Synthesis of deoxy and alanine-substituted derivatives of a T cell stimulating glycopeptide—An investigation of conditions for cleavage from the solid phase and deprotection

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Little is known concerning how T cells recognize glycopeptides presented by MHC molecules on antigen-presenting cells. In order to probe the specificity of helper T cells elicited on immunization of mice with glycopeptide **1**, which has the disaccharide galabiose [Galα(1→4)Galβ] *O*-linked to serine 56 in the hen egg lysozyme peptide HEL(52–61), we have prepared three sets of glycopeptides. These are: (*i*) the 6- and 6′-deoxygalabiose analogs of **1**, (*ii*) two glycopeptides in which the galabiose moiety of **1** has been replaced by galactose and lactose, respectively, and (*iii*) an alanine-scan series of **1** in which all amino acid residues, apart from 54 and 56, were replaced by alanine, one by one. Two deoxygenated galabiose donors, activated either as an anomeric trichloroacetimidate or as a β-acetate, were used for glycosylation of Fmoc-Ser-OPfp. The resulting, and other, glycosylated amino acids were then used as building blocks in solid-phase synthesis of the target glycopeptides. It was found that improved yields of glycopeptides could be obtained if cleavage from the solid phase was performed at 40 °C instead of at room temperature. In the final base-catalyzed deprotection of the carbohydrate moiety, removal of *O*-benzoyl groups was accompanied by substantial β-elimination. For one of the glycopeptides even deacetylation required carefully controlled conditions in order to avoid β-elimination.

Introduction

Processing of protein antigens into shorter peptides is an important event in eliciting a response from the immune system of higher vertebrates to infections caused by bacteria and viruses.[1–4] Proteins from such infectious agents are internalized by antigen-presenting cells, such as macrophages and B cells, and degraded into peptides which are usually 14–18 amino acids long. The peptides are then complexed by class II Major Histocompatibility Complex (MHC) molecules and transported to the cell surface, where the complexes are displayed to the surrounding environment. Recognition of the complexes by circulating helper T cells results in an immune response directed toward the antigen, which serves to eliminate the infection from the organism.

Carbohydrate antigens usually give a weak, T cell independent immune response, an observation which is explained by the inability of carbohydrates to bind MHC molecules.[5,6] Very little is known about the immune response towards glycoproteins and, to date, only a few examples of naturally processed glycopeptides presented by MHC molecules are known.[7–9] The only well characterized system involves processing of type II collagen from joint cartilage into a set of glycopeptides, which are recognized by autoimmune helper T cells in a mouse model for rheumatoid arthritis.[7,8] In addition, a number of model studies with synthetic neoglycopeptides show how mono- or small oligosaccharides can be attached to immunogenic peptides without loss of MHC binding (reviewed in references [10–12]). On immunization of mice some of these glycopeptides gave T cells which specifically recognized the carbohydrate moiety.[6,13–18] Compound **1** is such a glycopeptide[14] which consists of residues 52–61 from hen egg lysozyme [HEL(52–61)] and has a β-D-galabiose unit *O*-linked to Ser[56] which replaces the native Leu[56] (Chart 1). To allow more detailed studies of the interactions in the complex of **1** with the I-A^k class II MHC molecule and the T cell receptor we have now synthesized the 6- and 6′-deoxygalabiose analogs of **1**. Two glycopeptides, one with galactose and the other with lactose *O*-linked to Ser[56] in HEL(52–61), have also been synthesized, as well as an alanine-scan series of **1** in which all amino acid residues of **1**, apart from numbers 54 and 56, were replaced by alanine, one by one.

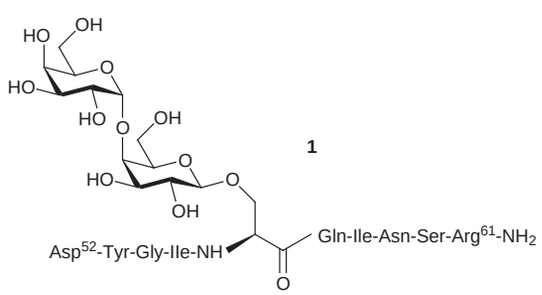
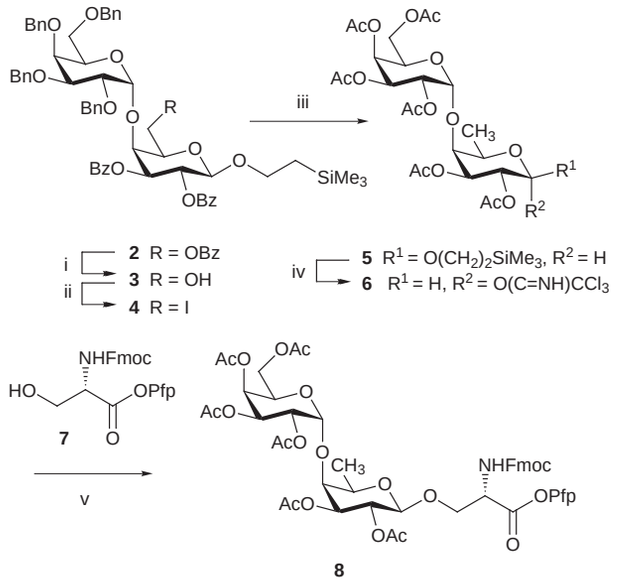


Chart 1 Immunization of mice with neoglycopeptide **1** elicited helper T cells which were specific for the carbohydrate moiety of **1**.[14,51]

Results and discussion

Synthesis of the 6-deoxy-β-D-galabiosylserine building block

2-(Trimethylsilyl)ethyl (TMSEt) galabioside **2**[19] was regioselectively debenzoylated at O-6 using methanolic sodium methoxide to give **3** (34%, Scheme 1). Unchanged **2** was recovered and debenzoylated once more thereby raising the total yield of **3** to 42%. Treatment[20] of **3** with triphenylphosphine, imidazole and iodine in toluene gave 6-deoxy-6-iodogalabioside **4** (89%). Reduction of the iodogalabioside, and



Scheme 1 *Reagents and conditions* (*and yields*): i) NaOMe, MeOH, 0 °C (42%); ii) I_2, imidazole, Ph_3P, toluene, 80 °C (89%); iii) H_2, Pd/C, HOAc; then NaOMe, MeOH; then Ac_2O, pyridine (63%); iv) TFA, CH_2Cl_2; then CCl_3CN, DBU, 0 °C (60%); v) AgOTf, CH_2Cl_2, 0 °C (39%).

simultaneous cleavage of the benzyl ether protecting groups by catalytic hydrogenation over Pd/C, gave a crude product which was used in the next step without purification. Subsequent removal of the benzoyl protective groups with methanolic sodium methoxide, followed by acetylation with acetic anhydride and pyridine, gave 6-deoxy derivative **5** (63% yield from **4**).

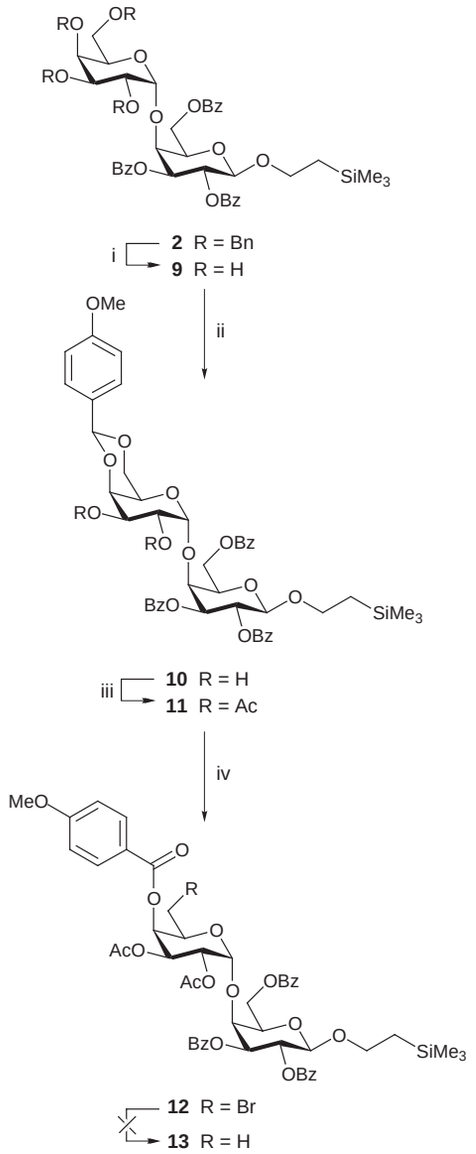
Others[21] have reported problems involving interglycosidic bond cleavage when converting deoxygenated TMSEt-glycosides into the corresponding anomeric acetates. In order to avoid this potential side-reaction, **5** was treated with trifluoroacetic acid (TFA) in dichloromethane to give the corresponding hemiacetal,[22] which was converted[23] into trichloroacetimidate **6** (60%) using trichloroacetonitrile and DBU. Glycosyl donor **6** was then used in a silver trifluoromethanesulfonate-promoted glycosylation of Fmoc-Ser-OPfp **7** to give the glycosylated building block **8** (39% after purification by normal-phase HPLC).

Synthesis of the 6′-deoxy-β-D-galabiosylserine building block

The first attempt to prepare a 6′-deoxy-β-D-galabiosyl building block started with hydrogenolysis of the benzyl ethers of **2** to give derivative **9** (Scheme 2). Protection of the 4′- and 6′-hydroxy groups as a 4′,6′-methoxybenzylidene acetal (see **10**), followed by acetylation gave fully protected disaccharide **11**. Regioselective opening of the methoxybenzylidene acetal[24] by treatment with copper(II) bromide, tetrabutylammonium bromide (TBAB) and DDQ in dichloromethane gave the bromo sugar **12** (74% yield from **2**).

Several attempts at reductive removal of the bromine atom in **12** were made, but none of them was successful. Treatment with tributyltin hydride, or with hydrogen over Pd/C, failed to give 6′-deoxy derivative **13** and unchanged **12** was recovered. This was also the case when hydrogenation was attempted at high pressure (100 psi). The difficulties in reduction of **12** may be due to steric hindrance from the bulky protecting groups. Attempted removal of the protecting groups with sodium methoxide, however, gave a 3′,6′-anhydro derivative by intramolecular substitution at C-6′ by O-3′. Due to these problems an alternative route to the 6′-deoxy building block was developed.

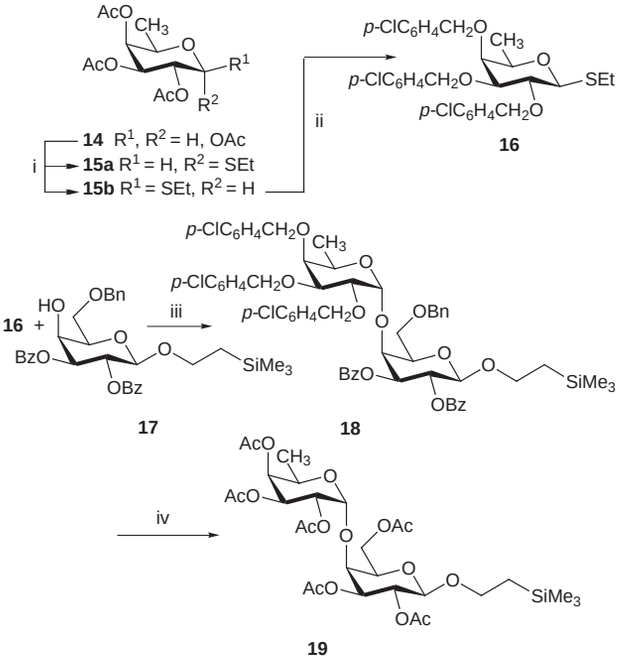
Peracetylated D-fucose **14** was used as starting material in



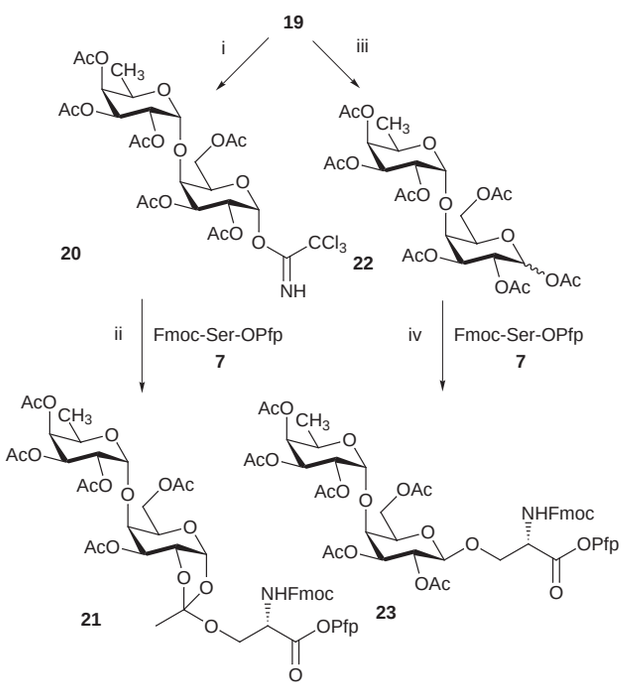
Scheme 2 *Reagents and conditions* (*and yields*): i) H_2, Pd/C, HOAc (98%); ii) *p*,α,α-trimethoxytoluene, *p*-TsOH, CH_3CN (90%); iii) Ac_2O, pyridine (96%); iv) Bu_4NBr, $CuBr_2$, DDQ, CH_2Cl_2, 90 °C (oil-bath) (87%).

this route (Scheme 3). Treatment of **14** with ethanethiol and boron trifluoride–diethyl ether gave thioglycosides **15a** (15%, α-anomer) and **15b** (65%, β-anomer). The β-glycoside **15b** was deacetylated and then treated with *p*-chlorobenzyl chloride and sodium hydride in DMF to give glycosyl donor **16** (47%). The *p*-chlorobenzyl protecting group was used since Kiessling and Pohl[25] recently reported superior yields when employing fucosyl donors protected with *p*-chlorobenzyl instead of ordinary benzyl groups. Glycosylation of acceptor **17**[22] with fucosyl donor **16** under promotion by *N*-iodosuccinimide (NIS) and triflic acid (TfOH) at −78 °C gave **18** in good yield (78%). Hydrogenolysis of the *p*-chlorobenzyl groups over Pd/C, followed by debenzoylation in methanolic sodium methoxide and acetylation, gave the TMSEt 6′-deoxygalabioside **19** (60%).

TMSEt-glycoside **19** was treated with TFA in dichloromethane to give the corresponding hemiacetal,[22] which was converted into trichloroacetimidate **20** using trichloroacetonitrile and DBU (Scheme 4). Attempted silver trifluoromethanesulfonate-promoted glycosylation of Fmoc-Ser-OPfp **7** by imidate **20** predominantly gave orthoester **21** instead of the desired building block **23**. Despite problems reported previously[21] TMSEt-glycoside **19** could be treated with acetic anhydride and



Scheme 3 *Reagents and conditions* (*and yields*): i) EtSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2, −10 °C, (15%, α-anomer), (65%, β-anomer); ii) NaOMe, MeOH; then NaH, *p*-$ClC_6H_4CH_2Cl$, DMF (47%); iii) NIS, TfOH, Et_2O–THF (5:1), −78 °C (78%); iv) H_2, Pd/C, HOAc; then NaOMe, MeOH; then Ac_2O, pyridine (60%).



Scheme 4 *Reagents and conditions* (*and yields*): i) TFA, CH_2Cl_2; then CCl_3CN, DBU, 0 °C (64%); ii) AgOTf, CH_2Cl_2, 0 °C; iii) $BF_3{\cdot}Et_2O$, Ac_2O, toluene, 0 °C (83%); iv) $BF_3{\cdot}Et_2O$, CH_3CN (37%).

boron trifluoride–diethyl ether in toluene at 0 °C to give peracetate **22** (83% yield, α:β = 1:8). Fmoc-Ser-OPfp **7** was then glycosylated with donor **22** under boron trifluoride–diethyl ether promotion[26] to give the desired 6′-deoxy building block **23** (37% after purification by normal-phase HPLC).

Synthesis of glycopeptides having 6- and 6′-deoxygalabiose moieties

Residues Arg[61] to Gln[57] of the 6-deoxygenated glycopeptide **24** (*cf.* Chart 2) were assembled on solid phase in an automatic peptide synthesizer constructed in our laboratory and operating

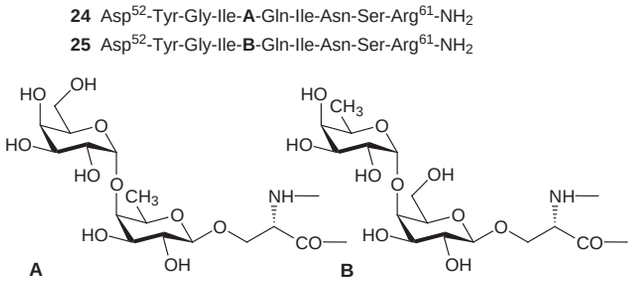


Chart 2

essentially as described previously by others.[27] Coupling of glycosylated building block **8** and the rest of the amino acids (Ile[55] to Asp[52]) was performed manually after removal of the peptide–resin from the synthesizer. All residues in the 6′-deoxyglycopeptide **25** were attached manually. The glycopeptides were prepared as *C*-terminal amides on a polystyrene resin grafted with polyethylene glycol spacers (TentaGel S NH_2) functionalized with the Rink linker {*p*-[α-(fluoren-9-ylmethoxyformamido)-2,4-dimethoxybenzyl]phenoxyacetic acid},[28,29] using DMF as solvent. N^{α}-Fmoc amino acids (4 equiv.) were coupled as benzotriazolyl esters[30] using 1-hydroxybenzotriazole (HOBt) and *N*,*N*′-diisopropylcarbodiimide (DIC) for activation. The glycosylated building block **8** (1.1 equiv.) was coupled in the presence of HOBt, whereas building block **23** (1.3 equiv.) was coupled in the presence of 1-hydroxy-7-azabenzotriazole[31] (HOAt). All couplings were monitored using Bromophenol Blue as indicator of unacylated amino groups.[32] The N^{α}-Fmoc protecting groups were removed with 20% piperidine in DMF. Cleavage of the glycopeptides from the resin with simultaneous deprotection of the side chains of the amino acids was affected by treatment with TFA containing water, thioanisole, and ethanedithiol as scavengers for 2 h at room temperature.[33] Purification by reversed-phase HPLC gave *O*-acetylated glycopeptides, de-*O*-acetylation of which turned out to be a crucial step. Careful addition of methanolic sodium methoxide (pH 8.5–9.0 on moist pH paper), and monitoring of the reaction with HPLC, were necessary in order to avoid β-elimination of the carbohydrate moiety from both glycopeptides. Purification of the glycopeptides by reversed-phase HPLC gave pure **24** and **25** in 8 and 15% yield, based on the capacity of the resin. The structures of glycopeptides **24** and **25** were confirmed using fast-atom bombardment mass spectroscopy (FABMS), amino acid analysis and ^{1}H NMR spectroscopy (Table 1).

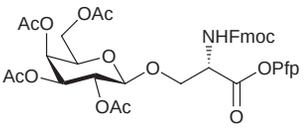
Synthesis of glycopeptides having galactose and lactose moieties

Synthesis of glycopeptides **28** and **29** (Chart 3) was performed in the peptide synthesizer essentially as described for glycopeptide **24**. The glycosylated building blocks **26**[34] (1.6 equiv.) and **27**[35] (2.8 equiv.) were coupled to the peptide–resin in the presence of HOBt. Cleavage from the resin and deacetylation as described for glycopeptides **24** and **25** gave galactosylated **28** (7%) after purification by reversed-phase HPLC. Deprotection of *O*-benzoylated **29** turned out to be more problematic. A substantially higher concentration of methanolic sodium methoxide had to be employed in order to obtain complete cleavage of the benzoyl protecting groups and a significant amount of β-elimination of the carbohydrate moiety could not be avoided. The reaction was monitored with analytical reversed-phase HPLC in order to minimize loss of product due to elimination, and glycopeptide **29** was obtained after purification by preparative reversed-phase HPLC. The β-eliminated peptide was also isolated and its structure was confirmed by FABMS [$(M + H)^+$ 1133. Calc. *m*/*z*, 1133]. Glycopeptides **28** and **29** were characterized by FABMS amino acid analysis and ^{1}H NMR spectroscopy (Table 1). Importantly, the ^{1}H NMR spectrum of **29** could be

Table 1 ^{1}H NMR chemical shifts for the *O*-linked glycopeptides **24**, **25**, **28**, **29**, **31** and **34**[a]

	24	**25**	**28**	**29**	**31**	**34**
Asp[52] or Ala[52]						
α-H	4.10	4.01	4.03	4.08	3.91	4.06
β-H	2.60, 2.70	2.51, 2.60	2.55, 2.65	2.59, 2.69	1.38	2.55, 2.68
Tyr[53]						
NH	8.78	8.59		8.77		8.76
α-H	4.52	4.43	4.47	4.51	4.46	4.48
β-H	2.91, 3.06	2.82, 2.96	2.87, 3.02	2.90, 3.05	2.89, 2.97	2.88, 3.02
Arom	6.79, 7.10	6.70, 7.03	6.76, 7.06	6.79, 7.10	6.75, 7.06	6.76, 7.04
Gly[54]						
NH	8.43	8.25	8.41	8.44	8.39	8.42
α-H	3.84[b]	3.75[b]	3.80[b]	3.84[b]	3.80[b]	3.80[b]
Ile[55]						
NH	8.11	7.90	8.06	8.10	8.07	8.07
α-H	4.17	4.07	4.12	4.15	4.14	4.13
β-H	1.86	1.76	1.81	1.84	1.80	1.81
γ-H_2	1.14, 1.42	1.04, 1.30	1.10, 1.38	1.15, 1.40	1.10, 1.38	1.10, 1.39
γ-H_3	0.8	0.78	0.80	0.8	0.8	0.8
γ-H_3	0.8	0.78	0.84	0.8	0.8	0.8
Ser[56]						
NH	8.64	8.44	8.65	8.66	8.66	8.61
α-H	4.56	4.48	4.52	4.56	4.54	4.54
β-H	3.82, 4.12	3.75, 4.06	3.81, 4.11	3.86, 4.13	3.81, 4.12	3.83, 4.12
Gln[57] or Ala[57]						
NH	8.44	8.22	8.38	8.38	8.44	8.34
α-H	4.30	4.21	4.26	4.30	4.29	4.22
β-H	1.93, 2.03	1.83, 1.94	1.89, 2.00	1.92, 2.04	1.89, 1.98	1.29
γ-H_2	2.29[b]	2.19[b]	2.26[b]	2.29[b]	2.26[b]	
δ-NH_2	6.95, 7.61	6.76, 7.40	6.90, 7.55	6.93, 7.57	6.94, 7.59	
Ile[58]						
NH	8.39	8.13	8.29	8.31	8.38	8.30
α-H	4.05	3.97	4.00	4.04	4.03	4.00
β-H	1.80	1.70	1.76	1.79	1.78	1.76
γ-H_2	1.14, 1.42	1.03, 1.32	1.10, 1.38	1.15, 1.40	1.10, 1.38	1.10, 1.39
γ-H_3	0.8	0.75	0.77	0.8	0.8	0.8
δ-H_3	0.8	0.75	0.80	0.8	0.8	0.8
Asn[59]						
NH	8.68	8.46	8.64	8.66	8.67	8.62
α-H	4.70	4.61	4.66	4.70	4.69	4.66
β-H	2.72, 2.83	2.62, 2.72	2.68, 2.79	2.71, 2.82	2.68, 2.78	2.67, 2.79
γ-NH_2	6.96, 7.68	6.78, 7.49	6.93, 7.66	6.96, 7.67	6.93, 7.65	6.93, 7.66
Ser[60]						
NH	8.44	8.21	8.41	8.42	8.44	8.42
α-H	4.34	4.17	4.30	4.34	4.29	4.31
β-H	3.80, 3.88	3.70, 3.76	3.76, 3.84	3.79, 3.87	3.76, 3.84	3.76, 3.84
Arg[61]						
NH	8.41	8.21	8.36	8.40	8.40	8.37
α-H	4.26	4.17	4.22	4.25	4.24	4.22
β-H	1.72, 1.86	1.62, 1.77	1.7, 1.8	1.72, 1.86	1.69, 1.82	1.69, 1.83
γ-H_2	1.60[b]	1.50[b]	1.55[b]	1.60[b]	1.56[b]	1.56[b]
δ-H_2	3.15[b]	3.05[b]	3.11[b]	3.15[b]	3.11[b]	3.11[b]
δ-NH	7.20[b]	7.04[b]	7.16[b]	7.19[b]	7.19[b]	7.16[b]
α-$CONH_2$[c]	7.23, 7.52	7.04, 7.37	7.21, 7.49	7.23, 7.51	7.21, 7.58	7.21, 7.50
Galβ						
H-1	4.38	4.32	4.32	[d]	4.38	4.38
H-2	3.47	3.39	3.46		3.48	3.49
H-3	3.67	3.59	3.56		3.66	3.66
H-4	3.77	3.80	3.81		3.93	3.92
H-5	3.76	n.d.[e]	n.d.[e]		n.d.[e]	n.d.[e]
H-6	1.28[b]	n.d.[e]	n.d.[e]		n.d.[e]	n.d.[e]
Galα						
H-1′	4.88	4.70			4.90	4.85
H-2′	3.78	3.61			n.d.[e]	3.72
H-3′	3.84	3.70			3.76	3.77
H-4′	3.95	3.61			3.91	3.90
H-5′	4.35	4.27			4.25	4.26
H-6′	3.62[b]	3.99[b]			3.59[b]	3.58[b]

[a] Spectra were recorded at 500 or 600 MHz for solutions in H_2O–D_2O (9:1; pH 5.0) at 278 K using HOD ($\delta_H = 4.98$) as internal standard. The spectrum of glycopeptide **25** was recorded at 293 K instead of at 278 K. [b] Degeneracy has been assumed. [c] C-Terminal amide. [d] Chemical shifts for the lactose moiety in glycopeptide **29**: 4.34 (H-1′), 3.47 (H-2′), 3.59 (H-3′), 3.85 (H-4′), 3.63 (H-5′), 3.79 and 3.88 (H_2-6′), 4.45 (H-1), 3.28 (H-2), 3.58 (H-3), 3.60 (H-4), 3.54 (H-5), 3.74 and 3.93 (H_2-6). [e] Not determined.



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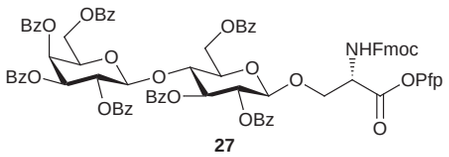
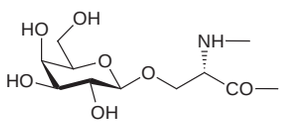


27

28 Asp[52]-Tyr-Gly-Ile-**A**-Gln-Ile-Asn-Ser-Arg[61]-NH_2
29 Asp[52]-Tyr-Gly-Ile-**B**-Gln-Ile-Asn-Ser-Arg[61]-NH_2



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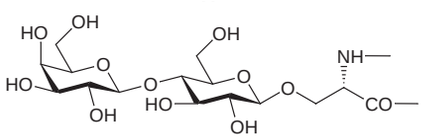


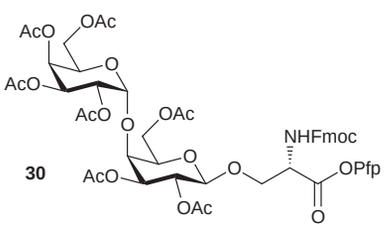
B

Chart 3

completely assigned, and minor peaks resulting from impurities were not observed, revealing that the peptide stereocenters in **29** had not undergone epimerization[36] during removal of the *O*-benzoyl groups.

Synthesis of alanine-substituted glycopeptides

Synthesis of glycopeptides **31**–**38** (Chart 4) was performed in



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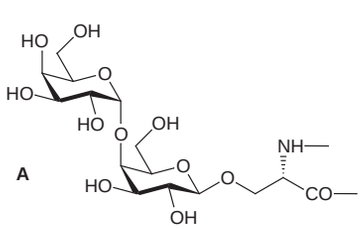
31 **Ala**[52]-Tyr-Gly-Ile-**A**-Gln-Ile-Asn-Ser-Arg[61]-NH_2
32 Asp[52]-**Ala**-Gly-Ile-**A**-Gln-Ile-Asn-Ser-Arg[61]-NH_2
33 Asp[52]-Tyr-Gly-**Ala**-**A**-Gln-Ile-Asn-Ser-Arg[61]-NH_2
34 Asp[52]-Tyr-Gly-Ile-**A**-**Ala**-Ile-Asn-Ser-Arg[61]-NH_2
35 Asp[52]-Tyr-Gly-Ile-**A**-Gln-**Ala**-Asn-Ser-Arg[61]-NH_2
36 Asp[52]-Tyr-Gly-Ile-**A**-Gln-Ile-**Ala**-Ser-Arg[61]-NH_2
37 Asp[52]-Tyr-Gly-Ile-**A**-Gln-Ile-Asn-**Ala**-Arg[61]-NH_2
38 Asp[52]-Tyr-Gly-Ile-**A**-Gln-Ile-Asn-Ser-**Ala**[61]-NH_2



A

Chart 4

the automatic peptide synthesizer essentially as described for glycopeptide **24**. For all of these glycopeptides the glycosylated building block **30**[26] (1.1–1.2 equiv.) was coupled in the presence of HOBt. Cleavage of the glycopeptides from the resin, deacetylation and purification by HPLC was performed as described for **24**, to give glycopeptides **31**–**38** in 11–28% yield. Glycopeptides **31**–**38** were characterized by FABMS and amino acid analysis. In view of their close structural similarity, and the fact that they were prepared under identical conditions, only the structures of **31** and **34** were confirmed by ^{1}H NMR spectroscopy (Table 1).

Conditions for cleavage from the solid phase and deacylation

Inspection of the reversed-phase analytical HPLC chromatogram for each of the 12 glycopeptides described in the present paper revealed that the crude product obtained after cleavage from the solid phase contained almost no peptide impurities. In spite of this the target glycopeptides were obtained in only ~10–30% overall yields after removal of the *O*-acyl protective groups and purification by preparative reversed-phase HPLC. It should, however, be pointed out that the yields have been corrected for the presence of moisture, which usually accounts for ~30% of the freeze-dried glycopeptide material. We reasoned that the rather low yields might result from incomplete cleavage from the solid phase, β-elimination on removal of the *O*-acyl protective groups, loss of material during purification by preparative reversed-phase HPLC, or a combination of these factors.

Gel-phase ^{19}F NMR spectroscopy has recently been shown to be an efficient tool for rapid monitoring of transformations of compounds attached to a solid phase using an ordinary NMR spectrometer.[37–39] This is due to the fact that commonly used solid supports do not contain fluorine. In addition, ^{19}F chemical shifts are spread over a wide frequency range and they are also sensitive to structural transformations in the vicinity of the fluorine atom. The HEL(52–61)-derived peptide–resin **39** was therefore prepared and used to investigate to what extent the peptide was cleaved from the solid phase under various conditions (Fig. 1). In **39** the *p*-fluorophenyl residue located at position 53 in the peptide allows monitoring of the cleavage while the *m*-fluorophenylalanine residue, which is linked directly to the solid phase, serves as an internal reference (Fig. 1a). Cleavage of **39** was performed with the same mixture of TFA and scavengers as used in the synthesis of the different glycopeptides but it was carried out either at room temperature or at 40 °C for various periods of time. Interestingly, the conditions employed for the glycopeptides, *i.e.* cleavage for 2 h at room temperature, resulted in ~20% of the peptide remaining attached to the resin after cleavage (Fig. 1b). In contrast, cleavage for 2 h at 40 °C (Fig. 1c) or for 12 h at room temperature (data not shown) led to complete removal of the peptide from the solid phase. Thus, even though the half-life for cleavage of the Rink linker in solution has been determined to be only 9 min at room temperature,[29] a substantially prolonged reaction time, or preferably elevation of the temperature to 40 °C, is necessary in order to effect complete cleavage of peptides and glycopeptides attached to a solid phase. We have no reason to believe that this conclusion depends on how the linker is attached to the solid phase or on the nature of the solid phase. It should be pointed out that use of elevated temperature for cleavage is most likely suitable also when combinatorial libraries of other types of compounds are prepared on solid phase using the Rink linker.

The choice between acetyl and benzoyl groups for protection of the carbohydrate moiety is clearly of importance for the success of the final deprotection step. When the *O*-benzoyl groups were removed to give lactose glycopeptide **29** a more concentrated sodium methoxide solution had to be employed and substantial β-elimination could not be avoided even when the deprotection was monitored by HPLC. Problems with β-elimination during debenzoylation have also been reported in several other syntheses of glycopeptides,[40–43] as well as in a

H-Asp[52]-*p*F-Phe-Gly-Ile-Leu-Gln(Trt)-Ile-Asn(Trt)-Ser(tBu)-Arg(Pmc)[61]-Rink linker-*m*F-Phe-Tenta Gel

39

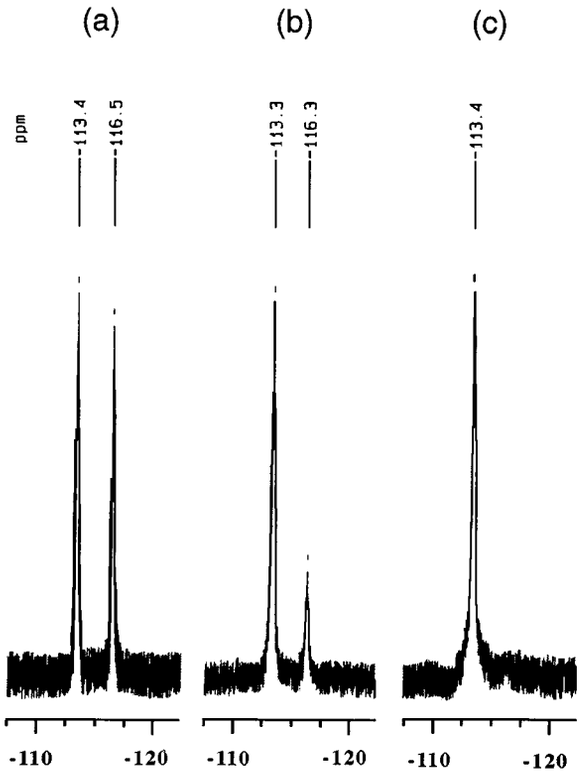


Fig. 1 Gel-phase ^{19}F NMR spectra obtained in $CDCl_3$ of (a) peptide–resin **39**, (b) **39** after cleavage for 2 h at room temperature with TFA employing water, thioanisole, and ethanedithiol as cation-scavengers, and (c) **39** after cleavage with the same reagent for 2 h at 40 °C. The resonance at −113.4 ppm originates from the *m*-F-Phe residue used as reference whereas the resonance at −116.5 ppm is derived from the *p*-F-Phe residue in the peptide part of **39**. Integration revealed that ~20% of the peptide was still attached to the resin when cleavage was performed for 2 h at room temperature.

model study.[36] In contrast a recent study described the successful removal of *O*-benzoyl protective groups from the carbohydrate moieties of a large number of glycopeptides.[44] It is not clear if the β-elimination that sometimes occurs is due to differences in the structure of the carbohydrate and/or the peptide moieties of the glycopeptides, or to slight variations in the reaction conditions employed. However, it appears desirable to avoid *O*-benzoyl protective groups if this is possible. The *O*-acetyl protective groups could in all cases be removed from the glycopeptides under less basic conditions than required for the benzoyl groups. Interestingly, in several cases when the deacetylations were monitored by analytical reversed-phase HPLC a product which was assumed to be derived from β-elimination began to be formed in parallel with the disappearance of the last traces of acetylated glycopeptide. However, formation of such a by-product was only significant for glycopeptide **31**, in which Asp[52] has been replaced by Ala, and in this case β-elimination was confirmed by FABMS. In efforts to avoid the final deacylation step, and thereby the risk of β-elimination, acid-labile silyl and *p*-methoxybenzyl ethers, as well as isopropylidene groups,[45–48] have been employed in glycopeptide synthesis.

Some reports have described substantial loss of material due to irreversible adsorption of glycopeptides to the reversed phase during purification by HPLC.[49,50] This factor probably contributes to the low yields for some of the glycopeptides, for instance **24** (8%), which has a 6-deoxygalabiose moiety, and the galactosylated **28** (7%). Since attempts to avoid this problem by using eluents other than aqueous acetonitrile have not been successful, losses can at present be minimized only by limiting the number of purifications by reversed-phase HPLC.

In view of the above, the influence of the conditions used for cleavage, deacetylation and purification on the overall yield was investigated for glycopeptide **36**. Cleavage was performed with the usual mixture of TFA and scavengers but it was carried out for 2 h at 40 °C. Deacetylation of the resulting, crude *O*-acetylated **36** was performed in methanolic sodium methoxide with careful monitoring by analytical reversed-phase HPLC. Purification using preparative HPLC then gave **36** in an overall yield of 33%, as compared to 21% when cleavage was performed for 2 h at room temperature and when *O*-acetylated **36** was purified by HPLC. The investigations with fluorinated peptide–resin **39** suggest that part of the yield increase obtained for **36** can be explained by the more efficient cleavage at 40 °C. In addition, it is assumed that omission of one purification by reversed-phase HPLC also contributed to the increased yield.

Immunological studies

The glycopeptides described in the present work have been evaluated for binding to I-A^k class II MHC molecules, as well as for their ability to stimulate helper T cell hybridomas obtained from mice immunized with the galabiosylated glycopeptide **1**.[51] These studies revealed that Asp[52] was the most important residue in anchoring of **1** to the class II MHC molecule. Less critical MHC-binding was provided by residues Ile[55], Gln[57], and Ile[58]. Furthermore, the T cell receptor of the hybridomas was found to make specific contacts with the galabiose moiety of **1**. This was established by the inability of the 6′-deoxygalabiosyl (**25**), galactosyl (**28**), and lactosyl (**29**) analogues of **1** to stimulate helper T cell hybridomas elicited by **1**. In addition to the galabiose moiety, residues Tyr[53] and Asn[59] were essential in contacting the T cell receptor. In general, these results are in excellent agreement with the structure of the complex between I-A^k and the peptide HEL(47–62) which was recently determined by X-ray crystallography.[52]

Experimental

General methods and materials

All reactions were carried out under an inert atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH_2Cl_2 and acetonitrile were distilled from calcium hydride. THF, diethyl ether and toluene were distilled from sodium–benzophenone. MeOH and pyridine were dried over 3 Å 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.

The ^1H and ^{13}C NMR spectra were recorded with a Bruker DRX-360, DRX-400 or ARX-500 spectrometer, for solutions in $CDCl_3$ [residual $CHCl_3$ (δ_H 7.27) or $CDCl_3$ (δ_C 77.23) as internal standard], CD_3OD [residual CD_2HOD (δ_H 3.31) or CD_3OD (δ_C 49.15) as internal standard] at 300 K. The ^1H NMR spectra of glycopeptides **24**, **25**, **28**, **29**, **31**, and **34** were recorded with a Bruker ARX-500 or DRX-600 spectrometer for solutions in a 9:1 mixture of H_2O and D_2O [H_2O (δ_H 4.98) as internal standard] at 278 or 300 K. First-order chemical shifts and coupling constants were obtained from one-dimensional spectra, and proton resonances were assigned from COSY,[53] TOCSY,[54] and ROESY[55] experiments. *J*-Values are given in Hz. 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. Optical rotations were measured on a Perkin-Elmer 343 polarimeter, and $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside[19] **2**, N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester[56] **7**, 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranoside[22] **17**, N^{α}-(fluoren-9-ylmethoxycarbonyl)-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-L-serine pentafluorophenyl ester[34] **26**, N^{α}-(fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-serine pentafluorophenyl ester[35] **27**, and N^{α}-(fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galacto-pyranosyl)-β-D-galactopyranosyl]-L-serine pentafluorophenyl ester[26] **30** were prepared according to the indicated literature methods.

Analytical normal-phase HPLC was performed on a Kromasil silica column (100 Å; 5 μm; 4.6 × 250 mm) with a flowrate of 2 ml min^{-1} and detection at 254 nm. Preparative purifications were performed on a Kromasil silica column (100 Å; 5 μm; 20 × 250 mm) with a flowrate of 16 ml min^{-1}.

2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside 3

Methanolic sodium methoxide (2 M; 0.6 ml) was added to a solution of 2-(trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galactopyranoside[19] **2** (1.31 g, 1.17 mmol) in CH_2Cl_2–MeOH (1:3; 24 ml) at 0 °C. The reaction was monitored by TLC and was terminated after 3 h by addition of acetic acid (120 μl). Concentration to dryness followed by chromatography on silica gel with heptane–EtOAc (3:1) gave **3** (397 mg, 34%). Starting material **2** (527 mg, 40%) was recovered and debenzoylated, as described above, to give more of **3** (97 mg, 8%), thus raising the total yield of **3** to 494 mg (42%). Compound **3** had: $[\alpha]_D^{20}$ +73 (*c* 0.5 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.05 (9 H, s, $SiMe_3$), 0.8–1.0 (2 H, m, CH_2CH_2Si), 3.59 (1 H, m, OCH_2CH_2), 4.05 (1 H, dd, *J* 3.1, 10.0, H-2′), 4.07 (1 H, m, OCH_2CH_2), 4.13 (1 H, dd, *J* 2.7, 10.3, H-3′), 4.39 (1 H, d, *J* 2.5, H-4), 4.71 (1 H, d, *J* 7.8, H-1), 4.78 (1 H, d, *J* 3.3, H-1′), 5.33 (1 H, dd, *J* 2.8, 10.6, H-3), 5.67 (1 H, dd, *J* 7.8, 10.6, H-2); δ_C(100 MHz; $CDCl_3$) 101.2 and 101.5 (C-1, -1′); HRMS (FAB): Calc. for $C_{59}H_{66}NaO_{13}Si$: 1033.4170 (M + Na^+). Found: *m*/*z*, 1033.4167.

2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-6-deoxy-6-iodo-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-β-D-galacto-pyranoside 4

Triphenylphosphine (327 mg, 1.25 mmol), imidazole (117 mg, 1.73 mmol), and iodine (317 mg, 1.25 mmol) were added to a solution of **3** (485 mg, 0.48 mmol) in toluene (10 ml) at 80 °C.[20] After 1 h, the mixture was diluted with toluene (20 ml) and the solid residue was dissolved in acetone (10 ml). The combined organic phases were washed successively with saturated aqueous $NaHCO_3$ (50 ml) and water (50 ml), dried, and concentrated to dryness. Chromatography on silica gel with heptane–EtOAc (8:1) gave **4** (477 mg, 89%), $[\alpha]_D^{20}$ +72 (*c* 0.5 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.01 (9 H, s, $SiMe_3$), 0.8–1.1 (2 H, m, CH_2CH_2Si), 2.96 (1 H, dd, *J* 4.9, 8.4, H-5′), 3.43 (1 H, dd, *J* 8.5, 9.5, H-6′), 3.63 (1 H, dd, *J* 6.5, 10.3, H-6), 3.68 (1 H, m, OCH_2CH_2), 3.87 (1 H, t, *J* 6.8, H-5), 4.36 (1 H, dd, *J* 5.0, 9.6, H-6′), 4.53 (1 H, br s, H-4), 4.72 (1 H, d, *J* 7.8, H-1), 4.95 (1 H, d, *J* 3.5, H-1′), 5.18 (1 H, dd, *J* 2.9, 10.6, H-3), 5.72 (1 H, dd, *J* 7.8, 10.6, H-2); δ_C(100 MHz; $CDCl_3$) 101.1 and 101.9 (C-1, -1′) (Found: C, 63.8; H, 6.2. $C_{59}H_{65}IO_{12}Si$ requires C, 63.2; H, 5.9%); HRMS (FAB): Calc. for $C_{59}H_{65}INaO_{12}Si$: 1143.3188 (M + Na^+). Found: *m*/*z*, 1143.3192.

2-(Trimethylsilyl)ethyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-fucopyranoside 5

Pd/C (10%; 200 mg) was added to a solution of **4** (447 mg, 0.40 mmol) in HOAc (20 ml). The mixture was hydrogenated at 400 kPa for 2 h, filtered through Celite, and concentrated. The solid residue was dissolved in MeOH (10 ml) and methanolic sodium methoxide (2 mM; 500 μl) was added to the solution, which was then stirred at room temperature for 20 h. HOAc (200 μl) was then added and the solution was concentrated. The solid residue was dissolved in acetic anhydride (10 ml) and pyridine (10 ml). After being stirred for 18 h, the solution was concentrated. Chromatography on silica gel with heptane–EtOAc (2:1) gave **5** (170 mg, 63%), $[\alpha]_D^{20}$ +85 (*c* 0.4 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 0.00 (9 H, s, $SiMe_3$), 0.8–1.1 (2 H, m, CH_2CH_2Si), 1.34 (3 H, d, *J* 6.5, H_3-6), 2.00, 2.04, 2.04, 2.07, 2.07, and 2.14 (6 s, each 3 H, 6 × Ac), 3.53 (1 H, m, OCH_2CH_2), 3.71 (1 H, q, *J* 6.5, H-5), 3.87 (1 H, d, *J* 2.5, H-4), 3.99 (1 H, m, OCH_2CH_2), 4.10 (1 H, dd, *J* 5.9, 10.8, H-6′), 4.17 (1 H, dd, *J* 8.1, 10.8, H-6′), 4.45 (1 H, d, *J* 7.8, H-1), 4.58 (1 H, t, *J* 7.5, H-5′), 4.86 (1 H, dd, *J* 2.9, 10.8, H-3), 5.08 (1 H, d, *J* 3.7, H-1′), 5.15 (1 H, dd, *J* 7.9, 10.7, H-2), 5.17 (1 H, dd, *J* 3.5, 10.8, H-2′), 5.44 (1 H, dd, *J* 3.4, 11.0, H-3′), 5.56 (1 H, dd, *J* 1.1, 3.3, H-4′); δ_C(100 MHz; $CDCl_3$) 99.2 and 101.0 (C-1, -1′); HRMS (FAB): Calc. for $C_{29}H_{46}NaO_{16}Si$: 701.2453 (M + Na^+). Found: *m*/*z*, 701.2460.

2,3-Di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyrano-syl)-α-D-fucopyranosyl trichloroacetimidate 6

TFA (4 ml) was added to a solution of **5** (158 mg, 0.23 mmol) in CH_2Cl_2 (2 ml) at 0 °C. After the mixture had been stirred for 1.5 h, *n*-propyl acetate (12 ml) was added, and the solution was co-concentrated several times with toluene. Chromatography on silica gel with heptane–EtOAc (1:2) gave the corresponding hemiacetal (114 mg, 0.20 mmol), which was dissolved in CH_2Cl_2 (8 ml). The solution was cooled to 0 °C, before trichloro-acetonitrile (650 μl, 6.48 mmol) and DBU (40 μl, 0.27 mmol) were added. After being stirred for 1.5 h the solution was concentrated and the residue was chromatographed on silica gel with heptane–EtOAc–Et_3N (2:1:0.15) to give **6** (100 mg, 60%), δ_H(400 MHz; $CDCl_3$) 1.28 (3 H, d, *J* 6.6, H_3-6), 2.02, 2.04, 2.05, 2.10, 2.12, and 2.16 (6 s, each 3 H, 6 × Ac), 4.29 (1 H, q, *J* 6.6, H-5), 4.58 (1 H, t, *J* 6.8, H-5′), 5.15 (1 H, d, *J* 3.7, H-1′), 5.23 (1 H, dd, *J* 3.7, 11.0, H-2′), 5.43 (1 H, dd, *J* 3.3, 11.0, H-3′), 5.57 (1 H, dd, *J* 1.2, 3.3, H-4′), 8.62 (1 H, s, HN).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)-β-D-fucopyrano-syl]-L-serine pentafluorophenyl ester 8

A mixture of **6** (94 mg, 0.13 mmol), N^{α}-(fluoren-9-ylmethoxy-carbonyl)-L-serine pentafluorophenyl ester **7** (88 mg, 0.18 mmol) and powdered molecular sieves (AW-300, 200 mg) in dichloromethane (10 ml) was stirred for 50 min at room temperature. The reaction mixture was then cooled to 0 °C and protected from light, before silver trifluoromethanesulfonate (106 mg, 0.41 mmol) was added. The reaction mixture was stirred for 1 h at 0 °C and was then allowed to attain room temperature during 1 h. The mixture was diluted with CH_2Cl_2 (50 ml), filtered (Celite), and washed with saturated aqueous $NaHCO_3$ (50 ml). The organic layer was dried, concentrated and the residue was chromatographed on silica gel with heptane–EtOAc (2:1). Preparative normal-phase HPLC (gradient; 100% hexane ⟶ 15% ethanol in hexane in 120 min) gave **8** (54 mg, 39%), $[\alpha]_D^{20}$ +40 (*c* 0.2 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 1.31 (3 H, d, *J* 6.5, H_3-6), 1.98, 2.02, 2.02, 2.05, 2.10, and 2.15 (6 s, each 3 H, 6 × Ac), 3.75 (1 H, q, *J* 6.6, H-5), 3.92 (1 H, d, *J* 2.7, H-4), 4.05 (1 H, dd, *J* 3.8, 10.5, Ser-Hβ), 4.27 (1 H, t, *J* 6.9, H-5′), 4.41 (1 H, dd, *J* 3.5, 10.2, Ser-Hβ), 4.55 (1 H, d, *J* 8.0, H-1), 4.87 (1 H, dd, *J* 3.4, 8.3, Ser-Hα), 4.92 (1 H, dd, *J* 2.7, 10.8, H-3), 5.12 (1 H, d, *J* 3.6, H-1′), 5.20 (1 H, dd,

J 3.6, 11.0, H-2′), 5.21 (1 H, dd, *J* 8.0, 10.9, H-2), 5.44 (1 H, dd, *J* 3.3, 11.0, H-3′), 5.58 (1 H, d, *J* 3.3, H-4′), 5.86 (1 H, d, *J* 8.2, Ser-NH); δ_C(100 MHz; $CDCl_3$) 98.8 (C-1′), 100.9 (C-1); HRMS (FAB): Calc. for $C_{48}H_{48}F_5NNaO_{20}$: 1076.2588 ($M + Na^+$). Found: *m*/*z*, 1076.2599.

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-(α-D-galacto-pyranosyl)-β-D-galactopyranoside 9

Pd/C (10%; 500 mg) was added to a solution of **2** (2.28 g, 2.04 mmol) in HOAc (20 ml). The mixture was hydrogenated at 400 kPa for 24 h, filtered through Celite, and co-concentrated several times with toluene, to give **9** (1.52 g, 98%), $[a]_D^{20}$ +71 (*c* 0.4 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.10 (9 H, s, $SiMe_3$), 0.8–1.0 (2 H, m, CH_2CH_2Si), 3.61 and 4.00 (each 1 H, m, OCH_2CH_2), 4.49 (1 H, d, *J* 2.9, H-4), 4.70 (1 H, dd, *J* 7.1, 11.3, H-6), 4.76 (1 H, d, *J* 7.8, H-1), 4.85 (1 H, dd, *J* 6.8, 11.3, H-6), 5.12 (1 H, d, *J* 3.6, H-1′), 5.32 (1 H, dd, *J* 2.9, 10.6, H-3), 5.72 (1 H, dd, *J* 7.8, 10.6, H-2); δ_C(100 MHz; $CDCl_3$) 101.0 and 101.3 (C-1, -1′) (Found: C, 60.8; H, 6.3. $C_{38}H_{46}O_{14}Si$ requires C, 60.5; H, 6.0%).

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-[4,6-*O*-(*p*-methoxybenzylidene)-α-D-galactopyranosyl]-β-D-galacto-pyranoside 10

Toluene-*p*-sulfonic acid (23 mg, 0.12 mmol) was added to a solution of **9** (1.50 g, 1.98 mmol), *p*,α,α-trimethoxytoluene (0.61 ml, 4.1 mmol) and dry CH_3CN (30 ml). The reaction mixture was stirred at room temperature for 23 h before triethylamine (100 μl) was added. The solution was concentrated and the solid residue was chromatographed on silica gel with heptane–EtOAc (1 : 1 ⟶ 1 : 3) to give **10** (1.56 g, 90%), $[a]_D^{20}$ +44 (*c* 0.4 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.10 (9 H, s, $SiMe_3$), 0.8–1.1 (2 H, m, CH_2CH_2Si), 2.52 (1 H, br s, OH-3′), 2.74 (1 H, d, *J* 5.9, OH-2′), 2.90 (1 H, dd, *J* 1.0, 12.8, H-6′), 3.18 (1 H, dd, *J* 1.5, 12.8, H-6′), 3.64 (1 H, m, OCH_2CH_2), 3.9–4.1 (1 H, m, H-2′), 3.9–4.1 (1 H, m, OCH_2CH_2), 4.1–4.2 (1 H, m, H-3′), 4.22 (1 H, dd, *J* 0.9, 3.4, H-4′), 4.54 (1 H, d, *J* 3.0, H-4), 4.78 (1 H, dd, *J* 8.0, 11.1, H-6), 4.78 (1 H, d, *J* 7.9, H-1), 4.87 (1 H, dd, *J* 6.3, 11.2, H-6), 5.14 (1 H, d, *J* 3.7, H-1′), 5.18 (1 H, dd, *J* 3.0, 10.7, H-3), 5.73 (1 H, dd, *J* 7.8, 10.7, H-2); δ_C(100 MHz; $CDCl_3$) 101.2, 101.3 and 102.2 (C-1, -1′ and OCO) (Found: C, 63.7; H, 6.3. $C_{46}H_{52}O_{15}Si$ requires C, 63.3; H, 6.0%).

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3-di-*O*-acetyl-4,6-*O*-(*p*-methoxybenzylidene)-α-D-galactopyranosyl]-β-D-galactopyranoside 11

Compound **10** (1.40 g, 1.61 mmol) was dissolved in acetic anhydride (20 ml) and pyridine (20 ml). After being stirred for 24 h, the solution was concentrated. Chromatography on silica gel with heptane–EtOAc (2 : 1) gave **11** (1.47 g, 96%), $[a]_D^{20}$ +94 (*c* 0.5 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.01 (9 H, s, $SiMe_3$), 0.8–1.1 (2 H, m, CH_2CH_2Si), 2.07 and 2.15 (2 s, each 3 H, 2 × Ac), 3.08 and 3.44 (2 H, 2 d, *J* 12.7, H_2-6′), 3.66 (1 H, m, OCH_2CH_2), 3.78 (3 H, s, OCH_3), 4.08 (1 H, m, OCH_2CH_2), 4.48 (1 H, d, *J* 2.8, H-4), 4.53 (1 H, dd, *J* 7.7, 11.0, H-6), 4.54 (1 H, br s, H-4′), 4.80 (1 H, d, *J* 7.8, H-1), 4.83 (1 H, dd, *J* 6.3, 10.8, H-6), 5.18 (1 H, dd, *J* 3.0, 10.8, H-3), 5.20 (1 H, d, *J* 3.9, H-1′), 5.41 (1 H, dd, *J* 3.4, 11.1, H-3′), 5.55 (1 H, dd, *J* 3.5, 11.1, H-2′), 5.71 (1 H, dd, *J* 7.8, 10.8, H-2); δ_C(100 MHz; $CDCl_3$) 100.5 (C-1′), 100.6 (OCO), 100.8 (C-1) (Found: C, 63.1; H, 6.1. $C_{50}H_{56}O_{17}Si$ requires C, 62.8; H, 5.9%).

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-benzoyl-4-*O*-[2,3-di-*O*-acetyl-6-bromo-6-deoxy-4-*O*-(*p*-methoxybenzoyl)-α-D-galacto-pyranosyl]-β-D-galactopyranoside 12

Compound **11** (500 mg, 0.52 mmol), TBAB (200 mg, 0.62 mmol), $CuBr_2$ (100 mg, 0.45 mmol) and crushed molecular sieves (4 Å, 500 mg) were suspended in CH_2Cl_2 (10 ml). The solution was stirred for 1 h at room temperature before 2,3-dichloro-5,6-dicyano-*p*-benzoquinone DDQ (230 mg, 1.01 mmol) was added. The solution was refluxed at 90 °C for 18 h, cooled to room temperature, diluted with EtOAc (50 ml) and filtered (Celite). The filtrate was washed with saturated aqueous $NaHCO_3$ (50 ml). The organic layer was dried, concentrated, and the solid residue was chromatographed on silica gel with heptane–EtOAc (3 : 1) to give **12** (469 mg, 87%), $[a]_D^{20}$ +100 (*c* 0.5 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.05 (9 H, s, $SiMe_3$), 0.8–1.1 (2 H, m, CH_2CH_2Si), 1.97 and 2.13 (2 s, each 3 H, 2 × Ac), 3.02 (2 H, d, *J* 7.0, H_2-6′), 3.65 (1 H, m, OCH_2CH_2), 3.86 (3 H, s, OCH_3), 4.07 (1 H, m, OCH_2CH_2), 4.14 (1 H, br t, *J* 6.6, H-5), 4.63 (1 H, t, *J* 7.1, H-5′), 4.80 (1 H, d, *J* 7.6, H-1), 4.83 (1 H, dd, *J* 7.1, 11.5, H-6), 5.30 (1 H, dd, *J* 3.6, 10.3, H-2′), 5.33 (1 H, d, *J* 3.8, H-1′), 5.36 (1 H, dd, *J* 2.6, 10.4, H-3), 5.59 (1 H, dd, *J* 3.2, 10.7, H-3′), 5.71 (1 H, dd, *J* 7.5, 10.2, H-2), 5.90 (1 H, d, *J* 3.2, H-4′); δ_C(100 MHz; $CDCl_3$) 98.4 (C-1′), 100.8 (C-1) (Found: C, 58.3; H, 5.5. $C_{50}H_{55}BrO_{17}Si$ requires C, 58.0; H, 5.4%).

1,2,3,4-Tetra-*O*-acetyl-D-fucopyranose 14

D-Fucopyranose (0.97 g, 5.9 mmol) was dissolved in a mixture of acetic anhydride (20 ml) and pyridine (20 ml). The solution was stirred at 100 °C for 1.5 h and then concentrated. Chromatography of the residue on silica gel with heptane–EtOAc (2 : 1) gave **14** as an α/β mixture (1.93 g, 98%), δ_H(400 MHz; $CDCl_3$) 1.15 (d, *J* 6.5, H-6), 1.22 (d, *J* 6.4, H-6), 1.95-2.20 (m, Ac), 3.95 (dd, *J* 6.7, 12.5, H-5), 4.26 (dd, *J* 6.5, 13.0, H-5), 5.0–5.4 (m, H-2, -3 and -4), 5.68 (d, *J* 8.3, H-1(β-anomer)), 6.34 (d, *J* 2.8, H-1(α-anomer)); δ_C(100 MHz; $CDCl_3$) 90.1 (C-1(α-anomer)), 92.3 (C-1(β-anomer)).

Ethyl 2,3,4-tri-*O*-acetyl-1-thio-α- and -β-D-fucopyranoside 15a and 15b

Compound **14** (1.89 g, 5.70 mmol) and ethanethiol (0.65 ml, 8.8 mmol) were dissolved in CH_2Cl_2 (15 ml). The solution was cooled to −10 °C before $BF_3{\cdot}Et_2O$ (3.5 ml, 27.8 mmol) was added during 30 min. After 3.5 h at −10 °C the solution was allowed to attain room temperature during 1.5 h. It was then diluted with CH_2Cl_2 (100 ml), and washed successively with ice–water (130 ml), saturated aqueous $NaHCO_3$ (100 ml) and brine (100 ml). The organic layer was dried, concentrated and the solid residue was chromatographed on silica gel with heptane–EtOAc (4 : 1 to 2 : 1) to give **15a** (0.28 g, 15%) and **15b** (1.24 g, 65%): Compound **15a** had $[a]_D^{20}$ +226 (*c* 0.8 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 1.14 (1 H, d, *J* 6.5, H-6), 1.24 (3 H, t, *J* 7.4, CH_3), 1.97, 2.05 and 2.15 (3 s, each 3 H, 3 × Ac), 2.4–2.7 (2 H, m, SCH_2CH_3), 4.47 (1 H, q, *J* 6.6, H-5), 5.20 (1 H, dd, *J* 3.2, 10.8, H-3), 5.24 (1 H, dd, *J* 5.5, 10.8, H-2), 5.27 (1 H, br d, *J* 3.2, H-4), 5.68 (1 H, d, *J* 5.3, H-1); δ_C(100 MHz; $CDCl_3$) 82.0 (C-1); FABMS: Calc. for $C_{14}H_{22}NaO_7S$: 357.1 ($M + Na^+$). Found: *m*/*z*, 357.0.

Compound **15b** had $[a]_D^{20}$ −13 (*c* 0.4 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 1.20 (3 H, d, *J* 7.4, H_3-6), 1.26 (3 H, t, *J* 7.5, CH_3), 1.96, 2.04 and 2.15 (3 s, each 3 H, 3 × Ac), 2.6–2.8 (2 H, m, SCH_2CH_3), 3.80 (1 H, q, *J* 6.4, H-5), 4.44 (1 H, d, *J* 9.9, H-1), 5.03 (1 H, dd, *J* 3.4, 10.0, H-3), 5.20 (1 H, t, *J* 10.0, H-2), 5.25 (1 H, br d, *J* 3.0, H-4); δ_C(100 MHz; $CDCl_3$) 83.6 (C-1) (Found: C, 50.4; H, 6.7. $C_{14}H_{22}O_7S$ requires C, 50.3; H, 6.6%).

Ethyl 2,3,4-tris-*O*-(*p*-chlorobenzyl)-1-thio-β-D-fucopyranoside 16

Methanolic sodium methoxide (2 M; 2.5 ml) was added to a solution of **15b** (1.00 g, 2.99 mmol) in MeOH (25 ml). The solution was stirred for 2 h and then concentrated. The crude material was dissolved in DMF (20 ml) and the solution was cooled to 0 °C before sodium hydride (60% in mineral oil; 480 mg, 12.0 mmol) and *p*-chlorobenzyl chloride (3.37 g, 20.9

mmol) in DMF (5 ml) were added. After being stirred for 1 h, the solution was diluted with toluene (100 ml), washed with water (100 ml), and concentrated. The solid residue was chromatographed on silica gel with heptane–EtOAc (6:1) to give **16** (824 mg, 47%), $[\alpha]_D^{20}$ +18 (*c* 0.3 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 1.24 (3 H, d, *J* 6.4, H_3-6), 1.30 (3 H, t, *J* 7.4, CH_3), 2.65–2.85 (2 H, m, SCH_2CH_3), 3.45–3.55 (2 H, m, H-3 and -5), 3.59 (1 H, dd, *J* 0.7, 2.9, H-4), 3.75 (1 H, t, *J* 9.4, H-2), 4.38 (1 H, d, *J* 9.6, H-1), 4.64 (1 H, d, *J* 11.9, *p*-$ClC_6H_4CH_2O$), 4.65 (2 H, s, *p*-$ClC_6H_4CH_2O$), 4.70 (1 H, d, *J* 10.6, *p*-$ClC_6H_4CH_2O$), 4.85 (1 H, d, *J* 10.3, *p*-$ClC_6H_4CH_2O$), 4.88 (1 H, d, *J* 11.7, *p*-$ClC_6H_4CH_2O$); δ_C(100 MHz; $CDCl_3$) 85.0 (C-1) (Found: C, 60.2; H, 5.6. $C_{29}H_{31}Cl_3O_4S$ requires C, 59.9; H, 5.4%).

2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-4-*O*-[2,3,4-tris-*O*-(*p*-chlorobenzyl)-α-D-fucopyranosyl]-β-D-galactopyranoside 18

Compound **16** (949 mg, 1.63 mmol), 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-β-D-galactopyranoside **17** (802 mg, 1.39 mmol) and NIS (310 mg, 1.38 mmol) were dissolved in diethyl ether (20 ml) and THF (4 ml). The solution was protected from light and cooled to −78 °C, before TfOH (30 µl, 0.34 mmol) was added. The solution was stirred for 1.5 h, diluted with CH_2Cl_2 (120 ml) and washed successively with a mixture of saturated aqueous $Na_2S_2O_3$ (50 ml), saturated aqueous $NaHCO_3$ (50 ml) and water (2 × 100 ml). The organic phase was then dried, concentrated, and the solid residue was chromatographed on silica gel with heptane–EtOAc (6:1). The product was re-chromatographed with CH_2Cl_2–EtOH (1:0 to 10:1) to give **18** (1.19 g, 78%), $[\alpha]_D^{20}$ +85 (*c* 0.5 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) −0.01 (9 H, s, $SiMe_3$), 0.62 (1 H, d, *J* 6.5, H-6′), 0.85–1.05 (2 H, m, CH_2CH_2Si), 3.56 (1 H, br s, H-4′), 3.63 (1 H, m, OCH_2CH_2), 4.05 (1 H, m, OCH_2CH_2), 4.12 (1 H, dd, *J* 2.7, 10.3, H-3′), 4.20 (1 H, q, *J* 6.0, H-5′), 4.35 (2 H, br s, *p*-$ClC_6H_4CH_2O$), 4.40 (1 H, br d, *J* 2.8, H-4), 4.49 (1 H, d, *J* 11.6, *p*-$ClC_6H_4CH_2O$), 4.55 (1 H, d, *J* 12.1, *p*-$ClC_6H_4CH_2O$), 4.70 (1 H, d, *J* 7.8, H-1), 4.78 (1 H, d, *J* 11.6, *p*-$ClC_6H_4CH_2O$), 4.93 (1 H, d, *J* 3.5, H-1′), 5.23 (1 H, dd, *J* 2.9, 10.7, H-3), 5.72 (1 H, dd, *J* 7.8, 10.7, H-2); δ_C(100 MHz; $CDCl_3$) 99.8 and 101.1 (C-1′, -1) (Found: C, 64.7; H, 6.0. $C_{59}H_{63}Cl_3O_{12}Si$ requires C, 64.5; H, 5.8%).

2-(Trimethylsilyl)ethyl 2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl)-β-D-galactopyranoside 19

Pd/C (10%; 1.00 g) was added to a solution of **18** (1.16 g, 1.06 mmol) in HOAc (30 ml). The mixture was hydrogenated at 400 kPa for 4 h, filtered through Celite, and concentrated to give crude 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzoyl-4-*O*-α-D-fucopyranosyl-β-D-galactopyranoside (673 mg). The solid residue (643 mg) was dissolved in MeOH (20 ml) and methanolic sodium methoxide (2 M; 3 ml) was added to the solution, which was then stirred at room temperature for 30 h before Duolite (H^+-exchanger) was added. When the solution was neutral, it was filtered, co-concentrated with toluene, and the resulting solid residue was dissolved in acetic anhydride (10 ml) and pyridine (10 ml). After being stirred for 18 h, the solution was concentrated and chromatographed on silica gel with heptane–EtOAc (3:2) to give **19** (407 mg, 60%), $[\alpha]_D^{20}$ +72 (*c* 0.4 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 0.00 (9 H, s, $SiMe_3$), 0.85–1.05 (2 H, m, CH_2CH_2Si), 1.17 (1 H, d, *J* 6.5, H-6′), 1.96, 2.05, 2.05, 2.07, 2.08 and 2.15 (6 s, each 3 H, 6 × Ac), 3.54 (1 H, m, OCH_2CH_2), 3.76 (1 H, br t, *J* 6.7, H-5), 3.98 (1 H, m, OCH_2CH_2), 4.04 (1 H, br d, *J* 2.1, H-4), 4.14 (1 H, dd, *J* 6.9, 10.8, H-6), 4.4–4.5 (2 H, m, H-5′ and -6), 4.46 (1 H, d, *J* 7.8, H-1), 4.74 (1 H, dd, *J* 2.8, 10.8, H-3), 4.91 (1 H, d, *J* 3.7, H-1′), 5.1–5.2 (2 H, m, H-2 and -2′), 5.3–5.4 (2 H, m, H-3′ and -4′); δ_C(100 MHz; $CDCl_3$) 99.6 and 100.4 (C-1′, -1) (Found: C, 51.7; H, 6.9. $C_{29}H_{46}O_{16}Si$ requires C, 51.3; H, 6.8%).

2,3,6-Tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl)-β-D-galactopyranosyl trichloroacetimidate 20

TFA (5 ml) was added to a solution of **19** (150 mg, 0.22 mmol) in CH_2Cl_2 (5 ml) at 0 °C. After stirring of the mixture for 3.5 h, *n*-propyl acetate (10 ml) was added and the reaction mixture was co-concentrated several times with toluene. Chromatography on silica gel with heptane–EtOAc (2:3) gave the corresponding hemiacetal (117 mg, 0.20 mmol). The solid residue (106 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (8 ml). The solution was cooled to 0 °C, before trichloroacetonitrile (600 µl, 5.98 mmol) and DBU (40 µl, 0.27 mmol) were added. After being stirred for 2 h the solution was concentrated and the solid residue was chromatographed on silica gel with heptane–EtOAc–Et_3N (2:1:0.15) to give **20** (92 mg, 64%), δ_H(400 MHz; $CDCl_3$) 1.17 (3 H, d, *J* 6.5, H_3-6′), 1.98, 2.03, 2.04, 2.10, 2.12 and 2.17 (6 s, each 3 H, 6 × Ac), 4.28 (1 H, br d, *J* 2.6, H-4), 4.47 (1 H, q, *J* 6.4, H-5′), 4.94 (1 H, d, *J* 3.7, H-1′), 5.23 (1 H, dd, *J* 3.6, 10.3, H-2′), 5.26 (1 H, dd, *J* 2.7, 11.2, H-3), 5.33–5.40 (2 H, m, H-3′ and -4′), 5.45 (1 H, dd, *J* 3.7, 11.1, H-2), 6.58 (1 H, d, *J* 3.7, H-1), 8.67 (1 H, s, HN); δ_C(100 MHz; $CDCl_3$) 93.6 and 99.3 (C-1, -1′).

3,6-Di-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl)-α-D-galactopyranose-1,2-diyl N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-alanin-3-yl orthoacetate pentafluorophenyl ester 21

A solution of **20** (85 mg, 0.12 mmol), Fmoc-Ser-OPfp **7** (71 mg, 0.14 mmol) and powdered molecular sieves (AW-300, 200 mg) in CH_2Cl_2 (10 ml) was stirred for 1 h at room temperature. The solution was cooled to 0 °C and protected from light before silver trifluoromethanesulfonate (88 mg, 0.34 mmol) was added. The reaction mixture was stirred for 1.5 h at 0 °C, then allowed to attain room temperature over a period of 1 h. The solution was diluted with CH_2Cl_2 (50 ml), filtered (Celite), and washed with saturated aqueous $NaHCO_3$ (50 ml). The organic layer was dried, concentrated, and chromatographed on silica gel with heptane–ethyl acetate (3:1 to 1:1) to give **21** (65 mg, with traces of impurities), δ_H(400 MHz; $CDCl_3$) 1.15 (3 H, d, *J* 6.4, H_3-6′), 1.70 (3 H, s, CH_3CO_3), 1.98, 2.08, 2.09, 2.17, and 2.18 (5 s, each 3 H, 5 × Ac), 3.90 (1 H, dd, *J* 3.2, 9.6, Ser-Hβ), 4.34 (1 H, q, *J* 6.5, H-5), 4.94 (1 H, br d, *J* 8.9, Ser-Hα), 4.99 (1 H, d, *J* 3.0, H-1′), 5.07 (1 H, dd, *J* 2.4, 6.6, H-3), 5.72 (1 H, d, *J* 9.0, Ser-NH), 5.78 (1 H, d, *J* 4.3, H-1); δ_C(100 MHz; $CDCl_3$) 96.8 and 98.1 (C-1, -1′).

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl)-D-galactopyranose 22

Compound **19** (191 mg, 0.28 mmol) was dissolved in a mixture of acetic anhydride (4.5 ml) and toluene (4.5 ml). The solution was cooled to 0 °C before $BF_3{\cdot}Et_2O$ (150 µl, 1.19 mmol) was added. After being stirred for 18 h, the solution was diluted with CH_2Cl_2 (50 ml), and washed successively with saturated aqueous $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried, concentrated, and chromatographed on silica gel with heptane–EtOAc (1:1) to give **22** (144 mg, 83%) as an α,β-mixture (1:8), δ_H(400 MHz; $CDCl_3$) **22-α** 1.16 (3 H, d, *J* 6.5, H_3-6′), 4.94 (1 H, d, *J* 3.6, H-1′), 6.36 (1 H, d, *J* 3.7, H-1); **22-β** 1.18 (3 H, d, *J* 6.5, H_3-6′), 1.98, 2.05, 2.05, 2.08, 2.09, 2.14, and 2.16 (7 s, each 3 H, 7 × Ac), 3.92 (1 H, br t, *J* 6.5, H-5), 4.11 (1 H, d, *J* 2.5, H-4), 4.13 (1 H, dd, *J* 6.1, 11.3, H-6), 4.41 (1 H, dd, *J* 6.9, 11.3, H-6), 4.47 (1 H, q, *J* 6.7, H-5′), 4.82 (1 H, dd, *J* 2.7, 10.7, H-3), 4.92 (1 H, d, *J* 3.6, H-1′), 5.32 (1 H, dd, *J* 8.0, 10.7, H-2), 5.70 (1 H, d, *J* 8.0, H-1); δ_C(100 MHz; $CDCl_3$) **22-α** 89.9 and 99.6 (C-1, -1′); **22-β** 92.0 and 99.9 (C-1, -1′).

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-3-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-α-D-fucopyranosyl)-β-D-galactopyranosyl]-L-serine pentafluorophenyl ester 23

Compound **22** (138 mg, 0.22 mmol) and Fmoc-Ser-OPfp **7** (145

mg, 0.29 mmol) were dissolved in CH_3CN (5 ml) before $BF_3{\cdot}Et_2O$ (70 μl, 0.56 mmol) was added. After being stirred for 1 h at room temperature, the solution was diluted with dichloromethane (50 ml), and washed successively with saturated aqueous $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried, concentrated, and chromatographed on silica gel with heptane–EtOAc (2 : 1 to 1 : 1). Preparative normal-phase HPLC chromatography (gradient; 100% hexane ⟶ 15% ethanol in hexane during 120 min) gave **23** (87 mg, 37%), $[\alpha]_D^{20}$ +40 (*c* 0.3 in $CHCl_3$); δ_H(400 MHz; $CDCl_3$) 1.18 (3 H, d, *J* 6.5, H_3-6′), 1.95, 2.02, 2.02, 2.07, 2.10, and 2.16 (6 s, each 3 H, 6 × Ac), 3.73 (1 H, br t, *J* 6.5, H-5), 4.03 (1 H, dd, *J* 3.4, 10.5, Ser-Hβ), 4.07 (1 H, br d, *J* 2.4, H-4), 4.10 (1 H, dd, *J* 6.6, 11.4, H-6), 4.25 (1 H, t, *J* 6.7, $OCOCH_2CH$), 4.51 (1 H, d, *J* 7.8, H-1), 4.78 (1 H, dd, *J* 2.6, 10.9, H-3), 4.86 (1 H, dd, *J* 3.5, 8.4, Ser-Hα), 4.93 (1 H, d, *J* 3.7, H-1′), 5.20 (1 H, dd, *J* 4.0, 10.1, H-2′), 5.21 (1 H, dd, *J* 8.6, 10.8, H-2), 5.33–5.40 (2 H, m, H-3′ and -4′), 5.78 (1 H, d, *J* 8.4, Ser-NH); δ_C(100 MHz; $CDCl_3$) 99.8 (C-1′), 100.9 (C-1) (Found: C, 55.0; H, 4.9; N, 1.5. $C_{48}H_{48}F_5NO_{20}$ requires C, 54.7; H, 4.6; N, 1.3%).

General procedure for solid phase peptide synthesis

Glycopeptides **28**, **29,** and **31**–**38** were synthesized in a custom made, fully automatic, continuous-flow peptide synthesizer constructed essentially as described.[27] A resin consisting of a cross-linked polystyrene backbone grafted with poly(ethylene glycol) chains (Tentagel S NH_2™, Rapp Polymere, Germany) was used for the syntheses. The resin was functionalized with the Rink amide linker {*p*-[α-(fluoren-9-ylmethoxyformamido)-2,4-dimethoxybenzyl]phenoxyacetic acid}[28,29] (Novabiochem, Läufelfingen, Switzerland). N^{α}-Fmoc-amino acids (Bachem, Switzerland) with the following protective groups were used: 2,2,5,7,8-pentamethylchroman-6-sulfonyl for arginine; triphenylmethyl (Trt) for asparagine and glutamine; *tert*-butyl for aspartic acid, serine and tyrosine. DMF was distilled before being used.

In the synthesis of glycopeptides **31**, **33**, **34** and **36**–**38**, 60 μmol of resin was used in the peptide synthesizer. The N^{α}-Fmoc-amino acids were activated as 1-benzotriazolyl esters.[30] These were prepared *in situ* by reaction of the appropriate N^{α}-Fmoc-amino acid (0.24 mmol), HOBt (0.36 mmol) and DIC (0.23 mmol) in DMF (1.3 mL). The glycosylated pentafluorophenyl (Pfp) ester **30** (72 μmol) was coupled in the presence of HOBt (0.36 mmol). After 45 min of activation Bromophenol Blue (45 nmol, 0.3 ml of a 0.15 mM solution in DMF) was added to the 1-benzotriazolyl ester solution, which was then recirculated through the column containing the resin. The acylation was monitored[32] by using the absorbance of Bromophenol Blue at 600 nm, and the peptide–resin was automatically washed with DMF after 1 h, or when monitoring revealed the coupling to be complete. N^{α}-Fmoc deprotection of the peptide resin was performed by a flow of 20% piperidine in DMF (2 ml min^{-1}) through the column for 12.5 min, and was monitored[57] using the absorbance of the dibenzofulvene–piperidine adduct at 350 nm. After completion of the N^{α}-Fmoc deprotection the peptide–resin was again washed automatically with DMF.

Glycopeptides **28**, **29**, **32** and **34** were synthesized essentially as described above using 80 μmol of resin. The Pfp ester **30** (88 μmol and 96 μmol, respectively) was used in the synthesis of **32** and **34**, whereas the Pfp esters **26** (0.13 mmol) and **27** (0.22 mmol) were used in the syntheses of **28** and **29**, respectively.

In the synthesis of glycopeptide **24**, residues Gln^{57}–Arg^{61} were coupled in the peptide synthesizer, as described above. The peptide–resin was then removed from the synthesizer and Pfp ester **8** (39 μmol), as well as the remaining amino acids, were coupled in a mechanically agitated reactor. Reagent solutions and DMF for washing were added manually to the reactor. The N^{α}-Fmoc-amino acids were coupled as benzotriazolyl esters. These were prepared, *in situ*, from the appropriate acid (0.14 mmol), HOBt (0.16 mmol) and DIC (0.14 mmol) in DMF (1 ml). After 30–60 min, the solution was added to the reactor containing the resin (35 μmol). Acylations were monitored by addition of Bromophenol Blue (89 nmol) to the reactor. N^{α}-Fmoc deprotection was effected by treatment with 20% piperidine in DMF (4 min flow). Glycopeptide **25** was synthesized in a mechanically agitated reactor, as described for **24**, using 60 μmol of resin and 82 μmol of Pfp ester **23**, which was coupled in the presence of HOAt (246 μmol).

After completion of the synthesis, the resins carrying the protected glycopeptides were washed with CH_2Cl_2 and dried under vacuum. For each glycopeptide–resin the glycopeptide was then cleaved from a portion of the peptide–resin (details are given for each glycopeptide) and the amino acid side chains were deprotected by treatment with TFA–water–thioanisole–ethanedithiol (87.5 : 5 : 5 : 2.5, ~20 ml/200 mg of glycopeptide–resin) for 2 h followed by filtration. Acetic acid (5 ml) was added to the filtrate, the solution was concentrated, and acetic acid (2 × 5 ml) was added again followed by concentration after each addition. The residue was triturated with diethyl ether (15 ml) which gave a solid, crude glycopeptide, which was dissolved in a mixture of acetic acid and water (10 ml) and freeze dried. Purification by preparative reversed-phase HPLC gave pure *O*-acetylated glycopeptides, which were deacetylated in methanolic sodium methoxide (details are given for each glycopeptide). Neutralization of the solution with Duolite (H^+-exchanger) or acetic acid, followed by concentration and purification of the solid residue by preparative reversed-phase HPLC, gave pure glycopeptides.

The glycopeptides were analyzed on a Kromasil C-8 column (100 Å, 5 μm, 4.6 × 250 mm) using a linear gradient of 0 ⟶ 80% of *B* in *A* over a period of 60 min with a flow rate of 1.5 ml min^{-1} and detection at 214 nm (solvent systems *A*: 0.1% aqueous TFA and *B*: 0.1% TFA in CH_3CN). Purification of the crude glycopeptides was performed on a Kromasil C-8 column (100 Å; 5 μm, 20 × 250 mm) using the same gradient and a flow rate of 11 ml min^{-1}.

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-fucopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 24

Synthesis, cleavage of the resin-bound glycopeptide (35 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **24** (10 mg). Deacetylation for 4 h 15 min with methanolic sodium methoxide (2 mM; 9 ml), neutralization, concentration and purification by preparative HPLC gave **24** (6.5 mg, 65% peptide content, 8% overall yield). FABMS: $(M + H)^+$ 1459 (Calc. *m*/*z*, 1459); amino acid analysis: Arg 0.99 (1), Asp 2.01 (2), Glu 0.98 (1), Gly 1.02 (1), Ile 2.02 (2), Ser 1.99 (2), Tyr 1.00 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-fucopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 25

Synthesis, cleavage of the resin-bound glycopeptide (33 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **25** (25 mg). Deacetylation of *O*-acetylated **25** (12 mg) for 2 h 15 min with methanolic sodium methoxide (8 mM; 10 ml), neutralization, concentration and purification by preparative HPLC gave **25** (5.6 mg, 62% peptide content, 15% overall yield). FABMS: $(M + H)^+$ 1459 (Calc. *m*/*z*, 1459); amino acid analysis: Arg 1.01 (1), Asp 2.01 (2), Glu 0.97 (1), Gly 1.02 (1), Ile 2.01 (2), Ser 1.98 (2), Tyr 1.00 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-(β-D-galactopyranosyl)-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 28

Synthesis, cleavage of the resin-bound glycopeptide (46 μmol),

and purification by preparative HPLC according to the general procedure gave *O*-acetylated **28** (15 mg). Deacetylation for 3.5 h with methanolic sodium methoxide (2 mM; 10 ml), neutralization, concentration and purification by preparative HPLC gave **28** (6.5 mg, 63% peptide content, 7% overall yield). FABMS: $(M + H)^+$ 1313 (Calc. *m*/*z*, 1313); amino acid analysis: Arg 1.01 (1), Asp 1.99 (2), Glu 0.99 (1), Gly 1.02 (1), Ile 1.99 (2), Ser 2.01 (2), Tyr 0.99 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 29

Synthesis, cleavage of the resin-bound glycopeptide (41 μmol), and purification by preparative HPLC according to the general procedure gave *O*-benzoylated **29** (45 mg). Debenzoylation of *O*-benzoylated **29** (5 mg) for 7 h with methanolic sodium methoxide (20 mM; 5 ml), neutralization, concentration and purification by preparative HPLC gave **29** (1.5 mg, ~22%, not corrected for peptide content). FABMS: $(M + H)^+$ 1475 (Calc. *m*/*z*, 1475).

L-Alanyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 31

Synthesis, cleavage of the resin-bound glycopeptide (36 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **31** (27 mg). Deacetylation of *O*-acetylated **31** (17 mg) for 4 h with methanolic sodium methoxide (2 mM; 17 ml), neutralization, concentration and purification by preparative HPLC gave **31** (8.1 mg, 74% peptide content, 18% overall yield). FABMS: $(M + H)^+$ 1431 (Calc. *m*/*z*, 1431); amino acid analysis: Ala 0.98 (1), Arg 1.00 (1), Asp 1.03 (1), Glu 1.00 (1), Gly 1.01 (1), Ile 2.00 (2), Ser 1.99 (2), Tyr 0.99 (1).

L-α-Aspartyl-L-alanylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 32

Synthesis, cleavage of the resin-bound glycopeptide (41 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **32** (36 mg). Deacetylation in saturated methanolic ammonia (54 ml) at room temperature for 5 h, concentration and purification by preparative HPLC gave **32** (14 mg, 25%, not corrected for peptide content). FABMS: $(M + H)^+$ 1383 (Calc. *m*/*z*, 1383); amino acid analysis: Ala 1.00 (1), Arg 1.03 (1), Asp 2.00 (2), Glu 1.03 (1), Gly 1.02 (1), Ile 1.97 (2), Ser 1.96 (2).

L-α-Aspartyl-L-tyrosylglycyl-L-alanyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 33

Synthesis, cleavage of the resin-bound glycopeptide (35 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **33** (20 mg). Deacetylation for 4 h with methanolic sodium methoxide (2 mM; 20 ml), neutralization, concentration and purification by preparative HPLC gave **33** (7.6 mg, 74% peptide content, 11% overall yield). FABMS: $(M + H)^+$ 1433 (Calc. *m*/*z*, 1433); amino acid analysis: Ala 0.98 (1), Arg 1.01 (1), Asp 2.02 (2), Glu 0.99 (1), Gly 1.01 (1), Ile 0.98 (1), Ser 2.01 (2), Tyr 0.99 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-alanyl-L-isoleucyl-L-asparaginyl-L-seryl-L-arginine amide 34

Synthesis, cleavage of the resin-bound glycopeptide (39 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **34** (25 mg). Deacetylation of *O*-acetylated **34** (24 mg) for 2 h 15 min with methanolic sodium methoxide (2 mM; 24 ml), neutralization, concentration and purification by preparative HPLC gave **34** (9.0 mg, 68% peptide content, 12% overall yield). FABMS: $(M + H)^+$ 1418 (Calc. *m*/*z*, 1418); amino acid analysis: Ala 1.01 (1), Arg 0.99 (1), Asp 1.99 (2), Gly 1.01 (1), Ile 2.02 (2), Ser 1.98 (2), Tyr 0.99 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-alanyl-L-asparaginyl-L-seryl-L-arginine amide 35

Synthesis, cleavage of the resin-bound glycopeptide (35 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **35** (29 mg). Deacetylation of *O*-acetylated **35** (19 mg) for 4 h with methanolic sodium methoxide (2 mM; 19 ml), neutralization, concentration and purification by preparative HPLC gave **35** (9.4 mg, 73% peptide content, 21% overall yield). FABMS: $(M + H)^+$ 1433 (Calc. *m*/*z*, 1433); amino acid analysis: Ala 0.97 (1), Arg 1.02 (1), Asp 2.02 (2), Glu 0.99 (1), Gly 0.99 (1), Ile 1.01 (1), Ser 2.02 (2), Tyr 1.00 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-alanyl-L-seryl-L-arginine amide 36

Synthesis was performed according to the general procedure.

(A) Cleavage, deacetylation and purification according to the general procedure. Cleavage of the resin-bound glycopeptide (36 μmol), and purification by preparative HPLC gave *O*-acetylated **36** (27 mg). Deacetylation of *O*-acetylated **36** (17 mg) for 4 h with methanolic sodium methoxide (2 mM; 17 ml), neutralization, concentration and purification by preparative HPLC gave **36** (9.0 mg, 74% peptide content, 21% overall yield).

(B) Using improved conditions for cleavage, deacetylation and purification. Cleavage of the resin-bound glycopeptide (24 μmol) was performed by treatment with TFA–water–thioanisole–ethanedithiol (87.5 : 5 : 5 : 2.5; 9.25 ml) for 2 h at 40 °C followed by filtration. The resin was washed with acetic acid (2 × 4 ml) and the combined organic solutions were concentrated. Acetic acid (3 ml) was added to the filtrate, the solution was concentrated, and acetic acid (3 ml) was added again followed by concentration. The residue was triturated with diethyl ether (6 ml) which gave a solid, crude glycopeptide, which was dissolved in a mixture of acetic acid (1 ml) and water (26 ml) and freeze dried to give crude, *O*-acetylated **36** (41 mg). Crude, *O*-acetylated **36** (21 mg) was deacetylated in methanolic sodium methoxide (3 mM; 10 ml) for 3.5 h. Neutralization of the solution with acetic acid (100 μl), followed by concentration and purification of the solid residue by preparative reversed-phase HPLC, gave **36** (7.3 mg, 78% peptide content, 33% overall yield). Glycopeptide **36** had FABMS: $(M + H)^+$ 1432 (Calc. *m*/*z*, 1432); amino acid analysis: Ala 0.99 (1), Arg 0.99 (1), Asp 1.02 (1), Glu 0.99 (1), Gly 1.01 (1), Ile 1.99 (2), Ser 1.98 (2), Tyr 1.02 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-alanyl-L-arginine amide 37

Synthesis, cleavage of the resin-bound glycopeptide (34 μmol), and purification by preparative HPLC according to the general procedure gave *O*-acetylated **37** (18 mg). Deacetylation of *O*-acetylated **37** (12 mg) for 4 h with methanolic sodium methoxide (2 mM; 12 ml), neutralization, concentration and purification by preparative HPLC gave **37** (7.0 mg, 76% peptide content, 16% overall yield). FABMS: $(M + H)^+$ 1459 (Calc. *m*/*z*, 1459); amino acid analysis: Ala 0.99 (1), Arg 1.01 (1), Asp 2.02 (2), Glu 0.98 (1), Gly 0.99 (1), Ile 2.02 (2), Ser 1.00 (1), Tyr 0.98 (1).

L-α-Aspartyl-L-tyrosylglycyl-L-isoleucyl-*O*-[4-*O*-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-L-seryl-L-glutaminyl-L-isoleucyl-L-asparaginyl-L-seryl-L-alanine amide 38

Synthesis and cleavage of the resin-bound glycopeptide (36 μmol) gave crude *O*-acetylated **38** (40 mg). Deacetylation of crude *O*-acetylated **38** (14 mg) for 4 h with methanolic sodium methoxide (2 mM; 14 ml), neutralization, concentration and purification by preparative HPLC gave **38** (6.1 mg, 79% peptide content, 28% overall yield). FABMS: $(M + H)^+$ 1390 (Calc. *m/z*, 1390); amino acid analysis: Ala 1.00 (1), Asp 2.00 (2), Glu 0.99 (1), Gly 1.00 (1), Ile 2.02 (2), Ser 2.00 (2), Tyr 1.00 (1).

Synthesis and cleavage of peptide–resin 39

Peptide–resin **39** was prepared from Tentagel S NH_2 resin (224 μmol g^{-1}; 750 mg, 170 μmol) by sequential coupling of Fmoc-*m*-fluoro-L-phenylalanine, Rink amide linker, standard Fmoc-protected amino acids, and Fmoc-*p*-fluoro-L-phenylalanine in a mechanically agitated reactor as described in the general procedure for glycopeptide synthesis. After completion of the synthesis the Fmoc group was removed by treatment with 20% piperidine in DMF. The resin was washed with both DMF and CH_2Cl_2 and then dried under vacuum to give peptide–resin **39** (1.15 g).

Three portions of peptide–resin **39** (~100 mg each, ~15 μmol) were cleaved by treatment with TFA–water–thioanisole–ethanedithiol (87.5 : 5 : 5 : 2.5; 7 ml/100 mg of **39**) for (*a*) 2 h at room temperature, (*b*) 12 h at room temperature and (*c*) 2 h at 40 °C (bath temperature). After filtration the resins were washed successively with HOAc and CH_2Cl_2 and then dried under vacuum. They were then transferred into an ordinary 5 mm NMR tube and suspended in $CDCl_3$. Gel-phase ^{19}F NMR spectra were recorded as described previously.[38]

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