New solid-phase oligosaccharide synthesis on glycopeptides bound to a solid phase

Hans Paulsen,[a] Axel Schleyer,[a] Nathalie Mathieux,[a] Morten Meldal[b] and Klaus Bock[b]

[a] *Institut für Organische Chemie, Universität Hamburg, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany*
[b] *Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark*

A solid-phase oligosaccharide synthesis has been developed which utilises glycopeptides bound to the solid phase. The products are glycopeptides elongated at the saccharide chain. The glycosyl acceptors, which are assembled by simple solid-phase glycopeptide synthesis, contain 2-azido-2-deoxy-α-D-galactose with unsubstituted 3-OH or 6-OH groups, which can be glycosylated. Suitable glycosyl donors are perbenzoylated trichloroacetimidates of D-galactose or 2-amino-2-deoxy-D-glucose, although the 2-amino group must be protected with Teoc. A corresponding additional saccharide unit is added with a β-glycosidic linkage. With disaccharide donors, glycopeptides with linear trisaccharide side chains are obtained. By performing the solid-phase glycosylation synthesis twice in succession glycopeptides with branched trisaccharide chains have been obtained.

Introduction

In view of the very good results obtained with the Merrifield method[1] for solid-phase synthesis of oligopeptides and oligonucleotides, it seemed reasonable to investigate whether this method is also suitable for the preparation of oligosaccharides. From the outset it was anticipated that limitations would be encountered, since oligosaccharide glycosylations are very complex reactions which are not comparable with a simple peptide coupling. Saccharides are polyfunctional compounds containing numerous hydroxy groups which can all participate in coupling reactions. Therefore combinations of protecting groups are needed to achieve a certain regioselectivity. The yields obtained for the glycoside coupling reactions are also inferior to those obtained for a peptide coupling even with the current state of the art. Special consideration must be given to the stereoselectivity at the anomeric centre, where the reaction must be directed towards the α- or β-glycoside. Despite these difficulties, several applications have been developed in recent years, since solid-phase synthesis considerably simplifies purification of intermediate products. Solid-phase oligosaccharide synthesis could also play an important role in the currently popular 'combinatorial chemistry'.[2]

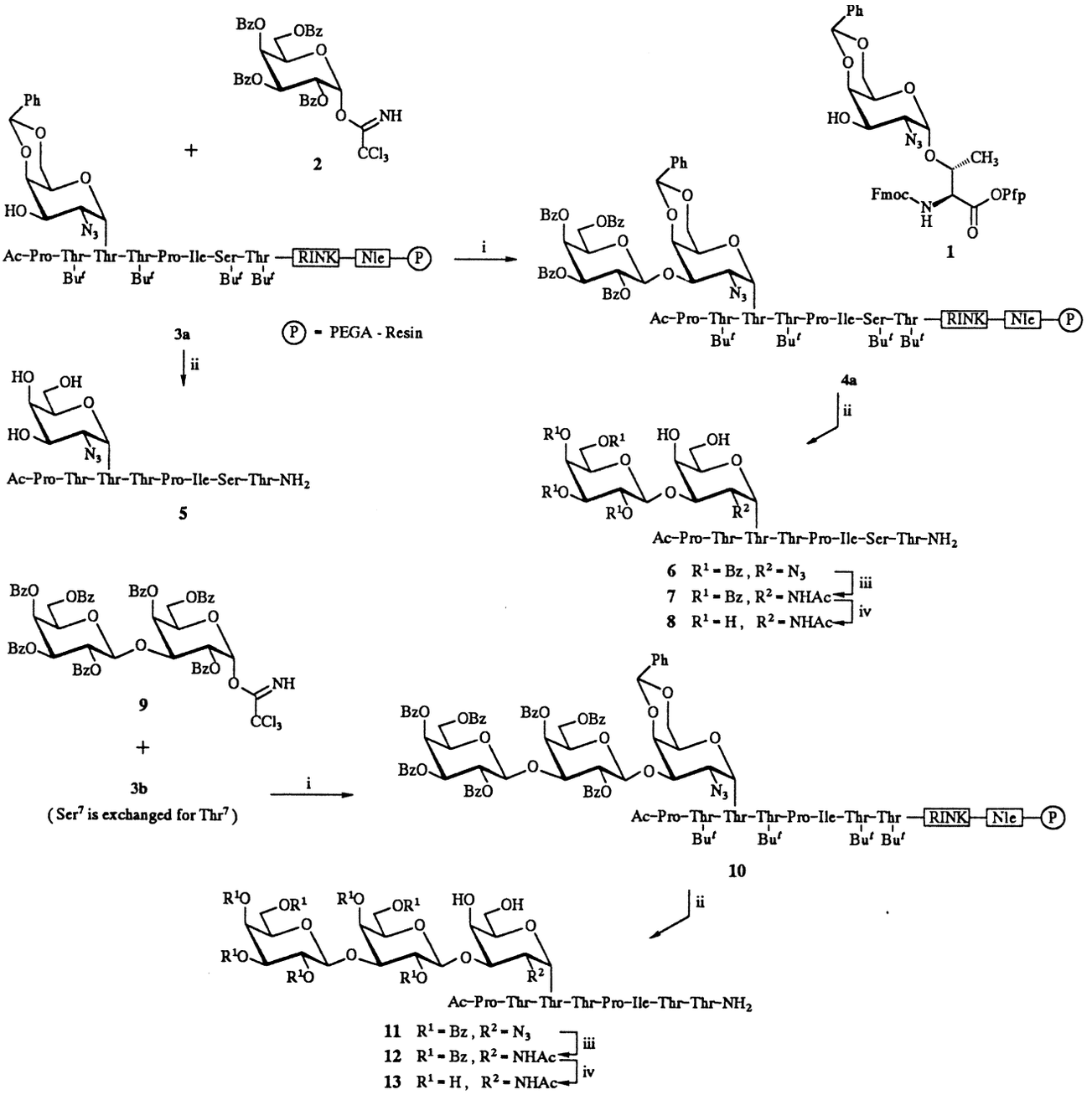
Schuerch[3] was the first to show that a saccharide unit bound to a solid phase can couple with a glycosyl donor with chain lenghthening. One of the methods developed in recent years using this improved coupling reaction is that of Danishefsky.[4–6] With this method, solid-phase-bound saccharides are lengthened by one saccharide unit by opening of the 1,2-epoxides (Brigl anhydrides), although care must be taken with the stereochemistry.[7] Khane[8] used an *S*-glycosyl sulfoxide as the glycosyl donor for coupling with solid-phase-bound saccharides. Like most solid-phase reactions, this method requires a large excess of glycosyl donor. Saccharides bound to glass surfaces were used by Wong[9] for an enzymic glycosylation. This method can be used to couple 5-*N*-acetylneuraminic acid to an oligosaccharide unit without the need for protecting-group chemistry. Consideration must be given to the fact that the capacity of glass surface phases is limited and therefore this method is suitable only for the preparation of minute amounts. A very interesting method has been developed by Krepinsky.[10,11] Use of soluble polyethers as the support for the solid-phase synthesis permits glycoside coupling to take place in an environment more conducive to this reaction. The saccharide is bound to a soluble polyether support and the chain is then lengthened by a saccharide unit by means of the trichloroacetimidate method.[12] After the product obtained is purified by precipitation, it can be redissolved for another reaction step. If similar donors are available and the repeated coupling steps lead if possible to 1,2-*trans*-linkages, this method can be used for constructing oligosaccharide chains, as shown, for example, by the heptasaccharide synthesis by van Boom.[13]

In the present paper, we are able to show that *O*-glycopeptides bound to solid phases can also be glycosylated with elongation of the saccharide chain. Although this method has not as yet been studied, it has the advantage that the glycosyl acceptor can be prepared according to established methods of solid-phase glycopeptide synthesis.[14] Glycopeptide synthesis takes place on the support material with well known reagents of peptide chemistry.[15,16] The subsequent glycosylation is problematical, since it cannot be performed in the solvents used for glycopeptide synthesis but requires anhydrous conditions in dichloromethane. This problem can, however, be solved and *O*-glycopeptides which are extended at the glycoside residue have been obtained as final products. These were cleaved from the resin using methods of glycopeptide synthesis and were then deprotected.

Results and discussion

Glycosylation of 3-OH

As target compounds for our synthesis we selected mucin core structures.[17] Mucins are highly glycosylated glycoproteins that play an important role in the respiratory and digestive tracts as protective substances and lubricants.[18] In cancer cells, the saccharide part of mucins is characteristically altered.[19] Mucins contain almost exclusively the *O*-glycopeptide type in which the first sugar molecule, 2-acetamido-2-deoxy-D-galactose, is always linked by an α-glycosidic bond to the hydroxy groups of threonine or serine. This basic structure occurs in all saccharide side chains. Other sugar moieties are bound *via* characteristic mucin core linkages to 2-acetamido-2-deoxy-α-D-galactose.[17,18] Some of these core structures can be prepared by means of the syntheses described here.



Scheme 1 *Reagents:* i, TMSOTf; ii, TFA, iii, Zn, Ac_2O, AcOH, THF; iv, NaOMe, MeOH

Building block **1**, which we have synthesized previously,[20] was used for construction of the solid-phase-bound glycosyl acceptor. In structure **1** 2-azido-2-deoxy-D-galactose was coupled to the threonine residue *via* an α-glycosidic linkage. The 3-OH group of the saccharide moiety was unsubstituted and served later as the glycosylation site of the acceptor. Using substrate **1**, a glycopeptide synthesis was carried out as already described,[15] affording product **3**. The selected amino acid sequence is a partial sequence of mucin MUC 2.[17] PEGA resin[21] served as the support, norleucine (Nle) as the internal standard and the Rink linker[22] as the linker. Coupling was with suitable Fmoc-amino acid pentafluorophenyl (Pfp) or 3,4-dihydro-4-oxo-1,2,3-benzotriazin-3-yl (Dhbt) esters. The N-terminal proline was protected by N-acetylation. Treatment of acceptor **3** with 95% trifluoroacetic acid (TFA) led to cleavage of the glycopeptide from the resin and concurrent removal of all Bu^t protecting groups as well as the benzylidene group of the saccharide residue. The resultant glycooctapeptide **5** was identical with a previously synthesized substance (see Scheme 1).[15]

Product **3** is a suitable glycosyl acceptor. Prior to glycosylation, resin with the bound glycopeptide was washed several times with dichloromethane and diethyl ether to remove the solvent used for synthesis of the peptide. It was then dried overnight at high vacuum. To enhance the reactivity of the resin, prior to reaction it was allowed to swell at ambient temperature under nitrogen and with exclusion of moisture for one hour in absolute dichloromethane. The trichloroacetimidate **2**[23] was used as the glycosyl donor. The analogous acetylated substance[24] was also tested but proved more labile than the benzoate and therefore gave less favourable results. The quality of the trimethylsilyl trifluoromethanesulfonate (TMSOTf) used as the catalyst proved to be extremely important for successful glycosylation. Best results were obtained with TMSOTf that had been freshly distilled within a few hours prior to use. A catalyst that had been stored for several days under nitrogen in the refrigerator gave unfavourable results in the glycosylation or sometimes no reaction at all. In contrast, TMSOTf which has been stored for longer

periods can be used freely for similar reactions in solution.[12]

Since an excess of donor **2** was always needed for this reaction, it was decided to calculate the yield on the basis of acceptor **3**. Since this requires knowledge of the glycopeptide content of compound **3**, the glycopeptide **5** was cleaved from compound **3** with TFA and the amount of free glycopeptide determined. This amount always serves as the basis for calculating the yield relative to acceptor **3**. The same procedure was used for all other acceptors described here.

The reaction conditions for treatment of the solid phase **3** suspended in stirred dichloromethane with donor **2** had to be carefully optimised. The progress of glycosylation of acceptor **3** to yield disaccharide **4** cannot be detected analytically on the resin, but the disappearance of the donor **2** can be followed chromatographically. Optimisation of the course of the reaction could be monitored by first cleaving the product from samples of the resin and then comparing the amount of the desired product **6** obtained with that of glycopeptide **5**, which was formed from unchanged compound **3**. It was found that optimal results were obtained if donor **2** was used in excess (8 mol equiv.). The reaction was performed in dichloromethane at −30 °C for 24 h. After coupling to give the linked peptide **4**, the disaccharide peptide **6** was obtained upon cleavage with TFA in 67% yield relative to the glycosyl acceptor **3**. The reaction proceeded stereoselectively with neighbouring-group participation and afforded only the β-glycosylated product. Therefore solid-phase oligosaccharide synthesis was possible according to the procedure described. The excess of glycosyl donor **2** could in some instances be reduced (from 8 to 5 mol equiv.). An even smaller excess decreased the yield dramatically. These results are similar to those of other solid-phase oligosaccharide syntheses in which comparable or sometimes even larger excessive concentrations are required for coupling.

To study the influence of the resin on the glycosylation reaction, the same sequence as that of acceptor **3** was prepared with other resins such as Polyhipe, Macrosorb and Tenta Gel as the solid phases. The Macrosorb and Tenta Gel compounds gave no glycosylation products. Surprising results were obtained with the acceptor bound to Polyhipe upon treatment with donor **2**. Although a glycosylation product was obtained under the same conditions and in about the same yield as with compound **3**, both the cleavage product **6** and the corresponding product with an α-glycosidic linkage to D-galactose were observed. The α:β product ratio was 1:2.3. After deprotection, the two anomers could be separated and identified. This result showed that contrary to expectations the type of support resin has an influence on the stereoselectivity of glycosylation.

To convert compound **6** into the target free compound **8** it was necessary to transform the azido group into an acetamido group. This could be done in one step by one of two pathways. Either compound **6** was treated with thioacetic acid[15] or it was reduced with activated zinc in acetic anhydride–acetic acid–tetrahydrofuran (THF).[20] The latter method usually gave better results and was therefore used for all the analogous compounds. From azide **6** was obtained compound **7**, from which the benzoyl groups were cleaved with catalytic amounts of sodium methoxide in methanol to give glycopeptide **8**. Compound **8** is a core 1 structure with a partial sequence of mucin MUC 2.[17,18]

The coupling reaction described was also applied to disaccharide donors. As the glycosyl donor we used the β-(1→3)-glycosidically linked disaccharide **9** consisting of two galactose moieties. This was made to react with the acceptor **3**[25] under conditions analogous to those for the reaction with compound **2**. Treating linked peptide **3** with disaccharide **9** in the presence of the TMSOTf catalyst at −25 °C for 12 h afforded product **10**. After cleavage with TFA, the trisaccharide **11** could be isolated in 36% yield relative to peptide resin **3**. Deprotection of azide **11** is analogous to that of the disaccharide. Reduction of the azido group with activated zinc provided the acetamide **12**, which after cleavage of the benzoyl groups afforded the desired octapeptide trisaccharide **13**.

Glycosylation at 6-OH

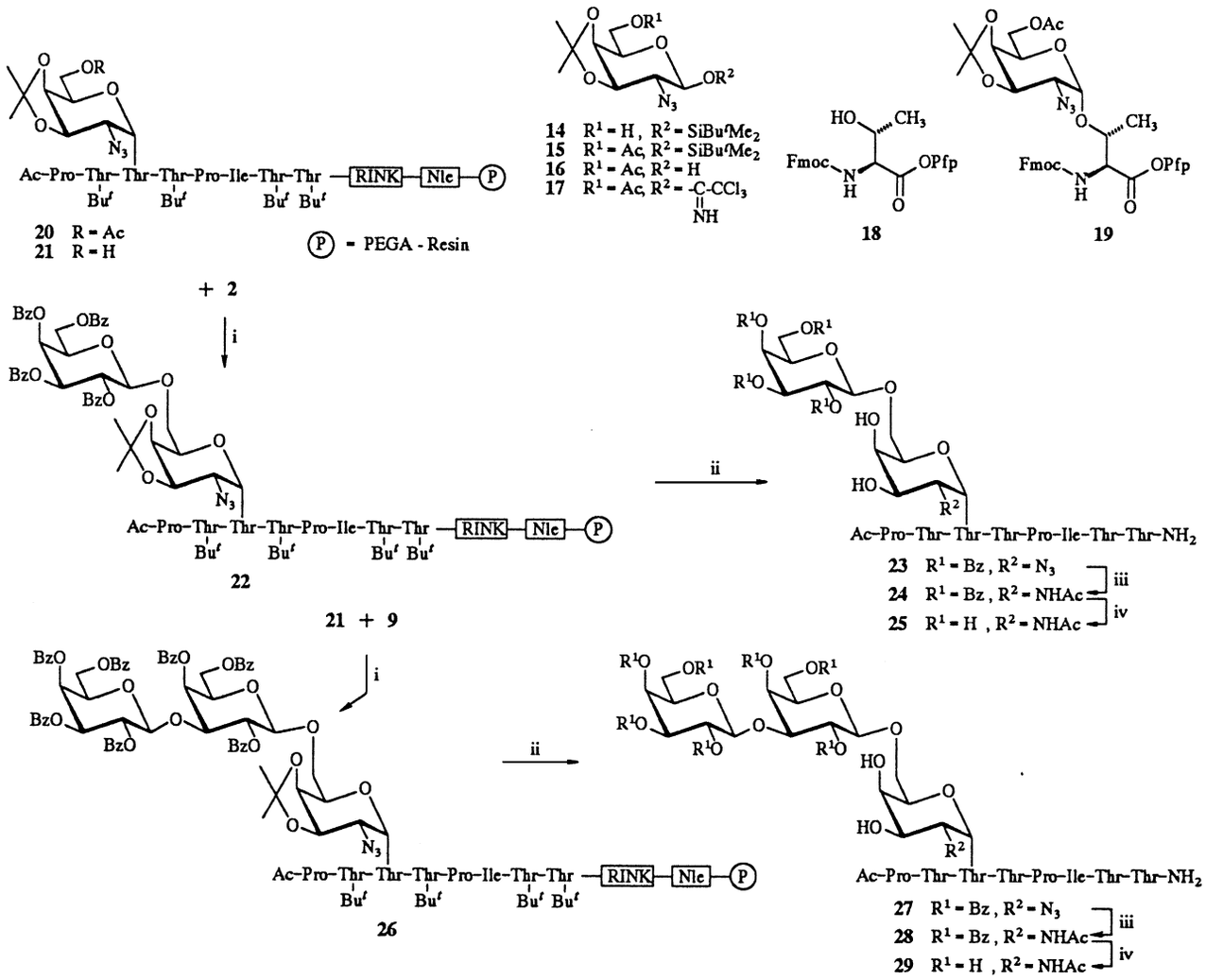
For glycosylation at the 6-OH group of the saccharide residue, a glycosyl acceptor **21** was needed which has a solid-phase-supported glycopeptide containing an unsubstituted 6-OH group on the sugar residue. Preparation of the acceptor **21** required a building block with a blocking pattern like that shown in structure **19**. Of the possible synthetic pathways to compound **19**, a synthesis starting from the well known *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-galactopyranoside[24] proved most effective. This was converted regioselectively with 2,2-dimethoxypropane and acid catalysis into the 3,4-isopropylidene compound **14**. After acetylation to compound **15**, the silyl group was cleaved with *tert*-butylammonium fluoride (TBAF) and the compound **16**, which is unsubstituted at the 1-OH position, was obtained. This was immediately treated with trichloroacetonitrile and the weak base K_2CO_3 to give the β-trichloroacetimidate **17** in good yield. The glycoside synthesis from compound **17** and the threonine derivative **18**[15] under TMSOTf catalysis gave only the desired α-glycosidically linked product **19**, making available a suitable building block for synthesis of linked resin **21**. Analogous to the preparation of compound **3**, a solid-phase glycopeptide synthesis was performed with glycosyl amino ester **19**, leading to the product **20**. The acetyl group of the saccharide residue of product **20** could be selectively removed by mild alkaline hydrolysis, yielding the glycosyl acceptor **21**, which was suitable for coupling reactions at 6-OH. Again the PEGA, Polyhipe, Macrosorb and Tenta Gel resins were all tested as solid-phase supports. In subsequent solid-phase glycoside syntheses, the PEGA resin once again gave the best results.

In monosaccharides, the primary hydroxy group 6-OH is usually more reactive than the secondary 3-OH group.[26] Accordingly, glycosylation should proceed more smoothly with acceptor **21** than with acceptor **3**. It was shown, however, that the reaction conditions for glycosylation of compound **21** with the proven glycosyl donor **2**[23] were the same as for acceptor **3** and afforded the same results. The difference in the reactivities of the two hydroxy groups is therefore insignificant when the acceptors are bound to the solid support. After drying, acceptor **21** was converted into **22** in 24 h by treatment with 8 mol equiv. of donor **2** in dichloromethane at −30 °C with TMSOTf as the catalyst. An excess of the donor **2** was required. If the excess was reduced to less than 5 mol equiv., the yield decreased dramatically. After cleavage of the glycopeptide from the solid phase, product **23** was obtained in 69% yield relative to acceptor **21**. Deprotection of compound **23** was performed by the usual method by reducing the azido group with activated zinc and *in situ* acetylation to give the acetamide **24**. After cleavage of the benzoyl group with a catalytic amount of sodium methoxide, the disaccharide octapeptide **25** was obtained (see Scheme 2).

The glycosyl acceptor **21** was treated with the disaccharide donor **9**.[25] In this case best results were obtained by treating compound **21** with 7 mol equiv. of donor **9** in the presence of the catalyst TMSOTf for 2 h at room temperature. The product **26** was obtained. After cleavage from the solid phase, the trisaccharide derivative **27** was isolated in 53% yield relative to substrate **21**. Glycosylation to yield compounds **22** and **26** resulted, in both cases, exclusively in formation of the β-glycosidically linked product. Deprotection of compound **27** was easily achieved by reduction of the azido group with activated zinc and acetylation to give compound **28**, and subsequent debenzoylation provided the trisaccharide octapeptide **29**.

Glycosylation with amino sugars

A solid-phase oligosaccharide synthesis with amino sugars is



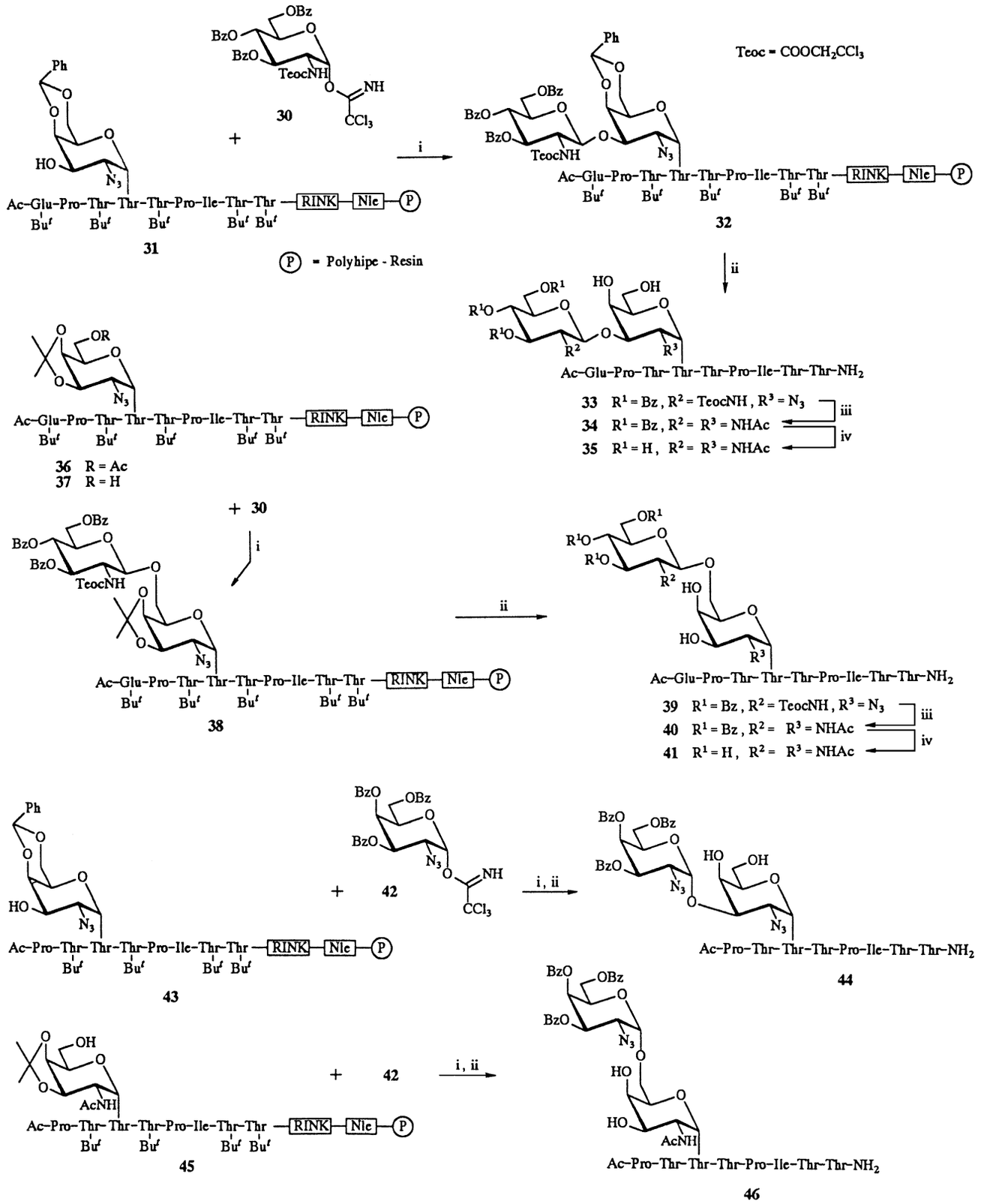
Scheme 2 *Reagents:* i, TMSOTf; ii, TFA; iii, Zn, Ac_2O, AcOH, THF; iv, NaOMe, MeOH

likewise an interesting possibility. In particular, the elongation of chains on the saccharide residues of compounds **3** and **21** with 2-acetamido-2-deoxy-β-D-glucose is of importance because it leads to partial sequences of important mucin core structures. In the case of glycosyl donors of 2-amino sugars, substitution at the 2-amino group is of importance. For the stereoselectivity of the β-glycosylation to be assured, it must be possible to cleave the protecting group under mild conditions, and formation of an oxazoline must be avoided. The phthalimido group used successfully for β-glycosylation[27] was not considered because the strongly alkaline conditions required for cleavage result in degradation of the glycopeptide. The trichloroethoxycarbonyl (Teoc),[28,29] *N*-dithiasuccinimido (N-Dts)[30] and allyloxycarbonyl (Alloc)[31] groups were tested as amino-protecting groups on a donor consisting of 2-amino-2-deoxy-D-glucose. The Teoc group in combination with the benzoyl protecting groups gave the best results. The imidate **30** was therefore a suitable glycosyl donor for selectively achieving a β-glycoside.

The glycosyl acceptor initially synthesized with building block **1** was slightly modified. In this case, Polyhipe proved favourable as the solid support. The reactions on Polyhipe proceeded stereoselectively, with no anomeric mixtures obtained, as was the case for the reaction of donor **2** with glycopeptides attached to Polyhipe. Moreover, an additional glutamic acid was coupled to the peptide sequence. Glutamic acid in this position permits a subsequent coupling of the final product with bovine albumin or other proteins which can then be used for immunisation to obtain monoclonal antibodies that react specifically with the corresponding glycopeptide structure.

After intensive drying of the Polyhipe-bound glycopeptide **31**, it was treated in dichloromethane with 8 mol equiv. of the glycosyl donor **30** and TMSOTf at −15 °C for 12 h to give product **32**. After cleavage of the resin, compound **33** was isolated in 62% yield relative to acceptor **31**, with only the β-(1→3) glycosidically linked product being obtained. For deprotection, it is important that it be possible to reduce the Teoc and azido groups simultaneously and to N-acetylate concurrently with activated zinc in acetic anhydride–acetic acid–THF, to ensure product **34** can be obtained in one step. Cleavage of the benzoyl group with catalytic amounts of sodium methoxide resulted in pentaol **35** (Scheme 3). This is a peptide sequence with a mucin core 3 structure.[17,18]

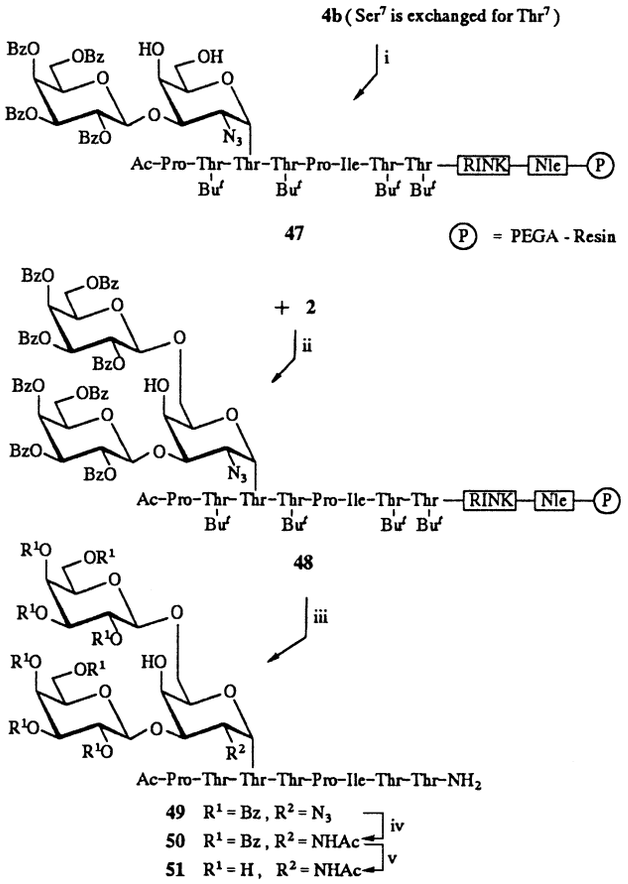
The 6-OH group of the saccharide side chain can be coupled with the donor **30** in a reaction similar to that of acceptor **31** with donor **30**. For this purpose the glycosyl acceptor **36**, which likewise contains glutamic acid at the N-terminal for protein coupling, was synthesized with building block **19** on the Polyhipe resin. After deacetylation of compound **36** to 6-OH product **37**, this was treated with 8 mol equiv. of donor **30** in the presence of the TMSOTf catalyst to give disaccharide **38**. After cleavage from the resin, compound **39** was obtained in 62% yield from acceptor **37**. For deprotection, the Teoc and azido groups were again simultaneously removed with activated zinc to give the N-acetylated compound **40**. Debenzoyl-



Scheme 3 *Reagents:* i, TMSOTf; ii, TFA; iii, Zn, Ac_2O, AcOH, THF; iv, NaOMe, MeOH

ation gave the β-(1→6)-glycosidically linked mucin core 6 structure **41**.[17]

Although problematic because the stereochemistry of the corresponding reaction to the α-glycoside without neighbouring-group participation is more difficult to control even in solution, an attempt was made to elongate the chain of the saccharide residue on the solid phase *via* an α-glycosidic linkage. As the donor, the trichloroacetimidate **42** of 2-azido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactose was chosen. It was important that compound **42** be stablilised by benzoyl groups. An analogous acetate gave no product on treatment with a solid-phase-bound acceptor. In the reaction of acceptor **43** with donor **42** at 0 °C and 12 h reaction time, a product was formed that after cleavage from the resin was shown by its ^{1}H NMR data to be the α-(1→3)-glycosidically linked disaccharide peptide **44**. At about 20% relative to the acceptor **43**, however, the yield was very low. In addition, an even greater excess of the donor (10 mol equiv.) must be used. Since, unlike the donors in the other reactions, donor **42** is very expensive, this procedure cannot be considered economical.



Scheme 4 *Reagents:* i, PTSA aq. MeOH; ii, TMSOTf; iii, TFA; iv, Zn, Ac_2O, AcOH, THF; v, NaOMe, MeOH

Acceptor **45** can be treated under analogous conditions with an excess of 10 mol equiv. of the glycosyl donor **42**. In this case, after cleavage from the resin the corresponding α-(1→6)-glycosidically linked disaccharide peptide **46** was obtained. Since the yield was only 18–20%, this reaction was not pursued further.

Synthesis of a branched trisaccharide octapeptide

Based on previous findings, it was demonstrated that after the first solid-phase disaccharide synthesis a second coupling to yield oligosaccharides is possible on the same solid phase. A modified product **4a** was used for this in which Ser-7 had been replaced by Thr-7 by varying the peptide synthesis. The first glycosylation was fully analogous to the reaction of acceptor **3** with donor **2** to give product **4a**. The disaccharide octapeptide is bound to PEGA resin. If compound **4a** was suspended in methanol–water 1:1 and treated with catalytic amounts of toluene-*p*-sulfonic acid (PTSA), the benzylidene group was removed selectively within 12 h without cleavage of the product from the support and compound **47**, which had two deprotected hydroxy groups, was obtained (see Scheme 4). It cannot be excluded that during the process a small proportion of the Bu^t groups was also cleaved. If so, the corresponding product cannot be detected on the resin and has no significance for the further reaction sequence, since it was found not to interfere with glycosylation and all Bu^t groups were eventually removed when the final product was cleaved from the resin.

After the resin-bound glycopeptide **47** had been dried, it was glycosylated to trisaccharide **48** under standard conditions with 8 mol equiv. of the glycosyl donor **2** at −30 °C in 12 h with catalysis by TMSOTf. After cleavage from the support, product **49** was obtained in 64% yield relative to acceptor **47**. Deprotection was achieved by the established method using reduction of the azido group with activated zinc and acetylation to give compound **50**. Subsequent cleavage of the benzoyl groups yielded glycopeptide **51**. The structures of compounds **49**–**51** were confirmed by two-dimensional ^{1}H NMR spectroscopy. The synthesis showed that the method could be extended and that even branched trisaccharide glycopeptides could be obtained by this pathway.

Attempts to glycosylate solid-phase bound peptides directly

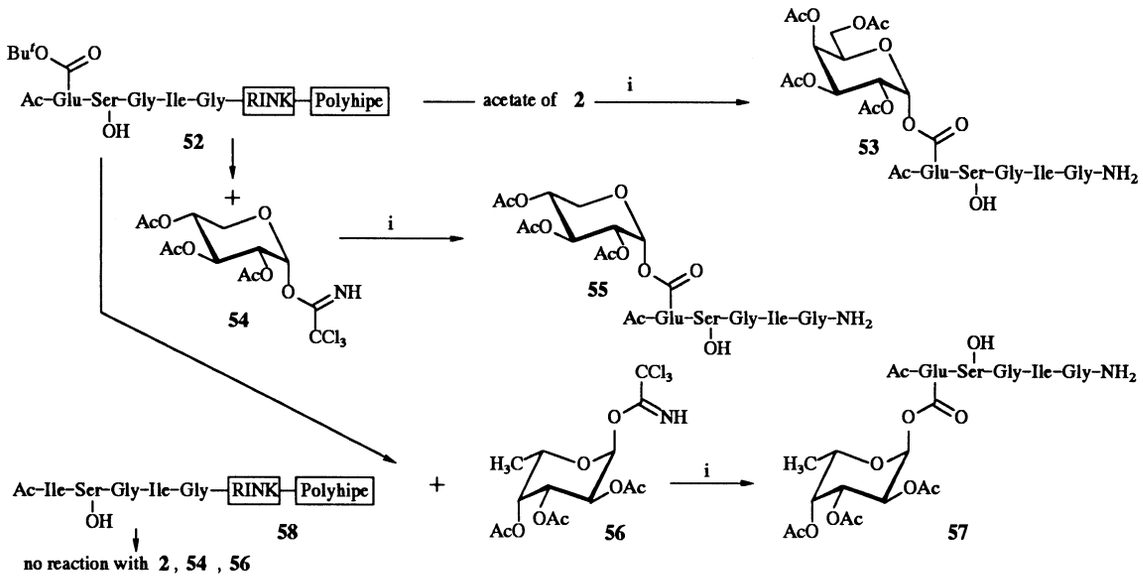
After it had been demonstrated that solid-phase-bound glycopeptides with free hydroxy groups on the oligosaccharide residue could be glycosylated with various glycosyl trichloroacetimidates, the question arose as to whether it was also possible to use this type of reaction directly to glycosylate free hydroxy groups of threonine or serine in simple peptides bound to the solid phase. To study this, the two peptides **52** and **58** were synthesized. Since the success of this type of reaction could be highly dependent on the type of solid support used, peptides **52** and **58** were bound to various supports including PEGA resin, Tenta Gel, Polyhipe and Macrosorb with Rink linker and polystyrene with Wang linker. The per-acetylated α-D-galactosyl trichloroacetimidate[24] corresponding to compound **2** as well as the per-acetylated trichloroacetimidates of D-xylose, **54**,[32] and L-fucose **56**[33] served as the glycosyl donors. The reaction of the glycosyl acceptors **52** and **58** on the different supports with the glycosyl donors **2** (as acetate), **54** and **56** were studied in the solvents dichloromethane, acetonitrile and nitromethane at temperatures varying from −50 to +20 °C. As the catalyst in all reactions, 0.10 mol equiv. of TMSOTf was used. The glycosyl donors were always used in an excess of 8 mol equiv. each.

No reaction was observed with compound **58** under any of the conditions or with any of the support resins studied. With Polyhipe-bound compound **52**, a small amount of product (10–20%) was formed with donor **2** (as the acetate) under standard conditions in dichloromethane. This amount of product could be dramatically increased by performing the reaction in acetonitrile at 20 °C in 2 h. After cleavage from the support, a glycosylated product could be isolated in 73% yield. Careful evaluation of the ^{1}H NMR spectrum showed, however, that glycosylation had not taken place at the hydroxy group of serine, but that the product was the glycosyl ester **53** of glutamic acid (see Scheme 5). Under the altered reaction conditions, the Bu^t ester of glutamic acid was presumably cleaved from acceptor **52**, with glycosylation of the free carboxy group of glutamic acid taking place to give product **53** after cleavage from the support resin. With catalytic amounts of sodium methoxide in methanol the saccharide part of compound **53** could be cleaved quantitatively, with the simple peptide and the 1-OH-unsubstituted saccharide being recovered.

In acetonitrile, the glycosyl donors **54** and **56** also reacted with acceptor **52**. With the xylose compound **54** at a reaction temperature of 10 °C the ester **55** was obtained after cleavage from the support in 81% yield relative to acceptor **52**. Like ester **53**, it has only an α-glycosidic linkage. The fucose compound **56** gave a coupling product at 0 °C. From this the α-glycosidically bound ester **57** was isolated after cleavage from the support in 46% yield. These examples demonstrate how resistant the hydroxy groups of serine in solid-phase-bound peptides are to glycosylation under the conditions described. An alternative, unexpected reaction was observed in the presence of Bu^t-protected glutamic acid under forcing reaction conditions.

Conclusions

A new method for solid-phase oligosaccharide synthesis has been presented which utilises solid-phase-bound glycopeptides as support. These were easily prepared from suitable building blocks according to general methods of solid-phase glycopeptide synthesis. Solid-phase-bound glycopeptides containing free hydroxy groups on the oligosaccharide residue could be glycosylated with different glycosyl trichloroacetimidates. The glyco-



Scheme 5 *Reagents:* i, TMSOTf, CH_3CN, TFA

syl acceptor contains differently substituted 2-azido-2-deoxy-α-D-galactose compounds. As glycosyl donors per-benzoylated trichloroacetimidates were used, which afforded β-glycosidic elongation of the saccharide chain. While per-acetylated trichloroacetimidates were too reactive to be used in solid-phase glycosylations, per-benzoylated donors gave good yields. In the case of 2-amino-2-deoxy-D-glucose, the 2-Teoc-NH-protecting group was used to achieve a β-glycosidic chain elongation. When disaccharide donors were used, glycopeptides with linear trisaccharide chains were obtained. Glycopeptides with branched trisaccharide chains were accessible by means of two subsequent solid-phase glycosylations. The matrix seems to influence the reactivity of hydroxy groups, and differences in the reactivity of 3-OH and 6-OH groups could not be detected. It has been found that formation of a 1,2-*trans* glycosidic bond occurs much more readily than does the corresponding 1,2-*cis* linkage, probably due to differences in reactivity and stability of the activated glycosyl donor.

Experimental

Materials and methods

All solvents were distilled at the appropriate pressure. Dimethylformamide (DMF) was analysed for free amines by addition of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) prior to use. Reagents for peptide synthesis were purchased as follows: Dhbt-OH and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) from Fluka; fluoren-9-ylmethoxycarbonyl (Fmoc)-amino acid-O-Pfp esters and Fmoc-protected Rink linker from NovaBiochem. ^{1}H NMR Spectra were recorded on a Bruker AMX 400 MHz spectrometer; δ values are in ppm and J values are in Hz (±0.3 Hz). Flash-column chromatography was performed on silica gel (ICN Biomedical, 12–16, 60 Å) with 1.5–6 bar pressure.† HPLC was performed on a Merck/Hitachi HPLC system with LiChrospher reversed-phase RP-18 columns (250 × 25 mm; 7 μm; flow rate 10 cm^3 min^{-1}) with buffer A (0.1% TFA in water) and buffer B (0.1% TFA in acetonitrile). FAB Mass spectra were recorded on a double-focused VG-Analytical 70-250 S mass spectrometer with *m*-nitrobenzyl alcohol matrix. Optical rotations were recorded on a Perkin-Elmer Polarimeter 241, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. IR Spectra were measured on a Perkin-Elmer FT–IR spectrometer 1720X spectrometer. Light petroleum refers to the fraction with distillation range 60–70 °C.

Multiple-column solid-phase synthesis

The solid-phase glycopeptide synthesis was performed on a polyethylene glycol dimethylacrylamide copolymer (PEGA) resin which was derivatised with norleucine as an internal reference amino acid, and *p*-[α-amino-(2,4-dimethoxyphenyl)methyl]phenoxyacetic acid (Rink linker) as an acid-labile amide linker. The Polyhipe resin with Rink linker was from NovaBiochem. All Fmoc cleavages were carried out under mild conditions by treatment of the resin with 20% piperidine in DMF. The Fmoc amino acids were introduced into the peptide chains as Pfp esters. In all cases, Dhbt-OH was added as an auxiliary nucleophile, in order to increase the rate of the coupling reaction and to allow us to follow the progress of the reaction visually by the disappearance of the strong yellow colour of the Dhbt-OH ammonium salt. The side chains of the non-glycosylated residues of Thr, the residues of Ser and the carboxylic group of Glu residues were protected as their *tert*-butyl ethers and esters, respectively. After attachment of the last amino acid and removal of Fmoc the terminal amino groups were acetylated with Ac_2O in DMF to give the resin-bound glycopeptides **3** and **20**. All glycopeptides were cleaved from the resin by treatment with 95% aq. TFA with concurrent removal of the Bu^t groups.

General procedure of the solid-phase glycosylation

The acceptor resin was washed with methanol (5 ×) and dichloromethane (5 ×) and was dried under vacuum for 24 h. A mixture of the acceptor resin, the donor imidate and 4 Å molecular sieves (beads *ca.* 2 mm) was dried *in vacuo* for 6 h. It was added to dry dichloromethane (2.5 cm^3) and the mixture was stirred at room temperature under nitrogen for 1 h. After cooling of this mixture to reaction temperature, fresh distilled TMSOTf was added. Reaction time was varied and is mentioned for each compound. After the reaction the resin was filtered off, washed successively with methanol (5 ×) and dichloromethane (5 ×) and dried. The resin was treated with 95% aq. TFA (2 cm^3) for 2 h at room temperature, then was filtered and washed with TFA (5 ×). The solution was concentrated and co-distilled first with toluene and then with methanol–toluene (1:3) *in vacuo*. The product was purified by preparative reversed-phase HPLC.

General procedure for the reduction

A solution of the glycopeptide in THF–acetic anhydride–acetic

† 1 bar = 10^5 Pa.

Table 1 ^{1}H NMR data (400 MHz) for the amino acid protons. Solvents: for the saccharide-part-blocked compounds, CD_3OD; for the completely deblocked compounds, D_2O. Internal reference H_2O. δ Values given in ppm, J values given (in parentheses) in ± 0.3 Hz

	Pro$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Pro$^{\alpha}$	Ile$^{\alpha}$	Ser$^{\alpha}$	Thr$^{\alpha}$	Pro$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Pro$^{\beta}$	Ile$^{\beta}$	Ser$^{\beta}$	Thr$^{\beta}$	Pro$^{\gamma}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Pro$^{\gamma}$	Ile$^{\gamma c}$	Ile$^{\gamma a}$	Ile$^{\gamma b}$	Thr$^{\gamma}$	Pro$^{\delta}$	Ile$^{\delta}$	Ac	Bz
6	4.43	4.75	4.78	4.64	4.39	4.15	4.42	4.41	1.84-1.98	4.12	4.20	4.18	1.84-1.98	1.78-1.88	3.63, 3.75	4.02	1.84-1.98	1.12	1.18	1.10	1.84-1.98	0.86	1.39-1.52	1.07-1.17	1.09	3.75	0.80	2.01	7.09-8.01
	(6.1) (9.0)	(6.6)	(3.6)	(4.6)	(4.8) (9.1)	(7.5)	(5.6) (5.8)	(3.0)	2.17-2.33	(6.6)	(6.6)	(6.1)	2.17-2.33		(11.4)	(6.1)	2.17-2.33				2.17-2.33								
7	4.33	4.46	4.44	4.35	4.33	4.10	4.37	4.30	1.84-2.02	4.04	4.18	4.14	1.84-2.02	1.71-1.87	3.64, 3.78	4.20	1.84-2.02	1.13	1.26	1.11	1.84-2.02	0.86	1.40-1.50	1.07-1.20	1.09	3.52-3.61	0.81	2.05	7.19-8.12
	(6.1) (8.9)	(5.6)	(2.0)	(4.2)	(5.1) (8.9)	(7.2)	(5.8)	(3.6)	2.11-2-21	(6.4)	(6.4)	(6.4)	2.11-2.21		(11.2)	(6.4)	2.11-2.21				2.11-2.21							1.92	
8	4.29	4.39	4.49	4.36	4.25	3.97	4.37	4.15	1.70-1.79	3.95	4.19	4.05	1.70-1.79	1.62-1.77	3.69, 3.65	4.05	1.70-1.79	1.08	1.07	1.08	1.70-1.79	0.80	1.29-1.39	1.03-1.19	1.04	3.48-3.54	0.71	1.89	-
	(4.6) (8.8)	(5.1)	(2.0)	(5.1)	(4.2) (8.6)	(7.1)	(5.8) (6.0)	(3.6)	2.09-2.18	(6.4)	(6.6)	(6.6)	2.09-2.18		(11.2)	(6.4)	2.09-2.18				2.09-2.18							1.99	

	Pro$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Pro$^{\alpha}$	Ile$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Pro$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Pro$^{\beta}$	Ile$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Pro$^{\gamma}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Pro$^{\gamma}$	Ile$^{\gamma c}$	Ile$^{\gamma a}$	Ile$^{\gamma b}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Pro$^{\delta}$	Ile$^{\delta}$	Ac	Bz
11	4.43	4.56	4.38	4.47	4.39	4.20	4.31	4.24	2.34-2.18	4.29	4.23	4.12	2.34-1.18	1.78-1.67	4.08	4.02	2.03-1.83	1.28	1.21	1.23	2.03-1.83	0.95	1.59-1.48	1.13	1.20	1.18	3.72-3.63	0.89	2.12	8.22-7.00
	(4.6) (9.2)	(5.1)	(3.6)	(4.2)		(7.1)	(5.6)	(4.9)	2.03-1.83				2.03-1.83					(6.6)	(6.7)	(7.1)					(6.6)	(6.6)				
12	4.52	4.38	4.46	4.29	4.45	4.18	4.24	4.21	2.38-2.12	4.31	4.36	4.16	2.38-2.12	1.71-1.54	4.12	4.13	2.00-1.78	1.31	1.29	1.27	1.98-1.86	0.98	1.62-1.51	1.12	1.26	1.23	3.77-3.64	0.91	2.08	8.18-7.07
	(4.6) (8.8)	(4.4)	(2.0)		(3.9) (9.0)	(7.5)	(4.3)	(5.3)	2.00-1.78				2.00-1.78					(6.6)	(6.6)	(6.1)					(6.1)	(6.1)			1.99	
13	4.28	4.38	4.54	4.31	4.21	4.24	4.28	4.17	2.42-2.21	4.25	4.28	4.04	2.42-2.21	1.87-1.74	4.11	4.09	2.07-1.81	1.22	1.25	1.23	2.02-1.89	0.99	1.63-1.48	1.09	1.20	1.16	3.66	0.89	2.06	
	(5.0) (8.3)	(5.0)	(3.1)	(5.1)	(4.6) (9.2)	(7.2)	(5.1)	(4.8)	2.07-1.81				2.07-1.81					(6.6)	(6.6)	(6.6)					(6.8)	(6.6)			1.99	
23	4.49	4.65	4.46	4.36	4.48	4.50	4.42	4.28	2.09-2.26	4.23	4.22	3.99	2.09-2.26	1.84-1.94	4.09	4.23	2.09-2.26	1.24	1.19	1.17	2.09-2.26	0.94	1.49-1.59	1.14-1.29	1.04	1.17	3.69-3.88	0.88	2.06	7.19-8.09
	(6.0) (9.1)	(6.0)	(3.0)	(4.1)	(4.6) (9.0)	(7.6)	(3.6)	(3.1)	1.94-2.07	(7.0)	(6.6)	(6.9)	1.94-2.07	(7.0)	(6.8)	(6.6)	1.94-2.07				1.94-2.07		(7.0)	(7.1)			3.44-3.62			
24	4.29	4.49	4.31	4.34	4.25	4.09	4.29	4.23	2.02-2.12	4.36	4.12	4.12	2.02-2.12	1.76-1.87	4.05	4.17	2.02-2.12	1.15	1.13	1.11	2.02-2.12	0.87	1.44-1.52	1.08-1.18	1.01	1.08	3.54-3.73	0.89	2.00	7.10-8.10
	(4.6) (9.0)	(5.6)	(3.1)	(3.6)	(4.6) (9.1)	(7.5)	(4.0)	(5.5)	1.87.1.98	(6.1)	(6.1)	(6.1)	1.87.1.98	(7.1)	(6.1)	(6.4)	1.87.1.98				1.87.1.98		(6.6)	(6.6)			3.38-3.52		1.92	
25	4.37	4.53	4.43	4.28	4.36	4.08	4.24	4.15	2.03-2.13	3.98	4.13	4.12	2.03-2.13	1.75-1.83	4.09	4.14	2.03-2.13	1.12	1.07	1.04	2.03-2.13	0.77	1.38-1.50	1.02-1.07	1.04	1.07	3.72-3.88	0.82	2.08	-
	(6.6) (8.1)	(5.6)	(3.1)	(3.5)	(6.6) (8.1)	(7.1)	(3.6)	(3.2)	1.83-1.91	(6.1)	(6.1)	(6.1)	1.83-1.91	(7.8)	(6.1)	(6.1)	1.83-1.91				1.83-1.91		(7.0)	(7.0)			3.46-3.56		1.95	
27	4.46	4.52	4.28	4.44	4.39	4.15	4.41	4.36	2.38-2.21	4.28	4.36	4.19	2.38-2.21	1.81-1.72	4.07	4.09	2.04-1.84	1.16	1.19	1.26	1.91-1.77	0.95	1.64-1.49	1.11	1.15	1.12	3.70-3.61	0.85	2.01	8.15-7.07
	(4.2) (9.1)	(5.3)	(1.8)	(6.2)		(7.0)	(4.2)		2.04-1.84				2.04-1.84					(6.6)	(6.6)	(6.6)					(6.6)	(6.6)				
28	4.44	4.57	4.41	4.47	4.41	4.31	4.44	4.38	2.31-2.12	4.33	4.29	4.08	2.31-2.12	1.74-1.60	4.25	4.14	2.03-1.85	1.21	1.15	1.17	2.05-1.91	0.96	1.65-1.52	1.09	1.17	1.19	3.68-3.60	0.84	2.09	8.26-7.12
	(6.1) (9.3)	(4.8)	(3.0)	(4.7)		(7.1)			2.03-1.85				2.03-1.85					(6.1)	(6.6)	(6.6)					(6.6)	(6.6)			1.98	
29	4.58	4.62	4.67	4.54	4.40	4.17	4.52	4.43	2.36-2.09	4.27	4.31	4.15	2.36-2.09	1.76-1.62	4.10	4.07	1.98-1.75	1.24	1.18	1.15	1.96-1.81	0.98	1.62-1.48	1.07	1.21	1.17	3.62-3.56	0.88	2.14	
	(4.6) (9.6)	(3.9)	(3.6)		(5.3) (8.7)	(7.1)		(5.4)	1.98-1.75				1.98-1.75					(6.6)	(6.7)	(6.6)					(6.6)	(6.6)			2.01	
44	4.55	4.69	4.56	4.58	4.48	4.20	4.39	4.35	2.09-2.22	4.09	4.27	4.14	2.09-2.22	1.80-1.91	4.20	4.19	2.09-2.22	1.23	1.27	1.07	2.09-2.22	0.92	1.38-1.48	1.23-1.33	1.21	1.20	3.87	0.86	1.93	7.28-7.61
	(5.1) (8.2)	(5.6)	(3.0)	(5.6)	(5.0) (9.1)	(7.5)	(3.5)	(3.6)	1.80-1.91	(6.6)	(6.1)	(6.4)	1.80-1.91	(7.2)	(6.6)	(6.6)	1.80-1.91				1.80-1.91		(7.0)	(7.1)						
46	4.53	4.55	4.52	4.48	4.48	4.23	4.30	4.21	2.08-2.18	3.97	4.28	4.11	2.08-2.18	1.78-1.88	4.10	4.07	2.08-2.18	1.21	1.33	1.21	2.08-2.18	0.90	1.33-1.44	1.01-1.11	1.03	1.02	3.68-3.82	0.84	1.86	7.25-7.75
	(4.6) (8.6)	(5.6)	(1.9)	(5.2)	(5.2) (9.0)	(7.7)	(3.6)	(3.6)	1.88-1.98	(6.6)	(6.4)	(6.6)	1.88-1.98	(7.1)	(6.6)	(6.4)	1.88-1.98				1.88-1.98		(7.0)	(6.9)					1.91	
49	4.36	4.51	4.33	4.33	4.34	4.16	4.28	4.20	1.74-1.83	3.97	4.09	4.09	1.74-1.83	1.61-1.70	3.75	4.15	1.74-1.83	1.12	1.06	1.03	1.74-1.83	0.83	1.39-1.48	1.04-1.09	0.85	1.07	3.45-3.60	0.75	1.97	6.94-8.01
	(3.5) (7.1)	(5.4)	(3.6)	(4.0)	(5.0) (8.1)	(7.1)	(3.6)	(3.6)	2.13-2.22	(6.1)	(6.1)	(6.1)	2.13-2.22	(7.1)	(6.4)	(6.1)	2.13-2.22				2.13-2.22		(7.0)	(7.0)						
50	4.27	4.32	4.51	4.27	4.32	4.05	4.23	4.15	1.79-1.88	3.95	4.17	4.10	1.79-1.88	1.69-1.78	4.07	3.79	1.79-1.88	1.03	1.03	1.01	1.79-1.88	0.87	1.32-1.42	0.98-1.08	1.01	0.84	3.63-3.80	0.73	1.94	7.01-8.08
	(5.0) (8.6)	(3.6)	(3.5)	(4.1)	(4.2) (8.2)	(7.2)	(3.6)	(3.0)	1.48-2.08	(5.1)	(6.1)	(5.1)	1.48-2.08	(7.2)	(6.1)	(6.1)	1.48-2.08				1.48-2.08		(7.1)	(7.1)			3.33-3.58		2.00	
51	4.55	4.65	4.73	4.62	4.50	4.28	4.54	4.40	1.92-2.02	4.21	4.44	4.30	1.92-2.02	1.92-2.02	4.32	4.33	1.92-2.02	1.32	1.35	1.33	1.92-2.02	1.01	1.55-1.65	1.25-1.36	1.01	1.27	3.69-3.76	0.96	2.10	-
	(4.6) (8.1)	(5.6)	(2.9)	(5.6)	(5.1) (7.6)	(7.6)	(4.6)	(3.6)	2.33-2.43	(5.6)	(6.6)	(5.8)	2.33-2.43	(7.1)	(6.1)	(6.1)	2.33-2.43				2.33-2.43		(7.6)	(7.6)			3.43-3.64		2.19	

	Glu$^{\alpha}$	Pro$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Pro$^{\alpha}$	Ile$^{\alpha}$	Thr$^{\alpha}$	Thr$^{\alpha}$	Glu$^{\beta}$	Pro$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Pro$^{\beta}$	Ile$^{\beta}$	Thr$^{\beta}$	Thr$^{\beta}$	Glu$^{\gamma}$	Pro$^{\gamma}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Pro$^{\gamma}$	Ile$^{\gamma c}$	Ile$^{\gamma a}$	Ile$^{\gamma b}$	Thr$^{\gamma}$	Thr$^{\gamma}$	Pro$^{\delta}$	Ile$^{\delta}$	Ac	Bz
33	4.65	4.52	4.58	4.52	4.53	4.50	4.19	4.25	4.25	2.07-2.15	2.15-2.23	4.04	4.07	4.11	2.15-2.23	1.82-1.90	4.13	4.13	2.45	2.15-2.23	1.05	1.15	1.01	2.15-2.23	0.81	1.44-1.52	1.15-1.63	1.01	1.01	3.72-3.82	0.74	1.90	7.27-7.78
	(5.1) (9.2)	(8.9)	(5.1)	(2.5)	(5.1)	(6.0)(8.0)	(7.5)	(6.1)	(6.1)		1.95-2.03	(6.6)	(6.1)	(6.6)	1.95-2.03	(7.1)	(6.6)	(6.6)		1.95-2.03				1.95-2.03		(7.6)	(7.6)						
34	4.60	4.49	4.60	4.53	4.49	4.41	4.15	4.37	4.20	2.06-2.14	2.20-2.30	3.96	4.12	4.04	2.20-2.30	1.89-1.96	4.07	4.08	2.45	2.20-2.30	1.16	1.18	1.04	2.20-2.30	0.88	1.50-1.60	1.19-1.25	0.98	0.98	3.76-3.84	0.90	2.04, 1.94	7.33-7.83
	(5.0) (9.2)	(6.0) (8.6)	(5.6)	(2.5)	(4.1)	(6.1) (7.9)	(7.2)	(3.6)	(3:1)		1.95-2.03	(6.6)	(7.1)	(6.1)	1.95-2.03	(7.1)	(6.6)	(6.6)		1.95-2.03				1.95-2.03		(7.6)	(7.6)					1.85	
35	4.66	4.53	4.42	4.58	4.37	4..40	4.16	4.32	4.18	2.15-2.22	2.20-2.28	3.97	4.03	4.15	2.20-2.28	1.82-1.92	4.10	4.11	2.45	2.20-2.28	1.13	1.13	1.11	2.20-2.28	0.79	1.49-1.57	1.15-1.23	1.11	1.05	3.70-3.82	0.74	1.95, 1.97	-
	(4.8) (9.6)	(5.1) (9.1)	(5.1)	(2.2)	(5.6)	(6.2) (9.0)	(8.0)	(4.6)	(4.1)		2.15-2.22	(7.1)	(7.1)	(7.1)	2.15-2.22	(7.6)	(7.1)	(6.6)		2.15-2.22				2.15-2.22		(7.2)	(7.2)					1.99	
39	4.68-4.72	4.55	4.70	4.55	4.55	4.51	4.20	4.39	4.3	2.07-2.15	2.19-2.25	4.12	4.27	4.23	2.19-2.25	1.85-1.90	4.23	4.23	2.45	2.19-2.25	1.21	1.27	1.21	2.19-2.25	0.97	1.54-1.62	1.21-1.30	1.19	1.19	3.76-3.81	0.91	1.97	7.33-7.82
	(9.8) (5.1)	(6.0) (8.0)	(5.6)	(3.1)	(4.1)	(7.0) (8.1)	(7.2)	(4.1)	(3.1)		1.97-2.04	(6.6)	(6.6)	(6.8)	1.97-2.04	(7.1)	(6.8)	(6.8)		1.97-2.04				1.97-2.04		(7.5)	(7.5)						
40	4.46	4.50	4.54	4.53	4.43	4.41	4.17	4.40	4.24	2.09-2.17	2.12-2.20	4.08	4.17	4.17	2.12-2.20	1.80-1.90	4.17	4.20	2.48	2.12-2.20	1.19	1.19	1.15	2.12-2.20	0.93	1.57-1.61	1.16-1.21	1.12	1.12	3.74-3.80	0.79	1.91, 1.93	7.31-7.82
	(5.1) (9.1)	(8.6) (5.9)	(5.6)	(3.0)	(4.6)	(6.1) (7.7)	(7.2)	(4.6)	(3.5)		1.85-1.95	(6.6)	(6.6)	(6.6)	1.85-1.95	(7.4)	(6.6)	(6.6)		1.85-1.95				1.85-1.95		(7.6)	(7.6)					, 1.94	
41	4.61	4.48	4.52	4.58	4.42	4.36	4.16	4.37	4.26	2.02-2.12	2.21-2.31	4.04	4.15	4.13	2.21-2.31	1.82-1.92	4.03	4.15	2.41	2.21-2.31	1.19	1.19	1.15	2.21-2.31	0.87	1.47-1.51	1.12-1.22	1.13	1.13	3.67-3.79	0.81	1.92, 1.94	-
	(4.1) (9.6)	(6.1) (7.8)	(5.5)	(1.6)	(5.1)	(6.0) (8.1)	(8.1)	(4.6)	(4.1)		1.81-1.92	(6.6)	(6.6)	(6.6)	1.81-1.92	(7.4)	(6.6)	(6.6)		1.81-1.92				1.81-1.92		(7.6)	(7.6)					1.95	

Table 2 ^{1}H NMR data (400 MHz) for the saccharide protons. Solvents: for the saccharide-part-blocked compounds, CD_3OD; for the completely deblocked compounds, D_2O. Internal reference H_2O. δ Values given in ppm, J values given (in parentheses) (± 0.3 Hz) are couplings to the lower numbered proton in the case of dd or ddd

	1-H	2-H	3-H	4-H	5-H	6-H^a	6-H^b	1′-H	2′-H	3′-H	4′-H	5′-H	6′-H^a	6′-H^b	1″-H	2″-H	3″-H	4″-H	5″-H	6″-H^a	6″-H^b
6	4.97	3.57	4.12	4.16	3.52-3.59	3.55	3.41	5.33	5.76	5.67	5.91	4.42	4.40-4.60								
	(3.6)	(11.2)	(2.6)	(0.8)	(5.1) (m)	(11.7)		(7.6)	(10.2)	(3.6)	(0.8)	(5.1) (6.0)									
7	4.65	4.27	3.85	4.14	3.41	3.53	3.40	5.19	5.80	5.76	5.98	4.46	4.40-4.60								
	(3.6)	(11.2)	(2.6)	(0.8)	(7.6) (4.6)	(11.7)		(7.6)	(11.2)	(2.1)	(0.7)	(4.6) (6.1)									
8	4.59	4.04	3.85	4.01	3.86	3.44-3.60	3.44-3.60	4.50	3.48	3.56	3.84	3.47	3.81	3.76							
	(3.6)	(11.2)	(3.1)	(1.0)	(5.0) (6.8)	(11.2)		(7.7)	(9.7)	(3.6)	(0.8)	(5.9) (6.1)	(11.2)								
11	4.93	3.40	3.99	4.12	3.47	3.33	3.28	5.12	5.69	4.56	6.06	4.63	4.58-4.51	4.58-4.51	5.29	5.54	5.58	5.85	4.52	4.58-4.51	4.58-4.51
	(3.6)	(11.2)	(3.1)	(1.5)				(8.1)	(10.1)	(3.6)					(7.6)	(10.2)	(3.0)				
12	4.67	4.08	3.81	4.17	3.31	3.41	3.37	5.03	5.71	4.71	5.98	4.64	4.61	4.48	5.17	5.51	5.64	5.69	4.54	4.63	4.44
	(3.6)	(11.2)	(3.0)					(7.6)	(10.7)	(3.0)					(7.6)	(10.7)	(3.1)				
13	4.58	4.14	3.86	4.06	3.76	3.60	3.51	4.14	3.33	3.54	3.72	3.92	3.77	3.48	4.25	3.48	3.71	3.86	4.01	3.74	3.54
	(4.1)	(11.1)	(3.0)					(7.6)	(10.8)	(3.6)					(8.1)	(10.7)	(3.0)				
23	4.90	3.49	3.77	3.76	3.59	4.06	3.89	5.09	5.68	5.75	5.98	4.56	4.64	4.50							
	(3.5)	(11.2)	(3.0)	(0.8)	(m)	(11.7)		(7.1)	(10.2)	(3.0)	(0.8)	(5.5)	(11.2)								
24	4.68	4.07	3.62	3.67	3.96	4.02	3.79	5.01	5.60	5.65	5.90	4.48	4.55	4.36							
	(4.0)	(10.7)	(3.1)	(0.8)	(4.6) (7.6)	(10.7)		(7.7)	(10.6)	(2.5)	(0.8)	(6.5) (5.6)	(11.2)								
25	4.87	4.15	3.93	4.07	4.24	4.11	3.89	4.47	3.56	3.68	3.72	3.73	3.82	3.78							
	(3.8)	(10.8)	(3.1)	(0.8)	(4.5 7.5)	(11.3)		(7.6)	(10.2)	(3.7)	(0.8)	(7.6) (4.5)	(11.7)								
27	4.96	3.31	3.68	3.88	4.01	4.08	3.88	5.21	5.71	4.78	6.12	4.60	4.58	4.39	5.41	5.83	5.74	5.91	4.63	4.55	4.33
	(3.6)	(10.7)	(3.0)					(7.8)	(10.7)	(3.0)					(8.1)	(11.0)	(3.6)				
28	4.71	4.18	3.59	3.72	3.75	4.10	3.95	5.15	5.67	4.84	5.99	4.68	4.54-4.41	4.54-4.41	5.34	5.75	5.69	5.78	4.62	4.54-4.41	4.54-4.41
	(3.6)	(11.2)	(3.5)					(7.6)	(10.9)	(3.1)					(8.0)	(11.2)	(3.6)				
29	4.74	4.23	3.85	4.01	4.12	4.14	4.06	4.38	3.48	3.41	3.76	4.01	3.81	3.66	4.30	3.54	3.81	3.84	3.98	3.83	3.74
	(3.6)	(10.7)	(3.0)					(7.6)	(11.2)	(3.0)					(8.1)	(10.9)	(3.4)				
33	5.00	3.65	3.90	4.07	3.70	3.52	3.38	4.92	3.92	5.61	5.37	4.12	4.49	4.32							
	(4.1)	(10.7)	(3.1)	(m)	(4.6) (7.1)	(11.7)		(8.7)	(10.3)	(9.7)	(9.7)	(3.1) (4.0)	(12.2)								
34	4.71	4.28	3.75	4.03	3.75	3.62	3.49	4.92	3.92	5.61	5.37	4.12	4.49	4.32							
	(3.6)	(11.2)	(3.1)	(0.8)	(4.5) (7.1)	(11.4)		(7.7)	(10.1)	(9.7)	(3.0)	(3.6) (4.6)	(11.8)								
35	4.68	4.15	3.88	4.17	3.85-3.95	3.75	3.65	4.50	3.61	3.49	3.38	3.35	3.83	3.68							
	(3.6)	(10.2)	(3.0)	(1.0)	(m)	(11.7)		(8.6)	(10.2)	(10.0)	(10.0)	(3.1) (5.0)	(12.2)								
39	5.05	3.56	3.98	3.85	3.74-3.84	3.83	3.76	4.86	3.94	5.71	5.54	4.20	4.61	4.48							
	(4.0)	(10.2)	(3.1)	(m)	(m)	(m)		(8.6)	(9.9)	(9.9)	(9.9)	(3.6) (5.0)	(11.7)								
40	4.81	4.17	3.77	3.80	3.74	3.84	3.76	4.83	4.17	5.63	5.52	4.20	4.56	4.20							
	(3.8)	(11.0)	(3.0)	(m)	(m)	(m)		(8.6)	(9.8)	(10.0)	(10.0)	(3.0) (5.0)	(11.3)								
41	4.75	4.01	3.79	3.88	4.00	3.70-3.80	3.70-3.80	4.46	3.65	3.42	3.32	3.36	3.84	3.61							
	(4.1)	(11.2)	(3.1)	(m)	(m)	(m)		(8.7)	(10.0)	(10.0)	(10.0)	(3.0) (5.0)	(11.2)								
44	5.15	3.78	4.49	4.23	3.92	3.69	3.68	5.55	4.29	5.78	6.21	4.20	4.27	4.19							
	(3.6)	(10.2)	(2.8)	(1.0)	(4.6) (7.1)	(12.0)		(3.6)	(10.8)	(2.5)	(0.8)	(5.1) (7.1)	(11.6)								
46	4.77	4.31	4.37	3.79	4.14	4.12	3.71	5.13	4.00	5.63	5.86	4.33	4.28	4.23							
	(3.6)	(10.6)	(3.0)	(0.8)	(3.9) (6.5)	(12.2)		(3.8)	(9.8)	(2.8)	(1.0)	(4.8) (6.8)	(11.7)								
49	4.76	3.45	3.91	4.09	3.74	3.84	3.73	5.11	5.71	5.62	5.88	4.45	4.52	4.37	4.49	5.65	5.60	5.86	4.43	4.49	4.35
	(3.6)	(10.7)	(3.1)	(0.8)	(4.6) (6.5)	(10.7)		(7.7)	(10.1)	(2.1)	(0.8)	(4.6) (7.2)	(11.7)		(7.8)	(10.1)	(3.0)	(0.8)	(8.0) (6.6)	(10.7)	
50	4.61	3.95	4.34	4.05	4.14	4.08	3.77	4.93	5.63	5.58	5.86	4.40	4.44	4.31	4.86	5.63	5.56	5.79	4.40	4.43	4.28
	(4.0)	(10.9)	(3.5)	(0.8)	(6.1) (7.6)	(10.8)		(7.1)	(10.2)	(2.5)	(0.8)	(7.1) (5.1)	(11.2)		(6.6)	(10.2)	(3.5)	(0.8)	(7.6) (5.6)	(11.2)	
51	4.88	4.31	4.10	4.12	4.30	4.13	3.92	4.41	3.58	3.71	3.98	3.74	3.85	3.73	4.52	3.57	3.68	3.97	3.69	3.82	3.74
	(3.6)	(10.8)	(3.0)	(0.8)	(4.5) (8.0)	(11.2)		(7.6)	(9.8)	(3.1)	(0.8)	(7.6) (5.6)	(11.7)		(7.6)	(1.2)	(3.1)	(0.8)	(7.1) (5.1)	(11.2)	

acid (8:3:1) was treated with activated zinc powder (activation with 2% $CuSO_4$ in water for 5 min). The mixture was stirred for 10 min at room temp. and then was filtered and washed with THF (5 × 1 ml) and acetic acid (5 × 0.5 ml) before being concentrated and co-distilled with toluene and then with methanol–toluene (1:3) *in vacuo*. The residue was purified by preparative reversed-phase HPLC.

General procedure for the deprotection

A mixture of glycopeptide in absolute methanol (1 cm^3) and sodium methoxide (0.1 M, 5 drops) at pH 8–9 was stirred for 6 h at room temperature. The solution was neutralised by addition of acetic acid (5 drops), concentrated, and co-distilled with toluene *in vacuo*.

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-seryl-L-threoninamide 6.** The acceptor **3** (50 mg, 7.0 μmol), the imidate **2**[23] (42 mg, 56 μmol) and TMSOTf (1 mm^3) were treated together at −30 °C for 24 h and then treated under the reaction conditions for the general procedure for solid-phase glycosylation. The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→60:40 (10 min)→20:80 (20 min)→5:95 (10 min)→2:98 (5 min)]. *Compound* **6** (5.7 mg, 67%) [Found: MH^+ (FAB-MS), 1623.5. $C_{77}H_{98}N_{12}O_{27}$ requires M, 1622.7].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-seryl-L-threoninamide 7.** Compound **6** (3 mg, 1.8 μmol) was reduced following the general procedure for the reduction and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→50:50 (30 min)→5:95 (10 min)]. *Compound* **7** (2.2 mg, 75%) [Found: MH^+ (FAB-MS), 1639.9. $C_{79}H_{102}N_{10}O_{28}$ requires M, 1638.7].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(β-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-seryl-L-threoninamide 8.** Compound **7** (2.2 mg, 1.3 μmol) was deprotected following the general procedure for the deprotection and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→80:20 (20 min)→50:50 (20 min)→5:95 (10 min)]. *Compound* **8** (1.3 mg, 82%) [Found: MH^+ (FAB-MS), 1224.0. $C_{51}H_{86}N_{10}O_{24}$ requires M, 1222.6].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-*O*-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 11.** The acceptor **3** (45 mg, 6.2 μmol), imidate **9**[25] (53 mg, 44 μmol) and TMSOTf (1.3 mm^3) were treated under the reaction conditions for the solid-phase glycosylation at −25 °C for 12 h. The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→40:60 (10 min)→20:80 (40 min)]. *Compound* **11** (4.6 mg, 35%) [Found: M + Na (FAB-MS), 2135.5. $C_{105}H_{122}N_{12}O_{35}$ requires M, 2110.81].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-*O*-[(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 12.** Compound **11** (3 mg, 1.4 μmol) was reduced as described in the general procedure for the reduction and purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→40:60 (10 min)→20:80 (40 min)]. *Compound* **12** (2.3 mg, 77%) [Found: MH^+ (FAB-MS), 2128.2. $C_{107}H_{126}N_{10}O_{36}$ requires M, 2126.83].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(β-D-galactopyranosyl)-(1→3)-(β-D-galactopyranosyl)-(1→3)-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 13.** Compound **12** (2.1 mg, 1 μmol) was deprotected as described in the general procedure for the deprotection and purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→85:15 (20 min)→50:50 (30 min)] to give the pure deprotected glycopeptide. *Compound* **13** (1.2 mg, 88%) [Found: MH^+ (FAB-MS), 1399.7. $C_{58}H_{98}N_{10}O_{29}$ requires M, 1398.65].

***tert*-Butyldimethylsilyl 2-azido-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranoside 14**

To a solution of *tert*-butyldimethylsilyl 2-azido-2-deoxy-β-D-galactopyranoside (300 mg, 0.94 mmol) in 2,2-dimethoxypropane (5 cm^3) was added anhydrous PTSA (15 mg). After 48 h at room temp. the solution was neutralised with triethylamine and concentrated. The residue was co-distilled *in vacuo* with toluene (3 ×) and dissolved in methanol–water (10:1; 10 cm^3). After 6 h the solution was concentrated and co-distilled with toluene (3 ×). The residue was chromatographed on a silica column (10 g) with light petroleum–ethyl acetate (10:1→4:1→2:1) to give *compound* **14** (290 mg, 76%), $[\alpha]_D^{20}$ + 128 (*c* 0.5, $CHCl_3$); δ_H($CDCl_3$; Me_4Si) 4.15 (1 H, d, $J_{1,2}$ 8.1, 1-H), 3.73 (1 H, dd, $J_{5,6a}$ 7.5, $J_{6a,6b}$ 10.6, $J_{6a,OH}$ 2.3, 6-H^a), 3.56 (1 H, dd, $J_{5,6b}$ 4.9, $J_{6b,OH}$ 7.3, 6-H^b), 3.51 (1 H, dd, $J_{2,3}$ 8.1, $J_{3,4}$ 5.3, 3-H), 3.45 (1 H, d, $J_{4,5}$ 2.2, 4-H), 3.37 (1 H, dd, 2-H), 3.12 (1 H, ddd, $J_{5,6b}$ 4.9, 5-H), 1.17 (3 H, s, isopropylidene CH_3), 1.02 (3 H, s, isopropylidene CH_3), 0.89 (9 H, s, CMe_3) and 0.07 (6 H, s, $SiMe_2$) (Found: C, 50.2; H, 8.1; N, 11.6. $C_{15}H_{29}N_3O_5Si$ requires C, 50.12; H, 8.13; N, 11.69%); ν_{max}(KBr)/cm^{-1} 2115 (N_3).

***tert*-Butyldimethylsilyl 6-*O*-acetyl-2-azido-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranoside 15**

A mixture of compound **14** (290 mg, 0.71 mmol), acetic anhydride (3 cm^3) and 4-(dimethylamino)pyridine (5 mg) was stirred at room temp. for 24 h. The solution was concentrated and the residue was co-distilled with toluene. The residue was chromatographed on a silica gel column (20 g) and eluted with light petroleum–ethyl acetate (20:1→10:1→5:1) to give *compound* **15** (261 mg, 91%), $[\alpha]_D^{20}$ + 129 (*c* 1.0, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 2114 (N_3); δ_H($CDCl_3$; Me_4Si) 4.26 (1 H, d, $J_{1,2}$ 8.2, 1-H), 4.17 (2 H, m, $J_{6a,6b}$ 12.1, 6-H_2), 3.89 (1 H, d, $J_{3,4}$ 5.3, $J_{4,5}$ 2.3, 4-H), 3.78 (1 H, ddd, $J_{5,6a} = J_{5,6b}$ = 6.0, 5-H), 3.70 (1 H, dd, $J_{2,3}$ 8.1, 3-H), 3.16 (1 H, dd, 2-H), 1.91 (3 H, s, $COCH_3$), 1.39 (3 H, s, isopropylidene CH_3), 1.17 (3 H, isopropylidene CH_3), 0.78 (9 H, s, Bu^t) and 0.01 (6 H, s, $SiMe_2$) (Found: C, 50.8; H, 7.75; N, 10.4. $C_{17}H_{31}N_3O_6Si$ requires C, 50.85; H, 7.78; N, 10.46%).

6-*O*-Acetyl-2-azido-2-deoxy-3,4-*O*-isopropylidene-β-D-galactopyranosyl trichloroacetimidate 17

A solution of compound **15** (200 mg, 0.5 mmol) in dry dichloromethane was stirred with TBAF (1.3 mol equiv., 360 mm^3) at −15 °C to give compound **16**. After 30 min, trichloroacetonitrile (2.50 mmol) and dry K_2CO_3 (200 mg) were added. The mixture was stirred at −15 °C for 10 min (extension of the reaction time led predominantly to the α-anomer). The solution was concentrated and the residue was co-distilled with toluene. The residue was chromatographed on a silica gel column (20 g) and eluted with light petroleum–ethyl acetate (20:1→10:1→5:1) to give *compound* **17** (240 mg, 62%), $[\alpha]_D^{20}$ + 48 (*c* 1.0, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 2114 (N_3); δ_H($CDCl_3$; Me_4Si) 5.66 (1 H, d, $J_{1,2}$ 3.4, 1-H), 4.50 (1 H, dd, $J_{2,3}$ 7.6, $J_{3,4}$ 5.5, 3-H), 4.44 (1 H, ddd, $J_{4,5}$ 2.8, $J_{5,6a}$ 4.2, $J_{5,6b}$ 7.0, 5-H), 4.39 (1 H, dd, $J_{6a,6b}$ 11.7, 6-H^a), 4.31 (1 H, d, 4-H), 4.27 (1 H, dd, 6-H^b), 3.77 (1 H, dd, 2-H), 2.06 (3 H, s, $COCH_3$), 1.56 (3 H, s, isopropylidene CH_3) and 1.38 (3 H, s, isopropylidene CH_3) (Found: C, 36.2; H, 4.0; N, 12.9. $C_{13}H_{17}Cl_3N_4O_6$ requires C, 36.17; H, 3.97; N, 12.98%).

***N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*O*-(6-*O*-acetyl-2-azido-2-deoxy-3,4-*O*-isopropylidene-α-D-galactopyranosyl)-L-threonine pentafluorophenyl ester 19**

A mixture of compound **17** (220 mg, 0.48 mmol), the acceptor **18**[15] and activated powdered 4 Å molecular sieves in dry dichloromethane was stirred for 1 h at room temp. under nitro-

gen. The mixture was cooled to 0 °C, and TMSOTf (8 mm^3, 48 μmol) was added. The cooling process was stopped and the solution was stirred for another 6 h. Triethylamine (2 drops) was added, the solution was concentrated, and the residue was co-distilled with toluene. The residue was purified by silica gel column chromatography (20 g) and eluted with toluene–ethyl acetate (10:1→5:1→2:1) to give *compound* **19** (240 mg, 62%), $[\alpha]_D^{20}$ +41 (*c* 0.5, $CHCl_3$); ν_{max}(KBr)/cm^{-1} 2113 (N_3); δ_H($CDCl_3$; Me_4Si) 7.28–7.80 (8 H, m, 8 × Fmoc-H), 5.79 (1 H, d, $J_{NH,CH\alpha}$ 9.2, Thr-NH), 4.75 (1 H, dd, $J_{CH\alpha,CH\beta}$ 2.2, Thr-CH$^{\alpha}$), 5.09 (1 H, d, $J_{1,2}$ 3.9, 1-H), 4.56 (1 H, m, $J_{CH\beta,CH\gamma}$ 6.3, Thr-CH$^{\beta}$), 4.51 (1 H, dd, $J_{5,6a}$ 7.3, $J_{6a,6b}$ 10.7, 6-H^{a}), 4.40 (1 H, dd, $J_{5,6b}$ 7.5, 6-H^{b}), 4.38 (1 H, dd, $J_{2,3}$ 6.8, $J_{3,4}$ 5.8, 3-H), 4.33 (2-H, m, $J_{CH,CH2}$ 2.1, $J_{CH2a,CH2b}$ 8.8, Fmoc-CH_2), 4.29 (1 H, m, Fmoc-CH), 4.26 (1 H, ddd, $J_{4,5}$ 1.7, 5-H), 4.21 (1 H, d, 4-H), 3.64 (1 H, dd, 2-H), 2.08 (3 H, s, $COCH_3$), 1.51 (3 H, s, isopropylidene CH_3), 1.45 (3 H, d, Thr-CH$^{\gamma}$) and 1.35 (3 H, s, isopropylidene CH_3) (Found: C, 55.8; H, 4.1; N, 7.2. $C_{36}H_{33}F_5N_4O_{10}$ requires C, 55.67; H, 4.28; N, 7.21%).

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 23.** The acceptor **21** (50 mg, 7.5 μmol) was glycosylated with imidate **2** (46 mg, 60 μmol) by TMSOTf (1 mm^3) at −30 °C for 24 h following the general procedure for the solid-phase glycosylation. The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→60:40 (10 min)→20:80 (20 min)→5:95 (10 min)→2:98 (5 min)]. *Compound* **23** (5.7 mg, 71%) [Found: MH$^+$ (FAB-MS), 1637.9. $C_{78}H_{100}N_{12}O_{27}$ requires M, 1636.7].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 24.** Compound **23** (4.5 mg, 2.8 μmol) was reduced following the general procedure for the reduction and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→50:50 (30 min)→5:95 (10 min)]. *Compound* **24** (3.3 mg, 72%) [Found: MH$^+$ (FAB-MS), 1653.9. $C_{80}H_{104}N_{10}O_{28}$ requires M, 1652.7].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(β-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 25.** Compound **24** (3.5 mg, 2.9 μmol) was deprotected following the general procedure for deprotection and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→80:20 (20 min)→50:50 (20 min)→5:95 (10 min)]. *Compound* **25** (2.9 mg, 81%) [Found: MH$^+$ (FAB-MS), 1237.8. $C_{52}H_{88}N_{10}O_{24}$ requires M, 1236.6].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threonin amide 27.** The acceptor **21** (50 mg, 7.5 μmol), imidate **9** (57 mg, 47 μmol) and TMSOTf (1.4 mm^3) were treated following the general procedure for the solid-phase glycosylation. The reaction was carried out at 0 °C for 2 h. The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→40:60 (10 min)→20:80 (40 min)]. *Compound* **27** (7.3 mg, 52%) [Found: MH$^+$ (FAB-MS), 2111.8. $C_{105}H_{122}N_{12}O_{35}$ requires M, 2110.81].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-*O*-(2,4,6-tri-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 28.** Compound **27** (4 mg, 1.9 μmol) was reduced as described in the general procedure for the reduction and purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→40:60 (10 min)→20:80 (40 min)]. *Compound* **28** (2.9 mg, 72%) [Found: MH$^+$ (FAB-MS), 2128.3. $C_{107}H_{126}N_{10}O_{36}$ requires M, 2126.83].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(β-D-galactopyranosyl)-(1→3)-*O*-(β-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 29.** Compound **28** (5.8 mg, 2.7 μmol) was deprotected as described for the deprotection and was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→85:15 (20 min)→50:50 (30 min)] to give the pure *deprotected glycopeptide* **29** (3.1 mg, 81%) [Found: MH$^+$ (FAB-MS), 1400.0. $C_{58}H_{98}N_{10}O_{29}$ requires M, 1398.65].

1,3,4,6-Tetra-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose

Benzoyl chloride (8.65 ml, 74.1 mmol) was given dropwise to a solution of 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose[28] (3.5 g, 9.88 mmol) in pyridine (200 cm^3) at 0 °C. The cooling process was stopped and the mixture was stirred for 12 h at room temperature. Chloroform (300 cm^3) and water (200 cm^3) were added and the mixture was washed successively with saturated aq. $NaHCO_3$ (100 cm^3, 3 ×) and water (100 cm^3, 3 ×). The solution was concentrated and co-distilled with toluene (3 ×) in vacuum. The residue was purified by silica gel column chromatography (250 g) and eluted with light petroleum–ethyl acetate (10:1→5:1→2:1) to give the title compound (5.41 g, 71%), δ_H($CDCl_3$; Me_4Si) 7.32–8.21 (20 H, m, benzoyl H), 6.66 (1 H, d, $J_{2,NH}$ 3.6, NH), 5.92 (1 H, dd, $J_{2,3}$ 10.2, $J_{3,4}$ 9.7, H-3), 5.87 (1 H, dd, $J_{4,5}$ 9.7, H-4), 5.48 (1 H, d, $J_{1,2}$ 9.7, H-1), 4.72 [1 H, d, J(CH_2) 12.2, Teoc H], 4.60 (1 H, ddd, H-2), 4.59 (1 H, dd, $J_{5,6a}$ 2.6, $J_{6a,6b}$ 11.7, H^{a}-6), 4.51 (1 H, dd, $J_{5,6b}$ 6.6, H^{b}-6) and 4.46 (2 H, d, Teoc H and H-5).

3,4,6-Tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose

To a solution of 1,3,4,6-tetra-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranose (5 g, 6.5 mmol) in THF (150 cm^3) was added ethanolamine (15 cm^3). The mixture was stirred at room temperature for 2.5 h. The residue was purified by silica gel column chromatography (200 g) and eluted with light petroleum–ethyl acetate (10:1→5:1→2:1) to give the title compound (3.38 g, 78%), δ_H($CDCl_3$; Me_4Si) 7.43–8.21 (15 H, m, benzoyl H), 6.00 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 9.7, H-3), 5.87 (1 H, dd, $J_{4,5}$ 10.2, H-4), 5.48 (1 H, d, $J_{2,NH}$ 10.1, NH), 5.60 (1 H, d, $J_{1,2}$ 3.6, H-1), 4.82 [1 H, d, J(CH_2) 12.2, Teoc H], 4.79 (1 H, dd, $J_{5,6a}$ 3.0, $J_{6a,6b}$ 12.2, H^{a}-6), 4.75 (1 H, m, $J_{5,6b}$ 4.2, H-5), 4.56 (1 H, dd, H^{b}-6), 4.46 (1 H, d, Teoc H), 4.60 (1 H, ddd, H-2) and 3.89 (1 H, m, OH).

3,4,6-Tri-*O*-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α-D-glucopyranosyl trichloroacetimidate 30

To a mixture of 3,4,6-tri-*O*-benzoyl-2-deoxy-2-trichloroethoxycarbonylamino-β-D-glucopyranose (3.0 g, 4.50 mmol) and trichloroacetonitrile (4.4 cm^3) in dry dichloromethane containing molecular sieves (3 Å) was added 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) (0.225 cm^3, 1.50 mmol). The mixture was stirred for 45 min at room temperature and concentrated in vacuum. The residue was purified by silica gel column chromatography (200 g) and eluted with light petroleum–ethyl acetate (10:1→5:1→2:1) to give the title compound **30** (2.59 g, 71%), δ_H($CDCl_3$; Me_4Si) 9.00 (1 H, s, NH), 7.47–8.18 (15 H, m, benzoyl H), 5.71 (1 H, d, $J_{1,2}$ 3.6, H-1), 5.98 (1 H, dd, $J_{2,3}$ = $J_{3,4}$ = 9.7, H-3), 5.87 (1 H, dd, $J_{4,5}$ 9.5, H-4), 5.56 (1 H, d, NH), 4.79 [1 H, d, J(CH_2) 12.2, Teoc H], 4.65–4.77 (3 H, H_2-6 and H-5) and 4.61 (1 H, ddd, H-2).

***N*$^{\alpha}$-Acetyl-L-glutamyl-L-prolyl-L-threonyl-*O*-[*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-trichloroethoxycarbonylamino-β-D-glucopyranosyl)-(1→3)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 33.** The glycosylation of acceptor **31** (80 mg, 11.2 μmol) with imidate **30** (74 mg, 89.6 μmol) was performed as described in the general procedure for the solid-phase glycosylation and the reaction was carried out at −15 °C for 12 h.

The residue was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→60:40 (20 min)→20:80 (20 min)→2:98 (10 min)]. *Compound* **33** (9.3 mg, 70%) [Found: MH^+ (FAB-MS), 1819.3. $C_{79}H_{105}Cl_3N_{14}O_{29}$ requires M, 1818.6].

***N*$^{\alpha}$-Acetyl-L-glutamyl-L-prolyl-L-threonyl-*O*-[*O*-(2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threonin amide 34.** Compound **33** (4.3 mg, 2.4 μmol) was reduced as described in the general procedure and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 70:30→60:40 (5 min)→2:98 (35 min)→2:98 (10 min)]. *Compound* **34** (2.4 mg, 58%) [Found: MH^+ (FAB-MS), 1719.9. $C_{80}H_{110}N_{12}O_{30}$ requires M, 1718.7].

***N*$^{\alpha}$-Acetyl-L-glutamyl-L-prolyl-L-threonyl-*O*-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→3)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 35.** Compound **34** (2.4 mg, 1.4 μmol) was deprotected as described in the general procedure and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→80:20 (15 min)→50:50 (30 min)→5:95 (10 min)]. *Compound* **35** (2.2 mg, 88%) [Found: MH^+ (FAB-MS), 1407.9. $C_{59}H_{98}N_{12}O_{27}$ requires M, 1406.7].

***N*$^{\alpha}$-Acetyl-L-glutamyl-L-prolyl-L-threonyl-*O*-[*O*-(3,4,6-tri-*O*-benzoyl-2-deoxy-2-trichloroethoxycarbonylamino-β-D-glucopyranosyl)-(1→6)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 39.** The acceptor **37** (80 mg, 11.2 μmol), imidate **30** (73 mg, 89.6 μmol) and TMSOTf (2 mm^3) were allowed to react together at −15 °C for 12 h as described in the general procedure for the solid-phase glycosylation. The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→60:40 (20 min)→20:80 (20 min)→2:95 (10 min)]. *Compound* **39** (4.2 mg, 62%) [Found: MH^+ (FAB-MS), 1819.5. $C_{79}H_{105}Cl_3N_{14}O_{29}$ requires M, 1818.6].

***N*$^{\alpha}$-Acetyl-L-glutamyl-L-prolyl-L-threonyl-*O*-[*O*-(2-acetamido-3,4,6-tri-*O*-benzoyl-2-deoxy-β-D-glucopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 40.** Compound **39** (4.2 mg, 2.4 μmol) was reduced as described in the general procedure and the product was purified by preparative reversed-phase HLPC [buffer A–buffer B 70:30→60:40 (5 min)→2:98 (35 min)→2:98 (10 min)]. *Compound* **40** (2.8 mg, 68%) [Found: MH^+ (FAB-MS), 1719.5. $C_{80}H_{110}N_{12}O_{30}$ requires M, 1718.7].

***N*$^{\alpha}$-Acetyl-L-glutamyl-L-prolyl-L-threonyl-*O*-[*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 41.** Compound **40** (2.8 mg, 1.6 μmol) was deprotected as described in the general procedure and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 95:5→80:20 (15 min)→50:50 (30 min)→5:95 (10 min)]. *Compound* **41** (1.9 mg, 83%) [Found: MH^+ (FAB-MS), 1407.9. $C_{59}H_{98}N_{12}O_{27}$ requires M, 1406.7].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2-azido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-(2-azido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 44.** The glycosylation of acceptor **43** (50 mg, 7.5 μmol) with imidate **42** (75 mg, 0.11 mmol) and TMSOTf (4 mm^3) was carried out at 0 °C for 12 h as described in the general procedure. The residue was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→60:40 (20 min)→20:80 (20 min)→2:98 (10 min)]. *Compound* **44** (1.7 mg, 24%) [Found: MH^+ (FAB-MS), 1558.9. $C_{71}H_{95}N_{15}O_{25}$ requires M, 1557.7].

Resin 47. Resin **4b** (50 mg, 7.0 μmol) with methanol–water (10:1; 2 cm^3) was stirred for 2 h at room temp. PTSA was added (to pH 1.5–2.0) and the mixture was stirred for 12 h. The resin was filtered, washed with methanol (5 ×) and dichloromethane (5 ×) and dried in vacuum for 12 h.

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-[*O*-(2-azido-3,4,6-tri-*O*-benzoyl-2-deoxy-α-D-galactopyranosyl)-(1→6)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 46.** The acceptor **45** (50 mg, 7.5 μmol), the imidate **42** (75 mg, 0.11 mmol) and TMSOTf (4 mm^3) were treated together at 0 °C for 12 h as described in the general procedure for the glycosylation. The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→60:40 (20 min)→20:80 (20 min)→2:95 (10 min)]. *Compound* **46** (1.4 mg, 20%) [Found: MH^+ (FAB-MS), 1554.9. $C_{73}H_{99}N_{13}O_{26}$ requires M, 1573.7].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-{*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)]-(2-azido-2-deoxy-α-D-galactopyranosyl)}-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 49.** The glycosylation of acceptor **47** (50 mg, 7.0 μmol) with imidate **2** (45 mg, 60 μmol) and TMSOTf (2 mm^3) was carried out at −30 °C for 24 h as described in the general procedure. The residue was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→50:50 (10 min)→20:80 (30 min)→2:98 (20 min)]. *Compound* **49** (5.0 mg, 64%) [Found: MH^+ (FAB-MS), 2216.0. $C_{112}H_{126}N_{12}O_{36}$ requires M, 2214.8].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-{*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→3)-[*O*-(2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl)-(1→6)]-(2-acetamido-2-deoxy-α-D-galactopyranosyl)}-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 50.** Compound **49** (4.0 mg, 1.8 μmol) was reduced as described in the general procedure and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 80:20→50:50 (10 min)→2:98 (20 min)]. *Compound* **50** (2.9 mg, 72%) [Found: MH^+ (FAB-MS), 2232.0. $C_{114}H_{130}N_{10}O_{37}$ requires M, 2230.9].

***N*$^{\alpha}$-Acetyl-L-prolyl-L-threonyl-*O*-{*O*-(β-D-galactopyranosyl)-(1→3)-[*O*-(β-D-galactopyranosyl)-(1→6)]-(2-acetamido-2-deoxy-α-D-galactopyranosyl)}-L-threonyl-L-threonyl-L-prolyl-L-isoleucyl-L-threonyl-L-threoninamide 51.** Compound **50** (2.9 mg, 1.3 μmol) was deprotected as described in the general procedure and the product was purified by preparative reversed-phase HPLC [buffer A–buffer B 100:0→80:20 (30 min)→50:50 (10 min)→5:95 (10 min)]. *Compound* **51** (1.4 mg, 78%) [Found: MH^+ (FAB-MS), 1399.9. $C_{58}H_{98}N_{10}O_{29}$ requires M, 1398.7].

***N*$^{\alpha}$-Acetyl-*O*-(2,3,4,6-tetraacetyl-α-D-galactopyranosyl)-L-glutamyl-L-serylglycyl-L-isoleucylglycinamide 53.** A mixture of the acceptor resin **52** (50 mg, 7.5 μmol), the per-acetylated imidate of compound **2** [24] (30 mg, 60 μmol) and 4 Å molecular sieves were dried in vacuum for 12 h. Dry acetonitrile (2 cm^3) was added and the mixture was stirred at room temperature under nitrogen for 1 h. TMSOTf (1 mm^3) was added and the mixture was stirred for another 2 h. After the reaction the resin was filtered off washed successively with methanol (5 ×) and dichloromethane (5 ×) and dried. The resin was treated with 95% aq. TFA (2 cm^3) for 2 h at room temperature, then was filtered off and washed with TFA (5 ×). The solution was concentrated and co-distilled first with toluene and then with methanol–toluene (1:3). The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 90:10→50:50 (20 min)→20:80 (20 min)→5:95 (10 min)]. *Compound* **53** (3.5 mg, 78%) [Found: MH^+ (FAB-MS), 833.7. $C_{34}H_{52}O_{18}N_6$ requires M, 832.3]; δ_H(MeOD; Me_4Si) 6.31 (1 H, d, $J_{1,2}$ 3.1, H-1), 5.51 (1 H, dd, $J_{4,5}$ 0.8, H-4), 5.37 (1 H, dd, $J_{2,3}$ 10.7, $J_{3,4}$ 3.1, H-3), 5.33 (1 H, dd, H-2), 4.48 (1 H, ddd, $J_{5,6a}$ 4.6, $J_{5,6b}$ 7.0, H-5), 4.38 (1 H, dd, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 5.6, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta b}$ 7.6, Ser CH^{α}), 4.36 (1 H, dd, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 4.8, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta b}$ 9.1, Glu CH^{α}), 4.18 (1 H, dd, $J_{6a,6b}$ 11.2, H^a-6), 4.13 (1 H, dd, H^b-6), 4.11 (1 H, d, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta}$ 7.4, Ile CH^{α}), 3.91 (2 H, d, $J_{\mathbf{CH}\alpha a,\mathrm{CH}\alpha b}$ 10.2, Gly CH^{α}), 3.83 (2 H, d, $J_{\mathbf{CH}\alpha a,\mathrm{CH}\alpha b}$ 10.2, Gly CH^{α}), 3.81 (2 H, dd, $J_{\mathbf{CH}\beta a,\mathrm{CH}\beta b}$ 11.0, Ser CH^{β}), 2.59 (2 H, m, Glu CH^{γ}), 2.12 (1 H, m, Glu CH^{β}), 1.98 (1 H, m, Glu CH^{β}), 1.94–2.03 (12 H, 4 s, 4 × $COCH_3$), 1.83 (1 H, m, $J_{\mathbf{CH}\beta,\mathrm{CH}\gamma c}$ 7.5, Ile CH^{β}), 1.39 (1 H, m, $J_{\mathbf{CH}\gamma a,\mathrm{CH}\delta}$ 7.5, Ile $CH^{\gamma a}$), 1.12 (1 H, m, $J_{\mathbf{CH}\gamma b,\mathrm{CH}\delta}$ 7.5, Ile $CH^{\gamma b}$), 0.85 (3 H, d, Ile $CH^{\gamma c}$) and 0.79 (3 H, t, Ile CH^{δ}).

***N*$^{\alpha}$-Acetyl-*O*-(2,3,4-triacetyl-α-D-xylopyranosyl)-L-glutamyl-L-serylglycyl-L-isoleucylglycinamide 55.** A mixture of the acceptor resin **52** (50 mg, 7.5 μmol), the per-acetylated imidate **54**[32] (23 mg, 60 μmol) and 4 Å molecular sieves were dried in vacuum for 12 h. It was treated with dry acetonitrile (2 cm^3) and the mixture was stirred at room temperature under nitrogen for 1 h. TMSOTf (1 mm^3) was added and the mixture was stirred for 1 h. After the reaction the resin was filtered off, washed successively with methanol (5 ×) and dichloromethane (5 ×) and dried. The resin was treated with 95% aq. TFA (2 cm^3) for 2 h at room temperature and then filtered off, and washed with TFA (5 ×). The solution was concentrated, and co-distilled first with toluene and then with methanol–toluene (1:3). The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 90:10→50:50 (20 min)→20:80 (20 min)→5:95 (10 min)].*Compound* **55** (3.4 mg, 81%) [Found: MH^+ (FAB-MS), 761.5. $C_{31}H_{48}N_6O_{16}$ requires M, 760.3]; δ_H(MeOD; Me_4Si) 6.18 (1 H, d, $J_{1,2}$ 3.5, H-1), 5.36 (1 H, dd, $J_{2,3}$ 9.7, $J_{3,4}$ 9.4, H-3), 5.11 (1 H, dd, H-2), 5.07 (1 H, m, $J_{4,5a}$ 6.1, $J_{4,5b}$ 10.2, H-4), 4.38 (1 H, dd, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 5.1, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta b}$ 6.1, Ser CH^{α}), 4.35 (1 H, dd, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 5.6, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta b}$ 9.1, Glu CH^{α}), 4.12 (1 H, d, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta}$ 7.1, Ile CH^{α}), 3.95 (1 H, m, H^a-5), 3.92 (2 H, d, $J_{\mathbf{CH}\alpha a,\mathrm{CH}\alpha b}$ 11.0, Gly CH^{α}), 3.84 (2 H, d, $J_{\mathbf{CH}\alpha a,\mathrm{CH}\alpha b}$ 11.2, Gly CH^{α}), 3.81 (2 H, dd, $J_{\mathbf{CH}\beta a,\mathrm{CH}\beta b}$ 11.2, Ser CH^{β}), 3.74 (1 H, m, H^b–5), 2.60 (2 H, m, Glu CH^{γ}), 2.14 (2 H, m, Glu CH^{β}), 1.98, 2.00 and 2.01 (9 H, 3 s, 3 × $COCH_3$), 1.83 (1 H, m, $J_{\mathbf{CH}\beta,\mathrm{CH}\gamma c}$ 6.8, Ile CH^{β}), 1.38 (1 H, m, $J_{\mathbf{CH}\gamma a,\mathrm{CH}\delta}$ 7.4, Ile $CH^{\gamma a}$), 1.12 (1 H, m, $J_{\mathbf{CH}\gamma b,\mathrm{CH}\delta}$ 7.4, Ile $CH^{\gamma b}$), 0.85 (3 H, t, Ile $CH^{\gamma c}$) and 0.79 (3 H, t, Ile CH^{δ}).

***N*$^{\alpha}$-Acetyl-*O*-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-L-glutamyl-L-serylglycyl-L-isoleucylglycinamide 57.** A mixture of the acceptor resin **52** (50 mg, 7.5 μmol), the per-acetylated imidate **56**[33] (26 mg, 60 μmol) and 4 Å molecular sieves were dried in vacuum for 12 h. It was treated with dry acetonitrile (2 cm^3) and the mixture was stirred at room temperature under nitrogen for 1 h before being cooled to 0 °C, TMSOTf (1 mm^3) was added and the mixture was stirred for 6 h at 0 °C. After the reaction the resin was filtered off, washed successively with methanol (5 ×) and dichloromethane (5 ×) and dried. The resin was treated with 95% aq. TFA (2 cm^3) for 2 h at room temperature and then was filtered off, and washed with TFA (5 ×). The solution was concentrated and co-distilled first with toluene and then with methanol–toluene (1:3). The product was purified by preparative reversed-phase HPLC [buffer A–buffer B 90:10→50:50 (20 min)→20:80 (20 min)→5:95 (10 min)]. *Compound* **57** (2.1 mg, 47%) [Found: MH^+ (FAB-MS), 775.7. $C_{32}H_{50}N_6O_{16}$ requires M, 774.3]; δ_H(MeOD; Me_4Si) 6.21 (1 H, d, $J_{1,2}$ 3.5, H-1), 5.26 (1 H, dd, $J_{2,3}$ 10.6, H-3), 5.25 (1 H, dd, $J_{3,4}$ 3.1, $J_{4,5}$ 0.8, H-4), 5.11 (1 H, dd, H-2), 4.39 (1 H, dd, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 5.1, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta b}$ 7.4, Ser CH^{α}), 4.33 (1 H, dd, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 5.6, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta a}$ 8.1, Glu CH^{α}), 4.28 (1 H, dd, $J_{5,6}$ 6.7, H-5), 4.10 (1 H, d, $J_{\mathbf{CH}\alpha,\mathrm{CH}\beta}$ 7.1, Ile CH^{α}), 3.76–3.91 (2 H, m, Gly CH^{α}), 3.76–3.91 (2 H, m, Gly CH^{α}), 3.70 and 3.81 (2 H, dd, $J_{\mathbf{CH}\beta a,\mathrm{CH}\beta b}$ 11.2, Ser CH^{β}), 2.50 (2 H, m, Glu CH^{γ}), 2.08 (2 H, m, Glu CH^{β}), 1.92, 1.91 and 1.88 (9 H, 3 s, 3 × $COCH_3$), 1.83 (1 H, m, $J_{\mathbf{CH}\beta,\mathrm{CH}\gamma c}$ 7.0, Ile CH^{β}), 1.44 (1 H, m, $J_{\mathbf{CH}\gamma a,\mathrm{CH}\delta}$ 7.6, Ile $CH^{\gamma a}$), 1.11 (1 H, m, $J_{\mathbf{CH}\gamma b,\mathrm{CH}\delta}$ 7.6, Ile $CH^{\gamma b}$), 1.04 (3 H, d, H–6), 0.81 (3 H, t, Ile CH^{δ}) and 0.85 (3 H, d, Ile $CH^{\gamma c}$).

Acknowledgements

This research was supported by EU SCIENCE program (grant SCI*–CT 92-0765). We thank Dr V. Sinnwell for recording the NMR spectra.

References

1 B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149.
2 J. S. Früchtel and G. Jung, *Angew. Chem.*, 1996, **108**, 19; *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 17.
3 J. M. Frechet and C. Schuerch, *J. Am. Chem. Soc.*, 1971, **93**, 492; *Carbohydr. Res.*, 1972, **22**, 399.
4 S. J. Danishefsky, K. F. McClure, J. T. Randolph and R. B. Ruggeri, *Science*, 1993, **260**, 1307.
5 K. Suzuki. G. A. Sulikowski, R. W. Friesen and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1990, **112**, 8895.
6 J. T. Randolph, K. F. McClure and S. J. Danishefsky, *J. Am. Chem. Soc.*, 1995, **117**, 5712.
7 C. M. Timmers, G. A. van der Marel and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 609.
8 L. Yan, L. C. M. Taylor, R. Goodnow, Jr. and D. Khane, *J. Am. Chem. Soc.*, 1994, **116**, 6953.
9 M. Schuster, P. Wang, J. C. Paulson and C.-H. Wong, *J. Am. Chem. Soc.*, 1994, **116**, 1135.
10 S. P. Douglas, D. M. Whitefield and J. J. Krepinsky, *J. Am. Chem. Soc.*, 1991, **113**, 5095; 1995, **117**, 2116.
11 J. J. Krepinsky, in *Modern Methods in Carbohydrate Synthesis*, ed. S. H. Khan and R. A. O'Neill, Harwood Academic, Amsterdam, 1996, p. 194.
12 R. R. Schmidt and W. Kinzy, *Adv. Carbohydr. Chem. Biochem.*, 1994, **50**, 21.
13 R. Verduyn, P. A. M. van der Klein, M. Douwes, G. A. van der Marel and J. H. van Boom, *Recl. Trav. Chim. Pays-Bas*, 1993, **112**, 464.
14 S. Peters, T. Bielfeldt, M. Meldal, K. Bock and H. Paulsen, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1163.
15 H. Paulsen, T. Bielfeldt, S. Peters, M. Meldal and K. Bock, *Liebigs Ann. Chem.*, 1994, 369, 381.
16 H. Paulsen, S. Peters, T. Bielfeldt, M. Meldal and K. Bock, *Carbohydr. Res.*, 1995, **268**, 17.
17 I. Brockhausen, in *Glycoproteins*, ed. J. Montreuil, H. Schachter and J. F. G. Vliegenthart, Elsevier, Amsterdam, 1995, p. 201.
18 M. Verma and E. A. Davidson, *Glycoconjugate J.*, 1994, **11**, 172.
19 J. Taylor-Papadimetriou and A. A. Epenetos, *TIB TECH*, 1994, **12**, 227.
20 E. Meinjohanns, M. Meldal, A. Schleyer, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1996, 985.
21 L. R. Cameron, J. Holder, M. Meldal and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2895.
22 H. Rink, *Tetrahedron Lett.*, 1987, **28**, 3787.
23 S. Rio, J.-M. Beau and J.-C. Jacquinet, *Carbohydr. Res.*, 1991, **219**, 71.
24 G. Grundler and R. R. Schmidt, *Liebigs Ann. Chem.*, 1984, 1826.
25 S. Rio, J.-M. Beau and J.-C. Jacquinet, *Carbohydr. Res.*, 1993, **244**, 295.
26 H. Paulsen, *Angew. Chem.*, 1982, **94**, 184; *Angew. Chem., Int. Ed. Engl.*, 1982, **21**, 155.
27 R. U. Lemieux, T. Takeda and B. Y. Chung, ACS Symposium Series, 1976, vol. 39, p. 90.
28 T. M. Windholz and B. R. Jonston, *Tetrahedron Lett.*, 1967, 2555.
29 H. Paulsen and B. Helpap, *Carbohydr. Res.*, 1991, **216**, 289.
30 E. Meinjohanns, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1995, 405.
31 A. Vargas-Berenguel, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1994, 2615.
32 M. Mori, Y. Ito and T. Ogawa, *Carbohydr. Res.*, 1990, **195**, 199.
33 X.-B. Han and R. R. Schmidt, *Liebigs Ann. Chem.*, 1992, 817.

Paper 6/04123J
Received 12*th June* 1996
Accepted 5*th September* 1996