

Synthesis of Novel Tetrahydropyran-Based Dipeptide Isomers by Overman Rearrangement of 2,3-Didehydroglycosides

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Differently functionalized tetrahydropyran-based dipeptide isomers have been efficiently synthesized from 3,4,6-tri-*O*-acetyl-D-glucal. Analogues of the hsp65 p2–13 epitope of *Mycobacterium tuberculosis* and *Mycobacterium leprae* were prepared by replacement of the Ala–Tyr or Glu–Glu

moiety in the native dodecapeptide with the prepared dipeptide isomers.

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Introduction

Oligopeptides from natural sources form an important source of lead compounds for the development of new therapeutics. Important examples include the neuropeptides Leu- and Met-enkephalin,^[1] natural analgesics that exert their action through binding to the opioid receptor. Other oligopeptidic leads with therapeutic potential are represented by a variety of antigenic peptides, oligopeptides that modulate immune responses to bacterial or viral infections. Inappropriate antigen presentation can result in the development of (auto)immune diseases. Disturbance of the processing and presentation of selected antigenic peptides, which may be achieved by the application of modified analogues of peptides, is widely accepted as a promising strategy for the treatment of (auto)immune diseases, including type I diabetes, celiac disease, and multiple sclerosis.^[2–4]

A major drawback of the use of unmodified, naturally occurring oligopeptides in therapeutic applications is their intrinsic instability towards proteolytic activities. In this respect, the development of synthetic peptide-like compounds with enhanced physiological half-lives has attracted wide attention. One area of research comprises the development of analogues of peptide linkages, such as peptoids,^[5] sulfonamides,^[6] and olefins.^[7] A second approach entails the replacement of selected amino acid residues by so-called pep-

tide isomers, synthetic molecules that incorporate structural (e.g., conformational) and functional (e.g., amino acid side chain entities) elements of the native peptide in an essentially non-peptidic structure.^[8] In the context of the latter approach we have recently reported on the transformation of carbohydrate derivatives into peptide-like building blocks and their incorporation in a variety of linear and cyclic oligopeptides.^[9–14] As part of these research efforts, we disclosed the facile synthesis of a set of tetrahydropyran-based 2,6-*cis*- and *trans*-disposed peptide isomers **3** (Figure 1) and their incorporation in novel protein:farnesyltransferase^[9] and protein:geranylgeranyltransferase^[10] inhibitors. The key step in the synthetic sequence employed was the treatment of tri-*O*-acetyl-D-glucal **1** with trimethylsilyl cyanide in the presence of BF₃·Et₂O. Ensuing functional group manipulations of the resulting Ferrier rearrangement product **2** afforded dipeptide isomers **3**, possessing an additional hydroxy function attached to the tetrahydropyran core. We reasoned that utilization of this hydroxy function, which is also present in Ferrier rearrangement product **2** as the corresponding allylic alcohol, would give rise to a new set of tetrahydropyran-based dipeptide isomers **4**.

Here we report the synthesis of the four new Fmoc-protected, tetrahydropyran-based 2,5-*cis*- and 2,5-*trans* dipeptide isomers **5–8**. Key transformations en route to the target compounds entail the Ferrier rearrangement of tri-*O*-acetyl-D-glucal with different nucleophiles followed by Mitsunobu-mediated inversion of the secondary alcohol in **2** (in the cases of **7** and **8**) and final installment of the secondary amine through Overman rearrangement. The utility of dipeptide isomers **5–8** in solid-phase peptide synthesis is demonstrated by the preparation of analogues of antigenic

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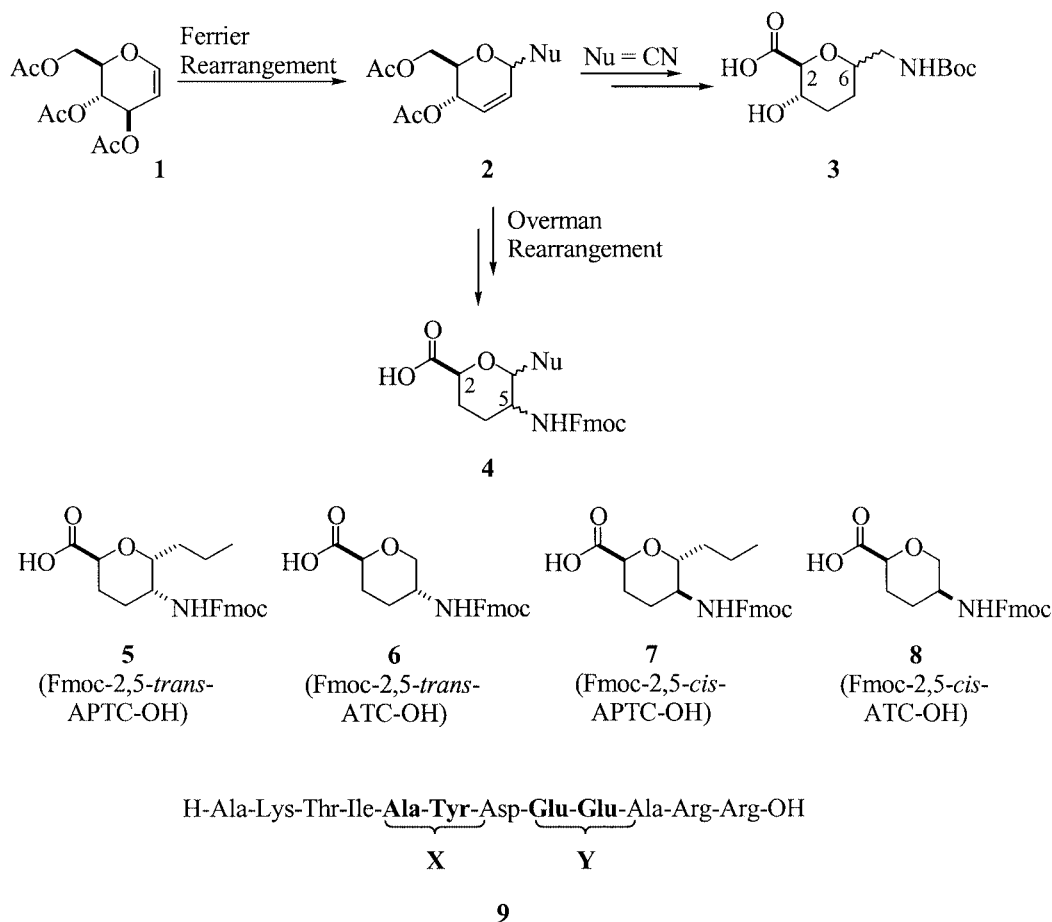


Figure 1. Schematic presentation of the synthesis of 2,6- and 2,5-disubstituted dipeptide isomers **3** and **4**, respectively, from tri-*O*-acetyl-D-glucal **1**; the novel dipeptide isomers **5–8** and antigenic peptide **9** from heat shock protein 65 of *Mycobacterium tuberculosis* and *Mycobacterium leprae*.

peptide **9**,^[15] originating from heat shock protein 65 (hsp65) from *Mycobacterium tuberculosis* and *Mycobacterium leprae*, in which either the Ala–Tyr or the Glu–Glu dipeptide is substituted.

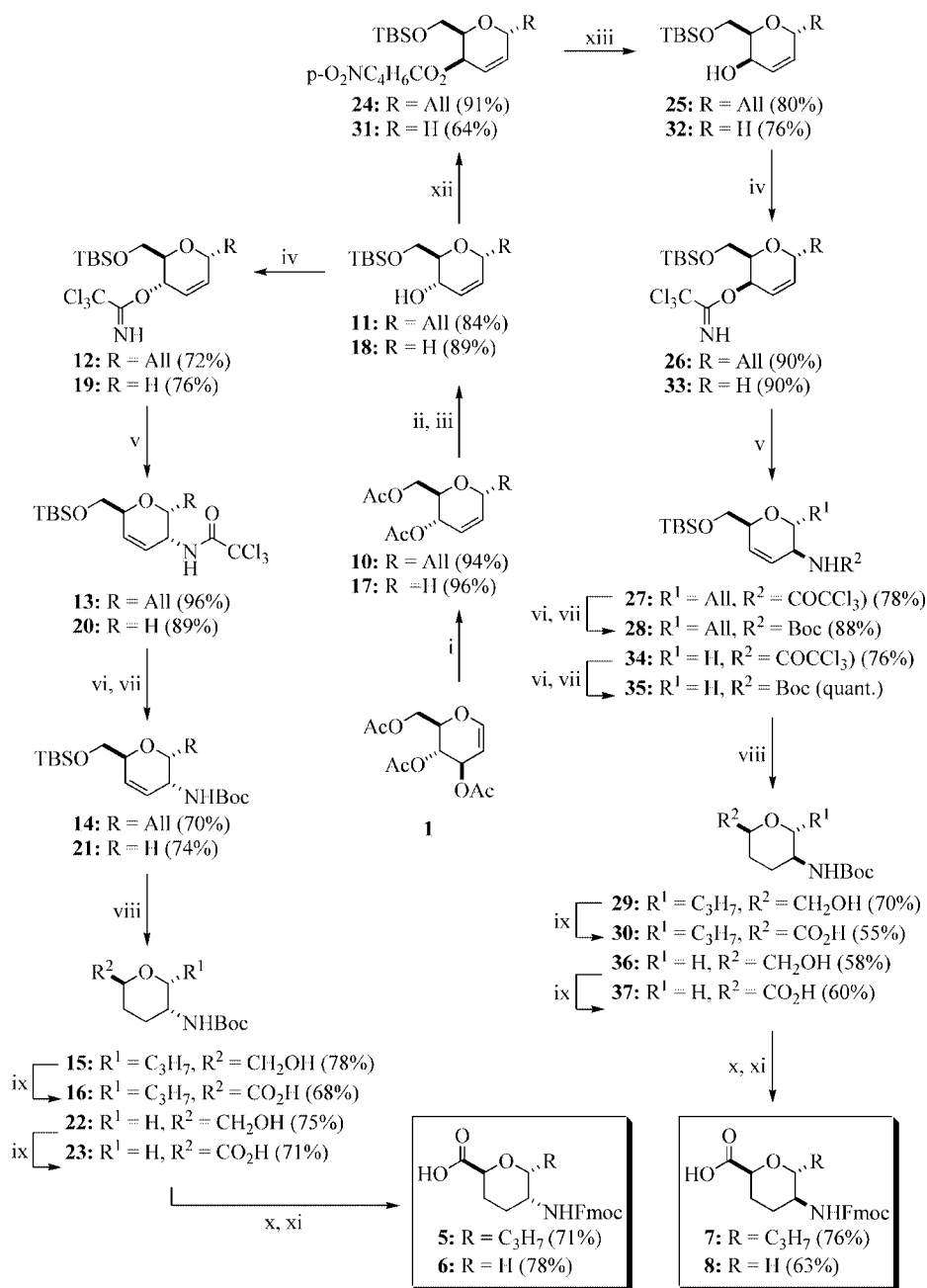
Results and Discussion

As the first research objective, the synthesis of 2,5-*trans* dipeptide isomer **5**, with a C-6 propyl functionality attached to the pyran core, was undertaken as follows. Ferrier rearrangement^[16] of tri-*O*-acetyl-D-glucal **1** (Scheme 1) with (allyl)trimethylsilane (All–TMS) in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ afforded *C*-glycoside **10**, which was converted into the known^[17] *C*-glycoside **11** by standard protective group manipulations (deacetylation, followed by selective protection of the primary alcohol as the *tert*-butyldimethylsilyl ether) in good yield. After formation of the trichloroacetimidate (DBU, trichloroacetonitrile, **11** to **12**), the installation of the secondary amine functionality was now effected by heating **12** at reflux in 1,2-dichlorobenzene in the presence of K_2CO_3 ,^[18] to give **13** through a [3,3]-sigmatropic Overman rearrangement.^[19] Deacylation of **13** (KOH, isopropanol)^[20] was followed by treatment of the

resulting amine with di-*tert*-butyl dicarbonate to give Boc-protected **14**. Hydrogenation of the olefins in **14** was accompanied by cleavage of the silyl ether^[21] to afford alcohol **15**, oxidation of which to the corresponding acid **16** was effected by treatment with a catalytic amount of ruthenium(III) chloride in the presence of sodium periodate.^[22] Subjection of Boc-protected **16** to 50% TFA in DCM and subsequent treatment with Fmoc–OSu gave the desired (2*S*,5*R*,6*R*)-5-[*N*-(9-fluorenylmethoxycarbonyl)amino]-6-propyl-tetrahydropyran-2-carboxylic acid (**5**) in 14% overall yield based on **1**.

(2*S*,5*R*)-5-[*N*-(9-Fluorenylmethoxycarbonyl)amino]-tetrahydropyran-2-carboxylic acid (**6**), with a hydrogen at the C-6 position, was prepared by a similar synthetic strategy. Application of triethylsilane as the incoming nucleophile in the Lewis acid-mediated Ferrier rearrangement of **1** furnished derivative **17** in 96% yield. Conversion of **17** into **20**^[23] and ensuing transformations into the target dipeptide isomer **6** went uneventfully (18% overall yield based on **1**), following the sequence of transformations as outlined for the synthesis of **5**.

Having accomplished the synthesis of 2,5-dipeptide isomers **5** and **6**, we set out on the preparation of the corre-

Scheme 1. Synthesis of dipeptide isomers **5**, **6**, **7** and **8**

Reagents and conditions: (i) BF₃·Et₂O, All-TMS (**10**) or triethylsilane (**17**), CH₂Cl₂, 0 °C; (ii) *t*BuOK, MeOH; (iii) TBS-Cl, pyridine; (iv) Cl₃CCN, DBU, CH₂Cl₂, 0 °C; (v) K₂CO₃, 1,2-DCB, Δ; (vi) KOH (3 equiv.), *i*PrOH; (vii) Boc₂O (1.2 equiv.), CH₂Cl₂; (viii) H₂, cat. Pd/C, MeOH; (ix) cat. RuCl₃·xH₂O, NaIO₄ (4.5 equiv.), CH₂Cl₂/H₂O/CH₃CN (1:1.5:1, v/v/v), 0 °C → room temp.; (x) TFA/CH₂Cl₂ (1:1, v/v); (xi) Fmoc-OSu (1.2 equiv.), 10% aq. NaHCO₃/1,4-dioxane (1:1, v/v), 0 °C → room temp.; (xii) *p*-NO₂-C₆H₄CO₂H, DEAD, PPh₃, THF; (xiii) KOH (1.5 equiv.), *i*PrOH.

sponding C-5 epimeric 2,5-*cis* dipeptide isomers **7** and **8**, respectively. We envisaged that the introduction of the C-5 epimeric amino functionality should be achievable by application of 2,3-anhydroglycosides **25** and **32** (that is, with the *D*-galacto instead of the *D*-gluco configuration) in the stereoselective Overman rearrangement reaction. In theory, these intermediates should be accessible by performing the Ferrier rearrangement of tri-*O*-acetyl-*D*-galactal. However, this process is known^[24] to be highly inefficient, both in

terms of yield and of stereoselectivity. We therefore elected to follow an alternative route, based on the Mitsunobu-mediated epimerization of intermediates **11** and **18**. As an example, treatment of **11** with *p*-nitrobenzoic acid under Mitsunobu conditions (DEAD, PPh₃, THF) furnished ester **24**, deacylation of which afforded alcohol **25**. Through the use of the same sequence of reactions as described for the transformation of alcohol **11** into **5**, the 2,5-*cis* dipeptide isomer **7** was obtained in 13% overall yield based on **11**.

The preparation of the hydrogen counterpart **8** was accomplished in a similar fashion. In this case, subsection of alcohol **18** to Mitsunobu reaction conditions at 0 °C resulted in the formation of an inseparable side product, possibly originating from S_N2' attack at the alkene in **18**. The amount of side product formed could be minimized by performing the reaction at −30 °C and by using only a small excess of the reagents. Deacylation of **31** resulted in the isolation of the desired allylic alcohol **32**, the configuration of which was ascertained by NOESY NMR analysis. The 2,5-*cis* dipeptide isomer **8** was obtained from **32** in 7% overall yield (based on **18**) by means of a sequence of reactions corresponding to those described for the conversion of alcohol **18** into **6**.

As the next research objective, the application of dipeptide isomers 2,5-*trans*-**5** and -**6** and 2,5-*cis*-**7** and -**8** in the solid-phase peptide synthesis of peptidomimetics **41–46** was undertaken (Scheme 2). Wang resin **38** was loaded with Fmoc-Arg(Pbf)-OH in the presence of *N,N'*-dicyclohexylcarbodiimide (DCC) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP), affording immobilized arginine derivative **39**. Deprotection of the amine function in **39**, followed by condensation of the appropriate amino acids with (benzotriazol-1-yl)oxy-tris(dimethylamino) phosphonium hexafluorophosphate (BOP) in the presence of 1-hydroxybenzotriazole (HOBt) and *N,N*-diisopropylethylamine (DiPEA) gave immobilized 5-(amino)tetrahydropyran-2-carboxylic acid-containing peptides **40a–c** (replacement of the Ala-Tyr dipeptide) and **40d–f** (replacement of Glu-Glu dipeptide). In all examples, the respective dipeptide isomers were incorporated uneventfully by means of the standard Fmoc-based solid-phase peptide synthesis procedures

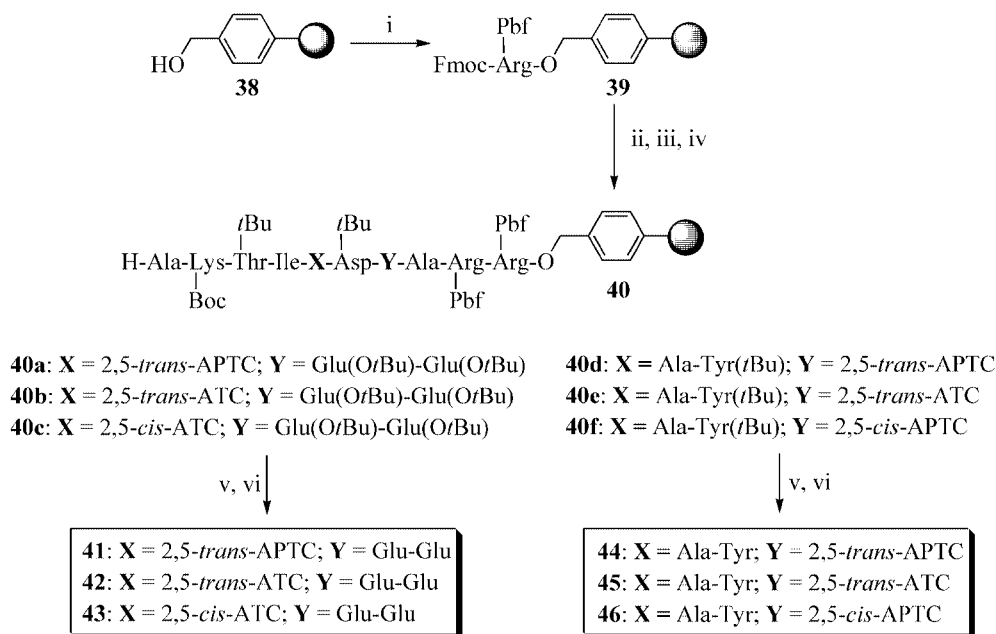
used.^[25] Cleavage of the immobilized modified peptides **40a–f** from the resin and simultaneous deblocking of the side chain protecting groups was accomplished by acidolysis. The obtained crude modified peptides **41–46** were purified by RP HPLC to give pure **41** (24%), **42** (26%), **43** (25%), **44** (22%), **45** (28%), and **46** (16%), as indicated by LC/MS analysis.

Conclusion

This paper describes the concise synthesis of 5-[*N*-(9-fluorenylmethoxycarbonyl)amino]tetrahydropyran-2-carboxylic acids 2,5-*trans*-**5** and -**6** and 2,5-*cis*-**7** and -**8** as suitably protected building blocks for the incorporation of novel dipeptide isomers in oligopeptides. The application of the Ferrier rearrangement allows the installment of different carbon, nitrogen, and oxygen functionalities, providing for a great variety of substituents at the C-6 of the pyran core. The building blocks **5–8** were effectively applied in the solid-phase assembly of modified peptides **41–46**, the design of which was based on the replacement of either dipeptide Ala-Tyr or Glu-Glu in the antigenic peptide **9**. The ability of peptides **41–46** to arrest hsp65-specific T cell response is currently being investigated and will be reported in due course.

Experimental Section

General Methods and Materials: Acetonitrile (Rathburn, HPLC grade), 1,2-dichloroethane (1,2-DCE), *i*PrOH, 1,4-dioxane, and



Scheme 2. Solid-phase synthesis of glycopeptides **41–46**

Reagents and conditions: (i) Fmoc-Arg(Pbf)-OH (3 equiv.), DCC (3.3 equiv.), DMAP (5 mol %), room temp., 2 h; (ii) repeat (10 ×) the following sequence: a. 20% piperidine in NMP, room temp., 5 min, b. Fmoc-AA-OH or building blocks **5–8** (5 equiv.), BOP (5 equiv.), HOBt (5 equiv.), DiPEA (10 equiv.), NMP, room temp., for amino acids: 1 h and for building blocks **5–8**: 2 h, c. Ac₂O/DiPEA/HOBt/NMP, room temp., 1 min; (iii) 20% piperidine in NMP, room temp., 5 min; (iv) TFA/TIS/H₂O (95:2.5:2.5, v/v/v), room temp., 2 h.

DCM (all Baker, p.a.) were stored over molecular sieves (4 Å). HPLC grade methanol (Biosolve) was stored over molecular sieves (3 Å). Solvents and reagents used in the solid-phase peptide synthesis were of peptide synthesis grade (Biosolve) and were used as received. Hyflo, di-*tert*-butyl dicarbonate, *N*-9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide (Fmoc-Osu), and *p*-nitrobenzoic acid were bought from Fluka and used as such. Anhydrous 1-hydroxybenzotriazole (HOBt) was purchased from Neosystem laboratoires (France), and (benzotriazol-1-yl)oxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) was obtained from Senn Chemicals. *p*-Benzyloxybenzyl alcohol resin (Wang resin) and the protected amino acids were bought from NovaBiochem (Switzerland). All other reagents were purchased from Acros. Reactions were performed under anhydrous conditions at room temperature unless otherwise stated. TLC analyses were performed on Merck plastic 60 F₂₅₄ silica gel sheets. Detection by UV (254 nm) was followed by spraying with one of the following solutions: (a) ammonium molybdate (25 g L⁻¹) and cerium(IV) sulfate (10 g L⁻¹) in 10% aq. sulfuric acid followed by charring, (b) aq. KMnO₄ (2% in aq. 1% K₂CO₃), or (c) 3% ninhydrin in ethanol, followed by charring. Column chromatography was performed with Fluka silica gel (230–400 mm mesh). Solvents used for column chromatography were of technical grade and were distilled prior to use. ¹H and ¹³C NMR spectra were recorded with a Bruker AC 200 instrument. Chemical shifts (delta) are given in ppm relative to TMS as an internal standard for ¹H NMR and ¹³C NMR spectroscopy. The HPLC purifications were performed on a BioCAD “Vision” automated HPLC system with an Alltima C₁₈ column (Alltech, 10.0 mm D × 250 mm L, 5 μ particle size) with use of the following buffers: A: H₂O, B: CH₃CN, and C: 1% aq. TFA. The system was eluted at 4.0 mL min⁻¹ and detection was performed at 214 nm unless stated otherwise. LC/MS analysis was performed on a Jasco HPLC system (detection simultaneously at 214 and 254 nm). An analytical Alltima C₁₈ column (Alltech, 4.0 mm D × 250 mm L, 5 μ particle size) was used. Buffers: A: H₂O, B: CH₃CN, and C: 0.5% aq. TFA, linear gradient 5 → 50% B in 20 min. Mass spectra were recorded with a Sciex API 165 mass instrument equipped with a custom-made Electrospray (ESI).

(2R,3S,6R)-3-*O*-Acetyl-2-(acetyloxymethyl)-6-allyl-3,6-dihydro-2H-pyran (10): Boron trifluoride–diethyl ether (3.8 mL, 30 mmol) was added dropwise to a cooled (–30 °C), stirred solution of 3,4,6-tri-*O*-acetyl-D-glucal (8.2 g, 30 mmol, dried by co-evaporation with 1,2-DCE, 2 × 15 mL) and (allyl)trimethylsilane (3 mL, 36 mmol) in DCM (100 mL). After TLC analysis showed the reaction to be complete, the reaction mixture was quenched by addition of NaHCO₃ (10%, 30 mL) and EtOAc (150 mL). The layers were separated, and the organic layer was washed with water and brine, dried (MgSO₄), filtered, and concentrated. Purification (silica gel, toluene/EtOAc, 100:0 → 75:25, v/v) afforded **10** (7.7 g, 94%) as a colorless oil. ¹³C NMR (CDCl₃): δ = 169.7 (2 × C=O acetyl), 132.3, 131.7 (C-4, C-5), 123.4 (=CH All), 116.9 (CH₂ All), 70.8, 62.9, 64.5 (C-2, C-3, C-6), 62.4 (CH₂O), 37.3 (CH₂ All), 20.1 (CH₃ acetyl) ppm. ¹H NMR (CDCl₃): δ = 5.92–5.71 (br. m, 3 H, 4-H, 5-H, =CH All), 5.14 (m, 2 H, =CH₂ All), 4.22 (m, 4 H, 2-H, 6-H, CH₂O), 3.90 (m, 1 H, 3-H), 2.37 (m, 2 H, CH₂ All), 2.01 (s, 6 H, 2 × CH₃ acetyl) ppm.

(2R,3S,6R)-6-Allyl-2-[(*tert*-butyldimethylsilyloxy)methyl]-3,6-dihydro-3-hydroxy-2H-pyran (11): Ferrier rearrangement product **10** (0.7 g, 4.3 mmol) was dissolved in MeOH (25 mL), *t*BuOK (25 mg) was added, and the reaction mixture was stirred for 2 h, after which TLC analysis (toluene/EtOAc, 1:1, v/v) showed the reaction to be complete. The reaction mixture was neutralized with DOWEX

(H⁺-form), filtered, and concentrated in vacuo. The crude diol (co-evaporated with pyridine) was dissolved in pyridine (20 mL) and the solution was cooled to 0 °C. A solution of *tert*-butyl(dimethyl)silyl chloride (0.65 g, 4.3 mmol) in pyridine (10 mL) was added dropwise, and the reaction mixture was stirred overnight and concentrated to dryness. The residue was dissolved in EtOAc (150 mL) and washed with NaHCO₃ (10%) and brine. The organic phase was dried (MgSO₄), filtered, and concentrated. Purification by silica gel chromatography (toluene/EtOAc, 100:0 → 90:10, v/v) afforded title compound **11** (1.0 g, 84%) as an oil. ¹³C NMR (CDCl₃): δ = 134.3, 129.7, 128.1 (C-4, C-5, =CH All), 117.0 (=CH₂ All), 71.8, 66.1, 65.1 (C-2, C-3, C-6), 64.4 (CH₂O), 37.7 (CH₂ All), 25.6 [(CH₃)₃C], 18.1 [(CH₃)₃CSi], –5.5 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 5.95–5.74 (m, 3 H, 4-H, 5-H, =CH All), 5.11 (m, 2 H, =CH₂ All), 4.15 (m, 2 H, 2-H, 3-H), 3.90 (m, 1 H, 6-H), 3.81 (m, 2 H, CH₂O), 2.58–2.26 (m, 2 H, CH₂ All), 0.89 [s, 9 H, (CH₃)₃C], 0.06 [s, 6 H, (CH₃)₂Si] ppm.

(2R,3S,6R)-6-Allyl-2-[(*tert*-butyldimethylsilyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2H-pyran (12): Alcohol **11** (4.8 g, 16.9 mmol) was coevaporated with 1,2-DCE (2 × 50 mL) and dissolved in DCM (70 mL). Under nitrogen, the solution was cooled to 0 °C and DBU (3.2 mL, 21.6 mmol) and Cl₃CCN (2.2 mL, 22.2 mmol) were added. After the mixture had been stirred for 1 hour, TLC analysis (EtOAc/toluene, 5:95, v/v) showed complete conversion of the alcohol. The reaction mixture was diluted with EtOAc (100 mL) and poured into aq. NH₄Cl (10%, 50 mL). The layers were separated, the organic layer was washed with water and brine, dried (Na₂SO₄), and filtered, and the solvents were evaporated. The residue was loaded onto a silica gel column, and elution with toluene/EtOAc (99:1 → 95:5, v/v), after concentration of the appropriate fractions, furnished title compound **12** (5.2 g) in 72% yield. ¹³C NMR (CDCl₃): δ = 161.5 (C=NH), 134.1, 133.1, 122.8 (C-4, C-5, =CH All), 117.2 (=CH₂ All), 91.3 (CCl₃), 72.1, 71.5, 69.5 (C-2, C-3, C-6), 62.5 (CH₂O), 37.7 (CH₂ All), 25.8 [(CH₃)₃C], 18.1 [(CH₃)₃CSi], –5.5 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 8.36 (s, 1 H, NH), 5.99–5.79 (m, 3 H, 4-H, 5-H, =CH All), 5.29 (m, 1 H, 3-H), 5.09 (m, 2 H, =CH₂ All), 4.30 (m, 1 H, 6-H), 3.92 (m, 1 H, 2-H), 3.81 (m, 2 H, CH₂O), 2.58–2.26 (m, 2 H, CH₂ All), 0.89 [s, 9 H, (CH₃)₃C], 0.06 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5R,6R)-6-Allyl-2-[(*tert*-butyldimethylsilyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6H-pyran (13): A solution of imide **12** (2.9 g, 6.8 mmol, dried by coevaporation with 1,4-dioxane) and K₂CO₃ (100 mg) in 1,2-dichlorobenzene (50 mL) was heated at reflux temperature (180 °C). After 5 h the [3,3]-sigmatropic rearrangement was complete, as shown by formation of a more polar compound (TLC analysis, EtOAc/toluene, 1:9, v/v). The reaction mixture was cooled to room temperature, filtered through Hyflo, and concentrated in high vacuum. Purification by silica gel column chromatography (light petroleum/EtOAc, 100:0 → 85:15, v/v) yielded **13** (2.8 g, 96%) as an oil. ¹³C NMR (CDCl₃): δ = 161.4 (C=O), 133.4, 131.2, 125.0 (C-3, C-4, =CH All), 117.2 (=CH₂ All), 92.5 (CCl₃), 74.6, 71.7 (C-2, C-6), 63.7 (CH₂O), 45.9 (C-5), 35.6 (CH₂ All), 25.8 [(CH₃)₃C], 18.0 [(CH₃)₃CSi], –5.5 [(CH₃)₂Si] ppm. ¹H NMR (HHCOSY, 300 MHz, CDCl₃): δ = 6.75 (d, *J* = 9.3 Hz, 1 H, NH), 6.06 (m, 2 H, 3-H, 4-H), 5.85 (m, 1 H, =CH All), 5.15 (m, 2 H, =CH₂ All), 4.34 (m, 2 H, 2-H, 5-H), 4.11 (m, 1 H, 6-H), 3.77 (m, 2 H, CH₂O), 2.33 (m, 2 H, CH₂ All), 0.89 [s, 9 H, (CH₃)₃C], 0.10 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5R,6R)-6-Allyl-5-[(*tert*-butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyloxy)methyl]-2,5-dihydro-6H-pyran (14): KOH (1.9 g, 34.2 mmol) was added to a solution of Overman rearrangement product **13** (4.9 g, 11.4 mmol) in *i*PrOH (50 mL). The reaction mix-

ture was stirred for 3 d (TLC analysis EtOAc/light petroleum, 1:9, v/v). Subsequently, the reaction mixture was diluted with DCM (100 mL), and di-*tert*-butyl dicarbonate (3.0 g, 13.7 mmol) was added. The reaction mixture was stirred overnight and the solvents were evaporated. The residue was purified by column chromatography (silica gel, light petroleum/EtOAc, 100:0 → 90:10, v/v) to yield **14** as a colorless oil (3.0 g, 70%, 2 steps). ¹³C NMR (CDCl₃): δ = 155.2 (C=O), 134.5, 129.0, 126.6 (C-3, C-4, =CH All), 116.4 (=CH₂ All), 78.7 (C_q Boc), 73.8 (C-6), 72.1 (C-2), 63.5 (CH₂O), 45.0 (C-5), 35.3 (CH₂ All), 28.0 [(CH₃)₃C Boc], 27.0 [(CH₃)₃C TBS], 17.8 [(CH₃)CSi], −5.8 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 5.90 (m, 3 H, 3-H, 4-H, =CH All), 5.04 (m, 2 H, =CH₂ All), 4.67 (d, 1 H, NH), 4.34 (m, 2 H, 2-H, 5-H), 4.12 (m, 1 H, 6-H), 3.87 (m, 2 H, CH₂O), 2.25 (m, 2 H, CH₂ All), 1.48 [s, 9 H, (CH₃)₃C Boc], 0.84 [s, 9 H, (CH₃)₃C TBS], 0.04 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5R,6R)-5-[(*tert*-Butoxycarbonyl)amino]-2-(hydroxymethyl)-6-propyl-tetrahydropyran (15): Olefin **14** (3.1 g, 8.0 mmol) was dissolved in MeOH (50 mL), and palladium on carbon (10%, 80 mg) was added. The suspension was repeatedly degassed under Ar. The reaction mixture was stirred overnight (16 h) under H₂, after which TLC analysis (EtOAc/toluene, 1:9, v/v) showed the complete consumption of the olefin. The catalyst was removed by filtration through Hyflo, and the filtrate was concentrated. The residue was loaded onto a silica gel column, and elution with toluene/EtOAc (95:5 → 50:50, v/v) afforded alcohol **15** (1.7 g, 78%) as an oil. ¹³C NMR (CDCl₃): δ = 155.5 (C=O), 79.0 (C_q Boc), 72.3 (C-6), 70.2 (C-2), 62.7 (CH₂O), 48.5, 46.4 (C-5), 33.8 (CH₂ Pr), 29.1, 28.5, 24.2, 23.4 (C-3, C-4), 17.4 (CH₃CH₂), 13.5 (CH₃) ppm.

(2S,5R,6R)-5-[(*tert*-Butoxycarbonyl)amino]-6-propyl-tetrahydropyran-2-carboxylic Acid (16): Alcohol **15** (0.68 g, 2.5 mmol) was dissolved in a mixture of DCM/CH₃CN/H₂O (1:1:1.45, v/v/v, 19 mL), and the mixture was cooled to 0 °C. With vigorous stirring, NaIO₄ (2.5 g, 11.7 mmol) and a catalytic amount of RuCl₃·xH₂O (6 mg) were added. After 3 h the reaction mixture was diluted with EtOAc (25 mL) and the solids were removed by filtration. The excess of NaIO₄ was removed by addition of MeOH (5 mL), and the obtained suspension was filtered. A solution of aq. NaHSO₃ (10%, 10 mL) was added to the filtrate, which resulted in the decolorization of the mixture. The pH was adjusted to 2 by addition of aq. HCl (1 M). The layers were separated and the aqueous layer was extracted with EtOAc (3 × 15 mL). The combined organic layers were dried (MgSO₄), filtered, and concentrated. The residue was dissolved in aq. NaHCO₃ (10%, 30 mL) and washed with EtOAc (2 × 10 mL). The water layer was acidified to pH 2 with HCl (1 M) and extracted with EtOAc (4 × 20 mL). The organic phase was dried over MgSO₄, filtered, and concentrated in vacuo. The obtained carboxylic acid **16** (0.48 g, 68%) was used in the next step without further purification. ¹³C NMR (CDCl₃): δ = 176.3 (C=O acid), 156.2 (C=O Boc), 79.5 (C_q Boc), 74.8, 72.4 (C-2, C-6), 46.7 (C-5), 33.6 (CH₂ Pr), 28.9 [(CH₃)₃C], 25.8, 21.3 (C-3, C-4), 18.3 (CH₃CH₂), 13.9 (CH₃) ppm.

(2S,5R,6R)-5-[N-(9-Fluorenylmethoxycarbonyl)amino]-6-propyl-tetrahydropyran-2-carboxylic Acid (5): Carboxylic acid **16** (0.48 g, 1.7 mmol) was added to a mixture of TFA/DCM (1:1, v/v, 20 mL), and the solution was stirred for 1 hour. The solvents were removed, and the residue was coevaporated twice with toluene (25 mL). The crude amino acid was taken up in 10% aq. NaHCO₃/1,4-dioxane (2:1, v/v, 30 mL) and cooled to 0 °C. A solution of Fmoc-OSu (0.69 g, 2.0 mmol) in 1,4-dioxane (10 mL) was slowly added. After the reaction mixture had been stirred overnight at room temperature, its pH was adjusted to 2 (HCl, 1 M) and the mixture was extracted with EtOAc (4 × 20 mL). After drying (MgSO₄) and con-

centration, crude **5** was isolated by silica gel column chromatography (light petroleum/EtOAc/AcOH, 3:1/0 → 0:98:2, v/v/v). Concentration of the appropriate fractions yielded dipeptide isoster **5** (0.49 g, 71%). ¹³C NMR (CDCl₃): δ = 173.2 (C=O acid), 156.2 (C=O Fmoc), 143.6, 141.1 (C_q Fmoc), 127.5, 126.8, 124.7, 119.7 (C_{arom} Fmoc), 74.2, 72.0 (C-2, C-6), 66.2 (CH₂ Fmoc), 47.4 (C-5), 47.0 (CH Fmoc), 25.1, 25.0 (C-3, C-4), 18.0 (CH₃CH₂), 13.7 (CH₃) ppm. ESI-MS: *m/z* = 410.0 [M + H]⁺; 432.0 [M + Na]⁺; 448.1 [M + K]⁺.

(2R,3S)-3-O-Acetyl-2-(acetyloxymethyl)-3,6-dihydro-2H-pyran (17): Triethylsilane (7.7 mL, 40 mmol) and BF₃·Et₂O (3.4 mL, 52 mmol) were added to a cooled solution (0 °C) of 3,4,6-tri-*O*-acetyl-D-glucal (10.9 g, 40 mmol, dried by co-evaporation with 1,2-DCE) in DCM (125 mL). The reaction mixture was stirred for 1 hour, after which TLC analysis (toluene/EtOAc, 1:1, v/v) showed complete conversion of the starting material. The reaction was quenched by addition of NaHCO₃ (1 M, 20 mL), the layers were separated, and the organic phase was washed with H₂O and brine, dried (MgSO₄), filtered, and concentrated. Purification of the residue by silica gel column chromatography (light petroleum/EtOAc, 75:25 → 60:40, v/v) afforded **17** as an oil (8.7 g, 96%). ¹³C NMR (CDCl₃): δ = 169.9, 169.5 (C=O acetyl), 128.9, 123.5 (C-4, C-5), 73.1, 64.6 (C-2, C-3), 64.2, 62.6 (C-6, CH₂O), 20.0, 19.8 (CH₃ acetyl) ppm. ¹H NMR (CDCl₃): δ = 5.95, 5.73 (2 × m, 2 H, 4-H, 5-H), 5.74 (m, 1 H, 3-H), 4.24 (m, 4 H, 6a-H, 6b-H, CH₂O), 3.74 (m, 1 H, 2-H), 2.10 (m, 6 H, 2 × CH₃ acetyl) ppm.

(2R,3S)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-hydroxy-3,6-dihydro-2H-pyran (18): Ferrier rearrangement product **17** (8.2 g, 38 mmol) was transformed into **18** (8.3 g, 89%) as described for **11**. ¹³C NMR (CDCl₃): δ = 128.0, 127.0 (C-4, C-5), 77.3, 66.1 (C-2, C-3), 65.0 (C-6, CH₂O), 25.7 [(CH₃)₃C], 18.1 [(CH₃)₃C], −5.7 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 5.81 (m, 2 H, 4-H, 5-H), 4.23 (m, 1 H, 3-H), 4.15 (m, 2 H, 6a-H, 6b-H), 3.95 (m, 1 H, CHHO), 3.68 (m, 1 H, CHHO), 3.37 (m, 1 H, 2-H), 3.08 (br. s, 1 H, OH), 0.91 [s, 9 H, (CH₃)₃C], 0.12 [s, 6 H, (CH₃)₂Si] ppm.

(2R,3S)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2H-pyran (19): Alcohol **18** (2.4 g, 10 mmol) was treated with DBU (1.9 mL, 13 mmol) and Cl₃CCN (1.3 mL, 13 mmol) according to the procedure described for **12**. Silica gel column chromatography (light petroleum/EtOAc/Et₃N, 94:5:1 → 90:9:1, v/v/v) afforded pure **19** (3.0 g, 76%). ¹³C NMR (CDCl₃): δ = 161.5 (C=NH), 130.0, 123.3 (C-4, C-5), 76.6, 69.8 (C-2, C-3), 65.3 (C-6), 63.0 (CH₂O), 25.8 [(CH₃)₃C], 18.3 [(CH₃)CSi], −5.4 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 8.39 (s, 1 H, NH), 5.96 (m, 2 H, 4-H, 5-H), 5.42 (dd, *J* = 2.9, *J* = 8.0 Hz, 1 H, 3-H), 4.25 (m, 2 H, 6a-H, 6b-H), 3.80 (m, 2 H, CH₂O), 3.69 (m, 1 H, 2-H), 0.89 [s, 9 H, (CH₃)₃C], 0.07 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5R)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6H-pyran (20): An Overman rearrangement of imide **19** (2.0 g, 5.1 mmol) as described for **13** furnished trichloroacetamide **20** (1.8 g, 89%). ¹³C NMR (CDCl₃): δ = 161.1 (C=O), 131.1, 124.4 (C-3, C-4), 73.9 (C-2), 64.9 (C-6), 64.1 (CH₂O), 44.7 (C-5), 25.5 [(CH₃)₃C], 17.9 [(CH₃)CSi], −5.7 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 6.76 (d, *J* = 8.0 Hz, 1 H, NH), 5.99 (m, 2 H, 3-H, 4-H), 4.43 (m, 1 H, 5-H), 4.16 (m, 2 H, 6a-H, 6b-H), 3.61 (m, 3 H, 2-H, CH₂O), 0.90 [s, 9 H, (CH₃)₃C], 0.08 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5R)-5-[(*tert*-Butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyloxy)methyl]-2,5-dihydro-6H-pyran (21): Deacylation of the trichloroacetamide **20** (2.3 g, 6.0 mmol) and subsequent protection of the amine with a Boc group according to the procedure de-

scribed for **14** furnished Boc-protected amine **21** (1.5 g, 74%). ^{13}C NMR (CDCl_3): δ = 155.1 (C=O), 130.0, 127.2 (C-3, C-4), 79.2 (C_q Boc), 74.1 (C-2), 66.7 (C-6), 64.2 (CH_2O), 44.0 (C-5), 28.2 [$(\text{CH}_3)_3\text{C}$ Boc], 25.7 [$(\text{CH}_3)_3\text{C}$ TBS], 18.1 [$(\text{CH}_3)_3\text{CSi}$], -5.5 [$(\text{CH}_3)_2\text{Si}$] ppm. ^1H NMR (CDCl_3): δ = 5.87 (m, 2 H, 3-H, 4-H), 4.55 (m, 1 H, 5-H), 4.11 (m, 2 H, 6a-H, 6b-H), 3.71 (m, 1 H, CHHO), 3.57 (m, 1 H, CHHO), 3.44 (m, 1 H, 2-H), 1.45 [$(\text{CH}_3)_3\text{C}$ Boc], 0.89 [s, 9 H, $(\text{CH}_3)_3\text{C}$ TBS], 0.06 [s, 6 H, $(\text{CH}_3)_2\text{Si}$] ppm.

(2S,5R)-5-[(tert-Butoxycarbonyl)amino]-2-(hydroxymethyl)-tetrahydropyran (22): Reduction of **21** (2.4 g, 6.0 mmol) by the procedure described for **15** afforded alcohol **22** in 75% yield (1.0 g). ^{13}C NMR (CDCl_3): δ = 155.3 (C=O), 78.9 (C_q Boc), 77.4 (C-2), 70.4 (C-6), 64.7 (CH_2O), 46.1 (C-5), 29.2, 26.3 (C-3, C-4), 27.8 [$(\text{CH}_3)_3\text{C}$] ppm. ^1H NMR (CDCl_3): δ = 4.40 (d, 1 H, NH), 4.11 (m, 1 H, 2-H), 3.47 (m, 4 H, 6a-H, 6b-H, CH_2O), 3.23 (m, 1 H, 5-H), 2.06, 1.72 (2 $\times$ m, 4 H, 3a-H, 3b-H, 4a-H, 4b-H), 1.44 [s, 9 H, $(\text{CH}_3)_3\text{C}$] ppm.

(2S,5R)-5-[(tert-Butoxycarbonyl)amino]tetrahydropyran-2-carboxylic Acid (23): Alcohol **22** (1.0 g, 4.5 mmol) was oxidized to the corresponding acid **23** (0.79 g, 71%) as described for **16**. ^{13}C NMR (CDCl_3): δ = 174.6 (C=O acid), 157.4 (C=O Boc), 81.7 (C_q Boc), 74.8 (C-2), 70.6 (C-6), 46.6, 45.4 (C-5), 29.3, 27.6, 27.2 (C-3, C-4), 28.2 [$(\text{CH}_3)_3\text{C}$ Boc] ppm. ESI-MS: m/z = 246.1 [$\text{M} + \text{H}$] $^+$; 267.8 [$\text{M} + \text{Na}$] $^+$.

(2S,5R)-5-[N-(9-Fluorenylmethoxycarbonyl)amino]tetrahydropyran-2-carboxylic Acid (6): **(2S,5R)-5-[(tert-butoxycarbonyl)amino]tetrahydropyran-2-carboxylic acid** (0.66 g, 2.7 mmol) was subjected to TFA/DCM (1:1, v/v) and subsequent treatment with Fmoc-OSu as described for **5**. After silica gel column chromatography, **6** was obtained in 78% yield over 2 steps (0.77 g). ^{13}C NMR ($\text{CDCl}_3/\text{MeOD}/\text{D}_2\text{O}$): δ = 174.2 (C=O acid), 157.2 (C=O Fmoc), 144.4, 141.9 (C_q Fmoc), 128.3, 127.7, 125.5, 120.5 (C_{arom} Fmoc), 75.7 (C-2), 73.4 (C-6), 70.6 (CH_2 Fmoc), 47.7 (CH Fmoc), 46.7 (C-5), 28.2, 25.9 (C-3, C-4) ppm. ESI-MS: m/z = 368.1 [$\text{M} + \text{H}$] $^+$; 390.4 [$\text{M} + \text{Na}$] $^+$; 406.3 [$\text{M} + \text{K}$] $^+$.

(2R,3R,6R)-6-Allyl-2-[(tert-butyldimethylsilyloxy)methyl]-3-O-(p-nitrobenzoyl)-3,6-dihydro-2H-pyran (24): A solution of DEAD (2.7 mL, 17.3 mmol) in THF (20 mL) was slowly added to a cooled (0 $^\circ\text{C}$) solution of alcohol **11** (3.3 g, 11.5 mmol) in THF (50 mL) containing triphenylphosphane (4.50 g, 17.3 mmol) and *p*-nitrobenzoic acid (2.1 g, 12.7 mmol). After the mixture had been stirred overnight, the solvent was removed and the residue was dissolved in EtOAc (100 mL) and washed with aq. NaHCO_3 (10%), water, and brine. The organic layer was dried over MgSO_4 , filtered, and concentrated. The residue was dissolved in diethyl ether/light petroleum (9:1, v/v) and stored at 4 $^\circ\text{C}$ overnight. The solids were removed by filtration and the filtrate was concentrated in vacuo. Crude **24** was loaded onto a silica gel column, and elution with toluene/EtOAc (99:1 $\rightarrow$ 98:2, v/v) afforded *p*-nitrophenyl ester **24** (4.5 g, 91%). ^{13}C NMR (CDCl_3): δ = 163.7 (C=O), 150.2 (C_q Ph- NO_2), 135.4 (C_q Ph), 135.5 (=CH All), 133.9, 130.4, 123.1, 121.5 (C_{arom} , C-4, C-5), 117.2 (=CH $_2$ All), 72.5, 70.0, 64.5 (C-2, C-3, C-6), 61.4 (CH_2O), 36.4 (CH_2 All), 25.4 [$(\text{CH}_3)_3\text{C}$], 17.7 [$(\text{CH}_3)_2\text{CSi}$], -5.9 [$(\text{CH}_3)_2\text{Si}$] ppm. ^1H NMR (CDCl_3): δ = 8.34 (m, 4 H, H_{arom}), 6.23 (m, 2 H, 4-H, 5-H), 5.99 (m, 1 H, =CH All), 5.43 (m, 1 H, 3-H), 5.24 (m, 2 H, =CH $_2$ All), 4.50 (m, 1 H, 2-H), 4.16 (m, 1 H, 6-H), 3.89 (m, 2 H, CH_2O), 2.67–2.35 (br. m, 2 H, CH_2 All), 0.90 [s, 9 H, $(\text{CH}_3)_3\text{C}$], 0.08 (s, 3 H, CH_3Si), 0.06 (s, 3 H, CH_3Si) ppm.

(2R,3R,6R)-6-Allyl-2-[(tert-butyldimethylsilyloxy)methyl]-3-hydroxy-3,6-dihydro-2H-pyran (25): Ester **24** (5.8 g, 13.4 mmol) was

dissolved in *i*PrOH (60 mL), KOH (1.1 g, 20.1 mmol) was added, and the reaction mixture was stirred overnight. The volume was reduced, and the remaining solution was diluted with ethyl acetate (150 mL) and washed with water and brine. After drying (MgSO_4), filtration, and concentration, the residue was purified by silica gel column chromatography (light petroleum/EtOAc, 99:1 $\rightarrow$ 96:4, v/v). Concentration of the appropriate fractions gave **25** in 80% yield (3.0 g). ^{13}C NMR (CDCl_3): δ = 134.0 (=CH All), 131.8, 126.4 (C-4, C-5), 116.7 (=CH $_2$ All), 76.4, 63.1 (C-2, C-3, C-6), 62.6 (CH_2O), 36.3 (CH_2 All), 25.4 [$(\text{CH}_3)_3\text{C}$], 17.8 [$(\text{CH}_3)_3\text{CSi}$], -5.7 [$(\text{CH}_3)_2\text{Si}$] ppm. ^1H NMR (CDCl_3): δ = 5.92 (m, 3 H, 4-H, 5-H, =CH All), 5.12 (m, 2 H, =CH $_2$ All), 4.18 (m, 1 H, 6-H), 3.78 (m, 4 H, 2-H, 3-H, CH_2O), 2.51–2.18 (br. m, 2 H, CH_2 All), 0.91 [s, 9 H, $(\text{CH}_3)_3\text{C}$], 0.09 (s, 3 H, CH_3Si), 0.06 (s, 3 H, CH_3Si) ppm.

(2R,3R,6R)-6-Allyl-2-[(tert-butyldimethylsilyloxy)methyl]-3-O-(trichloroacetimidoyl)-3,6-dihydro-2H-pyran (26): Conversion of alcohol **25** (3.0 g, 10.7 mmol) into imide **26** by the procedure described for **12** and purification by silica gel column chromatography (toluene/EtOAc, 99:1 $\rightarrow$ 95:5, v/v) afforded **26** (4.2 g, 90%). ^{13}C NMR (CDCl_3): δ = 161.9 (C=NH), 135.7 (=CH All), 134.2, 121.0 (C-4, C-5), 117.3 (=CH $_2$ All), 72.4, 71.1, 68.1 (C-2, C-3, C-6), 61.7 (CH_2O), 36.8 (CH_2 All), 25.8 [$(\text{CH}_3)_3\text{C}$], 18.1 [$(\text{CH}_3)_3\text{CSi}$], -5.4 [$(\text{CH}_3)_2\text{Si}$] ppm. ^1H NMR (CDCl_3): δ = 8.26 (br. d, 1 H, NH), 6.23–5.78 (m, 3 H, 4-H, 5-H, =CH All), 5.17 (m, 2 H, CH_2 All), 4.37 (m, 1 H, 3-H), 4.01 (m, 1 H, 6-H), 3.83 (m, 3 H, 2-H, CH_2O), 2.54–2.22 (br. m, 2 H, CH_2 All), 0.87 [s, 9 H, $(\text{CH}_3)_3\text{C}$], 0.05 (s, 3 H, CH_3Si), 0.03 (s, 3 H, CH_3Si) ppm.

(2S,5S,6R)-6-Allyl-2-[(tert-butyldimethylsilyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6H-pyran (27): The Overman rearrangement of **26** (4.2 g, 9.7 mmol) was performed as described for compound **13**. Silica gel column chromatography (toluene/EtOAc, 100:0 $\rightarrow$ 95:5, v/v) furnished **27** (3.2 g, 78%). ^{13}C NMR (CDCl_3): δ = 161.0 (C=O), 133.6, 131.6, 123.2 (=CH All, C-3, C-4), 117.4 (=CH $_2$ All), 92.3 (CCl_3), 74.2, 70.3 (C-2, C-6), 64.7 (CH_2O), 47.3 (C-5), 35.0 (CH_2 All), 25.7 [$(\text{CH}_3)_3\text{C}$], 18.1 [$(\text{CH}_3)_3\text{CSi}$], -5.5 [$(\text{CH}_3)_2\text{Si}$] ppm. ^1H NMR (CDCl_3): δ = 6.76 (br. d, J = 8.0 Hz, 1 H, NH), 6.03–5.77 (m, 3 H, 3-H, 4-H, =CH All), 5.15 (m, 2 H, =CH $_2$ All), 4.18 (m, 2 H, 2-H, 5-H), 3.92 (m, 1 H, 6-H), 3.73 (m, 2 H, CH_2O), 2.56–2.27 (br. m, 2 H, CH_2 All), 0.90 [s, 9 H, $(\text{CH}_3)_3\text{C}$], 0.07 [s, 6 H, $(\text{CH}_3)_2\text{Si}$] ppm.

(2S,5S,6R)-5-[(tert-Butoxycarbonyl)amino]-2-(hydroxymethyl)-6-propyl-tetrahydropyran (29): The Boc-protected Overman rearrangement product **28** (2.6 g, 88%, two steps) was obtained from **27** (3.2 g, 7.5 mmol) as described for **14**. Subsequent hydrogenation of **28** by the procedure performed for **15** yielded saturated alcohol **29** (1.3 g, 70%). ^{13}C NMR (CDCl_3): δ = 154.4 (C=O), 78.4 (C_q Boc), 76.0, 68.6 (C-2, C-6), 64.4 (CH_2O), 46.5 (C-5), 30.8 (CH_2 Pr), 27.4 [$(\text{CH}_3)_3\text{C}$ Boc], 22.4, 21.1 (C-3, C-4), 17.8 (CH_2CH_3), 13.0 (CH_3) ppm.

(2S,5S,6R)-5-[(tert-Butoxycarbonyl)amino]-6-propyl-tetrahydropyran-2-carboxylic Acid (30): Alcohol **29** (0.67 g, 2.5 mmol) was oxidized in a fashion similar to that described for compound **16** to yield Boc-protected dipeptide isomer **30** (0.41 g, 55%). ^{13}C NMR (CDCl_3): δ = 172.8 (C=O), 152.9 (C=O), 78.8 (C_q Boc), 74.7, 73.9 (C-6), 68.1, 67.5 (C-2), 47.7; 46.0 (C-5), 30.4 (CH_2 Pr), 25.8 [$(\text{CH}_3)_3\text{C}$ Boc], 23.3, 22.4 (C-3, C-4), 15.9 (CH_2CH_3), 11.3 (CH_3) ppm.

(2S,5S,6R)-5-[N-(9-Fluorenylmethoxycarbonyl)amino]-6-propyl-tetrahydropyran-2-carboxylic Acid (7): Boc-protected acid **30** (406 mg, 1.35 mmol) was transformed into title compound **7** (417 mg, 76%) by the procedure described for **5**. ^{13}C NMR

(CDCl₃): δ = 172.8 (C=O acid), 156.0 (C=O Fmoc), 143.3, 141.1 (C_q Fmoc), 127.5, 126.9, 124.7, 119.6 (C_{arom} Fmoc), 74.3, 72.1 (C-2, C-6), 65.9 (CH₂ Fmoc), 47.5 (C-5), 47.0 (CH Fmoc), 25.1, 25.0 (C-3, C-4), 17.9 (CH₃CH₂), 13.8 (CH₃) ppm. ESI-MS: m/z = 410.0 [M + H]⁺; 432.0 [M + Na]⁺; 448.1 [M + K]⁺.

(2R,3R)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-*O*-(*p*-nitrobenzoyl)-3,6-dihydro-2H-pyran (31): A solution of alcohol **18** (6.1 g, 25 mmol), triphenylphosphane (9.8 g, 37.5 mmol), and *p*-nitrobenzoic acid (4.6 g, 27.5 mmol) in THF (100 mL) was cooled to –30 °C. A solution of diisopropyl azodicarboxylate (5.4 mL, 27.5 mmol) in THF (20 mL) was added dropwise, and the reaction mixture was allowed to warm up slowly to room temperature. After stirring overnight, the mixture was diluted with EtOAc (150 mL) and poured into aq. NaHCO₃ (10%, 75 mL), and the layers were separated. The organic layer was washed with brine (75 mL), dried (MgSO₄), filtered, and concentrated to dryness. Purification by silica gel column chromatography (toluene) afforded a mixture of ester **31** and an unidentified side product (6.3 g, 64%). ¹³C NMR (CDCl₃): δ = 164.2 (C=O), 150.4 (C_q Ph–NO₂), 134.6 (C_q Ph), 134.6, 122.2 (C-4, C-5), 130.7, 123.2 (C_{arom}), 74.6 (C-3), 67.1 (C-6), 66.6 (C-2), 64.9 (CH₂O), 25.7 [(CH₃)₃C], 18.1 [(CH₃)CSi], –5.5 [(CH₃)₂Si] ppm.

(2R,3R)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-hydroxy-3,6-dihydro-2H-pyran (32): Ester **31** (3.2 g, 8.1 mmol) was dissolved in methanol (75 mL), and a catalytic amount of *t*BuOK (100 mg) was added. The resulting solution was stirred for 16 h and neutralized by addition of acetic acid. The solvent was removed by evaporation, and the residue was purified by silica gel column chromatography (toluene/EtOAc, 100:0 → 90:10, v/v) to give alcohol **32** (1.5 g, 76%). ¹³C NMR (CDCl₃): δ = 129.5, 126.6 (C-4, C-5), 76.3, 61.9 (C-2, C-3), 65.7 (C-6), 62.4 (CH₂O), 25.6 [(CH₃)₃C], 18.1 [(CH₃)₃CSi], –5.5 [(CH₃)₂Si] ppm. ¹H NMR (HHCOSY, 400 MHz, CDCl₃): δ = 6.01 (m, 1 H, 4-H), 5.90 (ddd, 1 H, *J*_{4,5} = 1.5, *J*_{5,6b} = 3.5, *J*_{5,6a} = 10.1 Hz, 5-H), 4.15 (m, 2 H, 6a-H, 6b-H), 3.91 (m, 1 H, 3-H), 3.80 (m, 2 H, CH₂O), 3.50 (dt, 1 H, *J*_{2,3} = 1.9, *J*_{2,CHHO} = *J*_{2,CHHO} = 6.3 Hz), 2.00 (br. s, OH), 0.87 [s, 9 H, (CH₃)₃C], 0.59 (s, 3 H, CH₃Si), 0.53 (s, 3 H, CH₃Si) ppm.

(2R,3R)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2H-pyran (33): Trichloroacetimidate **33** (2.2 g, 90%) was obtained from allylic alcohol **32** (1.5 g, 6.1 mmol) by the procedure described for **19**. ¹³C NMR (CDCl₃): δ = 161.1 (C=O), 132.4, 120.4 (C-4, C-5), 76.2, 67.8 (C-2, C-3), 64.9 (C-6), 61.1 (CH₂O), 25.1 [(CH₃)₃C], 17.4 [(CH₃)₃CSi], –6.2 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 8.27 (s, 1 H, NH), 6.17 (m, 2 H, 4-H, 5-H), 5.28 (m, 1 H, 3-H), 4.27 (m, 2 H, 6a-H, 6b-H), 3.81 (m, 3 H, 2-H, CH₂O), 0.88 [s, 9 H, (CH₃)₃C], 0.06 (s, 3 H, CH₃Si), 0.04 (s, 3 H, CH₃Si) ppm.

(2S,5S)-2-[(*tert*-Butyldimethylsilyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6H-pyran (34): Imidate **33** (2.2 g, 5.4 mmol) was subjected to thermal rearrangement as depicted for **20** to give trichloroacetamide **34** (1.6 g, 76%). ¹³C NMR (CDCl₃): δ = 160.1 (C=O), 132.8, 123.5 (C-3, C-4), 92.2 (CCl₃), 75.1 (C-2), 67.8, 64.9 (C-6, CH₂O), 44.9 (C-5), 25.7 [(CH₃)₃C], 18.2 [(CH₃)₃CSi], –5.5 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 6.90 (d, 1H, NH), 5.99 (m, 2 H, 3-H, 4-H), 4.28 (m, 1 H, 5-H), 4.13 (m, 1 H, 2-H), 3.72 (m, 4 H, 6a-H, 6b-H, CH₂O), 0.89 [s, 9 H, (CH₃)₃C], 0.06 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5S)-5-[(*tert*-Butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilyloxy)methyl]-2,5-dihydro-6H-pyran (35): Overman product **34** (2.4 g, 6.1 mmol) was converted into Boc-protected **35** (2.4 g, quant.) as described for **21**. ¹³C NMR (CDCl₃): δ = 154.9 (C=O),

130.9, 125.5 (C-3, C-4), 79.2 (C_q Boc), 74.8 (C-2), 68.7, 65.1 (C-6, CH₂O), 43.7 (C-5), 28.1 [(CH₃)₃C Boc], 25.7 [(CH₃)₃C TBS], 18.2 [(CH₃)₃CSi], –5.6 [(CH₃)₂Si] ppm. ¹H NMR (CDCl₃): δ = 5.86 (m, 2 H, 3-H, 4-H), 4.07 (m, 2 H, 2-H, 5-H), 3.61 (m, 4 H, 6a-H, 6b-H, CH₂O), 1.39 [s, 9 H, (CH₃)₃C Boc], 0.86 [s, 9 H, (CH₃)₃C TBS], 0.03 [s, 6 H, (CH₃)₂Si] ppm.

(2S,5S)-5-[(*tert*-Butoxycarbonyl)amino]tetrahydropyran-2-carboxylic Acid (37): Olefin **35** (6.1 mmol) was converted into alcohol **36** (0.83 g, 58%) by the procedure described for **22**. Oxidation of **36** (0.46 g, 2.0 mmol) according to the procedure described for **23** gave acid **37** (0.3 g, 60%). ¹³C NMR (CDCl₃): δ = 173.8 (C=O acid), 155.2 (C=O Boc), 79.1 (C_q Boc), 74.8 (C-2), 70.3 (C-6), 44.1 (C-5), 27.9 [(CH₃)₃C], 27.0, 23.6 (C-3, C-4) ppm.

(2S,5S)-5-[N-(9-Fluorenylmethoxycarbonyl)amino]tetrahydropyran-2-carboxylic Acid (8): Carboxylic acid **37** (0.20 g, 0.80 mmol) was subjected to TFA/DCM (1:1, v/v) followed by introduction of the Fmoc group to furnish **8** (0.19 g, 63%) as described for **6**. ¹³C NMR (CDCl₃): δ = 174.0 (C=O acid), 156.0 (C=O Fmoc), 143.6, 141.1 (C_q Fmoc), 127.5, 126.9, 124.9, 119.8 (C_{arom} Fmoc), 75.1 (C-2), 70.4 (C-6), 66.6 (CH₂ Fmoc), 46.9 (CH Fmoc), 44.9 (C-5), 27.2, 23.6 (C-3, C-4) ppm. ESI-MS: m/z = 368.1 [M + H]⁺; 390.0 [M + Na]⁺; 406.1 [M + K]⁺.

General Procedure for Solid-Phase Peptide Synthesis: The 5-[N-(9-fluorenylmethoxycarbonyl)amino]tetrahydropyran-2-carboxylic acid-containing peptides **41–46** were synthesized on an automatic peptide synthesizer (ABI, 443A, Applied Biosystems, division of Perkin–Elmer). The synthesis was performed by use of the FastMoc protocol supplied with the synthesizer. Each peptide was synthesized on a 50 μ mol scale starting from Wang resin loaded with *N*^α-(9-fluorenylmethoxycarbonyl)-*N*^ω-2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl-arginine (vide infra). The Fmoc-amino acids used in the synthesis were as follows: Fmoc–Ala–OH, Fmoc–Arg(Pbf)–OH, Fmoc–Asp(OtBu)–OH, Fmoc–Glu(OtBu)–OH, Fmoc–Ile–OH, Fmoc–Lys(Boc)–OH, Fmoc–Thr(*t*Bu)–OH and Fmoc–Tyr(*t*Bu)–OH.

Generally, the following steps were performed for each cycle:

(a) Removal of the Fmoc group by treatment with 20% piperidine in NMP (5 × 1 min).

(b) Coupling of the suitably protected amino acids by application of a fivefold excess. The amino acid was dissolved in NMP (0.5 mL) and 1 equiv. of BOP/HOBt (0.5 M BOP/0.5 M HOBt in NMP/DMF, 1:1, v/v, 0.5 mL) and 2.5 equiv. of DiPEA (1.25 M DiPEA in NMP, 0.5 mL) were added. The resulting solution was transferred to the reaction vessel and the suspension was shaken for 1 hour. In the case of the 5-[N-(9-fluorenylmethoxycarbonyl)amino]tetrahydropyran-2-carboxylic acids **5–8** the time of the coupling reaction was set to 2 h.

(c) Capping of the remaining amine functions. A capping solution (0.5 M acetic anhydride, 0.125 M DiPEA and 0.015 M HOBt in NMP, 1 mL) was added and the resulting suspension was shaken for 1 min.

When the synthesis was complete, the resin was removed from the reaction vessel and placed in a round-bottomed flask. A mixture of TFA/H₂O/TIS (95:2.5:2.5, v/v/v, 10 mL) was added, and the suspension was shaken for 2 h. Subsequently the resin was filtered and washed with TFA. The filtrate was diluted with toluene, and the solvents were evaporated to dryness. The residue was dried in high vacuum, dissolved in little TFA, and precipitated by dropwise ad-

dition to diethyl ether. The crude pyran-containing peptide was purified by RP HPLC.

Fmoc-Arg(Pbf)-Wang Resin (39): In a silylated flask (20% trimethylsilyl chloride in CHCl_3), *p*-benzyloxybenzyl alcohol resin **38**, (Wang resin, 0.5 g, 430 μmol , 0.86 mmol/g) was dried by coevaporation with 1,4-dioxane ($2 \times 15 \text{ mL}$). The resin was suspended in DCM (40 mL), *N*^u-Fmoc-*N*^G-2,2,4,6,7-pentamethyl-dihydrobenzofuran-5-sulfonyl-L-arginine (Fmoc-Arg(Pbf)-OH, 0.84 g, 1.3 mmol), dicyclohexylcarbodiimide (DCC, 0.29 g, 1.4 mmol), and 4-(dimethylamino)pyridine (DMAP, 8 mg, 0.07 mmol) were added, and the flask was rotated for 2 h. The resin was filtered and subsequently washed with DCM ($2 \times 15 \text{ mL}$), MeOH (15 mL), and DCM ($2 \times 15 \text{ mL}$), and the loading of the resin was repeated once more. The resin was dried in high vacuum and the loading of the resin (0.48 mmol/g, 56%) was determined by UV absorption by the following procedure. A sample of the resin (4–5 mg) was exactly weighed in a volumetric flask (10 mL). A solution of piperidine/DMF (1:4, v/v, 1 mL) was added, and the mixture was left for 10 min. The volume was adjusted to 10 mL by addition of MeOH (HPLC grade) and the UV absorption was measured between 250 and 350 nm. The loading of the resin was calculated from the formula: $L \text{ (mmol/g)} = (A \times \text{vol}) / (7.8 \times \text{wt})$, where *A* is the absorption value at 300 nm (secondary maximum), vol is the volume of the sample (10 mL), and wt is the weight of the resin (mg).

H-Ala-Lys-Thr-Ile-[(2*S*,5*S*,6*R*)-5-Amino-2-carboxy-6-propyl-tetrahydropyran]-Asp-Glu-Glu-Ala-Arg-Arg-OH (41): The synthesis of the pyran-containing peptide **41** was according to the general procedure for solid-phase peptide synthesis, followed by RP HPLC purification (linear gradient, 10 $\rightarrow$ 22% B in 19.6 min, 4.0 CV), furnishing **41** (20.6 mg, 24.4%, TFA salt). LC/MS analysis: $R_t = 11.08 \text{ min}$, ESI-MS: $m/z = 1358.2 \text{ [M + H]}^+$; 679.7 [M + 2H]^{2+} ; 453.5 [M + 3H]^{3+} ; 340.3 [M + 4H]^{4+} ; calcd. 1356.7.

H-Ala-Lys-Thr-Ile-[(2*S*,5*R*)-5-Amino-2-carboxytetrahydropyran]-Asp-Glu-Glu-Ala-Arg-Arg-OH (42): Pyran-containing peptide **42** was synthesized as described in the general procedure for solid-phase peptide synthesis. HPLC purification (linear gradient 13 $\rightarrow$ 17% B in 12.3 min, 2.5 CV) afforded 21.7 mg (26.4%, TFA salt). LC/MS analysis: $R_t = 7.90 \text{ min}$, ESI-MS: $m/z = 1316.0 \text{ [M + H]}^+$; 658.9 [M + 2H]^{2+} ; 439.6 [M + 3H]^{3+} ; calcd. 1314.7.

H-Ala-Lys-Thr-Ile-[(2*S*,5*S*)-5-Amino-2-carboxytetrahydropyran]-Asp-Glu-Glu-Ala-Arg-Arg-OH (43): The solid-phase synthesis of peptide **43** was performed according to the general procedure for SPPS. After RP HPLC purification (linear gradient 10 $\rightarrow$ 18% B in 14.7 min, 3.0 CV) the 2,5-*cis*-pyran-containing peptide **43** was obtained in 25% yield (20.6 mg, TFA salt). LC/MS analysis: $R_t = 8.19 \text{ min}$, ESI-MS: $m/z = 1316.2 \text{ [M + H]}^+$; 659.0 [M + 2H]^{2+} ; 439.6 [M + 3H]^{3+} ; 329.8 [M + 4H]^{4+} ; calcd. 1314.7.

H-Ala-Lys-Thr-Ile-Ala-Tyr-Asp-[(2*S*,5*R*,6*R*)-5-Amino-2-carboxy-6-propyl-tetrahydropyran]-Ala-Arg-Arg-OH (44): Purification of the crude peptide (29 mg, 21.7 μmol) by RP HPLC (linear gradient 14 $\rightarrow$ 23% B in 14.7 min, 3.0 CV) furnished **44** (7.99 mg, 22.1%, TFA salt). LC/MS analysis: $R_t = 8.07 \text{ min}$, ESI-MS: $m/z = 1334.1 \text{ [M + H]}^+$; 667.5 [M + 2H]^{2+} ; 445.4 [M + 3H]^{3+} ; calcd. 1332.8.

H-Ala-Lys-Thr-Ile-Ala-Tyr-Asp-[(2*S*,5*R*)-5-Amino-(2-carboxy)-tetrahydropyran]-Ala-Arg-Arg-OH (45): A sample of the crude peptide **45** (45 mg, $\pm 25 \mu\text{mol}$) was loaded onto a semipreparative RP HPLC column. Elution with buffer A: H_2O ; B: CH_3CN , C aq. 1% TFA with application of a linear gradient in B (13 $\rightarrow$ 19%, in 14.7 min, 3.0 CV) gave **45** (11.46 mg, 28%, TFA salt). LC/MS

analysis: $R_t = 10.68 \text{ min}$, ESI-MS: $m/z = 1291.9 \text{ [M + H]}^+$; 646.7 [M + 2H]^{2+} ; 431.5 [M + 3H]^{3+} ; calcd. 1290.7.

H-Ala-Lys-Thr-Ile-Ala-Tyr-Asp-[(2*S*,5*S*,6*R*)-5-Amino-2-carboxy-6-propyl-tetrahydropyran]-Ala-Arg-Arg-OH (46): The crude peptide **46** (35 mg, $\pm 29 \mu\text{mol}$) was purified by RP HPLC (linear gradient 14 $\rightarrow$ 23% B in 14.7 min, 3.0 CV) to afford **46** (7.67 mg, 16%, TFA salt). LC/MS analysis: $R_t = 11.88 \text{ min}$, ESI-MS 1334.0 [M + H]^+ ; 667.5 [M + 2H]^{2+} ; calcd. 1332.8.

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