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HEXAFLUOROACETONE AS PROTECTING AND ACTIVATING REAGENT: A NEW APPROACH TO O-GLYCOSIDES

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Abstract - *O*-Glycosylated hexafluoroacetone-protected amino acid derivatives have been synthesized starting from serine, threonine, 4-hydroxyproline and tyrosine. They represent a new class of building blocks suitable for a divergent approach to *O*-glycopeptides.

INTRODUCTION

Carbohydrates are building blocks *i.a.* of ribonucleic acids, glycoproteins, glycolipids, and glycophospholipids.¹ In many cases the carbohydrate moiety is the carrier of informations.² As a constituent of the cell membrane, glycoconjugates are responsible for important functions³⁻⁵ in cell-cell recognition, in cell-matrix interactions, and cell growth regulation, consequently also in the development of tumors.⁶ Furthermore, they play a significant role in the interaction with biologically active factors as enzymes, hormones, bacteriotoxines, and viruses.³

The carbohydrate units undergo permanent changes⁷ during the life cycle of a cell, influencing the biological properties of peptides and proteins.¹ In general, glycosylation increases proteolytic stability,⁸ enhances solubility, improves transmembrane transport properties, and decreases excretion rate,⁹ thus enhancing bioavailability. Since glycosylations can influence conformational flexibility of peptides, they play a significant role in peptide folding processes.¹⁰

* Dedicated to Prof. Dr. Pierre Potier on the occasion of his 70th birthday

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In many cases, the glycosylation of naturally occurring and non-natural peptides leads to a change in their activity profile. For instance, the analgesic activity of enkephalines¹¹ was increased by glycosylation which was mainly attributed to an improved passage of the glycosylated species across the blood/brain barrier.¹²

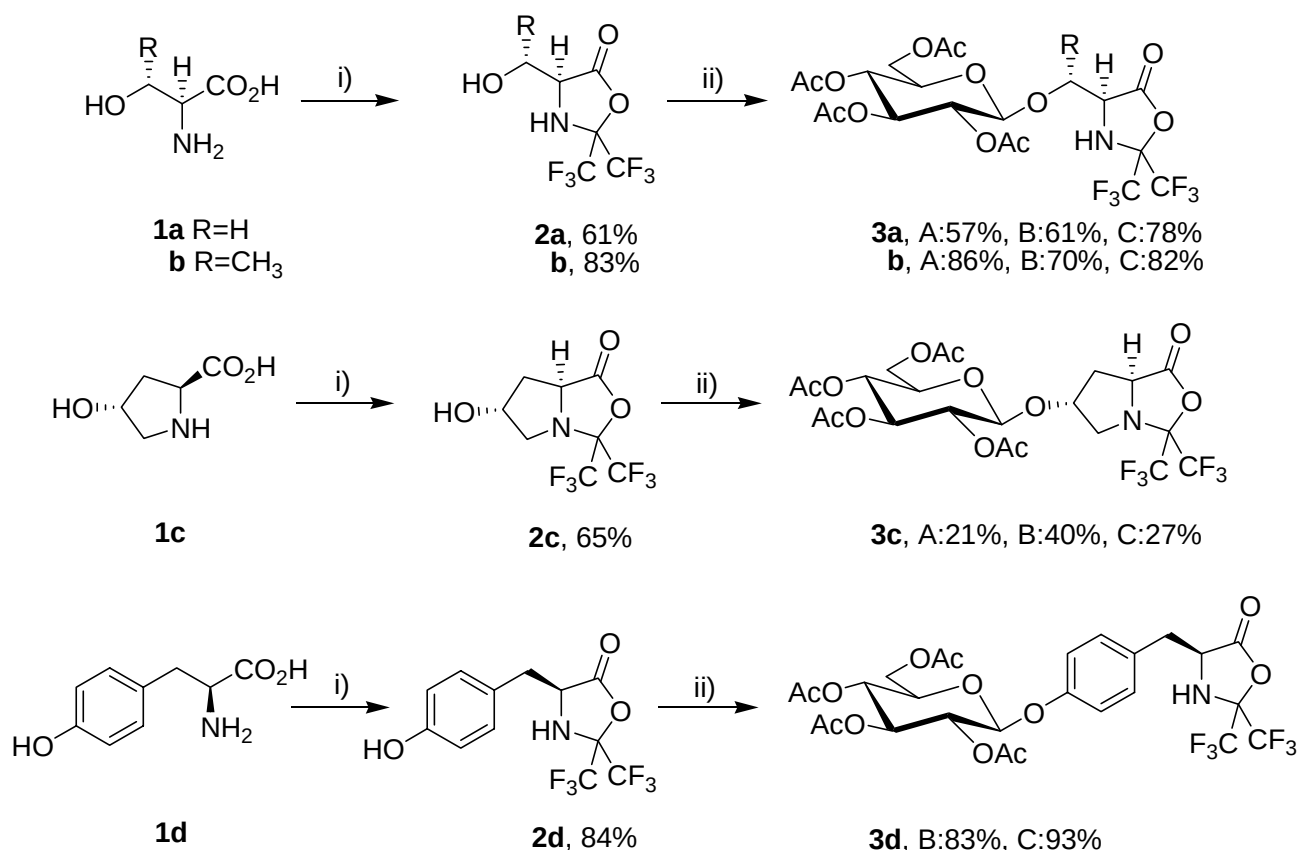
The convergent synthesis of *O*-glycosides by coupling reactions at the carbohydrate/peptide interface is problematic because of the generally poor solubility of peptides under glycosylation conditions and also because of regio- and stereochemical aspects.^{13,14} Therefore, the method of choice is an alternative strategy that involves the stepwise construction starting from *O*-glycosylated amino acid derivatives and small *O*-glycosylated peptide fragments, which can be assembled using solid phase techniques.¹⁵

Conventional syntheses of glycosylamino acids and peptides require orthogonal protection strategies for the α -amino and the α -carboxylic groups. After glycosylation of the unprotected hydroxy group, the carboxylic group is deprotected, activated, and finally the C-terminus is coupled with an amino acid derivative.

RESULTS AND DISCUSSION

The new protecting / activating strategy described herein offers some significant advantages: Introduction and cleavage of the protecting group occur under mild conditions. This resolves the issue of acid lability of the *O*-glycoside bond as well as the tendency of the *O*-glycosylated serine and threonine derivatives to undergo β -elimination in the presence of strong base. As an additional result, the new strategy offers the possibility to save steps. Moreover, the reaction can be monitored easily and quickly by ¹⁹F NMR spectroscopy without any loss of material.

Multifunctional α -amino acids such as serine, threonine, 4-hydroxyproline, and tyrosine react with hexafluoroacetone (HFA) in good yields to form 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones.¹⁶ The regioselective heterocyclization process allows a simultaneous protection of the α -amino and α -carboxylic groups, concomitantly an activation of the α -carboxylic group is achieved. Since an excess of hexafluoroacetone is normally used, the hydroxy groups of serine, threonine, and 4-hydroxyproline in the crude product are partially protected as hemiketals. However, the hexafluoroacetone can be readily cleaved by stirring a solution of the respective hemiketal in dichloromethane (DCM) in the presence of silica gel at room temperature. The progress of the deprotection process can be monitored efficiently by ¹⁹F NMR spectroscopy. The 2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-ones (**2**) can be stored for several months when kept below 0 °C in moisture-free environment.



Scheme 1: i) HFA, DMSO, rt; ii) *method A*: Ac₄- α -D-Glc-Br, Hg(CN)₂, CH₃CN; *method B*: Ac₄- β -D-Glc-OAc, BF₃·Et₂O, CH₂Cl₂; *method C*: Ac₄- β -D-Glc-O-C(=NH)CCl₃; BF₃·Et₂O, CH₂Cl₂.

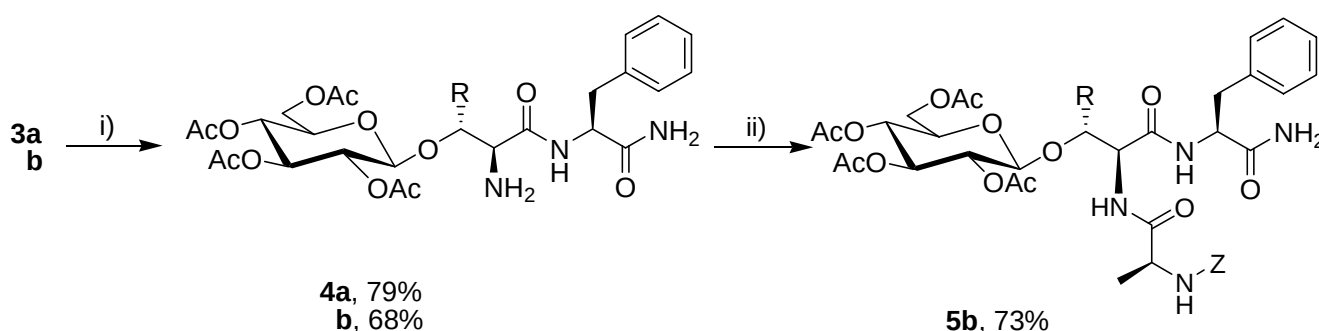
The HFA-protected amino acids (**2a-d**) were reacted with 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide / Hg(CN)₂ according to Helferich,¹⁷ with penta-*O*-acetyl- β -D-glucopyranose / BF₃·Et₂O according to Paulsen¹⁸ and with 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl trichloroacetimidate / BF₃·Et₂O according to Schmidt.¹⁹ Good to excellent yields of HFA-protected *O*-glycosylated amino acids (**3a-d**) indicate that the new protective group strategy can be applied successfully for all standard *O*-glycosylation procedures.²⁰

Compared to the glycosylation of HFA-protected Ser (**2a**) and Thr (**2b**), the yield of HFA-protected Hyp derivatives (**3c**) was surprisingly low (27%) even on using the trichloroacetimidate method. The main product in this case (55%) was 4-Ac-(HFA)-Hyp which indicates that an orthoester is formed as an intermediate.¹³

To date, only a few glycopeptides containing glycosyl-Tyr have been isolated from natural sources. β -D-Glucosyl-*O*-tyrosine itself was found as a tyrosine metabolite in *Lepidoptera*.²¹ The moderate yields (40%) in the glycosylation of 9-fluorenylmethoxycarbonyltyrosine-*O*-pentafluorophenyl ester (9-Fmoc-

Tyr-OPfp) have been attributed to the low nucleophilicity of the phenolic OH-group.²² O-Glycosylation of HFA-Tyr (**2d** → **3d**) via trichloroacetimidate method gives a yield of 93%.

The O-glycosylated oxazolidin-5-ones (**3a-d**) can be used directly as activated esters in peptide syntheses. When the lactone ring is opened with amino acid esters or amides, glycosylated dipeptide amides crystallize from the reaction mixture in good yields. The formation of the amido bond is coupled with the deprotection of the amino group, therefore an extension of the peptide chain at the N-terminus can be performed without any additional activation and deprotection steps.



Scheme 2: i) H-Phe-NH₂, EtOAc; ii) Z-Ala-OH, DIC, HOAt, CH₂Cl₂/DMF.

The glycosylated tripeptide Z-Ala-Thr(β-D-Ac₄-Glc)-Phe-NH₂ (**5b**) was obtained from Z-Ala-OH and **4b** on activation with diisopropylcarbodiimide (DIC) / 7-aza-1-hydroxy-1*H*-benzotriazole (HOAt) in 73% yield.

The protecting / activation concept disclosed herein allows the construction of O-glycosylated dipeptide (**1** → **2** → **3** → **4**) and tripeptide fragments (**1** → **2** → **3** → **4** → **5**) starting from hydroxy amino acids in only three or four synthetic steps, and therefore can be considered as a useful alternative to the pentafluorophenyl ester method.²³

EXPERIMENTAL

IR spectra were obtained on a Genesis ATI Mattson/Unicam FTIR spectrometer. ¹H NMR spectra were recorded at 300 and 400 MHz. Chemical shifts were reported in ppm relative to tetramethylsilane (TMS, δ = 0 ppm; *J* values are given in Hz (Hertz)). ¹³C NMR spectroscopy was performed at 75 and 100 MHz. ¹⁹F NMR spectra were recorded at 282 MHz with trifluoroacetic acid (TFA, δ = 0 ppm) as external standard. Melting points were determined on a Boëtius heating table. For C,H,N analyses a CHNO-Rapid-Elemental Analyzer (Hereaus) was used. For flash chromatography, silica gel (32 – 63

μm) was used with solvent systems given in the text. Organic solvents were dried and distilled prior to use.

Protocol 1 (Helferich variant of the Koenigs/Knorr method)¹⁷: To a stirred solution of the HFA-protected amino acid (**2**) (5.0 mmol) and 2,3,4,6-tetra-*O*-acetyl- α -D-glucopyranosyl bromide (2.06 g, 5.0 mmol) in acetonitrile (25 mL), $\text{Hg}(\text{CN})_2$ (1.26 g, 5.0 mmol) was added slowly under inertgas. After 24 h dichloromethane (DCM) (50 mL) was added, the undissolved material was filtered off, the organic layer was washed with saturated NaHCO_3 solution and water. The organic layer was dried (MgSO_4), the solvent evaporated in vacuo and the residue purified by flash chromatography.

Protocol 2 (Paulsen method)¹⁸: To a stirred mixture of the HFA-protected amino acid (**2**) (5.0 mmol) and penta-*O*-acetyl- β -D-glucopyranose (1.95 g, 5.0 mmol) in dry DCM (20 mL) $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (635 μL , 5.0 mmol) was added. The mixture was stirred for 24 h at rt, diluted with DCM (50 mL) and extracted with conc NaHCO_3 solution and water. The organic layer was dried (MgSO_4) and evaporated. The remaining residue was purified by flash chromatography.

Protocol 3 (R.R. Schmidt method)¹⁹: To a stirred mixture of the HFA-protected amino acid (**2**) (5.0 mmol) and 2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyltrichloroacetimidate (2.46 g, 5.0 mmol) in DCM (20 mL), $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (127 μL , 1.0 mmol) was added and the mixture stirred for 1 h at rt. After addition of DCM (20 mL) and water (10 mL) the mixture was stirred vigorously for 5 min. After separation, the organic layer was dried (MgSO_4) and evaporated under reduced pressure. The residue was purified by flash chromatography.

(4S)-4-(2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranosyloxymethyl)-2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-one (3a). 2,3,4,6-Tetra-*O*-acetyl- β -D-glucopyranosyl trichloroacetimidate (2.46 g, 5.0 mmol), **2a** (1.27 g 5.0 mmol) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (127 μL , 1.0 mmol) were reacted due to protocol 3. Purification by flash chromatography (petroleum ether / ethyl acetate, 2:1). Yield: 78% (2.28 g) **3a**, mp 173 – 174 °C. ^1H NMR (CDCl_3 , 300 MHz): δ = 2.01 (3H, s), 2.03 (3H, s), 2.06 (3H, s), 2.09 (3H, s), 3.69 – 3.75 (2H, m), 3.98 – 4.11 (3H, m), 4.20 – 4.21 (2H, m), 4.59 (1H, d, J = 8.0 Hz), 4.99 (1H, dd, J = 9.5 Hz, J = 8.0 Hz), 5.08 (1H, dd, J = 9.5 Hz, J = 9.5 Hz), 5.20 (1H, dd, J = 9.5 Hz, J = 9.5 Hz). ^{13}C NMR (acetone- d_6): δ = 20.5, 20.6, 55.9, 62.6, 68.5, 69.2, 71.7, 72.7, 73.6, 89.3 (sept, J = 34 Hz), 101.0, 121.4 (q, J = 286 Hz), 122.5 (q, J = 287 Hz), 169.7, 170.0, 170.2, 170.3, 170.8. ^{19}F NMR (acetone- d_6): δ = -1.85 (3F,

q, $J = 8.7$ Hz), - 2.38 (3F, q, $J = 8.7$ Hz). IR (KBr): $\nu = 3320, 1840, 1750\text{ cm}^{-1}$. MS (EI, 70 eV): $m/z = 584\text{ [M+H]}^+$, 583 $[\text{M}]^+$, 540 $[\text{M} - \text{CH}_3\text{CO}]^+$, 332 $[\text{M} - \text{C}_6\text{H}_3\text{NO}_3\text{F}_6]^+$, 331 $[\text{M} - \text{C}_6\text{H}_4\text{NO}_3\text{F}_6]^+$, 271, 169, 109, 81. Anal. Calcd for $\text{C}_{20}\text{H}_{23}\text{NO}_{12}\text{F}_6$: C, 41.18; H, 3.97; N, 2.40. Found: C, 41.24; H, 3.84; N, 2.35.

(4S)-4-[(1R)-1-(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyloxy)ethyl]-2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-one (3b). 2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyl trichloroacetimidate (2.46 g, 5.0 mmol), **2b** (1.34 g, 5.0 mmol) and $\text{BF}_3\cdot\text{Et}_2\text{O}$ (127 μL , 1.0 mmol) were reacted due to protocol 3. Purification by flash chromatography (toluene / ethyl acetate, 5:1). Yield: 82% (2.44 g) **3b**, mp 112 – 113 $^\circ\text{C}$. ^1H NMR (CDCl_3 , 300 MHz): $\delta = 1.38$ (3H, d, $J = 6.5$ Hz), 2.01 (3H, s), 2.02 (3H, s), 2.06 (3H, s), 2.07 (3H, s), 3.66 – 3.76 (3H, m), 4.06 – 4.13 (2H, m), 4.22 (1H, dd, $J = 12.5$ Hz, $J = 4.2$ Hz), 4.57 (1H, d, $J = 8.0$ Hz), 4.95 (1H, dd, $J = 9.6$ Hz, $J = 8.0$ Hz), 5.09 (1H, dd, $J = 9.7$ Hz, $J = 9.7$ Hz), 5.20 (1H, dd, $J = 9.7$ Hz, $J = 9.6$ Hz). ^{13}C NMR (CDCl_3): $\delta = 18.1, 20.4, 20.5, 20.8, 59.9, 61.3, 68.0, 71.2, 72.2, 72.6, 75.7, 89.3$ (sept, $J = 34$ Hz), 100.2, 120.2 (q, $J = 286$ Hz), 121.1 (q, $J = 287$ Hz), 169.2, 169.3, 169.4, 170.2, 170.7. ^{19}F NMR (CDCl_3 , 282 MHz): $\delta = -0.94$ (3F, q, $J = 8.6$ Hz, CF_3), -2.89 (3F, q, $J = 8.6$ Hz, CF_3). IR (KBr): $\nu = 3000 - 2940, 1830, 1750\text{ cm}^{-1}$. MS (EI, 70 eV): $m/z = 597\text{ [M]}^+$, 554 $[\text{M} - \text{CH}_3\text{CO}]^+$, 331 $[\text{M} - \text{C}_7\text{H}_6\text{NO}_3\text{F}_6]^+$, 271, 169, 109. Anal. Calcd for $\text{C}_{21}\text{H}_{25}\text{NO}_{12}\text{F}_6$: C, 42.22; H, 4.22; N, 2.34. Found: C, 42.22; H, 4.08; N, 2.36.

(5S,7R)-7-[2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyloxy]-2,2-bis(trifluoromethyl)-1-aza-3-oxabicyclo[3.3.0]octan-4-one (3c). 2,3,4,6-Tetra-O-acetyl- α -D-glucopyranosyl bromide (1.03 g, 2.5 mmol), **2c** (0.70 g, 2.5 mmol) and $\text{Hg}(\text{CN})_2$ (0.63 g, 2.5 mmol) were reacted due to protocol 1. Purification by flash chromatography (petroleum ether / ethyl acetate, 2:1). Yield: 21% (0.32 g) **3c**, mp 106 – 107 $^\circ\text{C}$. ^1H NMR (CD_3CN , 400 MHz): $\delta = 1.92$ (3H, s), 1.96 (3H, s), 1.98 (3H, s), 1.99 (3H, s), 2.11 (1H, ddd, $J = 13.8$ Hz, $J = 9.1$ Hz, $J = 4.8$ Hz), 2.31 (1H, ddd, $J = 13.8$ Hz, $J = 7.9$ Hz, $J = 1.6$ Hz), 3.31 (1H, dm, $J = 11.1$ Hz), 3.51 (1H, ddq, $J = 11.1$ Hz, $J = 4.2$ Hz, $J = 2.1$ Hz), 3.81 (1H, ddd, $J = 10.0$ Hz, $J = 5.3$ Hz, $J = 2.5$ Hz), 4.06 (1H, dd, $J = 12.3$ Hz, $J = 2.5$ Hz), 4.19 (1H, dd, $J = 12.3$ Hz, $J = 5.3$ Hz), 4.29 (1H, dd, $J = 9.1$ Hz, $J = 7.9$ Hz), 4.58 (1H, m), 4.72 (1H, d, $J = 8.1$ Hz), 4.86 (1H, dd, $J = 9.7$ Hz, $J = 8.1$ Hz), 5.00 (1H, dd, $J = 10.0$ Hz, $J = 9.8$ Hz), 5.21 (1H, dd, $J = 9.8$ Hz, $J = 9.7$ Hz). ^{13}C NMR (CD_3CN , 100 MHz): $\delta = 20.8, 20.9, 34.8, 55.7, 61.2, 62.7, 69.3, 71.8, 72.7, 73.3, 81.4, 92.3$ (m), 100.6, 121.0 (q, $J = 289$ Hz), 122.2 (q, $J = 287$ Hz), 170.3, 170.5, 170.9, 171.3, 172.1. ^{19}F NMR (CD_3CN , 282 MHz): $\delta = -3.09$ (3F, q, $J = 9.6$ Hz, CF_3), 5.48 (3F, q, $J = 9.6$ Hz, CF_3). IR (KBr): $\nu = 3454, 1833, 1756\text{ cm}^{-1}$. Anal. Calcd for $\text{C}_{22}\text{H}_{25}\text{NO}_{12}\text{F}_6$: C, 42.11; H, 4.02; N, 2.23. Found: C, 41.91; H, 3.90; N, 2.26.

(4S)-4-[4-(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyloxy)benzyl]-2,2-bis(trifluoromethyl)-1,3-oxazolidin-5-one (3d). 1,2,3,4,6-Penta-O-acetyl- β -D-glucopyranose (1.95 g, 5.0 mmol), **2d** (1.64 g, 5.0 mmol) and $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (635 μL , 5.0 mmol) were reacted due to protocol 2. Purification by flash chromatography (petroleum ether / ethyl acetate, 1:1). Yield: 83 % (2.74 g) **3d**, mp 155 -158 $^\circ\text{C}$ (ethanol / petroleum ether). ^1H NMR (DMSO-d_6 , 300 MHz): δ = 1.97 (3H, s), 2.01 (9H, s), 2.79 (1H, dd, J = 14.0 Hz, J = 8.0 Hz), 3.02 (1H, dd, J = 14.0 Hz, J = 4.5 Hz), 4.07 (1H, m), 4.20 (1H, m), 4.25 (1H, m), 4.43 (1H, m), 5.00 (1H, m), 5.05 (1H, m), 5.42 (1H, dd, J = 9.6 Hz, J = 9.6 Hz), 5.51 (1H, d, J = 8.0 Hz), 6.07 (1H, d, J = 6.7 Hz), 6.98 (2H, d, J = 8.7 Hz), 7.21 (2H, d, J = 8.7 Hz). ^{13}C NMR (DMSO-d_6 , 75 MHz): δ = 20.2, 20.2, 20.3, 55.1, 61.6, 68.1, 70.7, 71.9, 88.1 (sept, J = 33 Hz), 97.1, 116.0, 120.2 (q, J = 286 Hz), 120.9 (q, J = 289 Hz), 130.2, 130.9, 155.4, 169.0, 169.3, 169.5, 169.9, 171.3. ^{19}F NMR (DMSO-d_6 , 188 MHz): δ = -0.54 (3F, q, J = 9 Hz, CF_3), -2.12 (3F, q, J = 9 Hz, CF_3). IR (KBr) ν : 1830, 1732 cm^{-1} . Anal. Calcd for $\text{C}_{26}\text{H}_{27}\text{NO}_{12}\text{F}_6$: C, 47.35; H, 4.13; N, 2.12. Found: C, 47.00; H, 4.20; N, 1.99.

O-(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyl)serylphenylalanine amide (4a). A solution of **3a** (584 mg, 1.0 mmol) and phenylalanine amide (164 mg, 1.0 mmol) in ethyl acetate (20 mL) is stirred at rt until the starting material was consumed (3 d, ^{19}F NMR reaction control). The precipitate was filtered off and carefully washed with ethyl acetate and ether. Yield: 79% (0.46 g) **4a**, mp 183 $^\circ\text{C}$ (methanol). ^1H NMR (CDCl_3): 2.00 (3H, s), 2.03 (3H, s), 2.04 (3H, s), 2.09 (3H, s), 3.07 (1H, dm; J = 13.8 Hz), 3.16 (1H, dm, J = 13.8 Hz), 3.53 (1H, dd, J = 7.4 Hz, J = 4.8 Hz), 3.63 (1H, dd, J = 10.0 Hz, J = 7.4 Hz), 3.70 (1H, ddd, J = 9.8 Hz, J = 4.8 Hz, J = 2.4 Hz), 3.79 (1H, dd, J = 10.0 Hz, J = 4.8 Hz), 4.14 (1H, dd, J = 12.5 Hz, J = 2.4 Hz), 4.25 (1H, dd, J = 12.5 Hz, J = 4.8 Hz), 4.50 (1H, d, J = 7.7 Hz), 4.65 (1H, m), 4.92 (1H, dd, J = 9.7 Hz, J = 7.7 Hz), 5.04 (1H, dd, J = 9.8 Hz, J = 9.7 Hz), 5.19 (1H, dd, J = 9.7 Hz, J = 9.7 Hz), 5.64 (1H, s), 6.15 (1H, s), 7.25 (5H, m), 7.65 (1H, d, J = 8.1 Hz). ^{13}C NMR (CD_3OD): δ = 20.5, 20.6, 39.0, 55.4, 56.0, 63.1, 69.8, 72.8, 73.0, 73.2, 74.2, 101.9, 127.9, 129.5, 130.5, 138.3, 171.2, 171.3, 171.6, 172.4, 174.5, 175.8. IR (KBr): ν = 3400, 3020, 1726, 1688 cm^{-1} . MS (FAB): m/z = 604 $[\text{M} + \text{Na}]^+$. Anal. Calcd for $\text{C}_{26}\text{H}_{35}\text{N}_3\text{O}_{12}$: C, 53.70; H, 6.07; N, 7.23. Found: C, 53.68; H, 6.09; N, 7.10.

O-(2,3,4,6-Tetra-O-acetyl- β -D-glucopyranosyl)threonylphenylalanine amide (4b). A solution of **3b** (597 mg, 1 mmol) and phenylalanine amide (164 mg, 1 mmol) in ethyl acetate is stirred until the starting material was consumed (3 d, ^{19}F NMR reaction control). After evaporation of the solvent, the

residue was purified by flash chromatography (CHCl_3 / CH_3OH , 8:1). Yield: 68% (403 mg) **4b**, amorph, mp 75 -78 °C. ^1H NMR (CD_3OD): δ = 1.12 (3H, d, J = 6.5 Hz), 1.96 (3H, s), 1.97 (3H, s), 1.99 (3H, s), 2.02 (3H, s), 3.00 (1H, J = 14.0 Hz, J = 7.6 Hz), 3.13 (1H, dd, J = 14.0 Hz, J = 5.8 Hz), 3.28 (1H, d, J = 6.5 Hz), 3.86 (1H, m), 3.99 (1H, dq, J = 5.0 Hz, J = 6.5 Hz), 4.09 (1H, dd, J = 12.3 Hz, J = 4.8 Hz), 4.28 (1H, dd, J = 12.3 Hz, J = 2.2 Hz), 4.61 (1H, dd, J = 6.0 Hz, J = 8.0 Hz), 4.73 (1H, d, J = 8.0 Hz), 4.84 (1H, dd, J = 8.0 Hz, J = 9.6 Hz), 4.98 (1H, dd, J = 9.6 Hz, J = 9.9 Hz), 5.26 (1H, dd, J = 9.6 Hz, J = 9.6 Hz), 7.21-7.33 (5H, m). ^{13}C NMR (CD_3OD): δ = 16.8, 20.5, 20.6, 20.7, 38.9, 55.5, 60.3, 63.0, 69.8, 72.8, 74.1, 78.9, 100.6, 127.9, 129.5, 130.6, 138.2, 171.2, 171.3, 171.6, 172.4, 174.2, 175.7. MS (MALDI): m/z = 635.3 $[\text{M} + \text{K}]^+$, 618.2 $[\text{M} + \text{Na}]^+$.

***N*-Benzyloxycarbonylalanyl-*O*-(2,3,4,6-tetra-*O*-acetyl- β -D-glucopyranosyl)threonylphenylalanine amide (**5b**).** To a stirred solution of Z-Ala-OH (76.7 mg, 0.34 mmol) in DCM (20 mL) HOAt (46.8 mg, 0.34 mmol) and DIC (53.3 μL , 0.34 mmol) were added at rt. Then a solution of **4b** (100 mg, 0.17 mmol) in DMF (1 mL) was added. After 2 h the reaction mixture was concentrated in vacuo, the residue was redissolved in ethyl acetate (25 mL) and carefully washed with citric acid (10% solution), conc NaHCO_3 solution and water. The organic phase was dried (MgSO_4) and evaporated; the residue was purified by flash chromatography (CHCl_3 / CH_3OH , 10:1). Yield: 73% (101 mg) **5b**, crystalline solid, mp 190 °C. ^1H NMR (CD_3OD): δ = 1.12 (3H, d, J = 6.2 Hz), 1.30 (3H, d, J = 7.2 Hz), 1.93 (3H, s), 1.98 (3H, s), 2.00 (3H, s), 2.03 (3H, s), 3.04 (1H, dd, J = 14.0 Hz, J = 7.7 Hz), 3.17 (1H, dd, J = 14.0 Hz, J = 5.5 Hz), 3.90 (1H, m), 4.07 (1H, dd, J = 12.3 Hz, J = 5.3 Hz), 4.14 (1H, m), 4.28 (1H, dd, J = 12.3 Hz, J = 5.3 Hz), 4.45 (1H, d, J = 6.2 Hz), 4.60 (1H, m), 4.77 (1H, d, J = 8.0 Hz), 4.85 (1H, dd, J = 9.6 Hz, J = 9.6 Hz), 5.01 (1H, dd, J = 9.6 Hz, J = 9.6 Hz), 5.05/5.10 (2H, dd, J = 12.5 Hz, J = 12.5 Hz), 5.27 (1H, dd, J = 9.6 Hz, J = 9.6 Hz), 7.15-7.35 (10H, m). ^{13}C NMR (CD_3OD): δ = 16.5, 17.8, 20.5, 20.6, 20.7, 38.6, 52.3, 55.8, 58.6, 63.1, 67.8, 69.8, 72.8, 73.2, 74.1, 76.4, 100.8, 127.9, 128.9, 129.0, 129.5, 129.6, 130.5, 138.3, 138.3, 158.5, 171.3, 171.4, 171.6, 172.4, 175.5, 175.9, 176.9. MS (MALDI): m/z = 839.1 $[\text{M} + \text{K}]^+$, 823.2 $[\text{M} + \text{Na}]^+$.

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