 h of agitation at room temperature, the resin was filtered and then washed with a mixture of THF (3.0 mL) and aqueous LiOH (3.0 mL, 0.20 M)

(86) Compounds **1** and **2** were only sparingly soluble in several solvents, which made NMR analysis somewhat complicated. However, ^{1}H and ^{13}C NMR spectra with satisfactory resolution and acceptable signal-to-noise ratio could be obtained for **1** in D_2O and **2** in CD_3CO_2D (cf. Supporting Information).

(87) Flitsch, S. L.; Ulijn, R. V. *Nature* **2003**, *421*, 219−220.

followed by HOAc (2 × 5 mL). The filtrate was lyophilized and then purified with reversed-phase HPLC (gradient: 5→100% MeCN in H_2O, both containing 0.1% TFA, during 50 min) to give the α/*β*/α anomer **25*β*** (16.0 mg, 27%) and the corresponding α/α/α anomer **25α** (5.0 mg, 8%) as white solids. **25α**: $[\alpha]^{20}_D$ +37 (*c* 0.37, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.63−6.88 (m, 20 H; Ar*H*), 6.84 (s, 1 H; AcN*H*), 5.74 (s, 1 H; 3-FPhC*H*O_2), 5.45 (s, 1 H; 2-FPhC*H*O_2), 5.27 (d, *J* = 2.9 Hz, 1 H; H-1 or H-1″), 4.87 (d, *J* = 3.0 Hz, 1 H; H-1 or H-1″), 4.76 (s, 2 H; FPhC*H*$_2$O), 4.73 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.64 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.53 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.48 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.40 (br s, 1 H; OH), 4.33 (d, *J* = 7.7 Hz, 1 H; H-1′), 4.29 (d, *J* = 1.9 Hz, 1 H; H-4′), 4.08 (dd, *J* = 10.0, 3.2 Hz, 1 H; H-2 or H-2″), 3.73 (d, *J* = 2.9 Hz, 1 H; H-4 or H-4″), 3.56 (dd, *J* = 9.2, 2.4 Hz; H-3′), 2.06 (s, 3 H; *Ac*NH); ^{13}C NMR[88] (100 MHz, $CDCl_3$) *δ* 103.6, 99.9, 97.9, 96.0, 95.9, 80.7, 76.7, 75.7, 75.0, 74.2, 72.7, 70.5, 70.2, 69.2, 69.1, 69.0, 68.6, 66.8, 66.4, 65.4, 64.4, 63.3, 53.4, 20.7, 22.8; ^{19}F NMR (376 MHz, $CDCl_3$) *δ* −120.3, −119.5, −119.2, −114.9, −113.8; HRMS (FAB) calcd for $C_{57}H_{59}F_5NO_{18}$ 1140.3653 *m*/*z* $(M)^+$, observed 1140.3654.

25α: $[\alpha]^{20}_D$ +16 (*c* 0.15, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.66−6.85 (m, 20 H; Ar*H*), 5.73 (s, 1 H; 3-FPhC*H*O_2), 5.47 (s, 1 H; 2-FPhC*H*O_2), 5.29 (d, *J* = 2.0 Hz, 1 H; H-1 or H-1′ or H-1″), 5.24 (d, *J* = 2.8 Hz, 1 H; H-1 or H-1′ or H-1″), 4.87 (d, *J* = 3.2 Hz, 1 H; H-1 or H-1′ or H-1″); ^{13}C NMR[88] (100 MHz, $CDCl_3$) *δ* 102.9, 100.0, 98.0, 95.8, 95.0 81.5, 77.3, 75.8, 75.1, 74.2, 73.5, 73.2, 72.8, 70.8, 69.3, 68.6, 68.3, 67.3, 66.1, 65.3, 63.9, 63.0, 29.7, 20.5; ^{19}F NMR (376 MHz, $CDCl_3$) *δ* −120.5, −119.5, −119.5, −115.0, −113.9; HRMS (FAB) calcd for $C_{57}H_{59}F_5NO_{18}$ 1140.3653 *m*/*z* $(M)^+$, observed 1140.3656.

Carboxymethyl 6-*O*-(4-Fluorobenzyl)-4-*O*-{4,6-*O*-(2-fluorobenzylidene)-3-*O*-[2,3-di-*O*-(2-fluorobenzyl)-4,6-*O*-(2-fluorobenzylidene)-α-D-galactopyranosyl]-α/*β*-D-galactopyranosyl}-α-D-glucopyranoside (26α/26*β*). Resin **4** (69 *μ*mol) was treated with THF (3.0 mL) and aqueous LiOH (4.0 mL, 0.20 M) as described for **25α/25*β***. Purification with reversed-phase HPLC (gradient: 0→100% MeCN in H_2O, both containing 0.1% TFA, during 50 min) gave the α/*β*/α anomer **26*β*** (28.2 mg, 37%) and 14 mg of the corresponding α/α/α anomer **26α** as white solids. Compound **26α** was ∼80% pure and the yield of **26α** was estimated to ∼14%. **26*β***: $[\alpha]^{20}_D$ −110 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.65−6.88 (m, 20 H; Ar*H*), 5.75 (s, 1 H; 3-FPhC*H*O_2), 5.45 (s, 1 H; 2-FPhC*H*O_2), 5.28 (d, *J* = 2.6 Hz, 1 H; H-1 or H-1″), 4.86 (d, *J* = 3.3 Hz, 1 H; H-1 or H-1″), 4.75 (s, 2 H; FPhC*H*$_2$O), 4.73 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.64 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.53 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.48 (d, *J* = 11.9 Hz, 1 H; FPhC*H*$_2$O), 4.30 (d, *J* = 7.6 Hz, 1 H; H-1′), 3.56 (dd, *J* = 9.4, 2.5 Hz, 1 H; H-3′); ^{13}C NMR[88] (100 MHz, $CDCl_3$) *δ* 103.1, 99.9 (d, *J* = 1.7 Hz), 99.5, 95.9 (d, *J* = 3.7 Hz), 95.7, 79.6, 77.2, 75.6, 75.0, 74.1, 72.7, 72.6, 72.1, 71.6, 70.2, 69.2, 69.0, 68.3, 66.7, 66.4 (d, *J* = 3.7 Hz), 65.8, 65.4 (d, *J* = 4.0 Hz), 63.4; ^{19}F NMR (376 MHz, $CDCl_3$) *δ* −120.4, −119.5, −119.2, −114.8, −113.8; HRMS (FAB) calcd for $C_{55}H_{55}F_5O_{18}Na$ 1121.3207 *m*/*z* $(M + Na)^+$, observed 1121.3195.

26α: ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.67−6.87 (m, 20 H; Ar*H*), 5.73 (s, 1 H; 3-FPhC*H*O_2), 5.47 (s, 1 H; 2-FPhC*H*O_2), 5.34 (d, *J* = 3.0 Hz, 1 H; H-1 or H-1′ or H-1″), 5.24 (d, *J* = 3.4 Hz, 1 H; H-1 or H-1′ or H-1″), 4.84 (d, *J* = 3.6 Hz, 1 H; H-1 or H-1′ or H-1″), 4.38 (d, *J* = 2.7 Hz, 1 H; H-4′ or H-4″); ^{13}C NMR[88] (100 MHz, $CDCl_3$) *δ* 106.6, 100.0, 99.7, 95.8 (d, *J* 3.7 Hz), 81.4, 78.0, 77.2, 75.5, 75.2, 74.1, 73.6, 73.5, 72.9, 71.4, 70.8, 69.4, 69.3, 68.6, 66.3 (d, *J* = 3.3 Hz), 65.2 (d, *J* = 4.0 Hz), 63.8, 63.4; ^{19}F NMR (376 MHz, $CDCl_3$) *δ* −120.3, −119.4, −119.0, −115.0, −113.7; HRMS (FAB) calcd for $C_{55}H_{55}F_5O_{18}Na$ 1121.3207 *m*/*z* $(M + Na)^+$, observed 1121.3190.

Carboxymethyl 2-Acetamido-4-*O*-[3-*O*-(α-D-galactopyranosyl)-*β*-D-galactopyranosyl]-2-deoxy-α-D-glucopyranoside (1). A solution of compound **25*β*** (12.6 mg, 11.1 *μ*mol) in HOAc (1.5 mL) was treated with 10% Pd/C (15 mg) under hydrogen (3.7 atm) at room temperature for 12 h. The catalyst was removed by filtration (Hyflo-Supercel) and washed with HOAc (10 mL), MeOH (10 mL), and H_2O (10 mL). The combined filtrates were evaporated and lyophilizated from H_2O (10 mL) to afford **1** (6.1 mg, 91%) as a white solid: $[\alpha]^{20}_D$ +5 (*c* 0.8, DMSO); ^{1}H NMR (400 MHz, D_2O) *δ* 5.04 (s, 1 H; H-1 or H-1″), 4.77 (s, 1 H; H-1 or H-1″), 4.45 (d, *J* = 6.8 Hz, 1 H; H-1′); ^{13}C NMR (100 MHz, D_2O) *δ* 102.8, 97.0, 95.5, 78.6, 77.2, 75.1, 70.9, 70.8, 70.1, 69.9, 69.6, 69.3, 69.2, 68.3, 64.9, 61.1, 61.0, 59.9, 53.1, 22.0; MS (ESI) calcd for $C_{22}H_{38}NO_{18}$ 604.20 *m*/*z* $(M + H)^+$, observed 604.04.

Carboxymethyl 4-*O*-[3-*O*-(α-D-Galactopyranoside)-*β*-D-galactopyranosyl]-α-D-glucopyranoside (2). A solution of compound **26*β*** (5.1 mg, 4.6 *μ*mol) in HOAc (1.5 mL) was treated with 10% Pd/C (20 mg) under hydrogen (1 atm) at room temperature for 24 h. The catalyst was removed by filtration (Hyflo-Supercel) and washed with HOAc (10 mL). The combined filtrates were lyophilizated to afford **2** (2.5 mg, 97%) as a white solid: $[\alpha]^{20}_D$ +10 (*c* 0.1, HOAc); ^{1}H NMR (400 MHz, CD_3CO_2D) *δ* 5.18 (s, 1 H; H-1 or H-1″), 5.01 (d, *J* = 3.2 Hz, 1 H; H-1 or H-1″), 4.56 (d, *J* = 6.8 Hz, 1 H; H-1′), 4,18 (s, 1 H; H-4′), 4.14 (s, 1 H; H-4 or H-4″); ^{13}C NMR (100 MHz, CD_3CO_2D) *δ* 104.4, 100.0, 97.9, 81.2, 80.3, 75.7, 73.5, 72.4, 71.8, 71.8, 71.3, 70.9, 70.7, 69.9, 67.4, 66.0, 62.5, 62.2, 54.7; HRMS (FAB) calcd for $C_{20}H_{34}O_{18}Na$ 585.1643 *m*/*z* $(M + Na)^+$, observed 585.1633.

Acknowledgment. This work was funded by grants from the Swedish Research Council and the Göran Gustafsson Foundation for Research in Natural Sciences and Medicine. We also acknowledge financial support from the J. C. Kempe's Foundation.

Supporting Information Available: General methods and materials; experimental procedures for the synthesis of building blocks **5**, **6**, **7**, and **8**; and ^{19}F NMR, ^{1}H NMR, and ^{13}C NMR spectral data for all described compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(88) ^{13}C NMR resonances originating from the fluorinated protective groups are split by J_{C-F} couplings, resulting in a complex spectrum. Therefore signals downfield from 110.0 ppm are not reported.