Tetrahedron: *Asymmetry* 20 (2009) 902–909

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Aziridine ring opening as regio- and stereoselective access to *O*-glycosyl amino acids and their transformation into O-glycopeptide mimetics

Andreas Schäfer, Dirk Henkensmeier, Lars Kröger, Joachim Thiem *

Faculty of Science, Department of Chemistry, University of Hamburg, Martin-Luther-King-Platz 6, 20146 Hamburg, Germany

ARTICLE INFO

Article history:
Received 9 February 2009
Accepted 11 February 2009
Available online 28 March 2009

Dedicated to Professor George W. J. Fleet on the occasion of his 65th birthday

ABSTRACT

Glycosyl amino acid mimetics of the typical GalNAc-(1→*O*)-Ser/Thr motif of O-glycopeptides were synthesised. Starting from galactose a 1,5-anhydro derivative could be obtained and regio- and stereoselectively coupled to serine- or threonine-derived aziridine compounds, respectively. The corresponding Fmoc derivatives could be used to prepare two 13-mer glycopeptides of the mucin MUC1 carrying instead of Ser-2 or Th-5, the corresponding *O*-glcycosyl amino acid mimetics.

1. Introduction

The biochemistry of glycoproteins, which could be regarded as having had its origin in 1865 when Eichwald found evidence that various mucines, behave like typical proteins, but release carbohydrates under certain conditions.[1] Nevertheless, it took almost another 100 years before Ashwell et al. discovered the endogenous lectins, demonstrating that glycans are recognition signals and thereby connecting glycoprotein chemistry with molecular biology.[2] Nowadays it is well accepted that nearly all cells exhibit complex oligosaccharides on their surface, forming the glycocalix.[3] In the case of O-glycoproteins, the connecting amino acid must contain a hydroxyl group. Thus, the most common O-glycosylated hydroxy amino acids are L-serine and L-threonine. A typical motif of O-glycoproteins is the core A structure, β-D-Gal-(1→3)-α-D-GalNAc-(1→*O*)-Ser/Thr **1** and **2**, which is the carbohydrate component of the Thomsen-Friedenreich antigen (T-antigen) connected with tumour cells[4] (Fig. 1).

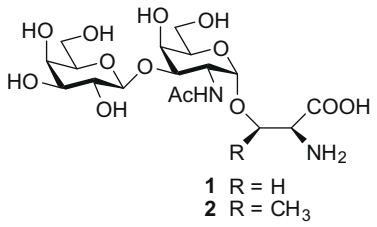


Figure 1.

Due to their important role in biological systems, carbohydrate structures should be promising in the search for new pharmaceuticals. However, a major drawback is the lability of the glycosidic bond. The half-time of carbohydrates in vivo is comparatively low, and oral application is nearly impossible.[5] Furthermore, the affinity to protein receptors is relatively low.[6]

At this stage the development and application of glycomimetics may be considered. Glycomimetics are compounds closely related to natural glycostructures in functional aspects but with selected structural variations. Glycomimetics of pharmaceutical interest, must show high receptor affinity, increased in vivo stability, easy access and improved pharmacological properties, for example, easier application and availability at the target cell.[7]

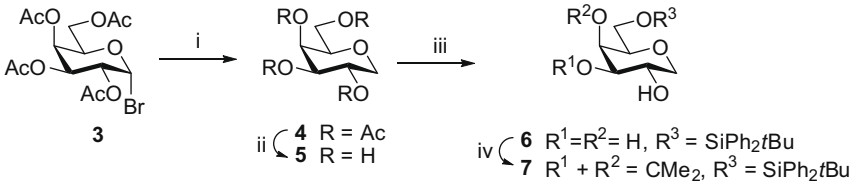
Since the hydrolysis of the glycosidic bond is a particular obstacle much work has been done to generate C-glycoside analogues.[8–11] Previously we could prepare a modified *N*-gluco-asparagine unit, in which the asparagine has been shifted from the anomeric centre to position 2 of the carbohydrate.[12] By changing the site of connection between the amino acid and the carbohydrate from the anomeric centre of an O-glycoside to another carbon atom, the type of bond changes from the labile glycosidic acetal to a stable ether bond. This family of compounds should show a significantly reduced lability towards hydrolysis, but, nevertheless, the majority of the galactose epitope is retained.

In this endeavour we wanted to prepare the decisive carbohydrate–amino acid linkage to mimic the structure in **1** or **2** thus attaching a galactose moiety via the 2-position to either serine or threonine. The resulting novel carbohydrate–amino acids should be checked for their potential as inhibitors of α-galactosidase (*Aspergillus niger*). Further, by simple transformation the Fmoc protocol could be used to build up model glycoproteins, for example, of the mucin type.

Therefore, we prepared the selectively protected galactitol **7**, with an unprotected hydroxyl group at position 2, and reacted it with the 'masked' serine **14** and threonine **15** in an acid-catalysed aziridine ring opening that proceeded regioselectively as well as stereoselectively.

* Corresponding author. Tel.: +49 40 42838 4241; fax: +49 40 42838 4325.
E-mail address: joachim.thiem@chemie.uni-hamburg.de (J. Thiem).

0957-4166/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2009.02.038

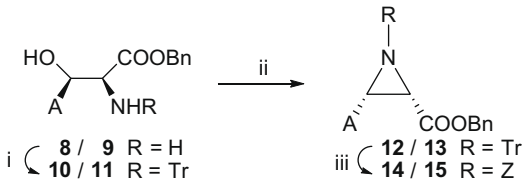


Scheme 1. Reagents and conditions: (i) Bu_3SnH, AIBN, toluene, 4 h, reflux (97%); (ii) NaOMe, MeOH, 1.5 h, rt (74%); (iii) TBDPSCl, pyridine, DMAP, 12 h, rt (81%); (iv) acetone, $(MeO)_2CMe_2$, TsOH, 1 h, reflux (78%).

2. Results and discussion

The galactitol **7** is readily available via a convenient four-step pathway, starting from peracetylated galactopyranosyl bromide **3**, which can be considered a bulk compound (galactose in acetic acid, AcBr, rt, 100%).[13] Radical dehalogenation with tributyl tinhydride led directly to the 1,5-anhydro-galactitol **4** in nearly quantitative yield (97%).[14] This step was followed by deprotection, employing the modified method of Zemplén,[15] in which **4** was treated with sodium methylate in methanol for 1.5 h. Desalting and neutralisation with Amberlite IR 120 (H^+), filtration and evaporation of the solvent yielded **5** as a colourless solid (74%). Selective blocking of the positions 3, 4 and 6 could be realised by first protecting the 6-position with the bulky *tert*-butyldiphenylsilyl group to yield compound **6** in 81% yield. Finally, the positions 3 and 4 could be protected as an isopropylidene acetal to afford the selectively protected 1,5-anhydrogalactitol **7** in a total yield of 45% over four steps (Scheme 1).

Intramolecular ring closure of vicinal amino alcohols is one general pathway leading to aziridines. Thus, the amino acids L-serine and L-threonine themselves should be suitable starting materials, since they naturally provide the required stereochemical information. Starting from the benzyl esters **8** and **9**, respectively, it takes only three steps to reach the N-activated aziridines.[16] The amino group was tritylated, applying tritylchloride and triethylamine in chloroform, yielding the compounds **10** (serine) and **11** (threonine) in 84% and 76%. Next, the hydroxyl group was transformed into a good leaving group by treatment with mesyl chloride and triethylamine in tetrahydrofuran. Without workup, the activated amino alcohols could be ring closed by raising the temperature to 60 °C. The aziridines **12** and **13** could be obtained in 86% and 63% yield, respectively. Finally, the electron-donating trityl group had to be exchanged for an electron-withdrawing group. This could be done in a two-step one-pot synthesis, first removing the trityl group with trifluoroacetic acid/methanol in chloroform. Then the solvents were evaporated and the aziridines treated with benzyl chloroformate/triethylamine to give the activated aziridines **14** and **15** in 74% yield (Scheme 2).



Scheme 2. Reagents and conditions: Serine series A = H **8**, **10**, **12**, **14**: (i) TrCl, NEt_3, $CHCl_3$ (84%); (ii) MsCl, Et_3N, THF, 48 h, 60 °C (86%); (iii) (1) CF_3CO_2H, MeOH, $CHCl_3$, −15 °C; (2) BnOCOCl, Et_3N. $CHCl_3$, 0 °C, 14 h (74%). Threonine series A = CH_3 **9**, **11**, **13**, **15**: (i) TrCl, Et_3N, $CHCl_3$ (76%); (ii) MsCl, NEt_3, THF, 48 h, 60 °C (63%); (iii) (1) CF_3CO_2H, MeOH, $CHCl_3$, −15 °C; (2) BnOCOCl, Et_3N; $CHCl_3$, 0 °C, 14 h (74%).

Baeyer strain combined with the electronegativity of the nitrogen atom explains the ability of aziridines to undergo ring opening under relatively mild conditions. However, since nitrogen is less electronegative than oxygen this is not as facile as epoxide opening.[17–21] There are only a few reports in which nucleophiles attack the C-2 of activated aziridines under Lewis acidic catalysis to yield β-amino acids.[21] In some cases, especially if the nucleophile is a Wittig-reagent or an organometallic compound, α/β mixtures are obtained,[22] however, most often nucleophiles attack at the C-3 position to afford α-amino acids. Generally, this is the case with amines, alcohols, carboxylic acids, thiols and indoles.[23] This regioselectivity can be explained by applying perturbational and HSAB (hard and soft acids and bases) theories.[24] Both, coulombic and molecular orbital interactions influence the reaction. Calculations of protonated aziridines corresponding to aziridines **14** and **15** show that the LUMO coefficient of C-2 is larger than that of C-3, which implies that a nucleophilic attack at C-2 should be favoured. Again, the impact of coulombic interactions increases with the hardness of the nucleophile. Thus, calculated charge distributions show that the positive charge at C-3 is twice as large as that at C-2. This fact, and in some cases steric factors as well, is the reason why hard nucleophiles such as alcohols attack at C-3.[21]

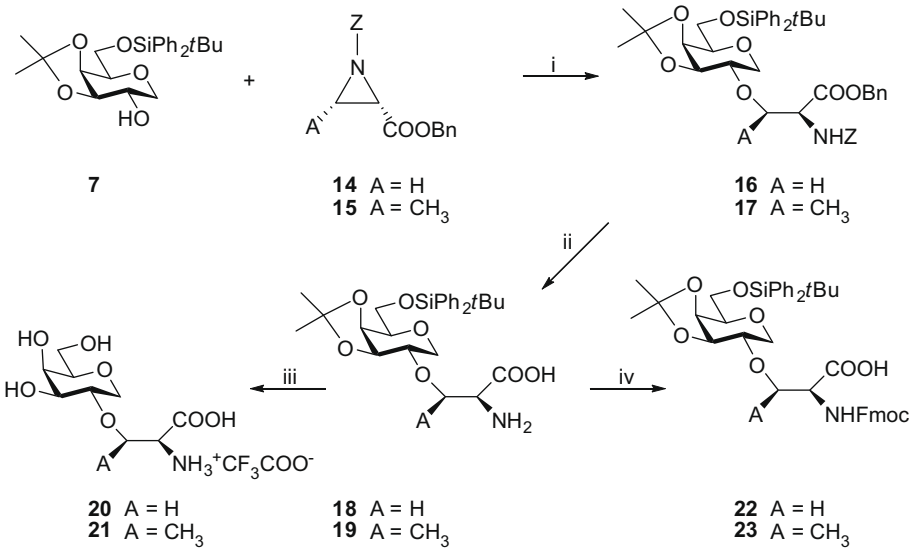
In keeping with this Nakajima et al. observed quantitative yields in aziridine openings with methanol and isopropanol, moderate yields with phenol but only low yields with a primary thiol, the more complex benzyloxycarbonyl protected cystein benzylester.[25,26]

As expected, the reaction between the selectively protected galactitol **7** and the amino acid-derived aziridines **14** and **15** showed high regioselectivity. By employing boron trifluoride etherate in chloroform, selective attack at the 3-position of the aziridines was observed and without optimisation the corresponding sugar amino acids were obtained in moderate yields of 54% **16** and 44% **17**. The moderate yields and the long reaction time of 48 h might be explained by steric hindrance (Scheme 3).

For deprotection of compounds **16** and **17** the *Z*-group and the benzyl ester could be removed almost quantitatively by hydrogenolysis on palladium/charcoal (10%) to give **18** and **19**. The remaining isopropylidene and *tert*-butyldiphenyl silyl groups could be removed in nearly 90% yield by treatment with trifluoroacetic acid to give the trifluoroacetates **20** and **21**.

The free glycosyl amino acids **20** and **21** were tested as potential inhibitors of the α-galactosidase from *A. niger*[27] with α-*p*NP-galactopyranoside as donor and of the β-galactosidase from *Escherichia coli*[28] with β-*p*NP-galactopyranoside as donor. Unfortunately, none of them showed any inhibitory effect for these enzymes. However, applying the aziridine ring opening method to a C-glycoside resulted in galactose-serine/threonine mimetics which showed significant inhibition of the α-galactosidase of *A. niger*.[29]

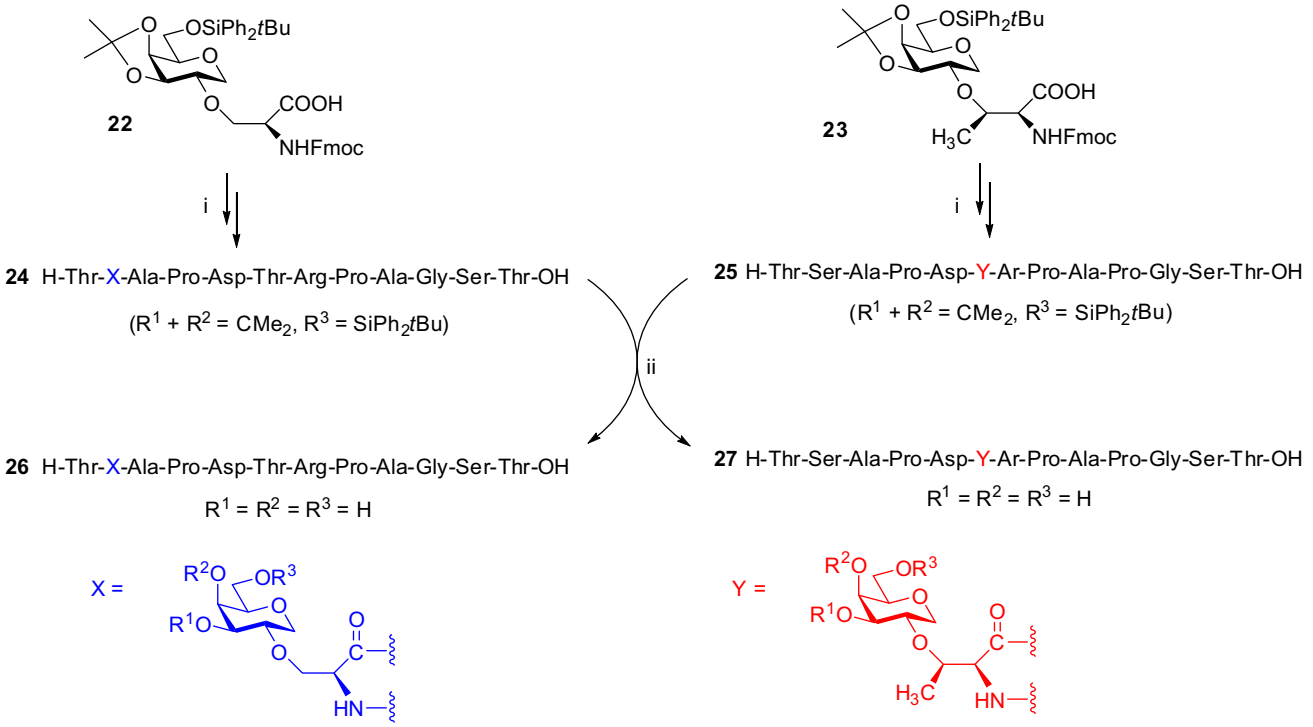
It was of interest to check the suitability of these modified *O*-glycosyl amino acid building units for use in glycopeptide solid phase synthesis. Thus, the preparation of certain model glycopeptides was addressed. Therefore, the required Fmoc-COOH protecting group pattern for amino acids had to be established. Treatment of the sugar-protected amino acid structures **18** and **19** with Fmoc-succinimidyl carbonate gave the required derivatives **22** and **23** in 86% and 83% yield, respectively.



Scheme 3. Reagents and conditions (A = H/A = CH_3): (i) $CHCl_3$, $BF_3{\cdot}Et_2O$, 16 h, rt (54%/44%); (ii) Pd–C/H_2, MeOH, 48 h, rt (91%/95%); (iii) CF_3CO_2H, 1 h, rt (86%/87%); (iv) Fmoc-OSuc, DMF, $NaHCO_3$, 30 min, 0 °C (86%/83%).

As model glycopeptide mimetics the peptide sequence of the human epithelial mucin MUC1 was selected.[30,31] The natural glycopeptide is carrying GalNAc saccharide bridgeheads α-linked to either Ser-2 or Thr-5, respectively. Thus, we introduced the novel mimetic building units either for Ser-2 or Thr-5.

The glycopeptide synthesis was performed following the classical batch method[32] employing Wang resin B1250 and the resin-attached 4-alkoxybenzyl alcohol group.[33] On acid treatment this labile linker will give the free carbonic acid at the C-terminus of the unblocked glycopeptides. The amino acid building units were employed as Fmoc-OPfp or Fmoc-ODhbt blocked and activated species. By the use of 3,4-dihydro-3-hydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt-OH) as a colour indicator, evidence for the complete cycle could be obtained. Attachment of the first amino acid was done by activation with 1-mesitylene-2-sulfonyl-3-nitro-1*H*-1,2,3-benzotriazol (MSNT).[34] Further coupling of the building units was done with TBTU activation[35] to give the derivatives **24** and **25**, respectively. Fmoc deblocking was performed with piperidine solution (20% in DMF), and recovery of the complete glycopeptide mimetic from the resin was effected with 95% aqueous trifluoroacetic acid. This simultaneously cleaved the remaining sugar blocking group to give the two 13-mer target model glycopeptide mimetics **26** and **27** in 48% and 43%, respectively, after HPLC purification. MALDI-Tof data for **26** and **27** showed $(M+H)^+$



Scheme 4. Reagents and conditions: (i) peptide synthesis protocol on Wang resin B1250 (cf. Section 4); (ii) CF_3CO_2H, 2 h, rt.

signals at m/z = 1403. Further detailed characterisation was performed by complete $^1H^1H$-TOCSY and $^1H^1H$-COSY spectra. Further biological tests will be reported in due course (Scheme 4).

3. Conclusion

An easy and straightforward access led to 1,5-anhydro-galactitol derivatives which could be used for the regio- and stereoselective opening of serine- or threonine-derived aziridines. Thus, mimetics of the GalNAc(1→*O*) serine/threonin sugar-amino acid linkage structure of *O*-glucosyl peptides were at hand. Their use in peptides synthesis could be realised and gave 13-mer glycopeptides mimetics of the human epithelial mucin MUC1.

4. Experimental

TLC was carried out on Silica Gel (60 GF 254, Merck) and on aluminium plates. Detection was by UV-light followed by charring with sulfuric acid in ethanol. Preparative column chromatography was performed on Silica Gel (60, 230–400 mesh, particle size 40–63 μm, Merck), using the flash technique. ^1H NMR spectra were recorded on a Bruker AMX 400 at 400 MHz or a Bruker AMX 500 at 500 MHz. ^{13}C NMR spectra were recorded on a Bruker AMX 400 at 100 MHz. NMR assignments were made using standard ^1H–^1H- and ^1H–^{13}C-COSY experiments. The connectivities of carbon atoms were given by DEPT experiments. Maldi-Tof mass spectra were taken on Bruker Biflex III with DHB or CCA as matrix in positive or negative reflector mode. ESI mass spectra were taken on Hewlett Packard Series 1100 MSD in positive mode at the given fragmentor voltage. Melting points were taken on an Olympus BH-polarising microscope with Mettler FP 82 heating plate and are uncorrected. Optical rotations were measured at 20 °C on a Perkin–Elmer model 241 polarimeter using a 1 dm cuvette. Evaporations were carried out at <45 °C under diminished pressure. Elemental analysis was provided by the Microanalytical Section, Department of Chemistry.

4.1. 1,5-Anhydro-6-*O*-*tert*-butyldiphenylsilyl-D-galactitol 6

1,5-Anhydro-D-galactitol **5**[36] (7.27 g, 44.3 mmol) was dissolved in pyridine (80 mL), treated with *t*-butyldiphenylchlorosilane (16.7 mL, 62.0 mmol) and dimethylaminopyridine (250 mg) and was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, residual pyridine was codistilled with toluene and the remaining oil was dissolved in ethyl acetate. The solution was washed with water and brine, dried over magnesium sulfate and filtered. Evaporation of the solvent and flash chromatography (petrol ether/ethyl acetate 1:2) gave **6** (14.44 g, 81%) as a colourless oil. $[\alpha]_D^{20} = +48.5$ (c 1, CH_2C1_2). ^1H NMR (400 MHz, $CDCl_3$) δ = 0.96 (s, 9H, *t*-Bu); 2.99 (dd, 1H, H-la); 3.30 (dd, 1H, H-5); 3.34 (dd, 1H, H-3); 3.74 (dd, 1H, H-6); 3.78 (dd, 1H, H-6′); 3.82 (ddd, 1H, H-2); 3.88 (dd, 1H, H-le); 4.00 (d, 1H, H-4); 7.27–7.34 (m, 6H, Ar); 7.56–7.61 (m, 4H, Ar). $J_{1a,1e}$ = 10.7, $J_{1a,2}$ = 10.7, $J_{1e,2}$ = 5.5, $J_{2,3}$ = 9.3, $J_{3,4}$ = 3.6, $J_{5,6}$ = 5.1, $J_{5,6'}$ = 6.0, $J_{6,6'}$ = 10.6 Hz. ^{13}C NMR (100 MHz, CDC1$_3$) δ = 18.13 (1C, $C(CH_3)_3$); 25.74 (3C, *t*-Bu); 62.47 (1C, C-6); 66.82 (1C, C-2); 68.74 (1C, C-4); 68.85 (1C, C-l); 74.68 (1C, C-3); 80.08 (1C, C-5); 126.78, 126.79, 128.57, 128.86, 131.83, 131.97, 133,79, 134.52, 134.59 (12C, Ar). Maldi-Tof (DHB, positive mode) 425 $(M+Na)^+$. Anal. Calcd for $C_{22}H_{30}O_5Si$: C, 65.64; H, 7.51. Found: C, 65.13; H, 7.36.

4.2. 1,5-Anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactitol 7

A solution of compound **6** (13.10 g, 32.5 mmol) in acetone (100 mL) was treated with 2,2-dimethoxypropane (20 mL, 163.2 mmol) and a catalytic amount of toluenesulfonic acid. The solution was refluxed for 1 h, the solvent was evaporated and the remaining syrup was purified by column chromatography (petrol ether/ethyl acetate 3:1) to give **7** (11.18 g, 78%) as a colourless syrup. $[\alpha]_D^{20} = +26.1$ (c 1, CH_2Cl_2). ^1H NMR (500 MHz, $CDCl_3$) δ = 0.97 (s, 9H, *t*-Bu); 1.28 (s, 3H, CH_3); 1.42 (s, 3H, CH_3); 2.83 (br s, 1H, OH); 3.01 (dd, 1H, H-la); 3.68–3.72 (m, 2H, H-2, H-5); 3.77–3.85 (m, 3H, H-le, CH_2-6); 3.90 (dd, 1H, H-3); 4.25 (dd, 1H, H-4); 7.26–7.33 (m, 6H, Ar); 7.59–7.64 (m, 4H, Ar). $J_{1a,1e}$ = 10.5, $J_{1a,2}$ = 10.5, $J_{2,3}$ = 7.0, $J_{3,4}$ = 5.78, $J_{4,5}$ = 2.3 Hz. ^{13}C NMR (100 MHz, $CDCl_3$) δ = 18.34 (1C, $C(CH_3)_3$; 25.20 (1C, CH_3); 25.74 (3C, *t*-Bu); 27.22 (1C, CH_3); 61.73 (1C, C-6); 67.04 (1C, C-l); 68.77 (1C, C-2); 72.09 (1C, C-4); 75.48 (1C, C-5); 78.36 (1C, C-3); 108.61 (1C, $C(CH_3)_2$); 126.57, 126.65, 126.82, 128.63, 128.66, 132.35, 132.44, 134.52, 134.60 (12C, Ar). Maldi-Tof (DHB, positive mode) 465 $(M+Na)^+$, 481 $(M+K)^+$. Anal. Calcd for $C_{25}H_{34}O_5Si$: C, 67.84; H, 7.75. Found: C, 67.13; H, 7.43.

4.3. (2*S*)-Benzyl-1-trityl-aziridine-2-carboxylate 12

A solution of *N*-trityl-L-serine benzyl ester **9** (20.3 g, 42.3 mmol) in anhydrous tetrahydrofuran (150 mL) under argon was cooled to 0 °C and triethylamine (12.9 mL, 92 mmol) was added. Within 10 min mesyl chloride (3.3 mL, 42.7 mmol) was added under vigorous stirring. Then the mixture was warmed to room temperature and stirred at 60 °C for another 48 h. After removal of the solvents the dry remainder was dissolved in ethyl acetate (200 mL), washed twice with 10% aqueous citric acid (50 mL each), three times with saturated aqueous sodium hydrogen carbonate (50 mL each), then dried over magnesium sulfate, filtered and evaporated. The solid raw material was crystallised from methanol to give **12** (15.33 g, 86%) as a colourless solid, mp 112 °C (Ref. 16 107 °C). $[\alpha]_D^{20} = -92.3$ (c 1, THF) (Ref. 16 107 °C). ^1H NMR (400 MHz, $CDCl_3$) δ = 1.34 (dd, 1H, H-3); 1.86 (dd, 1H, H-3′); 2.20 (dd, 1H, H-2); 5.12 (d, 1H, CH_{2a}-Bn); 7.12–7.42 (m, 20H, Ar). $J_{2,3}$ = 1.5, $J_{2,3'}$ = 2.5, $J_{3,3'}$ = 6.1, $J_{CH_2\text{-}Bn}$ = 12.2 Hz.

4.4. (2*S*,3*S*)-Benzyl-3-methyl-1-trityl-aziridine-2-carboxylate 13

Procedure, workup and purification were similar as those described for compound **12**. Material: *N*-trityl-L-threonine benzyl ester (15.0 g, 25.0 mmol), THF (100 mL), triethylamine (10.6 mL, 76.5 mmol), mesyl chloride (2.8 mL, 35.2 mmol). Yield of **13**: 9.5 g, 63%; colourless solid, mp 97 °C, $[\alpha]_D^{20} = -74.5$ (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) δ = 1.28 (d, 3H, CH_3); 1.55 (dq, 1H, H-3); 1.86 (d, 1H, H-2); 5.05 (d, 1H, CH_{2a}-Bn); 5.19 (d, 1H, CH_{2b}-Bn); 7.13–7.42 (m, 20H, Ar). $J_{2,3}$ = 6.6, J_{3,CH_3} = 5.6, $J_{CH_2\text{-}Bn}$ = 12.2 Hz.

4.5. (2*S*)-Benzyl-1-benzyloxycarbonyl-aziridine-2-carboxylate 14

A solution of compound **12** (11.33 g, 27.0 mmol) in chloroform (90 mL) and anhydrous methanol (20 mL) under argon was cooled to −14 °C. Under stirring trifluoroacetic acid (46 mL) was added. After 2 h the solvents were evaporated and the dry remainder was dissolved in chloroform (90 mL). At 0 °C triethylamine (9.58 mL, 68.6 mmol) and benzylchloroformiate (10.5 mL, 31.3 mmol, 50% solution in toluene) were added and stirred at this temperature for another 14 h. Then saturated aqueous sodium hydrogen carbonate (60 mL) was added, extracted with chloroform and evaporated to dryness. The oily product was purified by column chromatography on silica gel with petrol ether/ethyl acetate 6:1 to give **14** (6.25 g, 74%) as a colourless oil. $[\alpha]_D^{20} = -18.6$ (c 1, CH_2Cl_2) [Ref. 26 −20.0 (c 0.9, MeOH]. ^1H NMR (400 MHz, $CDCl_3$) δ = 2.39 (dd, 1H, H-3); 2.53 (dd, 1H, H-3′); 3.05 (dd, 1H, H-2);

4.97–5.07 (m, 4H, 2 × CH_2-Bn); 7.25–7.28 (m, 1OH, Ar). $J_{2,3}$ = 1.0, $J_{2,3'}$ = 3.1, $J_{3,3'}$ = 5.1 Hz.

4.6. (2*S*,3*S*)-Benzyl-1-benzyloxycarbonyl-3-methyl-aziridine-2-carboxylate 15

Procedure, workup and purification were similar as those described for compound **14**. Materials: Compound **13** (11.5 g, 26.5 mmol); chloroform (100 mL), methanol (25 mL), trifluoroacetic acid (50 mL). Yields of **15**: 6.4 g, 74%; colourless oil; $[\alpha]_D^{20} = -67.1$ (*c* 1, MeOH) [Ref. 26 −66.2]. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 1.24 (d, 3H, CH_3); 2.74 (dq, 1H, H-3); 3.14 (dd, 1H, H-2); 5.03 (d, 1H, CH_{2a}-Bn); 5.06 (d, 1H, CH_{2b}-Bn); 7.26–7.29 (m, 10H, Ar). $J_{2,3}$ = 6.6, J_{3,CH_3} = 5.6, $J_{CH_2\text{-}Bn}$ = 12.2 Hz.

4.7. N^{α}-Benzyloxycarbonyl-3-*O*-[1,5-anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactitol-2]-L-serine benzylester 16

The anhydro alditol compound **7** (850 mg, 1.92 mmol) and the aziridine derivative **14** (448 mg 1.44 mmol) were dissolved in dry chloroform (10 mL). The solution was degassed and maintained under high vacuum for 30 min. After flushing the flask with argon, the resulting syrup was redissolved in dry chloroform (2 mL) and treated with $BF_3{\cdot}Et_2O$ (10% in chloroform, 5 drops). The yellow solution was stirred for 16 h, treated with further 3 drops of catalyst and stirred again for 16 h. After diluting with chloroform, saturated sodium hydrogencarbonate was added, the organic phase was dried over magnesium sulfate and filtered. Evaporation of the solvent and column chromatography (petrol ether/ethyl acetate 6:1) yielded compound **16** as a colourless syrup (586 mg, 54%). $[\alpha]_D^{20} = +9.7$ (*c* 1, CH_2C1_2). ^{1}H NMR (500 MHz, $CDC1_3$) δ = 0.98 (s, 9H, *t*-Bu); 1.24 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); 2.85 (dd, 1H, H-la); 3.33 (ddd, 1H, H-2); 3.55 (ddd, 1H, H-5); 3.60 (dd, 1H, H-3); 3.69 (dd, 1H, H-β-Ser); 3.75, 3.76 (2 × dd, 2 × 1H, CH_2-6); 3.78 (dd, 1H, H-le); 4.11 (dd, 1H, H-4); 4.17 (dd, 1H, H′-β-Ser); 4.47 (ddd, 1H, H-α-Ser); 5.02–5.24 (m, 4H, 2 × CH_2-Bn); 5.54 (d, 1H, NH); 7.22–7.35 (m, 16H, H-Ar); 7.59–7.63 (m, 4H, H-Ar). $J_{1a,1e}$ = 11.4, $J_{1a,2}$ = 10.4, $J_{1e,2}$ = 5.7, $J_{2,3}$ = 1.9, $J_{3,4}$ = 6.3, $J_{4,5}$ = 2.1, $J_{5,6}$ = 6.0, $J_{5,6'}$ = 7.8, $J_{6,6'}$ = 9.8, $J_{NH,H\text{-}\alpha\text{-}Ser}$ = 8.8, $J_{H\text{-}\alpha\text{-}Ser,H\text{-}\beta\text{-}Ser}$ = 3.1, $J_{H\text{-}\alpha\text{-}Ser,H'\text{-}\beta\text{-}Ser}$ = 3.0, $J_{H\text{-}\beta\text{-}Ser,H'\text{-}\beta\text{-}Ser}$ = 9.7 Hz. ^{13}C NMR (100 MHz, $CDC1_3$) δ = 18.20 (1C, $C(CH_3)_3$); 25.15 (1C, CH_3); 25.73 (3C, *t*-Bu); 27.07 (1C, CH_3); 53.61 (1C, C-α-Ser); 61.68 (1C, C-6); 65.24 (1C, C-l); 66.08, 66.19 (2 × 1C, 2 × CH_2-Bn); 69.03 (1C, C-/3-Ser); 71.97 (1C, C-4); 75.26 (1C, C-5); 76.06 (1C, C-2); 76.88 (1C, C-3); 108.35 (1C, $C(CH_3)_2$); 126.57, 126.66, 127.11, 127.19, 127.38, 127.46, 127.51, 128.64, 128.68, 134.52, 134.59 (24C, Ar), 154.98 (1C, CONH), 169.07 (1C, CO). Maldi-Tof (DHB, positive mode) 754 $(M+Na)^+$, 776 $(M+Na)^+$, 793 $(M+K)^+$. Anal. Calcd for $C_{43}H_{51}NO_9Si$: C, 68.50; H, 6.82; N, 1.86. Found: C, 68.31; H, 6.77; N, 1.71.

4.8. N^{α}-Benzyloxycarbonyl-3-*O*-[1,5-anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactitol-2]-L-threonine benzylester 17

The reaction conditions, workup and purification were similar to those described for the synthesis of **16**. Materials: Compound **7** (1.5 g, 339 mmol), aziridine derivative **15** (735 mg, 2.26 mmol), chloroform (2 mL), $BF_3{\cdot}Et_2O$ as above. The eluent used for flash chromatography was petrol ether/ethyl acetate 8:1. The product **17** (740 mg, 44%) was obtained as a colourless syrup. $[\alpha]_D^{20} = +0.4$ (*c* 1, CH_2C1_2); ^{1}H NMR (400 MHz, $CDC1_3$) δ = 0.98 (s, 9H, *t*-Bu); 1.11 (d, 3H, CH_3-Thr); 1.22 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); 2.76 (dd, 1H, H-la); 3.39 (dd, 1H, H-3); 3.41 (dd, 1H, H-2); 3.49 (ddd, 1H, H-5); 3.66 (dd, 1H, H-le); 3.72, 3.74 (2 × dd, 2 × 1H, CH_2-6); 4.03 (dd, 1H, H-4); 4.31 (dd, 1H, H-α-Thr); 4.43 (dd, 1H, H-β-Thr); 5.05–5.24 (m, 4H, 2 × CH_2-Bn); 5.44 (d, 1H, NH); 7.21–7.37 (m, 6H, Ar); 7.59–7.64 (m, 4H, Ar). $J_{1a,1e}$ = 11.7, $J_{1a,2}$ = 9.7, $J_{1e,2}$ = 5.1, $J_{2,3}$ = 6.6, $J_{3,4}$ = 5.1, $J_{4,5}$ = 1.5, $J_{5,6}$ = 6.1, $J_{5,6'}$ = 7.9, $J_{6,6'}$ = 9.7, $J_{NH,H\text{-}\alpha\text{-}Thr}$ = 9.7, $J_{H\text{-}\alpha\text{-}Thr,H\text{-}\beta\text{-}Thr}$ = 2.5, $J_{H\text{-}\beta\text{-}Thr,CH_3\text{-}Thr}$ = 6.1 Hz. ^{13}C NMR (100 MHz, $CDC1_3$) δ = 15.12 (1C, CH_3-Thr); 18.21 (1C, $C(CH_3)_3$); 25.20 (1C, CH_3); 25.73 (3C, *t*-Bu); 27.08 (1C, CH_3); 58.04 (1C, C-α-Thr); 61.63 (1C, C-6); 66.12 (1C, C-l); 66.16 (2C, 2 × CH_2-Bn); 71.95 (1C, C-4); 72.36 (1C, C-β-Thr); 72.54 (1C, C-3); 75.27 (1C, C-5); 77.15 (1C, C-2); 108.25 (1C, $C(CH_3)_2$); 126.57, 126.67, 127.09, 127.17, 127.43, 127.50, 127.52, 127.80, 128.65, 128.69, 132.34, 134.52, 134.60, 155.77 (24C, Ar); 155.77 (1C, CONH); 169.57 (1C, COOH). Maldi-Tof (DHB, positive mode) 768 $(M+H)^+$, 790 $(M+Na)^+$, 806 $(M+K)^+$. Anal. Calcd for $C_{44}H_{53}NO_9Si$: C, 68.81; H, 6.96; N, 1.82. Found: C, 68.99; H, 7.01; N, 1.85.

4.9. 3-*O*-[1,5-Anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactitol-2]-L-serine 18

Compound **16** (325 mg, 0.43 mmol) was dissolved in dry methanol (20 mL), Pd/C (10%, 70 mg) was added and the flask flushed with hydrogen. After 48 h, the reaction was stopped by filtration. Evaporation of the solvent yielded **18** (207 mg, 91%) colourless syrup. $[\alpha]_D^{20} = +3.7$ (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 1.01 (s, 9H, *t*-Bu); 1.30 (s, 3H, CH_3); 1.45 (s, 3H, CH_3); 3.11 (dd, 1H, H-la); 3.33 (dd, 1H, H-α-Ser); 3.83 (ddd, 1H, H-2); 3.73 (dd, 1H, H-6); 3.78 (dd, 1H, H-6′); 3.83–3.88 (m, 3H, H-5, CH_2-β-Ser); 3.90 (dd, 1H, H-le); 4.13 (dd, 1H, H-3); 4.31 (dd, 1H, H-4); 7.45–7.50 (m, 6H, Ar); 7.66–7.69 (m, 4H, Ar). $J_{1a,1e}$ = 11.2, $J_{1a,2}$ = 10.2, $J_{1e,2}$ = 5.6, $J_{2,3}$ = 6.6, $J_{3,4}$ = 5.6, $J_{4,5}$ = 2.0, $J_{5,6}$ = 6.6, $J_{5,6'}$ = 6.6, $J_{6,6'}$ = 10.2, $J_{H\text{-}\alpha\text{-}Ser,H'\text{-}\beta\text{-}Ser}$ = 4.6 Hz. ^{13}C NMR (100 MHz, DMSO-d_6) δ = 19.14 (1C, $C(CH_3)_3$); 26.41 (1C, CH_3); 26.90 (3C, *t*-Bu); 28.28 (1C, CH_3); 54.80 (1C, C-α-Ser); 63.35 (1C, C-6); 65.80 (1C, C-l); 69.55 (1C, C-β-Ser); 73.15 (1C, C-4); 75.73 (1C, C-5); 76.43 (C-2); 77.49 (1C, C-3); 108.84 (1C, $C(CH_3)_2$); 128.21, 128.26, 130.25, 135.43, 135.46, (12C, Ar), 164.0 (lC,COOH). Maldi-Tof (DHB, positive mode) 530 $(M+H)^+$, 552 $(M+Na)^+$, 568 $(M+K)^+$.

4.10. 3-*O*-[1,5-Anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-D-galactitol-2]-L-threonine 19

Compound **17** (720 mg, 0.94 mmol) was dissolved in dry methanol (30 mL), Pd/C (10%, 150 mg) was added and the flask flushed with hydrogen. After 48 h, the reaction was stopped by filtration. Evaporation of the solvent gave **19** (481 mg, 95%) as a colourless syrup. $[\alpha]_D^{20} = -0.7$ (*c* 0.5, MeOH). ^{1}H NMR (400 MHz, MeOH-d_4) δ = 0.94 (s, 9H, *t*-Bu); 1.24 (s, 3H, CH_3); 1.25 (d, 3H, CH_3-Thr); 1.41 (s, 3H, CH_3); 3.07 (dd, 1H, H-la); 3.38 (dd, 1H, H-β-Thr); 3.58 (ddd, 1H, H-2); 3.67–3.76 (m, 3H, H-5, CH_2-6); 3.80 (dd, 1H, H-le); 4.07 (dd, 1H, H-3); 4.26 (dd, 1H, H-4); 4.35 (dd, 1H, H-α-Thr); 7.27–7.37 (m, 6H, Ar); 7.56–7.63 (m, 4H, Ar). $J_{1a,1e}$ = 11.3, $J_{1a,2}$ = 9.8, $J_{1e,2}$ = 5.4, $J_{2,3}$ = 6.4, $J_{3,4}$ = 5.9, $J_{4,5}$ = 1.5, $J_{NH,H\text{-}\alpha\text{-}Thr}$ = 6.2, $J_{H\text{-}\alpha\text{-}Thr,H\text{-}\beta\text{-}Thr}$ = 4.6, $J_{H\text{-}\beta\text{-}Thr,CH_3\text{-}Thr}$ = 5.0 Hz. ^{13}C NMR (100 MHz, MeOH-d_4) δ 15.43 (1C, CH_3-Thr); 18.45 (1C, $C(CH_3)_3$); 25.18 (1C, CH_3); 26.11 (3C, *t*-Bu); 27.16 (1C, CH_3); 59.86 (1C, C-β-Thr); 63.21 (1C, C-6); 66.56 (1C, C-l); 72.04 (C-α-Thr); 73.53 (1C, C-4); 74.12 (1C, C-2); 76.51 (1C, C-5); 77.90 (1C, C-3); 127.63, 127.68, 129.80, 135.57, 135.61 (12 C, Ar); 190.05 (lC,COOH). Maldi-Tof (DHB, positive mode) 544 $(M+H)^+$, 566 $(M+Na)^+$, 582 $(M+K)^+$.

4.11. 3′-*O*-[1,5-Anhydro-D-galactitol-2]-L-serine trifluoroacetate 20

A solution of compound **18** (150 mg, 0.28 mmol) in trifluoroacetic acid (95%, 1 mL) was stirred for 1 h. The trifluoroacetic acid was evaporated, the residue was dissolved in water and the solvent was removed to give **20** (88 mg, 86%) as a colourless solid. $[\alpha]_D^{20} = +17.4$

(*c* 1, H_2O). Mp 87 °C. ^1H NMR (400 MHz, $CDC1_3$) δ = 3.03 (dd, 1H, H-1a); 3.38 (ddd, 1H, H-5); 3.44–3.58 (m, 4H, H-2, H-3, CH_2-6); 3.77 (dd, 1H, H-4); 3.90–4.03 (m, 3H, H-1′, CH_2-β-Ser); 4.05 (dd, 1H, H-α-Ser). $J_{1a,1e}$ = 11.3, $J_{1a,2}$ = 10.2, $J_{3,4}$ = 3.0, $J_{4,5}$ = 1.1, $J_{5,6}$ = 4.5, $J_{5,6'}$ = 7.6, $J_{H\text{-}\alpha\text{-}Ser,H\text{-}\beta\text{-}Ser}$ = 3.7, $J_{H\text{-}\alpha\text{-}Ser,H\text{-}\beta'\text{-}Ser}$ = 4.8 Hz. ^{13}C NMR (100 MHz, $CDC1_3$) δ = 54.03 (1C, C-α-Ser); 61.68 (1C, C-6); 67.23 (1C, C-l); 68.06 (1C, C-β-Ser); 69.49 (1C, C-4); 73.53 (1C, C-2); 76.18 (1C, C-3); 79.70 (1C, C-5). ESI-MS (20 V, positive mode) 252 $(M+H)^+$.

4.12. 3′-*O*-[1,5-Anhydro-D-galactitol-2]-L-threonine trifluoroacetate 21

A solution of compound **19** (110 mg, 0.2 mmol) in trifluoroacetic acid (95%, 1 mL) was stirred for 1 h. The trifluoroacetic acid was evaporated, the residue was dissolved in water and the solvent was removed to give **21** (66 mg, 87%) as a colourless solid. $[\alpha]_D^{20} = +18.6$ (*c* 1, H_2O) mp 96 °C. ^1H NMR (400 MHz, $CDC1_3$) δ = 1.16 (d, 1H, CH_3-Thr); 3.02 (dd, 1H, H-la); 3.36 (ddd, 1H, H-5); 3.49 (dd, 1H, H-3); 3.52–3.53 (m, 2H, CH_2-6); 3.75 (d, 1H, H-α-Thr); 3.76 (dd, 1H, H-4); 3.90 (dd, 1H, H-le); 4.17 (dq, 1H, H-β-Thr). $J_{1a,1e}$ = 11.2, $J_{1a,2}$ = 10.2, $J_{1e,2}$ = 5.4, $J_{2,3}$ = 9.7, $J_{3,4}$ = 3.3, $J_{4,5}$ = 1.2, $J_{5,6}$ = 4.5, $J_{5,6'}$ = 7.6, $J_{H\text{-}\alpha\text{-}Thr,H\text{-}\beta\text{-}Thr}$ = 4.6, $J_{H\text{-}\beta\text{-}Thr,CH_3\text{-}Thr}$ = 6.5 Hz. ^{13}C NMR (100 MHz, $CDC1_3$) δ = 18.10 (1C, CH_3-Thr); 58.86 (1C, C-α-Thr); 61.67 (1C, C-6); 68.20 (1C, C-l); 69.47 (1C, C-4); 73.97 (1C, C-2/C-3); 74.47 (1C, C-β-Thr); 75.18 (1C, C-2/C-3); 79.60 (1C, C-5); 171.57 (1C, CO). ESI-MS (10 V, positive mode) 266 $(M+H)^+$.

4.13. N^{α}-Fluorenylmethoxycarbonyl-3-*O*-[1,5-anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-galactitol-2]-L-serine 22

Compound **18** (144 mg, 0.27 mmol) was dissolved in saturated aqueous $NaHCO_3$ (3 mL) and was treated at 0 °C with *N*-(9*H*-fluoren-9-yl-methoxycarbonyloxy)-succinimide in DMF (1 mL). The reaction mixture was stirred for another 30 min at room temperature, then diluted with water and extracted with ether and ethyl acetate. The aqueous solution was cooled to 0 °C and brought to pH 5 with 5 M HCl. The colourless precipitate was extracted with ethyl acetate, dried over $MgSO_4$ and evaporated to give the product **22** (201 mg, 99%) as a colourless oil. $[\alpha]_D^{20} = +19.7$ (*c* 1.0, MeOH). ^1H NMR (400 MHz, $CDCl_3$) δ = 0.97 (s, 9H, *t*-Bu); 1.26 (s, 3H, CH_3); 1.43 (s, 3H, CH_3); 2.96 (dd, 1H, H-1a); 3.42 (ddd, 1H, H-2); 3.64 (ddd, 1H, H-5); 3.68–3.79 (m, 4H, CH_2-6, CH_2-β-Ser); 3.84 (dd, 1H, H-1e); 3.92 (dd, 1H, H-3); 4.13 (vt, 1H, Fmoc-CH); 4.22 (dd, 1H, H-4); 4.32 (m, 2H, Fmoc-CH_2); 4.45 (m, 1H, CH-α-Ser); 5.68 (d, 1H, NH); 7.15–7.34 (m, 8H, Ar); 7.5–7.68 (m, 6H; Ar). $J_{1a,1e}$ = 11.0, $J_{1a,2}$ = 10.7, $J_{1e,2}$ = 5.9, $J_{2,3}$ = 6.9, $J_{3,4}$ = 5.6, $J_{4,5}$ = 1.9, $J_{5,6}$ = 6.4, $J_{5,6'}$ = 7.8, $J_{NH,H\text{-}\alpha\text{-}Ser}$ = 8.3 Hz. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 18.20 (1C, $C(CH_3)_3$); 25.12 (1C, CH_3); 25.74 (3C, *t*-Bu); 27.10 (1C, CH_3); 46.10 (1C, Fmoc-CH); 54.58 (1C, C-α-Ser); 61.72 (1C, C-6); 65.13 (1C, C-1); 66.20 (1C, CH_2-Fmoc); 69.03 (1C, C-β-Ser); 75.44 (1C, C-4); 76.23 (1C, C-2); 76.60 (1C, C-5); 76.88 (1C, C-3); 108.58 (1C, $C(CH_3)_2$); 123.73, 124.08, 126.06, 126.55, 126.66, 126.71, 128.65, 128.68, 132.38. 134.51, 134.59, 140.27, 142.65, 143.31, 155.20 (1C, CONH), 170.31 (1C, COOH). Maldi-Tof (DHB, positive mode) 774 $(M+Na)^+$, 791 $(M+K)^+$.

4.14. N^{α}-Fluorenylmethoxycarbonyl-3-*O*-[1,5-anhydro-6-*O*-*tert*-butyldiphenylsilyl-3,4-*O*-isopropylidene-galactitol-2]-L-threonine 23

Reaction conditions, workup and purification were similar to those described for **22**. Materials: compound **19** (304 mg, 0.56 mmol), 189 mg saturated aqueous $NaHCO_3$ (6 mL), DMF (4 mL) and *N*-(9*H*-fluoren-9-yl-methoxycarbonyloxy)-succinimide (189 mg, 0.56 mmol). Yield of **23** (356 mg, 83%) as a colourless solid, mp 75 °C, $[\alpha]_D^{20} = +22.9$ (*c* 1, MeOH). ^1H NMR (400 MHz, $CDCl_3$) δ = 0.98 (s, 9H, *t*-Bu); 1.12 (d, 3H, CH_3-Thr); 1.29 (s, 3H, CH_3); 1.50 (s, 3H, CH_3); 2.98 (dd, 1H, H-1a); 3.59 (ddd, 1H, H-2); 3.66 (ddd, 1H, H-5); 3.77 (dd, 1H, H-1e); 3.78–3.80 (m, CH_2-6); 3.93 (dd, 1H, H-3); 4.15 (t, 1H, CH-Fmoc); 3.98 (dd, 1H, H-β-Thr); 4.27 (dd, 1H, H-4); 4.35 (d, 2H, CH_2-Fmoc); 4.42 (dd, 1H, H-α-Thr); 5.58 (d, 1H, NH); 7.22–7.37 (m, 10H, Ar); 7.52–7.70 (m, 8H, Ar). $J_{1a,1e}$ = 11.0, $J_{1a,2}$ = 8.3, $J_{1e,2}$ = 5.9, $J_{2,3}$ = 7.2, $J_{3,4}$ = 5.3, $J_{4,5}$ = 2.0, $J_{5,6}$ = 6.0, $J_{5,6'}$ = 5.4, $J_{NH,H\text{-}\alpha\text{-}Thr}$ = 8.1 Hz, $J_{H\text{-}\alpha\text{-}Thr,H\text{-}\beta\text{-}Thr}$ = 3.5, $J_{H\text{-}\beta\text{-}Thr,CH_3\text{-}Thr}$ = 6.2 Hz. ^{13}C NMR (100.62 MHz, $CDCl_3$) δ = 15.12 (1C,

Table 1
^1H NMR of amino acid protons of glycopeptide **26**

	NH	H_{a1}	H_{a2}	H_{b1}	H_{b2}	H_{g1}	H_{g2}	H_{d1}	H_{d2}	NH	H-1a	H-1e	H-2	H-3			H-4	H-5	H-6	H-6′
Thr-1		3.91 (6.6)		4.17 (6.6)		1.28														
Ser-2	8.77 (6.6)	4.52 (4.5) (6.9)		3.81 (11.5)	3.79								3.13 (10.9) (11.0)	4.09 (5.0)	3.64	3.64	3.92	3.51 (4.3) (7.6)	3.92 (11.4)	3.67
Ala-3	8.39 (7.0)	4.60 (7.1)		1.39																
Pro-4	8.39 (6.0)	4.39[a]		2.31[a]	2.31[a]	2.04[a]	2.04[a]		3.83[a]	3.67										
Asp-5	8.55 (7.3)			2.94 (17.1)	2.86															
Thr-6	8.28 (8.62)	4.47 (3.2)		4.38 (6.5)		1.16														
Arg-7	8.23 (7.3)	4.67		1.86	1.72	1.67	1.62		3.23	3.23	7.13									
Pro-8		4.37		2.23	2.23	2.04[a]	2.04[a]			3.83[a]	3.65									
Ala-9	8.44 (5.7)	4.65 (7.1)		1.39																
Pro-10		4.38		2.31[a]	2.31[a]	2.04[a]	2.04[a]		3.83[a]	3.66										
Gly-11	8.37 (5.9) (6.0)	3.95 (17.0)	3.91																	
Ser-12	8.25 (7.2)	4.64 (5.9) (4.8)		3.87 (11.8)	3.85															
Thr-13	8.01 (7.9)	4.29 (4.2)		4.17 (6.6)		1.14														

[a] Signals not dispersed.

Table 2
^{1}H NMR of amino acid protons of glycopeptide **27**

	NH	H_{a1}	H_{a2}	H_{b1}	H_{b2}	H_{g1}	H_{g2}	H_{d1}	H_{d2}	NH	H-1a	H-1e	H-2	H-3			H-4	H-5	H-6	H-6′
Thr-1		3.76 (6.2) (6.3)		4.0 (6.4)		1.14														
Ser-2	8.62 (7.0)	4.35 (4.8) (6.5)		3.70 (11.6)	3.64															
Ala-3	8.26 (5.7)	4.40 (7.0)		1.18[a]																
Pro-4		4.23[a]		2.11[a]	1.75[a]	1.84[a]	1.84[a]			3.62[a]	3.47[a]									
Asp-5	8.37 (7.3)	4.6 (6.6) (6.9)		2.76 (17.1)																
Thr-6	8.03 (8.7)	4.22[a]		4.33[a]		1.01 (10.8)	(5.5)	(11.0)		(34.2)	(10.8)		2.99	3.90	3.78	3.55	3.45 (3.3)	3.35[a]	3.52[a]	3.78[a]
Arg-7	8.16 (7.0)	4.60[a]		1.66[a]	1.57[a]	1.50[a]	1.50[a]			3.03[a]	3.03[a]									
Pro-8		4.23[a]		2.08[a]	1.68[a]	1.82	1.82[a]			3.62[a]	3.42									
Ala-9	8.26 (5.6)	4.43 (7.0)		1.18[a]																
Pro-10		4.23[a]		2.11[a]	1.75[a]	1.84[a]	1.84[a]			3.62[a]	3.47[a]									
Gly-11	8.35 (6.0) (6.1)	3.85 (17.3) (5.3)	3.77																	
Ser-12	7.97 (7.2)	4.38[a]		3.74[a]	3.74															
Thr-13	7.93 (7.5)	3.89[a]		4.33[a]		0.93														

[a] Signals not dispersed.

*C*H$_3$-Thr); 18.19 (1C, *C*(CH$_3$)$_3$); 25.18 (1C, *C*H$_3$); 25.73 (3C, *t*-Bu); 27.10 (1C, *C*H$_3$); 46.14 (1C, *C*H-Fmoc); 56.58 (1C, C-α-Thr); 61.58 (1C, C-6); 65.68 (1C, C-1); 66.17 (1C, CH$_2$-Fmoc); 72.27 (1C, C-4); 73.21 (1C, C-β-Thr); 74.41 (1C, C-2); 75.55 (1C, C-5); 76.75 (1C, C-3); 108.75 (1C, *C*(CH$_3$)$_2$); 118.99, 124.08, 126.06, 126.60, 126.68, 126.73, 128.67, 134.51, 134.59, 140.28 (24C, Ar); 155.61 (1C, *C*ONH),; 172.50 (1C, *C*OOH). Maldi-Tof (DHB, positive mode): 788 (M+Na)$^+$, 804 (M+K)$^+$. Anal. Calcd for $C_{44}H_{51}NO_9Si$: C, 68.99; H, 6.72; N, 1.83. Found: C, 68.16; H, 6.83; N, 1.80.

4.15. H-L-Threonyl-3-*O*-[1,5-anhydro-galactitol-2]-Lseryl-Lalanyl-Lprolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-L-threonine 26

Glycopeptide formation was carried out by solid phase synthesis employing Wang resin B1250 (84.2 mg, 80 μmol). To attach the first amino acid to the *p*-hydroxy-benzyl alcohol linker the Wang resin B1250 (covering density 950 μmol/g) was swollen with dichloromethane (twice, 5 min) and dried. Coupling of the corresponding amino acid Fmoc derivatives was performed by employing MSNT (3 equiv) and methylimidazole (2.25 equiv) for 2 h. This was repeated for a second time. Following washing with dichloromethane and DMF the Fmoc group was cleaved with piperidine (20% in DMF) for 10 min (twice). Coupling of the following amino acids was performed by employing Fmoc-OPfd derivatives with addition of Dhbt (3 equiv) in DMF for 3 h. For coupling of **23** TBTU (1.5 equiv) and ethyldiisopropylamine (1.5 equiv) were added. After complete coupling and Fmoc deprotection washing with DMF and methanol was performed to give compound **24**. Final cleavage of the glycopeptides from the resin and simultaneous deprotection of the saccharide part was done with aqueous trifluoroacetic acid (95%) for 2 h. Solvents were removed from the combined filtrates in vacuo and codestillation with toluene/methanol 3:1 and final vacuum drying. RP-HPLC purification was used: 0.1% TFA in water/0.1% TFA in acetonitrile, 100:0→90:10 (5 min)→85:15 (5 min)→75:25 (5 min). ^{1}H NMR (500 MHz, H$_2$O/D$_2$O 9:1, pH 3.5) cf. Table 1. MALDI-Tof (positive mode, CCA) 1403 (M+H)$^+$.

4.16. H-L-Threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-3-*O*-[1,5-anhydro-galactitol-2]-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-L-threonine 27

Reaction conditions, workup and purification were similar to those described for the synthesis of **26**. ^{1}H NMR (500 MHz, H$_2$O/D$_2$O 9:1, pH 3.5) cf. Table 2. MALDI-Tof (positive mode, CCA) 1403 (M+H)$^+$.

Acknowledgements

Support of this work by the Deutsche Forschungsgemeinschaft (SFB 470, A5) and the Fonds der Chemischen Industrie is gratefully acknowledged.

References

1. Eichwald, E. *Ann. Chem. Pharmakol.* **1865**, *134*, 177.
2. Morell, A. G.; Irvine, R. A.; Sternlieb, I.; Scheinberg, I. H.; Ashwell, G. *J. Biol. Chem.* **1968**, *243*, 155–159.
3. *Glycoproteins*; Montreuil, J., Vliegenthart, J. F., Schachter, H., Eds.; Elsevier: Amsterdam, 1995.
4. Springer, G. F. *Science* **1984**, *224*, 1198–1206.
5. Wong, C.-H. *J. Chin. Chem. Soc.* **1999**, *46*, 271–281.
6. Toone, E. J. *Curr. Opin. Struct. Biol.* **1994**, *4*, 119–728.
7. Chapleur, Y. *Carbohydrate Mimics*; Wiley-VCH: Weinheim, 1998.
8. Wittmann, V.; Kessler, H. *Angew. Chem., Int. Ed. Engl.* **1993**, *105*, 1138–1140.
9. Levy, D. E.; Tang, C. *The Chemistry of C-Glycosides*; Pergamon Press, Elsevier, 1995.
10. Westermann, B.; Walter, A.; Florke, U.; Altenbach, H.-J. *Org. Lett.* **2001**, *3*, 1375–1378.
11. Schäfer, A.; Thiem, J. *J. Org. Chem.* **2000**, *65*, 24–29.
12. Schäfer, A.; Klich, G.; Schreiber, M.; Paulsen, H.; Thiem, J. *Carbohydr. Res.* **1998**, *373*, 107–116.
13. Ogura, K.; Arai, K.; Tsuch1Hashi, G.-I. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3669–3670.
14. Kocienski, P.; Pant, C. *Carbohydr. Res.* **1982**, *110*, 330–332.
15. Zemplen, G.; Pacsu, E. *Ber. Dtsch. Chem. Ges.* **1929**, *63*, 1613–1618.
16. Nakajima, K.; Takai, F.; Tanaka, T.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1978**, *51*, 1577–1578.

17. McCoull, W.; Davis, F. A. *Synthesis* **2000**, 1347–1365.
18. Sweeney, J. B. *Chem. Soc. Rev.* **2002**, *31*, 247–258.
19. Hu, X. E. *Tetrahedron* **2004**, *60*, 2701–2743.
20. Karban, J.; Kroutil, J. *Adv. Carbohydr. Chem. Biochem.* **2006**, *60*, 27–101.
21. Tanner, D. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 599–619.
22. Baldwin, J. E.; Adlington, R. M.; O'Neil, I. A.; Schofield, C.; Spivey, A. C.; Sweeney, J. B. *J. Chem. Soc., Chem. Commun.* **1989**, 1852–1854.
23. Dauban, P.; Dubois, L.; Dau, M. E. T. H.; Dodd, R. *J. Org. Chem.* **1995**, *60*, 2035–2043.
24. Dubois, L.; Mehta, A.; Tourette, A.; Dodd, R. H. *J. Org. Chem.* **1994**, *59*, 434–441.
25. Nakajima, K.; Neya, M.; Yamada, S.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 3049–3050.
26. Nakajima, K.; Oda, H.; Okawa, K. *Bull. Chem. Soc. Jpn.* **1983**, *56*, 520–522.
27. Bahl, O. P.; Agrawal, K. M. L. *J. Biol. Chem.* **1969**, *244*, 2970–2978.
28. Huber, E. E.; Gaunt, M. T. *Arch. Biochem. Biophys.* **1983**, *220*, 263–271.
29. Kröger, L.; Henkensmeier, D.; Schäfer, A.; Thiem, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 73–75.
30. Hase, S.; Nishimura, N.; Kawabata, S.; Iwanaga, S.; Ikenaka, T. *J. Biol. Chem.* **1990**, *265*, 872–883.
31. Hart, G. W.; Kreppel, L. K.; Comer, F. I.; Arnold, C. S.; Snow, D. M.; Ye, Z.; Cheng, X.; DellaManna, D.; Caine, D. S.; Earles, B. J.; Akimoto, Y.; Cole, R. N.; Hayes, B. K. *Glycobiology* **1996**, *6*, 711–716.
32. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149–2154.
33. Wang, S. S. *J. Am. Chem. Soc.* **1973**, *95*, 1328–1335.
34. Blankemeyer-Menge, B.; Nimtz, M.; Frank, R. *Tetrahedron Lett.* **1990**, *31*, 1701–1704.
35. Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gilessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927–1930.
36. Kondo, Y. *Carbohydr. Res.* **1983**, *141*, 324–327.