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Monitoring Solid-Phase Glycoside Synthesis with ^{19}F NMR Spectroscopy

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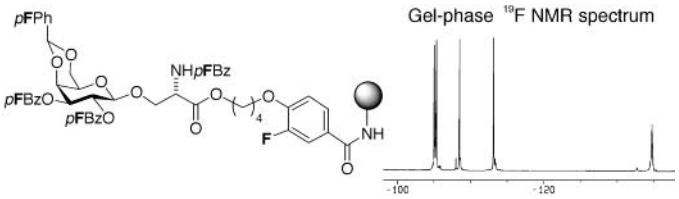
ABSTRACT



A simple and efficient method for monitoring and optimizing carbohydrate synthesis on polymeric support by using ^{19}F NMR spectroscopy is described. The method relies on the use of fluorinated variants of protective groups that are in common use in oligosaccharide synthesis.

Automated procedures for synthesis of oligopeptides and oligonucleotides have been of major importance in efforts to understand the biological roles of proteins and nucleic acids.[1] The functions of carbohydrates found in glycoconjugates are less well understood, and increasing attention is therefore being directed to development of protocols for solid-phase synthesis (SPS) of oligosaccharides.[2] One limitation of SPS is the difficulty in characterizing the outcome of complex reactions, such as glycosylations, when the product is still attached to the solid support. ^{13}C and ^{1}H NMR spectroscopy in combination with magic angle spinning, presaturation of support signals, or use of ^{13}C-enriched building blocks has been employed for monitoring solid-phase oligosaccharide synthesis.[3] However, these techniques either are expensive and time-consuming or require special equipment. Fluorinated reagents corresponding to the most common protective groups used in oligosaccharide synthesis, e.g., benzyl ethers, benzoates, and benzylidene acetals, are commercially available, in most cases at low price. Saccharide building blocks that carry fluorinated protective groups should therfore allow optimization of solid-phase oligosaccharide synthesis using gel-phase ^{19}F NMR spectroscopy. ^{19}F NMR spectroscopy has several favorable properties, including high sensitivity (the natural abundance of ^{19}F is 100%) and spreading of ^{19}F resonances over a wide spectral range as a result of the high polarizability of the ^{19}F nucleus. Moreover, since resins for SPS do not contain fluorine, disturbing signals from the solid support are not encountered. Previously, fluorinated building blocks or linkers have been utilized for quantification of reactions performed on solid support such as amide bond formation, reductive amination and cleavage of products,[4] or for encoding of combinatorial libraries.[5]

The properties of the linker must be chosen carefully considering the conditions to be used in SPS. It has been

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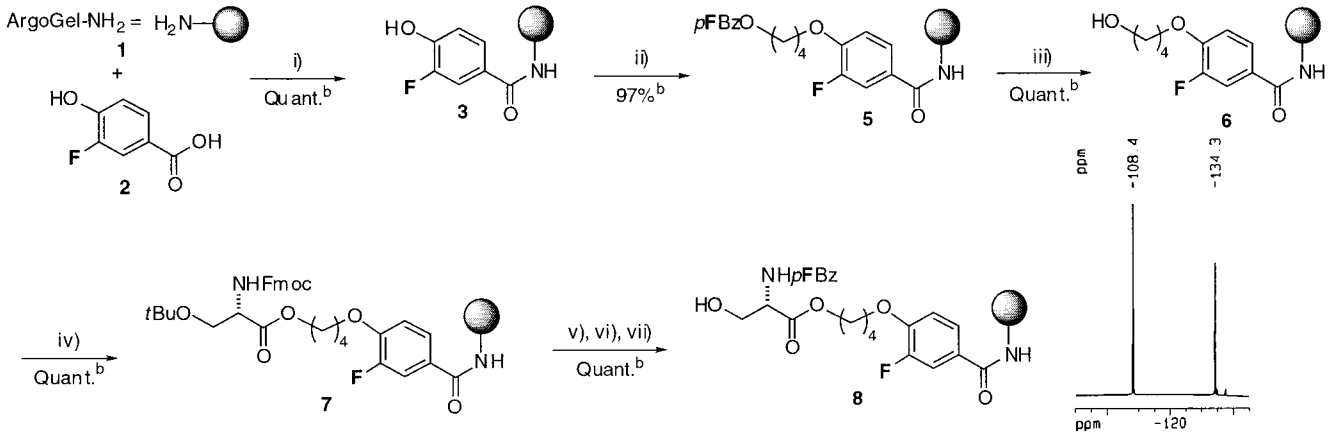
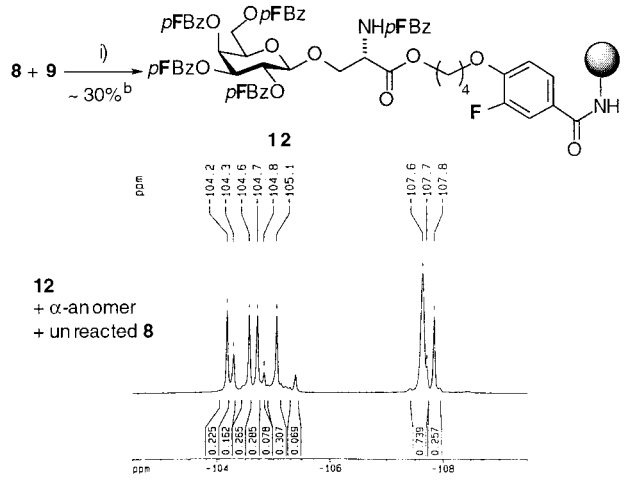
Scheme 1. Solid-Phase Synthesis of Linker and Serine-Based Acceptor[a]



[a] Conditions: (i) **2** (4 equiv), HOBt (6 equiv), DIC (3.9 equiv), DMF, 12 h, then 0.15 M NaOMe in $MeOH/CH_2Cl_2$, 2 h. (ii) $HO(CH_2)_4OpFBz$ (**4**, 15 equiv), DEAD (15 equiv), PPh_3 (15 equiv), −5 °C 3 h, then 0 °C 24 h. (iii) 50 mM NaOMe, $MeOH/CH_2Cl_2$, 3 h. (iv) FmocSer(*t*Bu)OH (4 equiv), MSNT (4 equiv), MeIm (3 equiv), CH_2Cl_2, 4 h. The coupling was repeated once. (v) 20% piperidine in DMF, 5 min. (vi) *p*-Fluorobenzoic acid (4 equiv), HOBt (6 equiv), DIC (3.9 equiv), DMF, then Ac_2O in CH_2Cl_2. (vii) TFA/H_2O (9:1), 3 h. [b]Yields are based on gel-phase ^{19}F NMR spectra. These were recorded in $CDCl_3$ with $CFCl_3$ (δ 0.00 ppm) as internal standard.

shown that insertion of a fluorine atom into the linker allows monitoring of coupling to and cleavage from the solid support.[4c,e] Since carbohydrate chemistry is normally carried out under acidic conditions a base-labile fluorinated linker (**6**, Scheme 1) was developed and used in this study. Synthesis of linker **6** started with coupling of **2** to the ArgoGel resin **1** (loading capacity, 0.38 mmol/g) to give **3** (Scheme 1). As judged by ^{19}F NMR spectroscopy[6] a few percent of the phenolic hydroxyl group in **3** was esterified by **2**. This side product was removed by treatment of resin **3** with methanolic sodium methoxide. Extension of resin **3** with 4-hydroxybutyl *p*-fluorobenzoate (**4**) under Mitsunobu conditions[7] furnished **5** in 97% yield, as estimated by integration of the resonances from the two fluorine atoms in **5**. In the subsequent step the *p*-fluorobenzoyl ester of **5** was cleaved with sodium methoxide in a mixture of methanol and dichloromethane to afford the resin bound linker **6** in quantitative yield. Fmoc-Ser(*t*Bu)-OH (4 equiv) was then coupled to **6** using 1-(2-mesitylenesulfonyl)-3-nitro-1,2,4-triazole (MSNT) as coupling reagent.[8] The fluorine resonance from product **7** was shifted 0.2 ppm upfield, as compared to **6**, which allowed the yield to be estimated to ~70%. Repeating the coupling under identical conditions pushed the conversion of **6** to **7** to completion. Cleavage of the Fmoc protective group with piperidine, followed by acylation of the liberated amino group with *p*-fluorobenzoic acid, introduced a second fluorine marker. Subsequent cleavage of the *tert*-butyl ether with trifluoroacetic acid/water furnished resin bound serine **8** in quantitative yield. The ^{19}F NMR spectrum of **8** displayed two distinct fluorine resonances (cf. Scheme 1).[9]

Scheme 2. Glycosylation Promoted by DMTST[a]



[a] Conditions: (i) **9** (3 equiv), DMTST (12 equiv), CH_2Cl_2, 20 h. [b]Yields are based on gel-phase ^{19}F NMR spectra. These were recorded in $CDCl_3$ with $CFCl_3$ (δ 0.00 ppm) as internal standard.

As outlined in Scheme 2 glycosylation of resin-bound acceptor **8** was first attempted with donor **9** (3 equiv, Figure 1) under promotion by dimethyl(methylthio)sulfonium triflate (DMTST).[10] Inspection of the ^{19}F NMR spectrum of the resin-bound product indicated that both stereoselectivity and

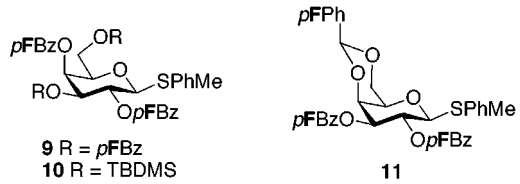
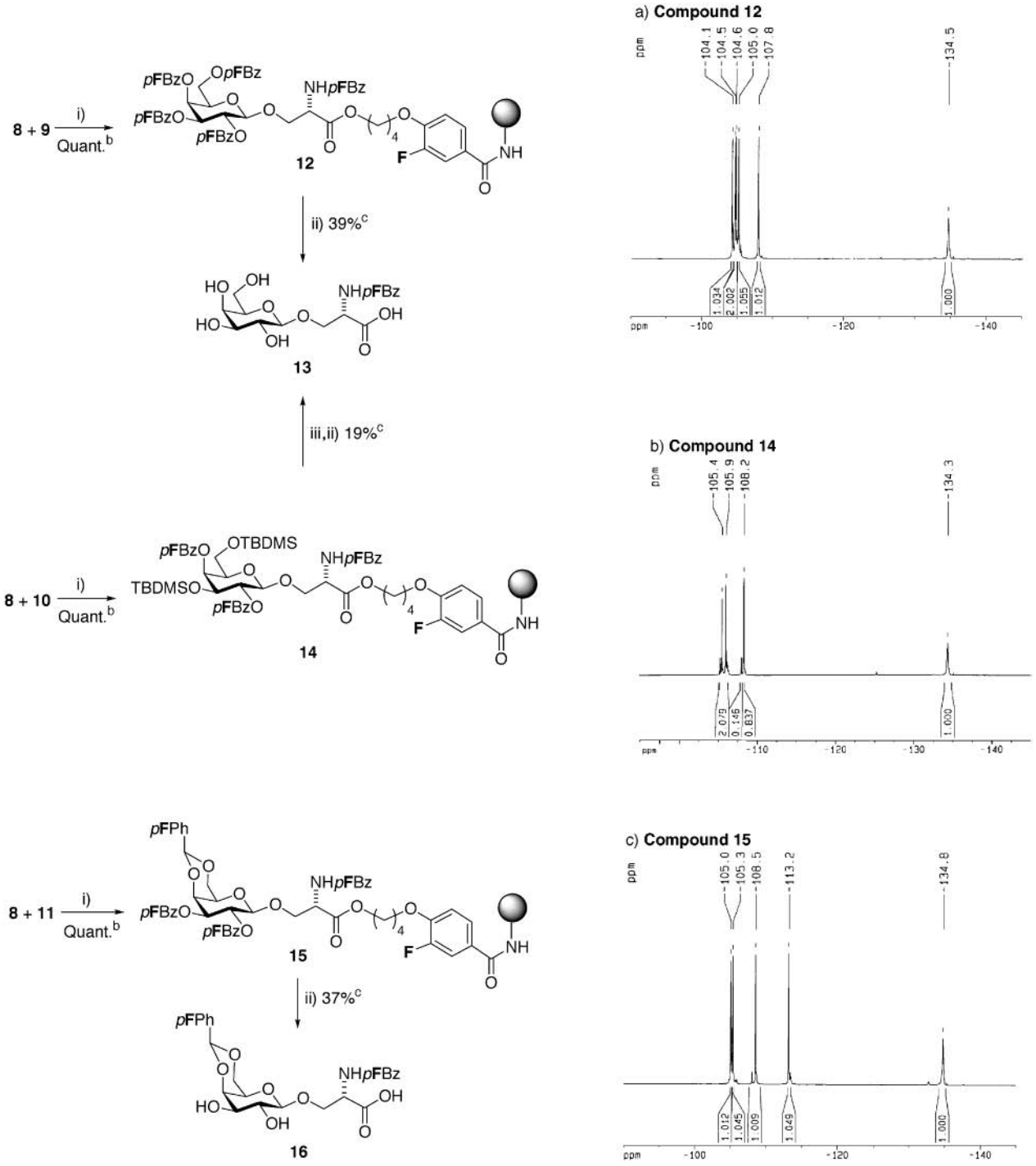


Figure 1.

Scheme 3. Glycosylation of Resin **8** with Different Galactosyl Donors[a]



[a] Conditions: (i) **9**, **10**, or **11** (5 equiv), NIS (5 equiv), TfOH (0.15 equiv), CH_2Cl_2, 3 h. (ii) 40 mM LiOH in H_2O/THF 3:1, 2 h. (iii) TFA/H_2O 9:1, 4 h. [b]Yields are based on gel-phase ^{19}F NMR spectra. These were recorded in $CDCl_3$ with $CFCl_3$ (δ 0.00 ppm) as internal standard. [c]Isolated yields are based on the capacity of the ArgoGel-NH_2 resin.

yield were unsatisfactory. The resonance at −107.6 ppm originates from the fluorobenzoyl amide in unreacted **8**, whereas the resonances at −107.8 and −107.7 ppm were assumed to be derived from **12** and the corresponding α-glycoside, respectively, suggesting a yield of 25−35%. The resonances at −104 to −105 ppm come from the fluorobenzoyl groups on the galactose moiety. Presumably, the weaker fluorobenzoate signals (−104.3, −104.8, and −105.9 ppm) originate from the α-anomer, indicating the α/β ratio to be ∼1:3.

When glycosylation of resin-bound **8** with **9** (5 equiv) was performed using the more powerful promoter system *N*-iodosuccinimide (NIS) and triflic acid (TfOH),[11] only one product was formed in quantitative yield according to ^{19}F

NMR spectroscopy.[12] This was assumed to be β-glycoside **12** (Scheme 3, spectrum a). When 3 equiv of donor **9** was employed in the glycosylation, **12** was obtained in ~68% yield as determined by integration of the gel-phase ^{19}F NMR spectrum. All four fluorine signals from the benzoyl groups in the galactose part were well separated, demonstrating the resolution of this analytical tool. Treatment of **12** with a solution of LiOH in a mixture of water and THF removed the benzoyl groups and led to release of **13** from the solid support (39% isolated yield based on resin capacity). The coupling constant between H1 and H2 of the galactose moiety ($^3J_{1,2}$ = 7.6 Hz) confirmed the β-glycosidic linkage to serine in **13** and thereby in **12**. These results encouraged us to investigate glycosylation of **8** with donors **10** and **11** (Figure 1), which have protective group patterns different than those of **9**. Glycosylation of **8** with **10** (5 equiv) promoted by NIS and TfOH afforded the disilylated galactoside **14** (Scheme 3, spectrum b). The doubling of the resonances from the fluorobenzoyl groups (−105 to −106 ppm), as well as the fluorobenzoyl amide (−108 ppm), suggested that the desired **14** was contaminated by the corresponding α-glycoside (~15%). Cleavage of the silyl groups of **14** with TFA/H_2O followed by saponification with LiOH, as for **12**, allowed **13** to be isolated in 19% yield, thus confirming that the β-glycoside **14** had been formed as the major product. Finally, reaction of **8** with thiogalactoside **11** (5 equiv) under activation with NIS and TfOH was achieved in quantitative yield to give **15** (Scheme 3, spectrum c). The three new fluorine signals in the ^{19}F NMR spectrum of **15** originate from the fluorobenzylidene protective group (−113.2 ppm) and the two benzoyl protective groups (−105.0 and −105.3 ppm). Debenzoylation and cleavage with LiOH gave the expected β-glycoside **16** (37% isolated yield, $^3J_{1,2}$ = 6.9 Hz).

Solid-phase carbohydrate synthesis is still in its infancy, and techniques for monitoring reactions "on bead" can be expected to have a large impact on development of new methology. The present work has demonstrated that gel-phase ^{19}F NMR spectroscopy, in combination with fluorinated protective groups, is useful for optimization of glycoside synthesis on solid support. For instance, thioglycosides were found to be excellent glycosyl donors for coupling to the hydroxyl group of serine linked to an ArgoGel resin, and promotion by NIS and TfOH was superior to use of DMTST. ^{19}F Resonances from *ortho*-, *meta*-, and *para*-fluorinated variants of benzyl ethers, benzoates, and benzylidene acetals attached to monosaccharides are spread over a wide spectral range (−104 to −121 ppm).[13] In addition, ^{19}F chemical shifts of compounds bound to solid supports closely match those of soluble references.[4a,14] Use of saccharide building blocks having fluorine-labeled protective groups, in combination with fluorinated linkers, should therefore allow convenient monitoring of solid-phase oligosaccharide synthesis without encountering problems with chemical shift overlap. Consequently, gel-phase ^{19}F NMR spectroscopy should find wide applications in solid-phase oligosaccharide synthesis.

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Supporting Information Available: Spectral data and experimental procedures for compounds **3**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) All gel-phase ^{19}F NMR spectra are ^{1}H-decoupled and were obtained after insertion of a suspension of the resin in a standard NMR tube. Spectra were recorded in a couple of minutes on an ordinary NMR instrument.

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(9) In the spectrum of **8** the resonance at −134.3 ppm originates from the fluorine atom in the phenolic linker, whereas the resonance at −108.4 ppm is derived from the *p*FBz group.

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(12) The fluorine resonances coincide with the set of major product resonances obtained in the glycosylation with DMTST (Scheme 2).

(13) *o*-, *m*-, and *p*-F-benzyl ethers attached to methyl α-D-glucopyranoside had the following ^{19}F chemical shifts: −119.0 to −119.4, −113.5 to −113.8, and −114.9 to −115.4 ppm, respectively. *o*-, *m*-, and *p*-F-perbenzoates of D-glucose appeared at −109.0 to −110.0, −111.6 to −112.6, and −103.9 to −105.5 ppm, respectively, whereas *o*-, *m*-, and *p*-F-benzylidene acetals on 4-methylphenyl 1-thio-β-D-galactopyranoside had their ^{19}F resonances at −121.1, −113.4 and −112.7 ppm, respectively.

(14) This was confimed by comparison of the ^{19}F chemical shifts of donors **9**−**11** with those of resins **12**, **14**, and **15** (cf. Supporting Information).