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Ring-closing metathesis for the synthesis of side chain knotted pentapeptides inspired by vancomycin

Hefziba T. ten Brink, Dirk T. S. Rijkers, Johan Kemmink, Hans W. Hilbers and Rob M. J. Liskamp*
Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, Utrecht University, P.O. Box 80082, 3508 TB Utrecht, The Netherlands. E-mail: R.M.J.Liskamp@pharm.uu.nl; Fax: +31 30 2536655; Tel: +31 30 2537396

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A versatile method for the synthesis of bicyclic side chain knotted peptides inspired by vancomycin is described. The synthetic approach is based on the incorporation of a central amino acid derivative **3** having two allylic groups—introduced by a Stille coupling—into pentapeptide **8** containing two allylated serine residues. Treatment of this bis-ring-closing metathesis precursor with 2nd generation Grubbs catalyst results in the formation of a bicyclic pentapeptide with the correct side chain to side chain connectivity pattern as observed in vancomycin: $i - 2 \rightarrow i, i \rightarrow i + 2$. Modelling studies using MacroModel hint at a cavity-like structure of the bicyclic pentapeptide which may bind suitable ligands.

Introduction

Nature has found many intriguing and elegant ways to reduce the flexibility of peptides in order to control shape. Reduction of the flexibility by disulfide bridges is the most well-known way to control the three dimensional structure.[1] One of the most sophisticated ways is probably "multiple side chain knotting", in which a number of side chains are tied together in such a way that conformational mobility is minimised. Outstanding examples in this respect are the glycopeptide antibiotics of which vancomycin is an important representative[2,3] (Fig. 1).

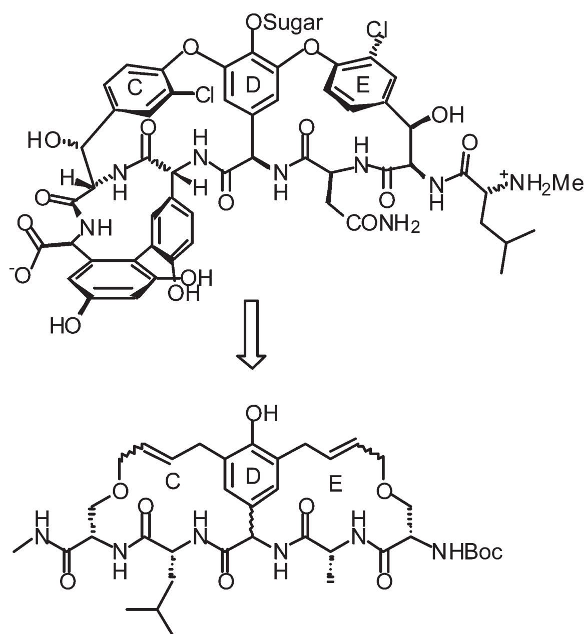

Fig. 1 Target bicyclic peptide as inspired by the vancomycin CDE ring system.

We are interested in applying selective C–C-coupling reactions in order to pre-organise peptides and ultimately to achieve control over their conformation. So far we have employed ring-closing metathesis (RCM) for this purpose[4] and other approaches for selective C–C-bond forming reactions to constrain the structure of peptides such as Sonogashira and Heck reactions are currently being explored in our laboratory.[5]

In the previously reported applications concerning ring-closing metathesis one might consider the alkene resulting from the metathesis reaction as a stable alternative of the disulfide bridge.[6] Here we wish to report the results of ring-closing metathesis towards the construction of a "multiple side chain knotted" system as is present in vancomycin. One of the aims is to develop vancomycin mimics, possibly possessing cavity or shell-like structures, which—because of their pre-organisation—may tightly bind other molecules. In the case of vancomycin mimics this may be D-Ala-D-Ala or D-Ala-D-lactate[7,8] but the variations of the building blocks used in the synthesis of our molecular constructs including combinatorial approaches might also allow the development of molecules capable of binding other biologically relevant targets.

So far only "*monocyclic*"—representing the right or the left macrocycle—mimics of vancomycin have been prepared.[9–11] Here we present a convenient approach which, for the first time, allows construction of the "*bicyclic* multiple side chain knotted" framework, featuring a Stille coupling[12] followed by tandem ring closing metathesis[13,14] (Scheme 1).

Results and discussion

An important design consideration in the synthesis of our target bicyclic pentapeptide **9** was the right choice of the central amino acid residue in the ring-closing metathesis precursor. This residue should possess two allylic groups, one for the macrocycle to be localised on the right and one for the macrocycle on the left hand side of the bicyclic target molecule. For this purpose, 4-hydroxyphenylglycine turned out to be a suitable starting compound (Scheme 1). After proper protection, the resulting amino acid derivative was easily iodinated on both the 3- and 5-positions by treatment with *N*-iodosuccinimide in acetone[15] to give **1**. In order to obtain good yields in the C–C-coupling reaction in which both iodine atoms were substituted, the hydroxyl function had to be converted into an electron withdrawing substituent by conversion into acetate **2** using acetic anhydride. Now **2** was subjected to a Stille coupling[12] with allyltributylstannane and $Pd(PPh_3)_2Cl_2$ in DMF at 100 °C to give **3** in 96% yield. Unfortunately, **3** was obtained as a racemate possibly due to the high reaction temperature. However, when the Stille coupling was carried out at a lower reaction temperature, racemization was not prevented but also resulted in a complex reaction mixture of mono- and dialkylated products, which was

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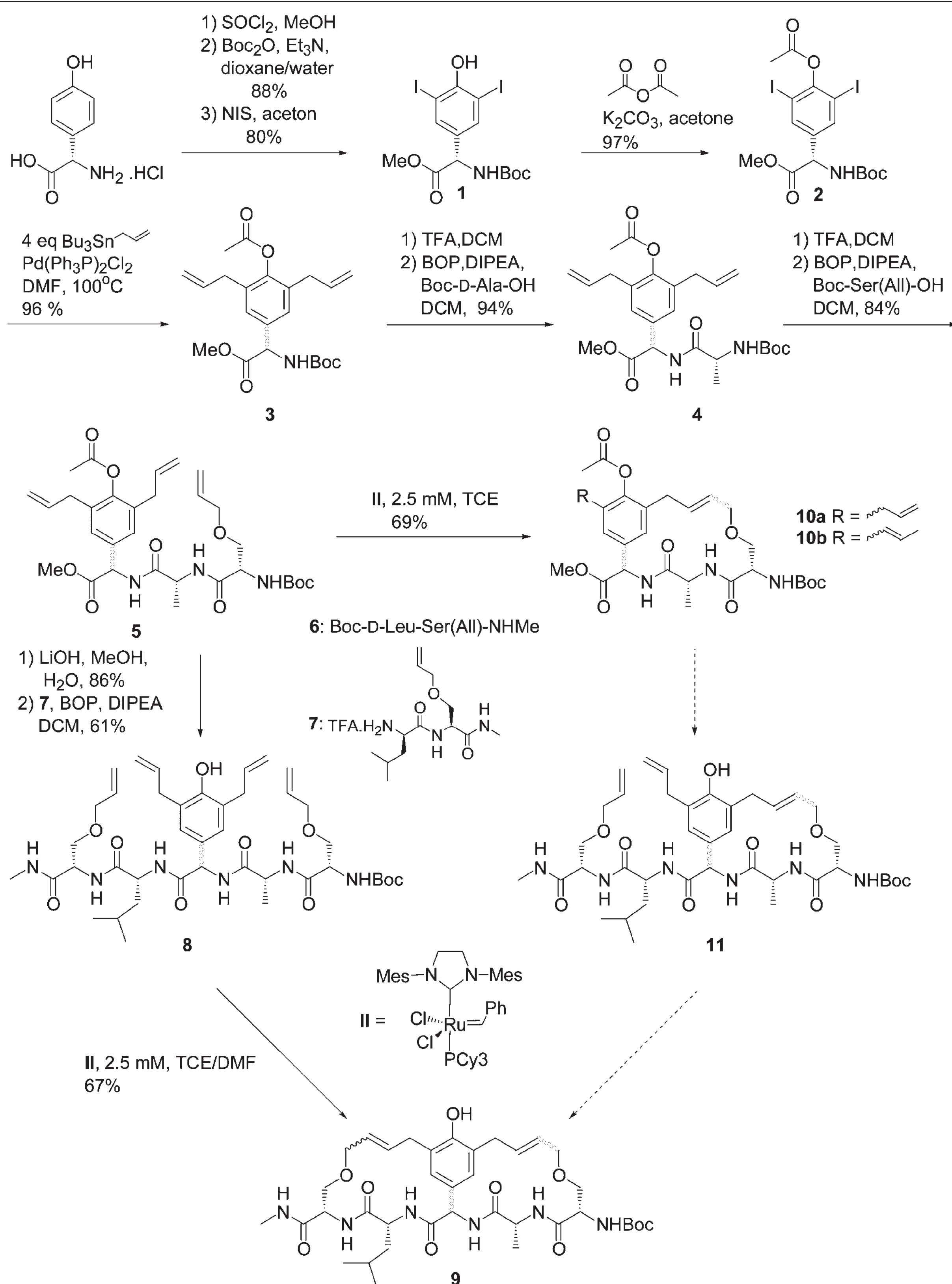

Scheme 1 Synthesis of bicylic pentapeptide **9** *via* a tandem ring-closing metathesis.

difficult to purify. Apparently, the temperature was not the only factor causing racemization and perhaps other factors include decomposition of DMF and basicity of the organometallic reagent or the racemization-proneness of the phenylglycine-derived ester. Nevertheless, bisallyl derivative **3** was treated with TFA to remove the Boc group followed by coupling of Boc-D-Ala-OH in the presence of BOP/DIPEA to afford dipeptide **4** in 94% yield. TFA treatment was repeated and the obtained TFA-salt was subjected to BOP-coupling with Boc-Ser(All)-OH[16] to yield tripeptide **5** (84%). In the first approach towards the synthesis of bicyclic pentapeptide **9**, tripeptide **5** was treated with 2nd generation Grubbs catalyst[17] in DCM in order to construct **10a** containing the macrocycle on the right. Unfortunately, treatment of cyclization precursor **5** resulted not only in the desired macrocycle **10a** but also the isomerized product **10b** was isolated as a major side product in a total yield of 69%.[18] This isomerization reaction[19] could be suppressed by the addition of hydride scavengers,[4b] albeit not completely and it was difficult to separate the isomerized product **10b** from the desired product **10a**. Therefore, this route was abandoned and it

was decided to complete the synthesis of the pentapeptide first and then attempt tandem ring-closing metathesis.

Thus, tripeptide **5** was saponified followed by BOP-coupling with dipeptide **7** to give pentapeptide **8** in 52% yield (two steps). Treatment of ring-closing metathesis precursor **8** with 2nd generation Grubbs catalyst in TCE was carried out in the presence of a small amount DMF because of the poor solubility of **8**. In contrast to most RCM reactions a stoichiometric amount of catalyst was required since the used DMF deactivated part of the catalyst by coordination to ruthenium.[20] The cyclization reaction was monitored by mass spectrometry, since the differences in R_f-values of **8**, the monocyclic intermediates and the desired bicyclic product **9** were too small. Monitoring by HPLC was too slow because the reaction turned out to be complete within 10 min. The cyclization proceeded *via* a monocyclic intermediate with a molecular mass of 771 Da. During the course of the reaction, this peak disappeared completely and a new peak at $m/z = 743$ Th appeared, corresponding to the mass of bicyclic product **9**. After workup this bicyclic peptide **9** was isolated in 67% yield. In principle, a mass value of 771 Da could also be due to formation of a monocyclic intermediate in which one allyl functionality was isomerized. However, this intermediate was unlikely, since this would probably not be converted to bicyclic pentapeptide **9**.

Unfortunately, further structural characterisation by ^{1}H and ^{13}C NMR (COSY, TOCSY, HMBC, HSQC) was rather difficult since the product is probably composed of eight possible stereoisomers: *E*/*Z* for each of the double bonds and *R*/*S* for the central phenylglycine derivative. However, the mass data were very unambiguous since HR-MS and MALDI-TOF analyses confirmed the molecular mass to be $(M + H)^+$ 743.39 Da, thus any cyclic dimers could be rigorously excluded. Moreover, a strong long-range coupling between the ~O–C$\underline{H}_2$–CH= and the =CH–C$\underline{H}_2$-arom could be observed in a TOCSY spectrum which is characteristic for the correct side chain to side chain connectivity pattern. Thus, the NMR and mass data were unambiguous and other possibilities for ring-closing metathesis could be excluded.

In order to get some qualitative insight into the 3D-structure of bicyclic peptide **9**, the eight possible stereoisomers were subjected to a Monte Carlo conformational search procedure in combination with molecular mechanics calculations using MacroModel[21] to find the global minimum energy conformation of each stereoisomer. Modelling experiments suggest that the bicyclic peptide **9** possessing the *Z*,*S*,*Z* configuration was the isomer with the lowest energy. Assuming for the time being that this stereoisomer was formed in excess, its 3D-structure is depicted in Fig. 2, showing the cavity-like appearance, which might host suitable ligands.

In conclusion, a bicyclic macrocyclic ring-system inspired by the multiple side chain knotted structure of vancomycin was constructed by tandem ring-closing metathesis, thereby providing access to many peptidic systems where more control of the three dimensional structure is required or at least beneficial for *e.g.* binding properties. Furthermore, the synthetic route is amenable to combinatorial approaches since a variety of other amino acids can be introduced, variation of the stereochemistry of these building blocks is possible and a solid phase procedure is within reach.

Material and methods

General

Chemicals were obtained from commercial sources and used without further purification, unless stated otherwise. DIPEA was distilled consecutively from ninhydrin and KOH. Other dry solvents were obtained as peptide grade solvents from Biosolve and were stored on molecular sieves (4 Å). ^{1}H NMR spectra were recorded on a Varian G-300 (300 MHz) or a Varian INOVA-500 (500 MHz) spectrometer and chemical shifts are given in ppm (δ) relative to TMS or DMSO-d_6 (2.39 ppm).

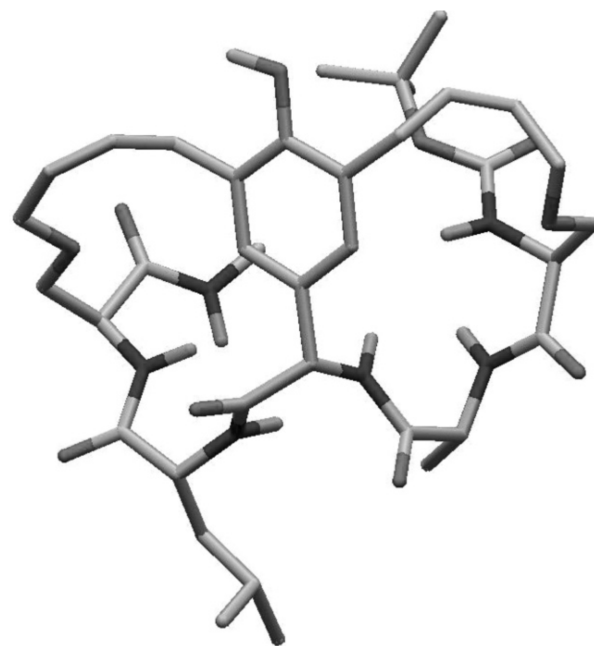

Fig. 2 Lowest energy conformation of bicyclic peptide **9**.

Peak assignments are based on ^{1}H NMR COSY spectra. ^{13}C NMR spectra were recorded on a Varian G-300 (75.5 MHz) or a Varian INOVA-500 (125 MHz) spectrometer and chemical shifts are given in ppm relative to $CDCl_3$ (77.0 ppm). The ^{13}C NMR spectra were recorded using the attached proton test (ATP) sequence (G-300) or using HMBC and HSQC sequences (I-500). Electrospray ionization mass spectrometry (EI-MS) was measured on a Shimadzu LCMS-QP8000 single quadruple bench-top mass spectrometer operating in a positive ionization mode. Analytical HPLC runs were performed on Shimadzu automated HPLC system equipped with a UV/VIS detector operating at 220/254 nm and an evaporative light scattering detector (Polymer Laboratories ELS 1000) on an Alltech Adsorbosphere C8 column (particle size 5 µm, pore size 90 Å, 250 × 4.6 mm) at a flow rate of 1 mL min^{-1} using a linear gradient from 100% buffer A (0.1% TFA in water) to 100% buffer B (0.085% TFA in acetonitrile/water 95 : 5 v/v) in 40 min. High resolution mass spectra (HR-MS) were measured on a Micromass Q-TOF hybrid mass spectrometer, with pentaphenylalanine as reference. MALDI-TOF analysis was performed on a Kratos Axima CFR apparatus, with bradykinin(1–7) as external reference and α-cyano-4-hydroxycinnamic acid as matrix. Optical rotations were measured at 20 °C using a Jasco P-1010 polarimeter. Elemental analyses were performed by Kolbe Microanalytisches Labor (Mülheim an der Ruhr, Germany). R_f values were determined by thin layer chromatography (TLC) on Merck precoated silica gel 60 F_{254} glass plates. Spots were visualized by UV quenching, ninhydrin or Cl_2/TDM.[22] Column chromatography was performed on ICN Silica 60 Å (70–230 mesh).

***S*-N^α-(*tert*-Butyloxycarbonyl)-4-hydroxy-3,5-diiodo-phenylglycine methyl ester (1).** $SOCl_2$ (6 mL) was carefully added to MeOH (60 mL) at 0 °C and to this mixture *S*-4-hydroxy-phenylglycine (5 g, 29.9 mmol) was added portionwise. After refluxing for 4 h, the reaction was complete according to TLC and the solvent was evaporated *in vacuo.* Then, the crude product was dissolved in H_2O/dioxane (100 mL, 1 : 1 v/v), Boc_2O (6.52 g, 29.9 mmol) was added and TEA was added portionwise to keep the pH at 8.5. After stirring for 5 h the reaction was complete according to TLC and the solvents were removed at reduced pressure. The residue was dissolved in EtOAc and subsequently washed with 1 N $KHSO_4$, 1 M $NaHCO_3$, brine and dried (Na_2SO_4). Ethyl acetate was removed under reduced pressure and *S*-N^α-(*tert*-butyloxycarbonyl)-4-hydroxy-phenylglycine methyl ester was obtained as a white solid in 88% yield (7.39 g) after crystallization from hexane/EtOAc. R_f(2 : 1

EtOAc/hexane): 0.73; δ_H 1.44 (9H, s, Boc), 3.70 (3H, s, OMe), 5.22 (1H, d, αCH), 5.65 (1H, d, NH), 6.73 (2H, d, arom H), 7.01 (1H, broad s, OH), 7.15 (2H, d, arom H); δ_C 28.3, 52.7, 57.0, 80.6, 115.8, 127.9, 128.4, 155.1, 156.4, 172.0. This Boc-protected ester (1.5 g, 5.3 mmol) was dissolved in acetone (60 mL). The mixture was cooled to −79 °C and the the mixture was protected from light by aluminium foil. To this mixture a solution of *N*-iodosuccinimide (2.64 g, 11.74 mmol) in acetone (30 mL) was added dropwise over 5 h at −79 °C.[15] After stirring overnight the reaction was complete according to TLC and the solvent was evaporated *in vacuo.* The residue was dissolved in EtOAc, washed with a saturated solution of $Na_2S_2O_3$, H_2O, brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and the diiodo compound **1** was obtained as a yellowish solid (2.24 g, 80%) after purification by column chromatography (1 : 4 acetone/hexane). R_f(1 : 2 EtOAc/hexane): 0.76; $[\alpha]_D$ +100 (*c* 1, $CHCl_3$); δ_H 1.43 (9H, s, Boc), 3.74 (3H, s, OMe), 5.18 (1H, d, αCH), 5.67 (1H, d, NH), 5.95 (1H, broad s, OH), 7.66 (2H, s, 2-H, and 6-H); δ_C 28.2, 53.0, 55.5, 80.5, 82.5, 131.9, 137.8, 153.7, 154.5, 170.8.

***S*-*N*^α^-(*tert*-Butyloxycarbonyl)-4-acetoxy-3,5-diiodo-phenylglycine methyl ester (2).** Diiodo compound **1** (4.3 g, 8 mmol) was dissolved in acetone (100 mL) in the presence of K_2CO_3 (1.67 g, 12.1 mmol) and acetic anhydride (1.06 mL, 11.2 mmol) was added dropwise and the obtained reaction mixture was stirred for 2 h. The solvent was evaporated *in vacuo* and the residue was dissolved in EtOAc and the organic layer was washed with 1 N $KHSO_4$, 1 M $NaHCO_3$, brine and dried (Na_2SO_4). The solvent was removed under reduced pressure and acetate **2** was obtained as a white solid (1.63 g, 97%) after purification by column chromatography (1 : 2 EtOAc/hexane). R_f(1 : 4 acetone/hexane): 0.63; $[\alpha]_D$ +59 (*c* 1, $CHCl_3$); δ_H 1.44 (9H, s, Boc), 2.43 (3H, s, CH_3), 3.76 (3H, s, OMe), 5.24 (1H, d, αCH), 5.67 (1H, d, NH), 7.78 (2H, s, 2-H/6-H); δ_C 21.3, 28.2, 53.2, 55.6, 80.7, 90.7, 138.2, 138.3, 151.6, 154.5, 167.3, 170.3; ES-MS calcd for $C_{16}H_{19}I_2NO_6$: 575; found: *m*/*z* $[M + Na]^+$ 598; Anal. calcd for $C_{16}H_{19}I_2NO_6$: C, 33.41; H, 3.33; N, 2.44; found: C, 33.35; H, 3.47; N, 2.45%.

***RS*-*N*^α^-(*tert*-Butyloxycarbonyl)-4-acetoxy-3,5-bisallyl-phenylglycine methyl ester (3).** In a flame-dried nitrogen filled flask, acetate **2** (723 mg, 1.26 mmol) was dissolved in DMF (14 mL). After addition of allyltributylstannane (1.53 mL, 5 mmol) the mixture was heated to 100 °C under a weak nitrogen flow. Then $Pd(PPh_3)_2Cl_2$ (88 mg, 0.13 mmol) was added. After 2 h the reaction was complete according to TLC and the solvent was evaporated *in vacuo.* The residue was dissolved in EtOAc, and the solution was washed with dilute ammonia, brine and dried (Na_2SO_4). After removal of EtOAc under reduced pressure, bisallyl compound **3** was obtained as a yellowish oil (487 mg, 96%) after purification by column chromatography (hexane → 1 : 4 acetone/hexane). R_f(1 : 4 acetone/hexane): 0.43; HPLC showed that the product was 99% pure (based on both enantiomers) by ELSD; δ_H (300 MHz, $CDCl_3$) 1.43 (9H, s, Boc), 2.29 (3H, s, CH_3), 3.24 (4H, d, 3-CH_2/5-CH_2), 3.71 (3H, s, OMe), 5.04–5.11 (4H, m, CH_2-allyl), 5.26 (1H, d J = 6 Hz, αCH), 5.49 (1H, d J = 6 Hz, NH), 5.78–5.90 (2H, m, CH-allyl), 7.10 (2H, s, 2-H/6-H); δ_C (75.5 MHz, $CDCl_3$) 20.6, 28.2, 35.8, 52.7, 57.0, 80.2, 116.6, 127.1, 133.1, 134.4, 135.4, 147.5, 154.7, 168.9, 171.5; ES-MS calcd for $C_{22}H_{29}NO_6$: 403; found: *m*/*z* $[M + Na]^+$ 426; HR-MS $[M + NH_4]^+$ found: *m*/*z* 421.2343, calcd 421.2339.

***N*^α^-(*tert*-Butyloxycarbonyl)-D-alanyl-*RS*-4-acetoxy-3,5-bisallyl-phenylglycine methyl ester (4).** TFA (5 mL) was added to bisallyl compound **3** (560 mg, 1.39 mmol) dissolved in DCM (25 mL). After 2 h deprotection was complete as judged by TLC and the reaction mixture was evaporated to dryness and subsequently coevaporated with DCM (twice) to remove any residual TFA. The crude product was dissolved in DCM (50 mL) and to this solution BOP (633 mg, 1.43 mmol), Boc-D-Ala-OH (270 mg, 1.43 mmol) and DIPEA were added until pH 8.5 was reached. The coupling was complete after 2 h and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with 1 N $KHSO_4$, 1 M $NaHCO_3$, brine and dried (Na_2SO_4). Ethyl acetate was removed under reduced pressure and dipeptide **4** was obtained as an oil (620 mg, 94%) which slowly solidified (after purification by column chromatography (1 : 2 EtOAc/hexane → 1 : 1 EtOAc/hexane)). R_f(1 : 1 EtOAc/hexane): 0.89; HPLC showed that the product was 100% pure (based on two diastereomers) as judged by ELSD; δ_H (300 MHz, $CDCl_3$) 1.35–1.37 (3H, t, βCH_3-Ala), 1.42 and 1.45 (9H, double s, Boc), 2.30 (3H, s, CH_3), 3.24 and 3.38 (4H, 2 × d, 3-CH_2/5-CH_2), 3.71 and 3.73 (3H, double s, OMe), 4.24 (1H, broad s, αCH-Ala), 5.04–5.12 (5H, m, CH_2-allyl/NH-urethane), 5.50 and 5.78 (1H, d, αCH), 5.78–5.92 (1H, m, CH-allyl), 7.09 and 7.10 (3H, 2 × s, 2-H/6-H/NH-amide); δ_C (75.5 MHz, $CDCl_3$) 18.1, 20.6, 28.2, 34.9, 49.8, 52.8, 55.8, 80.2, 116.7, 127.2, 133.2, 133.8, 135.3, 136.9, 147.6, 168.8, 170.9, 171.3; ES-MS calcd for $C_{25}H_{34}N_2O_7$: 474; found: *m*/*z* $[M + H]^+$ 475, $[M + Na]^+$ 497, $[(M - C_4H_8) + H]^+$ 419, $[(M - C_5H_8O_2) + H]^+$ 375; HR-MS $[M + H]^+$ found: *m*/*z* 475.2437, calcd 475.2444; Anal. calcd for $C_{25}H_{34}N_2O_7$: C, 63.27; H, 7.22; N, 5.90; found: C, 63.19; H, 7.24; N, 5.79%.

***N*^α^-(*tert*-Butyloxycarbonyl)-seryl(allyl)-D-alanyl-*RS*-4-acetoxy-3,5-bisallyl-phenylglycine methyl ester (5).** Dipeptide **4** (590 mg, 1.24 mmol) was dissolved in DCM (25 mL) and TFA (5 mL) was added. After 2 h the deprotection was complete as judged by TLC and the reaction mixture was evaporated to dryness and subsequently coevaporated with DCM (twice) to remove any residual TFA. The crude product was dissolved in DCM (50 mL) and to this solution were added BOP (606 mg, 1.37 mmol), Boc-Ser(All)-OH (335 mg, 1.37 mmol) followed by DIPEA until pH 8.5 was reached. The coupling was complete after 2 h and the solvent was evaporated *in vacuo.* The residue was dissolved in EtOAc and the solution was washed with 1 N $KHSO_4$, 1 M $NaHCO_3$, brine and dried (Na_2SO_4). Subsequently, ethyl acetate was removed under reduced pressure. Tripeptide **5** was obtained as a colorless oil (626 mg, 84%) which crystallized after purification by column chromatography (1 : 2 EtOAc/hexane → 1 : 1 EtOAc/hexane). R_f(1 : 1 EtOAc/hexane): 0.37; HPLC showed that the product was 100% pure (based on two diastereomers) as judged by ELSD; δ_H (300 MHz, $CDCl_3$) 1.36–1.41 (3H, t, βCH_3-Ala), 1.44 (9H, s, Boc), 2.29 (3H, s, CH_3), 3.23 (4H, d, 3-CH_2/5-CH_2), 3.47–3.56 (1H, m, βCH_2-Ser (1H)), 3.70 and 3.72 (3H, double s, OMe), 3.77–3.91 (3H, m, ~O-CH_2-allyl/βCH_2-Ser (1H)), 4.27 (1H, broad s, αCH-Ser), 4.50–4.53 (1H, m, αCH-Ala), 5.05–5.24 (6H, m, CH_2-allyl), 5.41 (1H, broad s, NH-urethane), 5.45–5.49 (1H, m, αCH), 5.74–5.91 (3H, m, CH-allyl), 6.92 (1H, d, NH-amide (Ala)), 7.08 (2H, s, 2-H/6-H), 7.08 and 7.24 (1H, 2 × d, NH-amide); δ_C (75.5 MHz, $CDCl_3$) 17.9, 18.1, 28.1, 34.8, 48.6, 52.7, 54.2, 55.7, 55.9, 69.4, 72.0, 80.2, 116.6, 117.5, 127.1, 127.2, 133.1, 133.6, 133.7, 135.3, 147.5, 147.6, 155.3, 168.8, 170.2, 170.3, 170.8, 171.2; ES-MS calcd for $C_{31}H_{43}N_3O_9$: 601; found: *m*/*z* $[M + H]^+$ 602, $[M + Na]^+$ 624, $[(M - C_4H_8) + H]^+$ 546, $[(M - C_5H_8O_2) + H]^+$ 502; HR-MS $[M + H]^+$ found: *m*/*z* 602.3065, calcd 602.3077; Anal. calcd for $C_{31}H_{43}N_3O_9$: C, 61.88; H, 7.20; N, 6.98; found: C, 61.98; H, 7.34; N, 6.78%.

Monocyclic tripeptide (10a and 10b). Tripeptide **5** (100 mg, 0.166 mmol) was dissolved in TCE (66 mL) to obtain a final concentration of 2.5 mM. The solution was purged with nitrogen gas (25 min) and heated until reflux followed by the addition Ru catalyst (2nd generation Grubbs; 14 mg, 17 µmol). After 10 min ring-closing was complete according to TLC and EI-MS. Subsequently, the solvent was removed under reduced pressure and the residue was purified by column chromatography (2 : 1 EtOAc/hexane). The product was obtained as a mixture of **10a** and **10b** (1 : 1) as a colorless oil (66 mg, 69%). R_f(1 : 1 EtOAc/hexane): 0.11; δ_H (500 MHz, $CDCl_3$), for clarity the two different products are assigned together, only the allyl

function of the aromate in **10a** and **10b** are indicated separately, 1.35–1.49 (12H, m, Boc/βCH$_3$-Ala), 1.89 and 1.96 (1.5 H, double s, CH_3-CH = (**10b**)), 2.28–2.41 (3H, m, CH$_3$), 3.10–3.26 (3H, m, 3-CH$_2$/5-CH$_2$), 3.52–3.59 (1H, m, βCH$_2$-Ser (1H)), 3.68–3.81 (4H, m, OMe/~O-CH$_2$-allyl (1H)), 4.05 (2H, m, ~O-CH$_2$-allyl (1H)/βCH$_2$-Ser (1H)), 4.65 (1H, broad s, αCH-Ala), 5.08–5.11 (1H, m, CH$_2$-allyl (**10a**)), 5.30–5.80 (4H, m, -C*H*=C*H*-/NH-urethane/αCH), 5.85 (0.5 H, m, CH-allyl (**10a**)), 6.29 (1H, m, 2 × CH-allyl (**10b**)), 6.75 (1H, broad s, NH-amide (Ala)), 6.97–7.41 (3H, m, 2-H/6-H/NH-amide); ES-MS calcd for $C_{29}H_{39}N_3O_9$: 573, found: *m*/*z* $[M + H]^+$ 574, $[M + Na]^+$ 596, $[(M - C_4H_8) + H]^+$ 518, $[(M - C_5H_8O_2) + H]^+$ 474; HR-MS $[M + H]^+$ found: *m*/*z* 574.2724, calcd 574.2764.

***N*$^\alpha$-(*tert*-Butyloxycarbonyl)-D-leucyl-serine(allyl) methyl amide (6).** Boc-Ser(All)-OH (1.02 g, 4.65 mmol) was dissolved in DCM (50 mL). To this solution BOP (2.06 g, 4.65 mmol) and DIPEA (813 µL, 5.12 mmol) were added, followed by the addition of methylamine (0.8 M in THF, 577 µL, 4.65 mmol). After 2 h of stirring at room temperature the amide formation was complete according to TLC and the solvent was evaporated *in vacuo.* The residue was dissolved in EtOAc and this solution was washed with 1 N $KHSO_4$, 1 M $NaHCO_3$, brine and dried (Na_2SO_4). Ethyl acetate was removed under reduced pressure and after purification by column chromatography (DCM → 95:5 DCM/MeOH) Boc-Ser(allyl)-NHMe was obtained as a white solid (0.90 g, 75%). R_f(2 : 1 EtOAc/hexane): 0.43; δ_H (300 MHz, $CDCl_3$) 1.45 (9H, s, Boc), 2.83 (3H, d, NHCH_3), 3.51–3.57 (1H, m, βCH$_2$-Ser (1H)), 3.81 and 3.86 (1H, dd, βCH$_2$-Ser (1H)), 4.01 (2H,d, ~O-CH$_2$-allyl), 4.26 (1H, bs, αCH-Ser), 5.16–5.29 (2H, m, CH$_2$-allyl), 5.51 (1H, d, NH-urethane), 5.82–5.91 (1H, m, CH-allyl), 6.67 (1H, broad s, N*H*Me); δ_C ($CDCl_3$, 75.5 MHz) 26.2, 28.2, 53.8, 69.6, 72.0, 80.0, 117.3, 133.9, 155.4, 170.8.

Boc-Ser(All)-NHMe (254 mg, 0.98 mmol) was dissolved in DCM (3 mL) and 6 N HCl in diethyl ether (10 mL) was added. After 10 min Boc-removal was complete as judged by TLC and the reaction mixture was evaporated to dryness and subsequently coevaporated with DCM (twice) to remove any residual acid. The crude product was dissolved in DMF (15 mL) and BOP (563 mg, 1.27 mmol), Boc-D-Leu-OH (250 mg, 1.08 mmol) and DIPEA (312 µL, 1.96 mmol) were added. The coupling reaction was completed after 3 h and the solvent was removed under reduced pressure. The residue was dissolved in EtOAc and the solution was washed with 1 N $KHSO_4$, 1 M $NaHCO_3$, brine and dried (Na_2SO_4). Ethyl acetate was removed under reduced pressure and after purification by column chromatography (EtOAc/hexane 1 : 4 v/v → EtOAc/hexane 1 : 1 v/v) dipeptide **6** was obtained as a colorless oil (345 mg, 98%). R_f(95 : 20 : 3 $CHCl_3$/MeOH/AcOH): 0.85; R_f(60 : 45 : 20 $CHCl_3$/MeOH/ 25%NH_4OH): 0.50; HPLC showed that the product was 100% pure according to ELSD and UV; δ_H ($CDCl_3$, 300 MHz) 0.93 and 1.44 (6H, dd, δ/δ′CH$_3$-Leu), 1.45 (9H, s, Boc), 1.53–1.68 (3H, m, γCH-Leu/βCH$_2$-Leu), 2.76 (3H, d, NHCH_3), 3.54–3.58 (1H, m, βCH$_2$-Ser (1H)), 3.99–4.04 (4H, m, βCH$_2$-Ser (1H)/~O-CH$_2$-allyl/αCH-Leu), 4.57–4.60 (1H, m, αCH-Ser), 5.17–5.28 (2H, m, CH$_2$-allyl), 5.38 (1H, d, NH-urethane), 5.80–5.90 (1H, m, CH-allyl), 6.98 (1H, d, NH-amide), 7.24 (1H, broad s, N*H*Me); δ_C ($CDCl_3$, 75.5 MHz) 22.0, 22.6, 24.5, 26.2, 28.1, 40.3, 52.8, 53.9, 68.9, 72.0, 80.2, 117.3, 134.0, 156.2, 170.0, 172.7; ES-MS calcd for $C_{18}H_{33}N_3O_5$: 371; found: *m*/*z* $[M + H]^+$ 372, $[M + Na]^+$ 394, $[(M - C_4H_8) + H]^+$ 316, $[(M - C_5H_8O_2) + H]^+$ 272; HR-MS $[M + H]^+$ found: *m*/*z* 372.2518, calcd 372.2498; Anal calcd for $C_{18}H_{33}N_3O_5$: C, 58.20; H, 8.95; N, 11.31; found: C, 58.33; H, 8.86; N, 11.28%.

***N*$^\alpha$-(*tert*-Butyloxycarbonyl)-seryl(allyl)-D-alanyl-*RS*-4-hydroxy-3,5-bisallyl-phenylglycyl-D-leucyl-serine(allyl) methyl amide (8).** Tripeptide **5** (200 mg, 0.33 mmol) was dissolved in MeOH/H_2O (30 mL 5 : 1 v/v) and LiOH (32 mg, 0.76 mmol) was added. After 20 min saponification was complete according to TLC and the solvents were evaporated *in vacuo.* The residue was dissolved in H_2O and the aqueous solution was acidified with 1 N $KHSO_4$ and extracted with EtOAc. The collected organic layers were dried (Na_2SO_4) and ethyl acetate was removed under reduced pressure. The saponified tripeptide was obtained as a white solid (158 mg, 86%). δ_H (300 MHz, $CDCl_3$) 1.34–1.36 (3H, m, βCH$_3$-Ala), 1.41 and 1.42 (9H, double s, Boc), 3.53–3.69 (1H, m, βCH$_2$-Ser (1H)), 3.34 (4H, d, 3-CH$_2$/ 6-CH$_2$), 3.85–3.89 (3H, m, ~O-CH$_2$-allyl/βCH$_2$-Ser (1H)), 4.34 (1H, broad s, αCH-Ser), 4.74 (1H, broad s, αCH-Ala), 5.09–5.20 (6H, m, CH$_2$-allyl, 3 × 2H)), 5.40–6.01 (5H, m, CH-allyl (3 × 1H)/NH-urethane/αCH), 7.01 (2H, d, 2-H/6-H), 7.47 (1H, d, NH-amide (Ala)), 7.68 (1H, broad s, OH), 7.78 (1H, d, N*H*Me); δ_C (75.5 MHz, $CDCl_3$) 18.12, 28.1, 35.1, 48.6, 54.2, 56.0, 55.1, 69.3, 72.0, 80.4, 116.5, 117.4, 126.2, 127.2, 127.8, 133.7, 133.8, 136.1, 152.7, 152.8, 155.5, 170.7, 171.6, 173.1. Next, dipeptide **6** (135 mg, 0.36 mmol) was dissolved in DCM (2 mL) and TFA (2 mL). After 1 h Boc-removal was complete as jugded by TLC and the reaction mixture was evaporated *in vacuo* and coevaporated with DCM (twice) to remove any residual TFA. Without further purification the saponified peptide and TFA salt **7** were dissolved in DCM/DMF (15 : 2 v/v) followed by the addition of BOP (136 mg, 0.31 mmol) and DIPEA (176 µL, 1.12 mmol). The coupling was complete in 3 h. After removal of the solvents, the product was triturated with EtOAc until pentapeptide **8** was obtained as a white solid (151 mg, 61%). δ_H (300 MHz, DMSO-d$_6$) 0.65–0.86 (6H, m, δ/δ′CH$_3$-Leu), 1.16–1.22 (3H, t, βCH$_3$-Ala), 1.35 (12H, s, Boc/ γCH-Leu/βCH$_2$-Leu), 2.53 (3H, broad s, NHCH_3), 3.27–3.29 (4H, m, 3-CH$_2$/5-CH$_2$), 3.48 (4H, m, βCH$_2$-Ser (2 × 2H)), 3.89 (4H, m, ~O-CH$_2$-allyl (2 × 2H)), 4.16 (2H, broad s, αCH-Ser), 4.96–4.29 (2H, m, αCH-Ala and αCH-Leu), 4.96–5.23 (8H, m, CH$_2$ (allyl, 4 × 2H)), 5.37–5.34 (1H, m, αCH), 5.75–5.93 (4H, m, CH-allyl (4 × 1H)), 6.76 (1H, d, NH-urethane), 6.90–6.93 (2H, m, 2-H/6-H), 7.94–8.42 (4H, m, NH-amide (4 × H)); ES-MS calcd for $C_{41}H_{62}N_6O_{10}$: 798; found: *m*/*z* $[M + H]^+$ 799, $[M + Na]^+$ 821, $[(M - C_4H_8) + H]^+$ 743, $[(M - C_5H_8O_2) + H]^+$ 699; HR-MS $[M + H]^+$ found: *m*/*z* 799.4583, calcd 799.4605; Anal. calcd for $C_{41}H_{62}N_6O_{10}$: C, 61.63; H, 7.82; N, 10.52; found: C, 61.72; H, 7.75; N, 10.40%.

Bicyclic pentapeptide (9). Pentapeptide **8** (20 mg, 0.025 mmol) was dissolved in TCE (12 mL). The solution was purged with nitrogen gas (25 min) and heated to 80 °C followed by the addition of Ru catalyst (2nd generation Grubbs, 21 mg, 0.025 mmol) and DMF (0.5 mL). After 10 min the reaction was complete according to TLC and ES-MS. Subsequently, the solvent was removed under reduced pressure and the residue was purified by column chromatography (DCM → 5 : 95 MeOH/DCM). The tandem ring-closed pentapeptide **9** was obtained as a slightly brownish solid in 67% yield (13.4 mg). HPLC showed that the product was 92% pure (based on eight diastereomers) according to ELSD; δ_H (500 MHz, DMSO-d$_6$) 0.83–0.87 (6H, m, δ/δ′CH$_3$-Leu), 1.39 (9H, s, Boc), 1.39–1.58 (6H, m, βCH$_3$-Ala/γCH-Leu/βCH$_2$-Leu), 2.27–2.49 (3H, m, NHCH_3), 3.17–4.62 (16H, m, 3-CH$_2$/5-CH$_2$/βCH$_2$-Ser (2 × 2H)/~O-CH$_2$-allyl (2 × 2H)/αCH-Ser/αCH-Ala/αCH-Leu), 4.62–5.90 (5H, m, CH=CH (2 × 2H)/αCH), 6.75–8.59 (8H, m, NH-urethane, 2-H/6-H/NH-amide (Ser)/NHMe/NH-amide (Ala)/NH-amide (Leu)/NH-amide); ES-MS calcd for $C_{37}H_{45}N_6O_{10}$: 742; found: *m*/*z* $[M + H]^+$ 743, $[M + Na]^+$ 765, $[(M - C_4H_8) + H]^+$ 687, $[(M - C_5H_8O_2) + H]^+$ 643; HR-MS $[M + H]^+$ *m*/*z* 743.3969 calcd 743.3979; MALDI-TOF found: *m*/*z* $(M + H)^+$ 743.39.

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