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Gel-phase ^{19}F NMR spectral quality for resins commonly used in solid-phase organic synthesis; a study of peptide solid-phase glycosylations

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Received 31st March 2004, Accepted 7th May 2004

First published as an Advance Article on the web 25th May 2004

The spectroscopic properties for seven different commercial resins used in solid-phase synthesis were investigated with ^{19}F NMR spectroscopy. A fluorine-labeled dipeptide was synthesized on each resin, and the resolution of the ^{19}F resonances in $CDCl_3$, DMSO-d_6, benzene-d_6 and CD_3OD were measured with a conventional NMR spectrometer, *i.e.* without using magic angle spinning. In general, resins containing poly(ethylene glycol) chains (ArgoGel, TentaGel and PEGA) were found to be favorable for the ^{19}F NMR spectral quality. Three serine containing tri-, penta-, and heptapeptides were then prepared on an ArgoGel resin functionalized with a fluorine-labeled linker. The resin bound peptides were glycosylated utilizing a thiogalactoside glycosyl donor carrying fluorine-labeled protective groups. Monitoring of the glycosylations with gel-phase ^{19}F NMR spectroscopy allowed each glycopeptide to be formed in ~80% yield, using a minimal amount of glycosyl donor (3 × 2 equivalents). In addition, it was found that the glycosylation yields were independent of peptide length.

Introduction

Since the pioneering work of Merrifield,[1] solid-phase synthesis has been widely applied for preparation of oligopeptides[2–5] and oligonucleotides[6] on an individual basis or in the form of libraries.[7,8] In recent years the introduction of combinatorial chemistry and parallel synthesis has renewed the interest in solid-phase synthesis of, for instance, oligosaccharides,[9–13] glycopeptides[14–17] and especially for parallel synthesis of small "drug-like" molecules.[7,8,18–21] In essence, the advantages with solid-phase synthesis compared to traditional solution-phase synthesis is that excess of reagents can be used to drive reactions to completion, and that reagents and soluble by-products can simply be removed by filtration and washing of the polymeric support. These properties render solid-phase synthesis suitable for automation, which is important for speeding up the discovery process of new biologically active compounds.[22–24] A major limitation when performing reactions on solid phase is the difficulties in analyzing the outcome of complex reactions when the product is still attached to the polymeric support. In order to avoid problematic purifications of the final product, it should be possible to analyze formation and accumulation of side-products in each synthetic step.

IR spectroscopy[25] and MALDI-TOF mass spectrometry in combination with a color test have in some cases been successfully employed for quantification of solid-phase reactions.[26] Ideally, analytical NMR spectroscopic methods employed in solution-phase synthesis would constitute a powerful tool for monitoring solid-phase synthesis directly on the solid support.[27] Therefore approaches such as high resolution magic-angle-spinning (HR-MAS) NMR spectroscopy and gated decoupling ^{13}C NMR spectroscopy have been utilized to quantify reactions directly on the solid support.[28–32] However, these methods are somewhat restricted by cost and the requirement of specialized equipment. Identification of products may also be problematic if the reactions are incomplete or if large amounts of by-products have been formed. To address this issue, ^{19}F NMR spectroscopy has been developed as a versatile and straightforward method for monitoring solid-phase synthesis.[33–52] ^{19}F NMR spectroscopy has several advantages, including the lack of interfering background signals, high sensitivity (the natural abundance of ^{19}F is 100%), and distribution of ^{19}F resonances over a wide spectral range as a result of the high polarizability of the ^{19}F nucleus. Thus, analysis of reactions directly on the solid phase by using a fluorinated linker as an internal standard in combination with fluorinated building blocks,[43,48] or building blocks carrying fluorine-labeled protective groups,[44,50,51] provides easily interpreted spectra. These contain both quantitative and qualitative information, including yield and stereoselectivity, and are obtained in a couple of minutes with a conventional NMR spectrometer.

Although solid-phase synthesis is in common use in organic chemistry, the understanding of the physicochemical and spectroscopic properties of the resins used in synthesis remains somewhat limited.[53–56] HR-MAS ^{1}H NMR spectroscopy has been used to study the spectrum quality for commercial resins loaded with aspartic acid,[30] and in a comparison of PEG-grafted resins and PEG-cross-linked polymers.[57] At present, no other NMR techniques have been used to examine the spectroscopic properties of commonly used resins.

In view of the advantages that gel-phase ^{19}F NMR spectroscopy provides for monitoring reactions directly on the solid support, this article describes: 1) a study of the influence of resin structure, tether length, and solvent upon the ^{19}F NMR spectral quality for seven commercially available and commonly used resins and, 2) a study of *O*-glycosylation of resin-bound peptides of varying lengths with a thiogalactoside donor.

Results and discussion

In order to study the influence of the resin on the spectral quality, a *N*-acylated dipeptide was synthesized on seven commercially available amino-functionalized polymeric supports, *i.e.* polystyrene (**1**), ArgoPore (**2**), ArgoGel (**3**), TentaGel (**4**), NovaGel (**5**), PEGA (**6**) and SPAR-50 (**7**) (Fig. 1). The ^{19}F NMR spectrum of each peptide-resin was then recorded in four different solvents; $CDCl_3$, DMSO-d_6, benzene-d_6 and CD_3OD (Fig. 2). The resin-bound dipeptides **1**–**7** all contained four fluorine atoms as analytical markers which allowed comparison of the spectral qualities.[58] The two fluorine resonances from the terminal *o*,*p*-difluorobenzoyl group

DOI: 10.1039/b404802d

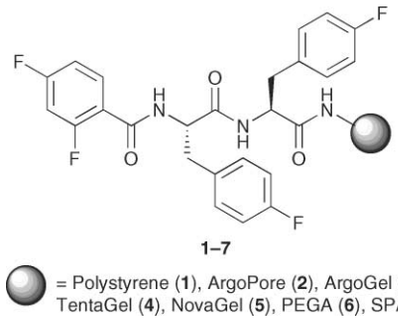


Fig. 1 Fluorinated dipeptides **1**–**7** prepared on seven commercial amino functionalized resins.

Table 1 Mean line widths (Hz) of the two fluorine resonances derived from the terminal *o*,*p*-difluorobenzoyl group of resins **1**–**7**[a]

Resin	$CDCl_3$	DMSO-d_6	Benzene-d_6	CD_3OD
Polystyrene **1**	nm	78	nm	nm
ArgoPore **2**	nm	nm	nm	nm
ArgoGel **3**	37	40	48	100
TentaGel **4**	27	39	40	102
NovaGel **5**	nm	81	nm	nm
PEGA **6**	38	35	116	80
SPAR-50 **7**	nm	148	nm	244

[a] Mean value of the line widths (in Hz) measured at half peak height. nm = not measured.

appeared between −103 and −106 ppm and between −108 and −109 ppm, respectively. The ^{19}F signals derived from the two *p*-FPhe residues appeared at −116 ppm. Comparison of the spectral quality for resins **1**–**7**, included evaluation of the separation between *p*-FPhe signals and the determination of the ^{19}F NMR line widths of the two fluorine signals derived from the terminal *o*,*p*-difluorobenzoyl group (Table 1).

With polystyrene resin **1**, which has 1% of divinyl benzene (DVB) cross-linking, a ^{19}F NMR spectrum of moderate quality could be generated only in DMSO-d_6 (Fig. 2). This classical Merrifield resin is frequently used for solid-phase synthesis due to its mechanical stability and high loading capacity. In general, the spectroscopic and physicochemical properties of this "gel-type" resin are highly dependent on the degree of DVB cross-linking,[59] in which a low degree of cross-linking favors the molecular mobility of the resin, and thus improves the spectral quality. This resin is available with 1 or 2% of DVB cross-linking to ensure adequate mechanical stability, swelling, and diffusion rate of the reagents in a wide range of solvents.[56,59,60] Although the spectral quality of the polystyrene resin is moderate, it has been successfully employed to monitor S_NAr reactions[34,61] and formation of tertiary amines[46] directly on the resin with gel-phase ^{19}F NMR spectroscopy. The more rigid ArgoPore resin **2**, which is based on a highly cross-linked macroporous polystyrene framework, did not generate an acceptable spectrum in any of the solvents (Fig. 2). The regional molecular mobility of this resin is restrained by the high DVB cross-linking, and thus the quality of the ^{19}F NMR spectra is low.

The ArgoGel resin **3** and TentaGel resin **4** produced high quality ^{19}F NMR spectra in $CDCl_3$, DMSO-d_6 and benzene-d_6, whereas use of CD_3OD resulted in spectra of moderate quality (Fig. 2). ArgoGel and TentaGel resins are polystyrene based resins that are grafted with poly(ethylene glycol) chains (PEG) on the hydrophobic core. The polystyrene core provides the resins with mechanical stability whereas the flexible PEG grafts results in excellent swelling properties in a wide variety of solvents. This type of resin swells well in both polar and non-polar solvents, and importantly, the organic moiety attached to the resins possesses a high molecular mobility. As a consequence both spin lattice relaxation and the chemical shift anisotropy are reduced and spectra with high quality are obtained in a wide variety of solvents.[30] The line-widths of the

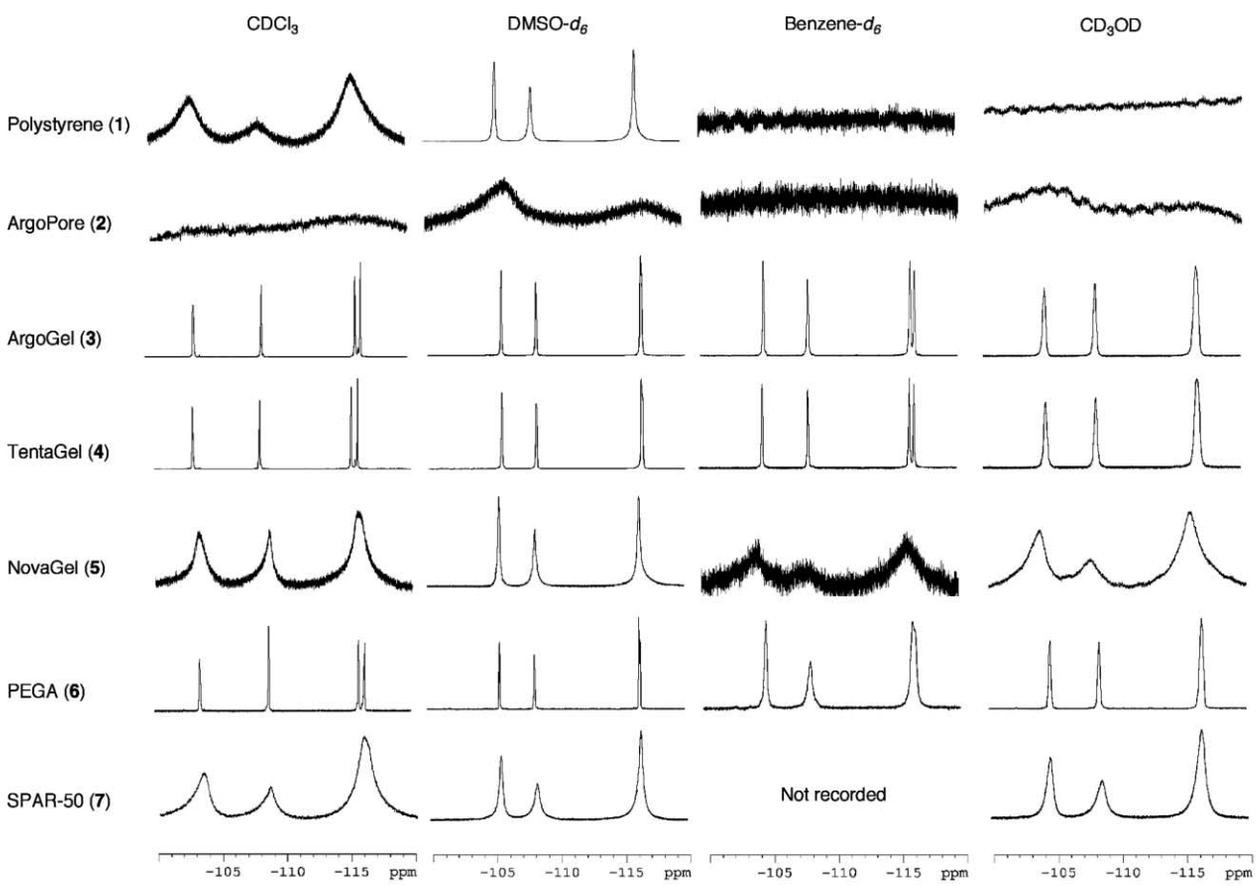
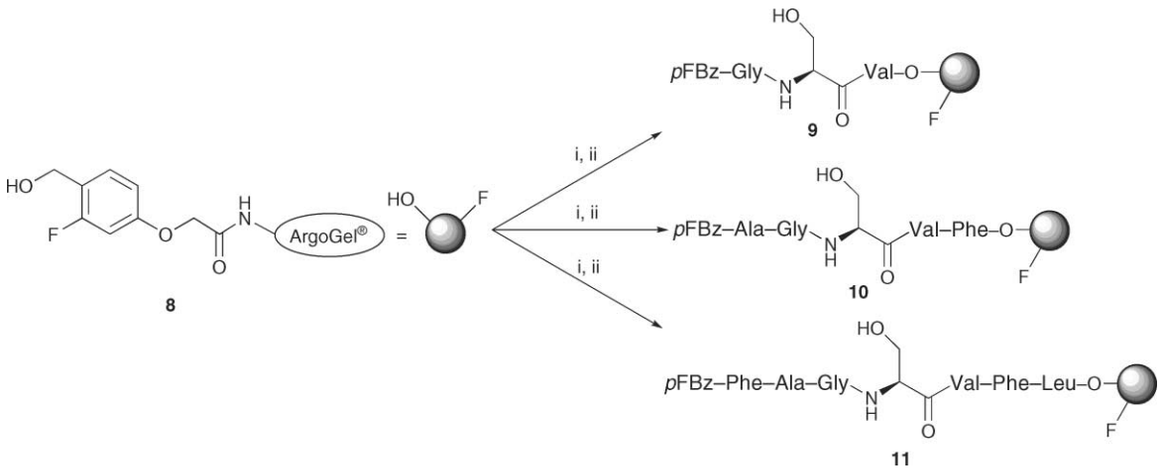


Fig. 2 ^{19}F NMR spectra of resins **1**–**7** recorded in $CDCl_3$, DMSO-d_6, benzene-d_6 and CD_3OD.



Scheme 1 Solid-phase synthesis of peptide resins **9**–**11** on resin **8**. *Reagents and conditions:* i) Fmoc-Ala-OH, Fmoc-Phe-OH or Fmoc-Leu-OH (4 equiv.), MSNT (4 equiv), MeIm (3 or 4 equiv.), CH_2Cl_2, RT. ii) Peptide synthesis according to the Fmoc protocol. MSNT = 1-(mesitylenesulfonyl)-3-nitro-1,2,4-triazole, MeIm = 1-methylimidazole.

two ^{19}F signals that originate from the terminal *o*,*p*-difluorobenzyl group revealed that the best spectra for both resins were obtained in $CDCl_3$, closely followed by DMSO-d_6, benzene-d_6 and CD_3OD (Table 1). Moreover, the ^{19}F resonances from the two *p*-FPhe residues were completely separated in both $CDCl_3$ and benzene-d_6. After a closer inspection, it could be concluded that the TentaGel resin **4** generally displayed a somewhat narrower line width than the ArgoGel resin **3** (Table 1).

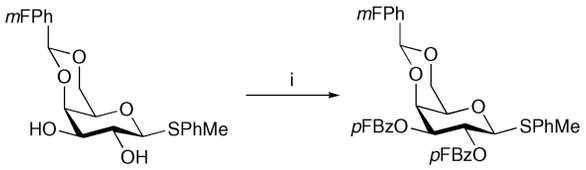
NovaGel is also a polystyrene PEG grafted copolymer that exhibits a loading capacity similar to the Merrifield resin. In addition, this resin has similar swelling characteristics as the TentaGel and ArgoGel resins. However, the location of the functional groups of the NovaGel resin is not at the end of the PEG chain, as for the TentaGel and ArgoGel resins, instead they are situated on the hydrophobic polystyrene core, just as for the Merrifield resin. Hence, the organic moiety attached to the resin will not possess the same molecular mobility as the TentaGel and Argogel resins, and accordingly, the spectral quality of the NovaGel resin **5** was in general low (Fig. 2). However, recording the spectrum in DMSO-d_6, produced a ^{19}F NMR spectrum of moderate quality comparable to that of polystyrene resin **1** (Table 1).

The polymeric supports PEGA and SPAR-50 are acrylamide based polymers that mainly have found uses within peptide synthesis and for enzymatic applications, due to their excellent swelling properties in both polar and non-polar solvents. The mechanical stability of this type of resin is however somewhat low since it lacks the stabilizing polystyrene core. PEGA is a poly-acrylamide support that is cross-linked with PEG chains, which gives the resin good spectroscopic and swelling properties in a wide range of solvents. As expected, the PEGA polymer **6** indeed produced ^{19}F NMR spectra of high resolution in both $CDCl_3$ and DMSO (Fig. 2, Table 1). However, four major resonances arises from one of the *p*-FPhe residues in the spectrum, these presumably originates from different stereoisomers of the secondary amine functionality and inhomogeneous distribution of the functional sites. Thus, only the *p*-FPhe that is attached to the resin-amino functionality is affected due to its close proximity to the stereocentre. Furthermore, just as for TentaGel and ArgoGel, the spectral quality decreased when recording in CD_3OD. The spectral quality for resin **6** was also poor when using benzene-d_6 as solvent. SPAR-50 is a poly-acrylamide polymer with large pore size, which is particularly suitable for enzymatic applications since the large pores facilitate the entrance of the enzyme into the polymer matrix. In contrast to PEGA, this polymer does not contain PEG tethers, and as expected, the molecular mobility of this polymer is low compared to PEGA. Hence, ^{19}F NMR spectra of low quality were obtained. This is consistent with a previous study, which used HR-MAS ^{1}H NMR spectroscopy to investigate the spectral quality of commercial resins.[30] These results imply that the influence from the PEG tether is the most important factor affecting the spectral quality, closely followed by the rigidity of the resin structure, *i.e.* the degree of cross-linking.

Thus, the ArgoGel and TentaGel resins have good qualities for monitoring solid-phase organic synthesis with gel-phase ^{19}F NMR spectroscopy. In addition, PEG containing resins exhibit excellent swelling properties, and high reaction rates for polar and ionic reagents.[56,57,60] Therefore we chose to evaluate solid-phase peptide glycosylations with the ArgoGel resin, since this resin displays better loading capacity (0.4 to 0.5 mmol g^{-1}) than TentaGel (0.2 to 0.3 mmol g^{-1}),[62] which is synthetically advantageous.

At present the most reliable method for synthesis of glycopeptides, is by solid-phase stepwise assembly of amino acids and glycosylated amino acid building blocks.[14–16] However, a strategy based on attachment of a carbohydrate moiety to free hydroxyl groups of serine, threonine, tyrosine, hydroxyproline or hydroxylysine residues in a preformed peptide is very appealing, since it offers possibilities to create diversity using different glycosyl donors at a late stage in the synthesis. Attempts to find protocols towards direct *O*-glycosylation of resin bound peptides are fairly rare and initial attempts using polystyrene as solid support gave the target glycopeptides in very low yields.[63–65] However, a few successful glycosylations on a polyoxyethylene-polyoxypropylene resin (POEPOP) by using trichloroacetimidates as glycosyl donor have been reported.[66,67] The difficulties encountered in glycosylation of peptides have been suggested to be due to the low solubility of peptides under the conditions used for glycosylation and/or adsorption of the Lewis acid by the peptide-amide functionalities.[68]

In an effort to find conditions for glycosylation of resin-bound peptides, tripeptide **9**, pentapeptide **10** and heptapeptide **11** were prepared on the linker-loaded ArgoGel resin **8** (Scheme 1). A fluorinated "Wang-type" linker was used to fulfil the analytical requirements and the ^{19}F signal derived from linker served as an internal standard throughout the syntheses.[36] In addition, the linker-peptide bond is essentially stable under the conditions used in glycosylations and peptide synthesis, but is readily cleaved under nucleophilic conditions or by acidolysis with trifluoroacetic acid (TFA).[36,38] Thioglycoside **13** (Scheme 2) was employed as glycosyl donor, since thioglycosides can be conveniently activated by soft Lewis acids or converted to other glycosyl donors.[69] Thiogalactoside building block **13** was prepared from 4,6-*O*-*m*-fluorobenzylidene protected thiocresyl galactoside **12**[51] in 98% yield by treatment with *p*-fluorobenzoyl chloride in pyridine (Scheme 2). Thioglycosides have recently been successfully employed in solid-phase



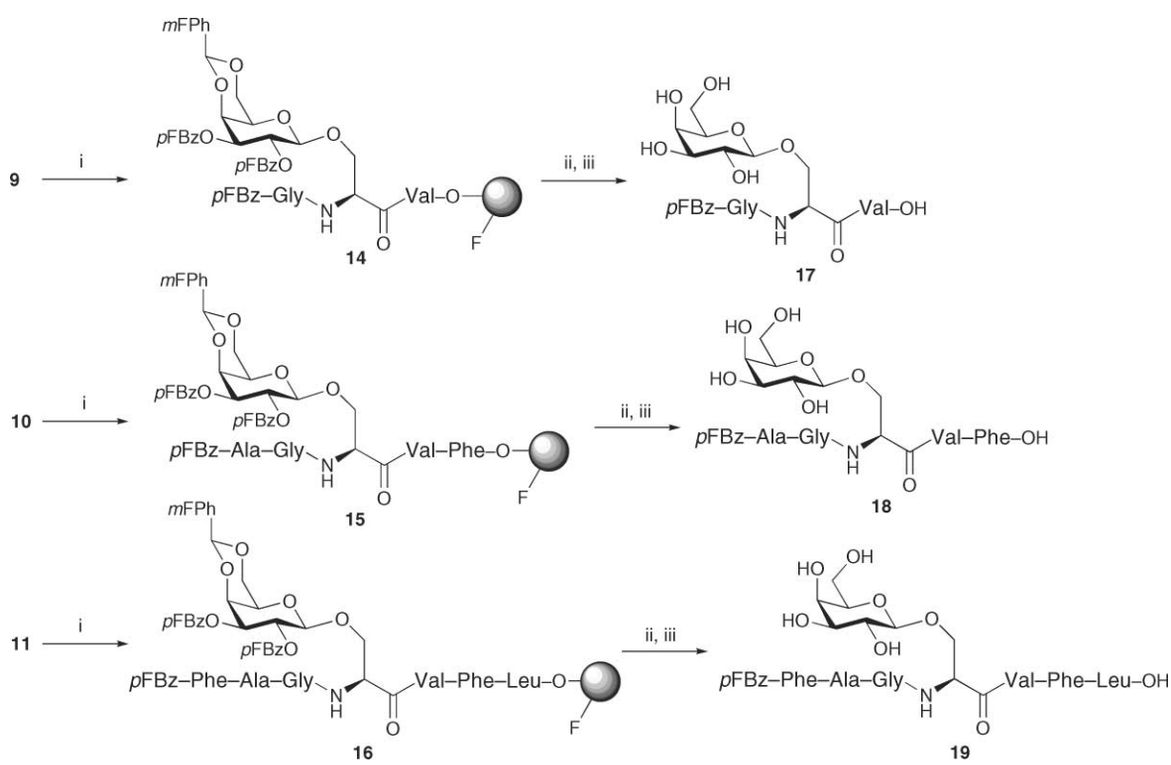
12 13

Scheme 2 Preparation of thiogalactoside donor **13**. *Reagents and conditions:* i) **12**, *p*FBzCl (2.6 equiv.), pyridine, RT.

glycosylations,[44,50,51,70–72] and it has been shown that *N*-iodosuccinimide (NIS) in combination with a catalytic amount of trifluoromethanesulfonic acid (TfOH) is an excellent promoter system.[73,74] Furthermore, in a previous study NIS was found to be superior to dimethyl(methylthio)sulfonium triflate (DMTST).[44]

The parallel solid-phase glycosylations of peptides **9**–**11** with thioglycoside **13** (2 equivalents) were carried out in CH_2Cl_2 under promotion by NIS and a catalytic amount of TfOH for two hours at room temperature (Scheme 3). Comparison of the integrals of the ^{19}F NMR signals from the carbohydrate protective groups with the ^{19}F resonances derived from the linker and the *N*-terminal *p*-fluorobenzoyl amide group of resins **14**–**16** revealed ~30% glycosylation of all the three peptides. Repeating the glycosylation once under identical conditions increased the yield to ~60% and after a third glycosylation the glycopeptides **14**–**16** were formed in ~80% yields (Fig. 3). Moreover, since the ^{19}F signals from each protective group appeared as a uniform singlet, it was assumed that only the desired β-galactosidic linkage was formed. Interestingly, the resin bound tripeptide **9** and glycosylated tripeptide **14** gave high quality ^{19}F NMR spectra in $CDCl_3$, whereas the penta- and heptapeptides **10** and **11** and the corresponding glycopeptides **15** and **16** furnished inadequate spectra in both $CDCl_3$ and benzene-d_6. This observation was assumed to originate from aggregation of these resin bound peptides and glycopeptides due to the low polarity of $CDCl_3$ and benzene-d_6.[68] However, when the solvent was changed to DMSO-d_6, the aggregation was thwarted and well resolved ^{19}F NMR spectra were obtained both for the resin bound peptides **10** and **11** and glycopeptides **15** and **16** (Fig. 3). These results strongly imply that aggregation of the resin bound peptides is not pivotal for the outcome of the glycosylation, when the reaction is carried out with a thioglycoside under activation by NIS and TfOH. The yields of the glycopeptide **14**–**16** were all the same after each consecutive glycosylation, although the glycosylations were performed in CH_2Cl_2, which is a less polar solvent than $CDCl_3$. Initially, saponification with aqueous LiOH in THF was used to cleave glycopeptide **14** from the solid phase. Careful monitoring of the cleavage of resin **14** with ^{19}F NMR spectroscopy and LC-MS analysis of the reaction mixture revealed that the hydrolysis was slow and that β-elimination of the carbohydrate moiety from the peptide was a severe problem. This was circumvented by acidolysis of resin **14**–**16** with TFA at 60 °C,[38] which resulted in cleavage of the glycopeptides from the linker with simultaneous removal of the benzylidene group (Scheme 3). Subsequently, the remaining *p*-fluorobenzoyl groups were saponified with a catalytic amount of LiOH (20 mM) in methanol without β-elimination.[75] This gave glycopeptides **17**, **18** and **19** in 27, 26 and 29% overall yields, respectively, based on the initial loading capacity of the amino ArgoGel resin (0.45 mmol g^{-1}). The β-galactosidic linkages of the isolated glycopeptides **17**–**19** were confirmed by the coupling constant between the H1 and H2 in the galactoside moieties ($^3J_{1,2} \approx 7$ Hz). However, coupling of the first amino acid of each of the three peptides with resin **8** resulted in ~20 to 35% epimerization, as determined by ^1H NMR spectroscopy of the isolated glycopeptides **17**–**19**. The epimerization could not be detected in the gel-phase ^{19}F NMR spectra, probably due to that the distance between the fluorine atom in the linker and the stereogenic center of the amino acid is too large. This indicates that proximity between a stereocenter and a fluorine label is required to obtain stereochemical information.

In conclusion, the ^{19}F NMR spectral quality for seven commercial resins were examined in four frequently used NMR solvents. It was found that flexible PEG-tethers grafted on a



Scheme 3 Solid-phase glycosylations of peptides **9**–**11** with thiogalactoside **13** followed by cleavage of glycopeptides **14**–**16** from the solid support and removal of the carbohydrate protective groups. *Reagents and conditions:* i) **13** (2 equiv.), NIS (2 equiv.), TfOH (0.2 equiv.), CH_2Cl_2, RT, the reactions were repeated twice. ii) TFA/H_2O (9/1, v/v), 60 °C. iii) LiOH (20 mM in H_2O/MeOH, 1/4, v/v), RT. NIS = *N*-iodosuccinimide, TfOH = trifluoromethanesulfonic acid.

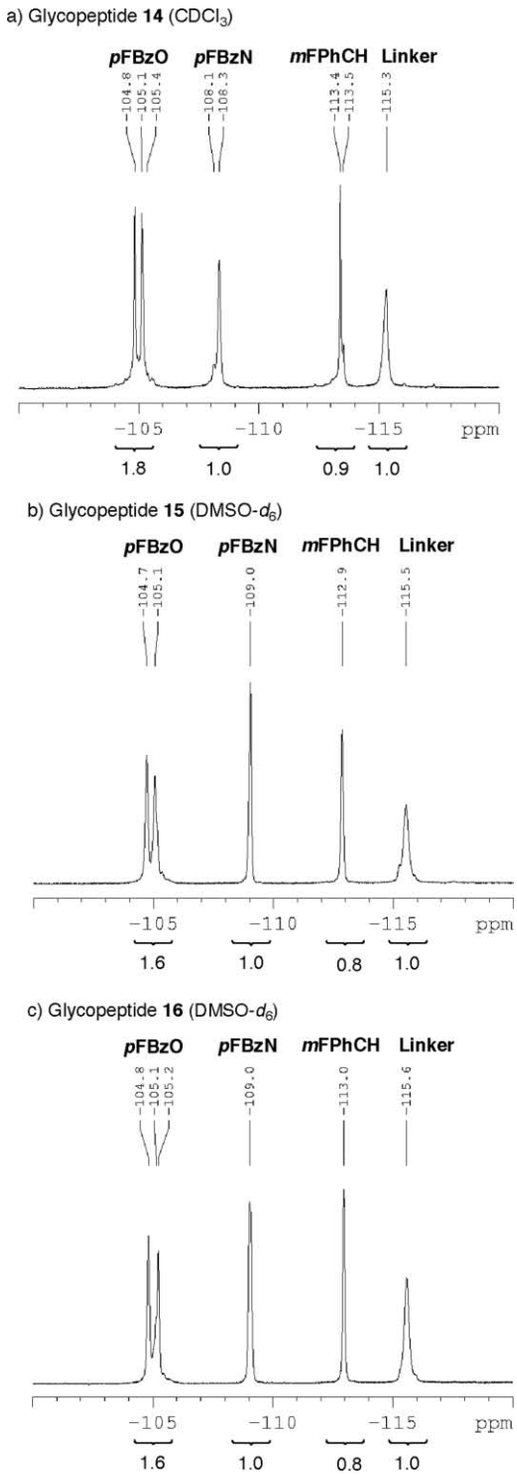


Fig. 3 ^{19}F NMR spectra of resin bound glycopeptides **14**–**16** after three identical glycosylations; (a) 90% conversion of resin **9**, (b) 80% conversion of resin **10**, (c) 80% conversion of resin **11**.

hydrophobic polystyrene core, or cross-linked into a polyacrylamide polymer, were essential to obtain good NMR spectroscopic properties. Glycosylations of resin-bound tri-, penta- and heptapeptides on an ArgoGel resin were successfully performed by employing a thiogalactoside glycosyl donor protected with fluorine-labeled protective groups. In this study it was found that neither the length nor aggregation of the peptides influenced the yield of the glycosylations. Analysis of the resin bound glycopeptides with gel-phase ^{19}F NMR spectroscopy, revealed that the yields could be increased to ~80% after three identical glycosylations that consumed in all six equivalents of glycosyl donor. Moreover, by using a saccharide building block carrying fluorine-labeled protective groups in combination with a fluorinated linker, both the anomeric purity and yield of the peptide glycosylations could be extracted from the ^{19}F NMR spectra.

Experimental

General methods and materials

All reactions were carried out at room temperature under an inert nitrogen atmosphere using dry, freshly distilled solvents. CH_2Cl_2 was distilled from calcium hydride. Organic solutions were dried over Na_2SO_4 before being concentrated. TLC was performed on Silica Gel F_{254} (Merck) and detection was carried out by examination under UV light and by charring with 10% sulfuric acid. Flash column chromatography was performed on Silica Gel (Matrex, 60 Å, 35–70 μm, Grace Amicon). Preparative HPLC separations were performed on a Beckman System Gold HPLC, using a Kromasil C-8 column (250 × 20 mm, 5 μm, 100 Å) with a flow rate of 11 mL min^{-1} and detection at 214 nm. Analytical HPLC was performed on a Beckman System Gold HPLC, using a Kromasil C-8 column (250 × 4.6 mm, 5 μm, 100 Å) with a flow rate of 1.5 mL min^{-1} and detection at 214 nm. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-400 or ARX-500 spectrometer for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.26 ppm), $CDCl_3$ (δ_C 77.0 ppm) as internal standard], [D_6]DMSO [residual [D_5]DMSO (δ_H 2.50 ppm), [D_6]DMSO (δ_C 39.51 ppm) as internal standard], or CD_3OD [residual CD_2HOD (δ_H 3.35 ppm), CD_3OD (δ_C 49.0 ppm) as internal standard] at 300 K. First order chemical shifts and coupling constants were determined from one-dimensional spectra and proton resonances were assigned from COSY, TOCSY, NOESY and HETCOR experiments. Proton resonances that could not be assigned are not reported. Proton resonances from the minor isomeric by-product of glycopeptide **17**–**19** are not reported. ^{13}C resonances that originated from the fluorinated protective groups were split by J_{C-F} coupling and therefore signals downfield from 110.0 ppm are not reported. Proton decoupled gel-phase ^{19}F NMR spectra were recorded with a Bruker DRX-400 spectrometer for resin suspensions in $CDCl_3$[$CFCl_3$ (δ_F 0.00 ppm) as internal standard] at 300 K. Two peaks appear in the spectra at around 0.00 ppm, one resonance originates from $CFCl_3$ inside the polymer the other resonance from $CFCl_3$ outside the polymer. The peak with the highest chemical shift was used as internal standard. Optical rotation was measured with a Perkin-Elmer 343 polarimeter and is given in 10^{-1} deg cm^2 g^{-1}. Mass spectra for glycopeptide **18** was recorded on a Waters Micromass ZQ using positive electrospray ionization (ES+). High resolution mass spectra were recorded on a JEOL SX102 A mass spectrometer. Ions for FABMS were produced by a beam of xenon atoms (6 keV) from a matrix of glycerol and thioglycerol.

Resin 1–7

Peptide syntheses were performed manually in a mechanically agitated reactor on commercial amino functionalized resins with Fmoc-*p*-fluorophenylalanine and *o*,*p*-difluorobenzoic acid as building blocks and *N*,*N*′-diisopropylcarbodiimide and 1-hydroxybenzotriazole according to previously reported procedures to give resins **1**–**7**;[76] resin **1** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −103, −109, −116; ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.6, −108.4, −116.5; resin **2** had: ^{19}F NMR (376 MHz, DMSO-d_6) δ −106, −117; resin **3** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.7, −108.9, −116.0, −116.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.7, −108.4, −116.5, −116.6; ^{19}F NMR (376 MHz, benzene-d_6) δ −104.9, −108.3, −116.3, −116.6; ^{19}F NMR (376 MHz, CD_3OD) δ −104.7, −108.7, −116.5; resin **4** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.7, −108.9, −116.0, −116.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.7, −108.4, −116.5, −116.6; ^{19}F NMR (376 MHz, benzene-d_6) δ −104.9, −108.4, −116.3, −116.7; ^{19}F NMR (376 MHz, CD_3OD)

δ −104.8, −108.7, −116.6; resin **5** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −103, −109, −116; ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.6, −108.4, −116.6; ^{19}F NMR (376 MHz, benzene-d_6) δ −104, −116; ^{19}F NMR (376 MHz, CD_3OD) δ −104, −108, −116; resin **6** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −103.5, −108.9, −115.9, −116.2, −116.3, −116.4, −116.4; ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.6, −108.3, −116.4, −116.5; ^{19}F NMR (376 MHz, benzene-d_6) δ −104.8, −108.3, −116.3; ^{19}F NMR (376 MHz, CD_3OD) δ −104.7, −108.5, −116.5; resin **7** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −103, −109, −116; ^{19}F NMR (376 MHz, DMSO-d_6) δ −105.5, −108.3, −116.4; ^{19}F NMR (376 MHz, CD_3OD) δ −104.6, −108.6, −116.3.

Resin 8

N,*N*′-Diisopropylcarbodiimide (0.24 mL, 1.55 mmol) was added to a solution of pentafluorophenol (0.43 g, 2.32 mmol) in EtOAc (30 mL) at 0 °C. After 30 min, a solution of 3-fluoro-4-(hydroxymethyl)phenoxyacetic acid[36] (0.31 g, 1.55 mmol) in EtOAc (40 mL) was added and the solution was stirred for 1 h and subsequently added to the ArgoGel-NH_2 resin (1.80 g, 0.77 mmol). After agitation for 16 h at room temperature the *N*-acylation was complete according to monitoring with bromophenol blue (0.19 mL, 2.0 mM in DMF). The resin was washed with EtOAc, MeOH and THF (5 × 10 mL each) to give resin **8**; ^{19}F NMR (376 MHz, $CDCl_3$) δ −117.2.

Resin 9–11

MSNT (0.16 g, 0.54 mmol) and the first amino acid (0.53 mmol) were dissolved in CH_2Cl_2 (3.0 mL), and added to ArgoGel resin **8** (0.13 mmol), functionalized with a fluorinated linker.[36] Methyl imidazole (31 μL, 0.39 mmol) was added and the mixture was mechanically agitated during 14 h. The resin was washed with CH_2Cl_2, DMF and CH_2Cl_2 (3 × 5 mL each), whereafter the resin was subjected to peptide synthesis according to previous reported procedures to give resin **9**, **10** and **11**;[76] resin **9** had: ^{19}F NMR (376 MHz, $CDCl_3$) δ −108.4, −115.2; resin **10** had: ^{19}F NMR (376 MHz, DMSO-d_6) δ −109.5, −116.1; resin **11** had: ^{19}F NMR (376 MHz, DMSO-d_6) δ −109.0, −115.5.

4-Methylphenyl 2,3-di-*O*-(4-fluorobenzoyl)-4,6-*O*-(3-fluorobenzylidene)-1-thio-β-D-galactopyranoside (13)

Compound **12**[51] (0.63 g, 1.61 mmol) was dissolved in pyridine (7.0 mL) and 4-fluorobenzoyl chloride (0.49 mL, 4.17 mmol) was added drop-wise over 5 min. The solution was stirred for 16 h at room temperature and then diluted with CH_2Cl_2 (100 mL), washed with sat. aqueous $NaHCO_3$ (2 × 50 mL) and H_2O (50 mL). The organic phase was concentrated and residual pyridine was removed by co-concentration with toluene. The resulting brown oil was purified by flash column chromatography (heptane/EtOAc, 5 : 1 ⟶ 4 : 1) to give **13** (1.00 g, 98%) as a colorless foam; $[\alpha]^{20}_D$ +28 (*c* 0.37, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04–7.91 (4H, m, Ar*H*), 7.51–7.48 (2H, d, *J* = 8.1 Hz, Ar*H*), 7.35–6.96 (10H, m, Ar*H*), 5.67 (1H, t, *J* = 9.9 Hz, H-2), 5.48 (1H, s, 3-FPhC*H*O_2), 5.33 (1H, dd, *J* = 10.0, 3.4 Hz, H-3), 4.89 (1H, d, *J* = 9.8 Hz, H-1), 4.55 (1H, d, *J* = 3.2 Hz, H-4), 4.45 (1H, dd, *J* = 12.4, 1.5 Hz, H-6), 4.08 (1H, dd, *J* = 12.4, 1.5 Hz, H-6), 3.75 (1H, s, H-5), 2.35 (3H, s, SPh*Me*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 100.0, 85.0, 74.1, 73.6, 69.7, 69.1, 67.2, 21.2; ^{19}F NMR (376 MHz, $CDCl_3$) δ −113.4, −105.5, −105.1; HRMS (FAB) calcd for $C_{57}H_{59}F_5NO_{18}$ 659.1322 *m*/*z* $(M + Na)^+$, observed 659.1328.

Resin 14

TfOH (0.77 μL, 8.6 μmol) was added to a suspension of resin **9** (43 μmol), NIS (19 mg, 85 μmol) and **13** (55 mg, 86 μmol) in CH_2Cl_2 (3.0 mL) in the absence of light. After 3 h agitation at room temperature, the resin was washed with CH_2Cl_2, THF, 20% piperidine in DMF, DMF and CH_2Cl_2 (5 × 3 mL each). The procedure was repeated twice to give resin **14** in 87% yield; ^{19}F NMR (376 MHz, $CDCl_3$) δ −104.8, −105.1, −108.3, −113.5, −115.3.

Resin 15

Resin **10** was treated as described for resin **14** to give resin **15** in 80% yield; ^{19}F NMR (376 MHz, DMSO-d_6) δ −104.8, −105.2, −109.0, −113.0, −115.6.

Resin 16

Resin **11** was treated as described for resin **14** to give resin **16** in 84% yield; ^{19}F NMR (376 MHz, DMSO-d_6) δ −104.7, −105.1, −109.0, −112.9, −115.5.

4-Fluorobenzoyl-glycyl-*O*-(β-D-galactopyranosyl)-L-seryl-L-valine (17)

A solution of trifluoroacetic acid in H_2O (9 : 1, 12 mL) was added to resin **14** (43 μmol) and the mixture was heated to 60 °C without stirring. After 3 h the resin was removed by filtration and washed with HOAc (2 × 5 mL). The filtrate was evaporated, whereafter HOAc (10 mL) was added and the solution was lyophilized. The residue was purified with reversed-phase HPLC (gradient: 10 ⟶ 100% MeCN in H_2O, both containing 0.1% TFA, during 60 min) and lyophilized. The resulting colorless solid was dissolved with MeOH (3.2 mL) and aqueous LiOH (0.8 mL, 0.1 M) was added drop-wise (5 min). After 1 h the solution was neutralized with HOAc, evaporated, diluted with HOAc (5 mL) and lyophilized. The residue was purified with reversed-phase HPLC (gradient: 0 ⟶ 100% MeCN in H_2O, both containing 0.1% TFA, during 60 min) and lyophilized to provide **17** (5.1 mg, 22%) as a colorless solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.95 (2H, m, Ar*H*), 7.31 (2H, t, *J* = 8.7 Hz, Ar*H*), Gly: 8.80 (1H, dd, *J* = 6.4 Hz, NH), 3.93 (2H, m, αH), Ser: 8.16 (1H, d, *J* = 7.8 Hz, NH), 4.63 (1H, m, αH), 3.95 (1H, m, βH), 3.63 (1H, m, βH), Val: 7.92 (1H, d, *J* = 7.5 Hz, NH), 4.13 (1H, m, αH), 2.06 (1H, m, βH), 0.89 (6H, m, γCH_3), Gal: 4.73 (1H, d, *J* = 7.3 Hz, H-1), 3.67 (1H, m, H-3), 3.58 (1H, m, H-4), 3.56 (1H, m, H-2); HRMS (FAB) calcd for $C_{23}H_{31}FN_3Na_2O_{11}$ 590.1733 *m*/*z* $(M + Na)^+$, observed 590.1730.

4-Fluorobenzoyl-L-alaninyl-glycyl-*O*-(β-D-galactopyranosyl)-L-seryl-L-valinyl-L-phenylalanine (18)

Resin **15** (43 μmol) was treated as described for **17** to give **18** (8.7 mg, 26%) as a colorless solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.98 (2H, dd, *J* = 5.5, 9.0 Hz, Ar*H*), 7.31 (2H, m, Ar*H*), Ala: 8.56 (1H, d, *J* = 7.6 Hz, NH), 4.52 (1H, q, *J* = 7.3 Hz, αH), 1.34 (3H, d, *J* = 7.3 Hz, βH), Gly: 8.18 (1H, d, *J* = 6.7 Hz, NH), 3.76 (2H, d, *J* = 6.7 Hz, αH), Ser: 8.48 (1H, d, *J* = 8.1 Hz, NH), 4.54 (1H, m, αH), 3.55 (2H, d, *J* = 6.4 Hz βH), Val: 7.87 (1H, d, *J* = 8.8 Hz, NH), 4.20 (1H, dd, *J* = 6.4, 8.8 Hz, αH), 1.99 (1H, m, βH), 0.83 (3H, d, *J* = 6.4 Hz, γCH_3), 0.77 (3H, d, *J* = 6.4 Hz, γCH_3), Phe: 8.18 (1H, d, *J* 7.6 Hz, NH), 4.41 (1H, m, αH), 3.04 (1H, m, βH), 2.88 (1H, m, βH), 7.26 (2H, d, *J* = 7.7 Hz, Ar*H*), 7.21 (3H, m, Ar*H*), Gal: 4.93 (1H, d, *J* = 6.8 Hz, H-1), 4.02 (1H, H-4), 3.91 (1H, H-5 or H-6), 3.76 (1H, t, *J* = 10.3, 6.3 Hz, H-2), 3.65 (1H, H-5 or H-6), 3.60 (1H, H-3), 3.54 (1H, H-5 or H-6); MS (ES+) calcd for $C_{35}H_{46}FN_5O_{13}$ 763.3 *m*/*z* $(M)^+$, observed 763.3.

4-Fluorobenzoyl-L-phenylalaninyl-L-alaninyl-glycyl-*O*-(β-D-galactopyranosyl)-L-seryl-L-valinyl-L-phenylalaninyl-L-leucine (19)

Resin **16** (11 μmol) was treated as described for **17** to give **19** (3.0 mg, 27%) as a colorless solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.85 (2H, m, Ar*H*), 7.26 (2H, m, Ar*H*), Phe: 8.58 (1H, d, *J* = 8.25 Hz, NH), 4.72 (1H, dd, *J* = 6.4, 10.2 Hz, αH), 3.15 (1H, m, βH), 2.96 (1H, m, βH), 7.24 (3H, m, Ar*H*), 7.16 (2H, d, *J* = 7.3 Hz, Ar*H*), Ala: 8.27 (1H, d, *J* = 6.4 Hz, NH), 4.34 (1H,

dq, J = 6.4, 12.9 Hz, αH), 1.27 (3H, d, J = 6.9 Hz, βH), Gly: 8.05 (1H, d, J = 9.0 Hz, NH), 3.80 (1H, m, αH), 3.73 (1H, m, αH), Ser: 8.03 (1H, d, J = 8.2 Hz, NH), 4.54 (1H, m, αH), 3.88 (1H, m, βH), 3.54 (1H, m, βH), Val 7.65 (1H, d, J = 8.7 Hz, NH), 4.14 (1H, m, αH), 1.90 (1H, m, βH), 0.74 (6H, d, J = 6.4 Hz, γCH_3), Phe 8.05 (1H, d, J = 6.5 Hz, NH), 4.57 (1H, m, αH), 3.06 (1H, m, βH), 2.77 (1H, m, βH), 7.35 (2H, d, J = 7.7 Hz, Ar*H*), 7.24 (3H, m, Ar*H*), Leu: 8.11 (1H, d, J = 9.6 Hz, NH), 4.22 (1H, m, αH), 1.51 (2H, dd, J = 8.7, 13.1 Hz, βH), 1.62 (1H, m, γH), 0.88 (3H, d, J = 6.5 Hz, δCH_3), 0.82 (3H, d, J = 6.5 Hz, δCH_3), Gal: 4.70 (1H, d, J = 6.4 Hz, H-1), 4.13 (1H, H-4), 3.63 (1H, H-5 or H-6), 3.51 (1H, H-5 or H-6), 3.34 (1H, H-3), 3.30 (2H, H-2 and H-5 or H-6); HRMS (FAB) calcd for $C_{50}H_{65}FN_7Na_2O_{15}$ 1068.4313 *m*/*z* $(M + Na)^+$, observed 1068.4324.

Acknowledgements

This work was funded by grants from the Swedish Research Council and the Göran Gustafsson Foundation for Research in Natural Sciences and Medicine.

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