Novel Ester-Linked Carbohydrate–Peptide Adducts: Effect of the Peptide Substituent on the Pathways of Intramolecular Reactions

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Carbohydrate–peptide conjugates in which D-glucose is linked through an ester linkage to the carboxy group of Tyr-Pro (**2**), Tyr-Pro-Phe (**5**) or Tyr-Pro-Phe-Val (**11**), through the C6 hydroxy group of the sugar moiety were synthesized to examine the utility of this type of monosaccharide modification for peptide prodrugs. Evidence is provided that glycoconjugates **2**, **5**, and **11** easily undergo intramolecular chemical transformations, subsequent to attack of the free *N*-terminal amino group at the peptide backbone or at the anomeric position of the D-glucose moiety, resulting in the formation of diketopiperazine **12**, glycosylamine **13**, or ketosugar derivative **15**. The data indicated that the length and structure of the peptide chain are the main factors that control the intramolecular reactions of the carbohydrate–peptide esters studied.

Introduction

Many potent biologically active peptides never become clinically useful agents because of their undesirable pharmaceutical and biopharmaceutical properties, such as low water solubility and low permeability through biological membrane barriers. Finding solutions to these problems is a contemporary issue, particularly because of the rapid development of biotechnology and combinatorial chemistry, and consequently the discovery of peptides and peptidomimetics with undesirable properties.[1,2] One possible strategy used to correct the physicochemical properties of peptides is by derivatization of the functional groups with carbohydrate moieties. Recent investigations revealed that the attachment of saccharides to bioactive peptides improves the immunizing performance,[3] increases resistance toward enzymatic degradation,[4] enables them to penetrate the blood-brain barrier,[5] or results in valuable receptor-mediated gene-delivery agents.[6] With the aim to improve the properties of peptides, in addition to modifications in which the carbohydrate residue is linked to the peptide at the anomeric position, glycoconjugates possessing an ester bond between the amino acid/peptide and one of the sugar hydroxyl groups were introduced as new tools in biomedical research. These monosaccharide esters seem to be able to increase the intestinal permeability,[7] and may be used as monomers in polycondensation reactions.[8] Furthermore, they can act as building blocks for the solid-phase combinatorial synthesis of libraries of novel glycopeptides[9] and glycosylation reactions based on peptide templates,[10] and are capable of altering receptor selectivity.[11]

In an effort to gain insight into the chemical reactivity of monosaccharide-related peptide esters and the role of the peptide substituent, we chose to study the chemical properties and the adducts formed by intramolecular reactions of sugar-peptide esters in which a dipeptide (Tyr-Pro), tripeptide (Tyr-Pro-Phe) or tetrapeptide (Tyr-Pro-Phe-Val) is linked to the C-6 hydroxy group of D-glucose. Since such carbohydrate esters contain a free amino group as well as a reducing sugar epitope, they also represent a model system for studying the Maillard reaction, a complex glycation reaction which contributes to the pathophysiological changes associated with diabetes and aging processes.[12,13]

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Results and Discussion

Syntheses of Monosaccharide Esters

Scheme 1 shows our general strategy for the synthesis of glycoconjugates in which D-glucose is linked to Tyr-Pro (**2**), Tyr-Pro-Phe (**5**), and Tyr-Pro-Phe-Val (**11**) by an ester bond involving the carboxy function of the peptide residue and the hydroxy group at the C-6 of the D-glucopyranose moiety. The key step in these syntheses was the coupling of the N-protected amino acid or the peptide with free D-glucose, which was carried out via pentachlorophenyl esters as activating species using imidazole as the catalyst.[14] Regioselective acylation was confirmed by NMR spectroscopy.

The monosaccharide ester **1** was prepared by esterification of D-glucose with dipeptide active ester Boc-Tyr(Boc)-Pro-OPCP. Tripeptide derivative **4** was obtained by reacting 6-*O*-(L-phenylalanyl)-D-glucopyranose (**3**)[15] with *N*-protected dipeptide Boc-Tyr(Boc)-Pro-OH, using TBTU as the coupling agent. For the synthesis of tetrapeptide ester **10**, the stepwise assembly of the peptide chain was performed after regioselective esterification of D-glucose with valine active ester Boc-Val-OPCP, resulting in the formation of compound **6**. Removal of the Boc group with TFA to yield valine ester **7**, and subsequent reaction with *N*-protected phenylalanine Boc-Phe-OH, using TBTU as the condensing agent, resulted in the protected dipeptide com-

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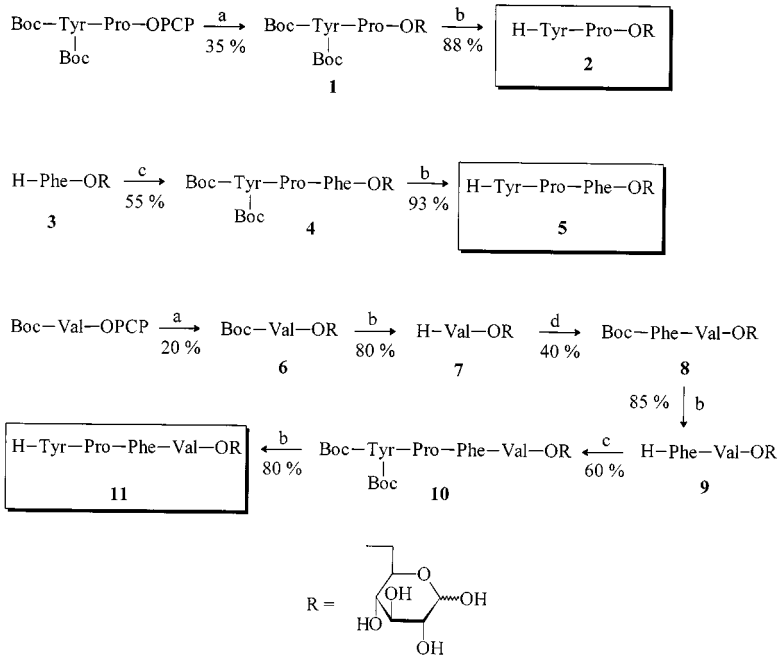


Scheme 1. Reagents and conditions: (a) D-glucose, imidazole, pyridine; (b) TFA/H_2O (9:1); (c) Boc-Tyr(Boc)-Pro-OH, TBTU, HOBt, DMF; (d) Boc-Phe-OH, TBTU, HOBt, DMF

pound **8**. To complete the peptide chain, the *N*-terminal Boc protecting group was removed with TFA, and product **9** was reacted with dipeptide Boc-Tyr(Boc)-Pro-OH in the presence of TBTU to yield tetrapeptide compound **10**. The resulting *N*-protected monosaccharide esters **1**, **4**, and **10** were treated with TFA/anisole to cleave the Boc group at the terminal tyrosine residue, resulting in the formation of the trifluoroacetate salts of the target compounds **2**, **5**, and **11**. The purity and identity of the synthetic monosaccharide esters were assessed by RP HPLC, elemental analysis and NMR spectroscopy.

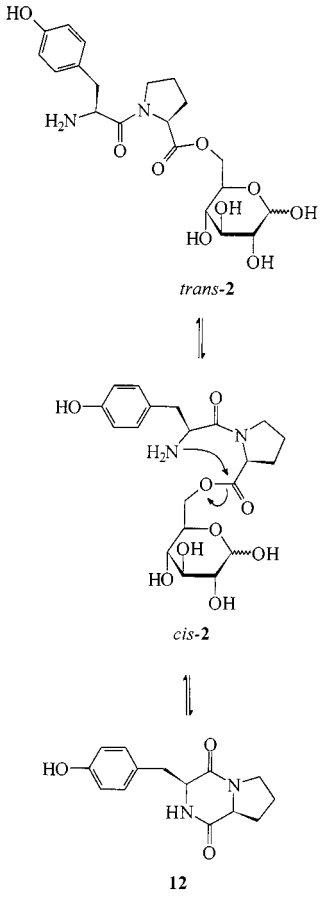
The ^{13}C NMR spectra of *N*-protected glycoconjugates **1**, **4**, and **10** in DMSO showed the presence of the α- and β-pyranose forms. An estimate of the equilibrium composition of the tautomeric forms in solution indicated that the β-pyranose form slightly prevails in dipeptide ester **1** (55%), whereas α-pyranose is the major form in tripeptide compound **4** (65%) and tetrapeptide derivative **10** (60%). The *cis* and *trans* conformations across the X-proline bond are often similar in energy and may both be present in solution. However, only the *trans* conformation of the Tyr-Pro amide bond was detected, based on the ^{13}C NMR shifts of the proline β- and γ-carbons[16,17] in monosaccharide ester **1**. Resonances of both, *trans* and *cis* isomers were observed in the NMR spectra of DMSO solutions of compounds **4** and **10**, with a *trans*-to-*cis* ratio of approximately 4:1.

According to the integration of the ^{13}C NMR signals of the unprotected monosaccharide esters **2**, **5**, and **11** in solutions of DMSO, the α ⇄ β anomeric equilibrium was shifted to the α-pyranose side in all compounds studied, with a 55:45 ratio for compounds **2** and **11**, whereas tripeptide compound **5** showed a 60:40 α/β ratio. The amount of the α-pyranose form found for D-glucose in DMSO solution is smaller[18] than that found for compounds **2**, **5**, and **11**, indicating the influence of C-6 esterification with a peptide moiety on the stabilization of the axial 1-OH group (α-anomer) of the sugar moiety in solution. When comparing the *cis*/*trans* ratio of esters **2**, **5** and **11** with those of the corresponding *N*-protected derivatives, in compound **2** only the existence of an isomer with the *trans* conformation at the Tyr-Pro bond was established, whereas a higher population of *cis* isomers (30%) was present in tri- and tetrapeptide derivatives **5** and **11**.

Intramolecular Reactions of Monosaccharide Esters of Di-, Tri-, and Tetrapeptides

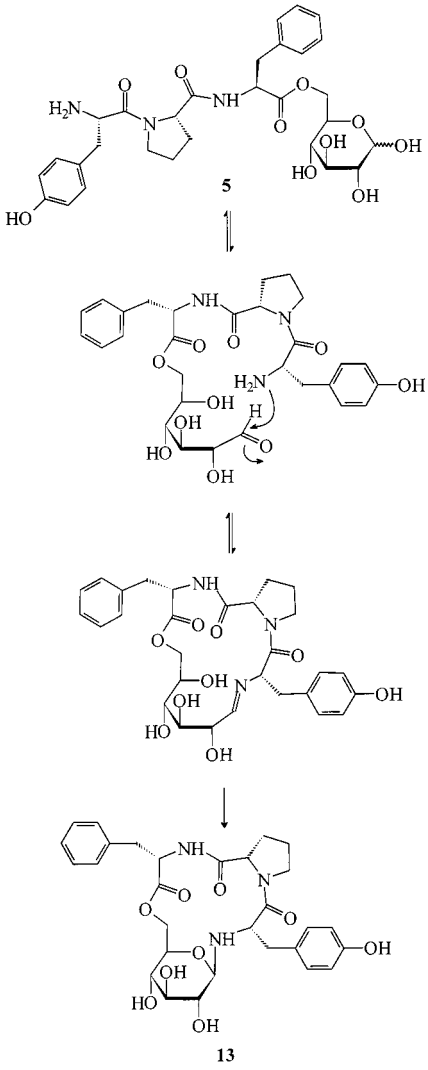
The chemical reactivity of monosaccharide esters **2**, **5**, and **11** was investigated in a pyridine/acetic acid (1:1) mixture. When dissolved in this solvent system and incubated at 50 °C for 2 days, dipeptide ester **2** afforded, after purification by semipreparative RP HPLC, diketopiperazine **12** (77%) (Scheme 2). *Cyclo*-(Tyr-Pro) (**12**) was also the only product generated in 48% yield by incubation of monosaccharide ester **2** in the same solvent at 4 °C for 2 days. Diketopiperazine formation by intramolecular nucleophilic addition of the terminal amino group to the carbonyl carbon of the second amino acid residue occurs easily in dipeptide esters because of good leaving groups (alcohol molecules).[19] Since a peptide bond is forced into the usually less favored *cis* conformation in the transition state, the cyclization reaction is promoted by *N*-alkylated amino acid or proline residues in the second position, because of their high propensity to form a *cis* peptide bond with the preceding residue. A pathway for the formation of diketopiperazine **12** from the ester **2** thus first requires a *trans* → *cis* isomerization. Although no *cis* isomer was present in a DMSO solution of **2**, as can be seen by NMR spectroscopy, we assume that the catalytic effect of acetic acid[20] mark-

edly increased the percentage of the *cis* isomer, thus ensuring a sufficiently high cyclization rate to diketopiperazine **12**, even at low reaction temperatures.



Scheme 2

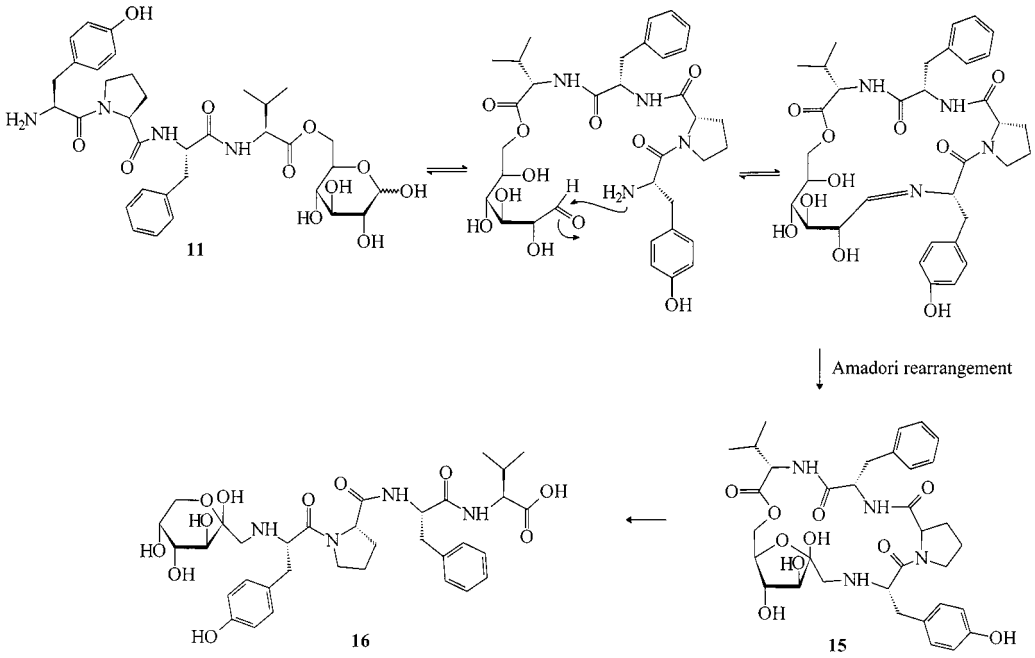
By heating 6-*O*-(Tyr-Pro-Phe)-D-glucopyranose (**5**) in pyridine/acetic acid for 24 h at 37 °C and at 50 °C, two different products were obtained. At 37 °C, by intramolecular cyclization of compound **5**, the major product isolated in a 52% yield was bicyclic *N*-(β-D-glucosyl)-tripeptide derivative **13** (Scheme 3). In contrast, incubation of **5** at a higher temperature (50 °C) gave *cyclo*-(Tyr-Pro) (**12**) (68%). Let us consider the mechanism of formation of glucosylamine derivative **13**: In the first step, the aldehyde group of the acyclic glucose moiety in compound **5** is attacked by the free amino terminus of the tripeptide residue to yield an open-chain iminium structure (Schiff base), which through intramolecular reaction of a 5-OH with C-1 then closes to give hemiacetal **13**. The fact that the incubation mixture of ester **5** at 37 °C contained, in addition to glucosylamine **13**, only traces of diketopiperazine, while incubation of **13** at 50 °C gave exclusively diketopiperazine **12**, suggests bicyclic compound **13** as the precursor of *cyclo*-(Tyr-Pro) (**12**). We can speculate that at higher temperatures, for steric reasons, the proline carbonyl carbon in bicyclic compound **13** is more vulnerable to nucleophilic attack by the secondary NH group of the tyrosine residue resulting in diketopiperazine **12** after cyclization and cleavage of the easily hydrolyzed *N*-glucosyl-amino acid bond[21].



Scheme 3

The Amadori rearrangement, transformation of glycosylamines into 1-amino-1-deoxy-2-keto-sugar derivatives, readily occurs with *N*-glycosylamino acids.[21] Surprisingly, attempts to rearrange glucosylamine tripeptide derivative **13** into the corresponding keto-sugar derivative, by heating **13** in a mixture of either pyridine/acetic acid or ethanol/diethyl malonate, were unsuccessful. However, heating of D-glucose with tripeptide Tyr-Pro-Phe at 37 °C for 2 days in pyridine/acetic acid (1:1) afforded the Amadori compound, *N*-(1-deoxy-D-fructos-1-yl)-Tyr-Pro-Phe (**14**) as the major isolated product (22%). The corresponding intermediate glucosylamine derivative of the tripeptide was not detected in the reaction mixture. It is rational to presume that inability of **13** to rearrange into the keto-sugar derivative is due to conformational reasons, and we are currently carrying out conformational analyses on compound **13** by using NMR spectroscopic techniques and molecular modeling, and the results will be published separately.

In order to gain insight into the stability of the bicyclic tripeptide glucosylamine derived from ester **5**, compound



Scheme 4

13 was dissolved in 0.1 M NH_4OH and 0.1 M HCl, and kept at room temperature for 3 days. As can be seen by means of RP HPLC, in the presence of base, **13** undergoes simultaneous hydrolysis of the ester and of the C−N glycoside bond to yield tripeptide Tyr-Pro-Phe. Under acidic conditions, glucosylamine **13** exists in equilibrium with the starting compound **5** owing to reversal of the amine−carbonyl reaction.

Incubation of monosaccharide ester **11** whose Tyr-Pro-Phe-Val amino acid sequence corresponds to the 51−54 fragment of human β-casein (found to possesses weak opioid activity)[22] in pyridine/acetic acid for 24 h at room temperature resulted in Amadori compound **15** (30%) (Scheme 4). The mechanism of the **11** → **15** transformation is rationalized as follows: The aldehyde of the open-chain form of the carbohydrate moiety in starting compound **11** is attacked by the free amino group of the *N*-terminal tyrosine residue. Instead of stabilization by formation of the glucosylamine derivative, the resulting cyclic Schiff base undergoes Amadori rearrangement to form the corresponding keto-sugar derivative **15** in the β-furanose form. In fact, the product of the intramolecular transformation of tetrapeptide ester **11** was analogous to the one we observed in our previous work on the monosaccharide ester related to the endogenous opioid pentapeptide.[23]

Hydrolysis of compound **15** (0.1 M NH_4OH) afforded *N*-(1-deoxy-D-fructopyranos-1-yl)-tetrapeptide **16** (22%) (Scheme 4), indistinguishable by spectroscopic data and optical rotation from compound **16** that was obtained by the reaction of D-glucose and Tyr-Pro-Phe-Val in pyridine/acetic acid for 3 days at 37 °C.

Conclusions

This study demonstrated that the structure and the length of the peptide residue determines the products formed from carbohydrate−peptide esters under conditions in which the balance of acidity and basicity in the reaction system controls the simultaneous and consecutive reactions.

Changes in the length of small glycopeptides with an identical *N*-terminal dipeptide sequence (Tyr-Pro, Tyr-Pro-Phe, Tyr-Pro-Phe-Val) attached at C-6 of a D-glucopyranose moiety, gave rise to specific chemical reactions, resulting in peptide backbone cyclization to form diketopiperazine, or in the corresponding glucosylamine and keto-sugar peptide derivatives. The factors which affect the overall reactivity of the peptide esters studied were the basicity of the *N*-terminal amine group, the *cis*−*trans* isomerization equilibrium of the Tyr−Pro amide bond, and the abundance of the acyclic (*aldehydo*) form of the sugar moiety in solution.

The data presented demonstrate that carbohydrate−peptide prodrugs containing free amino group(s) in the peptide part and a sugar moiety with an unsubstituted anomeric position may undergo a variety of reactions, which are to be taken into account when chemically manipulating the peptides.

Experimental Section

General: Melting points were determined on a Tottoli (Büchi) apparatus and are uncorrected. − Optical rotations were measured at 20 °C with an Optical Activity LTD automatic AA-10 Polarimeter. − Reactions were monitored by TLC on Silica Gel 60 F_{254} plates (Merck; Darmstadt, Germany) using detection with ninhydrin, chlorine−iodine reagent, or heating with H_2SO_4. − RP HPLC was performed on a Varian 9010 HPLC system with a Eurospher 100 reversed-phase C-18 semipreparative column (250 × 8 mm I.D., 5 μm) under isocratic conditions by using different concentrations of MeOH in 0.1% trifluoroacetic acid (TFA), flow rate 1.1 mL/min. − UV detection (Varian Model 9050 variable-wavelength UV/Vis detector) was performed at 280 nm. − NMR spectra were recorded on a Varian Gemini spectrometer, operating at 300.1 MHz for ^1H

and 75.5 MHz for ^{13}C nuclei in [D_6]DMSO or D_2O solutions at 25 °C. Chemical shifts, in ppm, are reported relative to TMS. − Elemental analyses were carried out at the Microanalytical Laboratory, Ruđer Bošković Institute.

6-*O*-(*N,O*-bis-*tert*-Butyloxycarbonyl-L-tyrosyl-L-prolyl)-D-glucopyranose (1): To a stirred solution of D-glucose (1260 mg, 7 mmol) in dry pyridine (30 mL), Boc-Tyr(Boc)-Pro-OPCP (1700 mg, 2.4 mmol) and imidazole (820 mg, 12 mmol) were added. The reaction mixture was stirred overnight at room temperature and the solvent was evaporated. To the residue, 10% citric acid (100 mL) and EtOAc (100 mL) were added. The organic layer was separated, and the water layer extracted with EtOAc (2 × 50 mL). The combined organic extracts were dried (Na_2SO_4), the solvent was evaporated, and the residue was purified by flash chromatography on silica gel (FCC) (EtOAc/EtOH/AcOH/H_2O 90:10:2:2) to yield after crystallization from EtOAc/hexane, ester **1** (540 mg, 35%), m.p. 135−140 °C. − $[\alpha]_D$ = +1.5 (*c* = 1, MeOH). − ^{13}C NMR ([D_6]DMSO): δ = 24.6 (Pro γ), 27.3 (OBoc CH_3), 28.2 (NHBoc CH_3), 28.6 (Pro β), 35.6 (Tyr β), 46.5 (Pro δ), 53.9 (Tyr α), 58.7 (Pro α), 64.6 (Glc C-6), 69.3 (αGlc C-5), 70.3 (βGlc C-4), 70.7 (αGlc C-4), 72.3 (αGlc C-2), 73.2 (αGlc C-3), 73.7 (βGlc C-5), 74.8 (βGlc C-2), 76.6 (βGlc C-3), 78.2 (NHBoc C), 83.2 (OBoc C), 92.5 (αGlc C-1), 97.2 (βGlc C-1), 121.2 (Tyr ε), 130.6 (Tyr δ), 135.9 (Tyr γ), 149.5, 151.6 (Boc CO), 155.6 (Tyr ζ), 170.6 (Pro CO), 172.2 (Tyr CO); anomer ratio α/β 45:55. − $C_{30}H_{44}N_2O_{13}$ (640.63): calcd. C 56.24, H 6.94, N 4.37; found C 56.32, H 6.86, N 4.45.

6-*O*-(L-Tyrosyl-L-prolyl)-D-glucopyranose (2): Compound **1** (500 mg, 0.78 mmol) was treated with TFA/water (9:1, 2 mL) in the presence of anisole (0.4 mL) for 1 h at room temperature. After addition of diisopropyl ether, the precipitate was collected by centrifugation and triturated several times with diisopropyl ether to yield **2** (380 mg, 88%), m.p. 118−125 °C. − $[\alpha]_D$ = +10.0 (*c* = 1, MeOH). − ^{13}C NMR ([D_6]DMSO): δ = 24.7 (Pro γ), 28.7 (Pro β), 35.5 (Tyr β), 46.6 (Pro δ), 52.7 (Tyr α), 59.0 (Pro α), 64.8 (Glc C-6), 69.4 (αGlc C-5), 70.3 (βGlc C-4), 70.7 (αGlc C-4), 72.3 (αGlc C-2), 73.0 (αGlc C-3), 73.7 (βGlc C-5), 74.9 (βGlc C-2), 76.6 (βGlc C-3), 92.5 (αGlc C-1), 97.2 (βGlc C-1), 115.6 (Tyr ε), 124.8 (Tyr γ), 131.1 (Tyr δ), 157.0 (Tyr ζ), 167.5 (Tyr CO), 171.6 (Pro CO); anomer ratio α/β = 55:45. − $C_{20}H_{28}N_2O_9{\cdot}CF_3COOH$ (554.39): calcd. C 47.66, H 5.28, N 5.05; found C 47.44, H 5.39, N 5.24. Desalting on a short (10 × 0.8 cm) Dowex 1 × 2, 200 (Ac) column and lyophilization gave ester **2**, which was used in subsequent experiments.

6-*O*-(*N,O*-bis-*tert*-Butyloxycarbonyl-L-tyrosyl-L-prolyl-L-phenylalanyl)-D-glucopyranose (4): The trifluoroacetate salt of 6-*O*-(L-phenylalanyl)-D-glucopyranose[15] (**3**) (440 mg, 1.0 mmol) was dissolved in dry DMF (5 mL) and neutralized by addition of NMM (0.1 mL, 1.0 mmol). To this solution were added Boc-Tyr(Boc)-Pro-OH (478 mg, 1.0 mmol), TBTU (330 mg, 1.1 mmol), HOBt (130 mg, 1.0 mmol), and NMM (0.1 mL, 1.0 mmol). The reaction mixture was stirred overnight at room temperature, and the solvent was evaporated. The residue was purified by FCC (EtOAc/AcOH/H_2O 70:2:2) to yield after crystallization from EtOAc/hexane ester **4** (400 mg, 55%), m.p. 120−125 °C. − $[\alpha]_D$ = −4.0 (*c* = 1, MeOH). − ^{13}C NMR ([D_6]DMSO): δ = 21.3 (*cis*Pro γ), 24.5 (*trans*Pro γ), 27.3 (OBoc CH_3), 28.2 (NHBoc CH_3), 28.9 (*trans*Pro β), 30.5 (*cis*-Pro β), 35.6 (*trans*Tyr β), 35.9 (*cis*Tyr β), 36.6 (*trans*Phe β), 36.7 (*cis*Phe β), 46.8 (Pro δ), 53.6 (Phe α), 54.0 (Tyr α), 59.39 (*trans*Pro α), 59.45 (*cis*Pro α), 64.8 (Glc C-6), 69.2 (αGlc C-5), 70.3 (βGlc C-4), 70.6 (αGlc C-4), 72.3 (αGlc C-2), 73.0 (αGlc C-3), 73.6 (βGlc C-5), 74.9 (βGlc C-2), 76.6 (βGlc C-3), 78.2 (NHBoc C), 83.2 (OBoc C), 92.6 (αGlc C-1), 97.2 (βGlc C-1), 121.2 (*trans*Tyr ε), 121.5 (*cis*Tyr ε), 128.5 (Phe ε), 129.5 (*trans*Phe δ), 129.2 (*cis*Phe δ), 130.6 (*trans*Tyr δ), 130.8 (*cis*Tyr δ), 136.1 (Tyr γ), 137.5 (Phe γ), 149.5, 151.7 (*trans*Boc CO), 149.8, 150.5 (*cis*Boc CO), 155.7 (Tyr ζ), 170.6 (*trans*Pro CO), 170.8 (*cis*Pro CO), 171.6 (Phe CO), 172.1 (Tyr CO); anomer ratio α/β = 65:35; *cis*/*trans* = 15:85. − $C_{39}H_{53}N_3O_{14}$ (787.81): calcd. C 59.45, H 6.79, N 5.34; found C 59.31, H 6.57, N 5.46.

6-*O*-(L-Tyrosyl-L-prolyl-L-phenylalanyl)-D-glucopyranose (5): Compound **4** (500 mg, 0.63 mmol) was treated in the same way as described for compound **2** to give, after precipitation with diisopropyl ether, pure **5**·TFA (410 mg, 93%), m.p. 135−140 °C. − $[\alpha]_D$ = +8.5 (*c* = 1, MeOH). − ^{13}C NMR ([D_6]DMSO): δ = 21.7 (*cis*Pro γ), 24.6 (*trans*Pro γ), 29.1 (*trans*Pro β), 31.4 (*cis*Pro β), 35.4 (*trans*Tyr β), 36.2 (*cis*Tyr β), 36.4 (*cis*Phe β), 36.7 (*trans*Phe β), 46.9 (Pro δ), 52.64 (*trans*Tyr α), 52.65 (*cis*Tyr α), 53.4 (*cis*Phe α), 53.6 (*trans*Phe α), 59.0 (*cis*Pro α), 59.5 (*trans*Pro α), 64.9 (Glc C-6), 69.3 (αGlc C-5), 67.0 (*cis*αGlc C-4), 70.3 (*trans*αGlc C-4), 70.5 (*cis*βGlc C-4), 70.6 (*trans*βGlc C-4), 72.3 (αGlc C-2), 73.1 (αGlc C-3), 73.6 (βGlc C-5), 74.9 (βGlc C-2), 76.6 (βGlc C-3), 92.6 (αGlc C-1), 97.2 (βGlc C-1), 115.6 (*trans*Tyr ε), 115.7 (*cis*Tyr ε), 124.3 (*cis*Tyr γ), 124.8 (*trans*Tyr γ), 126.8 (*trans*Phe ζ), 127.0 (*cis*Phe ζ), 128.6 (Phe ε), 129.3 (*cis*Phe δ), 129.5 (*trans*Phe δ), 130.6 (*cis*Tyr δ), 131.1 (*trans*-Tyr δ), 137.4 (*trans*Phe γ), 137.5 (*cis*Phe γ), 156.9 (*trans*Tyr ζ), 157.1 (*cis*Tyr ζ), 167.2, 170.88, 170.91, 171.5, 171.6 (Tyr, Pro, Phe CO); anomer ratio α/β = 60:40; *cis*/*trans* = 30:70. − $C_{29}H_{37}N_3O_{10}{\cdot}CF_3COOH$ (701.57): calcd. C 53.21, H 5.47, N 5.99; found C 53.18, H 5.57, N 5.88. Desalting was performed as described for monosaccharide ester **2**.

6-*O*-(*N*-*tert*-Butyloxycarbonyl-L-valyl)-D-glucopyranose (6): D-Glucose (8.1 g, 45 mmol) was treated with Boc-Val-OPCP (7.0 g, 15 mmol) and imidazole (5.1 g, 75 mmol) in the same way as described for compound **1** to give ester **6** (1.1 g, 20%), m.p. 140−145 °C (decomp.). − $[\alpha]_D$ = +27.0 (*c* = 1, MeOH). − $C_{16}H_{29}NO_9$ (379.37): calcd. C 50.65, H 7.72, N 3.69; found C 50.67, H 7.73, N 3.87.

6-*O*-(L-Valyl)-D-glucopyranose (7): Compound **6** was deprotected by using TFA as described in the preparation of **2**, affording pure **7**·TFA (80 mg, 80%), m.p. 75−80 °C (decomp.). − $[\alpha]_D$ = +27.0 (*c* = 1, MeOH). − $C_{11}H_{21}NO_7{\cdot}CF_3COOH$ (393.24): calcd. C 39.70, H 5.65, N 3.56; found C 39.76, H 5.88, N 3.59.

6-*O*-(*N*-*tert*-Butyloxycarbonyl-L-phenylalanyl-L-valyl)-D-glucopyranose (8): The TFA salt of compound **7** (1.96 g, 5 mmol) in dry DMF (10 mL) was neutralized by NMM (0.5 mL, 5 mmol), and to this solution Boc-Phe-OH (1.33 g, 5 mmol), TBTU (1.82 g, 5.5 mmol), HOBt (650 mg, 5 mmol), and NMM (0.5 mL, 5 mmol) were added. After stirring overnight at room temperature, the solvent was removed in vacuo and the residue purified by FCC (EtOAc/AcOH/H_2O 70:2:2) to yield, after crystallization from EtOAc/hexane, pure **8** (1.20 g, 40%), m.p. 135−140 °C (decomp.). − $[\alpha]_D$ = +16.0 (*c* = 1, MeOH). − $C_{25}H_{38}N_2O_{10}{\cdot}2\,H_2O$ (562.57): calcd. C 53.37, H 7.54, N 4.98; found C 53.50, H 7.31, N 5.10.

6-*O*-(L-Phenylalanyl-L-valyl)-D-glucopyranose (9): Compound **9** was obtained from the *N*-protected monosaccharide ester **8** (850 mg, 1.61 mmol) by using the same procedure as described for **2**. Precipitation by diisopropyl ether afforded **9**·TFA (710 mg, 80%), m.p. 130−135 °C (decomp.). − $[\alpha]_D$ = +15.5 (*c* = 1, MeOH). − $C_{20}H_{30}N_2O_8{\cdot}CF_3COOH$ (540.42): calcd. C 48.89, H 5.79, N 5.18; found C 48.53, H 6.04, N 5.38.

6-*O*-(*N,O*-bis-*tert*-Butyloxycarbonyl-L-tyrosyl-L-prolyl-L-phenylalanyl-L-valyl)-D-glucopyranose (10): Compound **10** was synthe-

sized from the ester **9** (710 mg, 1.3 mmol) and Boc-Tyr(Boc)-Pro-OH (620 mg, 1.3 mmol) as described above for the synthesis of compound **4**. Purification by FCC (EtOAc/AcOH/H_2O 70:2:2) and crystallization of the product from EtOAc/hexane afforded the title compound **10** (720 mg, 60%), m.p. 135−140 °C (decomp.). − $[\alpha]_D$ = −14.5 (*c* = 1, MeOH). − ^{13}C NMR ([D$_6$]DMSO): δ = 18.2, 19.1 (Val γ,γ′), 21.1 (*cis*Pro γ), 24.4 (*trans*Pro γ), 27.3 (OBoc CH_3), 28.2 (NHBoc CH_3), 28.8 (Pro β), 30.2 (Val β), 35.7 (Tyr β), 37.3 (Phe β), 46.8 (Pro δ), 53.5 (*trans*Phe α), 53.6 (*cis*Phe α), 53.9, 54.1 (Tyr α), 57.4 (Val α), 59.6 (*trans*Pro α), 60.0 (*cis*Pro α), 64.6 (Glc C-6), 69.2 (αGlc C-5), 70.3 (βGlc C-4), 70.7 (αGlc C-4), 72.3 (αGlc C-2), 73.1 (αGlc C-3), 73.6 (βGlc C-5), 74.9 (βGlc C-2), 76.6 (βGlc C-3), 78.2 (NHBoc C), 83.2 (OBoc C), 92.5 (αGlc C-1), 97.2 (βGlc C-1), 121.2 (*trans*Tyr ε), 121.4 (*cis*Tyr ε), 126.4 (Phe ε), 128.2 (Phe δ), 129.2 (*cis*Phe ζ), 129.5 (*trans*Phe ζ), 130.6, 130.7 (Tyr δ), 136.0 (Tyr γ), 137.9 (Phe γ), 149.5, 151.6 (*trans*Boc CO), 149.8, 151.5 (*cis*Boc CO), 155.7 (Tyr ζ), 170.8, 171.6, 171.7 (Tyr, Pro, Phe, Val CO); anomer ratio α/β = 60:40; *cis*/*trans* = 20:80. − $C_{44}H_{62}N_4O_{15}$·3 H_2O (940.98): calcd. C 56.16, H 7.30, N 5.96; found C 56.42, H 7.44, N 5.32.

6-*O*-(L-Tyrosyl-L-prolyl-L-phenylalanyl-L-valyl)-D-glucopyranose (11): Protected ester **10** (500 mg, 0.56 mmol) was deprotected as described for compound **2** to give pure **11**·TFA (370 mg, 80%), m.p. 145−148 °C (decomp.). − $[\alpha]_D$ = −0.5 (*c* = 1, MeOH). − ^{13}C NMR ([D$_6$]DMSO): δ = 18.2, 19.1 (Val γ,γ′), 21.7 (*cis*Pro γ), 24.5 (*trans*Pro γ), 29.2 (*trans*Pro β), 30.3 (Val β), 31.5 (*cis*Pro β), 35.6 (*trans*Tyr β), 36.4 (*cis*Tyr β), 37.2 (*cis*Phe β), 37.7 (*trans*Phe β), 46.9 (Pro δ), 53.6 (Phe α), 52.7 (Tyr α), 57.4 (*trans*Val α), 57.6 (*cis*Val α), 59.3 (*cis*Pro α), 59.7 (*trans*Pro α), 64.6 (Glc C-6), 69.3 (αGlc C-5), 70.4 (βGlc C-4), 70.7 (αGlc C-4), 72.4 (αGlc C-2), 73.1 (αGlc C-3), 73.7 (βGlc C-5), 74.9 (βGlc C-2), 76.7 (βGlc C-3), 92.6 (αGlc C-1), 97.3 (βGlc C-1), 115.6 (*trans*Tyr ε), 115.7 (*cis*Tyr ε), 124.3 (*cis*Tyr γ), 124.9 (*trans*Tyr γ), 126.5 (*trans*Phe ζ), 126.7 (*cis*Phe ζ), 128.3 (*trans*Phe ε), 128.4 (*cis*Phe ε), 129.3 (*cis*Phe δ), 130.62 (*trans*-Phe δ), 130.65 (*cis*Tyr δ), 131.2 (*trans*Tyr δ), 137.8 (*trans*Phe γ), 138.0 (*cis*Phe γ), 157.0 (*trans*Tyr ζ), 157.1 (*cis*Tyr ζ), 167.2, 167.4, 170.8, 171.1, 171.6, 171.8, 172.1 (Tyr, Pro, Phe, Val CO); anomer ratio α/β = 55:45; *cis*/*trans* = 30:70. − $C_{34}H_{46}N_4O_{11}$·CF_3COOH·3 H_2O (854.74): calcd. C 50.58, H 6.26, N 6.55; found C 49.73, H 5.99, N 6.03. − Desalting was performed as described for monosaccharide ester **2**.

***cyclo*-(Tyr-Pro) (12):** Monosaccharide ester **2** (50 mg, 0.11 mmol) was dissolved in dry pyridine/acetic acid (1:1, 50 mL), and the solution was kept in a closed round-bottomed flask for 24 h at 50 °C. The solvent was evaporated and the residue was purified by semi-preparative RP HPLC, using 40% MeOH/0.1% TFA as the eluent, to give diketopiperazine **12** (retention time 11.8 min), which was crystallized from CH_2Cl_2/petroleum ether (22 mg, 77%), m.p. 150−152 °C (ref.[24] m.p. 152−154 °C). − $[\alpha]_D$ = −79.5 (*c* = 1, MeOH).

***cyclo*-{*N*-[-6)-1-Deoxy-β-D-glucopyranos-1-yl]-L-tyrosyl-L-prolyl-L-phenylalanyl-(1→*O*} (13):** Tripeptide derivative **5** (50 mg, 0.08 mmol) was dissolved in dry pyridine/acetic acid (1:1, 50 mL) and the solution was kept in a closed round-bottomed flask for 24 h at 37 °C. The solvent was evaporated and the residue was purified by semi-preparative RP HPLC, using 40% MeOH/0.1% TFA as the eluent, to yield pure compound **13** as the trifluoroacetate salt (25 mg, 52%) (retention time 21.0 min), m.p. 160−165 °C (decomp.). − $[\alpha]_D$ = −50.0 (*c* = 1, MeOH). − ^{13}C NMR ([D$_6$]DMSO): δ = 20.9 (Pro γ), 31.8 (Pro β), 34.9 (Phe β), 36.2 (Tyr β), 46.0 (Pro δ), 53.6 (Phe α), 55.7 (Tyr α), 58.5 (Pro α), 63.4 (Glc C-6), 70.2 (Glc C-4), 71.7 (Glc C-2), 76.3, 76.5 (Glc C-3,5), 85.4 (Glc C-1), 115.3 (Tyr ε), 124.2 (Tyr γ), 126.3 (Phe ζ), 128.2 (Phe ε), 129.2 (Phe δ), 130.4 (Tyr δ), 138.4 (Phe γ), 156.5 (Tyr ζ), 166.1, 170.2, 170.3 (Tyr, Pro, Phe CO). − $C_{29}H_{35}N_3O_9$·CF_3COOH·3 H_2O (737.59): calcd. C 50.48, H 5.75, N 5.70; found C 50.77, H 5.42, N 5.34.

***N*-(1-Deoxy-D-fructos-1-yl]-L-tyrosyl-L-prolyl-L-phenylalanine (14):** To a solution of D-glucose (21 mg, 0.12 mmol) in dry pyridine/acetic acid (1:1, 25 mL) tripeptide Tyr-Pro-Phe (50 mg, 0.12 mmol) was added. The solution was kept for 2 d at 37 °C. After removal of the solvent, the residue was purified by semi-preparative RP HPLC, using 36.7% MeOH/0.1% TFA as the eluent, to provide pure compound **14** (retention time 14.7 min), which was crystallized from MeOH/diisopropyl ether (18 mg, 26%), m.p. 185−190 °C (decomp.). − $[\alpha]_D$ = −22.5 (*c* = 1, MeOH). − ^{13}C NMR (D_2O): δ = 21.1 (*cis*Pro γ), 24.0 (*trans*Pro γ), 28.5 (*trans*Pro β), 31.1 (*cis*Pro β), 34.2 (*trans*Tyr β), 35.4 (*cis*Tyr β), 35.9 (Phe β), 47.2 (*cis*Pro δ), 47.4 (*trans*Pro δ), 50.7 (α-*f*Fru C-1), 51.6 (β-*f*Fru C-1), 52.9 (β-*p*Fru C-1), 53.9 (*trans*Phe α), 56.5 (*cis*Phe α), 59.0 (Pro α), 59.8 (α-*f*Fru C-6), 60.1 (β-*f*Fru C-6), 60.5 (*cis*Tyr α), 60.6 (*trans*Tyr α), 63.5 (β-*p*Fru C-6), 68.3, 68.5 (β-*p*Fru C-5), 68.76, 68.82 (β-*p*Fru C-3), 69.1, 69.5 (β-*p*Fru C-4), 71.7 (α-*f*Fru C-4), 75.0 (β-*f*Fru C-4), 77.5 (β-*f*Fru C-3), 80.8 (β-*f*Fru C-5), 81.8 (α-*f*Fru C-5), 94.7, 94.8 (β-*p*Fru C-2), 98.2 (β-*f*Fru C-2), 100.9 (α-*f*Fru C-2), 115.4 (*trans*Tyr ε), 115.6 (*cis*Tyr ε), 124.1 (Tyr γ), 126.8 (*trans*Phe ζ), 127.0 (*cis*Phe ζ), 128.4 (Phe ε), 128.8 (*trans*Phe δ), 128.9 (*cis*Phe δ), 130.4 (*cis*Tyr δ), 130.9 (*trans*Tyr δ), 136.0 (*trans*Phe γ), 137.0 (*cis*Phe γ), 154.8 (*trans*-Tyr ζ), 155.1 (*cis*Tyr ζ), 166.2, 171.7, 172.5, 174.0, 174.3 (Tyr, Pro, Phe CO); anomer ratio β-*p*/β-*f*/α-*f* = 74:12:14; *cis*/*trans* = 70:30. − $C_{29}H_{37}N_3O_{10}$·CF_3COOH (701.57): calcd. C 53.21, H 5.47, N 5.99; found C 53.08, H 5.70, N 6.20.

***cyclo*-{*N*-[-6)-1-Deoxy-β-D-fructofuranos-1-yl]-L-tyrosyl-L-prolyl-L-phenylalanyl-L-valyl-(1→*O*} (15):** Tetrapeptide monosaccharide ester **11** (100 mg, 0.14 mmol) was dissolved in dry pyridine/acetic acid (1:1, 50 mL) and the solution was incubated in a closed round-bottomed flask for 24 h at room temperature. The solvent was evaporated and the residue was purified by semi-preparative RP HPLC, using 45.2% MeOH/0.1% TFA as the eluent, to give fractions enriched with **15** (retention time 21.4 min). Repeated RP HPLC purification with 40% MeOH/0.1% TFA as the eluent gave pure compound **15**, which was crystallized from MeOH/diisopropyl ether (30 mg, 30%), m.p. 135−140 °C (decomp.). − $[\alpha]_D$ = −3.5 (*c* = 1, MeOH). ^{13}C NMR ([D$_6$]DMSO): δ = 18.9, 19.1 (Val γ,γ′), 21.0 (Pro γ), 28.7 (Val β), 30.9 (Pro β), 34.1 (Tyr β), 35.5 (Phe β), 46.8 (Pro δ), 49.8 (Fru C-1), 53.0 (Phe α), 59.4 (Fru C-6), 59.2, 59.7, 60.0 (Tyr, Pro, Val α), 74.1 (Fru C-4), 78.1 (Fru C-5), 83.3 (Fru C-3), 100.4 (Fru C-2), 115.6 (Tyr ε), 123.6 (Tyr γ), 126.2 (Phe ζ), 128.0 (Phe ε), 128.8 (Phe δ), 130.5 (Tyr δ), 138.3 (Phe γ), 156.9 (Tyr ζ), 166.2, 170.5, 171.2, 171.4 (Tyr, Pro, Phe, Val CO). − $C_{34}H_{44}N_4O_{10}$·CF_3COOH (782.70): calcd. C 55.24, H 5.81, N 7.16; found C 55.10, H 5.88, N 7.34.

***N*-(1-Deoxy-β-D-fructopyranos-1-yl)-L-tyrosyl-L-prolyl-L-phenylalanyl-L-valine (16):** Bicyclic Amadori compound **15** (45 mg, 0.06 mmol) was dissolved in NH_4OH (0.1 M, 10 mL) and the solution was stirred for 8 h at room temperature. The solvent was removed in vacuo, and the residue was purified by RP HPLC, using 45.2% MeOH/0.1% TFA as the eluent, to yield pure compound **16** (retention time 13.3 min), which was crystallized from MeOH/diisopropyl ether (10 mg, 22%), m.p. 173−178 °C (decomp.). − $[\alpha]_D$ = −28.0 (*c* = 0.8, MeOH). − ^{13}C NMR (D_2O): δ = 17.5, 17.7 (*trans*Val γ,γ′), 18.6, 18.7 (*cis*Val γ,γ′), 21.9 (*cis*Pro γ), 24.8 (*trans*Pro γ), 29.3 (*trans*Pro β), 30.5 (*cis*Val β), 30.6 (*trans*Val β), 32.2 (*cis*Pro β), 35.0 (*trans*Tyr β), 36.2 (*cis*Tyr β), 37.26 (*trans*Phe

β), 37.34 (*cis*Phe β), 48.0 (*cis*Pro δ), 48.2 (*trans*Pro δ), 52.5 (*trans*-Fru C-1), 52.9 (*cis*Fru C-1), 55.5 (*cis*Phe α), 55.7 (*trans*Phe α), 59.8 (*cis*Pro α), 60.7 (*trans*Tyr α), 60.9 (*cis*Tyr α), 61.4 (*trans*Pro α), 64.3 (*trans*Fru C-6), 64.4 (*cis*Fru C-6), 69.1 (*trans*Fru C-5), 69.3 (*cis*Fru C-5), 69.56 (*trans*Fru C-4), 69.64 (*cis*Fru C-4), 69.9 (*cis*Fru C-3), 70.3 (*trans*Fru C-3), 95.5 (*cis*Fru C-2), 95.6 (*trans*Fru C-2), 116.2 (*trans*Tyr ε), 116.5 (*cis*Tyr ε), 124.9 (Tyr γ), 127.6 (*cis*Phe ζ), 127.9 (*trans*Phe ζ), 129.2 (*trans*Phe ε), 129.3 (*cis*Phe ε), 129.6 (*cis*Phe δ), 129.7 (*trans*Phe δ), 131.2 (*cis*Tyr δ), 131.7 (*trans*Tyr δ), 136.4 (*trans*-Phe γ), 137.3 (*cis*Phe γ), 155.7 (*trans*Tyr ζ), 155.9 (*cis*Tyr ζ), 166.9, 167.0, 172.4, 173.0, 173.1, 173.2, 174.9, 175.2 (Tyr, Pro, Phe, Val CO); *cis*/*trans* = 60:40. − $C_{34}H_{46}N_4O_{11}$·CF_3COOH (800.71): calcd. C 54.00, H 5.93, N 7.00; found C 53.18, H 5.83, N 6.76.

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