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

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Differently functionalized tetrahydropyran-based dipeptide isosters 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 isosters.

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 peptide isosters, 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 isosters **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 $BF_3{\cdot}Et_2O$. Ensuing functional group manipulations of the resulting Ferrier rearrangement product **2** afforded dipeptide isosters **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 isosters **4**.

Here we report the synthesis of the four new Fmoc-protected, tetrahydropyran-based 2,5-*cis*- and 2,5-*trans* dipeptide isosters **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 isosters **5–8** in solid-phase peptide synthesis is demonstrated by the preparation of analogues of antigenic

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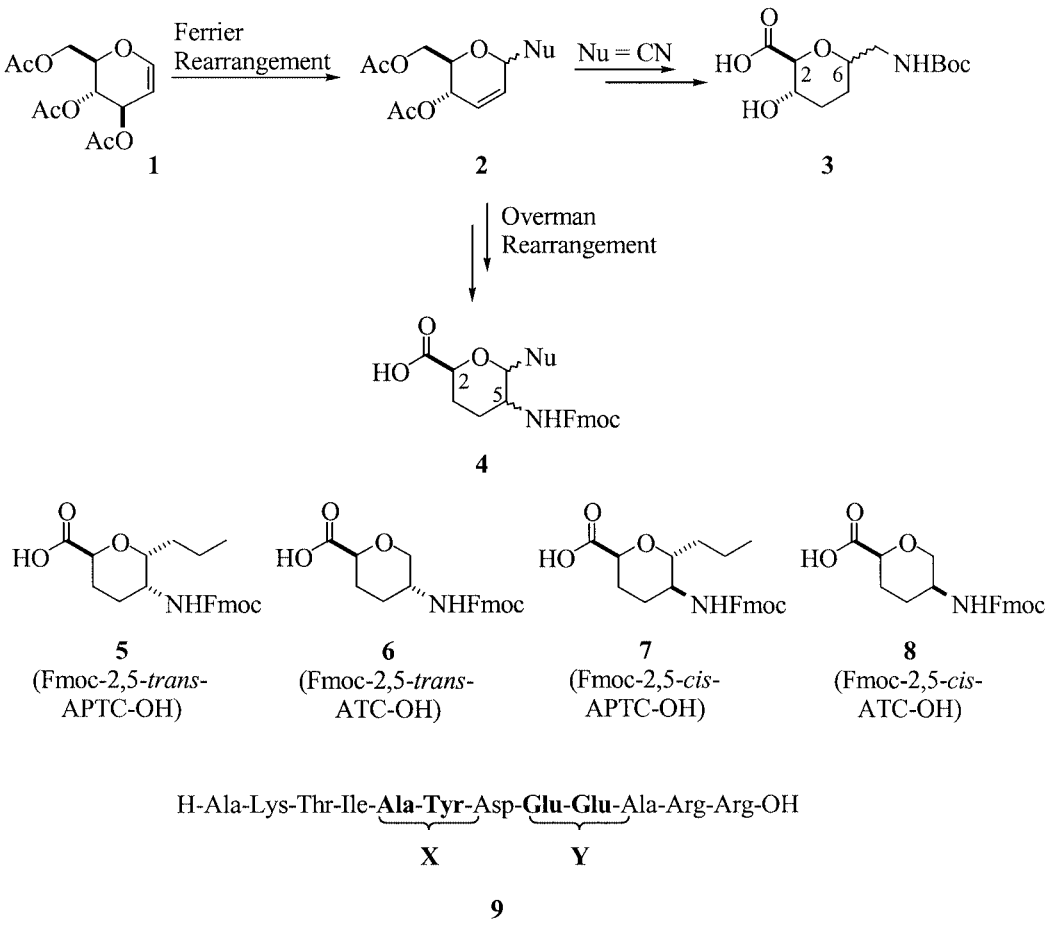

1 **2** **3** **4**

5 (Fmoc-2,5-*trans*-APTC-OH) **6** (Fmoc-2,5-*trans*-ATC-OH) **7** (Fmoc-2,5-*cis*-APTC-OH) **8** (Fmoc-2,5-*cis*-ATC-OH)

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

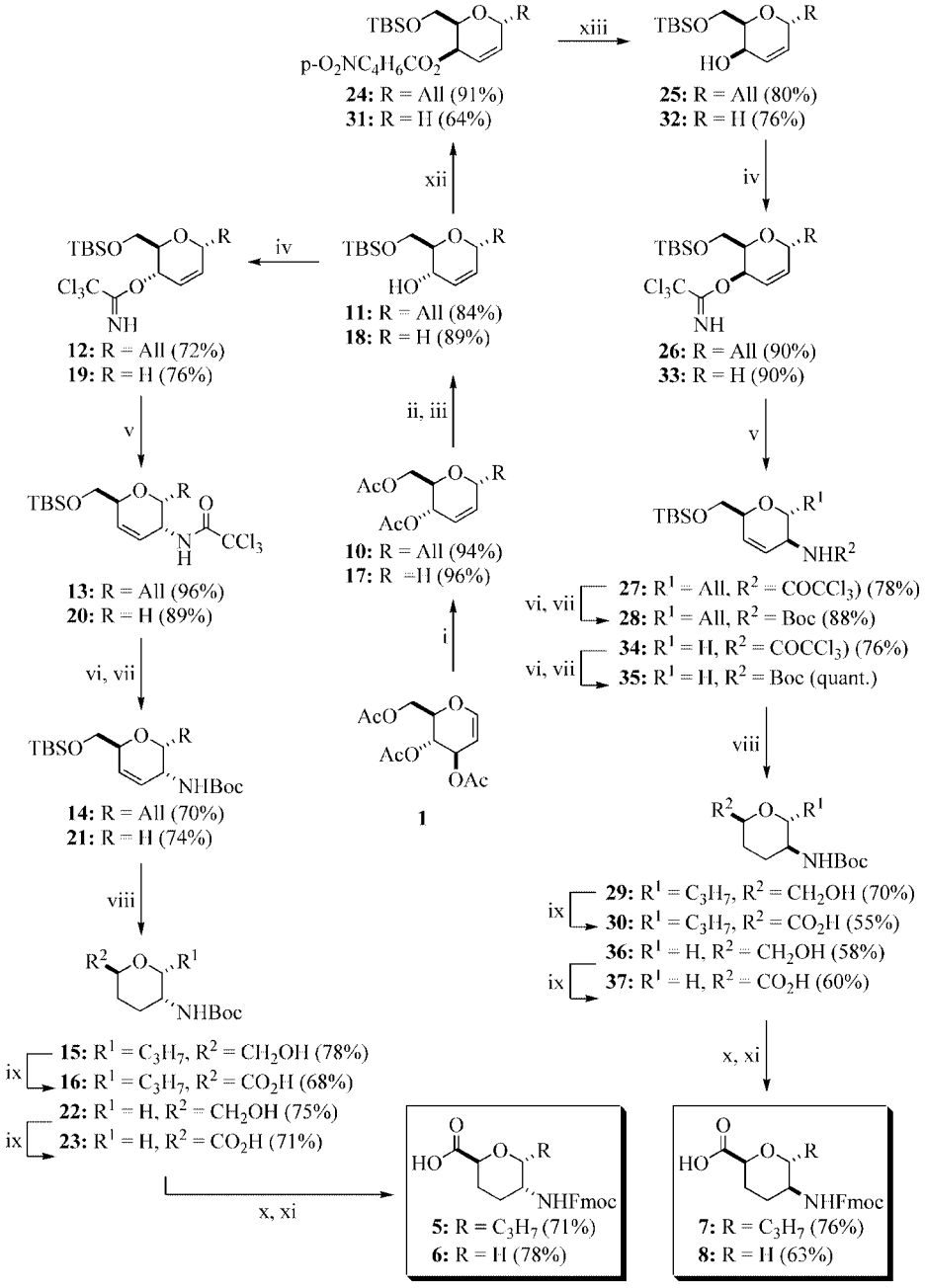
peptide **9**,[15] originating from heat shock protein 65 (hsp65) from *Mycobacterium tuberculosis* and *Mycobacterium leprae*, in which either the Ala–Tyr or the Glu–Glu dipeptide is substituted.

Results and Discussion

As the first research objective, the synthesis of 2,5-*trans* dipeptide isoster **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 $BF_3{\cdot}Et_2O$ 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 isoster **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 isosters **5** and **6**, we set out on the preparation of the corre-



Scheme 1. Synthesis of dipeptide isosters **5**, **6**, **7** and **8**
Reagents and conditions: (i) $BF_3{\cdot}Et_2O$, All−TMS (**10**) or triethylsilane (**17**), CH_2Cl_2, 0 °C; (ii) *t*BuOK, MeOH; (iii) TBS−Cl, pyridine; (iv) Cl_3CCN, DBU, CH_2Cl_2, 0 °C; (v) K_2CO_3, 1,2-DCB, Δ; (vi) KOH (3 equiv.), *i*PrOH; (vii) Boc_2O (1.2 equiv.), CH_2Cl_2; (viii) H_2, cat. Pd/C, MeOH; (ix) cat. $RuCl_3{\cdot}xH_2O$, $NaIO_4$ (4.5 equiv.), $CH_2Cl_2/H_2O/CH_3CN$ (1:1.5:1, v/v/v), 0 °C → room temp.; (x) TFA/CH_2Cl_2 (1:1, v/v); (xi) Fmoc−OSu (1.2 equiv.), 10% aq. $NaHCO_3$/1,4-dioxane (1:1, v/v), 0 °C → room temp.; (xii) *p*-$NO_2-C_6H_4CO_2H$, DEAD, PPh_3, THF; (xiii) KOH (1.5 equiv.), *i*PrOH.

sponding C-5 epimeric 2,5-*cis* dipeptide isosters **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, PPh_3, 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 isoster **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, subjection 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 isoster **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**.

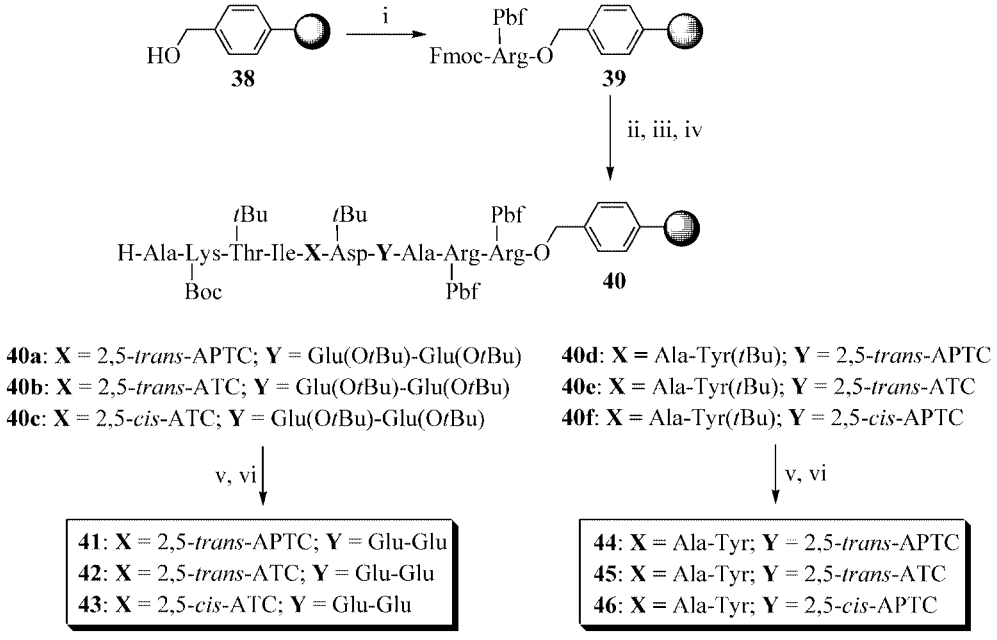
As the next research objective, the application of dipeptide isosters 2,5-*trans*-**5** and -**6** and 2,5-*cis*-**7** and -**8** in the solid-phase peptide synthesis of peptidomimetics **41**−**46** was undertaken (Scheme 2). Wang resin **38** was loaded with Fmoc−Arg(Pbf)−OH in the presence of *N*,*N*′-dicyclohexylcarbodiimide (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 isosters 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 isosters 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. Ac_2O/DiPEA/HOBt/NMP, room temp., 1 min; (iii) 20% piperidine in NMP, room temp., 5 min; (iv) TFA/TIS/H_2O (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 F_{254} 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 gL^{-1}) in 10% aq. sulfuric acid followed by charring, (b) aq. $KMnO_4$ (2% in aq. 1% K_2CO_3), 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 ^{13}C 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 ^{13}C NMR spectroscopy. The HPLC purifications were performed on a BioCAD "Vision" automated HPLC system with an Alltima C_{18} column (Alltech, 10.0 mm D × 250 mm L, 5µ particle size) with use of the following buffers: A: H_2O, B: CH_3CN, and C: 1% aq. TFA. The system was eluted at 4.0 $mLmin^{-1}$ and detection was performed at 214 nm unless stated otherwise. LC/MS analysis was performed on a Jacso HPLC system (detection simultaneously at 214 and 254 nm). An analytical Alltima C_{18} column (Alltech, 4.0 mm D × 250 mm L, 5µ particle size) was used. Buffers: A: H_2O, B: CH_3CN, 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).

(2*R*,3*S*,6*R*)-3-*O*-Acetyl-2-(acetyloxymethyl)-6-allyl-3,6-dihydro-2*H*-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 $NaHCO_3$ (10%, 30 mL) and EtOAc (150 mL). The layers were separated, and the organic layer was washed with water and brine, dried ($MgSO_4$), filtered, and concentrated. Purification (silica gel, toluene/EtOAc, 100:0 → 75:25, v/v) afforded **10** (7.7 g, 94%) as a colorless oil. ^{13}C NMR ($CDCl_3$): δ = 169.7 (2 × C=O acetyl), 132.3, 131.7 (C-4, C-5), 123.4 (=CH All), 116.9 (CH_2 All), 70.8, 62.9, 64.5 (C-2, C-3, C-6), 62.4 (CH_2O), 37.3 (CH_2 All), 20.1 (CH_3 acetyl) ppm. ^1H NMR ($CDCl_3$): δ = 5.92−5.71 (br. m, 3 H, 4-H, 5-H, =CH All), 5.14 (m, 2 H, =CH_2 All), 4.22 (m, 4 H, 2-H, 6-H, CH_2O), 3.90 (m, 1 H, 3-H), 2.37 (m, 2 H, CH_2 All), 2.01 (s, 6 H, 2 × CH_3 acetyl) ppm.

(2*R*,3*S*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-3,6-dihydro-3-hydroxy-2*H*-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 $NaHCO_3$ (10%) and brine. The organic phase was dried ($MgSO_4$), 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. ^{13}C NMR ($CDCl_3$): δ = 134.3, 129.7, 128.1 (C-4, C-5, =CH All), 117.0 (=CH_2 All), 71.8, 66.1, 65.1 (C-2, C-3, C-6), 64.4 (CH_2O), 37.7 (CH_2 All), 25.6 [(CH_3)$_3$C], 18.1 [(CH_3)$_3$*C*Si), −5.5 [(CH_3)$_2$Si] ppm. ^1H NMR ($CDCl_3$): δ = 5.95−5.74 (m, 3 H, 4-H, 5-H, =CH All), 5.11 (m, 2 H, =CH_2 All), 4.15 (m, 2 H, 2-H, 3-H), 3.90 (m, 1 H, 6-H), 3.81 (m, 2 H, CH_2O), 2.58−2.26 (m, 2 H, CH_2 All), 0.89 [s, 9 H, (CH_3)$_3$C], 0.06 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*R*,3*S*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2*H*-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 Cl_3CCN (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. NH_4Cl (10%, 50 mL). The layers were separated, the organic layer was washed with water and brine, dried (Na_2SO_4), 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. ^{13}C NMR ($CDCl_3$): δ = 161.5 (C=NH), 134.1, 133.1, 122.8 (C-4, C-5, =CH All), 117.2 (=CH_2 All), 91.3 (CCl_3), 72.1, 71.5, 69.5 (C-2, C-3, C-6), 62.5 (CH_2O), 37.7 (CH_2 All), 25.8 [(CH_3)$_3$C], 18.1 [(CH_3)$_3$*C*Si), −5.5 [(CH_3)$_2$Si] ppm. ^1H NMR ($CDCl_3$): 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, =CH_2 All), 4.30 (m, 1 H, 6-H), 3.92 (m, 1 H, 2-H), 3.81 (m, 2 H, CH_2O), 2.58−2.26 (m, 2 H, CH_2 All), 0.89 [s, 9 H, (CH_3)$_3$C], 0.06 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*R*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6*H*-pyran (13): A solution of imidate **12** (2.9 g, 6.8 mmol, dried by coevaporation with 1,4-dioxane) and K_2CO_3 (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. ^{13}C NMR ($CDCl_3$): δ = 161.4 (C=O), 133.4, 131.2, 125.0 (C-3, C-4, =CH All), 117.2 (=CH_2 All), 92.5 (CCl_3), 74.6, 71.7 (C-2, C-6), 63.7 (CH_2O), 45.9 (C-5), 35.6 (CH_2 All), 25.8 [(CH_3)$_3$C], 18.0 [(CH_3)$_2$*C*Si], −5.5 [(CH_3)$_2$Si] ppm. ^1H NMR (HHCOSY, 300 MHz, $CDCl_3$): 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, =CH_2 All), 4.34 (m, 2 H, 2-H, 5-H), 4.11 (m, 1 H, 6-H), 3.77 (m, 2 H, CH_2O), 2.33 (m, 2 H, CH_2 All), 0.89 [s, 9 H, (CH_3)$_3$C], 0.10 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*R*,6*R*)-6-Allyl-5-[(*tert*-butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilanyloxy)methyl]-2,5-dihydro-6*H*-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). ^{13}C NMR ($CDCl_3$): δ = 155.2 (C=O), 134.5, 129.0, 126.6 (C-3, C-4, =CH All), 116.4 (= CH_2 All), 78.7 (C_q Boc), 73.8 (C-6), 72.1 (C-2), 63.5 (CH_2O), 45.0 (C-5), 35.3 (CH_2 All), 28.0 [(CH_3)$_3$C Boc], 27.0 [(CH_3)$_3$C TBS], 17.8 [(CH_3)CSi], −5.8 [(CH_3)$_2$Si] ppm. ^{1}H NMR ($CDCl_3$): δ = 5.90 (m, 3 H, 3-H, 4-H, =CH All), 5.04 (m, 2 H, =CH_2 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, CH_2O), 2.25 (m, 2 H, CH_2 All), 1.48 [s, 9 H, (CH_3)$_3$C Boc], 0.84 [s, 9 H, (CH_3)$_3$C TBS], 0.04 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*R*,6*R*)-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 H_2, 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. ^{13}C NMR ($CDCl_3$): δ = 155.5 (C=O), 79.0 (C_q Boc), 72.3 (C-6), 70.2 (C-2), 62.7 (CH_2O), 48.5, 46.4 (C-5), 33.8 (CH_2 Pr), 29.1, 28.5, 24.2, 23.4 (C-3, C-4), 17.4 (CH_3CH_2), 13.5 (CH_3) ppm.

(2*S*,5*R*,6*R*)-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/CH_3CN/H_2O (1:1:1.45, v/v/v, 19 mL), and the mixture was cooled to 0 °C. With vigorous stirring, $NaIO_4$ (2.5 g, 11.7 mmol) and a catalytic amount of $RuCl_3 \cdot xH_2O$ (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 $NaIO_4$ was removed by addition of MeOH (5 mL), and the obtained suspension was filtered. A solution of aq. $NaHSO_3$ (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 ($MgSO_4$), filtered, and concentrated. The residue was dissolved in aq. $NaHCO_3$ (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 $MgSO_4$, filtered, and concentrated in vacuo. The obtained carboxylic acid **16** (0.48 g, 68%) was used in the next step without further purification. ^{13}C NMR ($CDCl_3$): 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 (CH_2 Pr), 28.9 [(CH_3)$_3$C], 25.8, 21.3 (C-3, C-4), 18.3 (CH_3CH_2), 13.9 (CH_3) ppm.

(2*S*,5*R*,6*R*)-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. $NaHCO_3$/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 ($MgSO_4$) and concentration, 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%). ^{13}C NMR ($CDCl_3$): 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 (CH_2 Fmoc), 47.4 (C-5), 47.0 (CH Fmoc), 25.1, 25.0 (C-3, C-4), 18.0 (CH_3CH_2), 13.7 (CH_3) ppm. ESI-MS: *m*/*z* = 410.0 $[M + H]^+$; 432.0 $[M + Na]^+$; 448.1 $[M + K]^+$.

(2*R*,3*S*)-3-*O*-Acetyl-2-(acetyloxymethyl)-3,6-dihydro-2*H*-pyran (17): Triethylsilane (7.7 mL, 40 mmol) and $BF_3 \cdot Et_2O$ (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 $NaHCO_3$ (1 M, 20 mL), the layers were separated, and the organic phase was washed with H_2O and brine, dried ($MgSO_4$), 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%). ^{13}C NMR ($CDCl_3$): 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, CH_2O), 20.0, 19.8 (CH_3 acetyl) ppm. ^{1}H NMR ($CDCl_3$): 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, CH_2O), 3.74 (m, 1 H, 2-H), 2.10 (m, 6 H, 2 × CH_3 acetyl) ppm.

(2*R*,3*S*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-3-hydroxy-3,6-dihydro-2*H*-pyran (18): Ferrier rearrangement product **17** (8.2 g, 38 mmol) was transformed into **18** (8.3 g, 89%) as described for **11**. ^{13}C NMR ($CDCl_3$): δ = 128.0, 127.0 (C-4, C-5), 77.3, 66.1 (C-2, C-3), 65.0 (C-6, CH_2O), 25.7 [(CH_3)$_3$C], 18.1 [(CH_3)$_3$C], −5.7 [(CH_3)$_2$Si] ppm. ^{1}H NMR ($CDCl_3$): 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, C*H*HO), 3.68 (m, 1 H, CH*H*O), 3.37 (m, 1 H, 2-H), 3.08 (br. s, 1 H, OH), 0.91 [s, 9 H, (CH_3)$_3$C], 0.12 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*R*,3*S*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2*H*-pyran (19): Alcohol **18** (2.4 g, 10 mmol) was treated with DBU (1.9 mL, 13 mmol) and Cl_3CCN (1.3 mL, 13 mmol) according to the procedure described for **12**. Silica gel column chromatography (light petroleum/EtOAc/Et_3N, 94:5:1 → 90:9:1, v/v/v) afforded pure **19** (3.0 g, 76%). ^{13}C NMR ($CDCl_3$): 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 (CH_2O), 25.8 [(CH_3)$_3$C], 18.3 [(CH_3)CSi], −5.4 [(CH_3)$_2$ Si] ppm. ^{1}H NMR ($CDCl_3$): 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, CH_2O), 3.69 (m, 1 H, 2-H), 0.89 [s, 9 H, (CH_3)$_3$C], 0.07 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*R*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6*H*-pyran (20): An Overman rearrangement of imidate **19** (2.0 g, 5.1 mmol) as described for **13** furnished trichloroacetamide **20** (1.8 g, 89%). ^{13}C NMR ($CDCl_3$): δ = 161.1 (C=O), 131.1, 124.4 (C-3, C-4), 73.9 (C-2), 64.9 (C-6), 64.1 (CH_2O), 44.7 (C-5), 25.5 [(CH_3)$_3$C], 17.9 [(CH_3)CSi], −5.7 [(CH_3)$_2$Si] ppm. ^{1}H NMR ($CDCl_3$): 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, CH_2O), 0.90 [s, 9 H, (CH_3)$_3$C], 0.08 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*R*)-5-[(*tert*-Butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilanyloxy)methyl]-2,5-dihydro-6*H*-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 [(CH_3)$_3$C Boc), 25.7 [(CH_3)$_3$C TBS), 18.1 [(CH_3)*C*Si], −5.5 [(CH_3)$_2$Si] ppm. ^{1}H 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, C*H*HO), 3.57 (m, 1 H, CH*H*O), 3.44 (m, 1 H, 2-H), 1.45 [(CH_3)$_3$C Boc), 0.89 [s, 9 H, (CH_3)$_3$C TBS), 0.06 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*R*)-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 [(CH_3)$_3$C] ppm. ^{1}H 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 × m, 4 H, 3a-H, 3b-H, 4a-H, 4b-H), 1.44 [s, 9 H, (CH_3)$_3$C] ppm.

(2*S*,5*R*)-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 [(CH_3)$_3$C Boc) ppm. ESI-MS: *m*/*z* = 246.1 [M + H]$^+$; 267.8 [M + Na]$^+$.

(2*S*,5*R*)-5-[*N*-(9-Fluorenylmethoxycarbonyl)amino]tetrahydropyran-2-carboxylic Acid (6): (2*S*,5*R*)-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 ($CDCl_3$/MeOD/D_2O): 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 [M + H]$^+$; 390.4 [M + Na]$^+$; 406.3 [M + K]$^+$.

(2*R*,3*R*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-3-*O*-(*p*-nitrobenzoyl)-3,6-dihydro-2*H*-pyran (24): A solution of DEAD (2.7 mL, 17.3 mmol) in THF (20 mL) was slowly added to a cooled (0 °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 °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 → 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 [(CH_3)$_3$C], 17.7 [(CH_3)*C*Si], −5.9 [(CH_3)$_2$ Si] ppm. ^{1}H 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, (CH_3)$_3$C], 0.08 (s, 3 H, CH_3Si), 0.06 (s, 3 H, CH_3Si) ppm.

(2*R*,3*R*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-3-hydroxy-3,6-dihydro-2*H*-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 → 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 [(CH_3)$_3$C], 17.8 [(CH_3)*C*Si], −5.7 [(CH_3)$_2$Si] ppm. ^{1}H 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, (CH_3)$_3$C], 0.09 (s, 3 H, CH_3Si), 0.06 (s, 3 H, CH_3Si) ppm.

(2*R*,3*R*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2*H*-pyran (26): Conversion of alcohol **25** (3.0 g, 10.7 mmol) into imidate **26** by the procedure described for **12** and purification by silica gel column chromatography (toluene/EtOAc, 99:1 → 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 [(CH_3)$_3$C], 18.1 [(CH_3)$_3$*C*Si], −5.4 [(CH_3)$_2$Si] ppm. ^{1}H 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, (CH_3)$_3$C], 0.05 (s, 3 H, CH_3Si), 0.03 (s, 3 H, CH_3Si) ppm.

(2*S*,5*S*,6*R*)-6-Allyl-2-[(*tert*-butyldimethylsilanyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6*H*-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 → 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 [(CH_3)$_3$C], 18.1 [(CH_3)$_3$*C*Si), −5.5 [(CH_3)$_2$Si] ppm. ^{1}H 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, (CH_3)$_3$C], 0.07 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*S*,6*R*)-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 [(CH_3)$_3$C Boc), 22.4, 21.1 (C-3, C-4), 17.8 (CH_2CH_3), 13.0 (CH_3) ppm.

(2*S*,5*S*,6*R*)-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 isoster **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 [(CH_3)$_3$C Boc), 23.3, 22.4 (C-3, C-4), 15.9 (CH_2CH_3), 11.3 (CH_3) ppm.

(2*S*,5*S*,6*R*)-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

($CDCl_3$): 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 (CH_2 Fmoc), 47.5 (C-5), 47.0 (CH Fmoc), 25.1, 25.0 (C-3, C-4), 17.9 (CH_3CH_2), 13.8 (CH_3) ppm. ESI-MS: *m*/*z* = 410.0 $[M + H]^+$; 432.0 $[M + Na]^+$; 448.1 $[M + K]^+$.

(2*R*,3*R*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-3-*O*-(*p*-nitrobenzoyl)-3,6-dihydro-2*H*-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. $NaHCO_3$ (10%, 75 mL), and the layers were separated. The organic layer was washed with brine (75 mL), dried ($MgSO_4$), 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%). ^{13}C NMR ($CDCl_3$): δ = 164.2 (C=O), 150.4 (C_q Ph−NO_2), 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 (CH_2O), 25.7 [(CH_3)$_3$C], 18.1 [(CH_3)*C*Si], −5.5 [(CH_3)$_2$Si] ppm.

(2*R*,3*R*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-3-hydroxy-3,6-dihydro-2*H*-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%). ^{13}C NMR ($CDCl_3$): δ = 129.5, 126.6 (C-4, C-5), 76.3, 61.9 (C-2, C-3), 65.7 (C-6), 62.4 (CH_2O), 25.6 [(CH_3)$_3$C], 18.1 [(CH_3)$_3$*C*Si], −5.5 [(CH_3)$_2$Si] ppm. ^1H NMR (HHCOSY, 400 MHz, $CDCl_3$): 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, CH_2O), 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, (CH_3)$_3$C], 0.59 (s, 3 H, CH_3Si), 0.53 (s, 3 H, CH_3Si) ppm.

(2*R*,3*R*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-3-*O*-(trichloroacetimidoyl)-3,6-dihydro-2*H*-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**. ^{13}C NMR ($CDCl_3$): 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 (CH_2O), 25.1 [(CH_3)$_3$C], 17.4 [(CH_3)$_3$*C*Si], −6.2 [(CH_3)$_2$Si] ppm. ^1H NMR ($CDCl_3$): 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, CH_2O), 0.88 [s, 9 H, (CH_3)$_3$], 0.06 (s, 3 H, CH_3Si), 0.04 (s, 3 H, CH_3Si) ppm.

(2*S*,5*S*)-2-[(*tert*-Butyldimethylsilanyloxy)methyl]-5-(trichloroacetamido)-2,5-dihydro-6*H*-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%). ^{13}C NMR ($CDCl_3$): δ = 160.1 (C=O), 132.8, 123.5 (C-3, C-4), 92.2 (CCl_3), 75.1 (C-2), 67.8, 64.9 (C-6, CH_2O), 44.9 (C-5), 25.7 [(CH_3)$_3$C], 18.2 [(CH_3)$_3$*C*Si], −5.5 [(CH_3)$_2$Si] ppm. ^1H NMR ($CDCl_3$): 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, CH_2O), 0.89 [s, 9 H, (CH_3)$_3$C], 0.06 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*S*)-5-[(*tert*-Butoxycarbonyl)amino]-2-[(*tert*-butyldimethylsilanyloxy)methyl]-2,5-dihydro-6*H*-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**. ^{13}C NMR ($CDCl_3$): 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, CH_2O), 43.7 (C-5), 28.1 [(CH_3)$_3$C Boc), 25.7 [(CH_3)$_3$C TBS), 18.2 [(CH_3)$_3$*C*Si], −5.6 [(CH_3)$_2$Si] ppm. ^1H NMR ($CDCl_3$): 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, CH_2O), 1.39 [s, 9 H, (CH_3)$_3$C Boc], 0.86 [s, 9 H, (CH_3)$_3$C TBS], 0.03 [s, 6 H, (CH_3)$_2$Si] ppm.

(2*S*,5*S*)-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%). ^{13}C NMR ($CDCl_3$): 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 [(CH_3)$_3$C], 27.0, 23.6 (C-3, C-4) ppm.

(2*S*,5*S*)-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**. ^{13}C NMR ($CDCl_3$): 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 (CH_2 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 μmol scale starting from Wang resin loaded with N^{α}-(9-fluorenylmethoxycarbonyl)-N^{ω}-2,2,4,6,7-pentamethyldihydrobenzofuran-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(O*t*Bu)−OH, Fmoc−Glu(O*t*Bu)−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/H_2O/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 × 15 mL). The resin was suspended in DCM (40 mL), N^{α}-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 × 15 mL), MeOH (15 mL), and DCM (2 × 15 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 (mmol/g) = (A × vol)/(7.8 × 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 → 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 min, ESI-MS: m/z = 1358.2 $[M + H]^+$; 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 → 17% B in 12.3 min, 2.5 CV) afforded 21.7 mg (26.4%, TFA salt). LC/MS analysis: R_t = 7.90 min, ESI-MS: m/z = 1316.0 $[M + H]^+$; 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 → 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 min, ESI-MS: m/z = 1316.2 $[M + H]^+$; 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 → 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 min, ESI-MS: m/z = 1334.1 $[M + H]^+$; 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, ± 25 μmol) 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 → 19%, in 14.7 min, 3.0 CV) gave **45** (11.46 mg, 28%, TFA salt). LC/MS analysis: R_t = 10.68 min, ESI-MS: m/z = 1291.9 $[M + H]^+$; 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, ±29 μmol) was purified by RP HPLC (linear gradient 14 → 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 min, ESI-MS 1334.0 $[M + H]^+$; 667.5 $[M + 2H]^{2+}$; calcd. 1332.8.

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