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Chemoenzymatic Synthesis of *O*-Glycopeptides Carrying the Tumor Associated T_N-Antigen Structure

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Abstract—The application of the lipase-catalyzed C-terminal deprotection of heptyl esters for the construction of acid- and base-labile *O*-glycopeptides carrying the characteristic structural element of the tumor associated T_N-antigen (GalNAc α→Ser/Thr) is described

Introduction

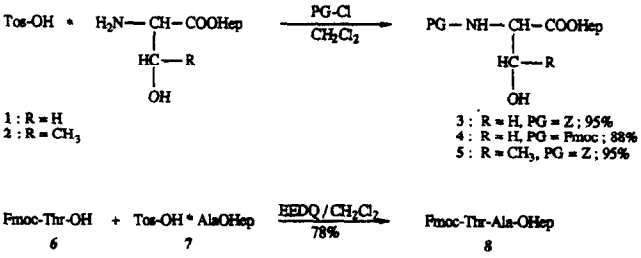
O-Glycoproteins exhibit important roles in numerous biological and immunological processes, e.g. they operate as surface receptors or as recognition components in the communication between cells, and they have also been described as tumor associated antigens. The investigation of biological processes in which glycoproteins are involved requires *O*-glycopeptides which contain the characteristic linkage region between the peptide and the carbohydrate part. However, the structural complexity and multifunctionality as well as the acid- and base-lability of the *O*-glycopeptides demand the application of numerous protecting groups which have to be orthogonally stable to each other and can selectively be removed under almost neutral conditions.[1] Enzymes often operate at neutral, weakly acidic or weakly basic p*H*-values and may combine a high selectivity for the reactions they catalyze together with a broad substrate tolerance. Therefore, enzymatic protecting group techniques[2] offer viable alternatives to classical chemical methods.[1] Thus, we have demonstrated that the N-terminal amino function and the C-terminal carboxy group of peptides can be selectively deprotected by means of penicillin G acylase[3] and lipases,[4] respectively. The lipase-mediated removal of heptyl (Hep) esters can advantageously be applied for the construction of model-*O*-glycopeptides.[5] It should be noted that proteases which, however, can attack peptide bonds have already been employed for the deprotection of glycosylated amino acids and peptides.[6]

This paper describes the application of the lipase-catalyzed C-terminal deprotection in the construction of acid- and base-labile *O*-glycopeptides carrying the characteristic structural element of the tumor associated T_N-antigen (GalNAc α→Ser/Thr).[1]

Dedicated to Prof. Dr Hans-Dieter Jakubke on the occasion of his 60th birthday.

Results and Discussion

To construct the required glycosyl acceptors **3**, **4** or **5** serine-**1** or threonine heptyl ester hydrotosylate **2** were protected at the N-terminus according to established procedures (Scheme I). In addition, the protected dipeptide **8** was prepared from the selectively blocked amino acids **6** and **7** by using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) as condensating reagent.[7]



Scheme I.

The serine- and threonine derivatives **3–5** and **8** were *O*-glycosylated by applying the thioglycoside **9** as glycosyl-donor in the presence of dimethyl(methylthio)-sulfoniumtriflate (DMTST).[8] The glycoconjugates **10–13** were formed in high yields as mixtures of anomers (α:β ratio ca. 2.5:1) which could be separated by flash chromatography. The 2-azido-α-glycoside **10a** was converted to the acetamido compound **14** by treatment with thioacetic acid.[9]

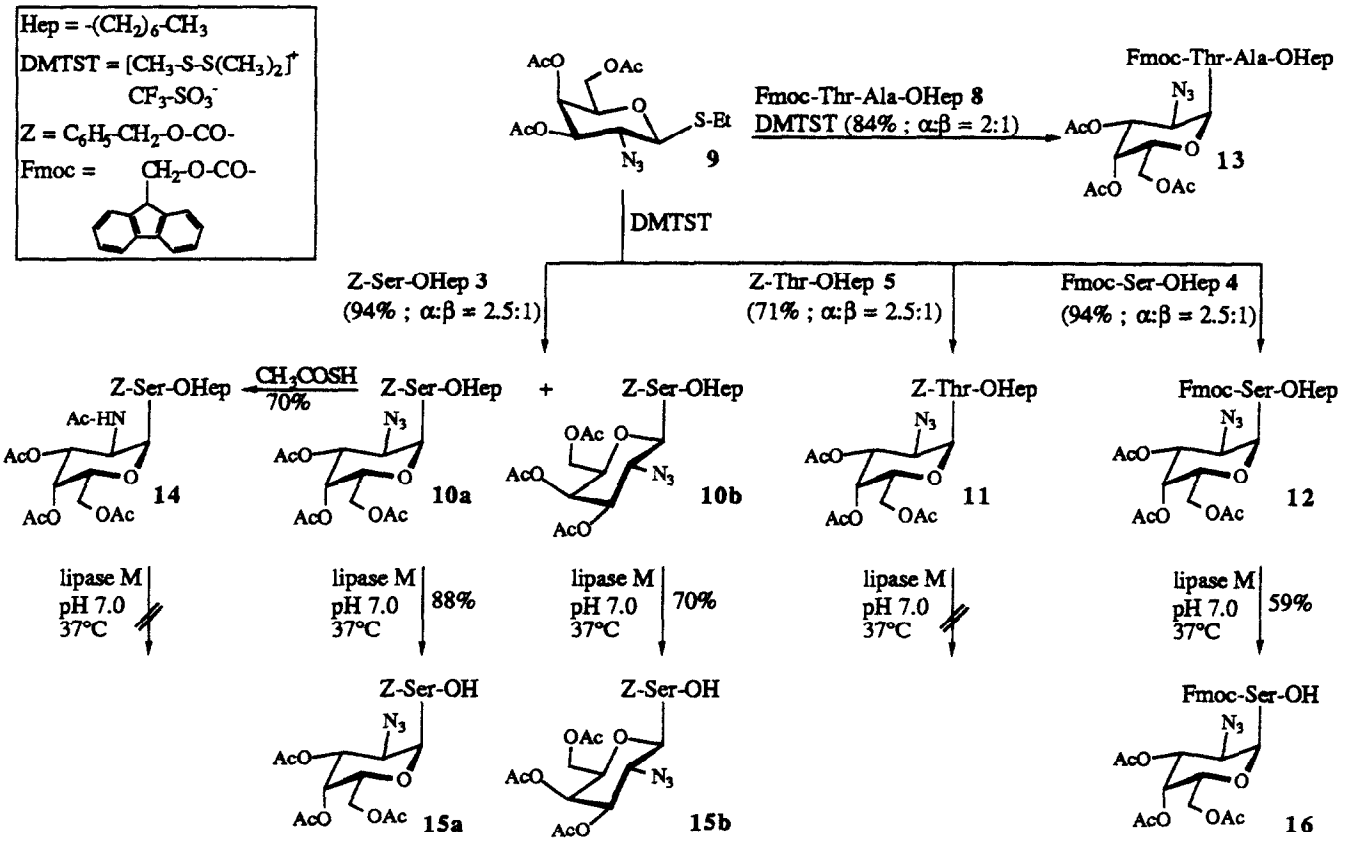
For the removal of the Hep-ester group from the glycoconjugates **10–14**, 15 different lipases originating from various biological sources were investigated (Table 1). These enzymes are commercially available, inexpensive and often do not require water soluble substrates since they preferentially operate at the interface between water and organic media. As lipases in general do not exhibit proteolytic activity, their application appears to be advantageous for the construction of peptides. In contrast, if proteases are employed for the removal of blocking

groups from peptides and glycopeptides hydrolysis of peptide bonds may occur.[2,6c]

Out of the 15 biocatalysts investigated, lipase M (Amano) from *Mucor javanicus* showed the most advantageous properties for the selective hydrolysis of the glycosylated amino acid heptyl esters. The enzyme accepts **10a, 10b** and **12** as substrates and selectively deblocks the C-terminal carboxy function at p*H* 7 and 37 °C in high yields to furnish the free acids **15a, 15b** and **16** on a mmol scale (Scheme II). In these enzymatic processes undesired reactions were not observed. The *N*-terminal urethanes, the azido functions, the base labile acetyl groups and the acid- and base-labile glycosidic linkages remained totally unaffected. The isolation of the acids **15** and **16** was achieved easily by simple extraction and subsequent chromatography. The obtained results demonstrate that the Z-protected serine glycosides **15** were obtained in higher yield than the corresponding Fmoc-derivative **16**. This is traced back to the less advantageous emulsifying properties caused by the hydrophobic Fmoc group which make compound **12** less accessible to the enzyme than **10**.

Table 1. Lipases investigated for the enzymatic removal of the heptyl esters from the glycopeptides **10–14**

name of lipase	origin
lipase AP (Amano)	*Aspergillus niger*
lipase M-AP (Amano)	*Mucor javanicus*
lipase P	*Pseudomonas fluorescens*
ipase F-AP (Amano)	*Rhizopus javanicus*
lipase N (Amano)	*Rhizopus niveus*
lipase AY (Amano)	*Candida cylindracea*
lipase G (Amano)	*Penicillium cyclopium*
lipase R (Amano)	*Penicillium roqueforti*
lipase from *Rhizopus niveus* (Fluka)	*Rhizopus niveus*
lipase from *Rhizopus delemar*	*Rhizopus delemar*
CCL (Sigma)	*Candida cyllindracea*
lipase WG (Sigma)	wheat germ
PPL (Sigma)	porcine pancreas
lipase from *Rhizopus arrhizus* (Boehringer Mannheim)	*Rhizopus arrhizus*
esterase: PLE-A (Amano)	pig liver

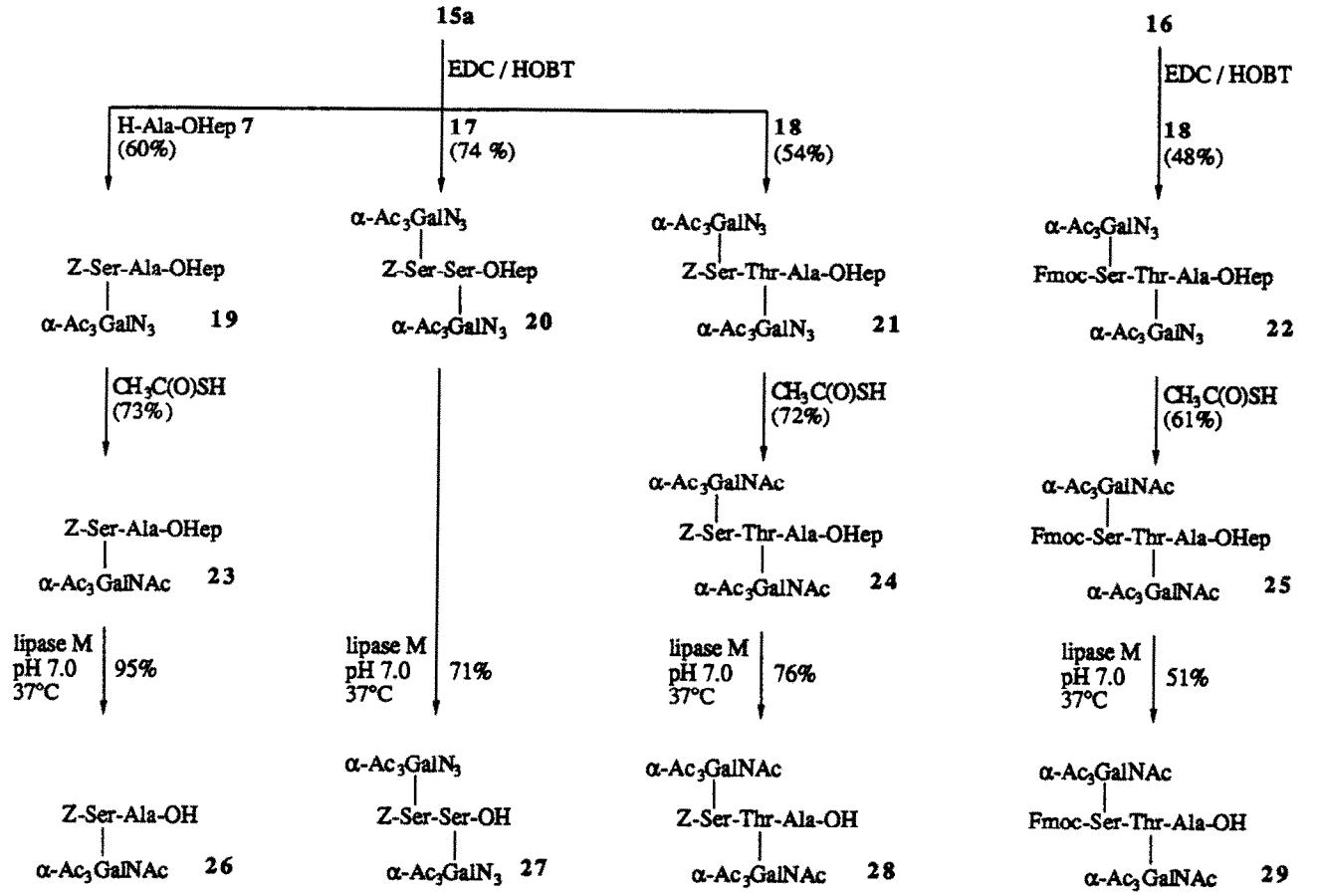


Scheme II.

Surprisingly, the threonine heptyl ester **11** was not hydrolyzed by any of the enzymes investigated. Since the solubility of **11** is comparable to **10**, the enhanced steric demand of the threonine residue in comparison to serine seems to be responsible for the inferior substrate properties. Also, the serine glycoside **14**, which differs from **10** only in the presence of an acetamido group instead of an azido function, could not be deprotected enzymatically by lipases. In this case, solubility problems cannot be responsible for the failure of the biocatalyzed transformation. Rather, the change in the preferred conformation of **14** compared to **10a** may be the reason, probably caused by a hydrogen bond between the acetamido-NH and the carbonyl C=O of the Hep ester or the urethane group. The removal of the Hep ester from the glycoside **14** was only possible by employing papain, according to a procedure described by Cantacuzène *et al.*[6b]

Whereas in all lipase-catalyzed transformations investigated for **10–12** no attack on the *O*-acetates of the carbohydrate occurred, from **14** the *O*-acetyl groups could be removed selectively by means of a lipase from wheat germ. In turn, the selective removal of *O*-acetates from carbohydrates using this enzyme proceeded without attack on the C-terminal ester. Furthermore the respective orthogonal cleavage of the Hep esters with papain without affecting the sugar protecting groups was also successful for more hydrophilic serine and threonine derivatives.[10]

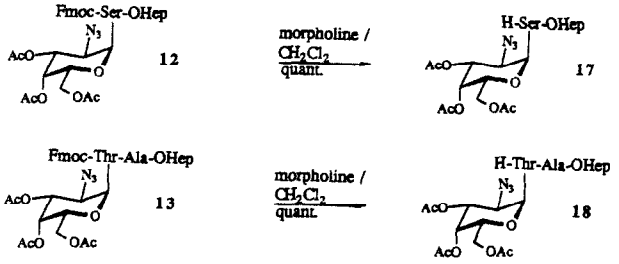
To construct complex multiple glycosylated glycopeptides, carrying the α-GalNAc→Ser(Thr) structural element characteristic for the tumor associated T_N antigen, the α-glycosides **15a** and **16** were converted into the glycopeptides **19–22** using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 1-hydroxybenzotriazole (HOBT) (Scheme III).[11, 12]



Scheme III.

The N-terminally deprotected glycoconjugates **17** and **18** required for this purpose were obtained from the fully protected Fmoc-glycoconjugates **12** and **13** by treatment with morpholine (Scheme IV).[13]

Deblocked compounds **17** and **18** were not isolated. After evaporation *in vacuo* of the morpholine they were used for the chain elongation reactions. The glycopeptides **19**, **21** and **22** carrying azido functions in the carbohydrate parts were converted to the respective acetamido compounds **23–25** by treatment with thioacetic acid.[9] The glycopeptide heptyl ester **23** and the multiple glycosylated esters **20**, **24** and **25** were then selectively deprotected at the C-terminus in high yields on a 100 mg – 1 g scale by applying lipase M (Scheme III). Even from these sterically demanding and sensitive substrates, the enzyme exclusively hydrolyzes the heptyl ester. Neither the urethanes and the amide bonds in the peptide part nor the *O*-acetates and the amides in the carbohydrate portions were attacked. Furthermore, the reaction conditions (p*H* 7, 37 °C) once more are so mild that the acid- and base-labile *O*-glycosides remained unaffected, i.e. neither β-elimination of the carbohydrates nor an anomerization of the glycosides was observed.



Scheme IV.

The successful hydrolysis of **20** which contains two hexoses attached to two amino acids which are immediately linked to each other is of particular interest. This example highlights that the lipase may accept even sterically very demanding serine-glycopeptides. Compounds **24** and **25** embody a glycosylated serine as well as a threonine $Ac_3GalNAc$ glycoside. In both cases, the presence of an additional alanine moiety ensures that neither the threonine γ-CH_3 nor the carbohydrate NH–Ac group prevent a successful enzymatic hydrolysis *(vide supra)*. Analogously to **12** and **10a** (see Scheme II), the Fmoc-protected glycopeptide **25** was deprotected in a lower yield than the corresponding *Z*-glycopeptide **24**. Once more the high hydrophobicity of the Fmoc-groups causes a substantial reduction of the solubility of the substrate and thus a limited accessibility of **25** to the lipase.

In conclusion, the enzymatic deprotection technique described in this paper can advantageously be applied for the construction of complex multifunctional and sensitive *O*-glycopeptides carrying the characteristic linkage region of the T_N antigen. The selectively deprotected glycopeptides **26–29** obtained by this technique are valuable intermediates for the construction of more complex glycoconjugates. Their application in the chemoenzymatic synthesis of oligopeptides embodying the determining linkage region of tumor-associated antigens will be reported on in due course.

Experimental Section

The general methods used have been described previously.[3–5] Lipase from *Mucor javanicus* was obtained from Amano Pharmaceutical Company.

N-*Benzyloxycarbonyl-L-serine heptyl ester 3*

To a solution of 9.4 g (0.025 mol) serine heptyl ester hydro-*p*-toluenesulfonate[4] **1** and 6.5 g (0.05 mol) *N*-ethyldiisopropylamine in 200 ml of dichloromethane is added dropwise 4.2 g (0.025 mol) of benzyl chloroformate at 0 °C within 30 min. After stirring at 0 °C for 1 h and at r.t. for 14 h the reaction mixture is extracted three times with 50 ml of 0.2 M HCl, 50 ml of $NaHCO_3$ solution and 50 ml of water. The organic layer is dried with $MgSO_4$ and concentrated *in vacuo*. The product is obtained from the residue by flash chromatography using petroleum ether/ethyl acetate (3:1, v/v) as eluent. Yield: 8.0 g (95%), waxy solid, $[\alpha]_{22}^{D}$ = +8.5° (c = 1.0, $CHCl_3$), R_f = 0.40 (petroleum ether/ethyl acetate 1:2). 90-MHz ^{1}H-NMR (DMSO-d_6): δ = 7.3 (m, 6H, NH, C_6H_5-), 5.0 (s, 2H, $C\underline{H}_2$–C_6H_5), 4.9 (t, 1H, OH), 4.2–3.9 (m, 3H, α-CH, OCH_2 Hep), 3.6 (m, 2H, β-CH_2 Ser), 1.7–1.1 (m, 10H, CH_2–$(C\underline{H}_2)_5$–CH_3), 0.85 (m, 3H, CH_3 Hep). $C_{18}H_{27}NO_5$ (337.4). Anal. calcd: C 64.07, H 8.07, N 4.15. Found: C 64.02, H 7.95, N 3.99.

N-*(9-Fluorenylmethoxycarbonyl)-L-serine heptyl ester 4*

To a solution of 12.0 g (0.032 mol) of serine heptyl ester-hydro-*p*-toluenesulfonate[4] **1** and 8.27 g (0.064 mol) *N*-ethyl-diisopropylamine in 200 ml of dichloromethane is added successively 8.38 g (0.032 mol) of 9-fluorenylmethyl chloroformate at 0 °C within 30 min. After stirring at 0 °C for 1 h and at r.t. for 14 h the reaction mixture is extracted three times with 50 ml of 0.2 M HC1, 50 ml of $NaHCO_3$ solution and 50 ml of water. The organic layer is dried with $MgSO_4$ and concentrated *in vacuo*. The product is obtained from the residue by flash chromatography using petroleum ether/ethyl acetate (3:1, v/v) as eluent. Yield: 12.0 g (88%), m.p.: 78 °C, $[\alpha]_{22}^{D}$ = +5.4° (c = 1.0, $CHCl_3$), R_f = 0.14 (petroleum ether/ethyl acetate 3:1). 200-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.77–7.25 (m, 8H, aromatic H), 5.80 (d, 1H, *J* = 6.8 Hz, NH), 4.43–4.39 (m, 3H, α-CH, OCH_2 Fmoc), 4.25–4.14 (m, 3H, OCH_2 Hep, 9-H Fmoc), 3.94 (bs, 2H, β-CH_2 Ser), 2.38 (bs, 1H, OH), 1.64 (m, 2H, OCH_2–$C\underline{H}_2$- Hep), 1.27 (m, 8H, -$(C\underline{H}_2)_4$–CH_3), 0.86 (m, 3H, CH_3 Hep). $C_{25}H_{31}NO_5$ (425.5). Anal. calcd: C 70.57, H 7.34, N 3.29. Found: C 71.06, H 7.37, N 3.16.

N-*Benzyloxycarbonyl-L-threonine heptyl ester 5*

As described for **3**, the protected threonine ester **5** (10.0 g, 95% yield) is obtained from 6.6 g (0.03 mol) of threonine heptyl ester toluenesulfonate[4] **2**. Oil, $[\alpha]_{22}^{D}$ = -8.9° (c = 1.0, $CHCl_3$), R_f = 0.45 (petroleum ether/ethyl acetate 1:2). 90-MHz ^{1}H-NMR (DMSO-d_6): δ = 7.3 (bs, 5H, C_6H_5-), 7.1 (d, 1H, NH), 5.0 (s, 2H, $C\underline{H}_2$–C_6H_5), 4.8 (t, 1H, OH), 4.0 (m, 4H, α-CH, β-CH, OCH_2 Hep), 1.8–1.0 (m, 13H, CH_2–$(C\underline{H}_2)_5$–CH_3, CH_3 Thr), 0.8 (m, 3H, CH_3 Hep). $C_{19}H_{29}NO_5$ (351.4). Anal. calcd: C 64.93, H 8.32, N 3.99. Found: C 65.18, H 8.19, N 4.13.

N-*(9-Fluorenylmethoxycarbonyl)-L-threonyl-L-alanine heptyl ester 8*

To a solution of 2.1 g (5.9 mmol) alanine heptyl ester hydro-*p*-toluoenesulfonate[4] **7** and 0.76 g (5.9 mmol) *N*-ethyl-diisopropylamine in 20 ml of dichloromethane, a solution of 2.0 g (5.9 mmol) Fmoc-threonine **6** and 2.2 g (8.8 mmol) 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) in 20 ml of dichloromethane is added. After stirring for 14 h at r.t. the reaction mixture is worked up as described for **3**. Yield: 2.33 g (78%), m.p.: 106 °C, $[\alpha]_{22}^{D}$ = -26.5° (c = 1.0, $CHCl_3$), R_f = 0.43 (petroleum ether/ethyl acetate 1:1). 90-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.80–7.25 (m, 8H, aromatic H), 6.80 (d, 1H, NH Ala),

5.72 (d, 1H, NH Ser), 4.61–4.05 (m, 8H, α-CH Ser, α-CH Thr, β-CH Thr, O–CH_2 Fmoc, 9-H Fmoc, OCH_2 Hep), 3.17 (broad, 1H, OH), 1.58 (m, 2H, OCH_2–CH_2-Hep), 1.39 (d, J = 7.3 Hz, 3H, CH_3 Ala), 1.29 (m, 8H, -$(CH_2)_4$–CH_3), 1.17 (d, J = 6.8 Hz, 3H, CH_3 Thr), 0.86 (m, 3H, CH_3 Hep). $C_{29}H_{38}N_2O_6$ (510.63), Anal. calcd: C 68.21, H 7.50, N 5.49. Found: C 67.88, H 7.47, N 5.94.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α- and β-D-galactopyranosyl)-L-serine heptyl ester ***10a/10b***

To a solution of 3.75 g (0.01 mol) ethyl-3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranoside **9** and 3.37 g (0.01 mol) *Z*-Ser-*O* Hep **3** in a mixture of 10 ml CH_2Cl_2 and 10 ml toluene are added 4 g molecular sieves 4 Å. After stirring for 1 h at r.t. 3.87 g (0.015 mol) dimethyl(methylthio)sulfoniumtriflate (DMTST) was added. After stirring for an additional 16 h the reaction mixture is neutralized by addition of 1.94 g (0.015 mol) of *N*-ethyldiisopropylamine, filtered and concentrated *in vacuo*. The anomers **10a** and **10b** are isolated by flash chromatography of the residue in a combined yield of 6.12 g (94%).

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine heptyl ester ***10a***

4.36 g (67%), amorphous, $[\alpha]_{22}^{D}$ = +88.9° (c = 1.0, $CHCl_3$), R_f = 0.58 (petroleum ether/ethyl acetate 2:1). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.3 (m, 5H, C_6H_5-), 5.81 (d, J = 7.9 Hz, 1H, NH), 5.39 (dd, $J_{3,4}$ = 2.7 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.23 (dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.3 Hz, 1H, 3-H), 5.13 (d, J = 12.2 Hz, 1H, OCH_{2a}–C_6H_5), 5.01 (d, J = 12.2 Hz, 1H, OCH_{2b}–C_6H_5), 4.92 (d, $J_{1,2}$ = 3.5 Hz, 1H, 1-H), 4.52 (m, 1H, α-CH Ser), 4.14 (m, 3H, OCH_2 Hep, 5-H), 4.07–3.96 (m, 4H, 6-$H_{a/b}$, β-CH_2–Ser), 3.58 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 11.2 Hz, 1H, 2-H), 2.10, 2.01, 1.98 (3s, 9H, 3 CH_3CO), 1.62 (m, 2H, OCH_2–CH_2- Hep), 1.26 (m, 8H, -$(CH_2)_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 50.3-MHz ^{13}C-NMR ($CDCl_3$): δ = 170.20, 169.8, 169.5 (CO), 156.0 (CO, urethane), 136.0 (*ipso*-C), 128.10, 127.99 (aromatic C), 99.05 (C-1), 69.54, 67.05, 66.19, (CH_2–C_6H_5, O–CH_2 Hep, β-C Ser), 67.93, 67.51, 67.22 (C-3, C-4, C-5), 61.62 (C-6), 57.43 (C-2), 54.48 (α-C Ser), 31.60, 28.76, 28.42, 25.69, 22.48 (5 CH_2 Hep), 20.46 (CH_3CO), 13.93 (CH_3 Hep). $C_{30}H_{42}N_4O_{12}$ (650.7). Anal. calcd: C 55.38, H 6.51, N 8.61. Found: C 55.45, H 6.64, N 8.44.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-L-serine heptyl ester ***10b***

1.76g (27%), amorphous, $[\alpha]_{22}^{D}$ = -12.9° (c = 1.1, $CHCl_3$), R_f = 0.48 (petroleum ether/ethyl acetate 2:1) 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.32 (m, 5H, C_6H_5-), 5.75 (d, J = 8.0 Hz, 1H, NH), 5.27 (dd, $J_{3,4}$ = 2.6 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.11 (s, 2H, OCH_2–C_6H_5), 4.71 (dd, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 3.4 Hz, 1H, 3-H), 4.50 (m, 1H, α-CH Ser), 4.30 (dd, J_1 = 2.94 Hz, J_2 = 10.38 Hz, 1H, β-CH_{2a}–Ser), 4.27 (d, $J_{1,2}$ = 8.05 Hz, 1H, 1-H), 4.15 (t, J = 6.73 Hz, 2H, OCH_2 Hep), 4.06 (d, J = 6.74 Hz, 2H, 6-$H_{a/b}$), 3.90 (dd, J_1 = 3.34 Hz, J_2 = 10.35 Hz, 1H, β-CH_{2b}–Ser), 3.74 (ddd, $J_{4,5}$ < 1Hz, $J_{5,6a}$ = 6.7 Hz, $J_{5,6a}$ = 6.8 Hz, 1H, 5-H), 3.60 (dd, $J_{1,2}$ = 8.0 Hz, $J_{2,3}$ = 10.9 Hz, 1H, 2-H), 2.10, 2.01, 2.01 (3s, 9H, 3 CH_3CO), 1.62 (m, 2H, OCH_2–CH_2- Hep), 1.26 (m, 8H, -$(CH_2)_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 170.19, 169.87, 169.61, 169.40 (CO), 155.82 (CO, urethane), 136.26 (*ipso*-C), 128.47, 128.11, 127.99 (aromatic C), 102.49 (C-1), 70.91, 70.76, 66.20 (C-3, C-4, C-5), 69.79, 66.99, 65.98, (CH_2–C_6H_5, O–CH_2 Hep, β-C Ser), 61.08 (C-6), 60.79 (C-2), 54.33 (α-C Ser), 31.60, 28.74, 28.40, 25.61, 22.48 (5 CH_2 Hep), 20.51, 20.46 (CH_3CO), 13.94 (CH_3 Hep). $C_{30}H_{42}N_4O_{12}$ (650.7). Anal. calcd: C 55.38, H 6.51, N 8.61. Found: C 54.92, H 6.31, N 8.50.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine heptyl ester ***11***

As described for **10**, the threonine glycoside **11** is obtained in 71 % yield (854 mg) from 632 mg (1.8 mmol) of *N*-benzyloxycarbonyl-L-threonine heptyl ester **5**. α:β ratio = 2.5:1, 610 mg (51%) α-anomer, oil, $[\alpha]_{22}^{D}$ = +62.4° (c = 1.0, $CHCl_3$), R_f = 0.48 (toluene/ethyl acetate 2:1). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.37–7.27 (m, 5H, C_6H_5-), 5.51 (d, J = 9.54 Hz, 1H, NH), 5.40 (d, $J_{3,4}$ = 2.3 Hz, 1H, 4-H), 5.19 (dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.2 Hz, 1H, 3-H), 5.13 (s, 2H, OCH_2–C_6H_5), 4.98 (d, $J_{1,2}$ = 3.71 Hz, 1H, 1-H), 4.43–4.36 (m, 2H, α-CH Thr, β-CH Thr), 4.20 (dt, $J_{4,5}$ = 0.77 Hz, $J_{5,6}$ = 6.5 Hz, 1H, 5-H), 4.12 (t, J = 6.8 Hz, 2H, OCH_2 Hep), 4.05 (d, J = 6.35 Hz, 2H, 6-$H_{a/b}$), 3.62 (dd, $J_{1,2}$ = 3.68 Hz, $J_{2,3}$ = 11.20 Hz, 1H, 2-H), 2.11, 2.01, 2.00 (3s, 9H, 3 CH_3CO), 1.61 (m, 2H, OCH_2–CH_2-Hep), 1.30 (d, J = 6.44 Hz, 3H, CH_3 Thr), 1.28–1.21 (m, 8H, -$(CH_2)_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 50.3-MHz ^{13}C-NMR ($CDCl_3$): δ = 170.17, 170.06, 169.83, 169.62 (CO), 156.70 (CO, urethane), 136.15 (*ipso*-C), 128.40, 128.02, 127.90 (aromatic C), 99.29 (C-1), 76.98 (β-C Thr), 68.26, 67.39, 66.96 (C-3, C-4, C-5), 67.39, 65.99 (CH_2–C_6H_5, O–CH_2 Hep), 61.67 (C-6), 58.69, 57.72 (C-2, α-C Thr), 31.56, 28.74, 28.32, 25.68, 22.44 (5 CH_2 Hep), 20.46 (CH_3CO), 18.38 (CH_3 Thr), 13.92 (CH_3 Hep). $C_{31}H_{42}N_4O_{12}$ (664.7). Anal. calcd: C 56.02, H 6.67, N 8.43. Found: C 55.77, H 6.53, N 8.32.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine heptyl ester ***12***

As described for **10**, the serine glycoside **12** is obtained in 94% yield (4.17 g) from 2.55 g (1.8 mmol) of *N*-(9-fluorenylmethoxycarbonyl)-L-serine heptyl ester **4**. α:β ratio = 2.5:1, 2.93g (66%) α-anomer, amorphous, $[\alpha]_{22}^{D}$ = +88.4° (c = 0.5, $CHCl_3$), R_f = 0.46 (petroleum ether/ethyl acetate 2:1). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.74–7.28 (m, 8H, aromatic H), 5.94 (d, J = 7.94 Hz, 1H, NH), 5.42 (dd, $J_{3,4}$ = 2.4 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.28 (dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.2 Hz, 1H, 3-H), 4.92 (d, $J_{1,2}$ =

3.44 Hz, 1H, 1-H), 4.54 (m, 1H, α-CH Ser), 4.38 (d, J = 7.3 Hz, 2H, OCH_2 Fmoc), 4.23 (t, J = 7.28 Hz, 1H, 9-H Fmoc), 4.18 (m, 3H, OCH_2 Hep, 5-H), 4.08–3.99 (m, 4H, 6-$H_{a/b}$, β-CH_2–Ser), 3.60 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 11.2 Hz, 1H, 2-H), 2.12, 2.04, 1.94 (3s, 9H, 3 CH_3CO), 1.65 (m, 2H, OCH_2–C$\underline{H}_2$- Hep), 1.33–1.25 (m, 8H, -(C$\underline{H}_2$)$_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 170.28, 169.86, 169.62, 169.46 (CO), 155.82 (CO, urethane), 143.77, 141.77, 127.70, 127.06, 125.08, 119.94 (aromatic C), 99.17 (C-1), 67.90, 67.46, 67.21 (C-3, C-4, C-5), 69.63, 67.31, 66.28 (OCH_2 Fmoc, O–CH_2 Hep, β-C Ser), 61.61 (C-6), 57.40 (C-2), 54.45 (α-C Ser), 47.07 (C-9 Fmoc), 31.61, 28.78, 28.44, 25.70, 22.50 (5 CH_2 Hep), 20.54, 20.49 (C$\underline{H}_3$CO), 13.97 (CH_3 Hep). $C_{37}H_{46}N_4O_{12}$ (738.8). Anal. calcd: C 60.15, H 6.28, N 7.58. Found: C 60.29, H 6.28, N 7.42.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine heptyl ester ***13***

As described for **10**, the threonine glycopeptide **13** is obtained in 84% yield (2.1 g) from 1.5 g (3.0 mmol) *N*-(9-fluorenylmethoxycarbonyl)-L-threonyl-L-alanine heptyl ester **8**. α:β ratio = 2:1, 1.4 g (56%) α-product, m.p.: 78 °C, $[\alpha]_{22}^{D}$ = +50.2° (c = 0.51, $CHCl_3$), R_f = 0.54 (toluene/ethyl acetate 5:2). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.74–7.26 (m, 9H, aromatic H, NH Ala), 5.95 (d, J = 6.48 Hz, 1H, NH Thr), 5.42 (d, $J_{3,4}$ = 2.4 Hz, 1H, 4-H), 5.34 (dd, $J_{2,3}$ = 10.88 Hz, $J_{3,4}$ = 3.17 Hz, 1H, 3-H), 5.24 (d, $J_{1,2}$ = 3.62 Hz, 1H, 1-H), 4.51 (m, 1H, α-CH Ala), 4.36 (d, J = 7.15 Hz, 2H, OCH_2 Fmoc), 4.32–4.04 (m, 8H, α-CH Thr, β-CH Thr, 9-H Fmoc, 5-H, 6-$H_{a/b}$, OCH_2 Hep), 3.92 (dd, $J_{1,2}$ = 3.65 Hz, $J_{2,3}$ = 10.85 Hz, 1H, 2-H), 2.13, 2.03, 2.00 (3s, 9H, 3 CH_3CO), 1.60 (m, 2H, OCH_2–C$\underline{H}_2$- Hep), 1.39 (d, J = 6.99 Hz, 3H, CH_3 Ala), 1.38–1.19 (m, 8H, -(C$\underline{H}_2$)$_4$–CH_3), 1.20 (d, J = 6.46 Hz, 3H, CH_3 Thr), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 172.16, 170.18, 169.91, 169.39, 167.68 (CO), 155.82 (CO, urethane), 143.70, 143.57, 141.21, 127.63, 126.99, 124.95, 119.92, 119.89 (aromatic C), 97.43 (C-1), 74.92 (β-C Thr), 69.46, 67.45, 67.05 (C-3, C-4, C-5), 67.13, 65.52 (OCH_2 Fmoc, O–CH_2- Hep), 61.60 (C-6), 58.40 (C-2), 56.68 (α-C Thr), 48.35 (α-C Ala) 47.06 (C-9 Fmoc), 31.56, 28.74, 28.48, 25.63, 22.43 (5 CH_2 Hep), 20.48 (C$\underline{H}_3$CO), 17.81 (CH_3 Ala), 15.56 (CH_3 Thr), 13.91 (CH_3 Hep). $C_{41}H_{53}N_5O_{13}$ (823.9). Anal. calcd: C 59.59, H 6.48, N 8.50. Found: C 59.56, H 6.48, N 8.45.

N-Benzyloxycarbonyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine heptyl ester ***14***

A mixture of 430 mg (0.66 mmol) of the 2-azidoglycoside **10a** and 5 ml of thioacetic acid is stirred for 16 h at r.t., concentrated *in vacuo* and the 2-acetamido compound **14** is isolated by flash chromatography with petroleum ether/ethyl acetate 4:1 in 70% yield (310 mg) as an amorphous solid. $[\alpha]_{22}^{D}$ = +81.3° (c = 1.2, $CHCl_3$), R_f = 0.40 (petroleum ether/ethyl acetate 1:3). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.30 (m, 5H, C_6H_5-), 5.87 (d, J = 8.4 Hz, 1H, NH), 5.83 (d, J = 9.6 Hz, 1H, NH), 5.31 (dd, $J_{3,4}$ = 2.93 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.09 (s, 2H, OCH_2–C_6H_5), 5.05 (dd, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 3.0 Hz, 1H, 3-H), 4.79 (d, $J_{1,2}$ = 3.3 Hz, 1H, 1-H), 4.51 (m, 2H, α-CH Ser, 2-H), 4.14–3.94 (m, 6H, 6-$H_{a/b}$, OCH_2 Hep, 5-H, β-CH_{2a}–Ser), 3.84 (dd, J_1 = 3.0 Hz, J_2 = 10.4 Hz, 1H β-CH_{2b}–Ser), 2.11, 1.98, 1.94, 1.91 (4s, 12H, 4 CH_3CO), 1.59 (m, 2H, OCH_2–C$\underline{H}_2$- Hep), 1.25 (m, 8H, -(C$\underline{H}_2$)$_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 170.82, 170.34, 170.23, 170.12 (CO), 155.82 (CO, urethane), 135.94 (*ipso*-C), 128.52, 128.26, 128.09 (aromatic C), 98.91 (C-1), 69.33, 66.15 (C$\underline{H}_2$–C_6H_5, O–CH_2- Hep, β-C Ser), 68.23, 67.17 (C-3, C-4, C-5), 61.85 (C-6), 54.39 (α-C Ser), 47.55 (C-2), 31.58, 28.73, 28.43, 25.69, 22.47 (5 CH_2 Hep), 23.03 (C$\underline{H}_3$CON), 20.63, 20.61, 20.60 (C$\underline{H}_3$COO), 13.95 (CH_3 Hep). $C_{32}H_{46}N_2O_{13}$ (666.7). Anal. calcd: C 57.65, H 6.95, N 4.20. Found: C 57.64, H 6.86, N 4.28.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine ***15a***

To a solution of 4 g of lipase M (Amano) from *Mucor javanicus* in 250 ml of 0.2 M sodium phosphate buffer (p*H* = 7.0) is added a solution of 2 g (3.1 mmol) of the heptyl ester **10a** in 10 ml of acetone and the reaction mixture is shaken gently at 37 °C for 24 h. After saturation with NaCl, the aqueous phase is extracted three times with 200 ml ethyl acetate, the combined organic layers are dried with $MgSO_4$ and concentrated *in vacuo*. The residue is purified by flash chromatography using first ethyl acetate as eluent and then ethyl acetate/ethanol 8:2 for the isolation of the product. Yield: 1.50 g (88%), amorphous solid, $[\alpha]_{22}^{D}$ = +146.9° (c = 1.1, $CHCl_3$), R_f = 0.33 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (DMSO-d_6): δ = 12.88 (bs, 1H, COOH), 7.87 (d, J = 8.6 Hz, 1H, NH), 7.33 (m, 5H, C_6H_5-), 5.32 (dd, $J_{2,3}$ = 11.1 Hz, $J_{3,4}$ = 3.3 Hz, 1H, 3-H), 5.28 (dd, $J_{3,4}$ = 3.1 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.08 (d, $J_{1,2}$ = 3.5 Hz, 1H, 1-H), 5.05 (d, J = 12.6 Hz, 1H, OCH_{2a}–C_6H_5), 5.02 (d, J = 12.6 Hz, 1H, OCH_{2b}–C_6H_5), 4.36 (t, J = 6.4 Hz, 1H, 5-H), 4.32 (ddd, J_1 = 3.6 Hz, J_2 = 5.3 Hz, J_3 = 8.8 Hz, 1H, α-CH Ser), 4 04 (dd, $J_{5,6a}$ = 5.5 Hz, $J_{6a,6b}$ = 11.2 Hz, 1H, 6-H_a), 3.97 (dd, $J_{5,6b}$ = 7.2 Hz, $J_{6a,6b}$ = 11.2 Hz, 1H, 6-H_b), 3.85 (dd, J_1 = 3.4 Hz, J_2 = 9.9 Hz, 1H, β-CH_{2a} Ser), 3.80 (dd, J_1 = 5,6 Hz, J_2 = 9.9 Hz, 1H, β-CH_{2b} Ser), 3.70 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 11.1 Hz, 1H, 2-H), 2.09, 1.97, 1.94 (3s, 9H, 3 CH_3CO). 100.6-MHz ^{13}C-NMR (DMSO-d_6): δ = 171.00, 169.90, 169.69, 169.23 (CO), 156.13 (CO, urethane), 136.87 (*ipso*-C), 128.22, 127.62 (aromatic C), 97.53 (C-1), 67.73, 65.49, (C$\underline{H}_2$–C_6H_5, β-C Ser), 67.62, 67.35, 66.27 (C-3, C-4, C-5), 61.34 (C-6), 56.84 (C-2), 53.93 (α-C Ser), 20.28, 20.26 (C$\underline{H}_3$CO). $C_{23}H_{28}N_4O_{12}$ (552.5). Anal. calcd: C 50.00, H 5.11, N 10.14. Found: C 49.99, H 5.19, N 9.92.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-L-serine ***15b***

As described for **15a**, the acid **15b** is obtained from 200 mg (0.31 mmol) of the ester **10b** in 70% yield (120 mg)

as amorphous solid. $[\alpha]_{22}^{D}$ = -9.9° (c = 1.0, $CHCl_3$), R_f = 0.22 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (DMSO-d_6): δ = 12.95 (bs, 1H, COOH), 7.41 (d, J = 8.3 Hz, 1H, NH), 7.30 (m, 5H, C_6H_5-), 5.16 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.03 (s, 2H, OCH_2–C_6H_5), 4.92 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, 3-H), 4.69 (d, $J_{1,2}$ = 8.1 Hz, 1H, 1-H), 4.30 (ddd, J_1 = 4.9 Hz, J_2 = 7.2 Hz, J_3 = 7.2 Hz, 1H, α-CH Ser), 4.14 (m, 1H, 5-H), 4.04 (m, 2H, 6-$H_{a/b}$), 3.97 (dd, J_1 = 7.2 Hz, J_2 = 10.5 Hz, 1H, β-CH_{2a} Ser), 3.88 (dd, J_1 = 4.6 Hz, J_2 = 10.5 Hz, 1H, β-CH_{2b} Ser), 3.65 (dd, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 10.7 Hz, 1H, 2-H), 2.10, 1.98, 1.97 (3s, 9H, 3 CH_3CO). 50.3-MHz ^{13}C-NMR (DMSO-d_6): δ = 170.02, 169.87, 169.74, 169.21 (CO), 155.82 (CO, urethane), 136.83 (*ipso*-C), 128.23, 127.69, 127.55 (aromatic C), 100.55 (C-1), 70.43, 69.91, 66.45 (C-3, C-4, C-5), 68.35, 65.54, (CH_2–C_6H_5, β-C Ser), 61.10 (C-6), 60.38 (C-2), 53.81 (α-C Ser), 20.34, 20.27 (CH_3CO). $C_{23}H_{28}N_4O_{12}$ (552.5). Anal. calcd: C 50.00, H 5.11, N 10.14. Found: C 50.31, H 5.17, N 9.80.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine ***16***

As described for **15a**, the acid **16** is obtained from 2.00 g (2.71 mmol) of the ester **12** in 59% yield (1.02 g) as amorphous solid. $[\alpha]_{22}^{D}$ = +101.5° (c = 0.5, CH_3OH), R_f = 0.54 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (DMSO-d_6): δ = 12.90 (bs, 1H, COOH), 8.00 (d, J = 8.6 Hz, 1H, NH), 7.89–7.29 (m, 8H, aromatic H), 5.36 (dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.4 Hz, 1H, 3-H), 5.31 (d, $J_{3,4}$ = 2.9 Hz, 1H, 4-H), 5.11 (d, $J_{1,2}$ = 3.5 Hz, 1H, 1-H), 4.37–4.21 (m, 5H, OCH_2 Fmoc, 9-H Fmoc, α-CH Ser, 5-H), 3.98 (dd, $J_{5,6a}$ = 7.1 Hz, $J_{6a,6b}$ = 11.2 Hz, 1H, 6-H_a), 3.93 (dd, $J_{5,6b}$ = 5.5 Hz, $J_{6a,6b}$ = 11.3 Hz, 1H, 6-H_b), 3.88 (dd, J_1 = 3.6 Hz, J_2 = 10.0 Hz, 1H, β-CH_{2a} Ser), 3.82 (dd, J_1 = 5.9 Hz, J_2 = 10.0 Hz, 1H, β-CH_{2b} Ser), 3.70 (dd, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 11.2 Hz, 1H, 2-H), 2.10, 1.99, 1.88 (3s, 9H, 3 CH_3CO). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 171.03, 169.91, 169.67, 169.31 (CO), 156.08 (CO, urethane), 143.74, 140.63, 127.56, 126.96, 125.22, 120.01 (aromatic C), 97.48 (C-1), 67.62, 67.35, 67.29 (C-3, C-4, C-5), 65.83 (OCH_2 Fmoc, β-C Ser), 61.42 (C-6), 56.82 (C-2), 53.93 (α-C Ser), 46.56 (C-9 Fmoc), 20.33, 20.28, 20.23 (CH_3CO). $C_{30}H_{32}N_4O_{12}$ (640.6). Anal. calcd: C 56.25, H 5.04, N 8.75. Found: C 56.89, H 5.32, N 8.14.

N-*Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-L-alanine heptyl ester* ***19***

A solution of 600 mg (0.92 mmol) of the carboxylic acid **15a**, 177 mg (0.92 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 249 mg (1.84 mmol) 1-hydroxybenzotriazole (HOBT) in 10 ml of dichloromethane/DMF (9:1) is stirred for 1 h at r.t. and a solution of 332 mg (0.92 mmol) alanine heptyl ester hydrotosylate **7** and 119 mg (0.92 mmol) of *N*-ethyl-diisopropylamine in 2 ml of dichloromethane is added. After stirring for an additional 48 h the reaction mixture is extracted twice with 5 ml of 0.1 M HCl and water, and the organic layers are dried with $MgSO_4$ and concentrated *in vacuo*. From the residue the glycodipeptide ester **19** is obtained by flash chromatography in 60% yield (400 mg) as a colourless oil. $[\alpha]_{22}^{D}$ = +94.4° (c = 0.54, $CHCl_3$), R_f = 0.32 (petroleum ether/ethyl acetate 2:1). 200-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.29 (bs, 5H, C_6H_5-), 6.98 (d, J = 6.2 Hz, 1H, NH Ala), 5.81 (d, J = 7.3 Hz, 1H, NH Ser), 5.35 (m, 2H, 3-H, 4-H), 5.07 (m, 3H, 1-H, OCH_2–C_6H_5), 4.47 (m, 1H, α-CH), 4.39 (m, 1H, α-CH), 4.23 (m, 1H, 5-H), 4.06 (m, 4H, 6-$H_{a/b}$, OCH_2 Hep), 4.00 (dd, 1H, β-CH_{2a} Ser), 3.70 (m, 2H, β-CH_{2b} Ser, 2-H), 2.09, 1.99, 1.97 (3s, 9H, 3 CH_3CO), 1.58 (m, 2H, OCH_2–CH_2- Hep), 1.37 (d, J = 7.2 Hz, 3H, CH_3 Ala), 1.24 (m, 8H, -$(CH_2)_4$–CH_3), 0.81 (m, 3H, CH_3 Hep). 50.3-MHz ^{13}C-NMR ($CDCl_3$): δ = 172.40, 170.39, 170.01, 169.50, 168.77 (CO), 155.83 (CO, urethane), 136.03 (*ipso*-C), 128.52, 128.21, 128.08 (aromatic C), 98.47 (C-1), 68.29, 67.66, 67.07 (C-3, C-4, C-5), 68.29, 67.19, 65.67, (CH_2–C_6H_5, O–CH_2 Hep, β-C Ser), 61.69 (C-6), 57.79 (C-2), 53.71 (α-C Ser), 48.48 (α-C Ala), 31.65, 28.83, 28.46, 25.72, 22.53 (5 CH_2 Hep), 20.60 (CH_3CO), 17.86 (CH_3 Ala), 14.04 (CH_3 Hep). $C_{33}H_{47}N_5O_{13}$ (721.8). Anal. calcd: C 54.92, H 6.56, N 9.70. Found: C 55.11, H 6.69, N 9.57.

N-*Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine heptyl ester* ***20***

A solution of 75 mg (0.1 mmol) of the Fmoc-protected serine glycoside **12** in a mixture of 1 ml of morpholine and 1 ml of dichloromethane is stirred for 2 h at r.t. and concentrated *in vacuo* by codistillation with toluene. The residue is dissolved in 2 ml of dichloromethane and a solution of 13.8 mg (0.072 mmol) of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and 20.0 mg (0.15 mmol) of 1-hydroxybenzotriazole (HOBT) in 5 ml dichloromethane/DMF 9:1 is added. After stirring at r.t. for 72 h the reaction mixture is worked up as described for **19**. Yield: 56 mg (74%), amorphous solid, $[\alpha]_{22}^{D}$ = +134.9° (c = 0.9, $CHCl_3$), R_f = 0.21 (petroleum ether/ethyl acetate 2:1). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.37–7.27 (m, 6H, C_6H_5-, NH), 6.01 (d, J = 7.94 Hz, 1H, NH), 5.40–5.36 (m, 3H, 3-H, 4-H, 4'-H), 5.26 (dd, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 3.2 Hz, 1H, 3'-H), 5.19–5.06 (m, 3H, OCH_2–C_6H_5, 1-H), 4.94 (d, $J_{1,2}$ = 3.5 Hz, 1H, 1'-H), 4.78 (m, 1H, α-CH Ser), 4.53 (m, 1H, α-CH Ser'), 4.24 (t, J = 6.4 Hz, 1H, 5-H), 4.19 (m, 1H, 5'-H), 4.14 (t, J = 6.8 Hz, 2H, OCH_2 Hep), 4.10–4.00 (m, 6H, 6-$H_{a/b}$, 6'-$H_{a/b}$, β-CH_2 Ser), 3.96 (dd, J_1 = 3.0 Hz, J_2 = 10.2 Hz, 1H, β-CH_{2a} Ser'), 3.84 (dd, J_1 = 5.5 Hz, J_2 = 10.1 Hz, 1H, β-CH_{2b} Ser'), 3.62–3.58 (m, 2H, 2-H, 2'-H), 2.11, 2.10 (2s, 6H, 2 CH_3CO), 2.00, 1.99 (2s, 9H, 3 CH_3CO), 1.89 (s, 3H, CH_3CO), 1.63 (m, 2H, OCH_2–CH_2- Hep), 1.39–1.19 (m, 8H, $(CH_2)_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 169.96, 169.84, 169.56, 169.10, 168.97 (CO), 156.0 (CO, urethane), 136.0 (*ipso*-C), 128.52, 128.31, 128.23 (aromatic C), 98.91, 98.70 (C-1, C-1'), 69.16, 68.21, 67.43, 66.31 (CH_2–C_6H_5, O–CH_2- Hep, β-C Ser, β-C Ser'), 68.08, 67.95, 67.62, 67.38, 67.03, 67.00 (C-3, C-3', C-4, C-4', C-5, C-5'), 61.68, 61.50 (C-6, C-6'), 57.65,

57.58 (C-2, C-2'), 54.37, 52.93 (α-C Ser, α-C Ser'), 31.61, 28.80, 28.41, 25.69, 22.51 (5 CH_2 Hep), 20.53 ($\underline{C}H_3CO$), 13.97 (CH_3 Hep). $C_{45}H_{62}N_8O_{21}$ (1051.03), FAB-MS (3-NOBA): 1051.6 $(M + H)^+$ calcd: 1051.4.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine heptyl ester ***21***

As described for **20** the glycopeptide heptylester **21** is obtained in 54% yield (615 mg) from 824 mg (1.0 mmol) of the Fmoc-protected threonine glycopeptide **13** and 553 mg (1.0 mmol) of the serine glycoside **15a**. m.p.: 59 °C, $[\alpha]_{22}^{D}$ = +88.5° (c = 0.51, $CHCl_3$), R_f = 0.63 (petroleum ether/ethyl acetate 1:2). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.44 (d, J = 6.52 Hz, 1H, NH), 7.33–7.28 (m, 6H, C_6H_5-, NH), 5.81 (d, J = 7.2 Hz, 1H, NH Ser), 5.39 (m, 2H, 4-H, 4'-H), 5.35 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, 3-H), 5.32 (dd, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 3.4 Hz, 1H, 3-H), 5.25 (d, $J_{1,2}$ = 3.7 Hz, 1H, 1-H), 5.13 (d, J = 12.2 Hz, 1H, OCH_{2a}–C_6H_5), 5.04 (d, J = 12.2 Hz, 1H, OCH_{2b}–C_6H_5), 5.00 (d, $J_{1,2}$ = 3.0 Hz, 1H, 1'-H), 4.49 (m, 2H, α-CH Ala, α-CH Thr), 4.41 (m, 1H, α-CH Ser), 4.27 (t, J = 6.8 Hz, 1H, 5-H), 4.21 (m, 2H, β-CH Thr, 5'-H), 4.17–4.00 (m, 6H, 6-$H_{a/b}$, 6'-$H_{a/b}$, OCH_2 Hep), 3.94 (dd, J_1 = 3.73 Hz, J_2 = 10.24 Hz, 1H, β-CH_{2a} Ser), 3.90 (dd, $J_{1,2}$ = 3.57 Hz, $J_{2,3}$ = 10.8 Hz, 1H, 2-H), 3.83 (dd, J_1 = 5.75 Hz, J_2 = 10.24 Hz, β-CH_{2b} Ser), 3.67 (dd, $J_{1,2}$ = 3.49 Hz, $J_{2,3}$ = 10.97 Hz, 1H, 2'-H), 2.12, 2.10, 1.992, 1.986, 1.98, 1.97 (6s, 18H, 6 CH_3CO), 1.59 (m, 2H, OCH_2–$C\underline{H}_2$- Hep), 1.38 (d, J = 7.24 Hz, 3H, CH_3 Ala), 1.35–1.18 (m, 8H, -$(C\underline{H}_2)_4$–CH_3), 1.15 (d, J = 6.44 Hz, 3H, CH_3 Thr), 0.84 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 172.19, 170.31, 170.24, 169.89, 169.55, 169.43, 168.99, 167.33 (CO), 156.11 (CO, urethane), 135.92 (*ipso*-C), 128.47, 128.19, 128.08 (aromatic C), 98.78, 97.47 (C-1, C-1'), 74.42 (β-C Thr), 69.36, 68.41, 67.54, 67.17, 67.13 (C-3, C-3', C-4, C-4', C-5, C-5'), 67.32, 65.48 ($\underline{C}H_2$–C_6H_5, O–CH_2- Hep, β-C Ser), 61.65 (C-6, C'-6), 58.75 (C-2), 57.60 (C-2'), 55.78 (α-C Thr), 54.59 (α-C Ser), 48.38 (α-C Ala), 31.60, 28.77, 28.46, 25.66, 22.46 (5 CH_2 Hep), 20.53, 20.49 ($\underline{C}H_3CO$), 17.73 (CH_3 Ala), 15.82 (CH_3 Thr), 13.93 (CH_3 Hep). $C_{49}H_{69}N_9O_{22}$ (1136.1). Anal. calcd: C 51.80, H 6.12, N 11.10. Found: C 51.61, H 6.29, N 11.01.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine heptyl ester ***22***

As described for **20** the glycopeptide heptylester **22** is obtained in 48 % yield (710 mg) from 1 g (1.2 mmol) of the Fmoc protected threonine glycopeptide **13** and 763 mg (1.2 mmol) of the serine glycoside **16**. Oil, $[\alpha]_{22}^{D}$ = +84.6° (c = 1.0, $CHCl_3$), R_f = 0.67 (petroleum ether/ethyl acetate 1:2). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.74–7.26 (m, 10H, aromatic H, NH Ala, NH Thr), 5.87 (d, J = 6.6 Hz, 1H, NH Ser), 5.43 (bs, 1H, 4-H), 5.41 (dd, $J_{3,4}$ = 3.2 Hz, $J_{4,5}$ = 0.8 Hz, 1H, 4'-H), 5.38 (dd, $J_{2,3}$ = 11.0 Hz, $J_{3,4}$ = 3.0 Hz, 1H, 3-H), 5.35 (dd, $J_{2,3}$ = 10.8 Hz, $J_{3,4}$ = 3.3 Hz, 1H, 3'-H), 5.28 (d, $J_{1,2}$ = 3.55 Hz, 1H, 1-H), 5.03 (d, $J_{1,2}$ = 3 Hz, 1H, 1'-H), 4.52–3.90 (m, 17H, α-CH Ser, α-CH Ala, α-CH Thr, OCH_2 Fmoc, β-CH Thr, 9-H Fmoc, 5-H, 5'-H, 6-$H_{a/b}$, 6'-$H_{a/b}$, OCH_2 Hep, β-CH_{2a} Ser, H-2), 3.87 (dd, J_1 = 7.7 Hz, J_2 = 10.7 Hz, 1H, β-CH_{2b} Ser), 3.71 (dd, $J_{1,2}$ = 3.06 Hz, $J_{2,3}$ = 11.1 Hz, 1H, 2'-H), 2.13, 2.12, 2.02, 2.01, 1.98, 1.97 (6s, 18H, 6 CH_3CO), 1.59 (m, 2H, OCH_2–$C\underline{H}_2$- Hep), 1.39 (d, J = 7.13 Hz, 3H, CH_3 Ala), 1.36–1.21 (m, 8H, -$(C\underline{H}_2)_4$–CH_3), 1.17 (d, J = 6.41 Hz, 3H, CH_3 Thr), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 172.23, 170.33, 170.24, 169.90, 169.52, 169.44, 168.90, 167.25 (CO), 156.0 (CO, urethane), 143.72, 143.64, 141.28, 127.73, 127.08, 125.01, 119.97 (aromatic C), 98.91, 97.54 (C-1, C-1'), 74.45 (β-C Thr), 69.44, 68.51, 67.59, 67.21 (C-3, C-3', C-4, C-4', C-5, C-5'), 68.66, 67.43, 65.52 (OCH_2 Fmoc, O–CH_2 Hep, β-C Ser), 61.71, 61.67 (C-6, C'-6), 58.68 (C-2), 57.67 (C-2'), 55.74 (α-C Thr), 54.54 (α-C Ser), 48.41 (α-C Ala), 47.08 (C-9 Fmoc), 31.64, 28.82, 25.70, 22.49 (CH_2 Hep), 20.55 ($\underline{C}H_3CO$), 17.78 (CH_3 Ala), 15.78 (CH_3 Thr), 13.97 (CH_3 Hep). $C_{56}H_{73}N_9O_{22}$ (1224.2). Anal. calcd: C 54.94, H 6.01, N 10.30. Found: C 54.94, H 5.96, N 10.28.

N-Benzyloxycarbonyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-L-alanine heptyl ester ***23***

A mixture of 290 mg (0.4 mmol) of the 2-azidoglycoside **19** and 5 ml of thioacetic acid is stirred for 16 h at r.t., concentrated *in vacuo* and the 2-acetamido compound **23** is isolated by flash chromatography with petroleum ether/ethyl acetate 2:1 in 73% yield (215 mg) as an amorphous solid. $[\alpha]_{22}^{D}$ = +62.4° (c = 1.0, $CHCl_3$), R_f = 0.10 (petroleum ether/ethyl acetate 1:1). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.30 (m, 5H, C_6H_5-), 6.86 (d, J = 7.0 Hz, 1H, NH), 6.38 (d, J = 9.5 Hz, 1H, NH), 5.80 (d, J = 7.8 Hz, 1H, NH Ser), 5.31 (d, $J_{3,4}$ = 2.6 Hz, 1H, 4-H), 5.07 (s, 2H, $OC\underline{H}_2$–C_6H_5), 5.06 (dd, $J_{2,3}$ = 11.26 Hz, $J_{3,4}$ = 3.28 Hz, 1H, 3-H), 4.89 (d, $J_{1,2}$ = 3.45 Hz, 1H, 1-H) 4.48–4.57 (m, 2H, α-CH, 2-H), 4.40 (m, 1H, α-CH), 4.14–4.02 (m, 5H, 5-H, 6-$H_{a/b}$, OCH_2 Hep), 3.79 (m, 2H, β-CH_2 Ser), 2.11, 1.97, 1.93, 1.92 (4s, 12H, 4 CH_3CO), 1.60 (m, 2H, OCH_2–$C\underline{H}_2$- Hep), 1.38 (d, J = 7.2 Hz, 3H, CH_3 Ala), 1.25 (m, 8H, -$(C\underline{H}_2)_4$–CH_3), 0.85 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 173.04, 170.51, 170.37, 170.26, 168.81 (CO), 135.85 (*ipso*-C), 128.52, 128.27, 128.05 (aromatic C), 98.62 (C-1), 68.46, 66.07 (CH_2–C_6H_5, O–CH_2- Hep, β-C Ser), 68.23, 67.26, 67.12 (C-3, C-4, C-5), 61.84 (C-6), 53.93 (α-C Ser), 48.29, 47.46 (α-C Ala, C-2), 31.59, 28.76, 28.36, 25.67, 22.46 (5 CH_2 Hep), 22.93 ($\underline{C}H_3CON$), 20.63, 20.57 ($\underline{C}H_3COO$), 18.13 (CH_3 Ala), 13.95 (CH_3 Hep). $C_{35}H_{51}N_3O_{14}$ (737.8). Anal. calcd: C 56.98, H 6.97, N 5.70. Found: C 56.74, H 7.03, N 5.51.

N-Benzyloxycarbonyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine heptyl ester **24**

A mixture of 340 mg (0.3 mmol) of the 2-azidoglycoside **21** and 5 ml of thioacetic acid is stirred for 16 h at r.t., concentrated *in vacuo* and the 2-acetamido compound **24** is isolated by flash chromatography with petroleum ether/ethyl acetate 1:4 in 72% yield (256 mg). m.p.: 82 °C, $[\alpha]_{22}^{D}$ = +96.6° (c = 1.0, $CHCl_3$), R_f = 0.43 (ethyl acetate/methanol 18:1). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.32–7.28 (m, 5H, C_6H_5-), 7.18 (d, *J* = 7.0 Hz, 2H, 2NH), 6.63 (d, *J* = 8.9 Hz, 1H, NH), 6.41 (d, *J* = 9.4 Hz, 1H, NH), 5.97 (d, *J* = 7.6 Hz, 1H, NH Ser), 5.34 (d, *J* = 2.5 Hz, 1H, 4-H), 5.26 (d, *J* = 3.4 Hz, 1H, 4'-H), 5.14–5.04 (m, 5H, OCH_2–C_6H_5, 1-H, 3-H, 3'-H), 4.88 (d, $J_{1,2}$ = 3.43 Hz, 1H, 1'-H), 4.52–4.46 (m, 5H, α-CH Ala, α-CH Thr, α-CH Ser, 2-H, 2'-H), 4.25–3.98 (m, 9H, β-CH Thr, 5-H, 5'-H, 6-$H_{a/b}$, 6'-H_a, OCH_2 Hep), 3.93 (dd, $J_{5,6'b}$ = 6.1 Hz, $J_{6'a,6'b}$ = 10.2 Hz, 1H, 6'-H_b), 3.78 (dd, J_1 = 5 Hz, J_2 = 10 Hz, 1H, β-CH_{2a} Ser), 3.73 (dd, J_1 = 7.5 Hz, J_2 = 10 Hz, 1H, β-CH_{2b} Ser), 2.12, 2.09, 1.98, 1.95, 1.92, 1.91 (6s, 24H, 8 CH_3CO), 1.59 (m, 2H, OCH_2–CH_2- Hep), 1.42 (d, *J* = 7.2 Hz, 3H, CH_3 Ala), 1.28–1.21 (m, 8H, -$(CH_2)_4$–CH_3), 1.18 (d, *J* = 6.3 Hz, 3H, CH_3 Thr), 0.84 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 173.12, 170.71, 170.63, 170.56, 170.50, 170.28, 169.60, 168.73 (CO), 155.9 (CO, urethane), 135.8 (*ipso*-C), 128.55, 128.32, 128.16 (aromatic C), 98.33, 98.98 (C-1, C-1'), 75.59 (β-C Thr) 67.66, 67.40, 66.26 (CH_2–C_6H_5, O–CH_2- Hep, β-C Ser), 68.19, 67.98, 67.26, 67.22, 67.19, 66.98 (C-3, C-3', C-4, C-4', C-5, C-5'), 61.99, 61.87 (C-6, C'-6), 56.09 (α-C Thr), 54.02 (α-C Ser), 48.51 (α-C Ala), 47.81, 47.51 (C-2, C-2'), 31.58, 28.75, 28.36, 25.66, 22.45 (5 CH_2 Hep), 22.98, 22.79 (CH_3CON), 20.63, 20.58, 20.52 (CH_3CO), 18.05 (CH_3 Ala), 16.78 (CH_3 Thr), 13.94 (CH_3 Hep). $C_{53}H_{77}N_5O_{24}$ (1168.2). Anal. calcd: C 54.49, H 6.64, N 5.99. Found: C 54.57, H 6.71, N 5.55.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galacto-pyranosyl)-L-threonyl-L-alanine heptyl ester **25**

A mixture of 625 mg (0.51 mmol) of the 2-azidoglycoside **22** and 25 ml thioacetic acid is stirred for 16 h at r.t., concentrated *in vacuo* and the 2-acetamido compound **25** is isolated by flash chromatography with petroleum ether/ethyl acetate 1:4 in 61 % yield (391 mg) as an amorphous solid. $[\alpha]_{22}^{D}$ = +90.1° (c = 1.0, $CHCl_3$), R_f = 0.1 (petroleum ether/ethyl acetate 1:4). 400-MHz ^{1}H-NMR ($CDCl_3$): δ = 7.71–7.24 (m, 9H, aromatic H, NH), 7.17 (d, *J* = 6.84 Hz, 1H, 1 NH), 6.62 (d, *J* = 8.78 Hz, 1H, NH), 6.49 (d, *J* = 9.17 Hz, 1H, NH), 5.97 (d, *J* = 6.59 Hz, 1H, NH Ser), 5.33 (bs, 1H, 4-H), 5.29 (d, *J* = 2.9 Hz, 1H, 4'-H), 5.13 (m, 2H, 1-H, 3-H), 5.07 (dd, $J_{2,3}$ = 11.6 Hz, $J_{3,4}$ = 2.5 Hz, 1H, 3'-H), 4.89 (bs, 1H, 1'-H), 4.59–4.01 (m, 16H, α-CH Ala, α–CH Thr, α-CH Ser, 2-H, 2'-H, β-CH Thr, OCH_2 Fmoc, 9-H Fmoc, 5-H, 5'-H, 6-$H_{a/b}$, 6'-H_a OCH_2 Hep), 3.94 (dd, $J_{5,6'b}$ = 6.3 Hz, $J_{6'a,6'b}$ = 10.3 Hz, 1H, 6'-H_b), 3.76–3.68 (m, 2H, β-$CH_{2a/b}$ Ser), 2.11, 2.09, 1.98, 1.95, 1.94, 1.93, 1.92, 1.91 (8s, 24H, 8 CH_3CO), 1.59 (m, 2H, OCH_2–CH_2- Hep), 1.41 (d, *J* = 7.07 Hz, 3H, CH_3 Ala), 1.38–1.24 (m, 8H, -$(CH_2)_4$–CH_3), 1.19 (d, *J* = 6.3 Hz, 3H, CH_3 Thr), 0.84 (m, 3H, CH_3 Hep). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 173.34, 170.64, 170.51, 170.45, 170.33, 169.60, 168.76 (CO), 155.94 (CO, urethane), 143.59, 141.24, 127.78, 127.08, 124.90, 120.02 (aromatic C), 99.18, 98.33 (C-1, C-1'), 75.78 (β-C Thr), 68.12, 67.94, 67.71, 67.27, 67.16, 66.35 (C-3, C-3', C-4, C-4', C-5, C-5', O–CH_2 Fmoc, O–CH_2 Hep, β-C Ser), 62.02 (C-6, C'-6), 55.99 (α-C Thr), 53.94 (α-C Ser), 48.47 (α-C Ala), 47.85, 47.50 (C-2, C-2'), 47.00 (C-9 Fmoc), 31.62, 28.79, 28.38, 25.69, 22.49 (5 CH_2 Hep), 23.06, 22.83 (CH_3CON), 20.68, 20.56 (CH_3CO), 18.10 (CH_3 Ala), 16.79 (CH_3 Thr), 14.00 (CH_3 Hep). $C_{60}H_{81}N_5O_{24}$ (1256.3). Anal. calcd: C 57.36, H 6.50, N 5.57. Found: C 57.80, H 6.30, N 5.23.

N-Benzyloxycarbonyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-L-alanine **26**

To a solution of 400 mg lipase M (Amano) from *Mucor javanicus* in 25 ml of 0.2 M sodium phosphate buffer (p*H* = 7.0) is added a solution of 70 mg (0.095 mmol) of the heptyl ester **23** in 1.25 ml of acetone and the reaction mixture is shaken gently at 37 °C for 24 h. After saturation with NaCl, the aqueous phase is extracted three times with 50 ml ethyl acetate, the combined organic layers are dried with $MgSO_4$ and concentrated *in vacuo*. The residue is purifled by flash chromatography using first ethyl acetate as eluent and then ethyl acetate/ethanol 5:1 for the isolation of the product. Yield: 58 mg (95%), m.p. 105 °C, $[\alpha]_{22}^{D}$ = +62.7° (c = 1.0, CH_3OH), R_f = 0.25 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (CD_3OD): δ = 7.40–7.33 (m, 5H, C_6H_5-), 5.42 (dd, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ < 1 Hz, 1H, 4-H), 5.24 (dd, $J_{2,3}$ = 11.42 Hz, $J_{3,4}$ = 3.22 Hz, 1H, 3H), 5.15 (s, 2H, OCH_2–C_6H_5), 4.96 (bs, H_2O), 4.49–4.42 (m, 3H, α-CH Ser, α-CH Ala, 2-H), 4.33 (t, $J_{5,6}$ = 6.5 Hz, 1H, 5-H), 4.14 (dd, $J_{5,6a}$ = 6.15 Hz, $J_{6a,6b}$ = 11.16 Hz, 1H, 6-H_a), 4.07 (dd, $J_{5,6b}$ = 7.0 Hz, $J_{6a,6b}$ = 11.14 Hz, 1H, 6-H_b), 3.98 (dd, J_1 = 5.0 Hz, J_2 = 10.3 Hz, 1H, β-CH_{2a} Ser), 3.82 (dd, J_1 = 4.6 Hz, J_2 = 10.2 Hz, 1H, β-CH_{2b} Ser), 2.17, 2.04, 1.98, 1.97 (4s, 12H, 4 CH_3CO), 1.47 (d, *J* = 7.25 Hz, 3H, CH_3 Ala). 100.6-MHz ^{13}C-NMR (CD_3OD): δ = 172.21, 172.13, 171.91, 171.78 (CO), 158 (CO, urethane), 138 (*ipso*-C), 129.52, 129.11, 128.96 (aromatic C), 99.66 (C-1), 69.88, 68.86, 68.15 (C-3, C-4, C-5), 69.43, 67.91 (CH_2–C_6H_5, β-C Ser), 63.00 (C-6), 55.75 (α-C Ser), 48.79 (α-C Ala, C-2), 22.74 (CH_3CON), 20.62, 20.53 (CH_3COO), 17.65 (CH_3 Ala). $C_{28}H_{37}N_3O_{14}$ (639.61). Anal. calcd: C 52.58, H 5.83, N 6.57. Found: C 52.58, H 5.95, N 6.60.

N-Benzyloxycarbonyl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-azido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-serine **27**

To a solution of 100 mg lipase M (Amano) from *Mucor javanicus* in 25 ml of 0.2 M sodium phosphate buffer (p*H*

= 7.0) is added a solution of 30 mg (0.029 mmol) of the heptyl ester **20** in 1.25 ml of acetone and the reaction mixture is shaken gently at 37 °C for 48 h. After saturation with NaCl, the aqueous phase is extracted three times with 50 ml of ethyl acetate, the combined organic layers are dried with $MgSO_4$ and concentrated *in vacuo*. The residue is purified by flash chromatography using first ethyl acetate as eluent and then ethyl acetate/ethanol 8:2 for the isolation of the product. Yield: 19 mg (71%), amorphous, $[\alpha]_{22}^{D}$ = +112.5° (c = 1.0, CH_3OH), R_f = 0.63 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (CD_3OD): δ = 7.44–7.33 (m, 5H, C_6H_5-), 5.50–5.43 (m, 4H, 3-H, 3'-H, 4-H, 4'-H), 5.21–5.08 (m, 4H, OCH_2–C_6H_5, 1-H, 1'-H), 4.79 (bs, 1H, α-CH Ser), 4.62 (t, J = 5.3 Hz, 1H, α-CH Ser'), 4.38 (t, J = 6.41 Hz, 2H, 5-H, 5'-H), 4.21–4.02 (m, 7H, 6-$H_{a/b}$, 6'-$H_{a/b}$, β-CH_2-Ser, β-CH_{2a}-Ser'), 3.91 (dd, J_1 = 5.9 Hz, J_2 = 9.7 Hz, 1H, β-CH_{2b}-Ser'), 3.78–3.73 (m, 2H, 2-H, 2'-H), 2.16 (s, 6H, 2 CH_3CO), 2.05, 2.04 (2s, 9H, 3 CH_3CO), 1.98 (s, 3H, CH_3CO). 100.6-MHz ^{13}C-NMR (CD_3OD): δ = 172.55, 172.21, 171.99, 171.58, 171.39 (CO), 158.2 (CO, urethane), 137.9 (*ipso*-C), 129.53, 129.13 (aromatic C), 99.90 (C-1, C-1'), 69.90, 69.54, 68.11 (CH_2–C_6H_5, β-C Ser, β-C Ser'), 69.70, 69.62, 69.08, 68.22, 68.19 (C-3, C-3', C-4, C-4', C-5, C-5'), 63.02, 62.79 (C-6, C-6'), 59.08, 58.94, 56.12 (C-2, C-2', α-C Ser, α-C Ser'), 20.68, 20.64, 20.46 (CH_3CO). $C_{38}H_{48}N_8O_{21}$ (952.83), FAB-MS (3-NOBA): 953.4 $(M + H)^+$ calcd: 953.5.

N-Benzyloxycarbonyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanine **28**

To a solution of 400 mg lipase M (Amano) from *Mucor jayanicus* in 25 ml of 0.2 M sodium phosphate buffer (p*H* = 7.0) is added a solution of 200 mg (0.17 mmol) of the heptyl ester **24** in 1.25 ml of acetone and the reaction mixture is shaken gently at 37 °C for 24 h. After saturation with NaCl, the aqueous phase is extracted three times with 50 ml of ethyl acetate, the combined organic layers are dried with $MgSO_4$ and concentrated *in vacuo*. The residue is purified by flash chromatography using first ethyl acetate as eluent and then ethyl acetate/ethanol 9:3 for the isolation of the product. Yield: 140 mg (76%), m.p.: 146 °C, $[\alpha]_{22}^{D}$ = +90.4° (c = 1.0, CH_3OH), R_f = 0.16 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (CD_3OD): δ = 7.44–7.32 (m, 5H, C_6H_5-), 5.43 (d, J = 2.2 Hz, 1H, 4-H), 5.41 (d, J = 2.4 Hz, 1H, 4'-H), 5.24–5.14 (m, 4H, OCH_2–C_6H_5, 3H, 3'-H), 4.99 (d, $J_{1,2}$ = 3.41 Hz, 1H, 1-H), 4.65–4.27 (m, 8H, 2-H, 2'-H, α-CH Ala, α-CH Thr, α-CH Ser, β-CH Thr, 5-H, 5'-H), 4.19–4.01 (m, 6-$H_{a/b}$, 6'-$H_{a/b}$, β-CH_{2a} Ser), 3.87 (dd, J_1 = 5.16 Hz, J_2 = 10.4 Hz, 1H, β-CH_{2b} Ser), 2.18, 2.17, 2.053, 2.049, 2.03, 1.983, 1.978, 1.969 (8s, 24H, 8 CH_3CO), 1.45 (d, J = 7.0 Hz, 3H, CH_3 Ala), 1.37 (d, J = 6.1 Hz, 3H, CH_3 Thr). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 172.60, 172.19, 172.11, 172.05, 171.86, 171.59 (CO), 158.0 (CO, urethane), 138.3 (*ipso*-C), 129.52, 129.11, 128.95 (aromatic C), 100.84, 99.83 (C-1, C-1'), 79.09 (β-C Thr) 69.40, 67.92 (CH_2–C_6H_5, β-C Ser), 70.47, 69.80, 68.96, 68.74, 68.28, 68.19 (C-3, C-3', C-4, C-4', C-5, C-5'), 63.28, 62.97 (C-6, C'-6), 57.88 (α-C Thr), 55.92 (α-C Ser), 48.78, 47.57, 47.35 (α-C Ala, C-2, C-2'), 22.82, 23.12 (CH_3CON), 20.69, 20.64, 20.57, 20.53 (CH_3CO), 19.14 (CH_3 Ala), 17.79 (CH_3 Thr). $C_{46}H_{63}N_5O_{24} * 2H_2O$ (1106.06). Anal. calcd: C 49.95, H 6.11, N 6.33. Found: C 49.96, H 5.95, N 6.66.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-seryl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galacto-pyranosyl)-L-threonyl-L-alanine **29**

To a solution of 1 g lipase M (Amano) from *Mucor javanicus* in 75 ml of 0.2 M sodium phosphate buffer (p*H* = 7.0) is added a solution of 500 mg (0.40 mmol) of the heptyl ester **25** in 3.75 ml of acetone and the reaction mixture is shaken gently at 37 °C for 48 h. After saturation with NaCl, the aqueous phase is extracted three times with 50 ml of ethyl acetate, the combined organic layers are dried with $MgSO_4$ and concentrated *in vacuo*. The residue is purified by flash chromatography using first ethyl acetate as eluent and then ethyl acetate/ethanol 8:2 for the isolation of the product. Yield: 237 mg (51%), m.p.: amorphous, $[\alpha]_{22}^{D}$ = +69.1° (c = 0.54, CH_3OH), R_f = 0.27 (ethyl acetate/methanol 2:1). 400-MHz ^{1}H-NMR (CD_3OD): δ = 7.85–7.34 (m, 8H, aromatic H), 5.41 (d, J = 2.5 Hz, 1H, 4-H), 5.38 (d, J = 2.2 Hz, 1H, 4'-H), 5.24–5.14 (m, 3H, 1-H, 3-H {dd, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 3.2 Hz}, 3'-H {dd, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 3.5 Hz}), 4.99 (d, $J_{1,2}$ = 3.12 Hz, 1H, 1'-H), 4.66–4.62 (m, 2H), 4.51–4.38 (m, 5H), 4.34–4.26 (m, 4H), 4.14 (dd, J_1 = 6.3 Hz, J_2 = 11.0 Hz, 1H), 4.08–3.98 (m, 4H), 4.66–3.98 (2-H, 2'-H, α-CH Ala, α-CH Thr, α-CH Ser, OCH_2 Fmoc, 9-H Fmoc, β-CH Thr, 5-H, 5'-H, 6-$H_{a/b}$, 6'-$H_{a/b}$, β-CH_{2a} Ser), 3.87 (dd, J_1 = 5.3 Hz, J_2 = 10.5 Hz, 1H, β-CH_{2b} Ser), 2.17, 2.16, 2.02, 2.00, 1.99, 1.98, 1.96, 1.92 (8s, 24H, 8 CH_3CO), 1.45 (d, J = 7.2 Hz, 3H, CH_3 Ala), 1.35 (d, J = 6.2 Hz, 3H, CH_3 Thr). 100.6-MHz ^{13}C-NMR ($CDCl_3$): δ = 173.41, 172.15, 172.09, 172.01, 171.83, 171.65 (CO), 158.4 (CO, urethane), 145.09 (C-4_a, C-4_b Fmoc), 142.57 (C-8_a, C-9_a Fmoc), 128.84, 128.22 (C-3/6 Fmoc), 126.17 (C-2/7, C-1/8 Fmoc), 121.00 (C-5, C-4 Fmoc), 100.91, 99.86 (C-1, C-1'), 79.40 (β-C Thr), 70.37, 69.76, 69.01, 68.70, 68.27, 68.18 (C-3, C-3', C-4, C-4', C-5, C-5'), 69.37, 63.38, 62.94 (OCH_2 Fmoc, β-C Ser, C-6, C'-6), 57.84 (α-C Thr), 55.87 (α-C Ser), 48.88, 48.42 (α-C Ala, C-2, C-2', C-9 Fmoc), 23.13, 22.83 (CH_3CON), 20.66, 20.61, 20.51 (CH_3CO), 19.32 (CH_3 Ala), 17.61 (CH_3 Thr). $C_{53}H_{67}N_5O_{24}$ (1158.1). Anal. calcd: C 54.97, H 5.83, N 6.05. Found: C 54.59, H 5.75, N 5.91.

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