Use of Fluorobenzoyl Protective Groups in Synthesis of Glycopeptides: *β*-Elimination of *O*-Linked Carbohydrates Is Suppressed†

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Fluorobenzoyl groups have been investigated as alternatives to acetyl and benzoyl protective groups in carbohydrate and glycopeptide synthesis. D-Glucose and lactose were protected with different fluorobenzoyl groups and then converted into glycosyl bromides in high yields (>80% over two steps). Glycosylation of protected derivatives of serine with these donors gave 1,2-trans glycosides in good yields (∼60−70%) and excellent stereoselectivity without formation of ortho esters. The resulting glycosylated amino acid building blocks were then used in solid-phase synthesis of two model *O*-linked glycopeptides known to be unusually sensitive to *β*-elimination on base-catalyzed deacylation. When either a 3-fluoro- or a 2,5-difluorobenzoyl group was used for protection of each of the two model glycopeptides the extent of *β*-elimination decreased from 80% to 10% and from 50% to 0%, respectively, as compared to when using the ordinary benzoyl group. Fluorobenzoyl groups thus combine the advantages of the benzoyl group in formation of glycosidic bonds (i.e., high stereoselectivity and low levels of ortho ester formation) with the ease of removal characteristic of the acetyl group.

Introduction

The choice of protective groups is crucial in order to succeed in synthesis of glycopeptides.[1−5] Today most glycopeptides are prepared on solid-phase according to the Fmoc-protocol.[6,7] This strategy utilizes the base-labile Fmoc-group[8] for N^{α}-protection of the amino acids in combination with side chain protective groups that are removed during cleavage from the solid phase with trifluoroacetic acid (TFA). As demonstrated recently the hydroxyl groups of the carbohydrate moieties can also be protected with groups that are cleaved by TFA, such as silyl- and methoxybenzyl groups or acetals.[9−14] Use of benzyl ethers, which are deprotected by hydrogenolysis,[15−18] or in two acid-catalyzed steps,[19] is another alternative that has found some use. However, in most syntheses of glycopeptides the hydroxyl groups of the carbohydrate are protected as acetyl- or benzoyl esters. These have the advantage of stabilizing the *O*-glycosidic bonds against acid-catalyzed degradation during cleavage from the solid-phase,[20,21] which is especially important for deoxysugars such as fucose.[20,22,23]

A disadvantage with using acetyl or benzoyl protective groups for carbohydrate moieties in glycopeptides is the need for a base-catalyzed deprotection step. This can cause *β*-elimination of carbohydrates linked to serine or threonine, as well as epimerization of peptide α-stereocenters. In the case of removal of *O*-acetyl groups these side-reactions occur only as rare exceptions,[23,24] since very mild conditions may be used for deprotection (e.g., 6 mM methanolic sodium methoxide).[25] *O*-Benzoyl groups may

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† Dedicated to Professor Joachim Thiem on the occasion of his 60th birthday.

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require higher concentration of base for removal (i.e., ~100 mM methanolic sodium methoxide), which leads to that β-elimination,[24,26] and epimerization of α-stereocenters[27] can occur more frequently. Unfortunately, the acetyl group has some disadvantages, as compared to the benzoyl group which prevents its exclusive use in glycopeptide synthesis. These are related to side reactions that may occur in glycoside synthesis, e.g., in glycosylation of serine or threonine. Thus, formation of ortho esters, an insufficient neighboring group effect that leads to a mixture of α- and β-glycosides, and migration of the acyl group from the glycosyl donor to the hydroxyl group being glycosylated are common when acetates are used as protective groups.[28,29]

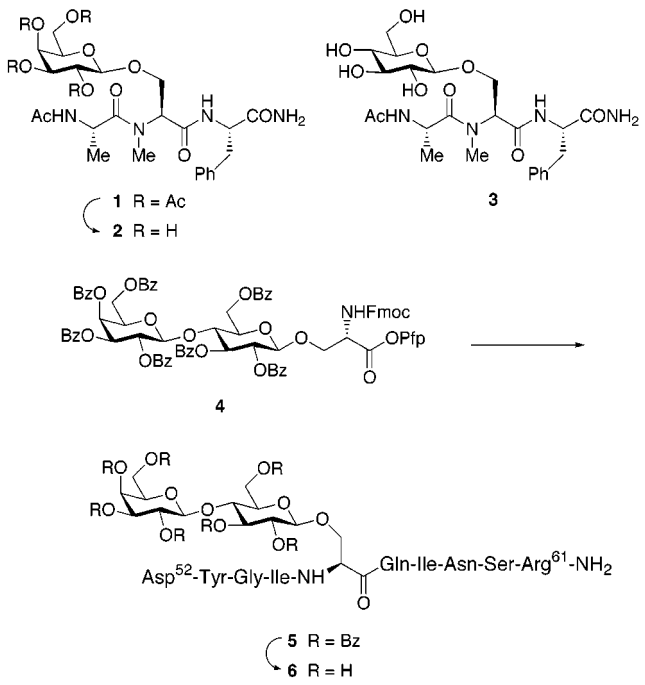
Parameters such as the solvent, structural features of the glycopeptide,[24] and the nature of the base also influence the outcome of base-catalyzed deprotection of glycopeptides. For instance, use of water as solvent has been found to give enhanced amounts of side-products, as compared to deacylation in organic solvents.[25] Ammonia,[27] sodium methoxide, hydrazine hydrate[30], or cyanide ion are all being used as bases, with methanolic sodium methoxide and ammonia being the most popular. Hydrazine hydrate and cyanide ion allow mild conditions for deprotection, but the fact that hydrazine is a suspected carcinogen and cyanide ion is very toxic may explain their limited use. In addition, hydrazine has been reported to attack *C*-terminal amides and the side-chains of asparagine thereby giving hydrazides.[23]

Deprotection of the carbohydrate hydroxyl groups is the last step in the synthesis of glycopeptides and the protective group pattern must therefore be chosen carefully before embarking on the synthesis. Since acetyl and benzoyl groups have different advantages in synthesis of *O*-linked glycopeptides, i.e., ease of removal for acetates and improved properties during formation of glycosidic bonds for benzoates, it would be of general interest if these advantages could be combined in an alternative acyl protective group. We now show that certain fluorinated benzoyl groups appear to fulfill this requirement and allow substantial improvements in preparation of two glycopeptides that are difficult to prepare and undergo large amounts of β-elimination during base-catalyzed deprotection.

Results and Discussion

Selection of Difficult Glycopeptides as Model Systems. We recently found that β-elimination could not be avoided during deacetylation of the *N*-methylated glycopeptide **1** to give **2** (Scheme 1).[31] Glycopeptide **2** is exceptionally sensitive to β-elimination since the *N*-methyl group attached to the glycosylated serine prevents formation of a protective aza-enolate on treatment with base.[31] Glycoproteins or glycopeptides found in nature do not contain galactose linked directly to serine, but the β-glucosyl serine linkage is found in proteins involved in

Scheme 1



blood coagulation and fibrinolysis.[32] Interestingly, attempts to remove *O*-benzoyl protective groups from synthetic glycopeptides having a β-glucosyl serine moiety were found to be accompanied by β-elimination.[26] Synthesis of glucosylated tripeptide **3**, in which the tendency for β-elimination is enhanced by *N*-methylation of the glycosylated serine, was therefore chosen as a first model for evaluation of novel acyl protective groups in the present study.

Glycodecapeptide **6** (Scheme 1) is derived from residues 52−61 of hen egg lysozyme (HEL) and has previously been prepared[24] from glycosylated serine **4** as part of an investigation of how T cells respond to glycopeptides.[33] In the synthesis of **6**, a substantial amount of β-elimination (~50%) could not be avoided during removal of the benzoyl protective groups from **5**.[24] In this case several attempts to prepare a building block corresponding to **4**, which carried acetyl instead of benzoyl protective groups, gave very low yields due to formation of a mixture of α- and β-glycosides as well as to formation of ortho esters.[34] Synthesis of glycopeptide **6** was therefore chosen as a second model for evaluation of alternative protective groups.

Selection and Preliminary Evaluation of Substituted *O*-Benzoyl Protective Groups. Introduction of electron-withdrawing substituents on the benzoyl group, such as halogen atoms, carbonyl, or nitro groups, should facilitate deprotection under basic conditions. It is less clear how such substituents would influence the properties of the benzoyl group when employed as the participating group in glycoside synthesis. The σ-values[35] of 4- and 3-fluorobenzoic acid (0.06 and 0.34, respectively) reveal that the electron withdrawing effect from a

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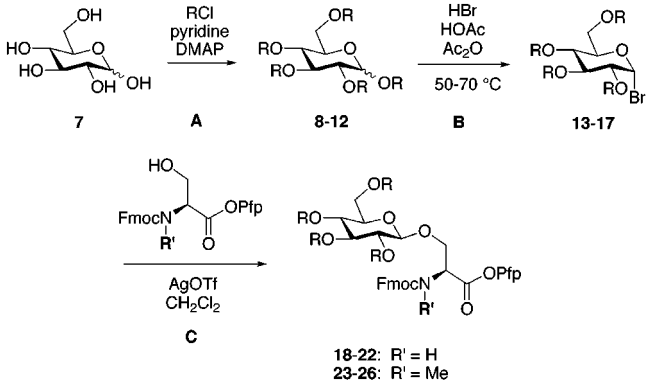
Table 1. Synthesis of β-D-Glucosyl Serine Building Blocks in Which the Glucose Moiety Is Protected with Benzoyl or Different Fluorobenzoyl Groups



R	step **A**[a]	step **B**[a]	step **C**[a] (**R′** = H)	step **C**[a] (**R′** = Me)
Bz	**8**: 82%	**13**: 97%[b]	**18**: 59%	**23**: 91%
2-FBz	**9**: 94%	**14**: 93%	**19**: 74%	
3-FBz	**10**: 83%	**15**: 96%	**20**: 71%	**24**: 87%
4-FBz	**11**: 90%	**16**: 89%	**21**: 51%	**25**: 86%
2,5-diFBz	**12**: 91%	**17**: 95%	**22**: 58%	**26**: 70%

[a] Yields were determined after purification by flash column chromatography. [b] Not purified by chromatography.

fluorine substituent may be large and depends on its location on the benzoyl group. In addition, the electron withdrawing effect is accumulated in di- and polyfluorinated benzoates. These observations, together with the commercial availability of a large number of fluorinated benzoyl chlorides and the chemical inertness of organofluorine compounds, focused our attention on evaluation of fluorinated benzoyl protective groups. An additional advantage with such protective groups is that ^{19}F NMR spectroscopy allows selective monitoring of reactions performed in solution or in solid-phase.[36−38] The properties of benzyl ether protective groups are often tuned by different substituents but, surprisingly enough, this concept has not been developed for benzoyl esters.[39−41]

A preliminary evaluation of fluorobenzoyl protective groups was obtained by comparing the rate of deprotection of glucose carrying 2-, 3-, and 4-fluorobenzoyl protective groups with that of perbenzoylated glucose **8**. To allow this study D-glucose (**7**) was protected by treatment with benzoyl chloride, or with 2-, 3-, or 4-monofluorobenzoyl chloride, to give the fully protected derivatives **8**−**11** as α/β-mixtures (Table 1). Fluorobenzoates **9**−**11** were then mixed, dissolved in MeOH-d_4, and introduced in an NMR tube before sodium methoxide was added. After mixing briefly ^{19}F NMR spectra were recorded every two minutes at room temperature. Since the resonances for fluorobenzoates **9**−**11** are well separated from each other, and from the three methyl fluorobenzoates formed on treatment with sodium methoxide, it was possible to monitor the progress of the deprotection. This showed that the 3-fluorobenzoyl group was most easily removed followed by the 2-fluoro- and finally the 4-fluorobenzoyl group. In addition, studies monitored by TLC revealed that perbenzoate **8** was deprotected somewhat slower than 4-fluorobenzoylated **11**. These results prompted a continued examination of fluorobenzoyl protective groups in glycopeptide synthesis. It was also decided to include the 2,5-difluorobenzoyl group, which combines the substitution pattern of the two most reactive monofluorobenzoates, in this investigation. Therefore, D-glucose was protected by treatment with 2,5-difluorobenzoyl chloride, as in the preparation of **8**−**11**, to give **12**.

Preparation of β-D-Glucosyl Serine Building Blocks which Carry Fluorobenzoyl Protective Groups. Fluorobenzoyl groups must not only allow facile removal in order to be of use in synthesis of glycopeptides; suitable glycosyl donors, e.g., glycosyl halides, in which the hydroxyl groups are protected with fluorobenzoyl groups must also be readily accessible. Furthermore, the glycosyl donors should allow amino acids to be glycosylated in high yields thereby giving building blocks that can be used for assembly of glycopeptides using standard techniques.

Perbenzoates **8**−**12** were transformed into glycosyl donors by treatment with hydrogen bromide in a mixture of acetic acid and acetic anhydride at 50−70 °C, which gave bromosugars **13**−**17** in 89−97% yields (Table 1). Just as for benzoylated glucosyl bromide **13**, the fluorobenzoylated bromosugars **14**−**17** could be purified by chromatography on silica gel without degradation and are stable at room temperature. Bromosugars **13**−**17** were then used to glycosylate N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester (Fmoc-Ser-OPfp)[42] under promotion by silver triflate in dichloromethane at temperatures ranging from −30 °C to room temperature.[26] In our hands, this gave the glucosylated serine building blocks **18**−**22** in 51−74% yields. The yields thus showed some variation depending on the type of fluorobenzoyl group being used, but did not differ substantially from those obtained when the ordinary benzoyl group was employed. The nature of the fluorobenzoyl protective group did, however, influence the reactivity of the glycosyl donor to a somewhat larger extent. For instance, the 3-fluorobenzoylated glucosyl bromide **15** did not react with Fmoc-Ser-OPfp at −30 °C, but gave **20** in 71% yield when allowed to attain room temperature. In contrast, the 2-fluorobenzoylated bromosugar **14** reacted smoothly at −30 °C to give building block **19** in an almost identical yield. Importantly, formation of α-glycosides and ortho esters corresponding to **19**−**22** were not observed in the glycosylations employing fluorobenzoylated **14**−**17**, nor was any benzoyl migration to serine detected. The fluorobenzoyl groups thus perform equally well as, or even better than, the ordinary benzoyl group in syntheses of bromosugars and in preparation of glycosylated amino acids.

Synthesis and Deprotection of Fluorobenzoylated Model Glycotripeptides. The influence of different fluorobenzoyl protective groups on the extent of β-elimination during base-catalyzed deprotection was first evaluated in preparation of model glycopeptide **3**,

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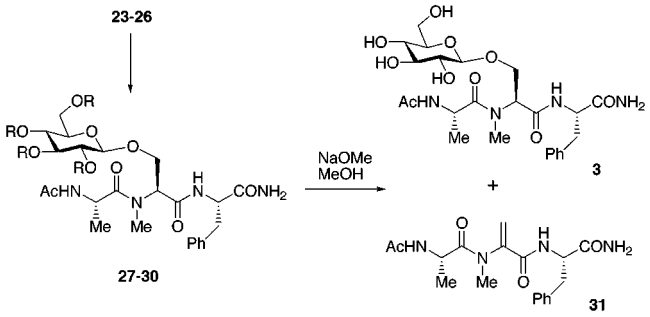
Table 2. Influence of the Type of Benzoyl Group Used for Protection of Glycopeptides 27−30 on the Amount of β-Elimination Obtained on Deacylation with Methanolic Sodium Methoxide



compound	R	reaction time	amount of **31**[a]
27	Bz	7 h	∼80%
28	3-FBz	1 h	∼20%
29	4-FBz	3 h	∼40%
30	2,5-diFBz	1 h	∼10%

[a] The reactions were allowed to proceed until all of the protected glycopeptide had been consumed. The extent of β-elimination to give **31** was then determined by integration of peaks in chromatograms obtained by analytical reversed-phase HPLC.

in which the glycosylated serine is *N*-methylated (Table 2). To allow this investigation N^{α}-(fluoren-9-ylmethoxycarbonyl)-N^{α}-methyl-L-serine pentafluorophenyl ester (Fmoc-NMe-Ser-OPfp)[31] was glycosylated with benzoylated bromosugar **13**, 3- and 4-monofluorobenzoylated **15** and **16**, as well as the 2,5-difluorobenzoylated **17**. This gave building blocks **23**−**26** in 70−91% yields when silver triflate was used as promoter under the same conditions as described for synthesis of **18**−**22** (Table 1). The 2-fluorobenzoyl group has a cleavage rate between the 3- and 4-fluorobenzoyl groups and was therefore not included in this study. Building blocks **23**−**26** were then used in assembly of glycopeptides **27**−**30** on a TentaGel S NH_2 resin functionalized with the Rink linker.[43,44] The use of 1-hydroxy-7-azabenzotriazole[45] (HOAt) in combination with *N*,*N*-diisopropylcarbodiimide as coupling reagent and 6 equiv of Fmoc-Ala-OH in the final coupling step was found to be important in order to give **27**−**30** in acceptable yields (48−68%). Glycopeptides **27**−**30** were then subjected to treatment with methanolic sodium methoxide (6 mM) in separate experiments. The low concentration of sodium methoxide was chosen in order to allow the progress of the deacylations to be reliably monitored by analytical reversed-phase HPLC. The reactions were allowed to progress until all of the protected glycopeptides had been consumed, after which the extent of β-elimination was determined by analytical reversed-phase HPLC. Deprotection of the benzoylated **27** resulted in predominant β-elimination (∼80%) to give **31**[31] (Table 2), the formation of which was reduced to ∼40% for 4-fluorobenzoylated **29**. β-Elimination was further reduced for the 3-fluorobenzoylated **28** (∼20%), whereas the 2,5-difluorobenzoylated **30** gave an even better result (∼10% of **31**). Thus, protection of the very base-labile glycopeptide **3** with either of the latter two fluorobenzoyl protective groups allowed the levels of β-elimination during deprotection to be decreased substantially, as compared to when using benzoyl protection. In this context it should also be pointed out that β-elimination could not be avoided on deprotection of the structurally related, *O*-acetylated glycopeptide **1** (cf. Scheme 1).[31]

Synthesis and Deprotection of Fluorobenzoylated Model Glycodecapeptides. The 4-fluoro- and 2,5-difluorobenzoyl groups were chosen for use in attempts to reduce the extent of β-elimination encountered during deprotection of **5** to give glycodecapeptide **6** (Scheme 1). These two fluorobenzoyl groups show the lowest and highest rates of base-catalyzed cleavage and therefore provide information on the limits of β-elimination that can be expected for the different fluorobenzoates, when employed in a more complex glycopeptide such as **6**. Synthesis of the fluorobenzoylated glycopeptides started with protection of lactose (**32**) by reaction with 4-fluorobenzoyl chloride and 2,5-difluorobenzoyl chloride, respectively, to give derivatives **33** and **34** in high yields (Table 3). Treatment of **33** and **34** with HBr in a mixture of acetic acid and acetic anhydride gave bromosugars **35** and **36**, which were then used to glycosylate Fmoc-Ser-OPfp. By using silver triflate as the promotor, building blocks **38** and **39** were obtained in 59% and 64% yields, respectively, which should be compared with 72% reported for **37** that carries ordinary benzoyl groups.[34] It is important to note that, in contrast to when acetyl groups were employed for protection of the lactosyl bromide, ortho ester formation or formation of α/β-mixtures was not detected in the synthesis of **38** and **39**. Use of building blocks **38** and **39** in solid-phase synthesis, under conditions reported previously,[24] then gave glycopeptides **40** and **41** (Table 4), which have the carbohydrate hydroxyl groups protected as 4-fluoro- and 2,5-difluorobenzoyl esters, respectively. Glycopeptide **5**, which carries benzoyl protective groups, was prepared as described previously.[24]

Glycopeptides **5**, **40** and **41** were treated with 20 mM LiOH in methanol in order to remove the protecting groups from the carbohydrate moiety and the progress of the reactions were monitored with analytical reversed-phase HPLC.[46] The benzoylated glycopeptide **5** was not fully deprotected even after 8 h of treatment with methanolic LiOH and ∼50% of the glycopeptide had undergone β-elimination to give **42**.[24,47] For glycopeptide **40**, which has 4-fluorobenzoyl protective groups, a small improvement was obtained but β-elimination still amounted to ∼40% when deprotection was complete after 8 h. In sharp contrast, deprotection of glycopeptide **41**, which carries 2,5-difluorobenzoyl groups, had reached completion in less than 30 min with no sign of β-elimination. This allowed glycopeptide **6** to be prepared in 41% yield by deprotection of **41**, as compared to 22% from benzoylated **5**.[24]

Benzoylated glycopeptide **5** and the 2,5-difluorobenzoylated **41** were also deprotected under milder conditions with saturated methanolic ammonia.[27] Glycopeptide **5** was not fully deprotected even after 48 h and ∼50% had undergone β-elimination. In comparison, **41** was fully deprotected in less than 2 h with no sign of β-elimination.

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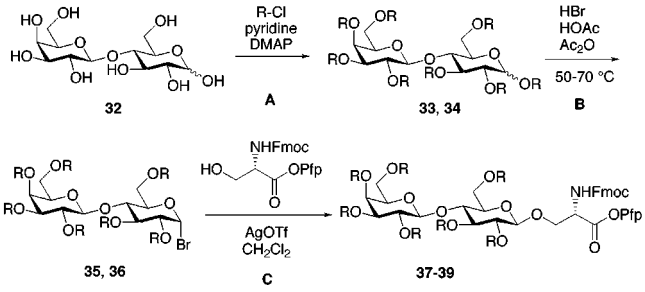
(46) Deprotection with LiOH in methanol gave HPLC chromatograms with sharper peaks as compared to when methanolic sodium methoxide was employed, explaining the use of LiOH in this study. The time required to reach completion of the reactions was, however, the same for LiOH as for sodium methoxide.
(47) The structure of the β-eliminated peptide **42** was confirmed by FABMS [calcd 1133 ($M + H^+$), found 1133].

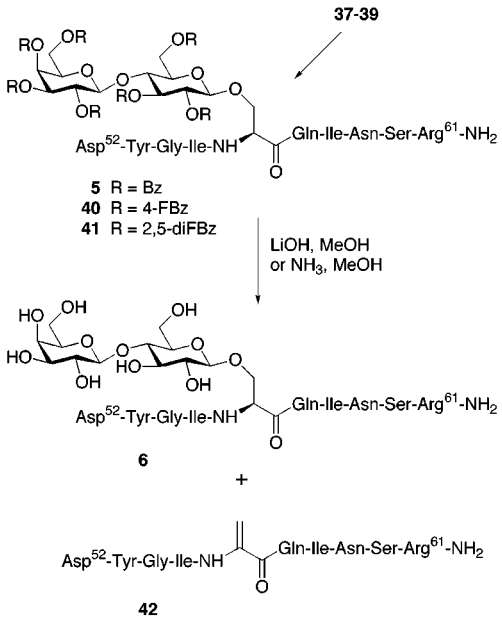
Table 3. Synthesis of β-D-Lactosyl Serine Building Blocks in Which the Lactose Moiety Is Protected with Benzoyl or Fluorobenzoyl Groups



R	step **A**[a]	step **B**[a]	step **C**[a]
Bz			**37**: 72%[b]
4-FBz	**33**: 99%	**35**: 91%	**38**: 59%
2,5-diFBz	**34**: 97%	**36**: 86%	**39**: 64%

[a] Yields were determined after purification by flash column chromatography. [b] Cf. Reference 34.

Table 4. Influence of the Type of Benzoyl Group Used for Protection of Glycopeptides 5, 41, and 42 on the Extent of β-Elimination during Base-Catalyzed Deacylation



compound	R	base	reaction time	amount of **42**[a]
5	Bz	LiOH	8 h	~50%
40	4-FBz	LiOH	8 h	~40%
41	2,5-diFBz	LiOH	<0.5 h	0%
41	2,5-diFBz	NH_3	2 h	0%

[a] The reactions were allowed to proceed until all of the protected glycopeptides had been consumed. The extent of β-elimination to give **42** was then determined by integration of peaks in chromatograms obtained by analytical reversed-phase HPLC.

Interestingly, when deprotection of **41** with methanolic ammonia was allowed to continue for 24 h, no sign of β-elimination to give **42** was observed. This reveals that a deprotected glycopeptide is substantially more stable against β-elimination than a fully protected or a partially deprotected one. A probable explanation for this observation is that the hydroxyl groups of the carbohydrate moiety, once deprotected, become partially ionized under the basic conditions used for deprotection. The resulting partial negative charge on the carbohydrate, especially when located at O-2, should prevent the development of a negative charge on O-1 which occurs during β-elimination. A similar effect could apply to glycopeptides containing carbohydrates with an acetamido group at C-2, which may give a protective "aza-enolate" upon treatment with base. Finally, it should be mentioned that epimerization of peptide α-stereocenters was not observed when **41** was deprotectied with methanolic ammonia during 24 h.

Conclusions. We have demonstrated that fluorobenzoyl groups are useful as protective groups in synthesis of *O*-linked glycopeptides. Just as for the ordinary acetyl and benzoyl group the fluorobenzoyl groups were readily attached to carbohydrates and allowed preparation of glycosyl donors in high yields. The fluorobenzoylated donors performed equally well in synthesis of glycosylated amino acids as when benzoyl groups were employed but better than when using acetyl groups. 1,2-Trans glycosides were thus obtained in high yields without formation of ortho esters or α-glycosides: side-products which are common when using acetyl protective groups. The rate of base-catalyzed deprotection of fluorobenzoylated glucose was found to depend on the number of fluorine atoms and on their position(s) on the benzoyl group giving the following order of decreasing deprotection rates: 2,5-difluoro→ 3-fluoro→ 2-fluoro→ 4-fluorobenzoyl. Consequently, base-catalyzed deprotection of *O*-linked glycopeptides carrying 3-fluoro- or 2,5-difluorobenzoyl groups was found to proceed substantially faster than when using the benzoyl group. Protection with either of these two fluorobenzoyl groups also allowed large reductions in the extent of β-elimination on deprotection of two base-labile glycopeptides which carried carbohydrates *O*-linked to serine. It thus appears that fluorobenzoyl groups combine the advantages of the benzoyl group in formation of glycosidic bonds with the ease of removal characteristic for the acetyl group. Use of fluorobenzoyl protecting groups should prove to be valuable in the synthesis of *O*-linked glycopeptides which are prone to undergo β-elimination and for glycopeptides which carry large carbohydrates that require prolonged reaction times in the final deacylation step.

Experimental Section

General Procedures. All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH_2Cl_2 was distilled from calcium hydride. MeOH and pyridine were dried over 3 and 4 Å molecular sieves, respectively. DMF was distilled and then sequentially dried over 3 Å molecular sieves. TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light and charring with aqueous sulfuric acid. Flash column chromatography was performed on silica gel (Matrex, 60 Å, 35−70 μm, Grace Amicon) with distilled solvents. Organic solutions were dried over Na_2SO_4 before being concentrated. 1,2,3,4,6-Penta-*O*-benzoyl-D-glucopyranose[48] (**8**) and 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl bromide[48] (**13**) were prepared as described below for compounds **9** and **14** and had data in agreement with the cited reference. N^{α}-(Fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester[42] and N^{α}-(fluoren-9-ylmethoxycarbonyl)-N^{α}-methyl-L-serine pentafluorophenyl ester[31] were prepared as described in the cited references.

The ^1H NMR spectra were recorded at 400 MHz for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27) as internal standard], CD_3OD [residual CD_2HOD (δ_H 3.31) as internal standard], or

(48) Ness, R. K.; Fletcher, H. G.; Hudson, C. S. *J. Am. Chem. Soc.* **1950**, *72*, 2200−2205.

acetone-d_6 [residual acetone-d_5 (δ_H 2.05) as internal standard] at 300 K. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra, whereas proton resonances were assigned from COSY[49] experiments. Resonances for aromatic protons and resonances that could not be assigned are not reported. Ions for positive FABMS were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thioglycerol. In the amino acid analyses, glutamine and asparagine were determined as glutamic acid and aspartic acid, respectively. Analytical and preparative HPLC separations were performed on Kromasil C-8 columns (100 Å, 50 μm, 4.6 or 20 × 250 mm, respectively) with flow rates of 1.5 and 11 mL/min, respectively, using the solvent systems A (aqueous 0.1% TFA) and B (0.1% TFA in MeCN) as eluants. UV detection was at 214 nm.

1,2,3,4,6-Penta-*O*-(2-fluorobenzoyl)-D-glucopyranose (9). D-Glucose (300 mg, 1.67 mmol) and 4-(dimethylamino)pyridine (40 mg, 0.33 mmol) were dissolved in pyridine (5 mL), after which 2-fluorobenzoyl chloride (1.50 mL, 12.6 mmol) was added droppwise over 15 min. The solution was stirred for 18 h and then methanol (2 mL) was added. After additional stirring for 1 h the solution was diluted with CH_2Cl_2 (70 mL) and washed with water (100 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried and concentrated. Flash cromatography of the residue on silica gel with heptane−EtOAc (3:1 → 2:1) gave **9** (1.23 g, 94%): ^{1}H NMR ($CDCl_3$) α/β-ratio 4.7:1, δ (α-anomer) 6.89 (d, 1 H, J = 3.6 Hz, H-1), 6.27 (t, 1 H, J = 10.0 Hz, H-3), 5.85 (t, 1 H, J = 9.7 Hz, H-4), 5.66 (dd, 1 H, J = 3.6, 10.2 Hz, H-2) 4.55−4.70 (m, 3 H, H-5 and H-6), δ (β-anomer) 5.99 (t, 1 H, J = 9.4 Hz, H-3); HRMS (FAB) calcd for $C_{41}H_{27}F_5NaO_{11}$ 813.1372 (M + Na^+), found 813.1367.

1,2,3,4,6-Penta-*O*-(3-fluorobenzoyl)-D-glucopyranose (10). Compound **10** was synthesized from D-glucose (300 mg, 1.67 mmol) as described for **9**, using 3-fluorobenzoyl chloride as acylating agent. Purification by flash column chromatography gave **10** (1.09 g, 83%): ^{1}H NMR ($CDCl_3$) α/β-ratio 6.5:1, δ (α-anomer) 6.84 (d, 1 H, J = 3.8 Hz, H-1), 6.23 (t, 1 H, J = 10.0 Hz, H-3), 5.82 (t, 1 H, J = 9.9 Hz, H-4), 5.67 (dd, 1 H, J = 3.8, 10.2 Hz, H-2), 4.50−4.65 (m, 3 H, H-5 and H-6), δ (β-anomer) 6.00 (t, 1 H, J = 9.5 Hz, H-3); HRMS (FAB) calcd for $C_{41}H_{27}F_5NaO_{11}$ 813.1372 (M + Na^+), found 813.1384.

1,2,3,4,6-Penta-*O*-(4-fluorobenzoyl)-D-glucopyranose (11). Compound **11** was synthesized from D-glucose (300 mg, 1.67 mmol) as described for **9**, using 4-fluorobenzoyl chloride as acylating agent. Purification by flash column chromatography gave **11** (1.19 g, 90%): ^{1}H NMR ($CDCl_3$) α/β-ratio 1.3:1, δ (α-anomer) 6.82 (d, 1 H, J = 3.7 Hz, H-1), 6.23 (t, 1 H, J = 10.0 Hz, H-3), 5.75−5.85 (m, 1 H, H-4), 5.66 (dd, 1 H, J = 3.8, 10.3 Hz, H-2), δ (β-anomer) 6.26 (d, 1 H, J = 8.1 Hz, H-1), 5.99 (t, 1 H, J = 9.6 Hz, H-3), 5.75−5.85 (m, 1 H, H-4); HRMS (FAB) calcd for $C_{41}H_{27}F_5NaO_{11}$ 813.1372 (M + Na^+), found 813.1381.

1,2,3,4,6-Penta-*O*-(2,5-difluorobenzoyl)-D-glucopyranose (12). Compound **12** was synthesized from D-glucose (300 mg, 1.67 mmol) as described for **9**, using 2,5-difluorobenzoyl chloride as acylating agent. Purification by flash column chromatography gave **12** (1.33 g, 91%): ^{1}H NMR ($CDCl_3$) α/β-ratio 2.0:1, δ (α-anomer) 6.86 (d, 1 H, J = 3.6 Hz, H-1), 6.17 (t, 1 H, J = 9.9 Hz, H-3), 5.70−5.80 (m, 1 H, H-4), 5.63 (dd, 1 H, J = 3.6, 10.2 Hz, H-2), δ (β-anomer) 6.24 (d, 1 H, J = 7.9 Hz, H-1), 5.93 (t, 1 H, J = 9.2 Hz, H-3), 5.70−5.80 (m, 2 H, H-2 and H-4); HRMS (FAB) calcd for $C_{41}H_{22}F_{10}NaO_{11}$ 903.0900 (M + Na^+), found 903.0920.

2,3,4,6-Tetra-*O*-(2-fluorobenzoyl)-α-D-glucopyranosyl Bromide (14). Compound **9** (600 mg, 0.76 mmol) was dissolved in a mixture of acetic acid (6 mL) and acetic anhydride (2.25 mL), after which HBr in acetic acid (33%, 6 mL) was added. The solution was heated to 50 °C and stirred for 4.5 h. It was then diluted with CH_2Cl_2 (70 mL) and washed with water (100 mL) followed by saturated aqueous $NaHCO_3$ (100 mL). The organic phase was dried and concentrated, and the residue was purified by flash column chromatography on silica gel with heptane−EtOAc (4:1 → 2:1) to give **14** (517 mg, 93%): $[\alpha]_D^{20}$ +109 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.88 (d, 1 H, J = 4.0 Hz, H-1), 6.23 (t, 1 H, J = 9.8 Hz, H-3), 5.82 (t, 1 H, J = 9.8 Hz, H-4), 5.30 (dd, 1 H, J = 4.1, 10.0 Hz, H-2), 4.70 (m, 1 H, H-5), 4.55−4.70 (m, 2 H, H-6); HRMS (FAB) calcd for $C_{34}H_{23}BrF_4NaO_9$ 753.0360 (M + Na^+), found 753.034. Anal. Calcd for $C_{34}H_{23}BrF_4O_9$: C, 55.8; H, 3.2. Found: C, 56.0; H, 3.3.

2,3,4,6-Tetra-*O*-(3-fluorobenzoyl)-α-D-glucopyranosyl Bromide (15). Compound **10** (700 mg, 0.89 mmol) was treated and purified as in the synthesis of **14** to give **15** (620 mg, 96%): $[\alpha]_D^{20}$ +112 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.84 (d, 1 H, J = 4.1 Hz, H-1), 6.21 (t, 1 H, J = 9.8 Hz, H-3), 5.78 (t, 1 H, J = 10.0 Hz, H-4), 5.33 (dd, 1 H, J = 4.1, 9.9 Hz, H-2), 4.73 (m, 1 H, H-5), 4.66 (dd, 1 H, J = 2.8, 12.6 Hz, H-6), 4.53 (dd, 1 H, J = 4.3, 12.6 Hz, H-6); HRMS (FAB) calcd for $C_{34}H_{23}BrF_4NaO_9$ 753.0360 (M + Na^+), found 753.0346. Anal. Calcd for $C_{34}H_{23}BrF_4O_9$: C, 55.8; H, 3.2. Found: C, 55.9; H, 3.4.

2,3,4,6-Tetra-*O*-(4-fluorobenzoyl)-α-D-glucopyranosyl Bromide (16). Compound **11** (800 mg, 1.01 mmol) was treated and purified as in the synthesis of **14** to give **16** (660 mg, 89%): $[\alpha]_D^{20}$ +107 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.83 (d, 1 H, J = 4.0 Hz, H-1), 6.19 (t, 1 H, J = 9.8 Hz, H-3), 5.76 (t, 1 H, J = 10.0 Hz, H-4), 5.31 (dd, 1 H, J = 4.0, 10.0 Hz, H-2), 4.71 (m, 1 H, H-5), 4.65 (dd, 1 H, J = 2.6, 12.6 Hz, H-6), 4.50 (dd, 1 H, J = 4.4, 12.6 Hz, H-6); HRMS (FAB) calcd for $C_{34}H_{23}BrF_4NaO_9$ 753.0360 (M + Na^+), found 753.0346. Anal. Calcd for $C_{34}H_{23}BrF_4O_9$: C, 55.8; H, 3.2. Found: C, 56.0; H, 3.4.

2,3,4,6-Tetra-*O*-(2,5-difluorobenzoyl)-α-D-glucopyranosyl Bromide (17). Compound **12** (664 mg, 0.75 mmol) was treated and purified as in the synthesis of **14** to give **17** (573 mg, 95%): $[\alpha]^{20}_D$ +100 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.85 (d, 1 H, J = 4.1 Hz, H-1), 6.17 (t, 1 H, J = 9.8 Hz, H-3), 5.75 (t, 1 H, J = 9.7 Hz, H-4), 5.29 (dd, 1 H, J = 4.1, 9.9 Hz, H-2), 4.68 (m, 1 H, H-5), 4.60−4.65 (m, 2 H, H-6); HRMS (FAB) calcd for $C_{34}H_{19}BrF_8NaO_9$ 824.9983 (M + Na^+), found 824.9979. Anal. Calcd for $C_{34}H_{19}BrF_8O_9$: C, 50.8; H, 2.4. Found: C, 51.0; H, 2.5.

***N*$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,4,6-tetra-*O*-(2-fluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (19).** *N*$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester[42] (Fmoc-Ser-OPfp, 120 mg, 0.24 mmol), silver trifluoromethanesulfonate (105 mg, 0.41 mmol), and powdered molecular sieves (3 Å, 300 mg) in CH_2Cl_2 (10 mL) were protected from light and cooled to −30 °C for 30 min. Bromosugar **14** (250 mg, 0.34 mmol) in CH_2Cl_2 (5 mL) was then added dropwise and the mixture was stirred for 2 h at −30 °C before being allowed to attain room temperature during 3 h. The mixture was diluted with CH_2Cl_2 (80 mL), filtered (Hyflo SuperCel), and washed with saturated aqueous $NaHCO_3$ (100 mL). The aqueous phase was extracted with CH_2Cl_2 (50 mL) and the combined organic phases were dried and concentrated. The residue was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) to give **19** (205 mg, 74%): $[\alpha]^{20}_D$ −0.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.92 (t, 1 H, J = 9.6 Hz, H-3), 5.82 (d, 1 H, J = 8.4 Hz, Ser-NH), 5.69 (t, 1 H, J = 9.7 Hz, H-4), 5.53 (dd, 1 H, J = 8.0, 9.6 Hz, H-2), 4.90−4.95 (m, 1 H, Ser-Hα), 4.90 (d, 1 H, J = 8.0 Hz, H-1), 4.50−4.60 (m, 1 H, Ser-Hβ), 4.15−4.20 (m, 1 H, H-5), 4.08 (dd, 1 H, J = 3.3, 10.3 Hz, Ser-Hβ); HRMS (FAB) calcd for $C_{58}H_{38}F_9NaNO_{14}$ 1166.2047 (M + Na^+), found 1166.2036. Anal. Calcd for $C_{58}H_{38}F_9NO_{14}$: C, 60.9; H, 3.4; N, 1.2. Found: C, 61.4; H, 3.5; N, 1.3.

***N*$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,4,6-tetra-*O*-(3-fluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (20).** Bromosugar **15** (250 mg, 0.34 mmol) was reacted with Fmoc-Ser-OPfp (120 mg, 0.24 mmol) at room temperature for 7 h as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (3:1) to give **20** (196 mg, 71%): $[\alpha]^{20}_D$ −0.7 (*c* 0.4,

(49) Derome, A. E.; Williamson, M. P. *J. Magn. Reson.* **1990**, *88*, 177−185.

$CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.90 (t, 1 H, J = 9.7 Hz, H-3), 5.60−5.70 (m, 2 H, Ser-NH and H-4), 5.50 (dd, 1 H, J = 8.0, 9.6 Hz, H-2), 4.85−4.90 (m, 2 H, Ser-Hα and H-1), 4.45−4.55 (m, 1 H, Ser-Hβ), 4.10−4.20 (m, 1 H, H-5), 4.05 (dd, 1 H, J = 3.5, 10.3 Hz, Ser-Hβ); HRMS (FAB) calcd for $C_{58}H_{38}F_9NaNO_{14}$ 1166.2047 (M + Na^+), found 1166.2068. Anal. Calcd for $C_{58}H_{38}F_9NO_{14}$: C, 60.9; H, 3.4; N, 1.2. Found: C, 60.5; H, 3.5; N, 1.2.

N^α-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (21). Bromosugar **16** (500 mg, 0.68 mmol) was reacted with Fmoc-Ser-OPfp (240 mg, 0.49 mmol) as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (3:1) to give **21** (282 mg, 51%): $[\alpha]_D^{20}$ −0.7 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.86 (t, 1 H, J = 9.7 Hz, H-3), 5.64 (t, 1 H, J = 9.8 Hz, H-4), 5.63 (d, 1 H, J = 8.2 Hz, Ser-NH), 5.46 (dd, 1 H, J = 7.9, 9.7 Hz, H-2), 4.86 (m, 1 H, Ser-Hα), 4.82 (d, 1 H, J = 7.8 Hz, H-1), 4.40−4.50 (m, 1 H, Ser-Hβ), 4.10−4.20 (m, 1 H, H-5), 4.03 (dd, 1 H, J = 3.4, 10.3 Hz, Ser-Hβ); HRMS (FAB) calcd for $C_{58}H_{38}F_9NaNO_{14}$ 1166.2047 (M + Na^+), found 1166.2019. Anal. Calcd for $C_{58}H_{38}F_9NO_{14}$: C, 60.9; H, 3.4; N, 1.2. Found: C, 60.5; H, 3.3; N, 1.2.

N^α-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (22). Bromosugar **17** (225 mg, 0.28 mmol) was reacted with Fmoc-Ser-OPfp (100 mg, 0.20 mmol) as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (3:1) to give **22** (143 mg, 58%): $[\alpha]^{20}_D$ −2.8 (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.85 (t, 1 H, J = 9.5 Hz, H-3), 5.73 (d, 1 H, J = 8.3 Hz, Ser-NH), 5.64 (t, 1 H, J = 9.7 Hz, H-4), 5.47 (dd, 1 H, J = 8.1, 9.7 Hz, H-2), 4.93 (m, 1 H, Ser-Hα), 4.90 (d, 1 H, J = 8.0 Hz, H-1), 4.50−4.55 (m, 1 H, Ser-Hβ), 4.10−4.15 (m, 1 H, H-5), 4.07 (dd, 1 H, J = 3.4, 10.3 Hz, Ser-Hβ); HRMS (FAB) calcd for $C_{58}H_{34}F_{13}NaNO_{14}$ 1238.1670 (M + Na^+), found 1238.1665. Anal. Calcd for $C_{58}H_{34}F_{13}NO_{14}$: C, 57.3; H, 2.8; N, 1.2. Found: C, 57.3; H, 3.1; N, 1.1.

N^α-(Fluoren-9-ylmethoxycarbonyl)-N^α-methyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-serine Pentafluorophenyl Ester (23). Bromosugar **13** (340 mg, 0.52 mmol) was reacted with N^α-(fluoren-9-ylmethoxycarbonyl)-N^α-methyl-L-serine pentafluorophenyl ester (184 mg, 0.36 mmol) at −30 °C for 3 h, as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (5:1) to give **23** (361 mg, 91%): $[\alpha]^{20}_D$ +3.4 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) rotamer ratio, ∼2:1, major rotamer = δ 5.93 (t, 1 H, J = 9.7 Hz, H-3), 5.70 (t, 1 H, J = 9.7 Hz, H-4), 5.57 (dd, 1 H, J = 7.9, 9.7 Hz, H-2), 4.98 (d, 1 H, J = 7.9 Hz, H-1), 4.15−4.25 (m, 1 H, H-5), 2.97 (s, 3 H, N-Me); minor rotamer = δ 5.91 (t, 1 H, J = 9.7 Hz, H-3), 5.68 (t, 1 H, J = 9.2 Hz, H-4), 5,49 (dd, 1 H, J = 8.1, 9.7 Hz, H-2), 4.60−4.70 (m, 1 H, H-1), 4.05−4.10 (m, 1 H, H-5), 2.88 (s, 3 H, N-Me); HRMS (FAB) calcd for $C_{59}H_{44}F_5NaNO_{14}$ 1108.2580 (M + Na^+), found 1108.2593. Anal. Calcd for $C_{59}H_{44}F_5NO_{14}$: C, 65.3; H, 4.1; N, 1.3. Found: C, 65.4; H, 4.4; N, 1.2.

N^α-(Fluoren-9-ylmethoxycarbonyl)-N^α-methyl-3-*O*-[2,3,4,6-tetra-*O*-(3-fluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (24). Bromosugar **15** (250 mg, 0.34 mmol) was reacted with N^α-(fluoren-9-ylmethoxycarbonyl)-N^α-methyl-L-serine pentafluorophenyl ester (123 mg, 0.24 mmol) at 0 °C for 2.5 h, as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (3:1) to give **24** (244 mg, 87%): $[\alpha]^{20}_D$ −5.1 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) rotamer ratio, ∼2:1, major rotamer = δ 5.88 (t, 1 H, J = 9.6 Hz, H-3), 5.67 (t, 1 H, J = 9.8 Hz, H-4), 5.53 (dd, 1 H, J = 8.1, 9.6 Hz, H-2), 4.98 (d, 1 H, J = 7.6 Hz, H-1), 4.15−4.25 (m, 1 H, H-5), 2.98 (s, 3 H, N-Me); minor rotamer = δ 5.80−5.90 (m, 1 H, H-3), 5.64 (t, 1 H, J = 10.1 Hz, H-4), 5,45 (br t, 1 H, J = 8.8 Hz, H-2), 4.60−4.70 (m, 1 H, H-1), 4.06 (m, 1 H, H-5), 2.90 (s, 3 H, N-Me); HRMS (FAB) calcd for $C_{59}H_{40}F_9NaNO_{14}$ 1180.2203 (M + Na^+), found 1180.2233. Anal. Calcd for $C_{59}H_{40}F_9NO_{14}$: C, 61.2; H, 3.5; N, 1.2. Found: C, 61.2; H, 3.5; N, 1.3.

N^α-(Fluoren-9-ylmethoxycarbonyl)-N^α-methyl-3-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (25). Bromosugar **16** (377 mg, 0.52 mmol) was reacted with N^α-(fluoren-9-ylmethoxycarbonyl)-N^α-methyl-L-serine pentafluorophenyl ester (184 mg, 0.36 mmol) at −30 °C for 1.5 h, as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (5:1 to 3:1) to give **25** (362 mg, 86%): $[\alpha]^{20}_D$ −6.7 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) rotamer ratio, ∼2:1, major rotamer = δ 5.84 (t, 1 H, J = 9.7 Hz, H-3), 5.64 (t, 1 H, J = 9.7 Hz, H-4), 5.50 (dd, 1 H, J = 7.9, 9.7 Hz, H-2), 4.96 (d, 1 H, J = 7.9 Hz, H-1), 4.10−4.20 (m, 1 H, H-5), 2.96 (s, 3 H, N-Me); minor rotamer = δ 5.82 (t, 1 H, J = 9.6 Hz, H-3), 5.63 (t, 1 H, J = 9.7 Hz, H-4), 5,43 (dd, 1 H, J = 8.0, 9.7 Hz, H-2), 4.68 (d, 1 H, J = 7.7 Hz, H-1), 4.06 (m, 1 H, H-5), 2.90 (s, 3 H, N-Me); HRMS (FAB) calcd for $C_{59}H_{40}F_9NaNO_{14}$ 1180.2203 (M + Na^+), found 1180.2212. Anal. Calcd for $C_{59}H_{40}F_9NO_{14}$: C, 61.2; H, 3.5; N, 1.2. Found: C, 61.2; H, 3.7; N, 1.2.

N^α-(Fluoren-9-ylmethoxycarbonyl)-N^α-methyl-3-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl)-β-D-glucopyranosyl]-L-serine Pentafluorophenyl Ester (26). Bromosugar **17** (275 mg, 0.34 mmol) was reacted with N^α-(fluoren-9-ylmethoxycarbonyl)-N^α-methyl-L-serine pentafluorophenyl ester (123 mg, 0.24 mmol) at −30 °C for 3 h, as described for **19**. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) followed by heptane−EtOAc (3:1) to give **26** (209 mg, 70%): $[\alpha]^{20}_D$ −4.1 (*c* 0.4, $CHCl_3$); ^1H NMR ($CDCl_3$) rotamer ratio, ∼2:1, major rotamer = δ 5.84 (t, 1 H, J = 9.6 Hz, H-3), 5.63 (t, 1 H, J = 9.6 Hz, H-4), 5.49 (dd, 1 H, J = 7.9, 9.6 Hz, H-2), 4.95 (d, 1 H, J = 7.9 Hz, H-1), 4.10−4.20 (m, 1 H, H-5), 3.02 (s, 3 H, N-Me); minor rotamer = δ 5.75−5.85 (m, 1 H, H-3), 5.59 (t, 1 H, J = 9.6 Hz, H-4), 5.39 (br t, 1 H, J = 8.9 Hz, H-2), 4.50−4.60 (m, 1 H, H-1), 3.97 (m, 1 H, H-5), 2.92 (s, 3 H, N-Me); HRMS (FAB) calcd for $C_{59}H_{36}F_{13}NaNO_{14}$ 1252.1826 (M + Na^+), found 1252.1855. Anal. Calcd for $C_{59}H_{36}F_{13}NO_{14}$: C, 57.6; H, 3.0; N, 1.1. Found: C, 57.8; H, 3.1; N, 1.2.

N^α-Acetyl-L-alanyl-N^α-methyl-3-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl)-L-seryl-L-phenylalanine Amide (27). Glycopeptide **27** was synthesized manually in a mechanically agitated reactor on a Tentagel S NH_2^{TM} resin (80 μmol) functionalized with the Rink amide linker, *p*-[α-(fluoren-9-ylmethoxyformamido)-2,4-dimethoxybenzyl]phenoxyacetic acid.[43] N^α-Fmoc-Phe-OH (0.24 mmol) was coupled as a 1-benzotriazolyl ester, prepared in situ by reaction with 1-hydroxybenzotriazole (HOBt, 0.36 mmol) and 1,3-diisopropylcarbodiimide (DIC, 0.23 mmol) in DMF (1.5 mL). The glycosylated serine derivative **23** (0.12 mmol) was coupled in the presence of HOBt (0.36 mmol). N^α-Fmoc-Ala-OH (0.48 mmol) was coupled with DIC (0.46 mmol) in the presence of HOAt (0.72 mmol). The couplings were monitored by addition of Bromophonol Blue[50] (200 nmol) to the reactor. Cleavage of the Fmoc-group was performed using 20% piperidine in DMF (4 min flow) and washing was done with DMF. After the final Fmoc cleavage the *N*-terminus was acetylated by the addition of acetic anhydride (0.4 mL) in DMF (1.5 mL). The glycopeptide resin was washed with DMF and dichloromethane and then dried under vacuum. Cleavage from the resin was affected with $CF_3CO_2H-H_2O$ (9:1) and glycopeptide **27** was obtained in 48% yield after purification by flash chromatography on silica gel with EtOAc−MeOH−HOAc (16:1:1) followed by reversed-phase HPLC. Glycopeptide **27** had the following characteristics: ^1H NMR (acetone-d_6) major rotamer = δ Ala 7.85−7.95 (m, 1 H, NH), 4.75 (t, 1 H, J = 6.6 Hz, H-α), 1.21 (d, 3 H, J = 6.9 Hz, Me); Ser 5.13 (dd, 1 H, J = 4.1, 10.3 Hz, H-α), 4.13 (t, 1 H, J = 10.6 Hz, H-β), 4.01 (dd, 1 H, J = 4.1, 10.7 Hz, H-β); Phe 8.44 (d, 1 H, J = 8.6 Hz, NH), 4.45−4.55 (m, 1 H, H-α), 3.28 (dd, 1 H, J = 3.6, 13.9 Hz, H-β), 2.91 (dd, 1 H, J = 11.5,

(50) Flegel, M.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1990**, 536−538.

13.9 Hz, H-β); Glc 5.98 (t, 1 H, J = 9.6 Hz, H-3), 5.69 (t, 1 H, J = 9.7 Hz, H-4), 5.44 (dd, 1 H, J = 8.1, 9.7 Hz, H-2), 5.25 (d, 1 H, J = 8.0 Hz, H-1), 4.50−4.60 (m, 1 H, H-5); minor rotamer = δ Ala 1.15 (d, 3 H, J = 6.8 Hz, Me); Ser 4.80 (m, 1 H, H-α), 4.26 (dd, 1 H, J = 8.6, 10.6 Hz, H-β); Phe 3.16 (dd, 1 H, J = 5.1, 14.2 Hz, H-β); Glc 6.01 (t, 1 H, J = 9.6 Hz, H-3), 5.75 (t, 1 H, J = 9.7 Hz, H-4), 5.24 (d, 1 H, J = 7.9 Hz, H-1). MS (TOF ES$^+$) calcd for $C_{53}H_{52}N_4O_{14}$ 957.4 (M + H$^+$), found 957.5.

***N*$^\alpha$-Acetyl-L-alanyl-*N*$^\alpha$-methyl-3-*O*-[2,3,4,6-tetra-*O*-(3-fluorobenzoyl)-β-D-glucopyranosyl]-L-seryl-L-phenylalanine Amide (28).** Glycopeptide **28** was prepared from Fmoc-Phe-OH, Fmoc-Ala-OH, and compound **24** as described for **27**. Purification, using the same conditions as for **27**, gave **28** in 68% yield. Glycopeptide **28** had the following characteristics: ^{1}H NMR (acetone-d_6) major rotamer = δ Ala 7.75−7.85 (m, 1 H, NH), 4.74 (t, 1 H, J = 6.6 Hz, H-α), 1.19 (d, 3 H, J = 6.9 Hz, Me); Ser 5.12 (dd, 1 H, J = 4.2, 10.2 Hz, H-α), 4.12 (t, 1 H, J = 10.5 Hz, H-β), 4.02 (dd, 1 H, J = 4.1, 10.6 Hz, H-β); Phe 8.39 (d, 1 H, J = 8.5 Hz, NH), 4.45−4.55 (m, 1 H, H-α), 3.27 (dd, 1 H, J = 3.6, 13.9 Hz, H-β), 2.90 (dd, 1 H, J = 11.5, 14.0 Hz, H-β); Glc 5.98 (t, 1 H, J = 9.5 Hz, H-3), 5.75 (t, 1 H, J = 9.6 Hz, H-4), 5.45 (dd, 1 H, J = 8.1, 9.6 Hz, H-2), 5.27 (d, 1 H, J = 8.0 Hz, H-1), 4.45−4.55 (m, 1 H, H-5); minor rotamer = δ Ala 1.14 (d, 3 H, J = 6.9 Hz, Me); Ser 4.80−4.85 (m, 1 H, H-α), 4.20−4.30 (m, 1 H, H-β); Phe 3.16 (dd, 1 H, J = 5.3, 14.2 Hz, H-β), 2.90−3.00 (m, 1 H, H-β); Glc 6.00−6.05 (m, 1 H, H-3), 5.80 (t, 1 H, J = 9.6 Hz, H-4), 5.24 (d, 1 H, J = 8.0 Hz, H-1). MS (TOF ES$^+$) calcd for $C_{53}H_{48}F_4N_4O_{14}$ 1029.3 (M + H$^+$), found 1029.4.

***N*$^\alpha$-Acetyl-L-alanyl-*N*$^\alpha$-methyl-3-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl-β-D-glucopyranosyl]-L-seryl-L-phenylalanine Amide (29).** Glycopeptide **29** was prepared from Fmoc-Phe-OH, Fmoc-Ala-OH, and compound **25** as described for **27**. Purification, using the same conditions as for **27**, gave **29** in 64% yield. Glycopeptide **29** had the following characteristics: ^{1}H NMR (acetone-d_6) major rotamer = δ Ala 7.89 (d, 1 H, J = 5.9 Hz, NH), 4.74 (t, 1 H, J = 6.6 Hz, H-α), 1.19 (d, 3 H, J = 6.9 Hz, Me); Ser 5.12 (dd, 1 H, J = 4.2, 10.3 Hz, H-α), 4.11 (t, 1 H, J = 10.5 Hz, H-β), 4.00 (dd, 1 H, J = 4.1, 10.7 Hz, H-β); Phe 8.44 (d, 1 H, J = 8.7 Hz, NH), 4.45−4.55 (m, 1 H, H-α), 3.23 (dd, 1 H, J = 3.5, 14.0 Hz, H-β), 2.90 (dd, 1 H, J = 11.6, 13.9 Hz, H-β); Glc 5.93 (t, 1 H, J = 9.5 Hz, H-3), 5.66 (t, 1 H, J = 9.7 Hz, H-4), 5.40 (dd, 1 H, J = 8.0, 9.6 Hz, H-2), 5.24 (d, 1 H, J = 8.0 Hz, H-1), 4.50−4.60 (m, 1 H, H-5); minor rotamer = δ Ala 1.14 (d, 3 H, J = 6.7 Hz, Me); Ser 4.82 (dd, 1 H, J = 5.4, 8.2 Hz, H-α), 4.21 (dd, 1 H, J = 8.3, 10.5 Hz, H-β); Phe 3.16 (dd, 1 H, J = 5.3, 14.2 Hz, H-β); Glc 5.96 (t, 1 H, J = 9.7 Hz, H-3), 5.72 (t, 1 H, J = 9.6 Hz, H-4), 5.22 (d, 1 H, J = 7.9 Hz, H-1). MS (TOF ES$^+$) calcd for $C_{53}H_{48}F_4N_4O_{14}$ 1029.3 (M + H$^+$), found 1029.4.

***N*$^\alpha$-Acetyl-L-alanyl-*N*$^\alpha$-methyl-3-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl-β-D-glucopyranosyl]-L-seryl-L-phenylalanine Amide (30).** Glycopeptide **30** was prepared from Fmoc-Phe-OH, Fmoc-Ala-OH and compound **26** as described for **27**. Purification, using the same conditions as for **27**, gave **30** in 48% yield. Glycopeptide **30** had the following characteristics: ^{1}H NMR (acetone-d_6) major rotamer = δ Ala 7.84 (d, 1 H, J = 5.9 Hz, NH), 4.77 (t, 1 H, J = 6.6 Hz, H-α), 1.22 (d, 3 H, J = 6.9 Hz, Me); Ser 5.15 (dd, 1 H, J = 4.4, 10.0 Hz, H-α), 4.11 (t, 1 H, J = 10.7 Hz, H-β), 4.04 (dd, 1 H, J = 4.3, 10.6 Hz, H-β); Phe 8.38 (d, 1 H, J = 8.7 Hz, NH), 4.45−4.55 (m, 1 H, H-α), 3.26 (dd, 1 H, J = 3.5, 13.9 Hz, H-β), 2.91 (dd, 1 H, J = 11.3, 14.0 Hz, H-β); Glc 5.93 (t, 1 H, J = 9.5 Hz, H-3), 5.72 (t, 1 H, J = 9.6 Hz, H-4), 5.42 (dd, 1 H, J = 8.1, 9.5 Hz, H-2), 5.22 (d, 1 H, J = 8.0 Hz, H-1), 4.55−4.65 (m, 1 H, H-5); minor rotamer = δ Ala 1.15 (d, 3 H, J = 6.8 Hz, Me); Ser 4.88 (dd, 1 H, J = 5.6, 8.6 Hz, H-α), 4.22 (dd, 1 H, J = 8.7, 10.2 Hz, H-β); Phe 3.17 (dd, 1 H, J = 5.1, 14.0 Hz, H-β), 2.95−3.00 (m, 1 H, H-β); Glc 5.96 (t, 1 H, J = 9.5 Hz, H-3), 5.74 (t, 1 H, J = 9.8 Hz, H-4), 5.19 (d, 1 H, J = 8.1 Hz, H-1). MS (TOF ES$^+$) calcd for $C_{53}H_{44}F_8N_4O_{14}$ 1101.3 (M + H$^+$), found 1101.4.

***N*$^\alpha$-Acetyl-L-alanyl-3-*O*-β-D-glucopyranosyl-L-seryl-L-phenylalanine Amide (3) and *N*$^\alpha$-acetyl-L-alanyl-(2-aminoprop-2-enoyl)-L-phenylalanine Amide (31).** The following general procedure was used for deprotection of glycopeptides **27**−**30**. The protected glycopeptide (20 mg) was dissolved in MeOH (20 mL), after which methanolic sodium methoxide (0.6 mL, 0.2 M) was added. The solution was stirred at room temperature and samples (each 50 μL) were taken from the solution. After addition of HOAc (50 μL) the samples were concentrated, dissolved in acetonitrile-water (1:1, 100 μL), and then analyzed with analytical reversed-phase HPLC using a linear gradient of 100% A to 100% B in 30 min. When all starting material had been converted to a mixture of **3** and **31**, the deprotection was quenched by addition of HOAc (200 μL) which gave pH ∼4−5. After concentration the residue was purified with reversed-phase HPLC using a linear gradient of 100% A to 100% B in 60 min to give **3** and **31**.

Compound **3** had: ^{1}H NMR (MeOH-d_4) major rotamer = δ Ala 4.90−4.80 (m, 1 H, H-α), 1.34 (d, 3 H, J = 6.9 Hz, Me); Ser 5.22 (dd, 1 H, J = 4.4, 9.5 Hz, H-α), 3.90−4.05 (m, 2 H, H-β,β'); Phe 8.49 (d, 1 H, J = 8.3 Hz, NH), 4.65−4.55 (m, 1 H, H-α), 3.00−2.90 (m, 1 H, H-β); Glc 4.27 (d, 1 H, J = 7.8 Hz, H-1), 3.08 (dd, 1 H, J = 7.8, 9.1 Hz, H-2); minor rotamer = δ Ala 4.70 (q, 1 H, J = 6.9 Hz, H-α), 1.23 (d, 3 H, J = 7.0 Hz, Me); Ser 5.13 (t, 1 H, J = 6.9 Hz, H-α), 4.05−3.90 (m, 2 H, H-β,β'); Phe 7.93 (d, 1 H, J = 7.8 Hz, NH), 4.65−4.55 (m, 1 H, H-α), 3.00−2.90 (m, 1 H, H-β); Glc 4.29 (d, 1 H, J = 8.5 Hz, H-1), 3.13 (dd, 1 H, J = 7.9, 9.1 Hz, H-2); HRMS (FAB) calcd for $C_{24}H_{36}O_{10}NaN_4$ 563.2329 (M + Na$^+$), found 563.2328.

Compound **31** had ^{1}H NMR data in agreement with data reported previously.[31]

1,2,3,6-Tetra-*O*-(4-fluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-β-D-galactopyranosyl]-D-glucopyranose (33). Reaction of D-lactose (1.00 g, 2.92 mmol) with 4-fluorobenzoyl chloride and purification, as described for **9**, gave **33** (3.82 g, 99%). Compound **33** had the following characteristics: ^{1}H NMR ($CDCl_3$) α-anomer δ 6.72 (d, 1 H, J = 3.8 Hz, H-1), 6.15 (m, 1 H, H-3), 5.65−5.75 (m, 1 H, H-2′), 5.58 (dd, 1 H, J = 3.8, 10.3 Hz, H-2), 5.40 (dd, 1 H, J = 3.2, 10.4 Hz, H-3′), 4.94 (d, 1 H, J = 7.9 Hz, H-1′), 4.25−4.35 (m, 1 H, H-4), 4.05−4.15 (m, 1 H, H-5); β-anomer δ 6.13 (d, 1 H, J = 8.2 Hz, H-1), 5.91 (t, 1 H, J = 9.3 Hz, H-3), 5.75 (d, 1 H, J = 3.3 Hz, H-4′), 5.65−5.75 (m, 2 H, H-2,2′), 5.41 (dd, 1 H, J = 3.4, 10.4 Hz, H-3′), 4.90 (d, 1 H, J = 7.9 Hz, H-1′), 4.25−4.35 (m, 1 H, H-4), 4.05−4.15 (m, 1 H, H-5); HRMS (FAB) calcd for $C_{68}H_{46}F_8NaO_{19}$ 1341.2404 (M + Na$^+$), found 1341.2421.

1,2,3,6-Tetra-*O*-(2,5-difluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl]-D-glucopyranose (34). Reaction of lactose (250 mg, 0.73 mmol) with 2,5-difluorobenzoyl chloride at 70 °C, as described for **9**, and purification gave **34** (1.03 g, 97%). Compound **34** had the following characteristics: ^{1}H NMR ($CDCl_3$) α-anomer δ 6.73 (d, 1 H, J = 3.7 Hz, H-1), 6.08 (t, 1 H, J = 9.8 Hz, H-3), 5.71 (dd, 1 H, J = 8.0, 10.3 Hz, H-2′), 5.45−5.50 (m, 2 H, H-2 and H-3′), 5.09 (d, 1 H, J = 8.0 Hz, H-1′), 4.48 (t, 1 H, J = 9.7 Hz, H-4), 4.30−4.40 (m, 1 H, H-5); β-anomer δ 6.10 (d, 1 H, J = 7.7 Hz, H-1), 5.84 (t, 1 H, J = 8.9 Hz, H-3), 5.80 (d, 1 H, J = 3.2 Hz, H-4′), 5.70 (dd, 1 H, J = 8.0, 10.3 Hz, H-2′), 5.65 (dd, 1 H, J = 7.8, 8.8 Hz, H-2), 5.48 (dd, 1 H, J = 3.3, 10.4 Hz, H-3′), 5.05 (d, 1 H, J = 8.0 Hz, H-1′), 4.55 (t, 1 H, J = 9.5 Hz, H-4), 4.05−4.10 (m, 1 H, H-5); HRMS (FAB) calcd for $C_{68}H_{38}F_{16}NaO_{19}$ 1485.1650 (M + Na$^+$), found 1485.1635.

2,3,6-Tri-*O*-(4-fluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-β-D-galactopyranosyl]-α-D-glucopyranosyl Bromide (35). Compound **33** (1.00 g, 0.76 mmol) was treated at room temperature for 4 h, as descibed for **14**, and purified to give **35** (870 mg, 91%): $[\alpha]^{20}_D$ +99 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, 1 H, J = 4.1 Hz, H-1), 6.09 (t, 1 H, J = 9.6 Hz, H-3), 5.74 (d, 1 H, J = 3.4 Hz, H-4′), 5.68 (dd, 1 H, J = 7.9, 10.4 Hz, H-2′), 5.39 (dd, 1 H, J = 3.4, 10.4 Hz, H-3′), 5.23 (dd, 1 H, J = 4.1, 9.9 Hz, H-2), 4.93 (d, 1 H, J = 7.9 Hz, H-1′), 4.59 (dd, 1 H, J = 3.8, 12.5 Hz, H-6), 4.53 (dd, 1 H, J = 2.0, 12.4 Hz, H-6), 4.44 (m, 1 H, H-5), 4.24 (t, 1 H, J = 9.7 Hz, H-4); HRMS (FAB) calcd for $C_{61}H_{42}BrF_7NaO_{17}$ 1281.1392 (M + Na$^+$), found 1281.1410. Anal. Calcd for $C_{61}H_{42}BrF_7O_{17}$: C, 58.2; H, 3.4. Found: C, 57.9; H, 3.6.

2,3,6-Tri-*O*-(2,5-difluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl]-α-D-glucopyranosyl Bromide (36). Compound **34** (900 mg, 0.62 mmol)

was treated at 50 °C for 2 h, as described for **14**, and purified to give **36** (732 mg, 86%): $[\alpha]^{20}_D$ +86 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.73 (d, 1 H, *J* = 4.1 Hz, H-1), 6.06 (t, 1 H, *J* = 9.5 Hz, H-3), 5.80 (d, 1 H, *J* = 3.4 Hz, H-4′), 5.70 (dd, 1 H, *J* = 8.0, 10.2 Hz, H-2′), 5.47 (dd, 1 H, *J* = 3.4, 10.3 Hz, H-3′), 5.15 (dd, 1 H, *J* = 4.1, 10.0 Hz, H-2), 5.08 (d, 1 H, *J* = 8.0 Hz, H-1′), 4.46 (t, 1 H, *J* = 9.5 Hz, H-4); HRMS (FAB) calcd for $C_{61}H_{35}BrF_{14}NaO_{17}$ 1407.0732 (M + Na^+), found 1407.0713. Anal. Calcd for $C_{61}H_{35}BrF_{14}O_{17}$: C, 52.9; H, 2.6. Found: C, 52.8; H, 2.7.

***N*$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-{2,3,6-tri-*O*-(4-fluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl}-L-serine Pentafluorophenyl Ester (38).** Bromosugar **35** (156 mg, 0.12 mmol) was reacted with Fmoc-Ser-OPfp (50 mg, 0.10 mmol) as described for **19**. The crude product was purified by repeated flash chromatography on silica gel with heptane−EtOAc (4:1 to 2:1) to give **38** (100 mg, 59%): $[\alpha]^{20}_D$ +22 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.76 (t, 1 H, *J* = 9.4 Hz, H-3), 5.72 (d, 1 H, *J* = 3.4 Hz, H-4′), 5.67 (dd, 1 H, *J* = 7.9, 10.4 Hz, H-2′), 5.52 (d, 1 H, *J* = 8.2 Hz, Ser-NH), 5.38 (dd, 1 H, *J* = 3.4, 10.3 Hz, H-3′), 5.34 (dd, 1 H, *J* = 7.5, 9.6 Hz, H-2), 4.86 (d, 1 H, *J* = 7.9 Hz, H-1′), 4.82 (m, 1 H, Ser-Hα), 4.67 (d, 1 H, *J* = 7.8 Hz, H-1), 4.35−4.45 (m, 1 H, Ser-Hβ), 4.10−4.20 (m, 1 H, H-4), 3.85−3.95 (m, 1 H, Ser-Hβ); HRMS (FAB) calcd for $C_{85}H_{57}F_{12}NaNO_{22}$ 1694.3079 (M + Na^+), found 1694.3110. Anal. Calcd for $C_{85}H_{57}F_{12}NO_{22}$: C, 61.1; H, 3.4; N, 0.8. Found: C, 61.0; H, 3.7; N, 0.9

***N*$^\alpha$-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-{2,3,6-tri-*O*-(2,5-difluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl}-L-serine Pentafluorophenyl Ester (39).** Bromosugar **36** (352 mg, 0.28 mmol) was reacted with Fmoc-Ser-OPfp (100 mg, 0.20 mmol) as described for **19** at 0 °C for 2 h. The crude product was purified by flash chromatography on silica gel with toluene−CH_3CN (30:1 → 25:1) to give building block **39** (232 mg, 64%): $[\alpha]^{20}_D$ +20 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.81 (d, 1 H, *J* = 3.3 Hz, H-4′), 5.77 (t, 1 H, *J* = 9.5 Hz, H-3), 5.72 (d, 1 H, *J* = 8.4 Hz, Ser-NH), 5.71 (dd, 1 H, *J* = 7.9, 10.3 Hz, H-2′), 5.50 (dd, 1 H, *J* = 3.4, 10.3 Hz, H-3′), 5.38 (dd, 1 H, *J* = 8.1, 9.5 Hz, H-2), 5.05 (d, 1 H, *J* = 8.0 Hz, H-1′), 4.90 (m, 1 H, Ser-Hα), 4.78 (d, 1 H, *J* = 7.9 Hz, H-1), 4.48 (dd, 1 H, *J* = 2.9, 10.2 Hz, Ser-Hβ), 4.35−4.45 (m, 1 H, H-4), 3.90−4.00 (m, 1 H, Ser-Hβ); HRMS (FAB) calcd for $C_{85}H_{50}F_{19}NaNO_{22}$ 1820.2419 (M + Na^+), found 1820.2410. Anal. Calcd for $C_{85}H_{50}F_{19}NO_{22}$: C, 56.8; H, 2.8; N, 0.8. Found: C, 56.7; H, 3.0; N, 0.8.

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-{2,3,6-tri-*O*-(4-fluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(4-fluorobenzoyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl}-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine Amide (40). Synthesis was performed on a TentaGel S NH_2 resin (30 μmol) as described previously,[24] followed by purification by preparative reversed phase HPLC, to give **40** (32 mg, 46% based on resin capacity). Glycopeptide **40** had the following characteristics: MS (TOF ES^+) calcd for $C_{109}H_{121}F_7N_{16}O_{34}$ 2330.8 (M + $2H^+$), found 2331.0.

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-{2,3,6-tri-*O*-(2,5-difluorobenzoyl)-4-*O*-[2,3,4,6-tetra-*O*-(2,5-difluorobenzoyl)-β-D-galactopyranosyl]-β-D-glucopyranosyl}-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine Amide (41). Synthesis was performed on a TentaGel S NH_2 resin (40 μmol) as described previously,[24] followed by purification by preparative reversed-phase HPLC, to give **41** (60 mg, 61% based on resin capacity). Glycopeptide **41** had the following characteristics: MS (TOF ES^+) calcd for $C_{109}H_{114}F_{14}N_{16}O_{34}$ 2456.7 (M + $2H^+$), found 2457.0.

General Procedure for the Deacylation of Glycopeptides 5, 40, and 41. The time-course of deacylation of the glycopeptides was first investigated on an analytical scale using methanolic lithium hydroxide or ammonia as base in the following manner: (a) Methanolic lithium hydroxide (0.1 mL, 0.2 M) was added to a solution of each of glycopeptides **5**, **40**, and **41** (1 mg) in MeOH (0.9 mL). The solutions were stirred at room temperature and samples (each 50 μL) were taken from the solutions. After addition of HOAc (50 μL) the samples were concentrated. The residues were dissolved in acetonitrile−water (1:1, 100 μL) and then analyzed with analytical reversed-phase HPLC (100% A to 100% B over 30 min). (b) Glycopeptides **5**, **40**, and **41** (1 mg) were also dissolved in saturated methanolic ammonia (1.5 mL) and the reactions were monitored in the same way.

On a preparative scale glycopeptide **41** (10 mg) was dissolved in methanolic lithium hydroxide (10 mL, 0.02 M). The solution was stirred for 1 h before HOAc (200 μL) was added. After concentration the residue was purified with preparative reversed-phase HPLC using a linear gradient of 100% A to 100% B in 60 min to give **6** (4.8 mg, 41% based on resin capacity and peptide content). Compound **6** had MS (FAB) and ^{1}H NMR data in agreement with those reported previously.[24]

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Supporting Information Available: Copies of ^{1}H NMR spectra for compounds **9**−**12**, **27**−**30**, **3**, **33**, and **34**. This material is available free of charge via the Internet at http://pubs.acs.org.

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