Dithiasuccinoyl (Dts) Amino-protecting Group used in Syntheses of 1,2-*trans*-Amino Sugar Glycosides

Ernst Meinjohanns,[a] Morten Meldal,*[,a] Hans Paulsen[b] and Klaus Bock[a]
[a] *Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10 DK-2500 Valby, Copenhagen, Denmark*
[b] *Institut für Organische Chemie, Martin-Luther-King-Platz 6, D-20146 Hamburg, Germany*

Suitable protected D-glucosamine derivatives possessing the *N*-dithiasuccinoyl (*N*-Dts) protecting group of the amino function have been applied in a new alternative route to 1,2-*trans*-glycosylation. The *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-dithiasuccinoylamino-β-D-glucopyranosyl) trichloroacetimidate **7** has been used to prepare corresponding β-glycosides in good yields promoted by Lewis acids. The *N*-dithiasuccinoyl protecting group was easy to remove by thiolysis using 2-sulfanylethanol or dithiothreitol or reductively using sodium boranuide, thus affording, after *N*-acetylation, the corresponding *N*-acetyl-β-D-glucosamine glycosides. It has been demonstrated that it is possible to reduce the Dts group in the presence of an azido group selectively by sodium boranuide or the Dts- and the azido group simultaneously by dithiothreitol, using diisopropylethylamine as a catalyst. The syntheses of the building blocks, N^{α}-Fmoc-Ser(Ac_3-β-D-GlcNDts)-OPfp **10** and N^{α}-Fmoc-Thr(Ac_3-β-D-GlcNDts)-OPfp **11**, suitable for the solid-phase glycopeptide synthesis of β-O linked GlcNAc bearing glycopeptides are described. Furthermore, the preparation of the N^{α}-Fmoc-Asn[Ac_3-β-D-GlcNAc-(1→4)-Ac_2-β-D-GlcNAc]-OPfp building block **19**, suitable for the solid-phase synthesis of *N*-glycopeptides, and the synthesis of the β-D-GlcNAc(1→6)-D-GalNAc disaccharide **27**, a mucin core structure, demonstrate the use of *N*-dithiasuccinoyl protection in oligosaccharide synthesis.

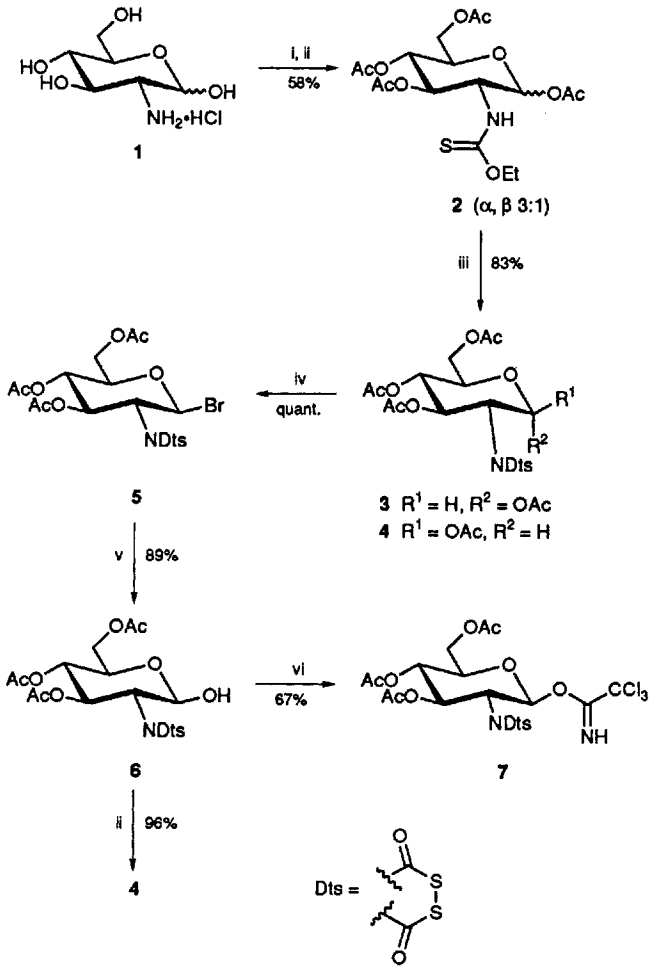
It is at present well established that the surface carbohydrates on glycoproteins and cell membranes are targets for biological recognition phenomena.[1] Glycoproteins are essential for the development and differentiation of complex organisms and are involved in inter- and intra-cellular interactions. In recent years the occurrence of *O*-GlcNAc residues β-*O*-glycosidically linked to serine and threonine has been reported[1,2] as a novel form of post-translational modification of intracellular proteins. The *O*-linked GlcNAc moieties are highly abundant on a number of intracellular proteins predominantly localized within the nucleo- and cyto-plasmatic compartments of eukaryotic cells.[3,4] *O*-GlcNAc-bearing proteins are furthermore found in a wide range of nuclear oncogens, chromatin proteins, nuclear pore proteins, cytoskeletal proteins and viral proteins, including transcriptional regulatory factors, kinases and tyrosine phosphatases. Post-translational *O*-GlcNAc modification of intracellular proteins may be crucial in blocking site-specific phosphorylation.[5–7] Studies have shown a reciprocal, so called 'yin-yang', relationship of *O*-GlcNAc and phosphorylation.[8] For elaborate studies of the structural and functional significance of these particular types of post-translational modifications the chemical synthesis of *O*-linked GlcNAc glycopeptides is essential. The currently most versatile approach to the synthesis of *N*- and *O*-glycopeptides is the stepwise solid-phase synthesis using glycosylated fluoren-9-ylmethoxycarbonyl (Fmoc) amino acid derivatives as building blocks.[9] An advantage of this strategy is the potential use of the protected and glycosylated amino acid building blocks for multiple-column syntheses of a wide range of glycopeptides.[10] Owing to the sensitivity of the serine and threonine *O*-glycosidic bond to both strong acids and bases only a limited number of orthogonal protecting groups for the sugar amino function may be utilized. The 1,2-*trans*-glycosylation of amino sugars requires glycosyl donors containing participating protective groups in the 2-position. Syntheses of 2-acetamidoglucopyranosyl amino acids have been performed by using the oxazoline,[11] the phthalimido,[11] the *N*-allyloxycarbonyl (Aloc)[12,13] and *N*-trichloroethoxycarbonyl (Teoc)[13,14] glucosamine derivatives as glycosyl donors. In the synthesis of N^{α}-Fmoc-Ser-(Ac_3-β-D-GlcNAc)-OPfp and N^{α}-Fmoc-Thr-(Ac_3-β-D-GlcNAc)-OPfp building blocks special problems occur depending on the nature of the 2-amino-protecting group of the corresponding glycosyl donors. The oxazoline procedure and more recent developments require high temperature and strongly acidic conditions and give good yields only with reactive acceptors.[11] Furthermore these glycosylation conditions are incompatible with the temperature-sensitive Pfp-esters. The use of the phthalimido group gives good yields and a high degree of stereoselectivity with most aglycones. Despite some recent improvements in the cleavage of the phthalimido group,[15,16] the deprotection still requires strongly basic conditions at elevated temperatures and incomplete deprotection is often observed. That means this sequence of reactions is not completely compatible with the alkali-labile character of the *O*-glycopeptides. Recent approaches are based on the use of the Aloc[12] and Teoc[14] amino-protecting groups. The 2-*N*-acyl protection leads to less reactive and stereoselective intermediates upon acidic activation. Furthermore both *N*-protective groups have to be chemoselectively removed and N-acetylated before incorporation as building blocks into the solid-phase glycopeptide synthesis. To be more flexible during the scheme of sequential solid-phase glycopeptide synthesis, an orthogonal amino-protecting group, which is easily removable during the solid-phase synthesis, is highly desirable. The dithiasuccinoyl (Dts) function, originally developed by Barany and Merrifield,[17] was conceived to satisfy the requirements of an orthogonally removable *N*-amino-protecting group of D-glucosamine derivatives. Although the application of *N*-Dts-protected amino acids in peptide syntheses have been described previously, this class of *N*-protection has not yet been investigated for amino sugars. The *N*-Dts protecting group is compatible with the Fmoc/Pfp strategy for the effective synthesis of glycopeptides and is rapidly and specifically removable under mild conditions by thiolysis during solid-phase synthesis. Furthermore the *N*-Dts

protection offers complete protection of the nitrogen atom, which avoids the undesirable formation of oxazoline by-products during the glycosylation reaction.

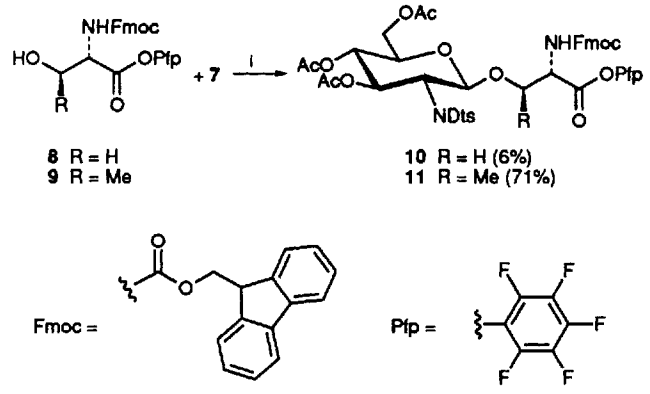
In the present paper the efficient use of *O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-dithiasuccinoylamino-β-D-glucopyranosyl) trichloroacetimidate **7** in glycosylation reactions as an important and reliable method for the preparation of 2-amino-2-deoxy-β-D-glucopyranosides is introduced.[18] The imidate **7** was successfully employed in the syntheses of the two new building blocks **10** and **11** and in the synthesis of the chitobiosyl-*N*-asparagine N^{α}-Fmoc-Asn-[Ac_3-β-D-GlcNAc-(1→4)-β-D-GlcNAc]-OPfp building block **19** to be used in preparation of β-GlcNAc-Ser/Thr-containing glycopeptides and in the synthesis of *N*-glycopeptides, respectively. Finally the disaccharide β-D-GlcNAc-(1→6)-D-GalNAc **27**, a mucin core structure, was synthesized by glycosylation of the 2-azidogalactose acceptor **24** with imidate **7** under trimethylsilyl trifluoromethanesulfonate (TMSOTf) catalysis and subsequent simultaneous reduction of the azido and the Dts group with dithiothreitol in the presence of diisopropylethylamine.

Results and Discussion

The original preparation of *N*-Dts derivatives of amino acids utilized ethoxythiocarbonyl derivatives of amino acids, which were treated in anhydrous solutions with chlorocarbonylsulfenyl chloride to form the desired heterocycle (*N*-Dts) with loss of ethyl chloride and hydrogen chloride.[17] Owing to the formation of HCl the presence of tertiary amines is favourable but not necessary. For the syntheses of the *N*-Dts derivatives **3** and **4** we followed an analogous reaction scheme. The ethoxythiocarbonyl derivative **2** was obtained by treatment of D-glucosamine **1** with *S*-carboxymethyl *O*-ethyl dithiocarbonate in methanol and subsequently peracetylation in acetic anhydride and pyridine. Compound **2** was obtained in an overall yield of 58% as a mixture of the two anomers in the ratio α:β = 3:1. In the next step chlorocarbonylsulfenyl chloride reacted in anhydrous dichloromethane at 0 °C instantaneously with compound **2** to afford an anomeric mixture of the *N*-Dts derivatives **3** and **4** in 83% yield. Ring closure to the 1,2,4-dithiazolidine-3,5-dione heterocyclic system in compounds **3** and **4** proceeded without formation of any by-products. Reaction of a mixture of compounds **3** and **4** with hydrogen bromide (33%) in acetic acid led to exclusive formation of the β-bromide **5** ($J_{1,2}$ 7.8 Hz at δ 6.37). It was not possible to purify the bromide **5** by silica gel chromatography due to its instability. The bromide **5** was then hydrolysed to hemiacetal **6** in acetone–water (3:1) in 89% yield. Only the β-anomer could be detected by ^{1}H NMR spectroscopy. The 1-H proton resonates at δ 5.67 with a coupling constant $J_{1,2}$ 8.1 Hz. At δ 4.42 ppm the corresponding signal for the 2-H proton was observed as a doublet of doublets. The β-anomer **6** is most likely stabilized by the presence of the *N*-Dts group at C-2. The reaction of hemiacetal **6** with trichloroacetonitrile in the presence of anhydrous potassium carbonate led to the imidate **7** in 67% (Scheme 1). The reaction led exclusively to the formation of the β-anomer; the α-imidate could be detected neither by TLC nor by ^{1}H NMR spectroscopy. The imidate **7** was investigated as glycosyl donor in different glycosylation reactions. Reaction of imidate **7** with the serine and threonine Pfp esters **8** and **9** in the presence of silver trifluoromethanesulfonate[19] without addition of neutralizing reagents gave stereoselectively the β-glycosides **10** and **11** in 64 and 71% yield, respectively (Scheme 2), after silica gel purification. Long reaction times (12–18 h) were required for completion of the glycosylation when using AgOTf as a promoter. These results emphasize that AgOTf can serve as an efficient promotor in glycosylation reactions with imidates. The application of the building blocks **10** and **11** for



Scheme 1 *Reagents:* i, $EtOC(S)SCH_2CO_2H$, MeOH; ii, Ac_2O, pyridine; iii, $ClSC(O)Cl$, CH_2Cl_2; iv, 33% HBr, HOAc; v, aq. acetone; vi, CCl_3CN, K_2CO_3, CH_2Cl_2

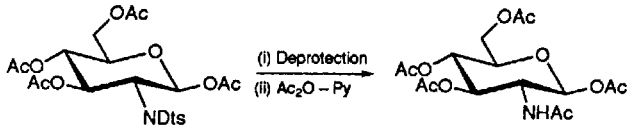


Scheme 2 *Reagents and conditions:* i, AgOTf, CH_2Cl_2, room temp.

glycopeptide synthesis of *O*-GlcNAc bearing glycopeptides is currently being submitted for publication.[20]

Effective deprotection procedures are essential for the broad application of *N*-Dts derivatives of amino sugars in oligosaccharide and glycopeptide synthesis. Therefore suitable reductive deprotection conditions with compound **4** were studied. Such investigations are a prerequisite for optimizing the conditions of oligosaccharide and solid-phase glycopeptide syntheses. The Dts amino-protecting group can be removed by thiols through an open-chain carbamoyl disulfide intermediate, which reacts further to give the free amino group.[17,21] Two

Table 1 Results of the reductive deprotection of the *N*-Dts compound and subsequent formation of the *N*-acetyl derivative



Entry	Reagent (concentration)	Base [concentration (mmol dm^{-3})]	Solvent	Yield[a] (%)
1	β-Sulfanylethanol	*N*,*N*-Diisopropylethylamine (500)	CH_2Cl_2	87
2	DTT		Pyridine	93
3	DTT (0.3 mol dm^{-3})	*N*,*N*-Diisopropylethylamine (0.1)	CH_2Cl_2	98
4	$NaBH_4$ (2 mol equiv.)		CH_2Cl_2–MeOH (1 : 1)	94
5	DTT (0.2 mol equiv.)	*N*-Ethylmorpholine (500)	MeCN	83
6	β-Sulfanylethanol (0.2 mol equiv.)	*N*,*N*-Diisopropylethylamine (500)	DMF	27
7	DTT (0.3 mol equiv.)	*N*,*N*-Diisopropylethylamine (0.1)	DMF	37

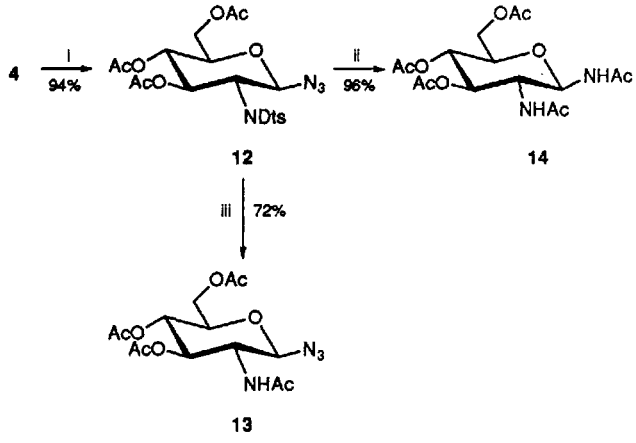
[a] The yields were determined after silica gel chromatography.

mole equivalents of thiol are oxidized to the disulfide. The reaction is driven by loss of two mole equivalents of gaseous carbonyl sulfide. Reducing agents other than thiols can also be effective for reductive deprotection of *N*-Dts groups.[22] In the following study the *N*-Dts derivative **4** was used to determine effective conditions for rapid thiolytic deprotection of the Dts group and subsequent N-acetylation (Table 1). Suitable conditions for quantitative removal of the Dts amino-protecting group include a wide range of reductive agents, *e.g.* (i) β-sulfanylethanol (0.2 mol dm^{-3})–diisopropylethylamine (0.5 mol dm^{-3}) in dichloromethane; (ii) dithiothreitol (DTT) (0.3 mol dm^{-3}) in neat pyridine; (iii) DTT (0.3 mol dm^{-3})–diisopropylethylamine (0.1 mmol) in dichloromethane; (iv) DTT (0.2 mol dm^{-3})–*N*-ethylmorpholine (0.5 mol dm^{-3}) in acetonitrile; and (v) sodium boranuide in dichloromethane–methanol. However, the choice of solvent was very important. In dimethylformamide (DMF) as solvent the rate of deprotection was very slow, and many side products were detected. By careful choice of thiol, base and solvent it has been possible to optimize the rate of reductive deprotection.

Furthermore, conditions for deprotection of the Dts group in the presence of azides were also investigated. To study the reduction of the Dts group in the presence of azido groups the model compound **12** was synthesized by tin(IV) chloride-catalysed reaction of β-acetate **4** with trimethylsilyl azide. The β-glycosyl azide **12** was obtained in 94% yield. It could be demonstrated that it is possible to reduce both the Dts and the azido group simultaneously, utilizing DTT as reducing reagent and diisopropylethylamine as a catalyst for the reaction. After *N*-acetylation the corresponding 1,2-bisacetamido compound **14** could be obtained in 96% yield (Scheme 3).

It has been reported that sodium boranuide in methanol does not reduce azides, but does reduce disulfides to thiols. By use of sodium boranuide in dichloromethane–methanol (1 : 1) it was possible to reduce selectively the *N*-Dts group of substrate **12** without effecting the azido group. After N-acetylation the azide **13** was obtained in 72% yield.

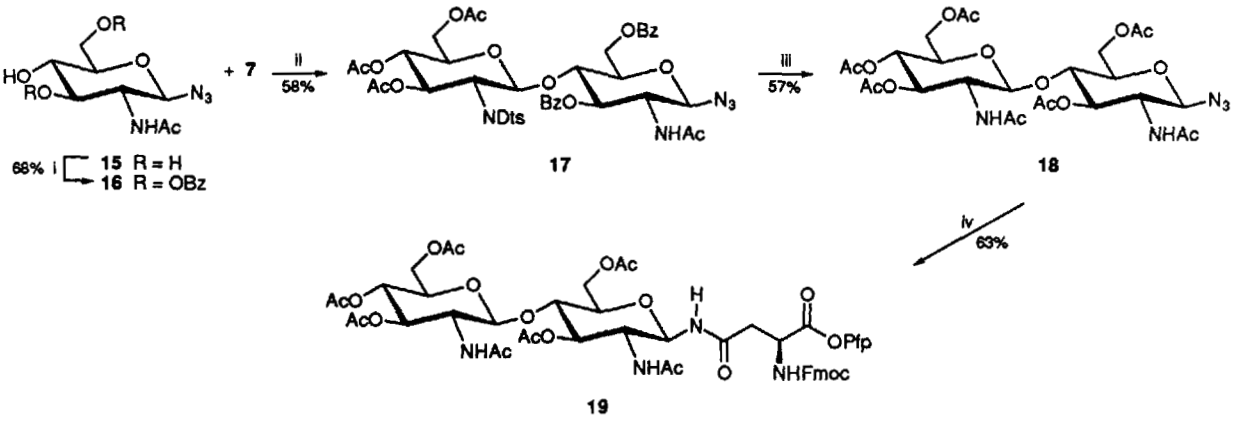
In the following syntheses of disaccharides the different *N*-Dts deprotection methods were successfully employed. First the chitobiosyl-*N*-asparagine building block **19** was synthesized by use of imidate **7** as glycosyl donor. The acceptor compound **16** was obtained by regioselective benzoylation of the previously described triol **15**[23] with benzoyl chloride in pyridine–dichloromethane (1 : 1) at −40 °C. This procedure afforded the GlcNAc-derivative **16** with a free hydroxy group in the 4-position in 68% yield. Reaction of acceptor **16** with imidate **7** under TMSOTf catalysis gave stereoselectively the β-linked disaccharide **17** in 58% yield. The conversion of *N*-Dts **17** into the acetamido compound **18** was performed by using sodium



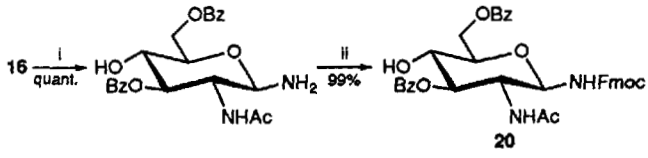
Scheme 3 *Reagents:* i, $TMSN_3$, $SnCl_4$, CH_2Cl_2; ii, (a) DTT, CH_2Cl_2; (b) Ac_2O, pyridine; iii, (a) $NaBH_4$, CH_2Cl_2, MeOH (1 : 1); (b) Ac_2O, pyridine

boranuide in dichloromethane–methanol followed by N-acetylation. The sodium boranuide efficiently reduced the Dts group without effecting the azide group. However, owing to the basic conditions some loss of acyl protecting groups was observed. Complete O-deacylation and subsequent peracetylation led to the acetylated chitobiosyl azide derivative **18** in 57% overall yield. The azido function was reduced with hydrogen on palladium/charcoal (5%) to the peracetylated chitobiosylamine and this was then coupled to the Fmoc-Asp(Cl)-OPfp[24] in the presence of *N*-ethylmorpholine at 0 °C to provide the chitobiosyl building block **19** (Scheme 4). After filtration through silica gel the desired asparagine building block **19**, containing a chitobiose moiety, was obtained in 63% yield.

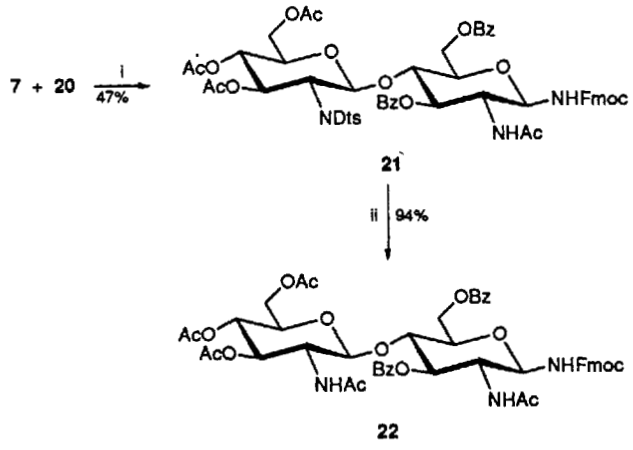
The preferred building blocks for the solid-phase glycopeptide syntheses are the N^{α}-Fmoc-protected glycosylated amino acids activated as their pentafluorophenyl esters. For a successful solid phase glycopeptide synthesis it is necessary that the deprotection of the *N*-Dts group does not effect the N^{α}-Fmoc group. Therefore the disaccharide **21** with the N^{α}-Fmoc protecting group was synthesized as a model compound and suitable conditions for reduction of the *N*-Dts group in the presence of the Fmoc group were investigated. The glycosyl azide **16** with a free hydroxy group in the 4-position was transformed into the glycosylamine by hydrogenation on palladium/charcoal (5%), the amino group was then protected with Fmoc and subsequently used as acceptor **20** in a glycosylation reaction with imidate **7** (see Scheme 5). The glycosylation reaction of compound **20** with imidate **7** was performed at room temperature with TMSOTf as catalyst. The



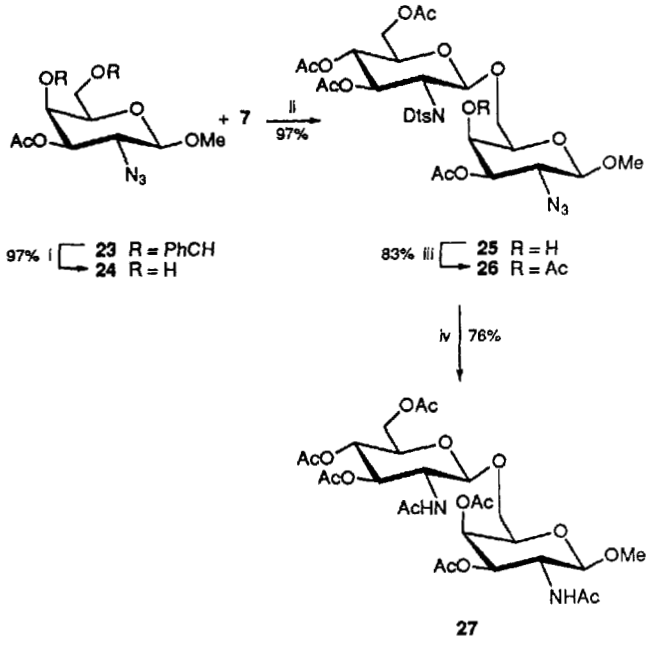
Scheme 4 *Reagents:* i, BzCl, pyridine, CH_2Cl_2; ii, TMSOTf, CH_2Cl_2; iii, (a) $NaBH_4$, CH_2Cl_2–MeOH; (b) Ac_2O, pyridine; iv, (a) H_2, Pd/C (10%); (b) Fmoc-Asp(Cl)-OPfp, *N*-ethylmorpholine, THF



Scheme 5 *Reagents:* i, H_2, Pd/C; ii, Fmoc-OSu, pyridine



Scheme 6 *Reagents:* i, TMSOTf, CH_2Cl_2; ii, (a) DTT, diisopropylethylamine, CH_2Cl_2; (b) Ac_2O, pyridine



Scheme 7 *Reagents and conditions:* i, 80% HOAc, 70 °C; ii, TMSOTf, CH_2Cl_2; iii, Ac_2O, pyridine; iv, DTT, diisopropylethylamine, CH_2Cl_2, MeOH; then Ac_2O, pyridine

corresponding disaccharide **21** was obtained in 47% yield. For the thiolysis of the Dts group we used DTT in the presence of diisopropylethylamine. Under these conditions the chitobiosyl precursor **22** was synthesized in 94% yield without any interference with the Fmoc group (Scheme 6). This result demonstrates that the *N*-Dts group is a valuable orthogonal protecting group in glycopeptide synthesis.

The Dts protection was also used in the synthesis of the peracetylated β-D-GlcNAc-(1→6)-D-GalNAc disaccharide **27**, which represents a moiety in the mucin core structure. The 4,6-*O*-benzylidene group in compound **23** was removed by treatment with aq. acetic acid (80%) to give the 4,6-diol **24** in 97% yield. Regioselective glycosylation of the 6-position was achieved in 97% yield by reaction of diol **24** with imidate **7** in the presence of TMSOTf at −30 °C. The regio- and stereo-selective formation of the β-(1→6) linkage in **24** was confirmed by ^1H NMR spectroscopy. The 1-H proton appears at δ 5.43 with a coupling constant $J_{1',2'} = 8.3$ Hz. After acetylation of the 4-OH group disaccharide compound **26** was converted into the peracetylated methyl β-D-GlcNAc-(1→6)-D-GalNAc glycoside **27** in one step by simultaneous reduction of the Dts and azido groups with DTT in dichloromethane in the presence of diisopropylethylamine (see Scheme 7). The overall yield after N-acetylation was 76%. The simultaneous reduction of Dts and azido groups with DTT may be a general approach to overcome the problems of azide reduction frequently observed during solid-phase glycopeptide synthesis. Further studies of the application of the dithiothreitol reduction in solid-phase glycopeptide synthesis are currently under investigation.

Conclusions.—The new strategy for the synthesis of β-D-glycosides of glucosamine complements the currently used oxazoline, phthalimido and *N*-alkoxycarbonyl procedures. The starting materials are easy to prepare and to handle. The β-glycosides are obtained stereoselectively and in good yields. The *N*-dithiasuccinoyl approach was easy to use under the classical glycosyl-donor activation with Lewis acids, the glycosylation occurred under mild conditions and was compatible with hydroxy-group protecting groups stable in acidic media. Furthermore, the cleavage of the *N*-dithiasuccinoyl group is perfectly selective and compatible with most *O*- and *N*-protecting groups, which represents an advantage over the previously reported methods applied in the field of 1,2-*trans*-glycosylation with 2-amino-2-deoxysugar derivatives. The problem of instability of the *N*-Dts group to nucleophiles can

be overcome in most situations by proper choice of the reaction conditions.

Experimental

General Procedures.—TLC was performed on Merck Silica Gel 60 F_{254} alumina sheets with detection by charring with sulfuric acid and by UV light when applicable. Vacuum liquid chromatography (VLC) was performed on Merck Silica Gel 60 H. All solvents were purchased from Labscan Ltd. (Dublin, Ireland). Dichloromethane was distilled from P_4O_{10} and was stored over molecular sieves 3 Å under argon in sealed vessels. Light petroleum was the 60–80 °C fraction. Concentrations were performed under reduced pressure at temperatures ≤40 °C. *O-tert*-Butyl N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-serine pentafluorophenyl ester and *O-tert*-butyl-N^{α}-(fluoren-9-ylmethoxycarbonyl)-L-threonine pentafluorophenyl ester were purchased from Bachem. Chlorocarbonylsulfenyl chloride (98%) and dithiothreitol (*threo*-1,4-disulfanylbutane-2,3-diol) (DTT) (99%) was purchased from Aldrich. Round-bottomed flasks for glycosylation reactions were either flame-dried or stored at 120 °C for 24 h prior to use. All reactions with silver triflate were carried out in the dark. M.p.s were measured on a Buchi melting point apparatus and are uncorrected. Microanalysis was generously carried out at Leo Pharmaceutical Products (Ballerup, Denmark). Optical rotations were recorded on a Perkin-Elmer 241 polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H NMR spectroscopy was performed on a Bruker AM 500 operating at 500.135 MHz. ^{13}C NMR spectra were recorded on a Bruker AM 500 operating at 125.76 MHz. Unless otherwise indicated all the NMR experiments were performed at 300 K in $CDCl_3$. Chemical shifts are given in ppm and referenced to internal $SiMe_4$ (0 ppm). Coupling constants are given in Hertz (±0.3 Hz). For all compounds the assignment of the ^{1}H NMR spectra was based on 2D proton–proton shift-correlation spectra. The assignment of ^{13}C NMR spectra was based on carbon–proton shift correlation spectra. The assigned ^{1}H NMR data are given in Tables 2 and 3 and the corresponding ^{13}C NMR data in Tables 4 and 5, respectively. ESMS spectra were recorded on a VG-Quatro instrument from Fisons.

Synthesis of S-*Carboxymethyl* O-*ethyl Xanthate.*—To a stirred and cooled solution of sodium hydroxide (13.6 g, 0.34 mol) in water (150 cm^3) was added chloroacetic acid (32.1 g, 0.34 mol) in small portions. This solution was then added to aq. potassium ethyl xanthate (54.5 g, 0.34 mol in 200 cm^3). After being stirred for a couple of minutes, the mixture was kept at room temperature overnight. The resulting solution of *S*-carboxymethyl *O*-ethyl xanthate was then acidified with conc. hydrochloric acid to obtain *S*-carboxymethyl *O*-ethyl xanthate in crystalline form (35.4 g, 57.9%), δ_H 1.49 (3 H, t, Me), 4.02 (2 H, s, CH_2CO_2H), 4.73 (2 H, q, CH_2) and 9.3 (1 H, br s, CO_2H).

1,3,4,6-*Tetra*-O-*acetyl*-2-*deoxy*-2-(*ethoxythiocarbonyl-amino*)-α,β-D-*glucopyranose* **2**.—D-Glucosamine hydrochloride **1** (10 g, 46.6 mmol) was added to a solution of sodium methoxide [from sodium (1.1 g, 48 mmol)] in methanol (50 cm^3) and this mixture was shaken at room temperature for 10 min. After filtration from precipitated sodium chloride, the filtrate was treated with *S*-carboxymethyl *O*-ethyl dithiocarbonate (8.36 g, 46.4 mmol). The mixture was kept at pH 10 (pH paper) by addition of supplementary 0.5 mol dm^{-3} sodium methoxide and was stirred at room temperature for 3 days; progress of the reaction was followed by TLC [chloroform–methanol–water (5:4:1)]. The mixture was then concentrated to dryness. After acetylation with acetic anhydride (25 cm^3) and pyridine (50 cm^3), the reaction mixture was concentrated to dryness, diluted with chloroform, and poured onto ice. The organic layer was washed successively with cold water, saturated aq. sodium hydrogen carbonate and water. Drying and concentration were followed by purification on silica gel with toluene–ethyl acetate (4:1). This afforded pure *compound* **2** (11.8 g, 58.4%) in the α:β ratio 2.66:1 (estimated by comparison of the integrals of the methyl signals within the ^{1}H NMR spectra). ^{1}H data are presented in Table 2 (Found: C, 46.45; H, 5.8; N, 3.2. $C_{17}H_{25}NO_{10}S$ requires C, 46.89; H, 5.79; N, 3.21%; M, 435.45).

1,3,4,6-*Tetra*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-α-D-*glucopyranose* **3** *and* 1,3,4,6-*Tetra*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranose* **4**.—Chlorocarbonylsulfenyl chloride (1 cm^3, 11.8 mmol) was added at 0 °C to a solution of compound **2** (5 g, 11.6 mmol) in dry dichloromethane (50 cm^3). Development of the reaction was monitored by TLC [toluene–ethyl acetate (2:1)]. After 30 min the reaction mixture was concentrated, redissolved in chloroform (100 cm^3), washed successively with 1 mol dm^{-3} hydrochloric acid (3 × 100 cm^3) and water (2 × 100 cm^3). The organic layer was then dried over magnesium sulfate and concentrated to provide the crude product. Purification was achieved by VLC [toluene–ethyl acetate (7:1)] and yielded pure title *compounds* **3** (2.46 g, 45.6%) and **4** (1.58 g, 29%) and a mixed fraction **3** + **4** (450 mg, 8.3%). ^{1}H and ^{13}C NMR data are presented in Tables 2 and 4, respectively (Found for compound **4**: C, 41.5; H, 5.3; N, 2.9. $C_{16}H_{19}NO_{11}S_2$ requires C, 41.29; H, 4.11; N, 3.01%; M, 465.46).

1,3,4,6-*Tetra*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranose* **4**.—Compound **6** (see later) (400 mg, 944.6 μmol) was dissolved in acetic anhydride (2.5 cm^3) and pyridine (5 cm^3). After 5 h at room temperature the reaction mixture was concentrated and several times coevaporated with toluene. Purification by VLC [toluene–ethyl acetate (7:1)] yielded the title compound **4** (420 mg, 95.5%), $[\alpha]_D^{25}$ +28.7 (*c* 1, $CHCl_3$). ^{1}H and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl Bromide* **5**.—The above mentioned derivatives **3** and **4** as a mixture of both anomers (4 g, 8.59 mmol) were treated at 0 °C with 33% hydrogen bromide in acetic acid (50 cm^3) for 3 h. The mixture was then poured into cold water and the aqueous layer was extracted with chloroform (3 × 100 cm^3). The organic layer was then neutralized with aq. sodium hydrogen carbonate, washed once with cold water and dried over magnesium sulfate. After concentration to dryness compound **5** was recovered as a yellow foam (4.1 g). Owing to its instability the bromide **5** was immediately used for the next reaction. ^{1}H NMR data are presented in Table 2.

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranose* **6**.—The bromide **5** (4.1 g, 8.4 mmol) was dissolved in acetone (60 cm^3)–water (20 cm^3) and the solution was stirred. After 3 h the hydrolysis of the bromide was complete. After concentration, and coevaporation with toluene (3 × 50 cm^3), compound **6** was obtained as a foam. The foam was dissolved in chloroform (100 cm^3) and the solution was washed 3 times with water and dried over magnesium sulfate. The organic solution was concentrated and then subjected to VLC [toluene–ethyl acetate (4:1)] to yield pure *compound* **6** (3.16 g, 88.5%). ^{1}H NMR data are presented in Table 2 (Found: C, 40.0; H, 4.2; N, 3.1. $C_{14}H_{17}NO_{10}S_2$ requires C, 39.71; H, 4.05; N, 3.31%; M, 423.42).

O-(3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*) *Trichloroacetimidate* **7**.—To a mixture of

Table 2 Selected ^{1}H NMR chemical shifts (ppm) for monosaccharide derivatives **2**–**7**, **10**–**14**, **16**, **20** and **24** measured at 500 MHz on solutions in $CDCl_3$ at 300 K. Coupling constants (Hz) in parentheses

	2	**3**	**4**	**5**	**6**	**7**	**10**	**11**	**12**	**13**	**14**	**16**	**20**	**24**
1-H	6.39 (3.6)	6.28 (3.2)	6.49 (8.7)	6.37 (7.8)	5.67 (8.1)	6.60 (8.4)	5.44 (8.3)	5.43 (8.3)	5.67 (9.0)	4.80 (9.4)	5.09 (8.3)	4.86 (9.3)	5.03 (9.0)	4.31 (8.0)
2-H	4.93 (10.3)	4.47 (11.5)	4.58 (9.2)	4.73 (10.0)	4.42 (9.8)	4.75 (9.3)	4.42 (10.8)	4.43 (10.6)	4.34 (9.0)	3.95 (10.0)	4.16 (10.2)	4.18 (10.6)	4.26 (9.3)	3.82 (10.7)
3-H	5.34 (9.8)	6.52 (9.5)	5.87 (9.3)	5.75 (9.8)	5.82 (9.7)	5.92 (9.1)	5.77 (9.0)	5.82 (9.2)	5.79 (10.3)	5.28 (9.2)	5.15 (9.2)	5.54 (9.0)	5.32 (8.5)	4.74 (3.0)
4-H	5.28 (9.8)	5.15 (9.6)	5.23 (10.0)	5.27 (10.1)	5.21 (10.2)	5.29 (9.8)	5.19 (10.0)	5.19 (9.7)	5.21 (10.1)	5.14 (9.2)	5.08 (10.2)	3.88 (9.9)	3.83 —	4.18 (0.2)
5-H	4.04 (4.1, 2.1)	4.30 (3.8, 1.7)	3.97 (4.5, 1.5)	3.81 (4.4, 2.1)	3.92 (3.9, 2.0)	4.03 (4.4, 2.2)	3.81 (2.3, 4.4)	3.83 (2.1, 4.3)	3.93 (4.4, 2.1)	3.83 (5.0, 2.0)	3.80 (4.3, 2.1)	3.95 (4.5, 2.15)	3.87 —	3.57 (4.6, 4.2)
6-H	4.31 (12.5)	4.37 (12.5)	4.36 (12.5)	4.32 (12.7)	4.31 (12.5)	4.40 (12.0)	4.28 (12.3)	4.26 (12.3)	4.33 (12.5)	4.32 (12.4)	4.33 (12.5)	4.77 (12.3)	3.83 (12.0)	3.97 (—)
6-H′	4.09	4.14	4.15	4.19	4.21	4.26	4.18	4.11	4.21	4.21	4.12	4.68	4.55	3.96
Ac	2.22, 2.13, 2.09, 2.08	2.15, 2.13, 2.07, 2.02	2.14, 2.13, 2.08, 2.04	2.17, 2.08, 2.02	2.17, 2.08, 2.03	2.16, 2.10, 2.05	2.14, 2.10, 2.06	2.08, 2.07, 2.05	2.15, 2.08, 2.03	2.06, 2.04, 2.03, 1.99	2.14, 2.12, 2.10, 2.02, 1.99	1.91	1.91	2.21
Other	6.62 (NH) (8.5) 4.47 (OCH_2) 1.32 (Me)				3.83 (OH) (4.8)	8.82 (NH)	4.88 (H^{α}) (4.0) 4.41 (H^{β}) (11.2) 4.06 (H^{β}) 5.58 (Fmoc) (7.8)	4.76 (H^{α}) (3.1) 4.57 (H^{β}) (6.29) 4.06 (H^{γ}) 5.58 (Fmoc) (8.8)		5.65 (NH) (9.0)	7.02 (NH^1) (8.3) 6.11 (NH^2) (8.0)	6.3 (NH^2) (9.3) 3.72 (OH)	6.45 (NH^2) (7.9) 3.52 (OH)	3.65 (OMe)
							Fmoc	Fmoc				Bz	Bz Fmoc	

Table 3 Selected ^{1}H NMR chemical shifts (ppm) and coupling constants (Hz, in parentheses) for disaccharide derivatives **17**, **18**, **21**, **22** and **25–27** measured at 500 MHz on solutions in $CDCl_3$ at 300 K

	17	**18**	**21**	**22**	**25**	**26**	**27**
1-H	4.88 (8.5)	4.60 (8.8)	4.97 (8.9)	4.93 (—)	4.23 (8.0)	4.28 (8.1)	4.31 (8.0)
2-H	3.43 (8.9)	4.08 (9.5)	4.26 (9.9)	4.33 (—)	3.74 (10.8)	3.64 (10.9)	3.67 (10.7)
3-H	5.35 (9.4)	5.14 (8.1)	5.48 (9.3)	5.21 (9.4)	4.72 (3.2)	4.81 (3.3)	4.79 (3.1)
4-H	3.79 (9.6)	3.82 (9.2)	4.27 (9.8)	4.06 (9.8)	4.02 (0.5)	5.29 (0.5)	5.38 (0.5)
5-H	3.92 (4.6, 1.9)	3.76 (2.4, 5.8)	4.23 (1.8, —)	3.98 (1.8, 2.5)	3.62 (6.8, 5.9)	3.73 (6.8, 5.7)	3.85 (—, —)
6-H[a]	4.82 (12.3)	4.42 (12.2)	4.75 (12.1)	4.70 (12.2)	4.01 (10.4)	3.78 (10.7)	3.87 (10.4)
6-H[b]	4.69	4.35	4.34	4.59	3.89	3.80	3.77
1′-H	6.75 (8.7)	4.61 (8.0)	5.65 (8.1)	4.89 (7.6)	5.43 (8.3)	5.40 (8.3)	4.92 (8.2)
2′-H	4.50 (10.1)	4.89 (10.4)	4.24 (9.8)	3.62 (9.8)	4.38 (10.6)	4.38 (10.6)	3.59 (10.5)
3′-H	5.85 (9.8)	5.23 (10.1)	5.58 (9.2)	5.51 (9.3)	5.75 (9.0)	5.71 (9.2)	5.44 (9.4)
4′-H	5.16 (9.8)	5.08 (10.2)	5.02 (9.8)	4.83 (9.6)	5.18 (10.1)	5.17 (9.5)	5.06 (9.7)
5′-H	4.83 (3.2, 1.9)	3.68 (4.4, 2.0)	3.25 (3.7, 2.1)	3.23 (3.6, 2.1)	3.82 (2.2, 4.7)	3.82 (1.9, 4.4)	3.74 (2.1, 4.7)
6′-H[a]	4.30 (12.6)	4.38 (12.0)	3.91 (12.5)	3.71 (12.5)	4.36 (12.3)	4.29 (12.4)	4.29 (12.4)
6′-H[b]	3.94	4.03	3.66	3.75	4.18	4.18	4.17
Ac	2.05, 2.03, 2.02, 1.97	2.03, 1.97, 1.93, 1.90, 1.89, 1.84, 1.79	2.03, 1.99, 1.96, 1.83	2.02, 1.98, 1.96, 1.89, 1.83	2.21, 2.16, 2.08, 2.03	2.16, 2.13, 2.06, 2.05, 2.01	2.17, 2.15, 2.12, 2.08, 2.06, 2.05, 1.99
Other		6.31 (NH) (8.8) 6.18 (NH) (9.3)	6.20 (NH) (7.7) 6.44 (NH) (8.3) Bz Fmoc	6.50 (NH) (7.4) 6.28 (NH) (7.6) 6.51 (NH) (7.6) Bz Fmoc	3.59 (OMe)	3.62 (OMe)	3.64 (OMe) Fmoc

Table 4 Selected ^{13}C NMR chemical shifts (ppm) for monosaccharide derivatives **3**, **4**, **7**, **10–12**, **14** and **24** measured at 125.77 MHz on solutions in $CDCl_3$ at 300 K

	3	**4**	**7**	**10**	**11**	**12**	**14**	**24**
C-1	89.82	88.76	92.56	97.15	94.54	84.36	80.34	103.46
C-2	59.15	59.73	59.77	60.51	60.81	60.20	53.42	73.62
C-3	66.01	69.31	69.20	69.29	69.21	69.20	67.63	73.46
C-4	69.36	68.10	68.25	68.59	68.69	68.20	72.94	67.86
C-5	61.27	72.63	72.82	72.16	72.00	73.99	73.47	62.86
C-6	70.37	61.32	61.36	61.56	61.59	61.52	61.71	60.11
C–*C*O	170.24, 169.66, 169.29 169.13	170.87, 170.32, 169.32, 169.13	170.6, 170.2, 169.8	170.59, 170.22, 169.30	170.6, 170.3, 169.3	170.57, 170.13, 169.22	172.00, 171.98, 170.87, 170.69, 169.26	171.32
Me	20.91, 20.67, 20.53 20.57	20.75, 20.69, 20.56, 20.37	20.72, 20.57, 20.42	20.59, 20.56, 20.39	21.45, 20.57, 20.43	20.67, 20.52, 20.32	23.35, 23.09, 20.72, 20.67, 20.57	20.97
S–CO	160.47, 162.67	164.3, 165.6	160.47	166.51, 165.94	166.32, 165.32	162.74, 160.68		
Other				54.29 (C^{α}) 68.90 (C^{β}) Fmoc Pfp	58.35 (C^{α}) 74.12 (C^{β}) 16.54(C^{γ}) Fmoc Pfp			57.45 (OMe)

compound **6** (1.1 g, 2.56 mmol) and trichloroacetonitrile (2.5 cm^3) in dry dichloromethane containing molecular sieves (3 Å) was added anhydrous potassium carbonate (1 g). The mixture was stirred overnight at room temperature and then was directly subjected to VLC [toluene–ethyl acetate (5:1)] to yield exclusively the β-*imidate* **7** (970 mg, 67%) (Found: C, 34.1; H,

Table 5 Selected ^{13}C NMR chemical shifts (ppm) for disaccharide derivatives **17**, **18**, **21**, **22** and **25–27** measured at 125.77 MHz on solutions in $CDCl_3$ at 300 K

	17	18	21	22	25	26	27
C-1	90.36	88.62	82.74	82.71	103.04	102.99	103.07
C-2	63.00	53.16	53.84	53.43	60.67	60.90	55.33
C-3	77.27	73.37	74.08	74.45	72.10	71.04	71.49
C-4	68.67	74.97	73.85	73.95	68.63	68.57	66.61
C-5	76.82	75.72	74.89	75.69	72.81	71.99	71.72
C-6	67.45	62.08	62.55	62.57	67.84	67.51	66.16
C-1′	88.75	101.29	96.25	100.40	97.14	96.70	99.77
C-2′	60.10	54.71	61.09	48.32	60.78	60.68	60.91
C-3′	69.55	73.14	69.12	68.06	69.39	69.39	71.38
C-4′	68.08	68.00	67.89	67.83	65.68	66.98	68.55
C-5′	72.01	71.93	71.84	71.80	73.62	72.23	71.87
C-6′	61.54	61.72	60.89	61.39	61.44	61.58	61.94
C–*C*O	172.31	171.01	172.04,	172.62,	170.84,	170.58,	170.63,
	172.29	170.84	170.42,	170.10,	170.22,	170.23,	170.53,
	169.33	170.54	170.09,	170.08,	169.97,	169.89,	170.12,
	167.07	170.47	169.05	169.32	169.26	169.65,	169.83,
	166.50	169.34	168.96			169.26	169.45,
							167.31,
							166.51
Me	20.62,	23.14,	23.09,	23.16,	20.96,	20.71,	20.29,
	20.56,	20.92,	20.67,	23.07,	20.70,	20.56,	20.73,
	20.39	20.57,	20.42,	20.67,	20.57,	20.37	20.61
		20.32,	20.24	20.49,	20.37	20.28	20.38
		20.18		20.24		20.18	20.29
S–CO	164.59		166.93,	165.98	167.89,	168.01,	
	162.14		165.83	166.38	166.59	166.49	
Other			Fmoc	Fmoc	57.09	57.38	57.40
					(OMe)	(OMe)	(OMe)
			Bz	Bz			
			Fmoc	Fmoc			

3.2; N, 4.75. $C_{16}H_{17}Cl_3N_2O_{10}S_2$ requires C, 33.85; H, 3.02; N, 4.93%; M, 567.81); $[\alpha]_D^{25}$ +35.3 (*c* 1.1, $CHCl_3$). ^1H NMR and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*)-L-*serine Pentafluorophenyl Ester* **10**.—*Method A*. Compound **8**[12] (500 mg, 1.007 mmol), the imidate **7** (637 mg, 1.107 mmol) and silver trifluoromethanesulfonate (302 mg, 1.007 mmol) were placed in a pre-dried flask. After evacuation at 0.1 mmHg the flask was filled with argon and dry dichloromethane (20 cm^3) was injected into the flask. The solution was stirred at room temperature for 12 h and then was directly subjected to VLC [ethyl acetate–light petroleum (2:3)] to yield the title compound **10** (620 mg, 63.8%).

Method B. Compound **8** (50 mg, 100 μmol), the imidate **7** (63.7 mg, 110 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask. After evacuation of 0.1 mmHg the flask was filled with argon. Dry dichloromethane (5 cm^3) was injected into the flask. The solution was cooled to −30 °C on solid CO_2 and a solution of trimethylsilyl trifluoromethanesulfonate in dry dichloromethane (100 mm^3 TMSOTf/5 cm^3 CH_2Cl_2) (100 mm^3) was injected. The reaction mixture was then allowed to warm to room temperature within 2 h. The mixture was then filtered through Celite and the filtrate was concentrated. VLC [ethyl acetate–light petroleum (2:3)] yielded the title compound **10** (47.6 mg, 48%).

Method C. Compound **8** (50 mg, 100 μmol), the imidate **7** (63.7 mg, 110 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask. After evacuation at 0.1 mmHg the flask was filled with argon. Dry dichloromethane (5 cm^3) was injected into the flask. The solution was cooled to 0 °C and a solution of boron trifluoride–diethyl ether in dry dichloromethane (100 mm^3 $BF_3{\cdot}Et_2O$/5 cm^3 CH_2Cl_2) (100 mm^3) was injected. The mixture was then allowed to warm to room temperature within 2 h. After filtration through Celite, the filtrate was concentrated and applied to VLC [ethyl acetate–light petroleum (2:3)]. This yielded pure β-*compound* **10** (27 mg, 27%), $[\alpha]_D^{22}$ −3.4 (*c* 0.5, $CHCl_3$) (Found: C, 51.1; H, 3.8; N, 3.0. $C_{38}H_{31}F_5N_2O_{14}S_2$ requires C, 50.78; H, 3.51; N, 3.12%; M, 898.79). ^1H and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

N$^{\alpha}$-(*Fluoren-9-ylmethoxycarbonyl*)-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*)-L-*threonine Pentafluorophenyl Ester* **11**.—*Method A*. Compound **9**[12] (500 mg, 979 μmol), the imidate **7** (618.3 mg, 1.08 mmol) and silver trifluoromethanesulfonate (294 mg, 1.08 mmol) were placed in a pre-dried flask, which was evacuated for several hours at 0.1 mmHg and filled with argon. Dry dichloromethane (30 cm^3) was injected into the flask. The mixture was stirred at room temperature overnight in the dark, filtered through Celite and then directly subjected to VLC [ethyl acetate–light petroleum (2:3)] to give exclusively the β-glycoside **11** (639 mg, 71%).

Method B. Compound **9** (50 mg, 97.9 μmol), the imidate **7** (61.8 mg, 108 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask, the flask was evacuated on an oil-pump and filled with argon and dry dichloromethane (3 cm^3) was added. The solution was cooled to −30 °C and a solution of trimethylsilyl trifluoromethanesulfonate in dry dichloromethane (TMSOTf/5 cm^3 CH_2Cl_2) (100 mm^3) was injected. The mixture was allowed to warm to room temperature within 2 h, filtered through Celite and concentrated. VLC [ethyl acetate–light petroleum (2:3)] yieled pure compound **11** (54 mg, 55%).

Method C. Compound **9** (50 mg, 97.9 μmol), β-imidate **7** (61.8 mg, 108 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask, the flask was evacuated on an oil-pump and filled with argon, dry dichloromethane (3 cm^3) was added and the mixture was cooled to 0 °C. Into this solution was then injected

boron trifluoride–diethyl ether (100 mm^3 $BF_3{\cdot}Et_2O$/5 cm^3 CH_2Cl_2) (100 mm^3). After 3 h the solution was filtered through Celite, concentrated and applied to VLC [ethyl acetate–light petroleum (2:3)]. This yielded the β-*glycoside* **11** (27 mg, 28%), $[\alpha]_D^{22}$ −25.2 (*c* 1, $CHCl_3$) (Found: C, 50.75; H, 3.9; N, 3.0. $C_{39}H_{33}F_5N_2O_{14}S_2$ requires C, 51.32; H, 3.64; N, 3.07%; M, 912.82). ^1H and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl Azide* **12**.—Into a solution of β-acetate **4** (420 mg, 902.3 μmol) in dry dichloromethane (10 cm^3) containing molecular sieves (3 Å) was first injected trimethylsilyl azide (169 mm^3, 1.29 mmol) and then tin(IV) chloride (63.5 mm^3, 537.5 μmol) under argon. The mixture was stirred at room temperature for 12 h and was then directly subjected to VLC [toluene–ethyl acetate (10:1)] to yield the β-*azide* **12** (380 mg, 94%), $[\alpha]_D^{22}$ −1.1 (*c* 1, $CHCl_3$) (Found: C, 37.1; H, 3.9; N, 11.8. $C_{14}H_{16}N_4O_9S_2$ requires C, 37.50; H, 3.60; N, 12.49%; M, 448.43). ^1H and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl Azide* **13**.—The β-azide **12** (50 mg, 111.5 μmol) was dissolved in dry dichloromethane (2 cm^3)–methanol (2 cm^3). To the reaction mixture was then added sodium boranuide (4.2 mg, 111.5 μmol). After 10 min, TLC [toluene–ethyl acetate (2:1)] showed complete disappearance of starting material **12**. The solution was then concentrated and the residue was dissolved in acetic anhydride (1 cm^3)–pyridine (2 cm^3). After being kept at room temperature for 2 h, the solution was concentrated, the residue was dissolved in chloroform (20 cm^3) and the solution was washed with water, dried over $MgSO_4$ and concentrated. The product was crystallized from ethyl acetate–light petroleum to give the title acetamide **13** (29.8 mg, 71.8%), m.p. 162–163 °C; $[\alpha]_D^{22}$ −42.6 (*c* 0.93, $CHCl_3$); δ_H 1.99 (3 H, s, NH*Ac*), 2.04 and 2.06 (9 H, 2 s, Ac), 3.83 (1 H, ddd, $J_{4,5}$ 9.2, $J_{5,6}$ 2.0, $J_{5,6}$ 5.0 5-H), 3.95 (1 H, ddd, $J_{1,2}$ 9.4, $J_{2,NH}$ 9.0, $J_{2,3}$ 10.0, 2-H), 4.21 (1 H, dd, $J_{6,6}$ 12.4, 6-H), 4.32 (1 H, dd, 6-H′), 4.8 (1 H, d, 1-H), 5.14 (1 H, $J_{3,4}$ 9.2, 4-H), 5.28 (1 H, dd, 3-H) and 5.65 (1 H, d, NH); δ_C 53.75 (C-2), 61.86 (C-6), 68.2 (C-4), 71.98 (C-3), 73.53 (C-5) and 88.1 (C-1).

1,2-*Bisacetamido*-3,4,6-*tri*-O-*acetyl*-1,2-*dideoxy*-β-D-*glucopyranose* **14**.—DTT (85 mg, 557.5 μmol) was added to a solution of the β-azido compound **12** (50 mg, 111.5 mmol) in dry dichloromethane (3 cm^3), diisopropylethylamine (0.005 cm^3) was injected and the mixture was stirred at room temperature for 30 min. The progress of the reaction was followed by TLC [chloroform–methanol (5:1)]. The mixture was concentrated under reduced pressure and the residue was dissolved in acetic anhydride (2 cm^3)–pyridine (4 cm^3). After 2 h at room temperature the N-acetylation mixture was concentrated under reduced pressure. The residue was purified by VLC [toluene–ethyl acetate (2:1)] to yield the *bis-amide* **14** (41.4 mg, 95.6%) (Found: C, 49.9; H, 6.0; N, 7.0. $C_{16}H_{24}N_2O_9$ requires C, 49.48; H, 6.23; N, 7.21%; M, 388.38). ^1H and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

2-*Acetamido*-3,6-*di*-O-*benzoyl*-2-*deoxy*-β-D-*glucopyranosyl Azide* **16**.—Benzoyl chloride (1.037 cm^3, 8.94 mmol) was added dropwise within 1 h to a solution of the triol **15**[23] (1 g, 4.06 mmol) in a mixture of dry dichloromethane (20 cm^3) and dry pyridine (20 cm^3) at −45 °C. The mixture was stirred at −45 °C for 3 h and was then allowed to warm to room temperature. After addition of methanol (1 cm^3) the solution was diluted with dichloromethane (100 cm^3) and the organic layer was washed successively with 5% hydrochloric acid, aq. $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated to dryness. VLC on silica gel with ethyl acetate–toluene (1:3) yielded the *title compound* **16** (1.25 g, 67.7%) (Found: C, 57.8; H, 5.0; N, 12.45. $C_{22}H_{22}N_4O_7$ requires C, 58.15; H, 4.88; N, 12.33%; M, 454.44). ^1H NMR data are presented in Table 2.

3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*-(1→4)-2-*acetamido*-3,6-*di*-O-*benzoyl*-2-*deoxy*-β-D-*glucopyranosyl Azide* **17**.—The imidate **7** (250 mg, 440 μmol), the acceptor **16** (200 mg, 440 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask. After evacuation with an oil-pump the flask was filled with argon, followed by dry dichloromethane (8 cm^3) by injection. After 30 min a solution of trimethylsilyl trifluoromethanesulfonate in dry dichloromethane (100 mm^3 TMSOTf/5 cm^3 CH_2Cl_2) (300 mm^3) was added. The solution was then filtered through Celite, concentrated, and subjected to silica gel. VLC [ethyl acetate–light petroleum (1:3 ⟶ 1:1)] gave pure *disaccharide* **17** (221 mg, 58.4%) (Found: C, 50.0; H, 4.45; N, 8.2. $C_{36}H_{37}N_5O_{16}S_2$ requires C, 50.20; H, 4.34; N, 8.15%; M, 859.85); $[\alpha]_D^{22}$ −36.8 (*c* 1.1, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*-(1→4)-2-*acetamido*-3,6-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl Azide* **18**.—Sodium boranuide (17.6 mg, 465.15 μmol) was added to a solution of compound **17** (200 mg, 232.5 μmol) in a dry mixture of dichloromethane (3 cm^3) and methanol (3 cm^3) at 0 °C. After 10 min, TLC [ethyl acetate–toluene (1:1)] showed complete disappearance of starting material. The solution was concentrated, the mixture was dissolved in methanol (5 cm^3) and the solution was adjusted to pH 10 (moist pH paper) by addition of sodium methoxide (1 mol dm^{-3}). After 6 h at room temperature the solution was neutralized with solid CO_2 and concentrated again. Peracetylation was achieved by dissolution of the residue in a mixture of acetic anhydride (4 cm^3) and pyridine (8 cm^3) while the temperature was kept at 6 °C for 6 h. Concentration under reduced pressure, dissolution in chloroform (25 cm^3), washing successively with 5% hydrochloric acid, aq. $NaHCO_3$ and water, drying over $MgSO_4$, and concentration yielded, after VLC [chloroform–methanol (100:1 ⟶ 50:1)], the *title compound* **18** (87 mg, 56.7%) (Found: C, 47.0; H, 5.4; N, 10.9. $C_{26}H_{37}N_5O_{15}$ requires C, 47.34; H, 5.65; N, 10.62%; M, 659.61); $[\alpha]_D^{22}$ −52.8 (*c* 0.6, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

N^{γ}-[2-*Acetamido*-4-O-(2-*acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*di*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*]-N^{α}-(*fluoren*-9-*ylmethoxycarbonyl*)-L-*asparagine Pentafluorophenyl Ester* **19**.—Compound **18** (50 mg, 75.8 μmol) was dissolved in ethyl acetate–methanol (3 cm^3; 1:1) and hydrogenated at atmospheric pressure in the presence of palladium on activated charcoal (Pd/C) (10 mg; 5%) for 2 h. The catalyst was filtered off and the solution was concentrated. The compound was homogeneous on TLC in chloroform–methanol (5:1), R_f 0.63; $[\alpha]_D^{22}$ −31.2 (*c* 1, $CHCl_3$).

The amine (45 mg, 71 μmol) was dissolved in tetrahydrofuran (THF) (10 cm^3) and *N*-ethylmorpholine (10 mm^3, 80 μmol) was added. The mixture was then added dropwise to a solution of N^{α}-Fmoc-Asp(Cl)-OPfp (43 mg, 80 μmol) in THF (1 cm^3) at 0 °C. *N*-Ethylmorpholine hydrochloride precipitated almost instantaneously and filtration of the reaction mixture through silica gel afforded the title compound **19** (50.8 mg, 63%). The resulting title compound **19** was pure according to ^1H and ^{13}C NMR spectroscopy and FAB-MS and was used without further purification for glycopeptide synthesis (Found: ESMS *m/z*, 1138.52 ($M + H^+$) and 1160.62 ($M + Na^+$). $C_{51}H_{53}F_5N_4O_{20}$ requires M, 1137.14).

2-*Acetamido*-3,6-*di*-O-*benzoyl*-2-*deoxy*-N-(*fluoren*-9-*yl*-*methoxycarbonyl*)-β-D-*glucopyranosylamine* **20**.—Azido glycoside **16** (106 mg, 233.2 μmol) was dissolved in methanol (3 cm^3) and the solution was stirred for 2 h under a flow of hydrogen in the presence of palladium on activated charcoal (10 mg; 5% Pd/C). Filtration of the catalyst, concentration of the solute and drying under reduced pressure on an oil-pump yielded the crude glycosylamine (99.4 mg), which was dissolved in pyridine (15 cm^3) without further purification. After addition of Fmoc-OSu (120 mg, 355.7 μmol) the mixture was stirred overnight at ambient temperature [TLC: ethyl acetate–toluene (1:1)], concentrated and co-concentrated with toluene. The residue was dissolved in dichloromethane (3 cm^3) and filtered through Celite. Purification by VLC on silica gel [ethyl acetate–toluene (2:3)] afforded the *title compound* **20** (150 mg, 98.9%), $[\alpha]_D^{22}$ −24 (*c* 1, $CHCl_3$) (Found: C, 68.6; H, 5.1; N, 4.2. $C_{37}H_{34}N_2O_9$ requires C, 68.30; H, 5.27; N, 4.31%; M, 650.69). ^1H data are presented in Table 2.

2-*Acetamido*-3,6-*di*-O-*benzoyl*-2-*deoxy*-N-(*fluoren*-9-*yl*-*methoxycarbonyl*)-4-O-(3,4,6-*tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*)-β-D-*glucopyranosylamine* **21**.—Imidate **7** (168 mg, 296 μmol), acceptor **20** (140 mg, 215 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask. After evacuation with an oil-pump the flask was filled with argon and dry dichloromethane (7 cm^3) was injected. The solution was stirred for 10 min at ambient temperature and then trimethylsilyl trifluoromethanesulfonate (300 mm^3) from a solution of trimethylsilyl trifluoromethanesulfonate in dry dichloromethane (100 mm^3 TMSOTf/5 cm^3 CH_2Cl_2) was injected into the flask. After 90 min, TLC [chloroform–methanol (20:1)] showed complete disappearance of the imidate **7**. The reaction mixture was diluted with dichloromethane (10 cm^3), filtered through Celite, concentrated, and purified by VLC [chloroform–methanol (120:1)] to yield disaccharide **21** (140 mg, 47%). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

2-*Acetamido*-4-O-(2-*acetamido*-2,3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*)-3,6-*di*-O-*benzoyl*-2-*deoxy*-N-(*fluoren*-9-*yl*-*methoxycarbonyl*)-β-D-*glucopyranosylamine* **22**.—Disaccharide **21** (100 mg, 99 μmol) and DTT (30.5 mg, 198 μmol) were dissolved in dry dichloromethane (5 cm^3) and diisopropylethylamine (20 mm^3) was added. The solution was then stirred at ambient temperature for 30 min. The thiolysis was followed by TLC [chloroform–methanol (25:1)]. After complete N-deprotection [TLC: chloroform–methanol (25:1)] the solvent was evaporated off under reduced pressure and the residue was dissolved in a mixture of acetic anhydride (2 cm^3) and pyridine (4 cm^3). The N-acetylation mixture was kept for 3 h at ambient temperature, concentrated, co-concentrated with toluene and purified by VLC [chloroform–methanol (100:1)] to achieve title compound **22** (94%). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

Methyl 3-O-*Acetyl*-2-*azido*-2-*deoxy*-β-D-*galactopyranoside* **24**.—Compound **23**[25] (107 mg, 306.2 μmol) was dissolved in 80% aq. acetic acid and the solution was kept at 70 °C for 3 h. The reaction mixture was concentrated, several times co-concentrated with toluene and subjected to silica gel. Purification by VLC [ethyl acetate–toluene (2:1)] yielded *diol* **24** (78 mg, 97%) (Found: C, 41.5; H, 5.9; N, 15.7. $C_9H_{15}N_3O_6$ requires C, 41.38; H, 5.79; N, 16.08%; M, 261.24); $[\alpha]_D^{22}$ +34.3 (*c* 1, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables 2 and 4, respectively.

Methyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*-(1→6)-3-O-*acetyl*-2-*azido*-2-*deoxy*-β-D-*galacto*-pyranoside **25**.—Imidate **7** (167, 294 μmol), diol **24** (70 mg, 267 μmol) and molecular sieves (3 Å) were placed in a pre-dried flask. After evacuation with an oil-pump, the flask was filled with argon and dry dichloromethane (5 cm^3). After the mixture had been stirred at −30 °C for 10 min, a trimethylsilyl trifluoromethanesulfonate solution (100 mm^3) (100 mm^3 TMSOTf/5 cm^3 CH_2Cl_2) was added into the reaction mixture. The solution was kept at −30 °C until the diol **24** disappeared on TLC [ethyl acetate–toluene (1:1)]. After dilution with dichloromethane (10 cm^3), filtration through Celite and concentration, the crude disaccharide **25** was subjected to VLC [ethyl acetate–toluene (1:3)] to yield *title compound* **25** (176 mg, 97%) (Found: C, 41.0; H, 4.7; N, 8.6. $C_{23}H_{30}N_4O_{15}S_2$ requires C, 41.44; H, 4.53; N, 8.40%; M, 666.64). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

Methyl 3,4,6-*Tri*-O-*acetyl*-2-*deoxy*-2-*dithiasuccinimido*-β-D-*glucopyranosyl*-(1→6)-3,4-*di*-O-*acetyl*-2-*azido*-2-*deoxy*-β-D-*galactopyranoside* **26**.—Disaccharide **25** (176 mg, 264 μmol) was dissolved in acetic anhydride (3 cm^3)–pyridine (6 cm^3) to achieve acetylation of the 4-position. After 2 h at ambient temperature the solution was concentrated and the residue was co-concentrated with toluene and subjected to VLC [ethyl acetate–toluene (1:3)], which yielded the desired *acetylated compound* **26** (156 mg, 83%) (Found: C, 42.1; H, 4.2; N, 8.1. $C_{25}H_{32}N_4O_{16}S_2$ requires C, 42.37; H, 4.55; N, 7.91%; M, 708.68); $[\alpha]_D^{22}$ +6.8 (*c* 1.1, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

Methyl 2-*Acetamido*-3,4,6-*tri*-O-*acetyl*-2-*deoxy*-β-D-*glucopyranosyl*-(1→6)-2-*acetamido*-3,4-*di*-O-*acetyl*-2-*deoxy*-β-D-*galactopyranoside* **27**.—DTT (44 mg, 282.2 μmol) and compound **26** (100 mg, 141 μmol) were dissolved in dichloromethane (6 cm^3). After addition of diisopropylethylamine (50 mm^3), the solution was stirred at ambient temperature for 30 min. The mixture was concentrated, then was co-concentrated with toluene and the residue was dissolved in a mixture of acetic anhydride (3 cm^3) and pyridine (6 cm^3) and kept at ambient temperature for 2 h. After completion of N-acetylation the mixture was concentrated and the residue was co-concentrated with toluene and subjected to VLC [chloroform–methanol (50:1)] to yield the *title compound* **27** (68 mg, 76%) (Found: C, 50.9; H, 6.6; N, 4.1. $C_{27}H_{40}N_2O_{15}$ requires C, 51.26; H, 6.37; N, 4.43%; M, 632.62); $[\alpha]_D^{22}$ +34.7 (*c* 1.0, $CHCl_3$). ^1H and ^{13}C NMR data are presented in Tables 3 and 5, respectively.

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References

1 A. Varki, *Glycobiology*, 1993, **3**, 97.
2 C.-R. Torres and G. W. Hart, *J. Biol. Chem.*, 1984, **259**, 3308.
3 G. W. Hart, R. S. Haltiwanger, G. D. Holt and W. G. Kelly, *Annu. Rev. Biochem.*, 1989, **58**, 841.
4 K. P. Kearse and G. W. Hart, *Arch. Biochem. Biophys.*, 1991, **290**, 543.
5 W. G. Kelly, M. E. Dahmus and G. W. Hart, *J. Biol. Chem.*, 1993, **268**, 10 416.
6 C. F. Chou, A. J. Smith and M. B. Omary, *J. Biol. Chem.*, 1992, **267**, 3901.
7 C. F. Chou and M. B. Omary, *J. Biol. Chem.*, 1993, **268**, 4465.
8 G. W. Hart, W. G. Kelly, M. A. Blomberg, E. P. Roquemore, L.-Y. D. Dong, L. Kreppel, T.-Y. Chou, D. Snow and K. Greis, in *Complex Carbohydrates in Drug Research. Structural and Functional Aspects*, ed. K. Bock and H. Clausen, Munksgaard, Copenhagen, 1993, p. 280.
9 S. Peters, T. Bielfeldt, M. Meldal, K. Bock and H. Paulsen, *J. Chem. Soc., Perkin Trans. 1*, 1992, 1163.
10 M. Meldal, in *Neoglycoconjugates: Preparation and Application*, ed. Y. C. Lee and R. T. Lee, Academic Press, San Diego, 1994, p. 149.

11 J. Banoub, P. Boullanger and D. Lafont, *Chem. Rev.*, 1992, **92**, 1167.
12 A. Vargas-Berenguel, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1994, 3287.
13 M. Schultz and H. Kunz, *Tetrahedron Lett.*, 1992, **33**, 5319.
14 T. Mukaiyama and K. Matsubara, *Chem. Lett.*, 1992, 1755.
15 O. Kanie, S. C. Crawley, M. M. Palcic and O. Hindsgaul, *Carbohydr. Res.*, 1993, **243**, 139.
16 J. Debenham, B. Fraser-Reid and P. M. Gross, *Abstracts of the XVIIth International Carbohydrate Symposium, Ottawa*, 1994, p. 200.
17 G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.*, 1977, **99**, 7363.
18 E. Meinjohanns, M. Meldal, H. Paulsen and K. Bock, *Abstracts of the XVIIth International Carbohydrate Symposium, Ottawa*, 1994, p. 260.
19 F. Rolla, *J. Org. Chem.*, 1982, **47**, 4327.
20 E. Meinjohanns, A. Vargas-Berenguel, M. Meldal, H. Paulsen and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1994, to be submitted.
21 G. Barany and R. B. Merrifield. *Anal. Biochem.*, 1979, **95**, 160.
22 G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.*, 1980, **102**, 3084.
23 L. Szilagyi and Z. Györgydeak, *Carbohydr. Res.*, 1985, **143**, 21.
24 I. Christiansen-Brams, M. Meldal and K. Bock, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1461.
25 H. Paulsen and M. Paal, *Carbohydr. Res.*, 1984, **135**, 53.

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