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Synthesis of T_N and T Antigen Glycopeptide Sequences of Tumor-Associated MUC-1 Using *S*-Pent-4-enyl Thioglycosides

Michael Leuck and Horst Kunz

Mainz, Institut für Organische Chemie der Universität

Dedicated to Professor Dr. Ekkehard Winterfeldt on the Occasion of his 65th Birthday

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Abstract. The syntheses of glycopeptides **34** and **38** with tumor associated T_N and T antigen structure containing a partial sequence of the tandem repeat unit of the polymorphic epithelial mucin MUC-1 were achieved *via* fragment condensations. Electrophilic activation of *S*-pent-4-enyl thioglycosides was applied for the construction of *O*-glycosyl amino acid and saccharide building blocks.

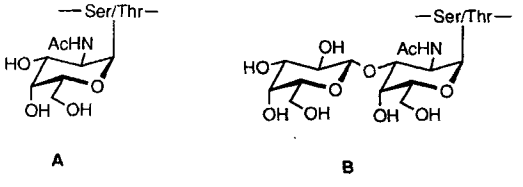
Polymorphic Epithelial Mucin (PEM) in Tumor Tissues

The polymorphic epithelial mucin (PEM) is an *O*-glycoprotein produced in normal as well as in transformed epithelial cells [1]. The complete primary structure of a tumor-associated PEM core protein named MUC-1 was elucidated by cloning a mammary carcinoma cell line (λgt 11) and DNA sequencing the cell clones [2]. According to this analysis, the PEM core protein contains a central portion of 30 to 90 tandem repeats each consisting of 20 amino acids. The threonine and serine residues of the repeat sequence constitute the potential glycosylation sites of the core protein. Many monoclonal antibodies directed against globulines from breast milk, transformed breast cells and tumor extracts recognize epitopes within the MUC-1 sequence **1**.

TAPPAHGVTSA**PDTRPAPGS** **1**

It was shown by epitope mapping that the antibodies bind to three to five amino acid residues of the PDTRPAP heptamer [3]. Two classes of antibodies were distinguished, one recognizing the DTR and the other the RPA sequence both sharing the central arginine residue. Glycosylation of the repeat sequence interfers with the immunological recognition. Therefore, antibodies directed against the unmasked core protein do not recognize mucins from normal tissues but rather tumor-associated mucins which contain short, incomplete saccharide side chains [4].

Because tumor cells exhibit lower *N*-acetylglucosaminetransferase activity and higher sialyl transferase activity [5, 6], T_N (**A**) and T antigen (**B**) as well as their sialylated derivatives are typically expressed on carcinoma cells [7, 8].



Scheme 1

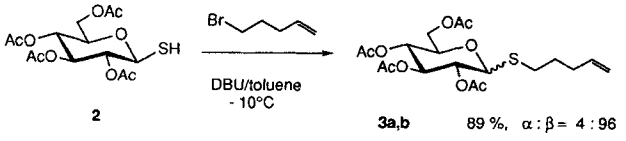
In consideration of these interesting tumor-immunological results, synthetic T_N and T antigen glycopeptides are attractive model compounds for the development of tumor selective vaccines [9]. Since early reports on monoclonal antibodies against tumor-associated T_N and T antigen glycoproteins particularly stressed on the cross-reactivity of these antibodies with asialoglycophorin [7], first syntheses of such glycopeptides [10, 11, 12, 13] were aimed at T_N and T antigen glycopeptides with *N*-terminal peptide sequences of glycophorin and their binding to carrier proteins [11]. It was re-

vealed by the immunological application of synthetic T antigen glycopeptides, that the recognition of the carbohydrate epitope is markedly influenced by the peptide sequence [14]. With regard to the afore-mentioned results with the tumor associated mucin MUC-1, glycopeptides combining the T_N and T antigen structure with the MUC-1 tandem repeat sequences are of particular interest. Solid phase syntheses of T_N antigen MUC-1 peptides have already been reported: The synthesis of the fivefold glycosylated tandem repeat was carried out on acid-sensitive SASRIN resin, but no yield and incomplete characterization was given for the final product [15]. High efficiency solid-phase synthesis of a T_N antigen glycononapeptide of MUC1 was achieved using an allylic anchor [16].

We report here on the synthesis of both, T_N and T antigen MUC-1 peptides according to a fragment condensation strategy in solution. For the construction of the saccharide portion of the T antigen series and of the *O*-glycosyl threonine building block of the T_N antigen series a novel variation of anomeric activation was used. It consists in the reaction of pent-4-enyl thioglycosides with soft electrophiles.

S-Pent-4-enyl Thioglycosides

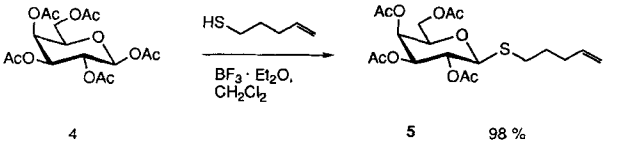
Electrophilic activation of thioglycosides has become an important tool in glycoside synthesis [17, 18]. On the other hand, *O*-pentyl glycosides are also useful glycosyl donors if their double bond is attacked by electrophiles and the subsequent cyclization generates the corresponding tetrahydrofuran as the anomeric leaving group [19]. Pentenyl-thioglycosides combine both activation principles. The preparation of these glycosyl donors is shown for three examples: The alkylation of 2,3,4,6-*tetra*-*O*-acetyl-1-thio-β-D-glucopyranose **2** [20] with 5-bromo-1-pentene in the presence of diaza-bicyclo-undecene (DBU) yields the *S*-pent-4'-enyl thioglycopyranoside **3**.



Scheme 2

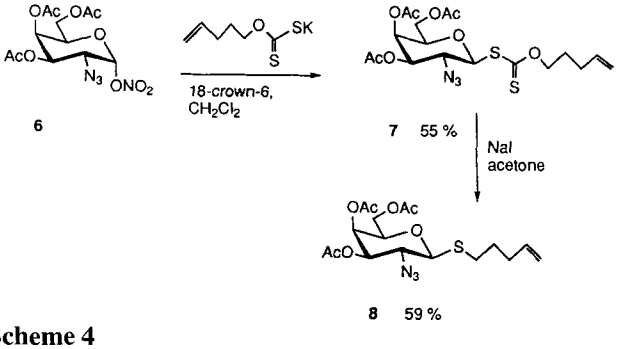
Since the intermediate thiolate is obviously prone to anomerization, the ratio of anomers of **3** depends upon the reaction temperature. In benzene at 22 °C, **3a** and **3b** are formed in a ratio of 48:52, while anomerization can be prevented by carrying out the reaction in toluene at −10 °C (**3a/3b** = 4:96).

Reaction of penta-*O*-acetyl-galactopyranose **4** with the light- and oxygen-sensitive 4-pentene-1-thiol [21] in the presence of borontrifluoride gives the 4-pentenyl-thiogalactopyranoside **5** as the pure β-anomer in high yield.



Scheme 3

The synthesis of the 2-azido-galactosyl thioglycoside required for the construction of T_N antigen glycopeptides is achieved by reaction of the galactose-derived azido nitrate **6** [22] with potassium 4-pentenyl xanthogenate obtained from 4-pentenol, potassium hydride and carbondisulfide in the presence of 18-crown-6. In analogy to the procedure for the corresponding ethyl xanthogenate [23], the formed *S*-glycosyl-*O*-pentenyl xanthogenate **7** is converted to the desired *S*-pentenyl 2-azido-galactoside **8** by treatment with sodium iodide in acetone under reflux.



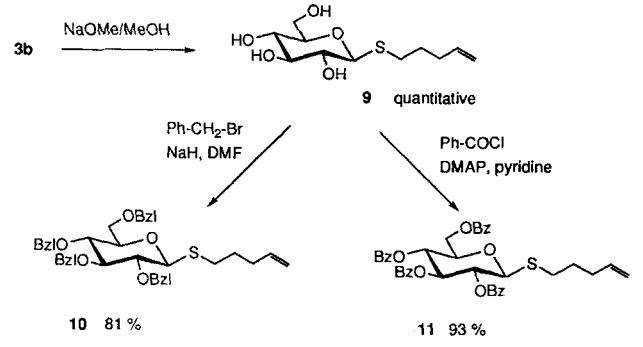
Scheme 4

S-Pentenylthioglycosides are stable under conditions required for a number of protecting group manipulations. For example, removal of the *O*-acetyl groups from **3b** by Zemplén transesterification quantitatively gives **9** which is subjected to benzylation to yield **10** or benzoylation to afford **11**.

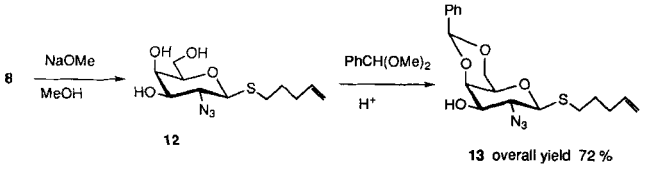
After de-*O*-acetylation of **8**, *O*-unprotected **12** is converted into the 4,6-benzylidene acetal **13** also without affecting the pentenyl-thioglycoside structure.

Galactosylation of **13** to furnish the T antigen disaccharide **14** is possible but accompanied by the formation of the transglycosylation product **5**.

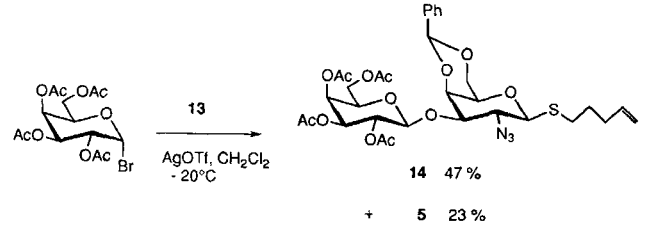
Similar transglycosylation reactions have been reported for other thioglycosides used as glycosyl acceptors [24]. Therefore, in further syntheses *S*-pentenylthioglycosides are only applied as glycosyl donors.



Scheme 5



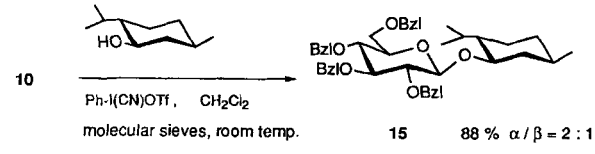
Scheme 6



Scheme 7

Glycosylation Using *S*-Pentenyl Thioglycosides

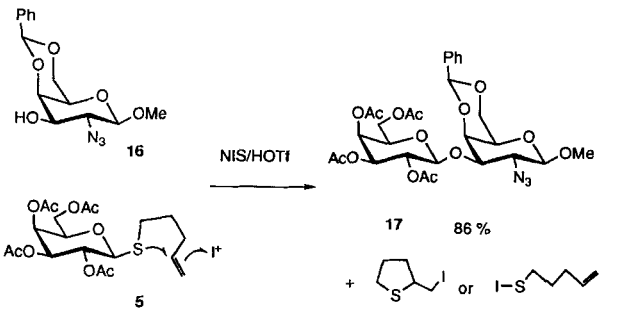
Soft electrophiles can attack *S*-pentenyl thioglycosides at the double bound or at the sulfur. Activation of **10** with phenyliodoniumcyanotriflate [25] forms a reactive glucosyl donor that glucosylates (–)-menthol to give menthyl glucoside **15** in high yield but low diastereoselectivity.



Scheme 8

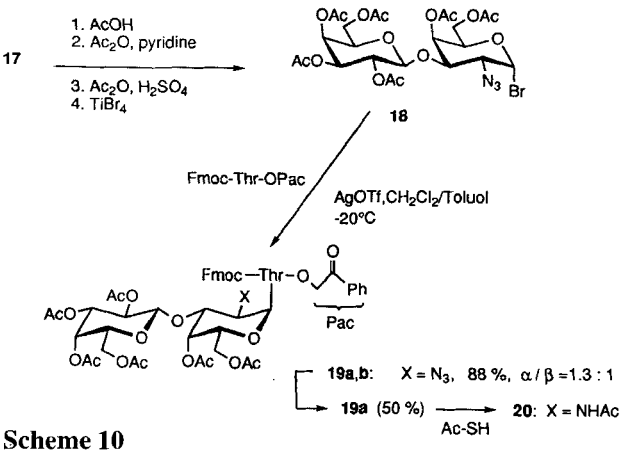
Activation of *O*-acyl protected thioglycosides is more difficult. Glycosylation of the known 2-azido-galactoside [26] **16** with *S*-pentenyl thiogalactoside **5** to give **17** is efficiently achieved using *N*-iodosuccinimide (NIS)/trifluoromethylsulfonic acid as the activating electrophile.

After separation by flash-chromatography, the product formed from the pentenylthio group can be detected by EI mass spectrometry. However, because the 2-iodomethyl-tetrahydrofuran and the sulfenyl iodide have identical mass, the localization of the electrophilic attack at the pentenyl thioglyoside remains unclear.



Scheme 9

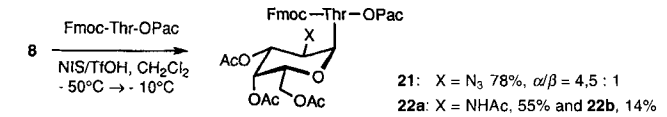
Compound **17** is converted into the T antigen disaccharide **18** by acidolysis of the benzylidene group, subsequent acetylation, acid-catalyzed acetolysis of the methyl glycoside, and treatment of the formed anomeric acetate with titanium tetrabromide according to known procedures [26].



Scheme 10

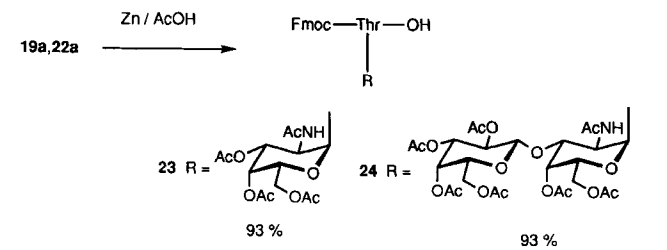
Reaction of the disaccharide bromide with 9-fluorenylmethoxycarbonyl-threonine phenacyl ester to give the disaccharide threonine conjugate **19** is achieved in analogy to the procedure described by Lüning *et al.* [27]. After chromatographic separation of the desired α-anomer **19a**, its azido group is converted to the acetamido group by treatment with thioacetic acid [28] to give the T antigen conjugate **20**.

For the synthesis of the corresponding T_N antigen threonine conjugate, the *S*-pentenyl azidogalactoside **8** is activated with NIS/trifluoromethylsulfonic acid and reacted with Fmoc-threonine phenacyl ester. *O*-Glycoconjugate **21** is obtained as a mixture of anomers (a/b = 4.5:1). After treatment with thioacetic acid [28], the formed acetamido galactose threonine conjugates **22** can be separated by flash-chromatography to yield the desired α-anomer **22a** [27].



Scheme 11

Selective cleavage of the phenacyl ester in the glycosylated threonine derivatives **19a** and **22a** is achieved in almost quantitative yield by reductive elimination using zinc in acetic acid [27].

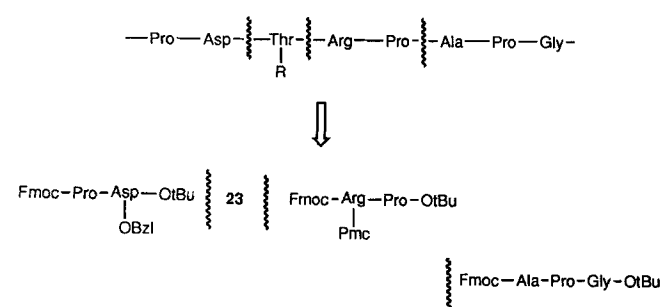


Scheme 12

The obtained *N*-Fmoc-protected T_N antigen (**23**) and T antigen building blocks can now be applied to the fragment condensation synthesis to furnish the MUC-1 glycopeptides.

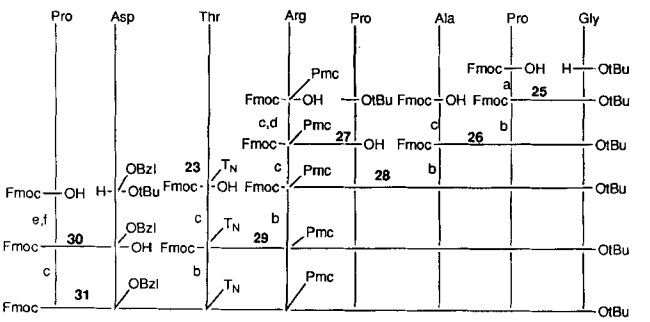
T_N Antigen MUC-1 Glycopeptides

In a convergent strategy four building blocks are synthesized and used for the construction of the T_N antigen glycooctapeptide: the C-terminal tripeptide, an arginine containing dipeptide, the T_N conjugate **23**, and the *N*-terminal dipeptide:



Scheme 13

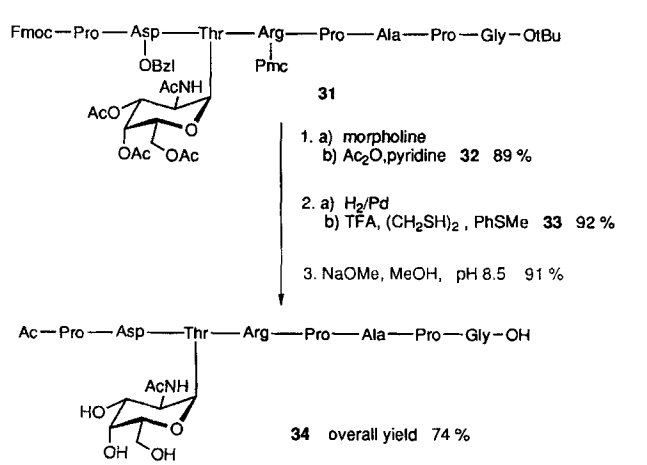
Common Fmoc/OtBu strategy is applied for the synthesis of the peptide fragments. Protection of the arginine guanidino function by the 2,2,5,7,8-pentamethylchromane-6-sulfonyl (Pmc) group [29] is considered advantageous in comparison to the analogous protection by the 4-methoxy-2,3,6-trimethylphenylsulfonyl (Mtr) group [16,30], because of the very mild deprotection conditions avoiding side-reactions. The β-carboxylic function of aspartic acid is protected as the benzylester. This sterically less demanding side chain is favourable during the coupling of the *N*-terminal dipeptide to the *O*-glycosylated threonine unit. At the same time, the sterically demanding glycosyl side chain prevents any transpeptidation rearrangements [16] during subsequent protecting group manipulations on the glycooctapeptides (Scheme 14).



Scheme 14

It is interesting to note, that the lowest yield during this strategy is obtained for the coupling of the arginine derivative to the proline ester (68%). In contrast, the couplings of **23** to give **29** and of **30** to the glycosylated unit to form **31** are achieved with yields of 79% and 86%, respectively.

Glycopeptide **31** is subjected to an exchange of the *N*-terminal Fmoc for an acetyl group to give **32**, subsequent removal of the aspartic acid benzyl ester and acidolysis of the Pmc- and O*t*Bu groups to yield **33**, and final removal of the *O*-acetyl groups by transesterification at a pH of 8.5 to furnish the T_N antigen MUC-1 glycooctapeptide **34**.

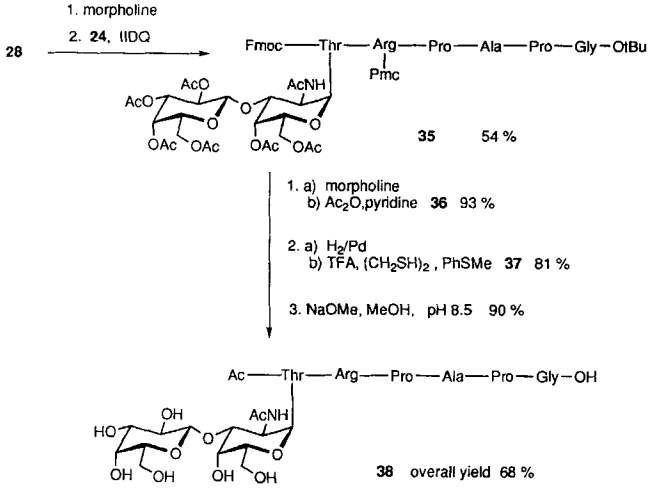


Scheme 15

All deprotecting reactions proceed with high yield and without side reactions. The structure of the T_N antigen MUC-1 conjugate **34** is ascertained by [^{1}H, ^{1}H]- and [^{1}H, ^{13}C]-COSY-NMR spectra and MALDI- mass spectrometry [33].

T Antigen MUC-1 Glycopeptide

The T antigen MUC-1 peptide conjugate is obtained by coupling the pentapeptide fragment **28** with the T antigen component **24**. Due to the sterically demanding structure of **24**, the yield of **35** is considerably lower than that for the corresponding T_N analogue **29**.



Scheme 16

The exchange of the *N*-terminal group and the deprotecting steps proceed efficiently. Ethane-1,2-dithiole proved to be most efficient as the scavenger of the *tert*-butyl cation during acidolytic treatment of **36**, while thioanisole accelerates the removal of the Pmc groups [29]. The structure of **38** is unequivocally ascertained by FAB- and MALDI mass spectrometry and by high-field NMR spectra including NOESY- and ROESY experiments. The ROESY-spectrum of **38** shows cross signals between the H-1 of the GalNAc and β-H and β-CH_3 of threonine on the one hand as well as between this H-1 and the methylene protons within the arginine side chain. These results suggest, that the glycosylation of the threonine induces a *cis*-peptide bond between threonine and arginine in glycopeptide **38**.

Glycopeptides **34** and **38** being exactly specified partial structures of tumor-associated mucin MUC-1 are now applicable to tumor-immunological investigations, e.g. probing of selective antibodies.

This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie.

Experimental

Melting points were measured on a Büchi apparatus and are uncorrected. ^{1}H-and ^{13}C chemical shifts (δ values) are given relative to tetramethylsilane. Mass spectra were recorded on a Finigan MAT-95 (FAB) or a Bruker-Reflex-MALDI TOF (MALDI) instrument [33]. Analytical HPLC was performed using a Knauer equipment (Knauer, Berlin, Germany) consisting of a WellChrom K 1000 Maxi Star 4000 pump, a DAD 2062 detector and an Eurospher-100, C8, 5 μm column and acetonitrile/water (gradient 1:99→100:0 within 42 min) as the eluent. Preparative HPLC was carried out using a Bishoff model 2200 pump and (A) Eurospher-100 (C8, 7μm) and (B) Eurospher-100 (C18, 7μm) columns from Knauer, Berlin, flow 20 ml/min, detection at λ 210 min. GPC (Gelpermeation chromatography) was performed on Sephadex LH-20 (200 g, Pharmacia). Flash-chromatography was carried out using silica gel 30–60 μm (Baker). For column chromatography, silica gel 0.063–0.200mm (Baker) was used. Tlc was performed on aluminum foil coated with silica gel 60 F_{254} or RP-8 F_{254} (E. Merck, Darmstadt). Optical rotation values were measured on a Perkin Elmer polarimeter 241. L-Amino acids were used in the corresponding syntheses. Reactions were carried out at room temperature if not indicated otherwise. Full NMR data are given in reference [34].

Pent-4'-enyl 2,3,4,6-tetra-O-acetyl-1-thio-β-D-gluco-yranoside (**3b**) and *Pent-4'-enyl 2,3,4,6-tetra-O-acetyl-1-thio-a-D-glucopyranoside* (**3a**)

To a solution of 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose **2** [20] (1.05 g, 2.9 mmol) in toluene (8.7 ml) kept at –10 °C under argon atmosphere were added DBU (0.43 ml, 2.9 mmol) and, subsequently, dropwise 5-bromo-pentene-1 (0.34 ml, 2.9 mmol). After stirring for 45 min, water (10 ml) was added, the organic layer was separated and the aqueous solution was extracted twice with dichloromethane (30 ml). The combined organic layers were diluted with dichloromethane (100 ml) and washed consecutively with 1M H_2SO_4, sat. $NaHCO_3$ and brine. After drying with $MgSO_4$ and evaporation of the solvent *in vacuo*, the remainder was subjected to flash-chromatography in petroleum ether/ethyl acetate (3:1) to yield the β-anomer **3b** (1.065 g, 85%) and the corresponding α-anomer **3a** (49 mg, 4%).

β-anomer **3b**: *m.p.* 62–63 °C, $[\alpha]_D^{22} = -26.0°$ (c 1, $CHCl_3$). R_f = 0,41 (petroleum ether/ethyl acetate 1:1). – 400 MHz ^{1}H NMR ($CDCl_3$): δ 5.74 (ddt, 1H, J_{trans} = 17.1 Hz, J_{cis} = 10.3 Hz, J_{vic} = 6.7 Hz, –CH=), 5.19 (t, 1H, J = 9.4 Hz, H-3), 5.36–4.94 (m, 2H =CH_2), 4.45 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1). 50.3 MHz. – ^{13}C GASPE-NMR ($CDCl_3$): δ 83.4 (C-1).

$C_{19}H_{28}O_9S$ (432.5) Calcd. C 52.77, H 6.63, S 7.41; Found C 52.58, H 6.69, S 7.72.

α-anomer **3a**: *m.p.* 68 °C; $[\alpha]_D^{22} = +176.5$ (c 1, $CHCl_3$); R_f = 0.45 (petroleum ether/ethyl acetate 1:1). – 400 MHz ^{1}H NMR ($CDCl_3$): δ 5.73 (ddt, 1H, J_{trans} = 17.0 Hz, J_{cis} = 10.3 Hz, J_{vic} = 6.8 Hz, –CH=), 5.63 (d, 1H, $J_{1,2}$ = 5.8 Hz, H-1), 5.33 (t, 1H, J = 9.8 Hz, H-3), 5.04–4.94 (m, 4H, 2-H, 4-H, =CH_2). 50.3 MHz. – ^{13}C NMR ($CDCl_3$): δ 81.89 (C-1).

S-Pent-4-enyl-isothiuronium bromide was prepared according to a varied procedure of Walling *et al.* [21] by stirring 5-bromo-pentene-1 (5 ml, 42 mmol) and thiourea (3.9 g, 51 mmol) in

dry ethanol (28 ml) for 1h under reflux. After evaporation of the solvent, the crude product was purified by flasch-chromatography (500 g of silica gel) in petroleum ether/acetone). Yield 7.2 g (76%), colorless crystals, *m.p.* 84 °C ([21] *m.p.* 91–93 °C).

Pent-4-ene-thiol was obtained from the isothiuronium bromide [21] (4 g, 15.7 mmol), sodium pyrosulfite (3.16 g, 16.4 mmol) and hydroquinone (0.05 g) in water (13 ml) and carbontetrachloride (carcinogenic!) (16 ml). The mixture was stirred and refluxed under argon atmosphere and protection from light for 30 min according to a procedure of Cerny *et al.* [35]. After separation, washing of the aqueous layer with carbontetrachloride, extraction of the combined organic solutions with brine and drying with $MgSO_4$, the solvent was evaporated under careful exclusion of oxygen. The obtained crude product was used for the synthesis of thioglycosides without purification.

Pent-4'-enyl-2,3,4,6-tetra-O-acetyl-1-thio-β-D-ga-lactopyranoside (**5**)

Penta-*O*-acetyl-β-D-galactopyranose **4** [36] (3.90 g, 10 mmol), pent-4-ene thiol (2.04 g, 20 mmol) and molecular sieves 4 Å (2g) in dichloromethane (60 ml) were stirred for 1 h at room temperature and with exclusion of light. Borontrifluoride etherate (1.9 ml, 15 mmol) was added dropwise. After 18 h stirring and incomplete conversion (tlc monitoring) pentenethiol (1.00 g, 9.8 mmol) and BF_3 etherate (1.9 ml, 15 mmol) were added dropwise. After stirring for 21 h, the mixture was neutralized by addition of sat. $NaHCO_3$ and filtered through Celite. The separated organic solution was washed with water, dried with $MgSO_4$ and concentrated *in vacuo*. Flash-chromatography in petroleum ether/ethyl acetate (3:1) gave **5** (4.24 g, 98%) as a colorless oil. $[\alpha]_D^{24} = -5.0°$ (c 1, $CHCl_3$); R_f = 0.46 (petroleum ether/ethyl acetate 2:1). 200 MHz. – ^{1}H NMR ($CDCl_3$): δ 5.70 (ddt, 1H, J_{trans} = 17.0 Hz, J_{cis} = 10.2 Hz, J_{vic} = 6.8 Hz, –CH=), 5.34 (dd, 1H, $J_{3,4}$ = 3.3 Hz, $J_{4,5}$ = 0.71 Hz, H-4); 5.15 (t, 1H, J = 9.9 Hz, H-2), 5.00–4.88 (m, 2H, =CH_2), 4.96 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.41 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1); 100.6 MHz. – ^{13}C GASPE-NMR ($CDCl_3$): δ 137.39 (–CH=), 115.22 (=CH_2), 84.09 (C-1).

$C_{19}H_{28}O_9S$	Calcd.	C 52.77,	H 6.53, S 7.41;
(432.5)	Found	C 52.85,	H 6.63, S 7.45.

S-(3,4,6-Tri-O-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl)-O-pent-4-enyl-dithiocarbonate (**7**)

a) *Potassium pent-4-enylxanthogenate*: To a stirred mixture of potassium hydride (2 g, 50 mmol) in dry tetrahydrofuran (45 ml) a solution of pent-4-enol was added cautiously and dropwise. After the evolution of hydrogen had deceased, carbondisulfide (4.4 ml, 73 mmol) was added dropwise. After 20 h stirring, dry diethyl ether (100 ml) was poured into the mixture. The precipitated crude product was collected by filtration and recrystallized from acetone/diethyl ether to give 7.7 g (80%), yellow powder, *m.p.* 200 °C (dec.).

b) **7**: This xanthogenate (6.0 g, 30 mmol), 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactosylnitrate [22] **6** (3.76 g, 10 mmol) and 18-crown-6 (528 mg, 2 mmol) in dichloromethane (100 ml) were stirred for 50 h. The mixture was poured into 200 ml of diethyl ether/water (1:1). The water layer was extracted twice with diethyl ether (50 ml). The combined organic solutions were washed with water, dried with $MgSO_4$ and concentrated *in vacuo*. The remaining product was purified by flash-chromatography in petroleum ether/ethyl acetate (4:1) to give **7**, yield 2.6 g (55%), yellow crystals, *m.p.* 68–69 °C, $[\alpha]_D^{22} = +2.9°$ (c 1, $CHCl_3$); R_f = 0.46 (petroleum ether/acetone 3:1). 200 MHz. – ^{1}H NMR ($CHCl_3$): δ 5.75 (ddt, 1H, J_{trans} = 17.0 Hz, J_{vic} = 6.6Hz, –CH=), 5.37 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4), 5.25 (d, 1H, $J_{1,2}$ = 10.7 Hz, H-1), 5.05–4.94 (m, 2H, =CH_2). – 50.3 MHz ^{13}C NMR ($CDCl_3$): δ 208.91 (C=S), 86.51 (C-1).

$C_{18}H_{25}N_3O_8S_2$	Calcd.:	C 45.46, H 5.30, N 8.84, S 13.49;
(475.5)	Found:	C 45.41, H 5.25, N 8.68, S 13.55.

Pent-4'-enyl 3,4,5-tri-O-acetyl-2-azido-2-deoxy-1-thio-β-D-galactopyranoside (**8**)

A mixture of **7** (2.35 g, 4.9 mmol) and sodium iodide (3.15 g, 21 mmol) in acetone (60 ml) was heated under reflux for 17 h. The acetone was evaporated *in vacuo*, and the residue was dissolved in 150 ml of water/dichloromethane (1:1). After separation, the water solution was extracted twice with dichloromethane (25 ml). The combined organic solutions were washed with water, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by flash-chromatography yielded **8**, 1.20 g (59%), yellowish oil, $[\alpha]_D^{25} = -42.0°$ (c 1, $CHCl_3$). R_f = 0.47 (petroleum ether/ethyl acetate 2:1). – 200 MHz-^{1}H NMR ($CDCl_3$): δ 5.70 (ddt, 1H, J_{trans} = 17.0 Hz, J_{cis} = 10.2 Hz, J_{vic} = 6.7 Hz, –CH=), 5.01–4.90 (m, 2H, =CH_2), 4.31 (d, 1H, $J_{1,2}$ = 10.1 Hz, H-1), 5.05–4.97 (m, 2H, =CH_2), 4.79 (dd, 1H, $J_{2,3}$ = 10.2 Hz, $J_{3,4}$ =3.3 Hz, H-3). – 50.3 MHz-^{13}C GASPE NMR ($CDCl_3$): δ 84.78 (C-1).

$C_{17}H_{25}N_3O_7S$	Calcd.:	C 49.15, H 6.06, N 10.11, S 7.72;
(415.5)	Found:	C 48.90, H 5.99m, N 10.16, S 7.75.

Pent-4'-enyl 1-thio-β-D-glucopyranoside (**9**)

A solution of **3b** (765 mg, 1.77 mmol) and sodium methanolate in methanol (0.22M, 3 ml) in methanol (10 ml) was stirred for 30 min at room temperature. The crude product **9** obtained after evaporation of methanol and methyl acetate *in vacuo* was used for further transformations.

Pent-4'-enyl 2,3,4,6-tetra-O-benzyl-1-thio-β-D-glucopyranoside (**10**)

The crude **9** was dissolved in dimethylformamide (25 ml). At 0 °C sodium hydride (2 g) and, after 15 min, benzyl bromide (6 ml) were added. After stirring for 1.5 h at 0 °C, methanol (5 ml) was added in order to solvolyse the excess of sodium hydride, and the mixture was poured into 160 ml of water/toluene (1:1). After separation the water solution was extracted twice with toluene (20 ml). The combined organic solutions were dried with $MgSO_4$ and concentrated *in vacuo*. Flash-chromatography in petroleum ether/acetone (13:1) gave **10**, yield 893 mg (81%), colorless crystals, *m.p.* 46 °C, $[\alpha]_D^{22}$ = +4.3° (c 1, $CHCl_3$), R_f = 0.48 (petroleum ether/acetone 5:1). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 7.37–7.15 (m, 20H, Ar-H), 5.76 (ddt, 1H, J_{trans} = 17.0 Hz, J_{cis} = 10.3 Hz, J_{vic} = 6.7 Hz, –CH=), 5.02 (dd, 1H, J_{gem} = 1.5 Hz, J_{trans} = 17.0 Hz, =CH_{trans}), 4.96 (dd, 1H, J_{gem} = 1.5 Hz, J_{cis} = 10.3 Hz, =CH_{cis}), 4.43 (d, 1H, $J_{1,2}$ = 9.8 Hz, H-1).

$C_{39}H_{44}O_5S$ (624.9) Calcd.: C 74.97, H 7.10, S 5.13; Found: C 74.89, H 7.38, S 5.52.

Pent-4'-enyl 2,3,4,6-tetra-O-benzoyl-1-thio-β-D-glucopyranoside (**11**)

The solution of **9** (1.77 mmol, *vide supra*) in 10 ml of methanol was stirred with ion-exchange resin Amberlite IR-120 (H^+ form, 2 g) for 5 min, filtered and the filtrate concentrated *in vacuo* to dryness. Traces of solvent were removed by lyophilization in high vacuum. The residue was dissolved in dry pyridine (10 ml). To this solution, benzoyl chloride (1.23 ml, 10.6 mmol) and 4-dimethylamino-pyridine (108 mg, 0.9 mmol) were added. The solution was stirred for 2 h at room teperature. After concentration *in vacuo*, and codistillation with toluene (10 ml), the remainder was dissolved in dichloromethane (50 ml), extracted with water (50 ml), 2N H_2SO_4 (50 ml), sat. $NaHCO_3$ (50 ml) and brine (50 ml). After drying with $MgSO_4$ and evaporation of the solvent, the crude **11** was purified by flash-chromatography in petroleum ether/acetone (6:1) to give **11**, yield 1.12 g (93%), *m.p.* 88–89 °C, $[\alpha]_D^{28} = +16.0°$ (c 1, $CHCl_3$), $R_f = 0.34$ (petroleum ether/acetone 2:1). – 200 MHz-^{1}H NMR, δ 8.15–7.80 (m, 8H, Ar-H_{ortho}); 4.86 (d, 1*H*, $J_{1,2}$ = 10.1 Hz, H-1). – 50.3 MHz-^{13}C GASPE-NMR ($CDCl_3$), δ 83.96 (C-1).

$C_{39}H_{36}O_9S$ (680.8) Calcd.: C 68.81, H 5.33; Found: C 68.93, H 5.35.

Pent-4'-enyl 2-azido-4,6-O-benzylidene-2-deoxy-1-thio-β-D-galactopyranoside (**13**)

To a *solution of* **8** (2.4 g, 5.8 mmol) in methanol (40 ml) was added 0.22M NaOMe in methanol (30 ml), and the mixture stirred for 30 min at room temperature. After neutralization with ion-exchange resin Amberlite IR-120 and filtration, the solvent was evaporated *in vacuo* and the residue dried in high vacuum. After dissolution of the crude product **12** in acetonitrile (40 ml), benzaldehyde dimethylacetale (1.28 ml, 8.5 mmol) and *p*-toluenesulfonic acid hydrate (50 mg, 0.3 mmol) were added and the mixture stirred for 30 min. After neutralization with triethylamine and addition of dichloromethane (150 ml), the solution was washed with brine (100 ml), dried with $MgSO_4$ and concentrated *in vacuo*. Flash-chromatography in petroleum ether/ethyl acetate (2:1) gave **13**, yield 1.57 g (72%), colorless oil, $[\alpha]_D^{22} = -41.1°$ (c 1, $CHCl_3$), $R_f = 0.22$ (petroleum ether/ethyl acetate 1:1). – 400 MHz-^{1}H NMR ($CDCl_3$) δ 5.78 (ddt, 1H, J_{trans} = 17.2 Hz, J_{cis} = 10.3 Hz, J_{vic} = 6.7 Hz, –CH=), 5.61 (d, 1H, $J_{3,OH}$ = 6.8 Hz, OH), 5.58 (s, 1H, PhC<u>H</u>), 4.45 (d, 1H, $J_{1,2}$ = 9.9 Hz, H-1). – 50.3 MHz-^{13}C GASPE-NMR ($CDCl_3$): δ 137.42 (-CH=), 83.47 (C-1).

Pent-4'-enyl 3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-azido-4,6-O-benzylidene-2-deoxy-1-thio-galactopyranoside (**14**)

A solution of **13** (377 mg, 1.0 mmol) 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide [37] (535 mg, 1.3 mmol) and molecular sieves 4Å (0.5 g) in dichloromethane (25 ml) was stirred for 30 min at room temperature. The mixture was cooled to –20 °C and a solution of silver triflate (411 mg, 1.6 mmol) in toluene (4 ml) was added dropwise. After stirring for 3 h at –20 °C, neutralization with triethylamine, dilution with dichloromethane (25 ml) and filtration, the filtrate was washed with sodium thiosulfate solution (10%) and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Flash-chromatography in petroleum ether/ethyl acetate (3:1→2:1) gave **14**, yield 334 mg (47%), oil, $[\alpha]_D^{24} = -1.7°$ (c 1, $CHCl_3$), $R_f = 0.21$ (petroleum ether/ethyl acetate 1:1). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 7.52 –7.31 (m, 5H, Ph), 5.74 (ddt, 1H, J_{trans} = 17.0 Hz, J_{cis} = 10.2 Hz, J_{vic} = 6.8 Hz, –CH=), 5.51 (s, 1H, PhC<u>H</u>), 5.38 (d, 1H, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.25 (dd, 1H, $J_{1',2'}$ = 7.9 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 4.79 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'). – 100.6 MHz-^{13}C GASPE-NMR ($CDCl_3$): δ = 137.67 (C_q, Ph), 137.40 (–CH=), 115.24 (=CH_2), 102.10 PhCH), 100.81 (C-1'), 84.09, (C-1).

$C_{32}H_{41}N_3O_{13}S$ (707.8) Calcd.: C 54.31, H 5.84, N 5.94, S 4.53; Found: C 54.28, H 5.84, N 5.83, S 4.48.

As a second product, **5** (101 mg, 23%, colorless oil) was isolated.

(–)-Menthyl 2,3,4,6-tetra-O-benzyl-α/β-D-glucopyranoside (**15**)

A mixture of **10** (200 mg, 0.3 mmol), (–)-menthol (60 mg, 0.38 mmol) and molecular sieves 4 Å (0.5g) in dichloromethane (10 ml) was stirred for 60 min. After addition of phenyliodonium cyanotrifluoromethanesulfonate [25] (155 mg, 0.45 mmol) dissolved in dichloromethane (10 ml), the mixture was stirred at room temperature for 3.5 h. The molecular sieves was filtered and washed with dichloromethane. The filtrate was extracted with 2M H_2SO_4, sat. $NaHCO_3$ and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by flash-chromatography in petroleum ether/acetone (13:1) gave **15**, yield 178 mg (88%), ratio of anomers according to HPLC (α:β 65:35), $R_f = 0.64$ (petroleum ether/acetone 2:1). – 200 MHz-^{1}H-NMR ($CDCl_3$): δ 7.33–7.10 (m, 20H, Ph), 5.03–4.42 (m, 9H, C<u>H</u>$_2$Ph, H-1α, H-1β), 4.06–3.93 (m, 0.7H, H-3α). – 50.3 MHz ^{13}C GASPE-NMR ($CDCl_3$): δ 100.73 (C-1β), 98.58 (C-1α), 74.73 (C-3'β), 70.23 (C-3'α), 48.69 (C-4'α), 48.06 (C-4'β), 25.21, 24.50 (C-8'α, C-8'β).

Methyl 3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-azido-4,6-O-benzylidene-2-deoxy-β-D-galactopyranoside (**17**)

A mixture of **5** (973 mg, 2.3 mmol), methyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside [26] **16** (460 mg, 1.5 mmol) and powdered molecular sieves (0.5 g) in dichloromethane (20 ml) was stirred for 30 min, then cooled to –25 °C. A cooled (–25 °C) mixture of *N*-iodosuccinimide (675 mg, 3 mmol) and trifluoromethanesulfonic acid (0.13 ml, 1.5 mmol) in dichloromethane/acetonitrile (7 ml, 4:3) was added. After stirring for 3 h at 0 °C, trimethylamine (2 ml) and dichloromethane (50 ml) were added, and the mixture was filtered. The filtrate was washed with sodium thiosulfate (10%) and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by flash-chromatography in petroleum ether/ethyl acetate (2:1) gave **17**, yield 824 mg (86%), amorphous, $[\alpha]_D^{24}$ = +7.3° (c 1, $CHCl_3$), [[26]: $[\alpha]_D^{25}$ = +7.7° (c 1, $CHCl_3$)]. – 400 MHz-^{1}H NMR ($CDCl_3$): δ = 7.52–7.31 (m, 5H, Ph), 5.53 (s, 1*H*, PhC<u>H</u>), 5.37 (d, 1H, $J_{3',4'}$ = 3.5 Hz, H-4'), 5.24 (dd, 1H, $J_{1',2'}$ = 8.0 Hz, $J_{2',3'}$ = 10.4 Hz, H-2'), 4.78 (d, 1H, $J_{1',2'}$ = 8.0 Hz, H-1'), 4.20 (d, 1H, $J_{3,4}$ = 3.5 Hz, H-4), 4.17 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1).

From first fractions of flash-chromatography, a residue was obtained that contained products formed from the leaving group: MS-EI: *m/z* 228 (0.3) [$C_5H_9IS^+$], 134 (6.9) [$C_5H_{10}O_2S^+$], 101 (22.9) [$C_5H_9S^+$].

4,6-Di-O-acetyl-3-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-α-D-galactopyranosylbromide (**18**)

was synthesized from **17** according to procedures described in reference [26] by treatment of 1.91 g (3.0 mmol) of **17** with aqueous acetic acid (80%, 100 ml), acetylation of the obtained debenzylidenated product with acetic anhydride/pyridine (24 ml, 2:1) and subsequent acetolysis of the methyl glycoside with acetic anhydride (10 ml) and acetic anhydride/conc. sulfuric acid (50:1, 10.2 ml) at −50 °C. Of the obtained product (1.58 g, 80%), 1.46 g (2.2 mmol) were reacted with $TiBr_4$ (1.0 g) in dichloromethane/ethyl acetate (10:1, 41 ml) to give **18**, yield 1.23 g (82%), $[\alpha]_D^{24}$ = +105.5° (c 1, $CHCl_3$), [[26] $[\alpha]_D^{26}$ = +76° (c 0.1, $CHCl_3$)].

N-(9-Fluorenylmethoxycarbonyl)-O-[4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-α-D-galactopyranosyl]threonine phenacyl ester (**19a**) and *N-(9-Fluorenylmethoxycarbonyl)-O-[4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-azido-2-deoxy-β-D-galactopyranosyl]-threonine phenacyl ester* (**19b**)

N-(9-Fluorenylmethoxycarbonyl)-L-threonine phenacyl ester [27] (781 mg, 1.7 mmol) was glycosylated using **18** (1169 mg, 1.7 mmol) in close analogy to a procedure described in reference [27] to give a mixture of anomers **19** which was separated by flash-chromatography first in petroleum ether/acetone (2.3:1) and then in petroleum ether/ethyl acetate (3:1) to yield.

a) the α-anomer **19a**, 907 mg (50%), amorphous, $[\alpha]_D^{23}$ = +35.7° (c 1, $CHCl_3$), [27]: $[\alpha]_D^{20}$ = +33° (c 0.4–0.7, $CHCl_3$), R_f = 0.24 (petroleum ether/ethyl acetate 1:1). – 400 MHz-^{1}H NMR (^{1}H,^{1}H-COSY, $CDCl_3$): δ 5.91 (d, 1*H*, $J_{\alpha,NH}$ = 9.4 Hz, NH), 5.54 (d, 1H J_{gem} = 16.4 Hz, CH^A-Pac), 5.48 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 5.46 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-4), 5.33 (d, 1H, J_{gem} = 16.4 Hz, CH^B-Pac), 5.33 (d, 1H, $J_{3',4'}$ = 3.2 Hz, H-4'), 4.71 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 1.39 (d, 3H, $J_{\beta,\gamma}$ = 6.4 Hz, γ-CH_3-Thr). – 100.6 MHz-^{13}C NMR ($CDCl_3$): δ 191.34 (C=O, Pac), 156.69 (OCONH), 101.41 (C-1'), 98.82 (C-1), 19.00 (γ-CH_3, Thr).

b) the β-anomer **19b**, 683 mg (38%), amorphous, $[\alpha]_D^{23}$ +11.3° (c 1, $CHCl_3$), [27]: $[\alpha]_D^{20}$ = +13° (c 0.4–0.7, $CHCl_3$), R_f = 0.21 (petroleum ether/ethyl acetate 1:1). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 4.70 (d, 1H, $J_{1',2'}$ = 7.8 Hz, H-1'), 4.61 (d, 1H, $J_{1,2}$ = 7.2 Hz, H-1). – 100.6 MHz-^{13}C-NMR ($CDCl_3$): δ 191.81 (C=O, Pac), 101.35 (C-1'), 100.47 (C-1), 18.17 (γ-CH_3, Thr).

N-(9-Fluorenylmethoxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl]-L-threonine phenacyl ester (**20**)

was synthesized by stirring **19a** (2.44 g, 2.3 mmol) with thioacetic acid (20 ml) for 24 h according to reference [27] to give after flash-chromatography 1.74 g (70%), amorphous, $[\alpha]_D^{23}$ = +23.5° (c 1, $CHCl_3$), [27]: $[\alpha]_D^{20}$ = +22° (c 0.4–0.7, $CHCl_3$), R_f = 0.37 (petroleum ether/acetone 1:1).

N-(9-Fluorenylmethoxcarbonyl)-O-(3,4,6-tri-O-acetyl-2-azido-2-deoxy-α/β-D-galactopyranosyl)-threoninephenacylester (**21**)

A mixture of Fmoc-threonine phenacyl ester [27] (335 mg, 0.73 mmol), *N*-iodosuccinimide (209 mg, 0.93 mmol) and molecular sieves (0.3 g) in dichloromethane (20 ml) was stirred for 30 min, then cooled to −50 °C. Trifluoromethanesulfonic acid (0.04 ml, 0.47 mmol) and, subsequently, a mixture of **8** (385 mg, 0.93 mmol) in dichloromethane (5 ml) were added. The mixture was stirred at −10 °C for 6 h. Triethylamine (1 ml) and dichloromethane (20 ml) were added. After filtration through Celite, the filtrate was washed with sodium thiosulfate solution (10%) and water, dried with $MgSO_4$ and concentrated *in vacuo*. Flash-chromatopgraphy in petroleum ether/ethyl acetate (2:1) yielded **21**, 440 mg (78%), amorphous; α/β 4.5:1 (according to ^{1}H NMR). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 5.53 (d, 0.82H, $J_{1,2}$= 3.5 Hz, H-1α), 4.76 (d, 0.18H, $J_{1,2}$ = 7.9 Hz, H-1β). – 100.6 MHz-^{13}C NMR ($CDCl_3$): δ 100.64 (C-1β), 98.75 (C-1α), 19.5 (γ-CH_3, Thr α), 18.30 (γ-CH_3, Thr β).

N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine- phenacylester (**22a**) *and N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-L-threonine phenacyl ester* (**22b**)

The anomeric mixture **21** (1.16 g, 1.5 mmol) was treated with thioacetic acid according to the procedure described in reference [27]. Purification by flash-chromatography in petroleum ether/acetone (1.2:1) resulted in the separation of the anomers to give

22a: Yield 651 mg (55%), amorphous, $[\alpha]_D^{25}$ = +21.4° (c 1, $CHCl_3$), [27]: $[\alpha]_D^{20}$ + 17° (c 0.4–0.7, $CHCl_3$), R_f = 0.38 (petroleum ether/acetone 1:1). – 400-MHz-^{1}H NMR ($CDCl_3$) δ 6.01 (d, 1H, $J_{2,NH}$ = 9.6 Hz, NHAc), 5.68 (d, 1H, $J_{\alpha,NH}$ = 9.5 Hz, NH-Thr), 5.42 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 5.41 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1). – 100.6 MHz-^{13}C GASPE-NMR ($CDCl_3$): δ 191.17 (C=O, Pac), 99.82 (C-1), 18.41 (γ-CH_3, Thr); and

22b: Yield 169 mg (14%), amorphous, $[\alpha]_D^{25}$ = +1.24° (c 1, $CHCl_3$), R_f = 0.29 (petroleum ether/acetone 1:1), – 400 MHz-^{1}H NMR ($CDCl_3$): δ 6.06 (d, 1H, $J_{2,NH}$ = 9.7 Hz, NHAc), 6.04 (d, 1H, $J_{\alpha,NH}$ = 8.8 Hz, NH-Thr), 5.30 (d, 1H, $J_{3,4}$ = 3.3 Hz, H-4), 5.22 (dd, 1H, $J_{2,3}$ = 11.3 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 4.85 (d, 1H, $J_{1,2}$ = 8.4 Hz, H-1). – 100.6 MHz-^{13}C GASPE-NMR ($CDCl_3$): δ 192.31 (C=O, Pac), 156.70 (OCONH), 99.92 (C-1), 18.46 (γ-CH_3, Thr).

$C_{41}H_{44}N_2O_{14}$ (778.8) Calcd.: C 62.43, H 5.62, N 3.55; Found: C 62.20, H 5.81, N 3.33.

N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonine (**23**)

was synthesized according to reference [27] by treatment of **22a** (630 mg, 0.8 mmol) with zinc (1.5 g) in 80% aqueous acetic acid (2 ml) for 2 h. Column-chromatography in ethyl-acetate/acetic acid (9:1) gave **23**, yield 497 mg (93%), amorphous, $[\alpha]_D^{23}$ = +63.7° (c 1.0, $CHCl_3$), [27]: $[\alpha]_D^{20}$ = +90° (c 0.4–0.7, $CHCl_3$), R_f = 0.38 (ethyl acetate/acetic acid 10:1).

N-(9-Fluorenylmethoxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl]-L-threonine (**24**)

was obtained from **19a** (1.62 g, 1.5 mmol) and zinc (3 g) in aqueous acetic (80%, 15 ml) according to reference [27]. Column-chromatography in ethyl acetate/acetic acid (10/1) yielded 1.33 g (93%) of **24**, amorphous $[\alpha]_D^{23} = +69.2°$ (c 1, $CHCl_3$), [27]: $[\alpha]_D^{20} = +67°$ (c 0.4–0.7, $CHCl_3$), $R_f = 0.41$ (ethyl acetate/acetic acid 10:1).

N-(9-Fluorenylmethoxycarbonyl)-L-prolylglycine tert-butyl ester (**25**)

was synthesized from Fmoc proline (1.86 g, 5.5 mmol), glycine *tert*-butylester hydrochloride [38] (0.84, 5.0 mmol), EEDQ (1.48 g, 6.0 mmol) in dichloromethane (20 ml) after addition of ethyldiisopropylamine (0.87 ml, 5 mmol), usual work-up and flash-chromatography. Yield 1.96 g (87%), amorphous, $[\alpha]_D^{28} = -46.1$ (c 1, $CHCl_3$) $R_f = 0.23$ (petroleum ether/ethyl acetate 1:1). – 200 MHz-^{1}H NMR ($CDCl_3$): δ 7.76–7.24 (m, 8H, Fmoc), 4.05–3.76 (m, 2H, CH_2-Gly), 3.36–3.32 (m, 2H, δ-CH_2-Pro), 1.42 (s, 9H, CH_3-*t*Bu) (two rotamers).

$C_{26}H_{30}N_2O_5$	Calcd.:	C 69.31, H 6.71, N 6.22;
(450.5)	Found:	C 69.26, H 6.82, N 6.16.

N-(9-Fluorenylmethoxycarbonyl)-L-alanyl-L-prolylglycine-tert-butylester (**26**)

A solution of **25** (1.0 g, 2.2 mmol) in dichloromethane (10 ml) and morpholine (5 ml) was stirred for 6 h. After evaporation of the solvents and codistillation of the residue with toluene (10 ml), the deblocked depeptide ester was lyophylized in high vacuum. The residue was dissolved in dichloromethane (15 ml), and the solution was added to a solution of Fmoc alanine (0.97 g, 3.1 mmol) and IIDQ [31] (0.9 ml, 3.1 mmol) in dichloromethane (10 ml) and dimethylformamide (3 ml) which had been stirred for 15 min. After stirring for 48 h, dichloromethane (50 ml) was added. Usual work-up and flash-chromatography in petroleum eether/acetone (2:1) yielded **26**, 0.99 g (86%), *m.p.* 151°C, $[\alpha]_D^{23} = -71.1°$ (c 1, $CHCl_3$), R_f = 0.24 (petroleum ether/acetone 2:1). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 7.75–7.27 (m, 8H, Fmoc), 4.63–4.61 (m, 1H, α-CH-Pro), 4.54 (qui, 1H, J = 7.1 Hz, α-CH-Ala), 3.88 (d, 2H, $J_{\alpha,NH}$ = 4.9 Hz, CH_2-Gly), 3.67–3.54 (m, 2H, δ-CH_2-Pro), 1.44 (s, 9H, CH_3-*t*-Bu), 1.41 (d, 3H, $J_{\alpha\beta}$ = 6.9 Hz, β-CH_3-Ala) (two rotamers).

N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ω}-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-proline- tert-butylester

was obtained from Fmoc-Arg(Pmc)-OH [29] (800mg, 1.2 mmol), H-Pro–O*t*Bu [39] (171 mg, 1.0 mmol) and IIDQ [31] (0.36 ml, 1.2 mmol) in dichloromethane (12 ml) within 24 h, as was described for **26**. Yield 552 mg (68%), $[\alpha]_D^{24} = -18.1°$ (c 1, $CHCl_3$), R_f = 0.11 (petroleum ether/acetone 2:1). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 7.74–7.26 (m, 8H, Fmoc), 6.17 (m, 3H, δ,ω-NH-Arg), 4.52–4.49 (m, 1H, α-CH-Pro), 4.39–4.29 (m, 3H, α-CH-Arg, CH_2-Fmoc), 3.63–3.51 (m, 2H, δ-CH_2-Pro), 3.21 (s, 2H, δ-CH_2-Arg), 2.58, 2.56 (2s, 6H, *o*-CH_3-Pmc), 1.43 (s, 9H, CH_3-*t*-Bu), 1.27 (s, 6H, $C(CH_3)_2$-Pmc).

N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ω}-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-proline (**27**)

A solution of Fmoc-Arg(Pmc)-Pro-O*t*Bu (*vide supra*) (1.78 g, 2.2 mmol) in formic acid (15 ml) was stirred for 7 d. After evaporation of formic acid, codistillation with toluene (20 ml) and lyophylization, the remainder was purified by flash-chromatography in chloroform/methanol/ acetic acid (40:2:1) to yield **27**, 1.45 g (87%), amorphous, $[\alpha]_D^{27} = -11.6°$ (c 1, $CHCl_3$), R_f = 0.28 ($CHCl_3$/MeOH/AcOH 40:2:1). – 400 MHz-^{1}H NMR ($CDCl_3$): δ 7.71–7.19 (m, 8H, Fmoc), 4.61–4.45 (m, 1H, α-CH-Pro), 4.30–4.09 (m, 4H, α-CH-Arg, CH_2-Fmoc, 9-CH-Fmoc), 3.94–3.57 (m, 2H, δ-CH_2-Pro), 1.74 (t, 2H, J_{vic} = 6.3 Hz, CH_2-Pmc), 1.25 (s, 6H, $C(CH_3)_2$-Pmc). – 100.6 MHz-^{13}C GASPE-NMR ($CDCl_3$): δ 156.18 (ω-C_q, Arg; OCONH), 73.53 ($\underline{C}(CH_3)_2$, Pmc), 66.91 (CH_2, Fmoc), 60.25 (α-CH, Pro), 52.19 (α-CH, Arg), (two rotamers). FAB-MS (NBA): *m/z* 761.0 [MH^+]

N^{α}-(9-Fluorenylmethoxycarbonyl)-N^{ω}-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine tert-butyl ester (**28**)

Fmoc Tripeptide *tert*-butylester **26** (1.51 g, 2.9 mmol) was stirred with morpholine (30 ml) for 2.5 h. After removal of morpholine as described for **25**, the remainder was dissolved in dichloromethane (15 ml) and added to a solution of **27** (2.2 g, 2.9 mmol) and IIDQ [31] (0.86 ml, 2.9 mmol) in dichloromethane (150 ml) which already had been stirred for 15 min. After 24 h, usual work-up and flash-chromatography in petroleum ether/acetone (1:2), 2.57 g (85%) of **28** were obtained as an amorphous solid, $[\alpha]_D^{27} = -30.7°$ (c 1, $CHCl_3$), R_f = 0.18 (petroleum ether/acetone 1:2). – 200 MHz-^{1}H NMR ($CDCl_3$): δ 6.28, 6.20 (2m, 3H, δ,ω-NH-Arg), 5.96 (d, 1H, $J_{\alpha,NH}$ = 7.5 Hz, α-NH-Arg), 4.67–4.48 (m, 3H, 2 α-CH-Pro, α-CH-Ala), 3.84–3.81 (m, 2H, CH_2-Gly), 3.70–3.45 (m, 4H, 2 δ-CH_2-Pro), 1.42 (s, 9H, CH_3-*t*-Bu), 1.35 (d, 3H, $J_{\alpha\beta}$ = 7.3 Hz, β-CH_3-Ala), 1.26 (s, 6H, $C(CH_3)_2$-Pmc). – 100.6 MHz-^{13}C NMR ($CDCl_3$): δ 155.83 (ω-C_q, Arg; OCONH), 81.73 (C_q, *t*-Bu), 73.35 ($\underline{C}(CH_3)_2$, Pmc), 60.38, 59.89 (2 α-CH, Pro), 51.99 (α-CH, Arg), 47.35, 47.15 (2 δ-CH_2, Pro), 46.98 (α-CH, Ala; 9-CH, Fmoc), 41.76 (CH_2, Gly), 40.55 (δ-CH_2, Arg), 18.26, 17.72, 17.19 (β-CH_3, Ala; *o*-CH_3, Pmc), 11.89 (*m*-CH_3, Pmc) (two rotamers). FAB-MS (NBA): *m/z* 1041.8 [MH^+].

N-(9-Fluorenylmethoxycarbonyl)-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-N^{ω}-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine tert-butyl ester (**29**)

Removal of the Fmoc group from **28** (708 mg, 0.68 mmol) was carried out with morpholine (10 ml) as described for **26**. A solution of the obtained amino component in dichloromethane (5 ml) was added to a solution of **23** (456 mg, 0.68 mmol) and IIDQ [31] (0.20 ml, 0.68 mmol) in dichloromethane (15 ml) which already had been stirred for 15 min. After stirring of the mixture for 16 h and subsequent addition of dichloromethane (50 ml), the solution was washed with 0.1N HCl (three times), sat. $NaHCO_3$ and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Purification by flash-chromatography in petroleum ether/acetone (1:3) gave the T_N antigen hexapeptide **29**, 790 mg (79%), amorphous, $[\alpha]_D^{25} = +11.5°$ (c 1, $CHCl_3$),

R_t = 37.4 min, R_f = 0.21 (petroleum ether/acetone 1:3). – 400 MHz-^{1}H NMR (DMSO-d_6): δ 6.63, 6.40 (2s, 3H, δ,ω-NH-Arg), 5.28 (m, 1H, H-4), 4.99 dd, 1H, $J_{2,3}$ = 11.5 Hz, $J_{3,4}$ = 3.0 Hz, H-3), 4.75 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.01 (d, 2H, $J_{\alpha,NH}$ = 6.0 Hz, CH_2-Gly), 3.68–3.41 (m, 4H, 2 δ-CH_2-Pro), 3.01 (m, 2H, δ-CH_2-Arg), 2.07, 2.00, 1.98, 1.87, 1.83 (5s, 15H, *m*-CH_3-Pmc, CH_3-Ac), 1.38 (s, 9H, CH_3-*t*-Bu), 1.22, 1.13 (2s, 6H, C(CH_3)$_2$-Pmc), 1.17 (d, 3H, $J_{\alpha,\beta}$ = 6.8 Hz, β-CH_3-Ala), 1.14 (d, $J_{\beta,\gamma}$ = 7.1 Hz, γ-CH_3-Thr). – 100.6 MHz-^{13}C NMR (DEPT-135, DMSO-d_6): δ 156.57, 155.92 (ω-C_q; Arg, OCONH), 119.99 (Fmoc), 98.56 (C-1), 80.47 (C_q, *t*-Bu), 75.95 (β-CH, Thr), 73.32 (C(CH_3)$_2$, Pmc), 59.29, 59.06 (2 α-CH, Pro), 58.15 (α-CH, Thr), 49.95 (α-CH, Arg), 46.71, 46.27, 46.13 (C-2; α-CH, Ala; 9-CH, Fmoc), 46.57, 46.44 (2 δ-CH_2, Pro), 41.28 (CH_2, Gly), 20.66 (CH_2C(CH_3)$_2$, Pmc), 20.39 (CH_3, Ac), 18.57, 18.01, 16.93, 16.78 (γ-CH_3, Thr; β-CH_3, Ala; *o*-CH_3, Pmc), 11.77 (*m*-CH_3, Pmc). – FAB-MS (glycerol): *m/z* 1472.1 [MH$^+$].

N-(9-Fluorenylmethoxycarbonyl)-L-prolyl-4-O-benzyl-L-aspartic acid tert-butyl ester

A solution of Fmoc-proline 2.77 g, 8.2 mmol), 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, 1.57 g, 8.2 mmol) and 1-hydroxy-benzotriazole [32] (2.22 g, 16.4 mmol) in dichloromethane (20 ml) and dimethylformamide (20 ml) was stirred for 1 h. A solution of H-Asp(OBzl)-OtBu [40] (1.9 g, 6.8 mmol) in dichloromethane (5 ml) was added. The mixture was stirred for 24 h, diluted with dichloromethane (100 ml), washed with 0.1M HCl, sat. $NaHCO_3$ and brine, dried with $MgSO_4$ and concentrated *in vacuo*. Flash-chromatography in petroleum ether/ethyl acetate (3:2) gave 3.50 g (86%) of the product, colorless crystals, *m.p.* 80 °C, $[\alpha]_D^{23}$ = –18.5° (c 1, $CHCl_3$), R_f = 0.32 (petroleum ether/ethyl acetate 1:1). – 200 MHz-^{1}H NMR ($CDCl_3$): δ 7.72–7.19 (m, 13H, Fmoc, Ph), 5.11–4.93 (m, 2H, PhC$\underline{H}_2$), 4.77–4.58 (m, 1H, α-CH-Pro), 3.05–2.78 (m, 2H, β-CH_2-Asp), 1.35, 1.30 (2s_b (CH_3-*t*-Bu) (two rotamers).

N-(9-Fluorenylmethoxycarbonyl)-L-prolyl-4-O-benzyl-L-aspartic acid (**30**)

A solution of Fmoc-Pro-Asp(OBzl)-O*t*Bu (2.6 g, 4.3 mmol) in dichloromethane/trifluoracetic acid (1:1, 40 ml) was stirred for 1 h. After evaporation of the solvents *in vacuo*, codistillation with toluene (20 ml) and lyophilization, the residue was purified by flash-chromatography in $CHCl_3$/MeOH/AcOH (70:2:1) to give **21**, yield 2.22 g (95%), *m.p.* 144 °C, $[\alpha]_D^{24}$ = –21.8° (c 1, $CHCl_3$), R_f = 0.35 ($CHCl_3$/MeOH/AcOH 40:2:1). – 400 MHz-^{1}H NMR (DMSO-d_6): δ 12.83 (s, 1H, COOH), 8.53 (d, 0.57H, $J_{\alpha,NH}$ = 8.2 Hz, NH-Asp, A), 8.32 (d, 0.43H, $J_{\alpha,NH}$ = 8.1 Hz, NH-Asp, B), 5.09 (s, 0.86H, PhC$\underline{H}_2$, B), 4.87 (s, 1.14H, PhC$\underline{H}_2$, A), 1.93–1.80 (m, 2H, γ-CH_2-Pro). Ratio of rotamers 1.3:1.0.

$C_{31}H_{30}N_2O_7$	Calcd:	C 68.62, H 5.57, N 5.16;
(542.6)	Found:	C 68.37, H 5.68, N 5.13.

N-(9-Fluorenylmethoxycarbonyl)-L-prolyl-4-O-benzyl-L-aspartyl-[O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)]-L-threonyl-N$^\omega$-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine tert-butyl ester (**31**)

Removal of the Fmoc group from **29** (760 mg, 0.51 mmol) was carried out by stirring with morpholine (10 ml) for 1 h as described for **26**. A solution of the amino-deblocked component in dichloromethane (5 ml) was added to a solution of **30** (280 mg, 0.51 mmol) and IIDQ [31] (0.15 ml, 0.51 mmol) in dichloromethane (15 ml) which had already been stirred for 15 min. The combined solutions were stirred for 22 h, diluted with dichloromethane (20 ml), washed with 0.1M HCl (three times), sat. $NaHCO_3$ and brine, dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by GPC on Sephadex LH-20 and, subsequently, by preparative HPLC on column A in acetonitrile/water (65/35). Yield 786 mg (86 %), amorphous, $[\alpha]_D^{25}$ = –18.0° (c 1, $CHCl_3$) R_t = 38.7 min; R_f = 0.46 ($CHCl_3$/MeOH 10:1). – 400 MHz-^{1}H NMR (DMSO-d_6): δ 8.53–6.95 (m, 19H, NH-Asp, NH-Thr, α-NH-Arg, NH-Ala, NH-Gly, AcN$\underline{H}$, Fmoc, Ph), 6.69, 6.42 (2s, 3H, δ,ω-NH-Arg), 3.04–2.99 (m, 2H, δ-CH_2-Arg), 1.38 (s, 9H, CH_3-*t*-Bu), 1.22 (s, 6H, C(CH_3)$_2$-Pmc), 1.16 (d, 3H, $J_{\alpha,\beta}$ = 6.2 Hz, β-CH_3-Ala), 1.10 (d, $J_{\beta,\gamma}$ = 5.6 Hz, γ-CH_3-Thr, A), 1.02 (d, $J_{\beta,\gamma}$ = 5.9 Hz, γ-CH_3-Thr, B). Ratio of rotamers 5:1. – 100.6 MHz-^{13}C NMR (DEPT-135, $CDCl_3$): δ 173.28, 172.20, 172.07, 171.64, 171.54, 171.36, 171.08, 170.87, 170.48, 170.29, 170.11, 169.28, 168.50 (C=O), 120.00 (Fmoc), 99.12 (C-1), 81.79 (C_q, *t*-Bu), 77.28 (β-CH, Thr), 73.47 (C(CH_3)$_2$, Pmc), 56.53 (α-CH, Thr), 51.95 (C-2), 50.55 (α-CH, Arg), 49.67 (α-CH, Asp), 47.51, 47.41 (3 δ-CH_2, Pro), 47.11, 46.78 (α-CH, Ala; 9-CH, Fmoc), 41.80 (CH_2, Gly), 28.00 (CH_3, *t*-Bu), 26.76, 26.71 (C($\underline{C}H_3$)$_2$, Pmc), 23.14 (CH_3, Ac), 21.40 ($\underline{C}H_2C(CH_3)_2$, Pmc), 20.73, 20.66, 20.42 (CH_3, Ac), 18.44, 18.35, 17.36 (γ-CH_3, Thr; β-CH_3, Ala; *o*-CH_3, Pmc), 12.05 (*m*-CH_3, Pmc). FAB-MS (glycerol): *m/z* 1774.1 [MH$^+$].

N-Acetyl-L-prolyl-(4-O-benzyl)-L-aspartyl-[O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-a-D-galactopyranosyl)]-L-threonyl-N^w-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine-tert-butyl-ester (**32**)

Removal of the Fmoc group from **31** (266 mg, 0.15 mmol) was carried out using morpholine (5 ml) within 45 min as was described for **29**. The obtained crude product was stirred in acetic anhydride/pyridine (1:3, 10 ml) for 45 min. After evaporation of the solvents *in vacuo*, codistillation with toluene (10 ml) and lyophilization, the residue was purified by GPC on Sephadex LH-20 and subsequent preparative HPLC (column A) in acetonitrile/water (55:45) to give 213 mg (89%) of **32**, amorphous, $[\alpha]_D^{23}$ = –16.8° (c 1, $CHCl_3$), R_t = 36.1 min, R_f = 0.38 ($CHCl_3$/MeOH 10:1). – 400 MHz-^{1}H NMR (^{1}H,^{1}H-COSY, DMSO-d_6): δ 8.19–8.17 (m, 1H, NH-Asp), 8.10–8.07 (m, 1H, NH-Gly), 7.95–7.93 (m, 1H, NH-Ala), 7.61–7.58 (m, 1H, α-NH-Arg), 7.34–7.24 (m, 6H, N$\underline{H}$Ac, Ph), 6.68, 6.41 (2s, 3H, δ,ω-NH-Arg), 5.07–5.06 (m, 3H, H-1, PhC$\underline{H}_2$), 3.68–3.64 (m, 2H, CH_2-Gly), 3.57–3.26 (m, 6H, 3 δ-CH_2-Pro), 2.97–2.86 (m, 1H, β-CH^A-Asp), 2.78–2.64 (m, 1H, β-CH^B-Asp), 1.38 (s, 9H, CH_3-*t*-Bu), 1.23 (s, 6H, C(CH_3)$_2$-Pmc), 1.17 (d, 3H, $J_{\alpha,\beta}$ = 6.9 Hz, β-CH_3-Ala), 1.11 (d, 3H, $J_{\beta,\gamma}$ = 6.5 Hz, γ-CH_3-Thr). – 100.6 MHz-^{13}C NMR (DEPT-135, $CDCl_3$): δ 117.71 (C_i, Pmc), 99.20 (C-1), 81.69 (C_q, *t*-Bu), 73.44 (C(CH_3)$_2$, Pmc), 68.67, 67.42, 66.76, (C-3, C-4, C-5, Ph$\underline{C}H_2$), 62.05 (C-6), 60.57, 60.30, 59.95 (3 α-CH, Pro), 56.77 (α-CH, Thr), 50.56 (α-CH, Arg), 50.01 (α-CH, Asp), 48.33, 47.39 (3 δ-CH_2, Pro), 47.24, 46.80 (C-2; α-CH, Ala), 41.77 (CH_2,

Gly), 32.89 (ArCH$_2$, Pmc), 23.12, 22.12 (CH$_3$, Ac), 21.37 (CH$_2$C(CH$_3$)$_2$, Pmc), 20.68, 20.63, 20.53 (CH$_3$, Ac), 18.38, 18.25, 17.31 (γ-CH$_3$, Thr; β-CH$_3$, Ala; *o*-CH$_3$, Pmc), 12.01 (m-CH$_3$, Pmc). FAB-MS (NBA): *m/z* 1593.8 [MH$^+$].

N-Acetyl-L-prolyl-L-aspartyl-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine hydrotrifluoracetate (**33**)

A solution of **32** (200 mg, 0.125 mmol) in methanol (15 ml) was hydrogenated under atmospheric pressure using Pd (black, 50 mg) for 30 min. After filtration through Celite, concentration of the filtrate *in vacuo*, drying in high vacuum, the remainder was dissolved in 10 ml of trifluoroacetic acid/dichloromethane/1,2-ethane-dithiol/thioanisole (45/45/5/5) and stirred for 2 h. After concentration *in vacuo*, codistillation with toluene (10 ml) and lyophylization, the crude product was purified by GPC on Sephadex LH-20 followed by preparative HPLC (column A) in acetonitrile/water (20/80) to give **33**, yield 149 mg (92%), amorphous, $[\alpha]_D^{23}$ −66.5° (c 1, MeOH), R_t = 22.7 min, R_f = 0.71 (RP-8, acetonitrile/water 1:1). – 400 MHz-^{1}H-^{13}C-COSY-NMR (D_2O, ratio of rotamers 7.8:1): δ 5.41 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-4), 5.18–5.13 (m, 1H, H-3), 5.03–5.01 (m, 1H, H-1), 4.83–4.69 (m, 1H, α-CH-Asp), 4.58–4.51 (m, 3H, α-CH-Thr, α-CH-Arg, α-CH-Ala), 4.46–4.32 (m, 5H, H-5, β-CH-Thr, 3 α-CH-Pro), 4.32 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10.9 Hz, H-2), 4.24–4.15 (m, 2H, H-6), 1.37 (d, 2.67H, $J_{\alpha,\beta}$ = 6.8 Hz, β-CH$_3$-Ala, A), 1.32 (d, 0.33H, $J_{\alpha,\beta}$ = 6.8 Hz, β-CH$_3$-Ala, B), 1.25 (d, 3H, $J_{\beta,\gamma}$ = 6.2 Hz, γ-CH$_3$-Thr). – 100.6 MHz-^{13}C NMR ((D_2O): δ 156.89 (ω-C$_q$, Arg), 98.74, 98.65 (C-1), 18.01 (γ-CH$_3$, Thr). MALDI-MS [α-cyano-4-hydroxy-cinnamicacid/(CCA)]: *m/z* 1181.8 [MH$^+$, cation].

N-Acetyl-L-prolyl-L-aspartyl-[O-(2-acetamido-2-deoxy-α-D-galactopyrano-syl)]-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine hydroacetate (**34**)

To a solution of **33** (60 mg, 46 mmol) in methanol (7ml) was added 1% NaOMe in methanol until pH 8.5 (pH paper) was achieved. The solution was stirred for 75 min, one drop of acetic acid was added and the mixture stirred with Amberlite IR-120 for 30 min. After filtration, the solvent was evaporated *in vacuo* to give **34**, yield 47 mg (91%), amorphous, $[\alpha]_D^{23}$ = −76.4° (c 1, H_2O), R_t = 2.47 min, R_f = 0.84 (RP-8, acetonitrile/water 3:7). 400 MHz-^{1}H-^{1}H-COSY-NMR (D_2O, ratio of rotamers 13:1): δ 4.87–4.79 (m, 1*H*, α-CH-Asp), 4.82 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.58–4.49 (m, 3H, α-CH-Thr, α-CH-Arg, α-CH-Ala), 4.41–4.34 (m, 4H, β-CH-Thr, 3 α-CH-Pro), 4.05 (dd, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 11.2 Hz, H-2), 3.99 (t, 1H, $J_{5,6}$ = 6.0 Hz, H-5), 3.93 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-4), 3.88 (dd, 1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 2.4 Hz, H-3), 3.78–3.47 (m, 10H, H-6, 3 δ-CH$_2$-Pro, CH$_2$-Gly), 3.23–3.14 (m, 2H, δ-CH$_2$-Arg), 2.85–2.63 (m, 2H, β-CH$_2$-Asp), 2.32–2.20, 2.13–1.77, 1.75–1.63 (3m, 25H, 3 β-CH$_2$-Pro, 3 γ-CH$_2$-Pro, β-CH$_2$-Arg, γ-CH$_2$-Arg, CH$_3$-Ac), 1.34 (d, 2.79H, $J_{\alpha,\beta}$ = 7.1 Hz, β-CH$_3$-Ala, A), 1.30 (d, 0.21H, $J_{\alpha,\beta}$ = 7.1 Hz, β-CH$_3$-Ala, B), 1.22 (d, 0.21H, $J_{\beta,\gamma}$ = 6.2 Hz, γ-CH$_3$-Thr, B), 1.21 (d, 2.79H, $J_{\beta,\gamma}$ = 6.2 Hz, γ-CH$_3$-Thr, A). – 100.6 MHz-^{1}H-^{13}C-COSY-NMR (D_2O): δ 174.33, 173.86, 173.55, 173.45, 173.31, 173.08, 171.26, 171.06 (C=O), 156.89 (ω-C$_q$, Arg), 98.65 (C-1), 75.48, 75.09 (β-CH, Thr), 71.42 (C-5), 68.65 (C-4), 68.10 (C-3), 61.43, 61.33 (C-6), 60.66, 60.26, 60.14 (3 α-CH, Pro), 57.24 (α-CH, Thr), 51.31 (α-CH, Arg), 50.93 (α-CH, Asp), 49.74 (C-2), 48.69, 47.77, 47.36, 47.14 (3 δ-CH$_2$, Pro), 47.65 (α-CH, Ala), 42.68 (CH$_2$, Gly), 40.63 (δ-CH$_2$, Arg), 31.76, 31.29 (β-CH$_2$, Asp), 29.96, 29.67, 29.36, 29.31 (3 β-CH$_2$, Pro), 27.56 (β-CH$_2$, Arg), 24.68, 24.57, 24.29, 24.18 (3 γ-CH$_2$, Pro; γ-CH$_2$, Arg), 22.33, 21.48, 21.38, 21.16 (CH$_3$, Ac), 18.39, 18.26 (γ-CH$_3$, Thr), 15.96, 15.48 (β-CH$_3$, Ala). – MALDI-MS (CCA): *m/z* 1055.3 [MH$^+$, cation].

N-(9-Fluorenylmethoxycarbonyl)-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl]-L-threonyl-N^ω-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine tert-butyl ester (**35**)

Removal of the Fmoc group from **28** (1.35 g, 1.3 mmol) in morpholine (20 ml) was carried out as described for the synthesis of **29**. A solution of the obtained amino component in dichloromethane (5 ml) was added to a solution of **24** (1.25 g, 1.3 mmol) and IIDQ [31] (0.4 ml, 1.3 mmol) in dichloromethane (50 ml) which already had been stirred for 15 min. Because **24** was not completely converted after 75 min, additional IIDQ (0.4 ml, 1.3 mmol) was added, and the mixture was stirred for 48 h. After dilution with dichloromethane (50 ml), subsequent washing with 0.1M HCl, sat. NaHCO$_3$ and brine, the organic solution was dried with MgSO$_4$ and concentrated *in vacuo*. Flash-chromatography in petroleum ether/acetone (2:3) resulted in recollection of **24** (467 mg, 38%) and isolation of the desired product **35**, yield 1.240 g (54%), amorphous, $[\alpha]_D^{23}$ = +10.9° (c 1, $CHCl_3$), R_t = 38.1 min, R_f = 0.44 ($CHCl_3$/MeOH 10:1). – 100.6 MHz-^{13}C NMR (DEPT-135, $CDCl_3$): δ 171.94, 171.61, 170.32, 170.26, 170.06, 169.90, 169.32, 168.47 (C=O), 156.72, 156.22 (ω-C$_q$, Arg, OCONH), 153.62 (C$_i$, Pmc), 143.63, 141.27 (C$_i$, Fmoc), 135.45, 134.79, 133.73, (C$_i$, Pmc), 127.77, 127.03, 125.00 (Fmoc), 123.84 (C$_i$, Pmc), 119.98 (Fmoc), 117.77 (C$_i$, Pmc), 100.86 (C-1'), 99.98 (C-1), 81.94 (C$_q$, *t*-Bu), 77.18 (β-CH, Thr), 73.50 (*C*(CH$_3$)$_2$, Pmc), 73.14, 70.53, 70.38, 69.27, 69.20, 67.73, 67.18 (C-3, C-4, C-5, C-2', C-3', C-4', C-5'), 67.29 (CH$_2$, Fmoc), 62.86 (C-6), 60.87 (C-6'), 60.00 (2 α-CH, Pro), 57.44 (α-CH, Thr), 50.83 (α-CH, Arg), 48.94 (C-2), 47.54, 47.43 (2 δ-CH$_2$, Pro), 47.15 (9-CH, Fmoc), 46.80 (α-CH, Ala), 41.73 (CH$_2$, Gly), 40.45 (δ-CH$_2$, Arg), 32.83 (ArCH$_2$, Pmc), 29.24, 29.05 (2 β-CH$_2$, Pro, β-CH$_2$, Arg), 27.92 (CH$_3$, *t*-Bu), 26.72, 26.63 (C(CH$_3$)$_2$, Pmc), 25.00 (2 γ-CH$_2$, Pro, γ-CH$_2$, Arg), 23.17 (CH$_3$, Ac), 21.35 (CH$_2$C(CH$_3$)$_2$, Pmc), 20.55, 20.45 (CH$_3$, Ac), 18.39, 17.30 (γ-CH$_3$, Thr; β-CH$_3$, Ala; *o*-CH$_3$, Pmc), 12.00 (m-CH$_3$, Pmc). – FAB-MS (glycerol): *m/z* 1760.0 [MH$^+$].

N-Acetyl-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl]-L-threonyl-N^ω-(2,2,5,7,8-pentamethylchromane-6-sulfonyl)-L-arginyl-L-prolyl-L-alanyl-L-prolyl-glycine tert-butyl ester (**36**)

Removal of the Fmoc group from **35** (400 mg, 0.227 mmol) in morpholine (6 ml, 1 h) and subsequent N-acetylation with acetic anhydride/pyridine (1:3, 10 ml, 45 min) was carried out as described for **32**. After purification by GPC on Sephadex

LH-20, 334 mg (93%) of **36** were obtained, amorphous, $[\alpha]_D^{23}$ = +25.4° (c 1, $CHCl_3$), R_t = 35.0 min, R_f = 0.21 ($CHCl_3$/MeOH 10:1). – 400 MHz-^{1}H NMR (^{1}H^{1}H-COSY, DMSO): δ 8.24 (d, 1H, $J_{\alpha,NH}$ = 7.9 Hz, NH-Thr), 8.07 (t, 1H, $J_{\alpha,NH}$ = 5.8 Hz, NH-Gly), 7.98 (d, 1H, $J_{\alpha,NH}$ = 6.9 Hz, α-NH-Arg), 7.83 (d, 1H, $J_{\alpha,NH}$ = 8.9 Hz, NH-Ala), 7.00 (d, 1H, $J_{2,NH}$ = 9.3 Hz, NHAc), 6.72, 6.41 (2m, 3H, δ,ω-NH-Arg), 5.27 (m, 1H, H-4), 5.23 (m, 1H, H-4'), 5.04–5.01 (m, 1H, H-3'), 4.84–4.75 (m, 2*H*, H-1', H-2'), 4.72 (m, 1H, H-1), 1.77 (t, 2H, J_{vic} = 6.4 Hz, CH_2-Pmc), 1.38 (s, 9H, CH_3-*t*-Bu), 1.17 (d, 3H, $J_{\alpha,\beta}$ = 6.8 Hz, β-CH_3-Ala), 1.09 (d, 3H, $J_{\beta,\gamma}$ = 5.9 Hz, γ-CH_3-Thr). 100.6 MHz. – ^{13}C NMR (DEPT-135, $CDCl_3$): δ 172.44, 171.94, 171.21, 170.80, 170.68, 170.61, 170.38, 170.29, 169.67, 168.80 (C=O), 156.52 (ω-C_q, Arg), 101.28 (C-1'), 100.25 (C-1), 82.26 (C_q, *t*-Bu), 77.15 (β-CH, Thr), 73.85 (C(CH$_3$)$_2$, Pmc), 23.54 (CH_3, Ac), 21.71 (CH_2C(CH$_3$)$_2$, Pmc), 20.93, 20.79 (CH_3, Ac), 18.82, 18.72, 17.64 (γ-CH_3, Thr; β-CH_3, Ala; *o*-CH_3, Pmc), 12.34 (m-CH_3, Pmc). – FAB-MS (NBA): *m/z* 1579.7 [MH$^+$].

N-Acetyl-O-[2-acetamido-4,6-di-O-acetyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-2-deoxy-α-D-galactopyranosyl]-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine hydrotrifluoracetate (**37**)

Removal of the *tert*-butylester and Pmc protecting groups from **36** (285 mg, 0.18 mmol) was carried out as described for **33**. Purification was achieved by preparative HPLC first on column A in acetonitrile/water (1:1) and, subsequently, on column B in acetonitrile/water (1:3). Yield 200 mg (81%), amorphous, $[\alpha]_D^{23}$ = –26.8° (c 1, MeOH), R_t = 27.6 min, R_f = 0.38 ($CHCl_3$/MeOH/AcOH 4:2:1). – 400 MHz-^{1}H NMR (D_2O, ^{1}H, ^{1}H- and ^{1}H, ^{13}C-COSY, ratio of rotamers 4:1): δ 5.38 (d, 1*H*, $J_{3,4}$ = 2.6 Hz, H-4), 5.34 (d, 1H, $J_{3',4'}$ = 3.4 Hz, H-4'), 5.09 (dd, 1H, $J_{2',3'}$ = 10.3 Hz, $J_{3',4'}$ = 3.4 Hz, H-3'), 4.91 (dd, 1H, $J_{1',2'}$ = 7.9 Hz, $J_{2',3'}$ = 10.3 Hz, H-2'), 4.80 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1), 4.79 (d, 1H, $J_{1',2'}$ = 7.9 Hz, H-1'), 4.55–4.47 (m, 1H, α-CH-Arg), 4.48 (q, 1H, $J_{\alpha,\beta}$ = 7.0 Hz, α-CH-Ala), 4.43 (d, 1H, $J_{\alpha,\beta}$ = 2.1 Hz, α-CH-Thr), 4.35–4.26 (m, 2H, 2 α-CH-Pro), 4.27 (t, 1H, $J_{5,6}$ = 7.0 Hz, H-5), 4.22 (dd, 1H, $J_{\alpha,\beta}$ = 2.1 Hz, $J_{\beta,\gamma}$ = 6.2 Hz, β-CH-Thr), 4.21 (dd, 1H, $J_{1,2}$ = 3.2 Hz, $J_{2,3}$ = 10.9 Hz, H-2), 4.15 (dd, 1H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6a}$ = 6.8 Hz, H-6a), 4.11–4.06 (m, H-3, H-5', H-6'), 4.01 (dd, 1H, $J_{6a,6b}$ = 11.8 Hz, $J_{5,6b}$ = 7.2 Hz, H-6b), 3.75–3.47 (m, 4H, 2 δ-CH_2-Pro), 3.66 (d, 2H, J_{gem} = 3.2 Hz, CH_2-Gly), 3.14–3.11 (m, 2H, δ-CH_2-Arg), 2.25–1.55 (m, 12H, 2 β-CH_2-Pro, 2 γ-CH_2-Pro, β-CH_2-Arg, γ-CH_2-Arg), 2.12, 2.10, 2.04, 2.03, 2.01, 1.93, 1.91 (7s, 24H, CH_3-Ac), 1.30 (d, 2.4H, $J_{\alpha,\beta}$ = 7.0 Hz, β-CH_3-Ala, A), 1.25 (d, 0.6H, $J_{\alpha,\beta}$ = 7.0 Hz, β-CH_3-Ala, B), 1.18 (d, 3H, $J_{\beta,\gamma}$ = 6.2 Hz, γ-CH_3-Thr). – 100.6 MHz-^{13}C NMR (D_2O): δ 176.28, 174.72, 173.77, 173.74, 173.56, 173.43, 173.25, 173.17, 172.81, 172.62, 171.41, 171.35, 171.18 (C=O), 157.03 (ω-C_q, Arg), 100.77 (C-1'), 98.79 (C-1), 76.19, 75.95 (β-CH, Thr), 74.21 (C-3), 71.25 (C-3'), 70.51 (C-4, C-5'), 69.64 (C-2'), 68.23 (C-4'), 67.68 (C-5), 63.42 (C-6), 61.96 (C-6'), 61.02, 60.86, 60.28 (2 α-CH, Pro), 57.52 (α-CH, Thr), 51.21 (α-CH, Arg), 49.13, 48.64, 48.09, 47.93, 47.85, 47.53 (C-2; 2 δ-CH_2, Pro; α-CH, Ala), 43.56 (CH_2, Gly), 40.74 (δ-CH_2, Arg), 29.50, (2 β-CH_2, Pro), 27.89 (β-CH_2, Arg), 24.82, 24.72, 24.52, 24.36 (2 γ-CH_2, Pro, γ-CH_2, Arg), 22.48, 21.92, 20.43, 20.40, 20.33, 20.16, 20.12 (CH_3, Ac), 18.38 (γ-CH_3, Thr), 16.04, 15.71 (β-CH_3, Ala). – MALDI-MS (CCA): *m/z* 1257.3 [MH$^+$, cation].

N-Acetyl-O-[2-acetamido-2-deoxy-3-O-(β-D-galactopyranosyl)-α-D-galactopyranosyl]-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolylglycine hydroacetate (**38**)

Removal of the *O*-acetyl groups from **37** (59 mg, 43 mmol) in methanol (5 ml) was carried out as described for **34**. Purification by preparative HPLC (column A) in acetonitrile/water (9:1) gave **38**, yield 41 mg (90%), amorphous, $[\alpha]_D^{23}$ = –63.3° (c 1, H_2O), R_t = 2.5 min, R_f = 0.77 (RP-8, acetonitrile/water 3:7). – 400 MHz-^{1}H NMR (D_2O, ^{1}H, ^{1}H- and ^{1}H, ^{13}C-COSY, ratio of rotamers 6.8:1): δ 4.80 (d, 1*H*, $J_{1,2}$ = 3.8 Hz, H-1), 4.61–4.57 (m, 1H, α-CH-Arg), 4.54 (q, 1H, $J_{\alpha,\beta}$ = 7.0 Hz, α-CH-Ala), 4.48 (d, 1H, $J_{\alpha,\beta}$ = 2.7 Hz, α-CH-Thr), 4.42 (d, 1H, $J_{1',2'}$ = 7.8-Hz, H-1'), 4.40 (dd, 1H, $J_{\alpha,\beta\alpha}$ = 4.7 Hz, $J_{\alpha,\beta\beta}$ = 8.5 Hz α-CH-Pro), 4.35 (dd, 1H, $J_{\alpha,\beta\alpha}$ = 5.9 Hz, $J_{\alpha,\beta\beta}$ = 8.2 Hz α-CH-Pro'), 4.28 (dd, 1H, $J_{\alpha,\beta}$ = 2.7 Hz, $J_{\beta,\alpha}$ = 6.3 Hz, β-CH-Thr), 4.20 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 11.2 Hz, H-2), 4.17 (d, 1H, $J_{3,4}$ = 2.8 Hz, H-4), 4.02 (t, 1H, $J_{5,6}$ = 6.0 Hz, H-5), 3.97 (dd, 1H, $J_{2,3}$ = 11.2 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 3.87 (d, 1H, $J_{3',4'}$ = 3.2 Hz, H-4'), 3.85–3.60 (m, 11H, H-6, H-5', H-6', CH_2-Gly, 2 δ-CH_2-Pro), 3.58 (dd, 1H, $J_{2',3'}$ = 10.0 Hz, $J_{3',4'}$ = 3.2 Hz, H-3'), 3.47 (dd, 1H, $J_{1',2'}$ = 7.8 Hz, $J_{2',3'}$ = 10.0 Hz, H-2'), 3.21–3.18 (m, 2H, δ-CH_2-Arg), 2.32–2.22, 2.06–1.79, 1.73–1.61 (3m, 12H, 2 β-CH_2-Pro, 2 γ-CH_2-Pro, β-CH_2-Arg, γ-CH_2-Arg), 2.10, 1.99, 1.89 (3s, 9H, CH_3-Ac), 1.37 (d, 2.6H, $J_{\alpha,\beta}$ = 7.0 Hz, β-CH_3-Ala, A), 1.31 (d, 0.4H, $J_{\alpha,\beta}$ = 7.0 Hz, β-CH_3-Ala, B), 1.26 (d, 3H, $J_{\beta,\gamma}$ = 6.3 Hz, γ-CH_3-Thr). – ROESY-NMR: cross peaks: H-1/γ-CH_3-Thr, H-1/β-CH-Thr, H-1/H-2, β-CH_2-Arg, γ-CH_2-Arg/H-1, α-CH-Thr/β-CH-Thr, H-1'/H-3, H-1'/H-3', H-1'/H-5'. – 100.6 MHz ^{13}C NMR (D_2O): δ 174.67, 173.78, 173.64, 173.47, 173.11, 171.42, 171.21 (C=O), 156.89 (ω-C_q, Arg), 104.64 (C-1'), 98.72 (C-1), 77.16 (C-3), 75.41 (β-CH, Thr), 75.09 (C-5), 72.67 (C-3'), 71.09 (C-5), 70.72 (C-2'), 68.88 (C-4), 68.72 (C-4'), 61.31, 61.14 (C-6, C-6'), 60.72, 60.10 (2 α-CH, Pro), 57.62 (α-CH, Thr), 51.02 (α-CH, Arg), 48.40 (C-2), 47.85, 47.79 (2 δ-CH_2, Pro), 47.71 (α-CH, Ala), 43.42 (CH_2, Gly), 40.59 (δ-CH_2, Arg), 29.35 (2 β-CH_2, Pro), 27.75 (β-CH_2, Arg), 24.67, 24.58 (2 γ-CH_2, Pro), 24.19 (γ-CH_2, Arg), 23.39, 22.42, 21.78 (CH_3, Ac), 18.43 (γ-CH_3, Thr), 15.90 (β-CH_3, Ala, B), 15.51 (β-CH_3, Ala, A). – MALDI-MS (CCA): *m/z* 1005.4 [MH$^+$, cation].

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Address for correspondence:
Prof. Dr. Horst Kunz
Institut für Organische Chemie
Johannes Gutenberg-Universität Mainz
J.-J.-Becher-Weg 18–20
D-55128 Mainz
E-mail:hokunz@goofy.zdv.uni-mainz.de