Tetrahedron: Asymmetry Vol. 4, No. 6, pp. 1205-1220, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00
Pergamon Press Ltd

Synthetic O-Glycopeptides as Model Substrates for Glycosyltransferases

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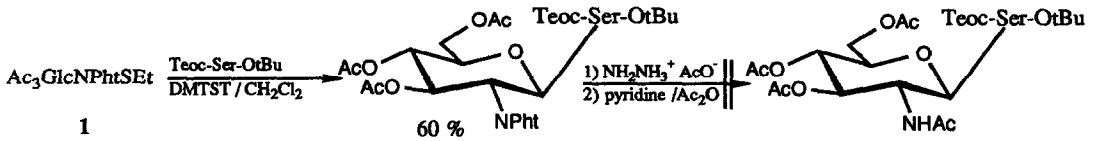
(*Received* 17 *February* 1993; *accepted* 14 *March* 1993)

Abstract: A new approach to O-glycopeptides of the glucosamine type is described. N-Urethane protected, peracetylated glucosamine is converted into its 1-thio (1-bromo) derivative and used for glycosylation of a variety of protected serine or threonine derivatives as acceptors. The urethane group is easily exchanged for the natural N-acetyl moiety and O-deacetylation is achieved with hydrazine/methanol. The resulting O-GlcNAc derivatives are subjected to an enzymatic galactosylation procedure using β-1,4-galactosyltransferase (EC 2.4.1.22) to furnish O-glycopeptides of the neolactosamine type.

The combined use of chemical and enzymatic methods is a promising strategy for the formation of complex molecules. An increasing number of enzymes and the corresponding methodologies are made available to synthetic chemists and the attractiveness of biocatalysts can easily be illustrated by numerous recent publications concerning preparative applications. Especially in the area of complex carbohydrate chemistry the use of enzymes extends the preparative repertoire and, thus, the attainable range of compounds.[1] Since the selective chemical formation of intersaccharidic bonds poses great synthetic demands and is usually accompanied by extensive protecting group manipulations, the use of glycosyltransferases, in particular, facilitates the transfer of carbohydrate moieties in a regio- and stereoselective fashion. Among these enzymes, β-1,4-galactosyltransferase (EC 2.4.1.22) is most thoroughly investigated in which its tolerance towards a broad range of acceptor substrates became obvious.[2] It is commercially available, and for large scale purposes the enzyme can be isolated in a few days from common bovine milk following the method established by Hill et al.[3] In several applications the preparative value of this enzyme was successfully proven by the galactosylation of oligosaccharides and N-glycopeptides bearing N-acetylglucosamine as terminating carbohydrate residue.[4a-c] We here report on the preparative enzymatic galactosylation of O-glycopeptides derived from glucosamine. Glycoproteins containing this linkage region widely occur in subcellular fractions of vertebrates while their function, e.g. in the nuclear envelope, remains unclear.[5]

In this context, an improved chemical synthesis of the required substrates, *i.e.* β-N-acetylglucosamine serine and threonine conjugates, was another matter of interest. The majority of O-glycopeptides of the N-acetylglucosamine type have been synthesized via haloglycosides[6a,b] or oxazolines.[7] Both methods are characterized

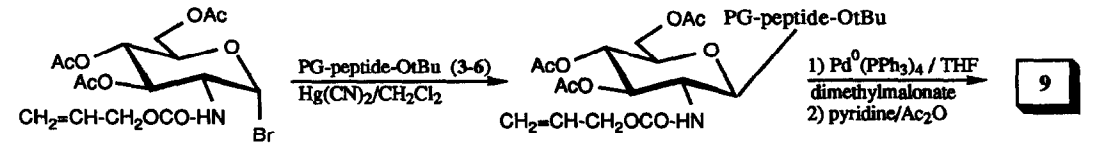
by acidic conditions as well as long reaction times and give low yields of the desired product. This holds true, especially if tert-butyl type protected amino acids are employed as acceptors. The application of such base-stable blocking groups, on the other hand, is inevitable if the O-GlcNAc derivatives should be selectively O-deacetylated to yield potential substrates for transferases. Thus, we first investigated the 2-deoxy-2-N-phthalimido-1-thioethyl-glucosamine **1** as donor in the glycosylation of serine derivatives. While this reaction proceeded stereoselectively and with high yield, monitoring of the subsequent O-/N-deacylation / acetylation by t.l.c. showed a number of side reactions and only traces of product (scheme 1).



Scheme 1

Progress in this field was achieved by the use of urethane protection for the glucosamine. In this sense, N-allyloxycarbonyl (Aloc) glucosaminyl bromide **2**, a useful donor for the glycosylation of alcohols and monosaccharides [8], smoothly reacted with protected serine tert-butyl esters **3**, **5**, **6**. It gave moderate yields with the threonine derivative **4**. Acceptors as **3** and **4** were usually synthesized[9] by tert-butylation (isobutene / sulfuric acid) of the hydroxy- and N-protected serine- and threonine intermediates with subsequent liberation of the hydroxy function. We have improved this strategy by applying an addition product formed from dicyclohexylcarbodiimide in tert-butanol[10a,b] which with Z-(Teoc-, Fmoc-) protected precursors gave the desired tert-butyl esters **3** (**4**) in high yield.

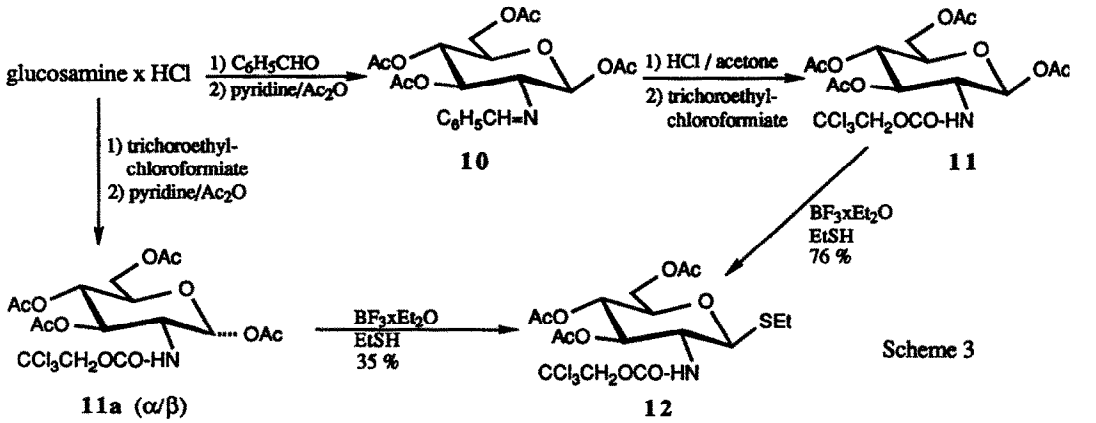
Cleavage of the allyl urethane in **8a-d** is accomplished by a palladium(0) catalyzed reaction in tetrahydrofuran using dimedone [11a] or dimethylmalonate [8] as nucleophile (scheme 2) whereas dimethyl barbituric acid, successfully employed in peptide chemistry [11b], led to a complicated purification after N-acetylation with pyridine / acetic anhydride.



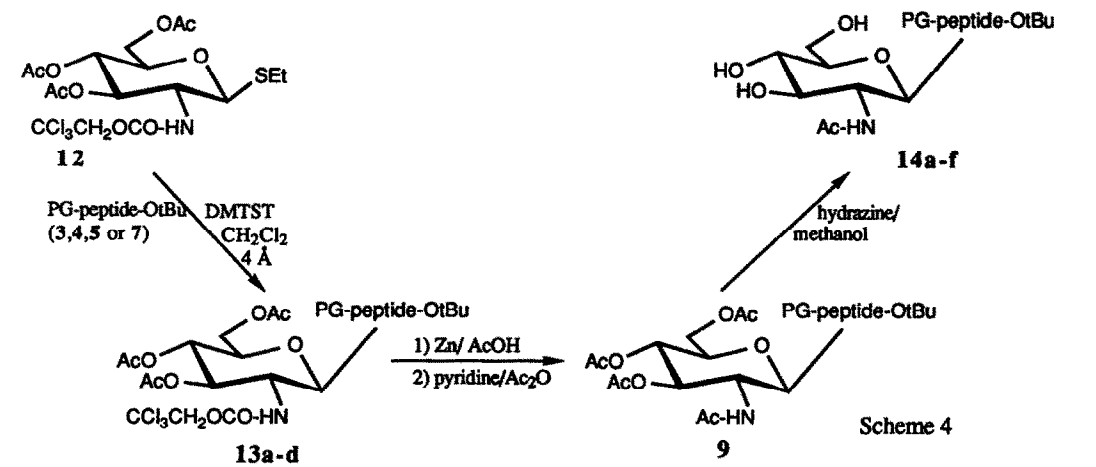
Scheme 2

It should be noted, that the donor **2** is unstable and has to be prepared directly before use. Furthermore, mercuric cyanide has to be used for activation. That is why we investigated the glucosamine thioglycoside **12** as an alternative glycosyl donor. It was synthesized stereoselectively according to known procedures[12] from glucosamine via the Schiff base **10**, which is acetylated, N-deprotected with HCl in acetone and reacted with trichloroethylchloroformiate to give peracetylated **11** (scheme 3). Subsequently, the thioethyl group was introduced with ethanethiol and boron trifluoride etherate to yield donor **12**. The introduction of the trichloroethyloxycarbonyl group is essential since the activation of thioglycosides with soft electrophiles interferes with the lability of the Aloc group towards this methodology.[13] Direct synthesis of **11** from glucosamine with

subsequent Lewis-acid promoted thioethylation gave unsatisfying results due to the low reactivity of the α-acetyl derivative **11a** (scheme 3).



Thiophilic activation of **12** was achieved using dimethyl(methylthio)sulfonium trifluoromethanesulfonate (DMTST)[14] and the desired glycopeptides **13a-d** were obtained stereoselectively to give yields up to 85 %. Conversion of the N-urethane protected compounds to the 2-N-acetyl derivatives was performed in an efficient two-step reaction employing activated zinc powder / glacial acetic acid for reductive elimination of the trichloroethyl moiety and pyridine / acetic anhydride for N-acetylation. All of the peracetylated glycopeptides **9a-e** revealed to be easily O-deacetylated applying hydrazine / methanol.[15] The resulting substrates **14a-f** were purified by HPLC to remove potential enzyme inhibitors.



NMR-data and elemental analysis of the isolated compounds were in agreement with the assumed structures.

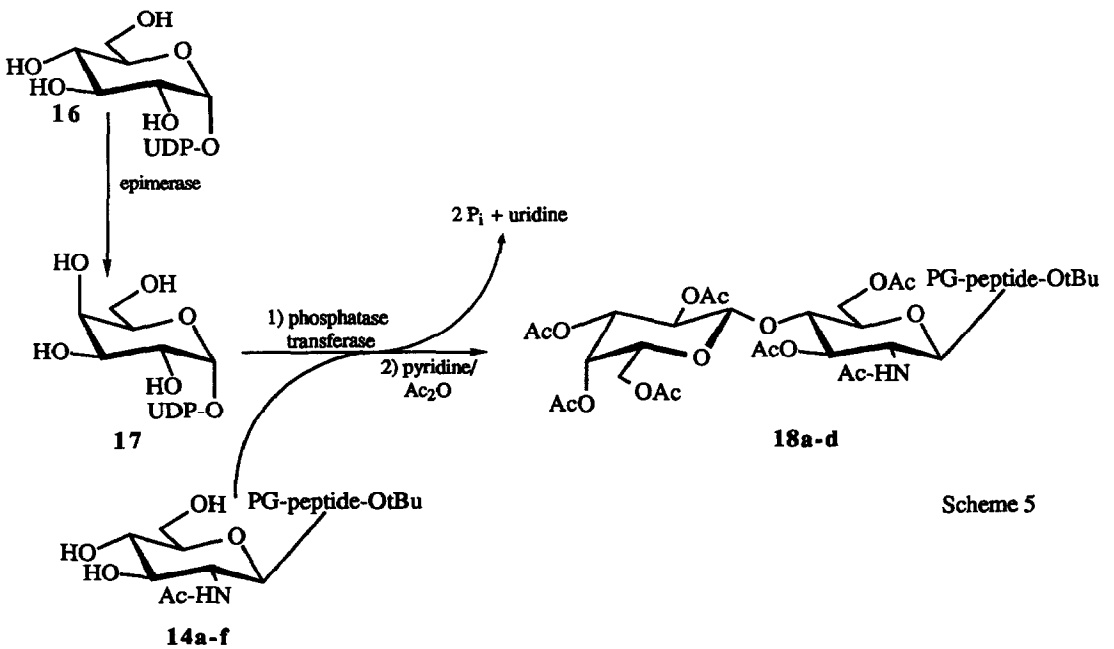
In conclusion (table 1), the obtained results prove the 2-N-Teoc-1-thioethyl glucosamine derivative **12** to be a stable and efficient glycosyl donor which allows efficient stereoselective glycosylations, in particular, with N-protected amino acid tert-butyl esters **3** (**4**).

Table 1 : Summarization of results

Peptide	Glycosylation with donor **2**	Glycosylation with donor **12**	$Ac_3GlcNAc$-derivatives	O-deacetylated substrates	Gal-β1,4-GlcNAc-peptides (Yields %)
Z-Ser-OtBu (**3**)	**8a** (45%)	**13a** (85%)	**9a**	**14a**	----
Z-Thr-OtBu (**4**)	**8b** (21%)	**13b** (55%)	**9b**	**14b**	----
Z-Ser-Ala-OtBu (**5**)	**8c** (54%)	**13c** (65%)	**9c**	**14c**	**18a** (52%)
Teoc-Ser-Ala-OtBu (**6**)	**8d** (55%)		**9d**	**14d**	**18b** (45%)
Z-Ser-Val-OtBu (**7**)		**13d** (55%)	**9e**	**14e**	**18c** (59%)
Z-Ala-Ser-Ala-OtBu			**9f**	**14f**	**18d** (56%)

For the synthesis of the N-terminal elongated glycopeptide **9f** (Z-Ala-Ser($Ac_3GlcNAc$)Ala-OtBu) the Z-group of **9c** was removed with palladium / hydrogen in methanol and the resulting amine **15** was condensed with Z-Ala-OH using water-soluble carbodiimide. Finally, O-deacetylation was accomplished to yield **14f**.

Enzymatic galactosylation of different substrates was performed by in situ generation of UDP-galactose **17** from UDP-glucose **16** employing UDP-glucose-4′-epimerase (EC 5.1.3.2) at pH 7.4 and 37°C. Alkaline phosphatase (EC 3.1.3.1) from calf intestinal mucosa was added to destroy uridinemonophosphate as transferase inhibitor and, thus, facilitates the equilibrium in the direction of the product formation (scheme 5).[16]



Scheme 5

Isolation of the products was achieved by acetylation and subsequent purification by flash chromatography and HPLC (RP 18, methanol / water). In one case, gel chromatography using Sephadex G15 (water) was the more convenient purification procedure. This method delivered different O-lactosamine derivatives of serine (**19a-d**). It should be mentioned that the O-glycosyl derivatives of threonine and serine tert-butylesters (**14a,b**) showed reduced reactivity in the enzymatic galactosylation. Therefore, the purification of the corresponding reaction mixtures turned out to be impossible.

In conclusion, a routinely applied enzymatic methodology as the one described here is useful and realizable even in laboratories of organic chemists. The reported results obtained with O-glycopeptides again prove β-1,4-galactosyltransferase to be an efficient tool in glycoconjugate synthesis. The present knowledge about the range of substrates accepted by the enzyme is convincing and has certainly not been exploited to its limit.

Experimental

General : Melting points were determined with a Büchi melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer-241 polarimeter. ^{1}H- and ^{13}C-NMR spectra were recorded at 400 MHz (^{1}H) and 100.6 MHz (^{13}C) with a Bruker WT 400 unless otherwise noted. All chemical shifts are given in ppm using tetramethylsilane as an internal standard (δ value). Preparative HPLC was performed on a Spherisorb Octyl (5μ) RP-Column (25 x 2 cm) using the 2140-UV/VIS-detector from LKB and a Bischoff 2200 pump (10ml/min). Gel chromatography on Sephadex G15 was monitored with a Uvicord 2510 (LKB) using the Pharmacia P 500 pump (1 ml/min). For flash chromatography silica gel 60 (30-60 μm, Baker) was employed while t.l.c analyses were performed with silica gel plates (60 F_{254}, E.Merck, Darmstadt, Germany), stained with a 1:1 mixture of 2n H_2SO_4 and a 0.2 % solution of 3-methoxy phenol in ethanol followed by heating. Enzymes were purchased from Sigma or (β-1,4-galactosyltransferase) isolated according to procedures described in the literature.[3)]

General procedure for the synthesis of N-benzyloxycarbonyl protected serine (threonine) tert-butyl esters (3, 4)[10a,b)] : A mixture of dicyclohexylcarbodiimide (9.3 g, 45 mmol), tert-butanol (4.4 g, 58 mmol) and copper-(I)-chloride (0.1 g, 1 mmol) was stirred for 5 d. The dark green suspension was then diluted with dry dichloromethane (30 ml) and the N-protected amino acid (14 mmol) in dichloromethane (30 ml) was added dropwise. The reaction was finished within 3 to 4 h (t.l.c : light petroleum / ethyl acetate 1:1). Precipitated urea was then removed by filtration. The organic layer was washed three times with sat. $NaHCO_3$ solution (100 ml), dried with $MgSO_4$ and concentrated *in vacuo*. The residue was taken up in ethyl acetate and further urea was removed after cooling the solution to - 28 °C. Flash chromatography on silica gel (light petroleum / ethyl acetate) yielded pure protected hydroxyl amino acids **3** (**4**).

N-Benzyloxycarbonyl-serine tert-butyl ester (3) : 73 %; $[\alpha]_D^{22}$ = - 13.7 (c=1.03, EtOH); m.p. 89-90 °C, Lit.[9)]: - 16.5 (c=1.03, EtOH); m.p. 93-95 °C. $C_{15}H_{21}O_5N$ (295.34); Anal. calcd.: C 61.00, H 7.17, N 4.74. Found: C 61.26, H 7.26, N,4.86.

N-Benzyloxycarbonyl-threonine tert-butyl ester (4) : 91 %; $[\alpha]_D^{22}$ = - 20.0 (c=1.0, $CHCl_3$); m.p. 62-63 °C, Lit[9]: $[\alpha]_D^{26}$ = - 20.6 (c=1.07, EtOH); m.p. 66-67 °C. $C_{16}H_{23}O_5N$ (309.36); Anal. calcd.: C 62.12, H 7.49, N 4.53. Found: C 62.03, H 7.42, N 4.72.

Synthesis of Dipeptides (5 - 7) : All peptides were synthesized using the EEDQ- (1-ethoxy-carbonyl-2-ethoxy-1,2-dihydro-quinoline) method.[17] Purification was achieved by flash chromatography (light petroleum / ethyl acetate) yielding 60-70 % of the desired dipeptides.

N-Benzyloxycarbonyl-seryl-alanine tert-butyl ester (5) : $[\alpha]_D^{22}$ = + 19.6 (c=1.0, $CHCl_3$); m.p. 57 °C; 90 MHz-^{1}H NMR ($CDCl_3$): 7.3 (m, 6 H, aromat./ NH-Ser), 6.2 (d, 1 H, NH-Ala), 5.1 (s, 2H, CH_2-benzyl), 3.5-4.5 (m, 4 H, CH-Ala, CH-Ser, CH_2-Ser), 1.4 (s, 9 H, $C(CH_3)_3$). $C_{18}H_{26}O_6N_2$ (366.41); Anal. calcd.: C 59.00, H 7.15, N 7.65. Found: C 58.35, H 7.04, N 7.55.

N-2,2,2-Trichloroethyloxycarbonyl-seryl-alanine tert-butyl ester (6) : $[\alpha]_D^{22}$ = - 33.2 (c=1.0, CH_3OH); m.p. 99-101 °C; 90 MHz-^{1}H NMR ($CDCl_3$): 6.9 (d, 1 H, NH-Ala), 6.1 (d, 1 H, NH-Ser), 4.72 (s, 2 H, CH_2-uret.), 3.5-4.5 (m, 4 H, CH-Ala, CH-Ser, CH_2-Ser), 1.43 (s, 9 H, $C(CH_3)_3$), 1.35 (d, J=7.6, 3 H, CH_3-Ala); $C_{13}H_{21}O_6N_2$ (407.22); Anal. calcd.: C 38.30, H 5.19, N 6.87, Cl 26.09. Found: C 38.34, H 4.92, N,6.89, Cl 26.02.

N-Benzyloxycarbonyl-seryl-valine tert-butyl ester (7) : colourless oil; $[\alpha]_D^{22}$ = -5.2 (c=1, $CHCl_3$); 90 MHz-^{1}H NMR ($CDCl_3$): 7.3 (m, 5 H, aromatic H), 7.1 (d, 1 H, NH-Val), 6.1 (d, 1 H, NH-Ser), 5.1 (s, 2 H, CH_2-benzyl), 3.5-4.5 (m, 5 H, α-CH-Ser, α-CH-Val, CH_2-Ser), 2.1 (m, 1 H, β–CH-Val), 1.42 (s, 9 H, $C(CH_3)_3$), 0.86 (2 d, 6 H, 2 x CH_3-Val).
$C_{20}H_{30}O_6N_2$ (394.47); Anal. calcd.: C 60.90, H 7.67, N 7.10. Found: C 60.86, H 7.62, N 6.97.

3,4,6-Tri-O-acetyl-2-N-allyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl bromide (2): This donor was prepared according to the procedure described by Boullanger et al.[8] in 3 steps from glucosamine hydrochloride. Neither the peracetylated intermediate (purified by flash chromatography, light petroleum / ethyl acetate 1:1) nor the crude bromide could be crystallized and the latter was immediately used without purification. 63 %; R_F = 0.7 (light petroleum / ethyl acetate 1:1).

1,3,4,6-Tetra-O-acetyl-2-N-(2,2,2-trichloroethyloxycarbonyl)amino-2-deoxy-β-D-glucopyranose (11) : Analogously to the procedure described in the literature[12] the peracetylated derivative **11** was isolated on a 100 mmolar scale by flash-chromatography with light petroleum / ethyl acetate 5:1-->1:5 as a colourless, amorphous solid. 74 %; $[\alpha]_D^{22}$ = + 59.3 (c=3.2, $CHCl_3$) , Lit.[10] : 48 %; $[\alpha]_D^{22}$ = + 15 (c=3.2, $CHCl_3$); 200-MHz-^{1}H NMR ($CDCl_3$): 5.72 (d, $J_{1,2}$ 8.6 Hz, 1 H, H-1), 4.7 (dd, J_{gem} 12.0 Hz, 2 H, CH_2-uret.).

3,4,6-Tri-O-acetyl-1-thioethyl-2-N-(2,2,2-trichloroethyloxycarbonyl)amino-2-deoxy-β-D-glucopyranose (12) : A solution of 3 g (6 mmol) of the peracetylated compound **11** in dry dichloro-

methane (30 ml) was stirred with molecular sieves 4 Å (3 g) for 30 min. The mixture was then cooled to - 10 °C and ethanethiole (0.93 g, 15 mmol) and 5.1 g (36 mmol) of boron trifluoride etherate were added in succession. After stirring at room temperature for 1 h the suspension was filtered with celite, washed with sat. $NaHCO_3$ solution and evaporated *in vacuo*. The remaining oil was crystallized from ethyl acetate / light petroleum. Yield: 2.4 g (76.5 %); $[\alpha]_D^{22}$ = - 46.7 (c=1, $CHCl_3$); m.p. 108 °C; ^1H NMR ($CDCl_3$): 5.21 (dd, $J_{3,4}$ 9.6 Hz, 1 H, H-3), 5.19 (d, $J_{NH,2}$ 9.1 Hz, 1 H, NH), 5.05 (dd, $J_{4/3} = J_{4/5}$ 9.6 Hz, 1 H, H-4), 4.78 (dd, J_{gem} 12.1 Hz, 2 H, CH_2-uret.), 4.60 (d, $J_{1/2}$ 10.3 Hz, 1 H, H-1), 4.22 (dd, $J_{6a/6b}$ 12.3 Hz, $J_{6a/5}$ 5.1 Hz, 1 H, H-6_a), 4.10 (dd, $J_{6b/6a}$ 12.3 Hz, $J_{6b/5}$ 2.2 Hz, 1 H, H-6_b), 3.75 (dd, $J_{2,1}$ 10.3 Hz, 1 H, H-2), 3.68 (ddd, 1 H, H-5), 2.69 (m, 2 H, S-CH_2), 2.04 (3s, 9 H, CH_3-acetyl), 1.24 (t, 3 H, CH_2-C<u>H</u>$_3$); 50.3-MHz-^{13}C NMR ($CDCl_3$): 170.7, 169.4 (C=O, acetyl), 154.0 (C=O, uret.), 95.3 (CCl_3), 84.3 (C-1), 75.7, 73.2, 68.5, 55.0 (C-2, C-3, C-4, C-5), 74.4 (CH_2-uret.), 62.3 (C-6), 24.2 (S-CH_2), 20.7 (CH_3-acetyl); $C_{17}H_{24}O_9N_1S_1Cl_3$ (524.80); Anal. calcd.: C 38.90, H 4.60, N 2.67, S 6.11, Cl 20.27. Found: C 38.86, H 4.62, N 2.62, S 6.09, Cl 20.28.

General procedure **: O-Glycosylation of Serine and Threonine Derivatives with 3,4,6-Tri-O-acetyl-2-N-allyloxycarbonyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide (2)** : Freshly synthesized donor **2** (5 mmol) and 5 mmol of the acceptor **3-6** were dissolved in 40 ml dry dichloromethane and stirred with 2 g of molecular sieves 4 Å at room temperature for 30 min. Mercuric cyanide (2 g, 8 mmol) was added and the mixture was stirred for another 3-4 h (t.l.c : light petroleum / ethyl acetate 1:1). After filtration with celite the organic layer was washed with 10 % potassium iodide (4 x 50 ml) and water, dried and evaporated. Purification of the remaining oil was achieved by flash chromatography.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-N-allyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-serine tert-butyl ester (8a) : Yield: 45 % (amorphous); $[\alpha]_D^{22}$ = - 14.3 (c=1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.31 (m, 5 H, CH, aromat.), 6.00 (d (broad), 1 H, NH), 5.80-5.90 (m, 1 H, CH_2=C<u>H</u>), 5.00-5.19 (m, 4 H, CH_2-benzyl, C<u>H</u>$_2$=CH), 4.99 (t, $J_{3/4}$ 9.7 Hz, 2 H, H-3), 4.56-4.61 (m, 3 H, H-4, C<u>H</u>$_2$-OCONH), 4.33-4.37 (m, $J_{\alpha\text{-CH-Ser}/CH_2\text{-Ser}}$ 3.5 Hz, 4.6 Hz, 1 H, α-CH-Ser), 4.16-4.23 (2 dd, $J_{6a/6b}$ 12.3 Hz, $J_{CH_{2a/b}\text{-Ser}}$ 10.1 Hz, 2 H, H-6a, CH_2-Ser_a), 4.08 (dd, J_{gem} 12.2 Hz, $J_{6b/5}$ 2.2 Hz, 1 H, H-6b), 3.84 (dd, $J_{CH_2\text{-Ser}/\alpha\text{-CH}}$ 2.9 Hz, 1 H, CH_{2b}-Ser), 3.62-3.67 (ddd, $J_{5/6a}$ 4.7 Hz, 1 H, H-5), 2.04, 1.99, 1.98 (3s, 9 H, CH_3-acetyl), 1.43 (s, 9 H, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$): 170.55, 170.49, 169.39, 167.98 (C=O, ester, amide), 155.67, 154.34 (C=O, uret.), 136.31 (ipso-C, aromat.), 132.57 (CH_2=C<u>H</u>), 129.12, 128.97, 128.78 (CH, aromat.), 117.76 (C<u>H</u>$_2$=CH), 100.67 (C-1), 82.84 (C<u>(</u>CH_3)$_3$), 72.02, 71.86, 68.57, 56.00 (C-2, C-3, C-4, C-5), 69.1 (CH_2-benzyl), 68.86 (CH_2=CH-C<u>H</u>$_2$O), 65.94 (CH_2-Ser), 62.04 (C-6), 54.69 (α-CH-Ser), 27.83 (C(C<u>H</u>$_3$)$_3$), 20.61, 20.56, 20.52 (CH_3-acetyl);
$C_{31}H_{41}O_{14}N_2$ (666.67); Anal. calcd.: C 55.85, H 6.35, N 4.20. Found: C 55.42, H 6.55, N 4.35.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-N-allyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-threonine tert-butyl ester (8b) : Yield: 21% (amorphous); $[\alpha]_D^{22}$ = - 1.1 (c=1, $CHCl_3$); 200 MHz-^1H NMR ($CDCl_3$) : 7.33 (m, 5 H, CH, aromat.), 5.71-5.82 (m, 1 H, C<u>H</u>=CH_2), 5.60 (d (broad), 1 H, NH), 5.09-5.28 (m, 5 H, CH_2-benzyl, C<u>H</u>$_2$=CH, H-3), 4.99 (t, $J_{4/3}$ 9.7 Hz, 1 H, H-4), 4.87 (m, 1 H), 4.38-4.62 (m, 3 H), 4.18-4.24 (m, J_{gem} 12 Hz, 2 H, H-6a), 4.05 (dd, 1 H, H-6b), 3.61-3.66 (m, 1 H, H-5), 3.37-3.41 (m, 1 H), 2.01, 1.99 (2s, 9 H, CH_3-acetyl), 1.43 (s, 9 H, C(CH_3)$_3$), 1.16 (d, $J_{CH_3\text{-}}$

Ala/α-CH 6.1 Hz, 3 H, CH_3-Ala); 50 MHz-^{13}C NMR ($CDCl_3$): 170.66, 170.56, 169.40, 168.84 (C=O, ester, amide), 156.81, 155.57 (C=O, uret.), 136.27 (ipso-C, aromat.), 132.43 ($\underline{C}H=CH_2$), 128.38, 127.92 (CH, aromat.), 117.61 ($\underline{C}H_2=CH$), 97.42 (C-1), 81.97 ($\underline{C}(CH_3)_3$), 73.18, 71.45, 68.45, 58.84 (C-2, C-3, C-4, C-5, β-CH-Thr), 66.87 (CH_2-benzyl), 65.65 ($\underline{C}H_2OCONH$), 61.90 (C-6), 56.12 (α-CH-Thr), 27.75 ($C(\underline{C}H_3)_3$), 20.54 (CH_3-acetyl), 15.81 (CH_3-Thr);
$C_{32}H_{46}O_{15}N_2$ (680.70); Anal. calcd.: C 55.01, H 6.31, N 4.21. Found: C 55.00, H 6.55, N 4.20.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-N-allyloxycarbonylamino-2-deoxy-α-D-glucopyranosyl)-seryl-alanine tert-butyl ester (8c) : Yield: 54 %; m.p. 126 °C; $[\alpha]_D^{22}$ = + 2.5 (c=0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): broad and poorly resolved signals; 7.25 (m, 5 H, CH-aromat), 6.84 (broad, 1 H, NH-Ala), 5.58-5.74 (broad, 3 H, NH-Ser, NH-uret., $\underline{C}H=CH_2$), 4.99 (s, 2 H, CH_2-benzyl), 1.96, 1.92, 1.91 (3s, 9 H, CH_3-acetyl), 1.39 (m, 12 H, $C(CH_3)_3$, CH_3-Ala); ^{13}C NMR ($CDCl_3$): 170.60, 170.40, 169.36, 169.20, 155.72 (C=O, ester, amide, uret.), 136.09 (ipso-C, aromat.), 132.53 ($\underline{C}H=CH_2$), 128.43, 128.10, 127.98 (CH, aromat.), 117.71 ($CH=\underline{C}H_2$), 101.15 (C-1), 82.42 ($\underline{C}(CH_3)_3$), 72.29, 71.93, 68.55, 55.91 (C-2, C-3, C-4, C-5), 69.57 (CH_2-Ser), 67.00 (CH-benzyl), 65.71 (CH_2O-allyl), 61.93 (C-6), 53.81 (α-CH-Ser), 48.96 (α-CH-Ala), 27.88 ($C(\underline{C}H_3)_3$), 20.56, 20.51 (CH_3-O-acetyl), 17.99 (CH_3-Ala).
$C_{34}H_{47}O_{15}N_3$ (737.76); Anal. calcd.: C 55.35, H 6.42, N 5.70. Found: C 55.23, H 6.21, N 5.78.

N-(2,2,2-Trichloroethyloxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-N-allyloxycarbonyl-amino-2-deoxy-α-D-glucopyranosyl)-seryl-alanine tert-butyl ester (8d) : 55% (amorphous), $[\alpha]_D^{22}$ = + 9.7 (c=1, $CHCl_3$); ^1H NMR ($CDCl_3$, broad signals) : 6.89 (broad, 1 H, NH-Ala), 5.99, 5.56 (broad, 2 H, NH-uret.1/2), 5.82-5.85 (m, 1 H, $\underline{C}H=CH_2$), 5.16-5.25 (m, 3 H, H-3, $\underline{C}H_2=CH$), 5.06 (t, $J_{4/3}$ 9.7 Hz, 1 H, H-4), 4.67 (s, 2 H, CH_2CCl_3), 4.38-4.64 (m, 4 H), 4.25 (dd, J_{gem} 12.3 Hz, $J_{6a/5}$ 5.1 Hz, 1 H, H-6a), 4.10 (d, J_{gem} 12 Hz, 1 h, H-6b), 4.02 (broad, 1 H), 3.66-3.70 (m, 3 H), 2.05, 1.99, 1.98 (3s, 9 H, CH_3-acetyl), 1.45 (s, 9 H, $C(CH_3)_3$), 1.36 (d, $J_{CH3\text{-}Ala/\alpha\text{-}CH}$ 6.1 Hz, 3 H, CH_3-Ala); ^{13}C NMR ($CDCl_3$): 172.43, 170.61, 170.43, 169.79, 168.73 (C=O, ester, amide), 156.10, 154.00, (C=O, uret.), 132.53 ($\underline{C}H=CH_2$), 117.89 ($CH=\underline{C}H_2$), 101.31 (C-1), 95.26 (CCl_3), 82.63 ($\underline{C}(CH_3)_3$), 74.69 ($\underline{C}H_2CCl_3$), 69.33 ($\underline{C}H_2OCONH$), 72.23, 72.10, 68.58, 56.05 (C-2, C-3, C-4, C-5), 65.84 (CH_2-Ser), 61.98 (C-6), 53.95, 49.04 (α-CH-Ser, α-CH-Ala), 27.93 ($C(\underline{C}H_3)_3$), 20.63, 20.54 (CH_3-acetyl), 18.13 (CH_3-Ala);
$C_{29}H_{42}O_{15}N_3Cl_3$ (779.02); Correct elemental analysis could not be obtained.

General procedure : **Glycosylation of Serine and Threonine Derivatives Using 3,4,6-Tri-O-acetyl-1-thioethyl-2-N-(2,2,2-trichloroethyloxycarbonyl)amino-2-deoxy-β-D-glucopyranose (12)** : A solution of thioglycoside **12** (535 mg, 1 mmol) and acceptor **3** (**4**, **5**, **7**) (1 mmol) in 50 ml dry dichloromethane was stirred for 30 min with 1g of molecular sieves 4 Å. Dimethyl(methylthio)sulfonium-trifluoromethansulfonate (DMTST)[12] (390 mg, 1.5 mmol) was added and the resulting suspension was stirred at room temperature for 2 h. After treatment with 155 mg of triethylamine the mixture was filtered, evaporated *in vacuo* and the resulting oil was purified by flash chromatography with light petroleum / ethyl acetate.

N-Benzyloxycarbonyl-O-[3,4,6-tri-O-acetyl-2-N-(2,2,2-trichloroethyloxycarbonyl)-amino-2-deoxy-β-D-glucopyranosyl)]-serine tert-butyl ester (13a) : Yield: 85% (amorphous);

$[\alpha]_D^{22}$ = + 10.9 (c=1, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.32 (m, 5 H, CH, aromat.), 5.67 (d, $J_{NH/H-2}$ 7.5 Hz, 1 H, NH-acetyl), 5.40 (d, $J_{NH/\alpha\text{-}CH\text{-}Ser}$ 8.5 Hz, 1 H, NH-Ser), 5.21 (t, $J_{3/4}$ 9.8 Hz, 1 H, H-3), 5.05 (dd, J_{gem} 12.0 Hz, 2 H, CH_2-benzyl), 4.98 (t, $J_{4/3}$ 9.8 Hz, 1 H, H-4), 4.62 (dd, J_{gem} 12.2 Hz, 2 H, CH_2-uret.), 4.56 (d, $J_{1/2}$ 7.9 Hz, 1 h, H-1), 4.34 (m, 1 H, α-CH-Ser), 4.24 (dd, J_{gem} 12.3 Hz, $J_{6a/5}$ 3.0 Hz, 1 H, H-6a), 4.17 (dd, J_{gem} 10.2 Hz, $J_{CH2a\text{-}Ser/\alpha\text{-}CH}$ 3.45 Hz, 1 H, CH_{2a}-Ser), 4.09 (dd, $J_{6b/5}$ 1.9 Hz, 1 H, H-6b), 3.75 (d, 1 H, CH_{2b}-Ser), 3.60 (m, 1 H, H-5), 3.51 (dd, 1 H, H-2), 2.02, 1.98, 1.96 (3s, 9H, CH_3-acetyl), 1.40 (s, 9 H, $C(CH_3)_3$);^{13}C NMR ($CDCl_3$): 170.50, 170.46, 169.37. 168.30 (C=O, ester, amide), 155.97, 154.04 (C=O, uret.), 136.18 (ipso-C, aromat.), 128.48, 128.16, 128.10 (CH, aromat.), 100.34 (C-1), 95.34 (CCl_3), 82.62 ($\underline{C}(CH_3)$, 74.38 ($\underline{C}H_2CCl_3$), 71.70, 68.56, 56.11, 54.45 (C-3, C-5, C-4, C-2, α-CH), 69.34 (CH_2-Ser), 66.96 (CH_2-benzyl), 61.93 (C-6), 27.80 ($C(\underline{C}H)_3$, 20.56 (CH_3-N-acetyl), 20.49 (CH_3-O-acetyl).
$C_{30}H_{41}O_{15}N_2Cl_3$ (776.02); Anal. calcd.: C 46.43, H 5.33, N 3.61. Found: C 46.05, H 5.39, N 3.65.

N-Benzyloxycarbonyl-O-[3,4,6-tri-O-acetyl-2-(N-2,2,2-trichloroethyloxycarbonyl)-amino-2-deoxy-β-D-glucopyranosyl)]-threonine tert-butyl ester (13b) : Yield : 55% (amorphous); $[\alpha]_D^{22}$ = - 8.4 (c=1, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.33 (m, 5 H, CH, aromat.), 5.54, 5.34 (2d, 2 H, 2 x NH), 5.28 (t, $J_{3/4}$ 9.7 Hz, 1 H, H-3), 5.08 (s, 2 H, CH_2-benzyl), 5.98 (t, $J_{4/3}$ 9.7 Hz, 1 H, H-4), 4.56-4.73 (m, 3 H), 4.41-4.43 (m, $J_{\beta\text{-}CH/CH3\text{-}Thr}$ 6.2 Hz, 1 H, β-CH-Thr), 4.20-4.31 (m, 2 H), 4.05 (dd, J_{gem} 12.1 Hz, 1 H, H-6b), 3.63-3.66 (m, 1 H, H-5), 3.40-3.46 (m, 1 H, H-2), 1.99, 1.98 (2s, 9 H, CH_3-acetyl), 1.42 (s, 9 H, $C(CH_3)_3$), 1.15 (d, $J_{CH3\text{-}Thr/\beta\text{-}CH}$ 6 2 Hz, 3 H, CH_3-Thr); ^{13}C NMR ($CDCl_3$): 170.65, 170.51, 169.39, 168.81 (C=O,ester, amide), 156.81, 153.96 (C=O, uret.), 136.37 (ipso-C, aromat.), 128.46, 128.04, 127.98 (CH, aromat.), 97.13 (C-1), 95.7 ($\underline{C}Cl_3$), 82.08 ($\underline{C}(CH_3)$), 74.45 ($\underline{C}H_2CCl_3$), 73.19, 71.69, 71.15, 68.55 (C-3, C-4, C-5, β-CH), 66.98 (CH_2-benzyl), 61.94 (C-6), 58.88, 56.47 (C-2, α-CH), 27.84 ($C(\underline{C}H_3)_3$), 20.55 (CH_3-acetyl), 15.83 (CH_3-Thr);
$C_{31}H_{41}O_{14}N_2Cl_3$ (772.03); Correct elemental analysis could not be obtained.

N-Benzyloxycarbonyl-O-[3,4,6-tri-O-acetyl-2-(N-2,2,2-trichloroethyloxycarbonyl)-amino-2-deoxy-β-D-glucopyranosyl)]-seryl-alanine tert-butyl ester (13c) : 65% (amorphous); $[\alpha]_D^{22}$ = + 5.9 (c=1, $CHCl_3$); 200 MHz-^1H NMR ($CDCl_3$): 7.32 (m, 5 H, CH, aromat.), 6.82 (broad, 1 H, NH-Ala), 6.00 (d, 1 H, NH), 5.58 (broad, 1 H, NH), 5.22 (t, $J_{3/4}$ 9.7 Hz, 1 H, H-3), 5.02-5.06 (m, 3 H, CH_2-benzyl, H-4), 4.74 (t, 1 H, H-4), 4.21-4.66 (m, 6 H), 3.98-4.12 (m, 1 H, H-5), 3.63-3.72 (m, 3 H), 2.04, 1.99, 1.98 (3s, 9 H, CH_3-acetyl), 1.47 (s, 9 H, $C(CH_3)_3$), 1.37 (d, $J_{CH3\text{-}Ala/\alpha\text{-}CH\text{-}Ala}$ 7.1 Hz, 3 H, CH_3-Ala); 50 MHz-^{13}C NMR ($CDCl_3$): 172.67, 170.69, 170.39, 169.44, 169.19 (C=O, ester, amide), 155.67, 154.32 (C=O, uret.), 136.03 (ipso-C, aromat.), 128.55, 128.26, 128.11 (CH, aromat.), 100.85 (C-1), 95.48 (CCl_3), 82.80 ($\underline{C}(CH_3)_3$), 74.39 ($CCl_3\underline{C}H_2$), 72.04, 71.90, 67.15, 56.10 (C-2, C-3, C-4, C-5), 69.41 (CH_2-benzyl), 68.49 (CH_2-Ser), 61.88 (C-6), 53.79, 49.10 (α-CH-Ser, α-CH-Ala), 27.94 ($C(\underline{C}H_3)_3$), 20.68, 20.59, 20.19 (CH_3-acetyl), 18.23 (CH_3-Ala);
$C_{33}H_{44}O_{15}N_3Cl_3$ (829.09); Anal. calcd.: C 47.81, H 5.35 N 5.07, Cl 12.83. Found: C 47.84, H 5.42, N 5.01, Cl 12.77.

N-Benzyloxycarbonyl-O-[3,4,6-tri-O-acetyl-2-(N-2,2,2-trichloroethyloxy-carbonyl)-amino-2-deoxy-β-D-glucopyranosyl)]-seryl-valine tert-butyl ester (13d) : Yield : 55%; m.p. 74

°C; $[\alpha]_D^{22}$ = + 8.6 (c=1, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.31 (m, 5 H, CH, aromat.), 6.78 (d, $J_{NH\text{-}Val/\alpha\text{-}CH}$ 7.7 Hz, 1 H, NH-Val), 5.84, 5.68 (2d, 2 H, NH-Ser, NH-acetyl), 5.29 (t, $J_{3/4}$ 9.8 Hz, 1 H, H-3), 5.07 (m, 3 H, CH_2-benzyl, H-4), 4.78 (d, 1 H), 4.67 (d, $J_{1/2}$ 7.5 Hz, 1 H, H-1), 4.47-4.49 (m, 3 H), 4.25 (dd, 1 H), 4.01-4.11 (m, 2 H), 3.62-3.71 (m, 2 H), 2.01, 2.00. 1.99 (3s, 9 H, CH_3-acetyl), 1.47 (s, 9 H, $C(CH_3)_3$), 0.90 (2s, 6 H, CH_3-Val); ^{13}C NMR ($CDCl_3$): 171.23, 170.66, 170.39, 169.44 (C=O, ester, amide), 155.76, 154.29 (C=O, uret.), 136.08 (ipso-CH aromat.), 128.53, 128.21, 128.09 (CH, aromat.), 100.94 (C-1), 95.45 (CCl_3), 82.59 ($\underline{C}(CH_3)_3$), 74.37 ($\underline{C}H_2$ CCl_3), 72.09, 71.84, 68.58, 57.96 (C-2, C-3, C-4, C-5), 69.64 (CH_2-benzyl), 67.11 (CH_2-Ser), 61.99 (C-6), 56.19, 53.93 (α-C-Ser, α-C-Val), 31.36 (β-C-Val), 28.04 ($C(\underline{C}H_3)_3$), 20.62, 20.55 (CH_3-acetyl), 18.64, 17.78 (CH_3-Val);
$C_{35}H_{48}O_{15}N_3Cl_3$ (829.09); Anal. calcd.: C 49.05, H 5.64, N 4.90. Found: C 49.09, H 5.50, N 4.92.

General procedure **: Exchanging N-allyloxycarbonyl for N-acetyl in the Glycopeptides 8a-d :** A solution of the N-urethane-protected compound **8a-d** (2 mmol) and 1.2 ml (14 mmol) dimethyl malonate in 30 ml of oxygen-free tetrahydrofuran under argon atmosphere was treated with 40 mg (0.035 mmol) of tetrakis(triphenylphosphine) palladium(0) for 24 h. After evaporation of the solution *in vacuo* the remaining oil was acetylated with pyridine / acetic anhydride 2 :1 (20 ml) at room temperature for 1 h. The resulting mixture was evaporated to dryness and the N-acetylated products were isolated by flash chromatography in light petroleum / ethyl acetate. Yield 75-85 %.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-serine tert-butyl ester (9a) : m.p. 134-136 °C; $[\alpha]_D^{22}$ = - 17.4 (c=1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.31 (m, 5 H, CH, aromat.), 5.80 (d, $J_{NH/2}$ 8.4 Hz, 1 H, NH-acetyl), 5.71 (d, $J_{NH/\alpha\text{-}CH}$ 7.75 Hz, 1 H, NH-Ser), 5.21 (t, $J_{3/4}$ 9.8 Hz, 1 H, H-3), 5.07 (s, 2 H, CH_2-benzyl), 4.98 (t, 1 H, H-4), 4.65 (d, $J_{1/2}$ 8.3 Hz, 1 H, H-1), 4.3 (dd, $J_{\alpha\text{-}CH/NH}$ 7.5 Hz, $J_{\alpha\text{-}CH/CH_2}$ 3.75 Hz, 1 H, α-CH-Ser), 4.18 (dd, $J_{6a/6b}$ 12.3 Hz, $J_{6a/5}$ 4.6 Hz, 1 H, $H\text{-}6_a$), 4.04-4.11 (m, 2 H, $H\text{-}6_b/CH_{2a}$-Ser), 3.81 (t, $J_{CH2b/CH2a}$ 10.25 Hz, 1 H, CH_{2b}), 3.72 (dd, $J_{2/1}$ 8.6 Hz, 1 H, H-2), 3.61 (ddd, $J_{5/6a}$ 4.6 Hz, $J_{5/6b}$ 6.7 Hz, $J_{5/4}$ 2.2 Hz, 1 H, H-5), 2.01, 1.97, 1.96 (3s, 9H, CH_3-O-acetyl), 1.83 (s, 3 H, CH_3-N-acetyl), 1.40 (s, 9 H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$): 170.71, 170.56, 170.44, 169.32, 168.49 (C=O amide, ester), 155.97 (C=O, uret.), 136.32 (ipso-C-aromat), 128.50, 128.17, 128.07 (CH, aromat.), 100.31 (C-1), 82.63 ($\underline{C}(CH_3)_3$, 72.3 (C-3), 71.79 (C-5), 68.97 (CH_2-Ser), 68.61 (C-4), 66.88 (CH_2-benzyl), 62.04 (C-6), 54.67 (α-CH-Ser), 54.58 (C-2), 27.84 ($C(\underline{C}H_3)_3$), 23.10 (CH_3-N-acetyl), 20.59, 20.53 (CH_3-O-acetyl).
$C_{29}H_{41}O_{13}N_2$ (625.65); Anal. calcd.: C 55.67, H 6.60, N 4.47. Found: C 55.61, H 6.58, N 4.66.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-α-D-glucopyranosyl)-seryl-alanine tert-butyl ester (9b) : m.p. 138 °C; $[\alpha]_D^{22}$ = + 4.4 (c=1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.31 (m, 5 H, CH, aromat.), 7.04 (broad, 1 H, NH-Ala), 6.18 (broad, 1 H, NH-acetyl), 5.73 (d, $J_{NH\text{-}Ser/\alpha\text{-}CH\text{-}Ser}$ 6.9 Hz, 1 H, NH-Ser), 5.20 (t, $J_{3/4}$ 9.5 Hz, 1 H, H-3), 5.06 (m, 3 H, CH_2-benzyl, H-4), 4.65 (d, $J_{1/2}$ 8.3 Hz, 1 h, H-1), 4.35-4.43 (m, 2 H), 4.23 (dd, J_{gem} 12.2 Hz, 1 H, H-6a), 4.09 (dd, 1 H, H-6b), 4.00 (dd, J_{gem} 10.5 Hz, 1 H, CH_{2a}-Ser), 3.90 (dd, 1 H, CH_{2b}-Ser), 3.67-3.74 (m, 2 H), 2.02, 1.99, 1.98 (3s, 9 H, CH_3-O-acetyl), 1.85 (s, 3 H, CH_3-N-acetyl), 1.45 (s, 9 H, $C(CH_3)_3$), 1.34 (d, $J_{CH3\text{-}Ala/\alpha\text{-}CH}$ 7.15 Hz, CH_3-Ala); ^{13}C NMR ($CDCl_3$): 172.03, 170.69, 170.59, 169.27, 169.15 (C=O, ester, amide), 155.89 (C=O,

uret.), 136.08 (ipso-C, aromat.), 128.43, 128.10, 127.93 (CH, aromat.), 100.91 (C-1), 82.20 ($\underline{C}(CH_3)_3$), 72.51, 71.94, 68.48, 54.13 (C-2, C-3, C-4, C-5), 68.96 (CH_2-benzyl), 66.98 (CH_2-Ser), 62.00 (C-6), 53.98, 48.97 (α-CH-Ser, α-CH-Ala), 27.88 ($C(\underline{C}H_3)_3$), 23.06 (CH_3-N-acetyl), 20.54, 20.52, 20.48 (CH_3-O-acetyl), 18.09 (CH_3-Ala);
$C_{32}H_{45}O_{14}N_3$ (695.72); Anal. calcd.: C 55.25, H 6.52, N 6.04, Found: C 54.76, H 6.54, N 6.06.

N-(2,2,2-Trichloroethyloxycarbonyl)-O-(3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-α-D-glucopyranosyl)-seryl-alanine tert-butyl ester (9c) : m.p. 159 °C; $[\alpha]_D^{22}$ = - 30.1 (c=1.0, CH_3OH); ^1H NMR ($CDCl_3$): 7.08 (d, $J_{NH\text{-}Ala/\alpha\text{-}CH}$ 7.2 Hz, 1 H, NH-Ala), 6.24 (d, $J_{NH/2}$ 8.4 Hz, 1 H, NH-acetyl), 6.06 (d, $J_{NH\text{-}uret./\alpha\text{-}CH\text{-}Ser}$ 7.2 Hz, 1 H, NH-Ser), 5.21 (t, $J_{3/4}$ 9.6 Hz, 1 H, H-3), 5.08 (t, 1 H, H-4), 4.65-4.71 (m, J_{gem} 12.2 Hz, 2 H, CH_2CCl_3, H-1), 4.38-4.43 (m, 2 H, α-CH-Ala, α-CH-Ser), 4.24 (dd, J_{gem} 12.3 Hz, $J_{6a/5}$ 5.2 Hz, 1 H, H-6a), 4.10 (dd, $J_{6b/5}$ 2.2, 1 H, H-6b), 3.89-4.02 (m, 2 H), 3.69-3.76 (m, 2 H), 2.05, 1.99, 1.99 (3s, 9 H, CH_3-O-acetyl), 1.90 (s, 3 H, CH_3-N-acetyl), 1.45 (s, 9 H, $C(CH_3)_3$), 1.35 (d, 3 H, CH_3-Ala); ^{13}C NMR ($CDCl_3$): 172.04, 170.81, 170.64, 170.59, 169.30, 168.61 (C=O, ester, amide), 154.16 (C=O, uret.), 101.03 (C-1), 95.25 ($CH_2\underline{C}Cl_3$), 82.38 ($\underline{C}(CH_3)_3$), 74.66 ($\underline{C}H_2CCl_3$), 72.39, 72.09, 68.41, 54.28 (C-2, C-3, C-4, C-5), 68.78 (CH_2-Ser), 62.01 (C-6), 54.10, 49.02 (α-CH-Ser, α-Ala), 27.93 ($\underline{C}(CH_3)_3$), 23.23 (CH_3-N-acetyl), 20.64, 20.57, 20.53 (CH_3-O-acetyl), 18.26 (CH-Ala);
$C_{34}H_{49}O_{14}N_3Cl_3$ (736.98); Anal. calcd.: C 44.00, H 5.47, N 5.70. Found: C 44.18, H 5.22, N 5.52.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-deoxy-2-acetamido-α-D-glucopyranosyl)-threonine tert-butyl ester (9d) : amorphous; $[\alpha]_D^{22}$ = - 23.3 (c=0.5, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.32 (m, 5 H, CH, aromat.), 5.72 (d, $J_{NH/2}$ 8.14 Hz, 1 H, NH-acetyl), 5.57 (d, $J_{NH/\alpha\text{-}CH}$ 9.1 Hz, 1 H, NH-Thr), 5.38 (t, $J_{3/4}$ 9.7 Hz, 1 H, H-3), 5.09 (dd, J_{gem} 12.4 Hz, 2 H, CH_2-benzyl), 4.97 (t, $J_{4/3}$ 9.7 Hz, 1 H, H-4), 4.80 (d, $J_{1/2}$ 8.25, 1 H, H-1), 4.35 (m, $J_{\beta\text{-}CH/CH_3\text{-}Thr}$ 6.3 Hz, 1 H, β-CH), 4.18-4.22 (m, 2 H, H-6a, α-CH), 4.04 (dd, J_{gem} 12.1 Hz, 1 H, H-6b), 3.66 (ddd, $J_{5/6b}$ 2.1 Hz, $J_{5/6a}$ 4.3 Hz, 1 H, H-5), 3.48 (ddd, 1 H, H-2), 1.99, 1.98, 1.97 (3s, 9 H, CH_3-O-acetyl), 1.85 (s, 3 H, CH_3-N-acetyl), 1.41 (s, 9 H, $C(CH_3)_3$), 1.14 (d, $J_{CH_3/\beta\text{-}CH}$ 6.3 Hz, 1 H, β-CH); ^{13}C NMR ($CDCl_3$): 170.71, 170.59, 170.35, 169.44, 168.97 (C=O, ester, amide), 156.80 (C=O, uret.), 136.52 (ipso-C, aromat.), 128.48, 128.07, 127.97 (CH, aromat.), 97.20 (C-1), 82.13 ($\underline{C}(CH_3)_3$), 73.65, 71.86, 71.63, 68.72 (C-3, C-4, C-5, β-CH), 66.89 (CH-benzyl), 62.06 (C-6), 59.07 (α-CH), 55.60 (C-2), 27.88 ($C(\underline{C}H_3)_3$), 23.27 (CH_3-N-acetyl), 20.65, 20.59 (CH_3-O-acetyl), 16.18 (CH_3-Thr); $C_{30}H_{42}O_{13}N_2$ (638.67); Anal. calcd.: C 56.42, H 6.63, N 4.39. Found: C 56.32, H 6.59, N 4.27.

General procedure: **Exchange of the N-Trichloroethyloxycarbonyl for the N-Acetyl Group in the Glycopeptides 13a-d :** Zinc powder was activated with 1n HCl, washed with water, methanol and diethyl ether and dried *in vacuo*. A solution of the N-urethane-protected compounds **13, 14, 15** or **16**) (1mmol) in glacial acetic acid (15 ml) was vigorously stirred with 1 g of freshly activated zinc powder for 12 h. The suspension was filtered through celite and evaporated *in vacuo*. The residue was then acetylated with pyridine / acetic anhydride 2 :1 (20 ml) for 1 h at room temperature. Work-up was carried out as described for **9a-d**. Yield 85-90 %.

N-Benzyloxycarbonyl-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-seryl-valine tert-butyl ester (9e) : m.p. 140 °C; $[\alpha]_D^{22}$ = + 25.8 (c=0.5, CH_3OH); ^1H NMR ($CDCl_3$): 7.30 (m, 5 H, CH, aromat.), 6.92 (d, $J_{NH\text{-}Val/\alpha\text{-}CH\text{-}Val}$ 8.1 Hz, 1 H, NH-Val), 6.16 (d, $J_{NH\text{-}Ser/\alpha\text{-}CH\text{-}Ser}$ 8.7 Hz, 1 H, NH-Ser), 5.77 (d, $J_{NH\text{-}acetyl/2}$ 6.9 Hz, 1 H, NH-acetyl), 5.21 (t, $J_{3/4}$ 9.6 Hz, 1 H, H-3), 5.02-5.06 (m, $J_{4/3}$ 9.6 Hz, 3 H, CH_2-benzyl, H-4), 4.66 (d, $J_{1/2}$ 8.3 Hz, 1 H, H-1), 4.43 (broad, 1 H), 4.36 (dd, $J_{\alpha\text{-}CH\text{-}Ser/NH\text{-}Ser}$ 8.5 Hz, $J_{\alpha\text{-}CH\text{-}Ser/CH_2\text{-}Ser}$ 4.7 Hz, 1 H, α-CH-Ser), 4.22 (dd, J_{gem} 12.2 Hz, $J_{6a/5}$ 5.3 Hz, 1 H, H-6a), 4.08 (d (broad), 1H), 3.99 (dd, 1 H), 3.87 (dd, 1 H), 3.70-3.74 (m, 2 H), 2.07-2.14 (m, 1 H, β-CH-Val), 2.01, 2.00, 1.98 (3s, 9H, CH-acetyl), 1.84 (s, 3 H, CH_3-N-acetyl), 1.45 (s, 9 H, $C(CH_3)_3$), 0.88 (q, $J_{CH_3\text{-}Val/\beta\text{-}CH\text{-}Val}$ 6.8 Hz, 6 H, CH_3-Val); ^{13}C NMR ($CDCl_3$): 170.83, 170.77, 170.63, 170.55, 169.43, 169.32 (C=O, ester, amide), 155.91 (C=O, uret.), 136.12 (ipso-C, aromat.), 128.47, 128.15, 128.03 (CH, aromat.), 101.10 (C-1), 82.26 ($\underline{C}(CH_3)_3$), 72.44, 72.05, 68.48, 57.87 (C-2, C-3, C-4, C-5), 69.30 (CH_2-Ser), 67.04 (CH_2-benzyl), 62.09 (C-6), 54.44 (α-CH-Ser), 54.10 (α-CH-Val), 31.37 (β-CH-Val), 28.01 ($C(\underline{C}H_3)_3$), 23.15 (CH_3-N-acetyl), 20.56, 20.65 (CH_3-O-acetyl), 18.78, 17.64 (CH_3-Val). $C_{34}H_{49}O_{14}N_3$ (723.77); Anal. calcd.: C 56.42, H 6.82, N 5.81, Found: C 56.85, H 6.85, N 6.27.

N-Benzyloxycarbonyl-alanyl-O-(3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl)-seryl-alanine-tert-butyl ester (9f) : The N-benzyloxycarbonyl-protected compound **9c** (480 mg, 0.69 mmol) was dissolved in 20 ml of methanol, treated with 50 mg of palladium / carbon (10 %) and stirred under hydrogen for 6 h. The mixture was filtered, evaporated *in vacuo* and the resulting crystalline amine (380 mg, 98 %) was directly used in the next step: A solution of N-benzyloxycarbonyl-alanine (151 mg, 0.68 mmol) in 25 ml of dry dichloromethane was stirred with 1-hydroxybenzotriazole (180 mg, 1.3 mmol) for 30 min. Then 130 mg (0.68 mmol) of 1-ethyl-3-(3-dimetylaminopropyl)carbodiimide (EDAC) and the free amine **15** (380 mg, 0.68 mmol) were added. The resulting suspension, homogenized by the addition of dry N,N'-dimethylformamide, was allowed to react for 3 d and was extracted with 10 % $NaHCO_3$, 0.5 n HCl and water (each 3 x 25 ml) and evaporated *in vacuo*.The resulting residue was purified by flash-chromatography in dichloromethane / methanol (30 :1) to give **22**, 238 mg (45 %); amorphous; $[\alpha]_D^{22}$ = - 23.8 (c=1.2, CH_3OH); ^1H NMR ($CDCl_3$): 7.02 (broad, 2 H, NH-peptide), 6.47 (broad, 1 H, NH-acetyl), 5.76 (broad, 1H, NH-uret.), 5.12-5.16 (m, 3 H, CH_2-benzyl, H-3), 5.05 (dd, $J_{4/3}$ 9.5 Hz, 1 H, H-4), 4.54-4.59 (m, 1 H), 4.51 (d, $J_{1/2}$ 8.3 Hz, 1 H), 4.33-4.38 (m, 1 H), 4.18-4.25 (m, 2 H), 4.18-4.25 (m, 2 H), 3.94-4.08 (m, 3 H), 3.80 (dd, 1 H), 3.55 (broad, 1 H, H-5), 2.04, 1.98 (2s, 9 H, CH_3-acetyl), 1.44 (s, 9 H, $C(CH_3)_3$), 1.38, 1.32 (2d, $J_{CH_3\text{-}Ala1/\alpha\text{-}CH}$ 7.05 Hz, $J_{CH3\text{-}Ala2/\alpha\text{-}CH}$ 7.14 Hz, 6 H, CH_3-$Ala_{1/2}$); ^{13}C NMR ($CDCl_3$): 172.75, 171.93, 170.83, 170.68, 169.29, 168.51 (C=O, ester, amide), 156.23 (C=O, uret.), 136.31 (ipso-C, aromat.), 128.53, 128.08, 127.71 (CH, aromat.), 101.29 (C-1), 82.23 ($\underline{C}(CH_3)_3$), 72.62, 72.02, 68.40, 54.15 (C-2, C-3, C-4, C-5), 52.66, 50.80, 49.07 (α-CH-Ser, 2 x α-CH-Ala), 27.94 ($C(\underline{C}H_3)_3$), 23.26 (CH_3-N-acetyl), 20.66, 20.60, 20.56 (CH_3-O-acetyl), 18.24, 18.12 (CH_3-Ala);
$C_{35}H_{50}O_{15}N_4$ (766.80); Anal. calcd.: C 54.82, H 6.57, N 7.30, Found: C 54.70, H 6.65, N 7.29.

General procedure : **O-Deacetylation of GlcNAc-Peptides 9a-f with Hydrazine** : A methanolic solution of the N-acetylated glycopeptides **9a-f** (0.15 mmol / 10 ml) was treated with 2 ml of hydrazine (85 %) for 1 h at room temperature. The mixture was cooled to 0 °C, acetone (10 ml) was added, the solution was brought to dryness *in vacuo* and codistilled with acetone (10 ml) three times. The remaining residue

was purified by preparative HPLC (methanol / water). After evaporation *in vacuo* and lyophilization pure **14a-f** was obtained.

N-Benzyloxycarbonyl-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl) serine tert-butyl ester (14a) : $[\alpha]_D^{22}$ = - 24.3 (c=0.45, CH_3OH); ^1H NMR (D_2O, broad signals): 7.39 (m, 5 H, CH, aromat.), 5.09 (dd, J_{gem} 12.2 Hz, 2 H, CH-benzyl), 4.79 (HDO), 4.47 (d, $J_{1/2}$ 8.3 Hz, 1 H, H-1), 4.24 (t (broad), 1 H), 4.07 (dd, 1 H), 3.85-3.88 (m, 2 H), 3.69 (dd, 1 H), 3.63 (t, 1 H), 3.45-3.50 (m, 1 H), 3.38 (d, 1 H), 1.92 (s, 3 H, CH_3-N-acetyl), 1.38 ($C(CH)_3$); ^{13}C NMR (D_2O): 174.35, 170.46 (C=O, ester, amide), 157.68 (C=O, uret.), 136.20 (ipso-C, aromat..), 128.74, 128.43, 127.83 (CH, aromat.), 100.78 (C-1), 84.23 ($\underline{C}(CH_3)_3$), 75.89, 73.65, 69.81, 55.32 (C-2, C-3, C-4, C-5), 68.24 (CH_2-benzyl), 67.14 (CH-Ser), 60.68 (C-6), 54.98 (α-CH), 27.05 ($C(\underline{C}H_3)_3$), 22.11 (CH_3-acetyl).
$C_{23}H_{34}O_{10}N_2$ x 0.5 H_2O (507.54); Anal. calcd.: C 54.43, H 6.95, N 5.52. Found: C 54.32, H 6.93, N 5.30.

N-Benzyloxycarbonyl-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-threonine tert-butyl ester (14b) : $[\alpha]_D^{22}$ = - 8.8 (c=1.1, CH_3OH); ^1H NMR (CD_3OD): 7.38 (m, 5 H, CH, aromat.), 5.16 (s, 2 H, CH_2-benzyl), 4.46 (d, $J_{1/2}$ 8.04 Hz, 1 H, H-1), 4.41 (m, $J_{\beta\text{-CH}/\alpha\text{-CH}}$ 3.4 Hz, $J_{\beta\text{-CH}/CH_3\text{-Thr}}$ 6.2 Hz, 1 H, β-CH), 4.14 (d, $J_{\alpha\text{-CH}/\beta\text{-CH}}$ 3.3 Hz, 1 H, α-CH), 3.91 (dd, J_{gem} 11.3 Hz, $J_{6a/5}$ 1.9 Hz, 1 H, H-6a), 3.66 (dd, J_{gem} 11.4 Hz, $J_{6b/5}$ 5.5 Hz, 1 H, H-6b), 3.57 (dd, $J_{3/4}$ 10.4 Hz, 1 H, H-3), 3.51 (dd, 1 H, H-4), 3.25-3.38 (m, H-2, H-5, CD_3OH), 1.99 (s, 3 H, CH_3-acetyl), 1.50 (s, 9 H, $C(CH_3)_3$), 1.19 (d, $J_{CH_3\text{-Thr}/\beta\text{-CH}}$ 6.6 Hz, 3 H, CH_3-Thr); ^{13}C NMR (CD_3OD): 173.93, 171.25 (C=O, ester, amide), 158.85 (C=O, uret.), 138.19 (ipso-C, aromat.), 129.46, 129.02, 128.89 (CH, aromat.), 100.09 (C-1), 83.34 ($\underline{C}(CH_3)_3$), 77.88, 75.58, 74.76, 72.39 (C-3, C-4, C-5, b-CH-Thr), 76.75 (CH_2-benzyl), 63.10 (C-6), 60.97, 57.59 (C-2, α-CH-Thr), 28.28 ($C(\underline{C}H_3)_3$), 23.02 (CH_3-acetyl), 17.03 (CH_3-Ala);
$C_{24}H_{36}O_{10}N_2$ x 0.5 H_2O (521.56); Anal. calcd.: C 55.27, H 7.15, N 5.37. Found: C 55.14, H 7.04, N 5.71.

N-Benzyloxycarbonyl-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-seryl-alanine tert-butyl ester (14c) : $[\alpha]_D^{22}$ = - 43.1 (c=1.0, CH_3OH); ^1H NMR (CD_3OD): 7.39 (m, 5 H, CH-aromat), 5.15 (s, 2 H, CH_2-benzyl), 4.47 (d, $J_{1/2}$ 8.4 Hz, 1 H, H-1), 4.38 (t, 1 H), 4.30 (dd, 1 H), 4.01 (dd, 1 H), 3.87-3.92 (m, 2 H), 3.65-3.73 (m, 2 H), 3.45-3.50 (m, 1 H), 3.32 (dd, 1 H), 1.97 (s, 3 H, CH_3-acetyl), 1.50 (s, 9 H, $C(CH)_3$), 1.40 (d, $J_{CH_3\text{-Ala}/\alpha\text{-CH}}$ 7.3 Hz, 3 H, CH_3-Ala); ^{13}C NMR (CD_3OD): 173.26, 172.61, 172.30 (C=O, ester, amide), 158.7 (C=O, uret.), 137.93 (ipso-C, aromat.), 129.32, 128.23, 127.97 (CH, aromat.), 102.78 (C-1), 82.85 ($\underline{C}(CH)_3$), 78.18, 76.21, 75.92, 57.18 (C-2, C-3, C-4, C-5), 71.26 (CH_2-benzyl), 68.08 (CH_2-Ser), 62.73 (C-6), 54.94, 50.99 (α-CH-Ser, α-CH-Ala), 28.24 ($C(\underline{C}H)_3$), 23.13 (CH_3-acetyl), 17.56 (CH_3-Ala);
$C_{26}H_{41}O_{12}N_3$ x H_2O (587.62); Anal. calcd.: C 53.14, H 7.03, N 7.15. Found: C 52.97, H 7.24, N 6.95.

N-Benzyloxycarbonyl-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-seryl-valine-tert-butyl ester (14d) : $[\alpha]_D^{22}$ = - 21.5 (c=0.6, CH_3OH); ^1H NMR (CD_3OD): 7.37 (CH, aromat.), 5.15 (CH_2-benzyl), 4.48 (d, $J_{1/2}$ 8.4 Hz, 1 H, H-1), 4.44 (dd, $J_{\alpha\text{-CH}/CH_{2a}}$ 6.2 Hz, $J_{\alpha\text{-CH}/CH_{2b}}$ 4.9 Hz, 1 H, α-CH-Ser), 4.24 (d, $J_{\alpha\text{-CH}/\beta\text{-CH}}$ 5.6 Hz, 1 H, α-CH-Val), 4.03 (dd, J_{gem} 10.6 Hz, 1 H, CH_{2a}-Ser), 3.84-3.93 (m, 2 H), 3.65-3.71 (m, 2 H), 3.49 (t, 1 H, H-2), 3.30-3.36 (m, 2 H + CD_3OH), 2.14-2.19 (m, 1 H, β-CH-Val),

1.98 (s, 3 H, CH_3-acetyl), 1.51 (s, 9 H, $C(CH_3)_3$), 0.99 (2s, 6 H, CH_3-Val); ^{13}C NMR ($CDCl_3$): 174.09, 172.39, 171.92 (C=O, ester, amide), 137.1 (ipso-C, aromat.), 129.50, 129.10, 129.01 (CH, aromat.), 102.23 (C-1), 83.03 ($\underline{C}(CH_3)_3$), 78.04, 75.88, 72.11, 59.86 (C-2, C-3, C-4, C-5), 69.38 (CH_2-benzyl), 67.91 (CH_2-Ser), 62.81 (C-6), 57.14, 56.56 (α-C-Ser, α-C-Val), 32.05 (β-C-Val), 28.31 ($C(\underline{C}H_3)_3$), 23.08 (CH_3-acetyl), 19.45, 18.48 (CH_3-Val);
$C_{28}H_{45}O_{12}N_3$ x H_2O (615.68); Anal. calcd.: C 54.62, H 7.37, N 6.83. Found: C 54.61, H 7.02, N 6.56.

N-Trichloroethyloxycarbonyl-O-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-seryl-alanine tert-butyl ester (14e) : $[\alpha]_D^{22}$ = - 29.9 (c=0.3, CH_3OH); ^1H NMR (DMSO-d_6): 8.25, 7.73, 7.52 (3d, 3 H, NH-Ala, NH-Ser, NH-acetyl), 5.03, 4.94 (2d, 2 H, OH-3, OH-4), 4.77 (Cl_3CCH_2), 4.53 (t, 1 H, OH-6), 4.35 (d, $J_{1/2}$ 8.46 Hz, 1 H, H-1), 4.21-4.24 (m, 1 H), 4.06-4.10 (m, 1 H), 3.66-3.74 (m, 3 H), 3.26-3.46 (m, 3 H + HDO), 3.02-3.12 (m, 2 H), 1.81 (s, 3 H, CH_3-acetyl), 1.37 (s, 9 H, $C(CH_3)_3$), 1.23 (d, $J_{CH_3\text{-}Ala/\alpha\text{-}CH}$ 7.3 Hz, 3 H, CH_3-Ala); ^{13}C NMR (DMSO-d_6): 171.41, 169.70, 168.76 (C=O, ester, amide), 154.15 (C=O, uret.), 100.50 (C-1), 95.50 (CCl_3), 80.47 ($\underline{C}(CH_3)_3$), 77.08, 74.03, 70.61, 55.03 (C-2, C-3, C-4, C-5), 73.61 ($CCl_3\underline{C}H_2$), 66.81 (CH_2-Ser), 61.10 (C-6), 54.70, 48.43 (α-CH-Ser, α-CH-Ala), 27.55 ($C(\underline{C}H_3)_3$), 23.03 (CH_3-acetyl), 16.87 (CH_3-Ala);
$C_{21}H_{34}O_{11}N_3Cl_3$ (610.87); Anal. calcd.: C 41.29, H 5.61, N 6.88, Cl 17.41, Found: C 38.81, H 5.50, N 7.19, Cl 17.40.

N-Benzyloxycarbonyl-alanyl-[O-(2-acetamido-2-deoxy-α-D-glucopyranose)]-seryl-alanine tert-butyl ester (14f) : m.p. 185 °C; $[\alpha]_D^{22}$ = - 97.3 (c=0.6, H_2O); ^1H NMR (CD_3OD): 7.40 (m, 5 H, CH, aromat.), 5.14 (dd, J_{gem} 12.5 Hz, 2 H, CH_2-benzyl), 4.61 (t, 1 H), 4.48 (d, $J_{1/2}$ 8.4 Hz, 1H, H-1), 4.27, 4,19 (2q, $J_{\alpha\text{-}CH\text{-}Ala_1/CH_3\text{-}Ala_2}$ 7.3 Hz, $J_{\alpha\text{-}CH\text{-}Ala_2/CH_3\text{-}Ala_2}$ 7.1 Hz, 2 H, α-CH-$Ala_{1/2}$), 3.93-4.00 (m, 3 H), 3.67-3.75 (m, 2 H), 3.48 (ddd, 1H), 2.02 (s, 3 H, CH_3-acetyl), 1.50 (s, 9 H, $C(CH_3)_3$), 1.41 (d, 3 H, CH_3-Ala_2), 1.38 (d, 3 H, CH_3-Ala_1); ^{13}C NMR (CD_3OD): 175.68, 174.05, 173.23, 173.39 (C=O, ester, amide), 158.46 (C=O, uret.), 138.14 (ipso-C, aromat.), 129.52, 129.01, 128.76 (CH, aromat.), 101.82 (C-1), 82.81 ($\underline{C}(CH_3)_3$, 78.15, 76.14, 72.05, 57.11 (C-2, C-3, C-4, C-5), 68.61 (CH_2-benzyl), 67.72 (CH_2-Ser), 62.71 (C-6), 54.20, 52.30, 50.46 (2 x α-CH, α−CH-Ser), 28.22 ($C(\underline{C}H_3)_3$), 23.16 (CH_3-acetyl), 18.07, 17.52 (2 x CH_3-Ala);
$C_{29}H_{46}O_{13}N_4$ x H_2O (658.71); Anal. calcd.: C 52.87, H 7.03, N 8.50. Found: C 52.80, H 7.04, N 8.49.

General procedure : **Enzymatic Galactosylation of the O-Glycopeptides 14a-f Catalyzed by β-1,4-Galactosyltransferase.** In a 2 ml microcentrifuge tube (Eppendorff) 30 μmol of the O-deprotected glycopeptide and UDP-glucose (40 mg, 60 μmol) were dissolved in 800 μl of cacodylate buffer (50 mM, pH 7.4, containing 1 mg bovine serum albumine, $MnCl_2$ (0.1 mmol) and NaN_3 (0.3 mmol)). 1.5 U UDP-glucose-4-epimerase and 6 U of alkaline phosphatase from calf intestinal mucosa were added and a small aliquote was taken. The reaction was started by the addition of 0.5 U β-1,4-galactosyltransferase and the tube was gently shaken at 37 °C until no further conversion was indicated by t.l.c (n-propanol / acetic acid / water 30:4 :1). After centrifugation, the remaining solution was lyophilised, acetylated with pyridine / acetic anhydride (3 ml, 2 : 1) for 12 h and evaporated to dryness *in vacuo*. Purification was achieved by preparative HPLC (methanol / water).

Alternatively, the valine derivative **19d** was purified without acetylation by gel chromatography on Sephadex G 15 using water as eluent.

N-Benzyloxycarbonyl-O-[3,6-di-O-acetyl-4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranosyl]-seryl-alanine tert-butyl ester (18a): Yield : 52 %; $[\alpha]_D^{22}$ = + 4.4 (c=1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, only assignable signals given): 7.32 (m, 5 H, CH, aromat.), 7.04 (broad, 1 H, NH-Ala), 6.45 (d, 1 H, NH-acetyl), 5.76 (broad, 1 H, NH-uret.), 5.03-5.11 (m, 4 H, CH_2-benzyl, H-4′, H-3), 4.88 (t, $J_{3/4}$ 9.4 Hz, 1 H, H-3), 2.10, 2.08, 2.06, 2.05, 2.04, 2.03 (CH_3-O-acetyl), 1.86 (s, 3 H, CH_3-N-acetyl), 1.45 (s, 9 H, $C(CH_3)_3$), 1.33 (d, $J_{CH3\text{-}Ala/\alpha\text{-}CH}$ 7.1 Hz, 3 H, CH_3-Ala); ^{13}C NMR ($CDCl_3$): 171.99, 171.36, 171.18, 170.96, 170.68, 169.15 (C=O, ester, amide), 156.2 (C=O, uret.), 136.06 (ipso-C, aromat.), 128.52, 128.21, 128.04 (CH, aromat.), 101.19, 101.09 (C-1, C-1′), 82.23 ($\underline{C}(CH_3)_3$, 73.45, 73.21, 72.78, 72.51, 72.24, 68.43, 53.97 (C-2 ---> C-5, C-2′- C-5′), 68.88 (CH_2-benzyl), 67.14 (CH_2-Ser), 62.63, 62.32 (C-6, C-6′), 53.44, 48.99 (α-CH-Ser, α-CH-Ala), 27.95 ($C(\underline{C}H_3)_3$), 23.07 (CH_3-N-acetyl), 20.90, 20.79 (CH_3-O-acetyl), 18.23 (CH_3-Ala).

N-2,2,2-Trichloroethyloxycarbonyl-O-[3,6-di-O-acetyl-4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranosyl]-seryl-alanine tert-butyl ester (18b): Yield : 45 %; $[\alpha]_D^{22}$ = + 4.4 (c=1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): 6.93 (d, $J_{NH\text{-}Ala/a\text{-}CH\text{-}Ala}$ 6.98 Hz, 1H, NH-Ala), 6.04, 5.98 (2d, 2 H, NH-uret., NH-acetyl), 5.33 (d, $J_{4/3}$ 2.9 Hz, 1 H, H-4′), 5.03-5.12 (m, 2 H, H-3, H-2′), 4.95 (dd, $J_{3'/4'}$ 3.3 Hz, $J_{3'/2'}$ 10.4 Hz, 1 H, H-3′), 4.71 (s, 2 H, CH_2-uret.), 4.37-4.51 (m, 5 H, H-1′, α-CH-Ala, α-CH-Ser), 3.99-4.15 (m, 5 H), 3.77-3.87 (m, 2 H), 3.68 (m, 1 H, H-2), 3.66 (m, 1 H, H-5), 2.13, 2.09, 2.05, 2.04, 1.95, 1.92 (6s, 21 H, CH_3-O/N-acetyl), 1.46 (s, 9 H, $C(CH_3)_3$), 1.37 (d, $J_{CH3\text{-}Ala/\alpha\text{-}CH\text{-}Ala}$ 7.1 Hz, 1 H, CH_3-Ala); ^{13}C NMR ($CDCl_3$): 170.69, 170.49, 169.99, 169.55 (C=O, ester, amide), 155.3 (C=O, uret.), 101.35, 101.07 (C-1, C-1′), 95.34 (CCl_3), 82.35 ($\underline{C}(CH_3)_3$), 75.80, 73.08, 72.80, 70.88, 70.80, 69.15, 66.65, 54.3 (C-2, C-2′, C-3, C-3′, C-4, C-4′, C-5, C-5′), 82.35 ($\underline{C}(CH_3)_3$), 74.75 ($\underline{C}H_2CCl_3$), 68.4 (CH_2-Ser), 62.17, 60.79 (C-6, C-6′), 53.60, 49.04 (α-CH-Ser, α-CH-Ala), 28.00 ($C(\underline{C}H_3)_3$), 23.28 (CH_3-N-acetyl), 20.79, 20.60, 20.47 (CH_3-O-acetyl), 18.39 (CH_3-Ala).

N-Benzyloxycarbonyl-O-[2-acetamido-2-deoxy-4-(β-D-galactopyranosyl)-α-D-glucopyrano-syl]-seryl-valine-tert-butyl ester (18c) : Yield : 59 %; $[\alpha]_D^{22}$ = - 21.5 (c=0.6, CH_3OH); ^1H NMR (CD_3OD): 7.39 (m, 5 H, CH, aromat.), 5.15 (dd, J_{gem} 12.4 Hz, 2 H, CH_2-benzyl), 4.50 (d, $J_{1/2}$ 8.2 Hz, 1 H, H-1), 4.41-4.49 (m, $J_{1'/2'}$ 7.7 Hz, 2 H, H-1), 4.24 (d, $J_{\alpha\text{-}CH\text{-}Val/\beta\text{-}CH\text{-}Val}$ 5.5 Hz, 1 H, α-CH-Val), 3.85-4.04 (m, 6 H), 3.57-3.75 (m, 6 H), 3.23-3.52 (m), 2.15-2.20 (m, 1 H, β-CH-Val), 1.97 (s, 3 H, CH_3-N-acetyl), 1.51 (s, 9 H, $C(CH_3)_3$), 0.99 (2s, 6 H, 2 x CH_3-Val); ^{13}C NMR (CD_3OD): 172.90, 172.35, 171.96 (C=O, ester, amide), 156.30 (C=O, uret.), 136.40 (ipso-C, aromat.), 129.53, 129.13, 129.03 (CH, aromat.), 104.66, 102.27 (C-1), 83.10 ($\underline{C}(CH_3)_3$), 81.12, 78.21, 77.96, 76.72, 75.00, 74.07 (C-2 ---> C-5, C-2′--->C-5′), 71.46 (CH_2-benzyl), 62.51 (CH_2-Ser), 62.03, 59.92 (C-6, C-6′), 56.78, 56.50 (α-CH-Ser, α-CH-Val), 32.08 (β-CH-Val), 28.35 ($C(\underline{C}H_3)_3$), 23.07 (CH_3-N-acetyl), 19.44, 18.51 (CH_3-Val).

N-Benzyloxycarbonyl-alanyl-O-[3,6-di-O-acetyl-4-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosyl)-2-acetamido-2-deoxy-α-D-glucopyranosyl]-seryl-alanine tert-butyl ester (18d):

Yield : 56 %; $[\alpha]_D^{22}$ = + 4.4 (c=1.1, $CHCl_3$); ^1H NMR ($CDCl_3$, only assignable signals given): 7.30-7.35 (m, 5 H, CH, aromat.), 7.08, 7.03 (2d, 2 H, NH-Ser, NH-Ala), 6.78 (d, 1 H, NH-acetyl), 5.84 (d, 1 H, NH-uret.), 5.04-5.14 (m, 4 H, H-4´, H-3, CH_2-benzyl), 4.88 (dd, $J_{3'/4'}$ < 1.5 Hz, $J_{3'/2'}$ 9.7 Hz, 1 H, H-3´), 4.61 (dd, 1 H), 2.10, 2.07, 2.06, 2.04 (4s, 18 H, CH_3-O-acetyl), 1.93 (s, 3 H, CH_3-N-acetyl), 1.43 (s, 9 H, $C(CH_3)_3$), 1.37, 1.30 (2d, 6 H, 2 x CH_3-Ala); ^{13}C NMR ($CDCl_3$): 173.08, 171.81, 171.24, 171.08, 170.88, 170.59, 168.75 (C=O, ester, amide), 156.29 (C=O, uret.), 136.41 (ipso-C, aromat.), 128.56, 128.03, 127.71 (CH, aromat.), 101.52, 101.28 (C-1, C-1´), 82.14 ($\underline{C}(CH_3)_3$), 76.64, 73.42, 72.74, 72.57, 72.27, 68.45, 53.37 (C-2 ---> C-5, C-2´---> C-5´), 68.50 (CH_2-benzyl), 66.92 (CH_2-Ser), 62.46, 62.29 (C-6, C-6´), 52.64, 50.66, 49.03 (α-CH-Ser, 2 x α-CH-Ala), 27.97 ($C(\underline{C}H_3)_3$), 23.25 (CH_3-N-acetyl), 20.98, 20.93, 20.86 (CH_3-O-acetyl), 18.11 (2 x CH_3-Ala).

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