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[19] This follows from the fact that $14 \times RT\ln s_0$(ABGY) $\approx 27 \times RT\ln s_0$(Scholtz peptides). ABGY and the 26-mer Ala-based peptide share similar (Aib + Ala) content (~60%). It should be pointed out that the difference in s_o for the two sets may also originate from a higher value of the helix nucleation parameter σ in Aib-based helices (held constant at 0.003 by us and Scholtz et al.[5]).
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Fluorinated Protective Groups for On-Resin Quantification of Solid-Phase Oligosaccharide Synthesis with ^{19}F NMR Spectroscopy

Mickael Mogemark, Mikael Elofsson,* and Jan Kihlberg*[a]

KEYWORDS:

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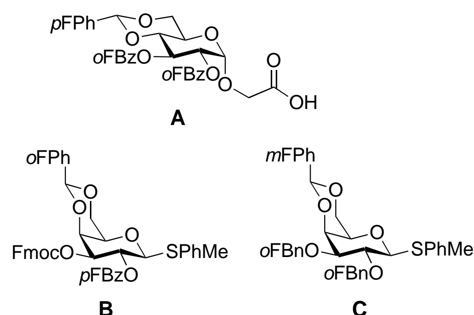
Solid-phase synthesis is an attractive approach for the generation of large numbers of compounds for biological studies, for instance in the pharmaceutical industry.[1] In addition, automated procedures for solid-phase synthesis of oligopeptides and oligonucleotides have been of major importance in developing an understanding of the functions of proteins and nucleic acids in the processes of life. As compared to knowledge about these two biopolymers, the roles played by carbohydrates in different glycoconjugates in nature are less well understood.[2] Unfortunately, the difficulties associated with the synthesis of oligosaccharides constitute a major impediment for the development of glycobiology.[3] For instance, the assembly of complex oligosaccharides on solid support or in solution is still a considerable challenge for all but a few laboratories.

Although major advances in the solid-phase synthesis of oligo- and polysaccharides have been made during the past few years,[4] the lack of simple and powerful analytical techniques for on-resin validation of the chemistry involved is a significant limitation.[5] In general, the use of nondestructive methods, such as IR and NMR spectroscopy, for elucidating reactions directly on the solid phase constitutes the most attractive approach.[6, 7] However, applications of these techniques in solid-phase oligosaccharide synthesis are rare and include the use of high-resolution magic-angle-spinning NMR spectroscopy,[8] as well as employment of ^{13}C-enriched acetyl protective groups.[9, 10] These methods are somewhat restricted by costs and the requirement for specialized NMR spectroscopy equipment. On the other hand, fluorinated reagents corresponding to the most common protective groups used in oligosaccharide synthesis are commercially available and are usually cheap. Use of saccharide building blocks that carry fluorinated protective groups should therefore allow optimization of solid-phase oligosaccharide synthesis by using gel-phase ^{19}F NMR spectroscopy. Gel-phase ^{19}F NMR spectroscopy has several favorable properties including high sensitivity (the natural abundance of ^{19}F is 100%) and a wide dispersion of the ^{19}F chemical shifts. Hence, magic-angle spinning is not required and high-quality spectra can be obtained in a couple of minutes with a conventional NMR spectrometer.[11–16]

To investigate the potential of ^{19}F NMR spectroscopy for monitoring solid-phase oligosaccharide synthesis we have undertaken the synthesis of the α-Gal epitope (Gal(α1-3)-Gal(β1-4)Glc), which is responsible for hyperacute rejection in xenotransplantation of porcine organs.[17] The synthetic sequence started with immobilization of glycoside **A** (Scheme 1) on the linker-loaded ArgoGel resin **1**[15] through an ester linkage[18] to afford resin **2** (Scheme 2). The outcome of the



Scheme 1. Monosaccharide building blocks used for solid-phase synthesis. Bn = benzyl, Bz = benzoyl, Fmoc = 9-fluorenylmethoxycarbonyl.

[a] Dr. M. Elofsson, Prof. J. Kihlberg, M. Mogemark
Organic Chemistry, Department of Chemistry
Umeå University, 90187 Umeå (Sweden)
Fax: (+46) 90-1388-85
E-mail: Mikael.Elofsson@chem.umu.se, Jan.Kihlberg@chem.umu.se

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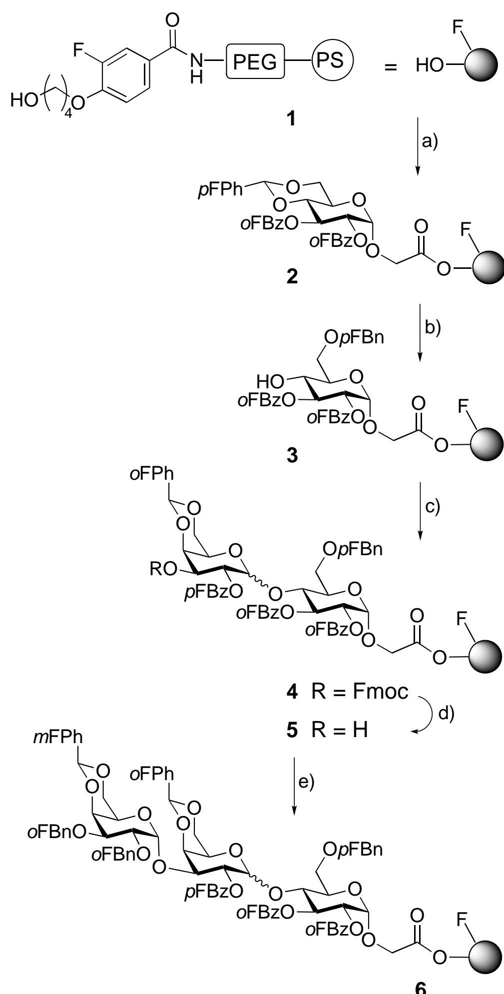

*Scheme 2. Solid-phase synthesis of resin **6**. Reagents and conditions: a) **A** (3.0 equiv), MSNT (3.0 equiv), MeIm (6 equiv), CH_2Cl_2, RT. b) $NaCNBH_3$ (50 equiv), HCl(g) in Et_2O, THF, RT. c) **B** (4.0 equiv), NIS (4.0 equiv), TfOH (0.2 equiv), CH_2Cl_2, RT, the reaction was repeated twice. d) Et_3N/CH_2Cl_2 (1:4 (v/v)), RT. e) **C** (5.4 equiv), NIS (5.4 equiv), TfOH (0.2 equiv), CH_2Cl_2, $-45\,^{\circ}C$. All steps were essentially quantitative as determined by integration of the gel-phase ^{19}F NMR spectra. MeIm = 1-methylimidazole, MSNT = 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole, NIS = N-iodosuccinimide, PEG = poly(ethylene glycol), PS = polystyrene, TfOH = trifluoromethane sulfonic acid, THF = tetrahydrofuran.*

reaction was analyzed with ^{19}F NMR spectroscopy and integration of the resonances from the fluorine atoms in the linker ($\delta = -134.4$ ppm), the 4,6-*O*-*p*-F-benzylidene group (-113.2 ppm) and the two *o*-F-benzoyl groups (-109.0 and -110.1 ppm) revealed the yield to be quantitative (Figure 1 a).[19] Resin-bound **2** was then converted into secondary alcohol **3** by regioselective opening of the 4,6-*O*-*p*-F-benzylidene acetal under reductive conditions with $NaCNBH_3$ in a solution of HCl(g) in diethyl ether.[20] After optimization with a model system, conditions were found that led to the complete disappearance of the resonance from the 4,6-*O*-*p*-F-benzylidene group (-113.2 ppm) of **2**, and the appearance of a single, new signal originating from the *p*-F-benzyl ether (-115.2 ppm, Figure 1 b) of **3**. To the best of our knowledge, this is the first example of performing this strategically important protective-group manipulation on solid support.

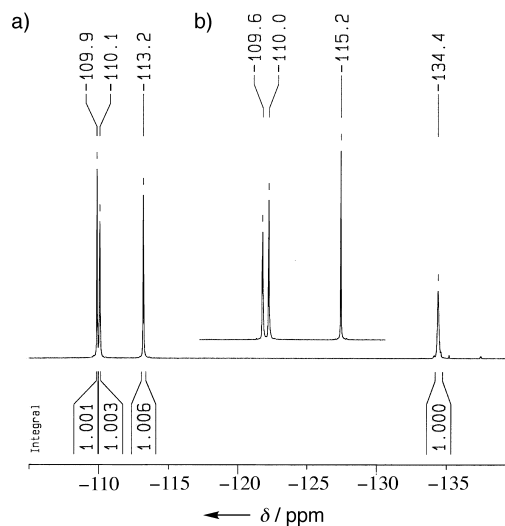

*Figure 1. Gel-phase ^{19}F NMR spectra of: a) resin **2** obtained after attachment of **A** to resin-bound linker **1**; b) resin **3** after stereoselective reduction of the benzylidene acetal. The spectra were recorded in $CDCl_3$ with $CFCl_3$ ($\delta = 0.00$ ppm) as the internal standard.*

The hydroxy group of **3** was glycosylated with thiogalactoside **B** (Scheme 1, 4 equivalents) by using NIS and TfOH as a promotor system.[21, 22] The ^{19}F NMR spectrum of resin **4** revealed that the glycosylation had not reached completion since resonances from unreacted **3** were still visible, and the yield could be estimated as $\approx 50\%$ (Figure 2 a). The *p*-F-benzoyl group, which is in close proximity to the newly formed glycosidic bond, displayed two resonances: a major one at -105.1 ppm and a minor peak at -105.9 ppm). It was assumed that this reflected the formation of an anomeric mixture, most likely in a 1:2 α/β ratio. All other resonances from the product were also split into 1:2 doublets.[23] Monitoring with ^{19}F NMR spectroscopy revealed that the glycosylation had to be repeated twice to achieve complete conversion of **3** into resin-bound disaccharide **4** (α/β 1:2, Figure 2 b). The Fmoc group at the 3′-position in **4** was then removed by treatment with triethylamine in dichloromethane to afford resin **5** in quantitative yield, as judged by ^{19}F NMR spectroscopy.[24]

α-Glycosylation of resin **5** with thiogalactoside donor **C** (Scheme 1, 5.4 equivalents) was carried out at reduced temperature with activation by NIS and TfOH to give resin **6**. The quantitative formation of a single anomer for the new glycosidic bond was evident from the appearance and integrals of the signals in the ^{19}F NMR spectrum of **6**. Cleavage from the solid phase under basic conditions allowed trisaccharides **7α** and **7β** to be isolated in $\approx 14\%$ and 37% yields, respectively (Scheme 3).[25] At this stage the configurations of the glycosidic bonds in **7α** and **7β** were confirmed by the values of the $^3J_{1,2}$ coupling constants. The ^{19}F resonances from the resin-bound trisaccharides **6** were spread out over a wide spectral range and the chemical shifts of **6** closely matched those of the isolated trisaccharides **7α** and **7β** (Table 1). Subsequent hydrogenation of **7β** over Pd/C at atmospheric pressure afforded the α-Gal epitope **8** (97%).

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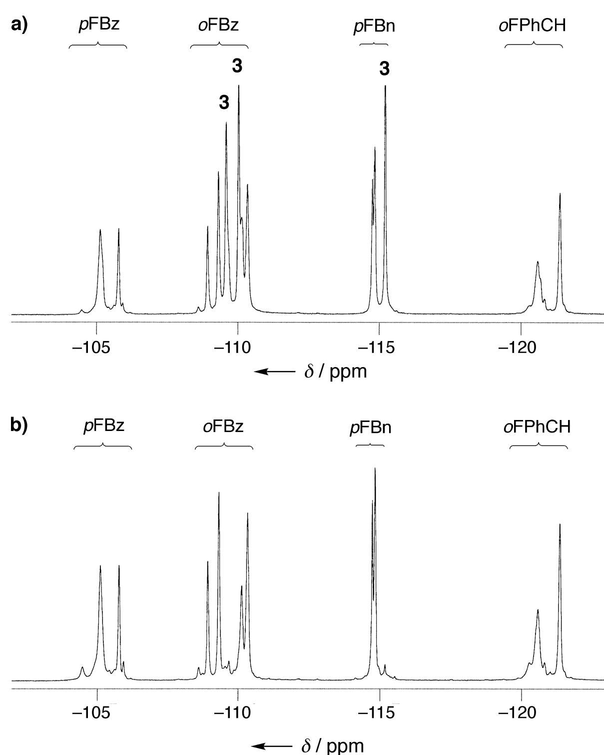
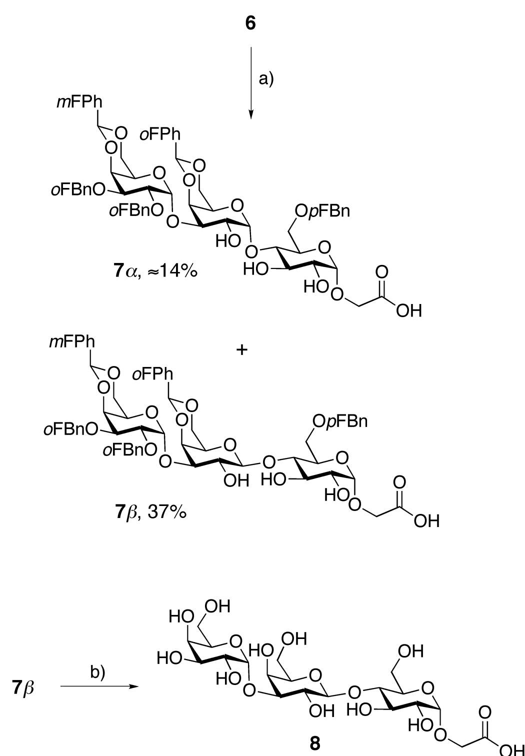


Figure 2. *Gel-phase* [19]*F NMR spectra of resins obtained in attempts to prepare* ***4*** *by glycosylation of* ***3****. a) A single glycosylation of* ***3*** *with 4 equivalents of* ***B*** *gave ≈50% conversion of* ***3****. b) Quantitative formation of resin* ***4*** *after three identical glycosylations. The spectra were recorded in $CDCl_3$ with $CFCl_3$ (δ = 0.00 ppm) as the internal standard.*



Scheme 3. *Cleavage from the solid phase and deprotection. Reagents and conditions: a) 0.11 M LiOH in H_2O/THF (4:3 (v/v)), RT, 51%. b) H_2 (1 atm), Pd/C (cat.), RT, 97%.*

Table 1. [19]*F NMR data (δ in ppm) of resin-bound trisaccharides* ***6*** *and the isolated trisaccharides* ***7α*** *and* ***7β****.*

Protective group	δ [ppm]			
	6β[a,b]	**7β**[b]	**6α**[a,c]	**7α**[c]
pFBz	−104.9		−105.6	
oFBz	−109.4		−108.9	
	−110.4		−110.1	
mFPhCH	−113.9	−113.8	−113.7	−113.7
pFBn	−114.8	−114.8	−114.9	−115.0
oFBn	−119.4	−119.2	−119.1	−119.0
	−119.6	−119.6	−119.4	−119.4
oFPhCH	−120.9	−120.4	−120.2	−120.3

[a] Gel-phase ^{19}F NMR shifts extracted from the spectrum of resin **6**. [b] β-Configuration for the Gal–Glc linkage. [c] α-Configuration for the Gal–Glc linkage.

In conclusion, the synthesis of trisaccharide **8** demonstrates that gel-phase NMR spectroscopy, in combination with fluorinated protective groups and linkers, is a simple and versatile method for on-resin quantification of solid-phase oligosaccharide synthesis. Thus, protective-group manipulations, as well as the anomeric purity and the yield in the formation of each glycosidic linkage, could be monitored directly on the resin. This allowed difficult glycosylations to be driven to completion (for example, the conversion of **3** into **4**) and formation of a 1,2-*cis* glycosidic linkage—a rare event in solid-phase oligosaccharide synthesis.[5, 26] Advantages of this analytical tool include the commercial availability of reagents that allow introduction of *ortho-*, *meta*, and *para*-fluorinated variants of benzyl ethers, benzoates and benzylidene acetals as well as the fact that their ^{19}F chemical shifts are spread over a wide spectral range (Table 1). It is important to note that the fluorinated protective groups employed in this study show the same overall reactivity during synthetic transformations (for example, reductive benzylidene opening, glycoside synthesis) and deprotection as the corresponding nonfluorinated variants,[27] but that some differences in reaction rates, for instance during removal of *O*-fluorobenzoates have been observed.[28] We believe that use of glycosyl donors, which carry different patterns of fluorinated protective groups, should be most useful in establishing conditions for complex steps in solid-phase oligosaccharide synthesis such as formation of α-glycosides and incorporation of sialic acid residues. By using carefully selected fluorinated protective groups it should also be possible to monitor solid-phase synthesis of large oligosaccharides without encountering problems with chemical shift overlap.

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[24] Removal of the Fmoc group in **4** induced a 0.2 ppm shift for the ^{19}F resonances derived from the *o*FPhCH and *p*FBz protective groups.

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