

# On-resin solid-phase synthesis of asparagine N-linked glycopeptides: use of *N*-(2-acetoxy-4-methoxybenzyl) (AcHmb) aspartyl amide-bond protection to prevent unwanted aspartimide formation

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The *N*-(2-acetoxy-4-methoxybenzyl) (AcHmb) backbone amide-protecting group has been applied in an on-resin solid-phase synthesis of asparagine N-linked glycopeptides. Backbone protection of the -Asp(OAllyl)-Ala- aspartyl amide bond suppressed the formation of aspartimide during both the initial chain assembly and the subsequent activation and glycosylation of the aspartyl  $\beta$ -carboxy group. A 1.1–1.25 mole excess of glycosylamine coupled quantitatively, with minimal side reaction, to the activated aspartyl  $\beta$ -carboxy group of the fully assembled backbone-protected peptide-resin. A parallel synthesis without backbone amide protection produced substantial aspartimide peptide, particularly during the initial chain assembly.

## Introduction

Glycosylation is the most common modification of proteins. In eukaryotes, most proteins that share a common processing pathway through the lumen of the endoplasmic reticulum, which include integral membrane proteins and most extracellular proteins, are glycosylated. Glycosylation introduces great structural diversity to the protein, a set of glycoforms may be generated from a single parent aglycone differing with respect to the number of oligosaccharides present, their structure and the glycosylation sites occupied.<sup>1</sup> Despite the ubiquity of this modification, no universal functional role has yet been discovered for protein glycosylation, although many putative roles have been suggested.<sup>2</sup> The presence of a glycan can modulate the properties of a protein such as its solubility, charge, immunogenicity and protease resistance. In addition it is assumed that it has a role in recognition mediated by carbohydrate-binding proteins. For example, in targeting of proteins to lysosomes, the elimination of serum glycoproteins and in cell adhesion.<sup>1</sup> It has been observed that some glycans are characteristic of certain cell types and developmental stages. Further, glycosyl modification has been observed during oncogenesis and has been implicated in metastasis.<sup>1</sup> Much recent interest was stimulated by the observation that oligosaccharides were involved in leukocyte recognition and binding to endothelia.<sup>1,3</sup> The possibility exists that carbohydrate recognition is involved in many other cellular processes.

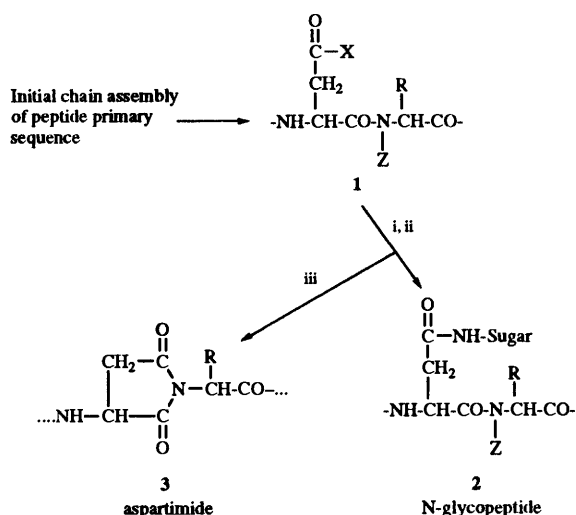
One of the commonest forms of attachment of an oligosaccharide to a protein is N-linkage through the side chain of asparagine. All N-linked glycoproteins share a common trimannosylchitobiosyl pentasaccharide core. N-linked proteins include antibodies, cell adhesion proteins and cell surface receptors.<sup>4,5</sup> Owing to the difficulty in expressing defined glycoproteins,<sup>5</sup> synthetic methods to yield glycopeptides as models to investigate the effects of glycosylation are desirable. Chitobiosylamine and 2-acetamido-1-amino-1,2-dideoxyglucopyranose have been used as model glycosylamines because of the limited availability of the larger naturally occurring oligosaccharides. Improvements in oligosaccharide synthesis should soon make the larger glycans available.<sup>6,7</sup>

For the rapid preparation of diverse sequences of glycopeptides, the solid-phase strategy offers great flexibility and automation. The preparation of glycopeptides is, however, complicated compared with standard peptide synthesis by the sensitive nature of glycosidic bonds. This sensitivity is an important consideration when selecting an appropriate solid-

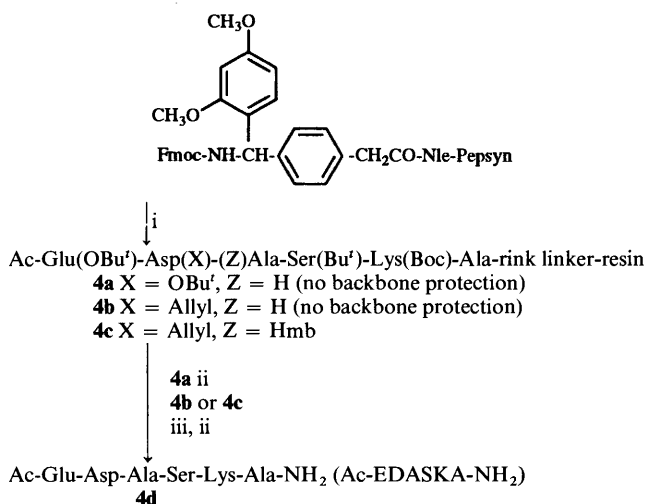
phase procedure, with mild reaction conditions being desired. Here, the fluoren-9-ylmethoxycarbonyl (Fmoc)/*tert*-butyl approach appears attractive, using relatively weak trifluoroacetic acid (TFA) as the final side-chain-protecting group and peptide-resin cleavage agent, compared with the harsh hydrofluoric acid conditions used in the *tert*-butoxycarbonyl (Boc)/benzyl approach.<sup>8</sup>

Currently two methods using the Fmoc/*tert*-butyl approach are used for the synthesis of N-linked glycopeptides.<sup>7</sup> The first makes use of preformed building blocks consisting of carboxy-activated, *N*<sup>α</sup>-Fmoc-protected asparagine to which the sugar of interest is N-linked through the  $\beta$ -carboxamide.<sup>9,10</sup> The second involves the coupling of an appropriate amino sugar with a side-chain-activated peptide aspartyl residue.<sup>11b–14</sup> Both methods are subject to limitations. For the former, there is the time-consuming preparation, isolation and characterisation of individual N-linked glycosylasparagine derivatives. With increasing complexity of the protected amino acid-sugar building blocks, coupling rates are often slow and, even with considerable excess of reagent, incorporation can be poor. This may often be prohibitive when the glycan moiety is of limited availability.<sup>10</sup> The latter method presents an additional level of complexity to traditional solid-phase peptide synthesis methods<sup>15</sup> and the basic strategy is depicted in Scheme 1. An orthogonal side-chain protecting group (Scheme 1, X), compatible with Fmoc/*tert*-butyl methodology, is required for the aspartyl  $\beta$ -carboxy group. This protecting group is retained during chain assembly and its selective removal enables  $\beta$ -carboxy-group activation of a particular aspartyl residue and subsequent coupling with a glycosylamine to give the desired N-glycopeptide (Scheme 1, 2). In this way, complete control of the glycosylation position is achieved. The commercially available allyl protecting group would seem to meet these requirements adequately. The allyl group (stable to repetitive treatment with base necessary for removal of Fmoc) has been widely applied in peptide chemistry<sup>16</sup> and is removed specifically by reaction with palladium-based reagents.

However, owing to the sensitive nature of the aspartyl  $\alpha$ -carboxy amide bond,<sup>17</sup> (hereafter simply referred to as the aspartyl amide bond) the seemingly simple strategy depicted in Scheme 1 contains several potential pitfalls. Until recently,  $\beta$ -*tert*-butyl ester side-chain protection of aspartic acid (Scheme 1, X = O*t*Bu and Z = H), routinely used in the Fmoc approach to peptide synthesis, was assumed to provide adequate protection of the aspartyl  $\beta$ -carboxy group. However, it has now unequivocally been shown that aspartimide formation



**Scheme 1** Pathway for N-linked glycosylation to aspartyl  $\beta$ -carboxy group, showing competing formation of aspartimide. See text for definitions of R, X and Z. *Reactions*: i, selective removal of aspartic acid  $\beta$ -carboxy-group protection 'X $\rightarrow$ OH'; ii, activation, glycosylamine 'OH $\rightarrow$ OAc'; iii, competing side reaction



**Scheme 2** Protecting-group strategies toward the preparation of Ac-EDASKA-NH<sub>2</sub> **4d**. *Reactions and reagents*: i, stepwise solid-phase peptide synthesis; ii, TFA, Et<sub>3</sub>SiH, water (90 : 5 : 5 v/v/v); iii, Pd(PPh<sub>3</sub>)<sub>4</sub>, NMM, AcOH, DMF, CHCl<sub>3</sub>

(Scheme 1, **3**) may occur with this protection strategy.<sup>18–20</sup> The extent of the aspartimide side-reaction is dependant upon the base employed for removal of Fmoc and the C-terminal residue of the aspartyl bond (especially prone are Gly, Ser, Thr, Asn, Gln). Additionally, earlier studies elucidated the importance of the nature of the aspartyl side-chain-protecting group with respect to formation of aspartimide. The performance of  $\beta$ -O-allyl side-chain protection in this context has not been previously reported. On steric considerations alone, it would be expected that formation of aspartimide would occur more readily with  $\beta$ -O-allyl protection compared with *tert*-butyl, and later we report here that this is indeed the case (see Results and discussion section). Also, once an efficient initial chain assembly has been performed, activation of the selectively side-chain-deprotected aspartyl residue, ready for glycosylamine coupling, can lead to formation of aspartimide.

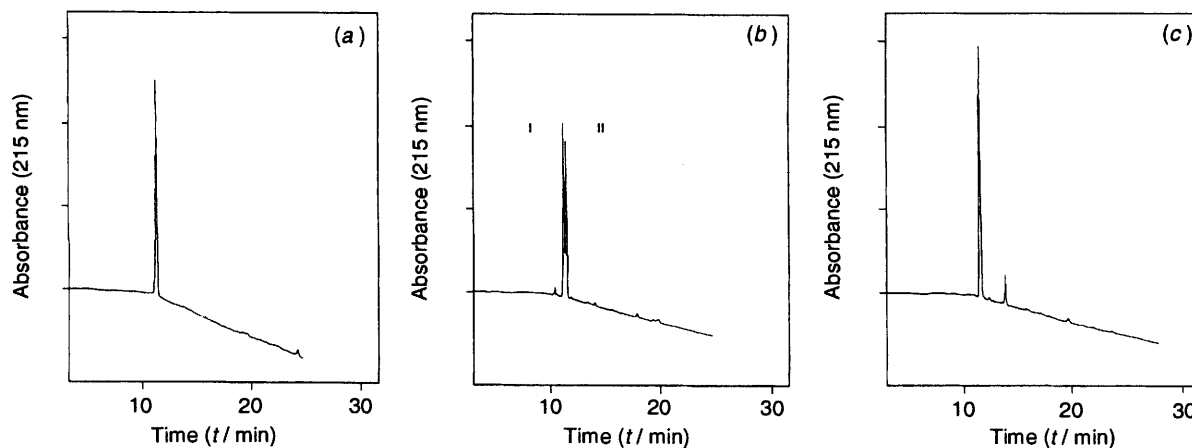
Several approaches to the problem of formation of aspartimide during glycopeptide synthesis have so far been advanced. One strategy has been to substitute glutamyl for aspartyl as the residue for glycosylation, a link so far unknown

to occur naturally but of potential use in the synthesis of peptide libraries.<sup>12,13</sup> Alternatively, the aspartyl amide bond contains a C-terminal proline residue.<sup>13</sup> Here the amide proton is removed and can no longer participate in formation of aspartimide. This technique, however, is not a general solution and a glycosylated aspartyl residue adjacent to a proline residue is thought to be xenobiotic.<sup>4</sup> The N-glycosylation of terminal positions is another strategy that avoids unwanted formation of aspartimide. A thorough investigation of the glycosylation reaction concluded with the recommendation of the use of a large excess of glycosylamine to act as base activator and substrate,<sup>11</sup> again prohibitive if only limited quantities of glycan are available. We have recently reported on a solution to the formation of aspartimide peptides under Fmoc/*tert*-butyl solid-phase synthesis conditions.<sup>20</sup> The mechanism of formation of aspartimide, under base-catalysed conditions (e.g., piperidine), is *via* abstraction of the aspartyl amide nitrogen proton followed by nucleophilic attack of the  $\beta$ -carboxy group. Our approach was to replace the amide nitrogen proton of the aspartyl bond with an acid-labile backbone-protecting group, 2-hydroxy-4-methoxybenzyl (Hmb) (Scheme 1, X = OBu, Z = Hmb, R = H, CH<sub>2</sub>CONHTrt). This modification of the backbone amide bond completely suppressed formation of aspartimide even in very sensitive sequences. We now report on an extension of this principle as applied to the solid-phase synthesis of N-linked glycopeptides through the 'on-resin' activation of the aspartyl  $\beta$ -carboxy group without formation of aspartimide.

## Results and discussion

Two major problems are associated with the synthesis of glycopeptides *via* the on-resin method illustrated in Scheme 1. First, the formation of aspartimide-containing peptides can arise during both the initial synthesis of the fully protected primary sequence and, following selective deprotection, during glycosylamine coupling to the appropriate aspartyl residue. Second, that the optimal solvents for dissolution of glycan may not always be compatible with peptide-resin solvation.<sup>15</sup> We therefore considered that backbone amide-bond substitution of the aspartyl residue destined for glycosylation would serve two functions. Not only would formation of aspartimide be prevented<sup>20</sup> but also that a range of solvents could potentially be used for the glycosylation reaction if required. Improved peptide-resin solvation would be expected because the major association of peptide chains (leading to their aggregation) is mediated through interchain hydrogen bonding (primarily *via* the secondary amide bonds of the peptide backbone). This is prevented by replacement of secondary by tertiary amide bonds, *i.e.* backbone-amide substitution, causing a disruption of the H-bonded network leading to a large increase in solubility.<sup>21,22</sup>

The peptide Ac-Glu-Asp-Ala-Ser-Lys-Ala-NH<sub>2</sub> (Scheme 2, **4d**) was chosen as a model sequence to examine the use of aspartyl  $\beta$ -O-allyl protection and backbone amide protection in N-linked glycopeptide synthesis. This sequence had previously been identified as being particularly susceptible to formation of aspartimide under conditions of the glycosylation reaction.<sup>11</sup> Initial studies investigated the propensity for formation of aspartimide using allyl side-chain protection of aspartic acid in comparison with the more usual *tert*-butyl. Syntheses were performed using standard pentafluorophenyl ester (OPfp) chemistry<sup>8</sup> on Kieselguhr-supported poly(dimethylacrylamide) through the Rink amide linker (Pepsyn KR). Analytical high-performance liquid chromatography (HPLC) of the crude product synthesized using *tert*-butyl protection (Scheme 2, **4a**) exhibited a single peak [Fig. 1(a)] upon acidic cleavage of the peptide from the support, that on MALDITOF-MS analysis, gave mass = 661.4 Da (theoretical mass = 660.7 Da). In contrast, a repeat of the synthesis using allyl side-chain protection (Scheme 2, **4b**), when analysed by HPLC, yielded



**Fig. 1** The effect of the substitution of *O*-allyl for *O*-*tert*-butyl in aspartyl side-chain-protection using the Fmoc/*tert*-butyl method illustrated by the synthesis of the peptide Ac-EDASKA-NH<sub>2</sub>, **4d**. (a) The peptide **4d** synthesized *via* standard Fmoc/*tert*-butyl solid-phase synthesis. (b) Products from the substitution of *O*-allyl for *O*-*tert*-butyl on the side chain of aspartyl. I is peptide **4d**, II is the aspartimide. (c) The peptide **4d** synthesized *via* substitution of *O*-allyl for *O*-*tert*-butyl on Asp and also including (Hmb)Ala backbone protection. HPLC conditions: Aquapore RP-300 C<sub>8</sub> column (250 × 4.6 mm), 0–40% B in A, linear gradient during 25 min (1.5 cm<sup>3</sup> min<sup>-1</sup>; 215 nm UV detection) where buffer A = 0.1% aq. TFA and buffer B = 90% acetonitrile–10% buffer A

two products [55:45 ratio, Fig. 1(b)] after removal of allyl protection and peptide-resin cleavage. Analysis of the two products by MALDITOF-MS showed that the later [Fig. 1(b), peak II] eluting species was the aspartimide peptide with mass = 642.6 Da. To confirm that aspartimide had arisen during assembly of the peptide and not during the palladium-catalysed removal of allyl protection, sequence **4b** was cleaved from the support by acidolysis whilst allyl protection was retained. Analytical HPLC of the product from allyl-substituted compound **4b** again showed two peaks (60:40 ratio), that on MALDITOF-MS analysis showed the former to be aspartimide with mass = 642.6 Da (see Experimental section for HPLC details). This result confirmed our earlier supposition that allyl protection was more likely to be prone to formation of aspartimide in comparison with *tert*-butyl. This finding should sound a cautionary note on the introduction of new synthetic strategies without a thorough investigation of potential side reactions.<sup>14</sup> A third synthesis of the test peptide again used allyl side-chain protection but this time Hmb substitution of the aspartyl amide bond was introduced (Scheme 2, **4c**). Following allyl-group removal and acidolytic cleavage of the peptide from the support, analytical HPLC showed a major peak [Fig. 1(c)], that gave on MALDITOF-MS analysis a mass = 660.9 Da (theoretical mass = 660.7 Da). The peptide from compound **4c** also co-eluted, on analytical HPLC, with peptide **4d** prepared utilising *tert*-butyl side-chain protection of aspartic acid (from peptide-resin **4a**). As observed previously, even for very sensitive sequences, formation of aspartimide is completely suppressed by backbone protection of the aspartyl amide bond. From this series of initial experiments, it is obvious that Hmb backbone substitution of the aspartyl bond is imperative for efficient assembly of the primary sequence containing Asp(OAllyl).

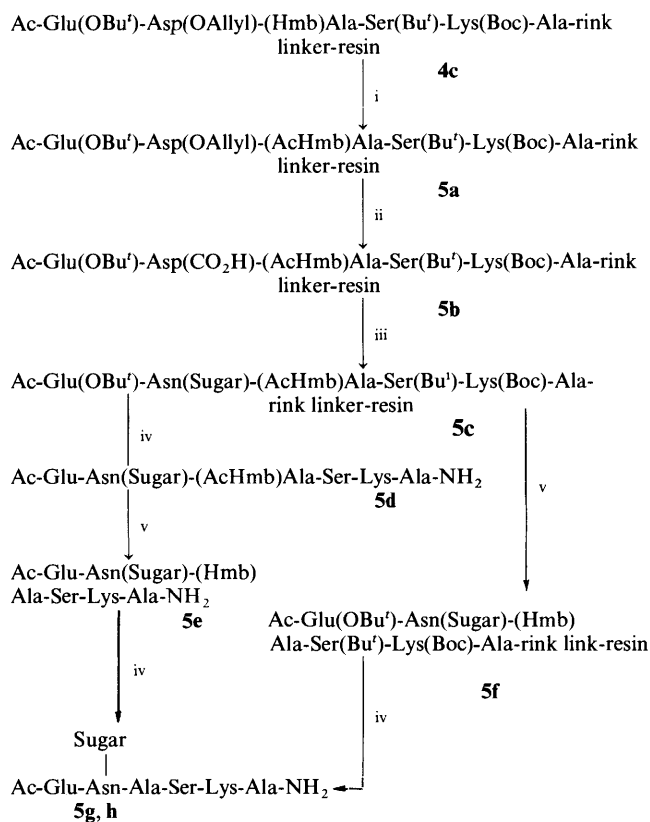
The basic strategy for synthesis of the primary sequence having been established, the coupling of the glycosylamine was next investigated. The basic scheme for glycosylamine coupling is outlined in Scheme 1 (1, Z = AcHmb). Scheme 3 shows the synthetic procedure in full. Our recent experience with fully protected backbone-substituted peptides indicated that superior results are obtained if the Hmb 2-hydroxy moiety is protected during its subsequent use in coupling reactions. Therefore, in order to prevent potential side reaction in our glycosylation scheme, the first step is to protect reversibly the Hmb hydroxy function (Scheme 3, **5a**). Our preferred method is to utilise simple acetylation (reversed by hydrazine-mediated cleavage of the acetyl group).<sup>23</sup> Thus, peptide-resin **4c** was treated with acetic anhydride (Ac<sub>2</sub>O) in the presence of diisopropylethyl-

amine (DIPEA) using dimethylformamide (DMF) as bulk solvent. To check on the progress of the acetylation reaction, a small quantity of peptide-resin **5a** was cleaved with a TFA/scavenger cocktail. Acetylation of the Hmb group (to 2-acetoxy-4-methoxybenzyl, AcHmb) renders it stable to TFA-based acidolytic cleavage.<sup>23</sup> Consequently, the AcHmb-substituted peptide is overall more hydrophobic than the parent, unsubstituted peptide and the two are invariably well separated on HPLC. The crude AcHmb-substituted peptide from compound **5a** showed a single major species by analytical HPLC with MALDITOF-MS analysis, giving mass = 879.1 Da (theoretical mass = 878.8 Da). Careful scrutiny of the analytical HPLC result did not reveal the presence of non-backbone-protected peptide, confirming full Hmb acetylation had been achieved.

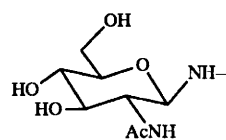
Peptide-resin **5a** was treated with palladium reagent in the presence of *N*-methylmorpholine (NMM)–AcOH,<sup>14</sup> giving peptide-resin **5b**. Completion of the reaction was confirmed by a small-scale cleavage and analytical HPLC analysis of the crude product [Fig. 2(a)]. The main peak showed a change in retention time compared with the allyl side-chain-protected peptide (*cf.* peptide from substrate **5a**) that was confirmed as the free aspartyl side-chain peptide from compound **5b** upon MALDITOF-MS analysis, giving mass = 839.0 Da (theoretical mass = 838.8 Da). No evidence for the allyl-protected peptide was observed by either HPLC or mass analysis.

The side-chain β-carboxylate of the aspartyl residue of peptide-resin **5b** was coupled with *N*-acetylglucosamine through activation with (benzotriazol-1-yl)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) reagent in the presence of *N*-hydroxybenzotriazole (HOBT) and DIPEA (1.1 mol equiv. of all reagents). HPLC analysis of the crude product from cleavage of peptide-resin **5c**, peptide **5d**, exhibited a new main species [Fig. 2(b)], and MALDITOF-MS analysis of the main species gave a mass = 1042.5 Da (theoretical mass = 1041.1 Da). Existing methods<sup>11</sup> require individual optimisation of each glycosyl coupling reaction; however, this was unnecessary in our procedure as formation of aspartimide was not a problem. It is noteworthy that this procedure requires only a 10% excess of reagents in order to drive the coupling reaction to completion, particularly important where limited quantities of glycosylamine are available. Previous reports have used several equivalents of glycosylamine to substitute for the basic component in the activation step in order to minimise formation of aspartimide.<sup>11–14</sup>

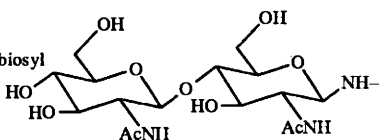
To obtain the final glycopeptide target structure, the backbone-protecting group must be de-*O*-acetylated in order to



**5g**; sugar = *N*-acetylglucosyl



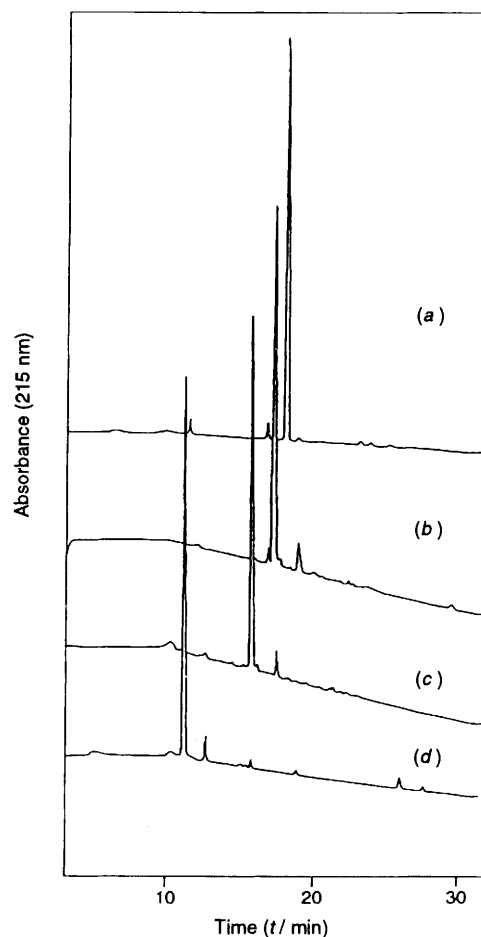
**5h**; sugar = *N,N'*-diacetyl-chitobiosyl



**Scheme 3** Synthetic routes for the glycosylated peptides **5g** and **5h**.  
**Reactions and reagents:** i, Hmb acetylation (Ac<sub>2</sub>O, DIPEA); ii, allyl removal [Pd(PPh<sub>3</sub>)<sub>4</sub>, NMM, AcOH, DMF, CHCl<sub>3</sub>]; iii, glycosyl coupling [BOP, HOBt, DIPEA, glycosylamine (1.1 mol equiv. each) in DMSO-DMF]; iv, acidolytic cleavage [TFA, water, Et<sub>3</sub>SiH, EDT (91 : 3 : 3 : 3 v/v/v/v)]; v, AcHmb deacetylation (N<sub>2</sub>H<sub>4</sub>, DMF)

re-establish its lability to TFA. De-O-acetylation is usually achieved through nucleophilic cleavage mediated by brief treatment with hydrazine hydrate. In order to establish minimum reaction conditions for the hydrazinolysis, investigations were performed in solution using peptide **5d** (sugar = *N*-acetylglucosyl). AcHmb-backbone-protected glycopeptide **5d** was subjected to hydrazine-mediated de-O-acetylation. Careful HPLC monitoring indicated that a 15 min reaction time was necessary for quantitative cleavage, giving peptide **5e** [Fig. 2(c)], with mass = 1000.0 Da (theoretical mass = 999.0 Da).

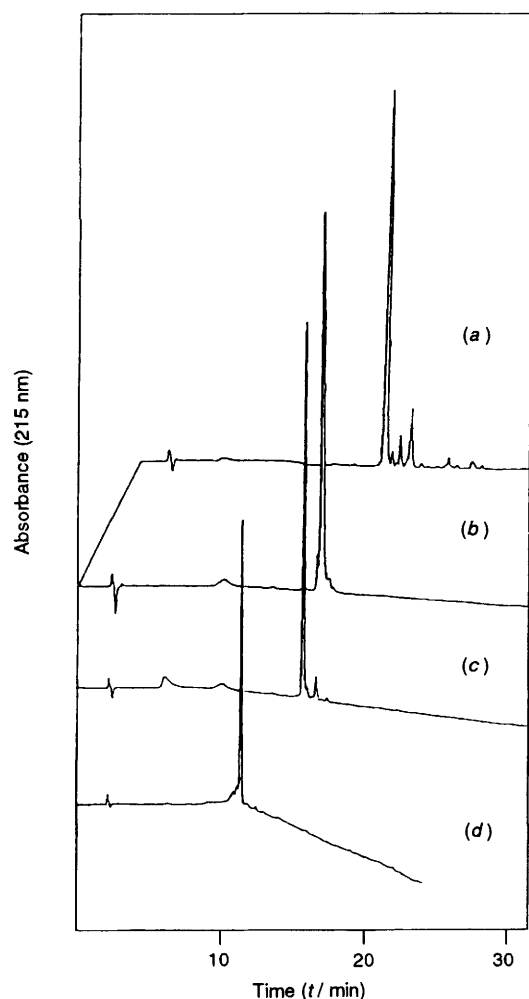
Several groups have reported that even the relatively weak TFA can lead to degradation of glycosidic bonds. However, the majority of glycosidic bonds of mono- and di-saccharides are stable during TFA cleavage of glycopeptides from a resin.<sup>10,24,25</sup> To confirm this, peptide **5e** was treated with TFA/scavenger mixture for 2 h to effect removal of Hmb. The final TFA-mediated acidolysis was carefully evaluated in solution with respect to glycoside stability. Peptide **5g** (solution method, see Experimental section for full details) exhibited a new major species on analytical HPLC analysis [Fig. 2(d)], with mass = 863.8 Da (theoretical mass = 862.9 Da). Obviously the glycosidic linkage is stable to the acidolysis



**Fig. 2** Analytical HPLC of intermediates in the synthesis of glycopeptide **5g** (Scheme 3). (a) Small scale-cleavage product from peptide-resin **5b**. (b) Glycosylated peptide containing AcHmb backbone protection **5d**. (c) Hmb backbone-protected glycopeptide **5e** from hydrazine treatment of **5d** in solution. (d) Fully deprotected glycopeptide **5g** by treatment of **5e** with TFA/scavenger mixture. HPLC conditions: Aquapore RP-300 C<sub>8</sub> column (250 × 4.6 mm), 0–40% B in A, linear gradient during 25 min (1.5 cm<sup>3</sup> min<sup>-1</sup>; 215 nm UV detection) where buffer A = 0.1% aq. TFA and buffer B = 90% acetonitrile–10% buffer A

conditions for removal of Hmb (it should be noted that these conditions are similar to those required for removal of other peptide side-chain-protecting groups and peptide cleavage from the resin).

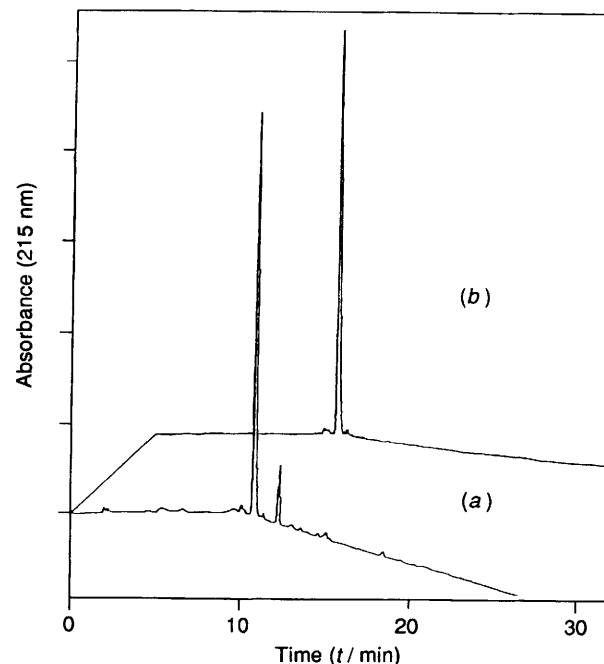
This protocol was repeated with the disaccharide *N,N'*-diacetylchitobiosylamine. The required β-1-amino sugar was prepared from commercially available chitobiose by using the Kochetkov method.<sup>26,27</sup> Peptide-resin **5b** was coupled with chitobiosylamine by utilising BOP-HOBt-DIPEA (1.25 mol equiv. each) for 4 h. The glycopeptide was cleaved from peptide-resin **5c** and analysed by analytical HPLC. The crude peptide (**5d**, sugar = *N,N'*-diacetylchitobiosyl) showed a single main species [Fig. 3(a)] with mass = 1243.5 Da (theoretical mass = 1244.3 Da). The main peak was isolated by preparative HPLC and re-analysed by analytical HPLC to confirm its purity [Fig. 3(b)]. Peptide **5d** was de-O-acetylated in solution following the optimal hydrazine hydrate treatment described above, to give compound **5e** [Fig. 3(c)]. The backbone-protected glycopeptide **5e**, containing two potentially cleavable glycosidic bonds, was treated with a TFA/scavenger-based cocktail to deprotect fully the glycopeptide to yield compound **5h**. Analytical HPLC analysis of product **5h** exhibited a single species [Fig. 3(d)], with mass = 1066.2 Da (theoretical mass = 1067.1 Da), confirming the stability of the glycosidic bonds to TFA/scavenger cleavage conditions. The glycopeptide **5h** was isolated in 16.4% overall yield.



**Fig. 3** Analytical HPLC of intermediates in the synthesis of glycopeptide **5h** (Scheme 3). (a) Crude and (b) purified product **5d** of glycosylation from **5b** with *N,N'*-diacetylchitobiosylamine. (c) Hmb backbone-protected glycopeptide **5e** from hydrazine treatment of **5d** in solution. (d) Fully deprotected glycopeptide **5h** by treatment of **5e** with TFA/scavenger mixture. HPLC conditions: Aquapore RP-300 C<sub>8</sub> column (250 × 4.6 mm), 0–40% B in A, linear gradient during 25 min (1.5 cm<sup>3</sup> min<sup>-1</sup>; 215 nm UV detection) where buffer A = 0.1% aq. TFA and buffer B = 90% acetonitrile–10% buffer A

Having established conditions, in solution, for de-O-acetylation of AcHmb and confirmed the stability of the glycosidic linkages to treatment with TFA, these procedures were applied to a complete solid-phase protocol in order to obtain the target glycopeptide, **5g**. Peptide-resin **5c** (Scheme 3) was treated with 5% hydrazine hydrate in DMF for 15 min to give peptide-resin **5f**, followed by acidolytic cleavage, for 2 h, (and simultaneous removal of the side-chain/backbone-protecting groups) of the glycopeptide from the support. The crude glycopeptide **5g** (all-on-resin method) was of good purity [90%, Fig. 4(a)] and was obtained in 30% overall yield after preparative HPLC purification [Fig. 4(b)].

Most N-linked glycoproteins so far examined have been shown to be linked through 2-acetamido-1-amino-1,2-dideoxyglucopyranose in the  $\beta$ -conformation. The preparation of glycosylamines exhibits a reversed anomeric effect, evident during the synthesis of the 1-amino sugar, which makes the  $\beta$ -conformation (equatorial amino group) preferred.<sup>28</sup> The condensation of glycosylamine with the side-chain of aspartic acid should lead to the required  $\beta$ -conformation, without the formation of the  $\alpha$ -(axial) amino-linked sugar. The glycosyl conformation of glycopeptides **5g** and **5h** was checked by <sup>1</sup>H NMR spectroscopy, with a large (9.6 Hz) coupling constant between the *N*-glycosyl C-1 proton and C-2 proton observed



**Fig. 4** Analytical HPLC of (a) crude and (b) purified glycopeptide **5g** from large-scale solid-phase preparation. HPLC conditions: Aquapore RP-300 C<sub>8</sub> column (250 × 4.6 mm), 0–40% B in A, linear gradient during 25 min (1.5 cm<sup>3</sup> min<sup>-1</sup>; 215 nm UV detection) where buffer A = 0.1% aq. TFA and buffer B = 90% acetonitrile–10% buffer A

indicating a *trans* proton configuration.<sup>11</sup> There was no evidence in the NMR spectrum for the presence of the  $\alpha$ -linked anomer, indicating that full control of the sugar C-1 stereochemistry had been achieved.

### Conclusions

The orthogonal (with respect to Fmoc/*tert*-butyl methodology) allyl side-chain-protecting group for aspartyl residues has been shown to be particularly prone to formation of aspartimide. This side reaction occurs in a sequence where *tert*-butyl would normally be adequate. It is completely suppressed by appropriate reversible N-substitution of the aspartyl amide bond with the acid-labile Hmb group. Backbone protection has led to a more flexible approach for the synthesis of glycopeptides mediated through on-resin coupling of an activated aspartyl  $\beta$ -carboxy function with glycosylamine. The formation of aspartyl side products usually associated with on-resin-based protocols are completely suppressed, allowing efficient use of valuable aminoglycosides. The methodology outlined above may be particularly important in synthesizing N-linked Asn glycopeptide libraries through the glycosylation of a common activated peptide-resin core with numerous glycans.

## Experimental

### Equipment, materials and methods

Continuous-flow Fmoc-polyamide methods reviewed by Atherton and Sheppard<sup>8</sup> were used exclusively. Fmoc amino acid pentafluorophenyl-activated esters (Novabiochem, Beeston, Nottingham, UK NG9 2JR) were used exclusively except for Ser(Bu<sup>t</sup>) which was coupled as the dihydroxobenzotriazine ester (Novabiochem, UK). *N,O*-BisFmoc-*N*-(2-hydroxy-4-methoxybenzyl)alanine pentafluorophenyl ester was prepared as previously detailed.<sup>29</sup> Pepsyn KR resin (containing the Rink linker) was purchased from Novabiochem, UK. All solvents were purified as previously described.<sup>8</sup>

Solid-phase peptide synthesis was performed on an LKB 'Biolynx' 4170 automated synthesizer programmed to perform acylation reactions (in DMF) for 45 min unless otherwise

stated, and Fmoc deprotection reactions (in 20% piperidine–DMF v/v) for 10 min. All chiral amino acids used were of the L-configuration. Amino acid side-chain protection was as follows: Lysine (*N*<sup>ε</sup>-*tert*-butoxycarbonyl, Boc), Serine (*tert*-butyl ether, Bu<sup>t</sup>), Aspartic and Glutamic acid (*tert*-butyl ester, OBU<sup>t</sup>). Peptide hydrolyses were performed at 110 °C for 18 h in HCl (6 mol dm<sup>-3</sup>) containing a trace of phenol, in evacuated, sealed tubes. Analysis of hydrolysis products was performed on a Beckman 7300 analyser. Separation was obtained using ion-exchange resin with manufacturer's buffer solutions and post-column detection by ninhydrin. Analytical HPLC was performed using a Brownlee Aquapore RP300 C8 column (250 × 4.6 mm). A 0–40% B in A gradient over a period of 25 min at (1.5 cm<sup>3</sup> min<sup>-1</sup>) was used unless otherwise stated, where A = 0.1% aq. TFA and B = 90% acetonitrile–10% A. Preparative HPLC was performed using a Vydac 214TP1022, protein C4 column (22 × 250 mm) at 10 cm<sup>3</sup> min<sup>-1</sup> flow rate and 215 nm UV detection, solvents A and B as above. MALDITOF-MS were obtained on a Kratos MALDI III bench-top linear–reflectron mass spectrometer. <sup>1</sup>H NMR spectra were obtained on a Bruker DRX 300 spectrometer. 2-Acetamido-1-amino-1,2-dideoxy-β-D-glucopyranose was purchased (Sigma) and 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-glucopyranosylamine (chitobiosylamine) was synthesized from *N,N'*-diacetylchitobiose (Fluka) by a published procedure.<sup>26,27</sup>

#### Preparation of Ac-Glu-Asp-Ala-Ser-Lys-Ala-NH<sub>2</sub> (Scheme 2, peptide 4d)

Peptide 4d was synthesized by standard Fmoc/*tert*-butyl solid-phase methods on Pepsyn KR resin, by three methods (see Scheme 2). Pepsyn KR resin (1.0 g, 0.1 mmol) was suspended in DMF for 10 min, the supernatant, containing any fine particles, was removed by decantation, and the resin was loaded onto the Biolynx synthesizer. The sequences were synthesized in a stepwise manner with activated residues coupled (0.5 mmol vials) under standard conditions except for peptide-resin 4c, where the *N,O*-bisFmoc-*N*-(2-hydroxy-4-methoxybenzyl)-alanine OPfp ester<sup>29</sup> (0.5 mmol) was coupled for 2 h. The subsequent *O*-allyl aspartic residue was coupled as its symmetric anhydride. The de-Fmoc resin was removed from the synthesizer, washed successively with DMF and diethyl ether, and dried *in vacuo* (over KOH pellets). The N-terminal Hmb-resin was suspended in a solution of the symmetrical anhydride (prepared by the method of Atherton and Sheppard<sup>8</sup>) of Fmoc-*O*-allyl aspartic acid (10 mol equiv., 0.25 mmol) in dichloromethane. After reaction for 2 h the resin was washed successively with DMF and diethyl ether, re-suspended in DMF, and loaded back onto the synthesizer, and the synthesis was continued for the addition of the glutamyl residue.

Peptide-resins 4a–c were then acetylated for 2 h in DMF by using acetic anhydride (205 mg, 2.0 mmol). Completeness of the acetylation was checked by using the ninhydrin reaction.<sup>8</sup>

Prior to cleavage, peptide-resins 4b and 4c were treated as described for peptide-resin 5b (see below) to remove allyl protection from aspartic acid. Peptide-resins 4a–c (25 mg each) were then cleaved using TFA–water–triethylsilane–ethane-1,2-dithiol (EDT) (91 : 3 : 3 : 3 v/v/v/v; 2 cm<sup>3</sup>) for 1 h. The cleaved resin was removed by filtration, washed with a little neat TFA, and the combined filtrates were sparged with N<sub>2</sub> to remove the bulk of the TFA. Ice-cooled diethyl ether (10 cm<sup>3</sup>) was added, precipitating the peptide; the suspension was cooled in acetone–solid CO<sub>2</sub> for 5 min and then centrifuged at 3000 rpm for 5 min. The ethereal solution was decanted from the peptide and further (5 × 10 cm<sup>3</sup>) diethyl ether extractions were performed. The residue was dried *in vacuo* and dissolved in 0.1% aq. TFA (500 mm<sup>3</sup>).

Compound 4d prepared from peptide-resin 4a gave the following quotients upon amino-acid analysis: Found (expected): Asp/Asn 1.03 (1), Ser 0.86 (1), Glu/Gln 1.08 (1), Ala

1.96 (2), Lys 1.07 (1). Analytical HPLC (Fig. 1a) showed the presence of a single peak of retention time 11.21 min with MALDITOF-MS at 661.4 Da (theoretical = 660.7 Da); δ<sub>H</sub>(300 MHz; D<sub>2</sub>O) 1.41–1.52 (8 H, m, 2 × Ala β-H<sub>3</sub>, Lys γ-H<sub>2</sub>), 1.67–2.15 (6 H, m, Lys β- and δ-H<sub>2</sub>, Glu β-H<sub>2</sub>), 2.07 (3 H, s, acetyl), 2.50 (2 H, t, Lys, ε-H<sub>2</sub>), 2.82 (1 H, dd, *J* 17 and 7.0, Asp, β-H<sup>a</sup>), 2.95 (1 H, dd, Asp β-H<sup>b</sup>), 3.03 (2 H, t, *J* 7.5, Glu γ-H<sub>2</sub>), 3.92 (2 H, Ser β-H<sub>2</sub>) and 4.27–4.45 (6 H, α-H).

Compound 4d from peptide-resin 4b gave upon HPLC analysis (Fig. 1b) two major species, at retention time 11.13 min with MALDITOF-MS at 661.4 Da (theoretical = 660.7 Da) and 11.27 min with MALDITOF-MS at 642.6 Da.

Compound 4d from peptide-resin 4c gave the following quotients upon amino-acid analysis: Found (expected): Asp/Asn 1.00 (1), Ser 0.88 (1), Glu/Gln 1.07 (1), Ala 2.04 (2), Lys 1.01 (1) and gave upon HPLC analysis [Fig. 1(c)] a major species with retention time 11.19 min with MALDITOF-MS at 662.0 Da (theoretical = 660.7 Da).

Peptide-resin 4b (25 mg) was treated with TFA/scavenger [to yield Asp(Oallyl) protected peptide] and the product was isolated as described above. The crude sample was dissolved in 0.1% aq. TFA (1000 mm<sup>3</sup>) and analysed by analytical HPLC, to show two major species, at retention time 11.32 min with MALDITOF-MS at 642.6 Da and 14.89 min with MALDITOF-MS at 702.3 Da (theoretical = 701.7 Da).

#### Preparation of Ac-Glu(OBU<sup>t</sup>)-Asp(Oallyl)-(AcHmb)Ala-Ser(Bu<sup>t</sup>)-Lys(Boc)-Ala-PepSyn KR (Scheme 3, Peptide-resin 5a)

Peptide-resin 4c was treated with acetic anhydride (205 mg, 2.0 mmol) and DIPEA (52.0 mg, 0.4 mmol) in DMF for 2 h. The peptide-resin was then thoroughly washed and dried. Peptide-resin 5a (25 mg) was cleaved using TFA–water–triethylsilane–ethane-1,2-dithiol (91 : 3 : 3 : 3 v/v/v/v; 2 cm<sup>3</sup>) for 1 h and isolated as previously described. Amino acid analysis of an aliquot of the crude peptide solution gave the following quotients: Asp/Asn 1.03 (1), Ser 0.86 (1), Glu/Gln 1.09 (1), Ala 2.02 (2), Lys 1.02 (1). Analytical HPLC, using a gradient of 10–40% B in A, gave a single peak of retention time 18.28 min. A sample was analysed by MALDITOF-MS and gave mass = 879.1 Da (theoretical = 878.8 Da).

#### Preparation of Ac-Glu(OBU<sup>t</sup>)-Asp(CO<sub>2</sub>H)-(AcHmb)Ala-Ser(Bu<sup>t</sup>)-Lys(Boc)-Ala-PepSyn KR 5b: removal of allyl protection

To peptide-resin 5a (Scheme 3) (500 mg, 0.05 mmol) were added tetrakis(triphenylphosphine)palladium(0) (87 mg, 0.075 mmol), DMF (1.25 cm<sup>3</sup>), CHCl<sub>3</sub> (1.25 cm<sup>3</sup>), AcOH (0.125 cm<sup>3</sup>) and NMM (50 mm<sup>3</sup>). Reaction was allowed to proceed for 4 h at room temperature with occasional agitation. The resin was washed once with DMF followed by DMF containing diethyldithiocarbamic acid sodium salt trihydrate (0.5% w/v), and DIPEA (0.5% v/v), to complex out residual palladium, and was finally washed with diethyl ether and dried *in vacuo*. Peptide-resin 5b (Scheme 3) (25 mg) was treated with TFA/scavenger mixture for 1 h and the peptide isolated as detailed above. The crude, dried peptide was dissolved in 0.1% aq. TFA (1000 mm<sup>3</sup>). Amino acid analysis of an aliquot of the peptide solution gave the following quotients: Found (expected): Asp/Asn 0.99 (1), Ser 0.87 (1), Glu/Gln 1.07 (1), Ala 2.03 (2), Lys 1.04 (1). Analytical HPLC gave a single peak retention time 17.98 min [Fig. 2(a)], with MALDITOF-MS giving mass = 839.0 Da (theoretical = 838.8 Da).

#### Preparation of Ac-Glu(OBU<sup>t</sup>)-Asn(sugar)-(AcHmb)Ala-Ser(Bu<sup>t</sup>)-Lys(Boc)-Ala-PepSyn KR 5c: N-glycosylation of backbone-protected peptide-resin 5b with 2-acetamido-1-amino-1,2-dideoxy-β-D-glucopyranose

Resin-bound peptide 5b (500 mg, 0.05 mmol) was suspended in a mixture of DMF–dimethyl sulfoxide (DMSO) (2:1 v/v) to

which were added BOP (24.3 mg, 0.055 mmol), HOBT (7.5 mg, 0.055 mmol), DIPEA (7.0 mg, 0.054 mmol) and 2-acetamido-1-amino-1,2-dideoxy- $\beta$ -D-glycopyranose (12.2 mg, 0.055 mmol, in DMSO). The coupling reaction mixture was left at room temperature with occasional mixing for 4 h. The resin was washed and dried as previously described.

#### Preparation of Ac-Glu-Asn(*N*-acetylglucosyl)-(AcHmb)Ala-Ser-Lys-Ala-NH<sub>2</sub> 5d

Peptide-resin **5c** (50 mg) was treated with TFA-water-triethylsilane-ethane-1,2-dithiol (91:3:3:3 v/v/v/v; 2 cm<sup>3</sup>) for 1 h and the peptide isolated as detailed above. The crude, dried peptide was dissolved in 0.1% aq. TFA (1000 mm<sup>3</sup>). Amino acid analysis of an aliquot of the peptide solution gave the following quotients: Found (expected): Asp/Asn 0.99 (1), Ser 0.88 (1), Glu/Gln 1.07 (1), Ala 2.00 (2), Lys 1.06 (1). Analytical HPLC showed a major peak with retention time 17.11 min (86%) and a minor peak with retention time 18.85 min (7%) [Fig. 2(b)]. Amino acid analysis of the first peak gave the following quotients: Asp/Asn 0.99 (1), Ser 0.87 (1), Glu/Gln 1.07 (1), Ala 2.00 (2), Lys 1.06 (1). MALDITOF-MS of the major component gave mass 1042.5 Da (theoretical = 1041.1 Da).

#### Preparation of Ac-Glu-Asn(*N*-acetylglucosyl)-(Hmb)Ala-Ser-Lys-Ala-NH<sub>2</sub> 5e

Lyophilised AcHmb glycopeptide **5d** was deacetylated in solution with DMF (50 mm<sup>3</sup>) containing 5% (w/w) hydrazine hydrate. After 15 min the glycopeptide was precipitated by the addition of ice-cold diethyl ether (35 cm<sup>3</sup>) and immersion of the mixture into acetone-solid CO<sub>2</sub> for 5 min followed by centrifugation at 3000 rpm for 5 min. The supernatant was removed and the peptide washed with further ice-cold diethyl ether (35 cm<sup>3</sup> × 5) under the same procedure. Analytical HPLC showed the presence of two peaks with retention time 15.70 min (88%) and 17.22 min (6.3%) [Fig. 2(c)]. Amino acid analysis of the major peak gave the following quotients: Asp/Asn 1.00 (1), Ser 0.89 (1), Glu/Gln 1.24 (1), Ala 1.96 (2), Lys 1.06 (1). MALDITOF-MS of the major component gave mass 1000.0 Da (theoretical = 999.0 Da).

#### Preparation of Ac-Glu-Asn(*N*-acetylglucosyl)-Ala-Ser-Lys-Ala-NH<sub>2</sub> 5g (solution method)

Backbone-protected glycopeptide **5e** was treated with TFA/scavenger mixture for 1 h and isolated as detailed above. The crude product was dissolved in 0.1% aq. TFA (1000 mm<sup>3</sup>), and analysed by analytical HPLC to give a major species (88%) with retention time 11.07 min [Fig. 2(d)]. Amino acid analysis gave the following quotients: Asp/Asn 0.99 (1), Ser 0.89 (1), Glu/Gln 1.05 (1), Ala 2.02 (2), Lys 1.07 (1). MALDITOF-MS of the major component gave mass 863.8 Da (theoretical = 862.9 Da).

#### Preparation of Ac-Glu(OBu<sup>t</sup>)-Asn(sugar)-(Hmb)Ala-Ser(Bu<sup>t</sup>)-Lys(Boc)-Ala-PepSyn KR 5f

Peptide-resin **5c** (Scheme 3) (450 mg) was treated with DMF containing hydrazine hydrate 5% (v/v) for 15 min and the resin was washed and dried as above.

#### Preparation of Ac-Glu-Asn(*N*-acetylglucosyl)-Ala-Ser-Lys-Ala-NH<sub>2</sub> 5g (solid-phase method)

Glycopeptide-resin **5f** (450 mg) was cleaved using TFA/scavenger mixture (10 cm<sup>3</sup>) and isolated as described above. The crude product was dissolved in 0.1% aq. TFA (1000 mm<sup>3</sup>), and analysed by analytical HPLC to show a major species (85%) with retention time 11.09 min [Fig. 4(a)]. The crude glycopeptide **5g** was purified by preparative HPLC using a gradient of 0–20% B in A and 5 × 2000 mm<sup>3</sup> injections. The purified product was lyophilised (yield 11.56 mg, 30%). Analytical HPLC [Fig. 4(b)] gave a single species with retention time 11.10 min (99%), and MALDITOF-MS at 863.4 Da;  $\delta_{\text{H}}$ (300 MHz; D<sub>2</sub>O) 1.41–1.53 (8

H, m, 2 × Ala  $\beta$ -H<sub>3</sub>, Lys  $\gamma$ -H<sub>2</sub>), 1.67–2.15 (6 H, m, Lys  $\beta$ - and  $\delta$ -H<sub>2</sub>, Glu  $\beta$ -H<sub>2</sub>), 2.05 (3 H, s, COCH<sub>3</sub>), 2.07 (3 H, s, COCH<sub>3</sub>), 2.50 (2 H, t, Lys  $\epsilon$ -H<sub>2</sub>), 2.79 (1 H, dd, Asn CH<sup>a</sup>), 2.92 (1 H, dd, Asn CH<sup>b</sup>), 3.04 (2 H, t, Glu  $\gamma$ -H<sub>2</sub>), 3.47–3.96 (8 H, m, Ser  $\beta$ -H<sub>2</sub>, sugar 2-, 3-, 4-, 5-, 6<sup>a</sup>-6<sup>b</sup>-H), 4.30–4.46 (6 H,  $\alpha$ -H) and 5.07 (1 H, d, J 9.6, 1-H).

#### Preparation of Ac-Glu(OBu<sup>t</sup>)-Asn(*N,N'*-diacetylchitobiosyl)-(AcHmb)Ala-Ser(Bu<sup>t</sup>)-Lys(Boc)-Ala-PepSyn KR 5c. *N*-glycosylation of backbone-protected peptide-resin **5b** with 2-acetamido-4-*O*-(2-acetamido-2-deoxy- $\beta$ -D-glycopyranosyl)-2-deoxy- $\beta$ -D-glycopyranosylamine

Peptide-resin **5b** (50 mg) was treated with 2-acetamido-4-*O*-(2-acetamido-2-deoxy- $\beta$ -D-glycopyranosyl)-2-deoxy- $\beta$ -D-glycopyranosylamine (2.65 mg, 6.25  $\mu$ mol) dissolved in DMSO (50 mm<sup>3</sup>), followed by 1.25 mol equiv. each of BOP (2.8 mg, 6.25  $\mu$ mol), HOBT (0.96 mg, 6.25  $\mu$ mol) and DIPEA (0.8 mg, 6.25  $\mu$ mol) added from stock solutions in DMSO. The glycosylation reaction was left for 4 h. Peptide-resin **5c** (Scheme 3) (40.0 mg) was cleaved using a TFA mixture for 1 h as above. Analytical HPLC [Fig. 3(a)] showed a major peak with retention time 17.03 min (72%) and a smaller peak at 18.85 min (10%). MALDITOF-MS of the major component gave mass = 1243.5 Da (theoretical = 1244.3 Da).

The cleaved peptide was purified by preparative HPLC using a gradient of 0–20% B in A and a 1000 mm<sup>3</sup> injection to yield peptide **5d**. Analytical HPLC [Fig. 3(b)] showed a single peak with retention time 17.05 min.

#### Preparation of Ac-Glu-Asn(*N,N'*-diacetylchitobiosyl)-(Hmb)Ala-Ser-Lys-Ala-NH<sub>2</sub> 5e

Peptide **5d** was lyophilised and then treated with a drop of DMF containing hydrazine hydrate (5% v/v) for 15 min, and re-precipitated as above. Analytical HPLC [Fig. 3(c)] showed the presence of a single peak, with retention time 15.74 min. Analysis by MALDITOF-MS gave mass = 1202.0 Da (theoretical = 1203.2 Da).

#### Preparation of Ac-Glu-Asn(*N,N'*-diacetylchitobiosyl)-Ala-Ser-Lys-Ala-NH<sub>2</sub> 5h

The backbone-protected glycopeptide **5e** was treated with TFA/scavenger mixture for 1 h and gave a single product by analytical HPLC, retention time 11.50 min [Fig. 3(d)]. Precipitation by addition of ice-cold diethyl ether yielded compound **5h** (0.7 mg, 16.4%). Analysis by MALDITOF-MS gave mass = 1066.2 Da (theoretical = 1067.1 Da). Amino acid analysis gave the following quotients: Asp/Asn 1.07 (1), Ser 0.9 (1), Glu 1.12 (1), Ala 1.88 (2), Lys 1.03 (1);  $\delta_{\text{H}}$ (300 MHz; D<sub>2</sub>O) 1.24–1.27 (8 H, m, 2 × Ala  $\beta$ -H<sub>3</sub>, Lys  $\gamma$ -H<sub>2</sub>), 1.53–1.8 (6 H, m, Lys  $\beta$ - and  $\delta$ -H<sub>2</sub>, Glu  $\beta$ -H<sub>2</sub>), 1.87, 1.90 and 1.92 (9 H, 3 × s, 3 × COCH<sub>3</sub>), 2.33 (2 H, t, Lys  $\epsilon$ -H<sub>2</sub>), 2.62 (1 H, dd, Asn CH<sup>a</sup>), 2.77 (1 H, dd, Asn CH<sup>b</sup>), 2.87 (2 H, t, Glu  $\gamma$ -H<sub>2</sub>), 3.35–3.38 (14 H, m, Ser  $\beta$ -H<sub>2</sub> + sugar H), 4.12–4.27 (6 H, m,  $\alpha$ -H), 4.45 (1 H, d, J 9.0, 1'-H) and 4.90 (1 H, d, J 9.5, 1-H).

## References

- 1 M. Fukuda, in *Molecular Glycobiology* ed. M. Fukuda and O. Hindsgaul, Oxford University Press, 1994, pp. 1–52.
- 2 A. Varki, *Glycobiology*, 1993, **3**, 97.
- 3 L. A. Lasky, *Science*, 1992, **258**, 964.
- 4 T. W. Rademacher, R. B. Parekh and R. A. Dwek, *Annu. Rev. Biochem.*, 1994, **57**, 785.
- 5 R. A. Dwek, *Biochem. Soc. Trans.*, 1995, **23**, 1.
- 6 T. Ogawa, *Chem. Soc. Rev.*, 1994, **23**, 397.
- 7 M. Meldal, *Curr. Opin. Struct. Biol.*, 1994, **4**, 710.
- 8 E. Atherton and R. C. Sheppard, in *Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press, 1989.
- 9 I. Christiansen-Brans, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1461.
- 10 L. Urge, D. C. Jackson, L. Gorbics, K. Wroblewski, G. Graczyk and L. Otvos, *Tetrahedron*, 1994, **50**, 2373.

- 11 (a) S. T. Cohen-Anisfeld and P. T. Lansbury, *J. Am. Chem. Soc.*, 1993, **115**, 10 531; (b) L. Urge and L. Otvos, *Lett. Pept. Sci.*, 1995, **1**, 207.
- 12 D. Vetter, D. Tumelty, S. K. Singh and M. A. Gallop, *Angew. Chem., Int. Ed. Engl.*, 1995, **34**, 60.
- 13 S. Y. C. Wong, G. R. Guile, T. W. Rademacher and R. A. Dwek, *Glycoconj. J.*, 1993, **10**, 227.
- 14 S. A. Kates, B. G. de la Torre, R. Eritja and F. Albericio, *Tetrahedron Lett.*, 1994, **35**, 1033.
- 15 M. Meldal, in *Neoglyconjugates*, Academic Press, 1994, pp. 145–183.
- 16 H. G. Garg, K. Von Dem Bruch and H. Kunz, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 277.
- 17 F. Marcus, *Int. J. Pept. Protein Res.*, 1985, **25**, 542.
- 18 E. Nicolas, E. Pedroso and E. Giralt, *Tetrahedron Lett.*, 1989, **30**, 497.
- 19 Y. Yang, W. V. Sweeney, K. Schneider, S. Thornqvist, B. T. Chait and J. P. Tam, *Tetrahedron Lett.*, 1994, **35**, 9689.
- 20 M. Quibell, D. Owen, L. C. Packman and T. Johnson, *J. Chem. Soc., Chem. Commun.*, 1994, 2343.
- 21 C. Hyde, T. Johnson, D. Owen, M. Quibell and R. C. Sheppard, *Int. J. Pept. Protein Res.*, 1994, **43**, 431.
- 22 M. Quibell, W. G. Turnell and T. Johnson, *J. Org. Chem.*, 1994, **59**, 1745.
- 23 M. Quibell, W. G. Turnell and T. Johnson, *Tetrahedron Lett.*, 1994, **35**, 2237.
- 24 L. Urge, L. Otvos, E. Lang, K. Wroblewski, L. Laczko and M. Hollosi, *Carbohydr. Res.*, 1992, **325**, 83.
- 25 K. B. Reimer, M. Meldal, S. Kusumoto, K. Fukase and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1993, 925.
- 26 L. M. Likhoshesterov, O. S. Novikova, V. A. Derevitskaja and N. K. Kochetkov, *Carbohydr. Res.*, 1986, **146**, C1.
- 27 E. Kallin, H. Lonn, T. Norberg and M. Elofsson, *J. Carbohydr. Chem.*, 1992, **8**, 597.
- 28 R. U. Lemieux, A. A. Pavia, J. C. Martin and K. A. Watanabe, *Can. J. Chem.*, 1969, **47**, 4427.
- 29 T. Johnson, M. Quibell and R. C. Sheppard, *J. Pept. Sci.*, 1995, **1**, 11.

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