

Synthesis of a β -turn mimetic suitable for incorporation in the peptide hormone LHRH

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Abstract—LHRH is a decapeptide hormone which plays a central role in neuroendocrinology. Conformational studies have suggested that LHRH may adopt a β -turn involving residues 5–8 when bound to its receptor. A β -turn mimetic with side chains corresponding to those of a Tyr-Gly-Leu-Orn tetrapeptide has therefore been synthesized for incorporation at positions 5–8 in LHRH. In the turn mimetic, residues i and $i+1$ are connected by a $\psi[\text{CH}_2\text{O}]$ isostere instead of an amide bond, while a covalent ethylene bridge replaces the hydrogen bond which is often found between residues i and $i+3$ in β -turns. The turn mimetic was assembled from three types of building blocks: an azido aldehyde, an Fmoc protected amino acid and a protected dipeptide amine.

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1. Introduction

Luteinizing hormone-releasing hormone (LHRH), pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂, plays a key role in the mammalian reproductive system.¹ The decapeptide hormone is secreted in pulses from the hypothalamus and stimulates the anterior pituitary gland to release the gonadotropic hormones, luteinizing hormone (LH) and follicle stimulating hormone (FSH). After the sequence of LHRH was determined in 1971,^{1–4} intensive studies directed towards obtaining knowledge of structure–activity relationships as well as receptor binding have been performed.^{5,6} Such studies are of interest in development of therapeutic agents for treatment of a variety of hormone dependent disorders, such as breast and prostate cancer.^{7,8} The use of peptides, such as LHRH, as drugs is often limited due to rapid degradation by proteolytic enzymes, low bioavailability and rapid secretion. Design of peptidomimetics which resemble the secondary structure of biologically active peptides is one way to circumvent these problems.^{9–11} Such, peptidomimetics are valuable not only for design of new pharmaceuticals, but also serve to help in elucidating the biologically active conformations of peptides, and provide additional structure–activity relationship data.^{12–14}

β -Turns are formed by four consecutive amino acid residues and constitute one of the most common secondary structure

elements found in peptides and proteins.^{15,16} In addition, β -turns have often been indicated as recognition elements involved in intermolecular interactions that regulate many important functions in organisms.^{17,18} Therefore, β -turns have received a great deal of attention and a large number of β -turn mimetics have been reported. In order to achieve high-affinity and selective binding to a targeted receptor, the conformation of the mimetic should accurately resemble that of the peptide backbone in the turn and the compound must also mimic side chains which are essential for receptor-binding.^{19,10} The existence of a β -turn involving residues Tyr⁵-Gly-Leu-Arg⁸ in LHRH has been suggested by conformational energy calculations²⁰ and various physiochemical methods.^{21,22} Furthermore, replacement of the Tyr⁵-Arg⁸ segment by a sterically hindered β -lactam,²³ which is a model of a type II' β -turn, resulted in an analogue that was more potent than LHRH both in vitro and in vivo. In addition, a highly potent and orally active nonpeptide antagonist at the human LHRH receptor based on a type II β -turn model has been developed.²⁴ Therefore, introduction of a β -turn mimetic having correct side chain functionalities will provide invaluable insight into the structural features essential for binding of LHRH to its receptor. As a first step towards this goal we now report an approach which provides access to a conformationally restricted β -turn mimetic for incorporation in place of residues 5–8 in LHRH.

2. Results and discussion

Our group recently reported results from a project aimed at design and synthesis of a novel type of β -turn mimetics, and

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exemplified the approach by incorporation of a mimetic into Leu-enkephalin.^{25,26} In these mimetics the amide bond between residue i and $i+1$ of the β -turn is replaced by a methylene ether isostere, while the $i \rightarrow i+3$ hydrogen bond which stabilizes the turn has been exchanged for a covalent ethylene bridge, thereby forming a 10-membered β -turn mimetic (cf. **1**, Fig. 1). We now describe the synthesis of β -turn mimetic **2** which has side-chains corresponding to those of a Tyr-Gly-Leu-Orn tetrapeptide (Fig. 2). Mimetic **2** is intended for incorporation at positions 5–8 in LHRH and in order to simplify the synthesis ornithine was chosen in place of arginine, which is found at position 8 in LHRH. This choice was made since it is possible to convert the side chain of ornithine to that of arginine on solid phase after incorporation of the mimetic in LHRH.²⁷ Mimetic **2** was designed to allow assembly from three building blocks; azido aldehyde **3**, dipeptide amine **4** and Fmoc-Leu-OH **5**. Building block **3** corresponds to Tyr and Gly at positions i and $i+1$ of the turn and the azido group serves as a precursor of the amino group of residue i . *N*-Alkylated amino acid derivatives, such as residue $i+3$ in mimetic **1** (Fig. 1), are more susceptible to epimerization during activation and coupling than the ordinary amino acids.^{28,29} Therefore, it was decided to avoid this potential problem by including Pro⁹ of LHRH when preparing the turn mimetic and dipeptide amine **4** was chosen instead of an ornithine building block. The *tert*-butyldiphenyl silyl protected hydroxyl group in **4** corresponds to the carboxyl group of the proline residue.

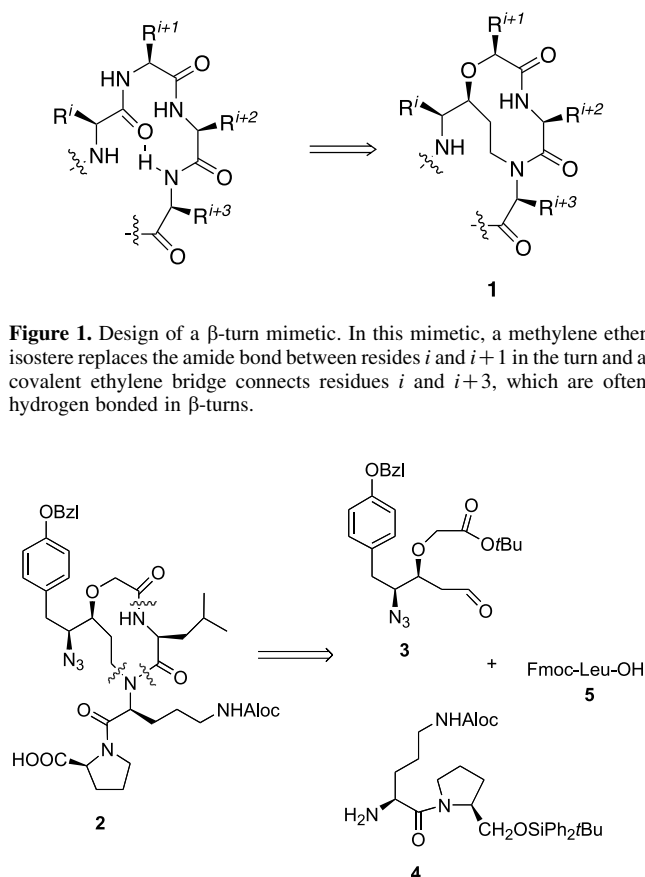


Figure 1. Design of a β -turn mimetic. In this mimetic, a methylene ether isostere replaces the amide bond between residues i and $i+1$ in the turn and a covalent ethylene bridge connects residues i and $i+3$, which are often hydrogen bonded in β -turns.

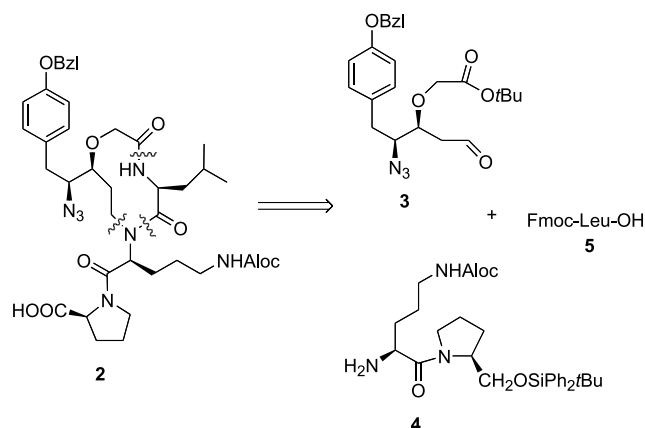
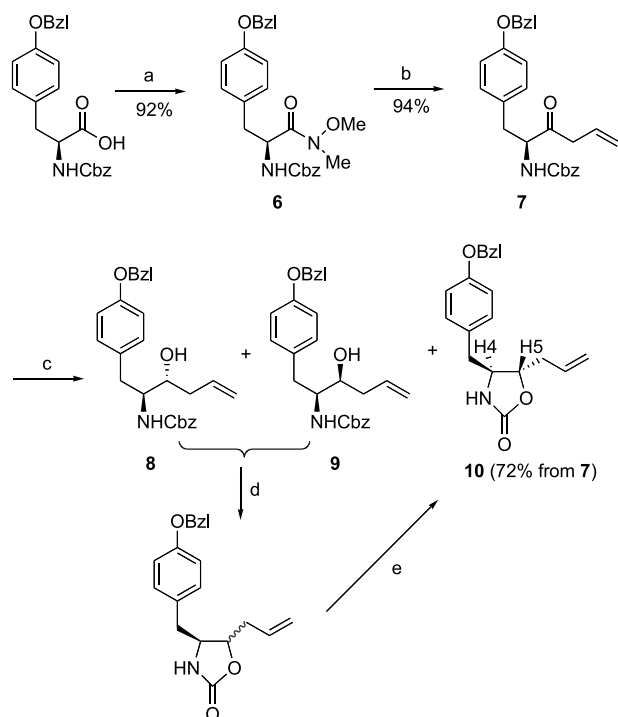
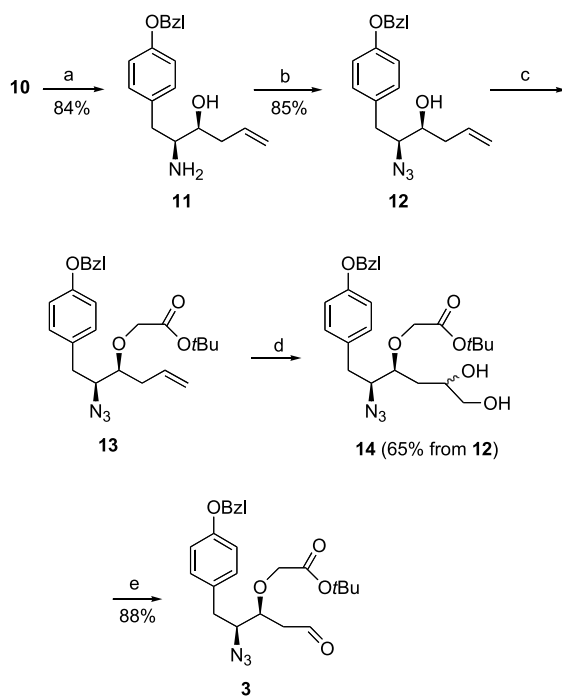


Figure 2. A retrosynthetic analysis shows that the target mimetic **2** may be assembled from three building blocks; azido aldehyde **3**, dipeptide amine **4** and Fmoc-Leu-OH (**5**).

The key building block, azido aldehyde **3**, was prepared from the commercially available Cbz-Tyr(Bzl)-OH (Schemes 1 and 2). This was transformed into a mixed anhydride by addition of isobutyl chloroformate and *N*-methyl



Scheme 1. (a) i BuOCOCi, NMM, MeONHMe·HCl, CH_2Cl_2 , $-15^\circ\text{C} \rightarrow \text{rt}$, 2 h, 92%. (b) Allylmagnesium bromide, THF, -78°C , 1.5 h, 94%. (c) K-Selectride, THF, -78°C 19 h. (d) and (e) THF:MeOH:aq KOH (7.5 N) (4:2:1), rt, 5 h, flash column chromatography, 72% of **10** over steps (c)–(e).



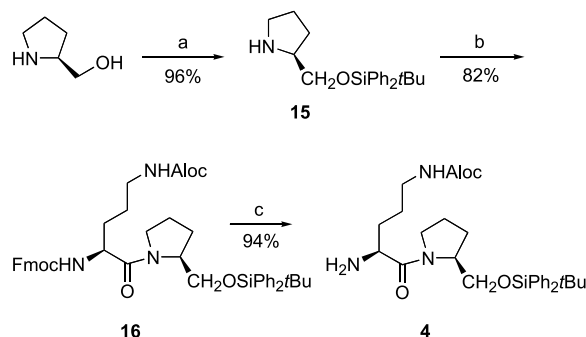
Scheme 2. (a) EtOH/KOH (aq, 1 M) (1:1), reflux, 6 h, 84%. (b) TiN_3 , DMAP, CuSO_4 , CH_2Cl_2 , rt, 2 h, 85%. (c) $\text{BrCH}_2\text{CO}_2t\text{Bu}$, Bu_4NHSO_4 , benzene:aq NaOH (50%) (1:1), rt, 1 h. (d) OsO_4 , NMO, THF/acetone: H_2O (1:1:1), rt, 2.5 h, 65% over two steps. (e) $\text{Pb}(\text{OAc})_4$, Na_2CO_3 , benzene, $0^\circ\text{C} \rightarrow \text{rt}$, 88%.

morpholine, followed by reaction with *N,O*-dimethylhydroxylamine to give Weinreb amide **6** (92%). Grignard reaction³⁰ of **6** with allylmagnesium bromide furnished ketone **7** (94%) which was reduced using K-selectride to give a mixture of **8**, **9**, and **10**. *Anti* and *syn* amino alcohols **8** and **9** were difficult to separate from each other by flash column chromatography, whereas *anti*-oxazolidinone **10** was readily separated from **8** and **9**. After removal of **10**, the mixture of **8** and **9** was converted to a mixture of *syn* and *anti*-oxazolidinones by treatment with aqueous KOH in a mixture of THF and methanol. Purification by chromatography then gave **10** in 72% overall yield from **7**. The stereochemistry of *anti*-oxazolidinone **10** was established by comparing the NOESY spectrum of **10** with that of the corresponding *syn*-isomer. The spectrum of **10** did not show any cross-peak for H-4 and H-5 in the oxazolidinone ring, whereas the *syn*-isomer showed a strong cross-peak between these two hydrogen atoms.

Oxazolidinone **10** was hydrolyzed under alkaline conditions to furnish amino alcohol **11** (Scheme 2). Azido transfer was then performed with freshly prepared triflyl azide in the presence of catalytic amount of CuSO₄ to give azido alcohol **12**.³¹ This transformation served the dual purposes of introducing an inert amino protective group as well as reducing the steric bulk in the vicinity of the hydroxyl group of **12**. Compound **12** decomposed on standing but could be purified by flash column chromatography if performed rapidly. Due to its instability, **12** was immediately alkylated with *tert*-butyl bromoacetate using phase-transfer catalysis,³² thus introducing the *i* + 1 residue of the turn mimetic. Compound **13** was even more labile than **12** and crude **13** was therefore oxidized immediately to diol **14** by osmium tetroxide catalysed dihydroxylation (65% from **12**). Cleavage of diol **14** with lead tetraacetate furnished the stable azido aldehyde **3** in 88% yield, thereby providing one of the building blocks required for assembly of the target β -turn mimetic.

Synthesis of a building block corresponding to **4** was first attempted for an Arg-Pro sequence. However, due to purification problems related to the guanidino group of arginine this approach had to be abandoned. Since ornithine can be converted to arginine late in synthetic routes,²⁷ an Orn-Pro dipeptide was chosen instead of Arg-Pro. A diketopiperazine was formed instead of the desired amine on cleavage of the Fmoc protecting group of Fmoc-Orn(Aloc)-Pro-OMe with morpholine. Therefore, prolinol was chosen as starting material and protected with *tert*-butyldiphenylsilyl chloride to give **15** in high yield (Scheme 3). Coupling of **15** with Fmoc-Orn(Aloc)-OH was then achieved by using HATU^{33,34} as coupling reagent in the presence of diisopropylethylamine in DMF. Finally cleavage of the Fmoc group of **16** furnished **4**, that is, the other key building block for synthesis of the β -turn mimetic.

Assembly of the β -turn mimetic began with reductive amination³⁵ of **3** with **4** using sodium triacetoxyborohydride as reducing agent to give **17** in almost quantitative yield. Introduction of Fmoc-Leu-OH **5** at position *i* + 2 in the mimetic turned out to be very difficult, most likely due to steric hindrance. Therefore, large efforts were made to find suitable conditions for acylation of **17** with **5**. When DIC

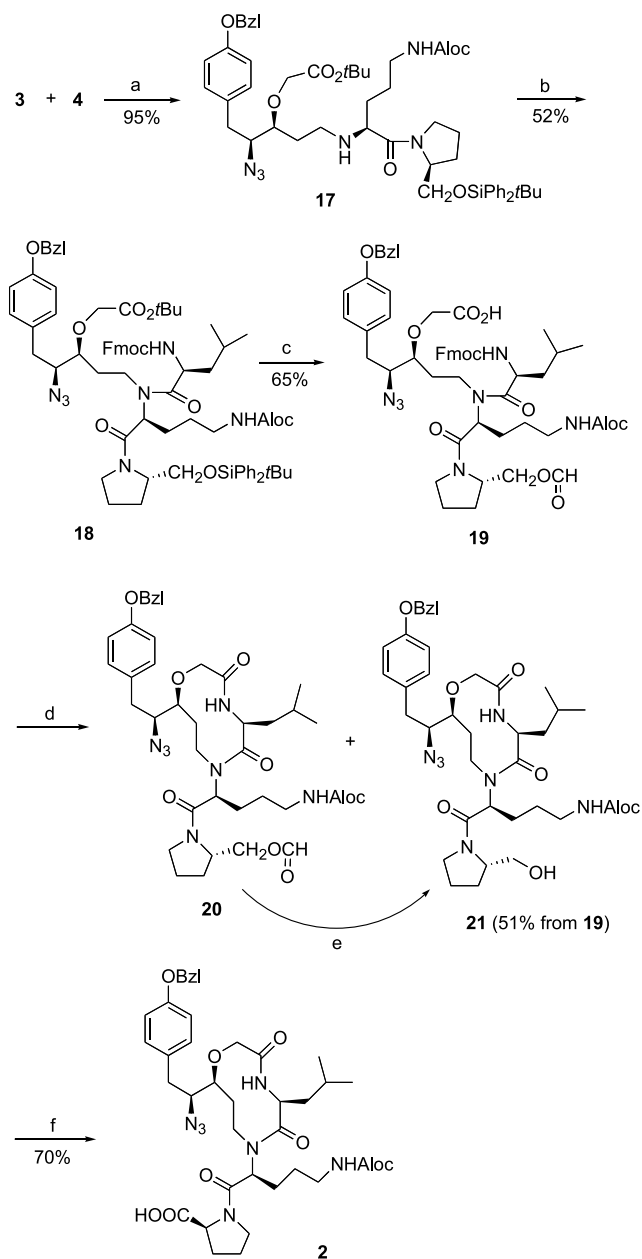


Scheme 3. (a) *t*BuPh₂SiCl, imidazole, CH₂Cl₂, rt, 3 h, 96%. (b) Fmoc-Orn(Aloc)-OH, HATU, DIEA, DMF, 0 °C → rt, 2 h, 82%. (c) Morpholine, rt, 3 h, 94%.

was used as coupling reagent under conditions found to be optimal for acylation of a secondary amine in a synthetic route leading to a γ -turn mimetic,³⁶ a very low yield of **18** was obtained as a mixture of diastereomers. Conversion of **5** into the corresponding acid chloride and coupling with **17** also led to a diastereomeric mixture of **18**, most likely due to epimerization of the stereogenic center of **5**. Instead **18** was obtained in enantiomerically pure form by use of HATU as coupling reagent in the presence of diisopropylethylamine. In contrast to what can be expected,³⁷ attempts to cleave the *tert*-butyl ester of **18** without removing the *tert*-butyldiphenylsilyl protecting group failed. The *tert*-butyldiphenylsilyl protecting group was found to be cleaved much faster than the *tert*-butyl ester in formic acid, but fortunately the resulting hydroxyl group was simultaneously protected as a formate so that **19** was formed. The carboxyl group of **19** was then activated as a pentafluorophenyl ester which was added to DBU in hot dioxane under high dilution conditions. This gave a mixture of **20** and **21** in a one-pot procedure³⁸ involving Fmoc deprotection followed by closure of the ten-membered ring and partial removal of the formate. Compound **20** was easily hydrolyzed to give **21**, which thereby was then obtained in 51% yield from **19** over the three steps. Finally Jones oxidation of **21** furnished the β -turn mimetic **2** which is ready for incorporation in LHRH using solid-phase synthesis (Scheme 4).

3. Conclusions

A β -turn mimetic with side-chains corresponding to a Tyr-Gly-Leu-Orn tetrapeptide, suitable for incorporation at positions 5–8 of the hormone LHRH, has been synthesized. The design of the mimetic is similar to a Tyr-Gly-Gly-Phe mimetic previously incorporated in Leu-enkephalin by us.^{25,26} Thus, residues *i* and *i* + 1 are connected by a ψ (CH₂O) isostere instead of an amide bond, while a covalent ethylene bridge replaces the hydrogen bond that is often found between the carbonyl group of residue *i* and the amino group of residue *i* + 3 in β -turns. In addition, we have now shown that it is possible to incorporate chiral amino acids at position *i* + 2 of this type of β -turn mimetics, that is, the strategy is not limited to glycine which was found at the *i* + 2 position in the Leu-enkephalin mimetic. After evaluation of several different procedures it was found that the *i* + 2 leucine could be coupled without racemization to a sterically hindered secondary amine using HATU as



Scheme 4. (a) NaBH(OAc)₃, DCE, rt, 45 min, 95%. (b) Fmoc-Leu-OH, HATU, DIEA, DMF, 0 °C → rt, 24 h, 52% (70% based on recovered **17**). (c) HCOOH, rt, 19 h, 65%. (d) PfpOH, DIC, EtOAc, 0 °C, 1.5 h, then DBU, dioxane, 100 °C, 4 h. (e) aq LiOH (1 M), THF/MeOH:H₂O (3:1:1), rt, 3 h, 51% for steps (d) and (e). (f) Jones oxidation, 3.5 h, 70%.

coupling reagent in the presence of diisopropylethylamine. In addition, the synthetic strategy was revealed to be compatible with amino acids having protected functionalities in their side-chains both at positions *i* and *i* + 4, such as those of tyrosine and ornithine in the present mimetic.

4. Experimental

4.1. General data

All reactions were carried out under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise stated. CH₂Cl₂ and THF were distilled from

calcium hydride and sodium-benzophenone, respectively. DMF was distilled and then dried over 3 Å molecular sieves. TLC was performed on Silica Gel 60 F₂₅₄ (Merck) with detection by UV light and charring with phosphomolybdic acid in EtOH. Flash column chromatography (eluents given in brackets) was performed on silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon).

¹H NMR spectra for compounds **2–21** were recorded at 400 MHz whereas ¹³C NMR spectra were obtained at 100 MHz for solution in CDCl₃ [residual CHCl₃ (δ_H 7.26 ppm) or CDCl₃ (δ_C 77.0 ppm) as internal standard] or in MeOH-*d*₄ [residual CD₂HOD (δ_H 3.31 ppm) or CD₃OD (δ_C 49.2 ppm) as internal standard] at 298 K. Elemental analysis was performed by Mikro Kemi AB, Uppsala, Sweden. Compounds lacking elemental analysis were characterized by high resolution MS and were >95% pure according to ¹H NMR spectroscopy. Ions for positive fast atom bombardment mass spectra were produced by a beam of Xenon atoms (6 keV) from a matrix of glycerol and thiolglycerol.

4.1.1. (S)-N-Methoxyl-N-methyl-2-benzyloxycarbonyl-amino-3-(4-benzyloxyphenyl)-propanoic amide (6). Isobutyl chloroformate (0.845 mL, 6.49 mmol) was added dropwise to a solution of Cbz-Tyr(Bzl)-OH (2.63 g, 6.49 mmol) and *N*-methyl morpholine (NMM, 1.57 mL, 14.3 mmol) in CH₂Cl₂ at –15 °C. After being stirred for 15 min, *N,O*-dimethylhydroxylamine·HCl (0.633 g, 6.49 mmol) was added. After a further 30 min the cooling bath was removed and the reaction mixture was stirred at room temperature for additional 1.5 h. The reaction mixture was poured into H₂O (20 mL) and extracted with CH₂Cl₂ (3 × 15 mL). The combined organic layers were washed sequentially with 0.2 M aq HCl, satd aq NaHCO₃ and brine, then dried with MgSO₄, filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 3:1 → 1:1) of the residue gave **6** as a colourless oil (2.69 g, 92%): [α]_D²⁰ = +9.3 (c 0.8, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ 7.30–7.45 (10H, m, Ph), 7.09 (2H, d, *J* = 8.5 Hz, Ph_{Tyr}), 6.90 (2H, d, *J* = 8.5 Hz, Ph_{Tyr}), 5.52 (1H, d, *J* = 8.6 Hz, NH), 5.04–5.13 (5H, m, CH₂Ph, CH₂Ph and CHNH), 3.68 (3H, s, OCH₃), 3.18 (3H, s, NCH₃), 3.05 (1H, ABX type dd, *J* = 5.8, 13.6 Hz, Tyr β-H), 2.88 (1H, ABX type dd, *J* = 7.2, 13.6 Hz, Tyr β-H); ¹³C NMR (100 MHz, CDCl₃) δ 171.9, 157.7, 155.7, 136.9, 136.3, 130.4, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.4, 114.7, 69.8, 66.6, 61.4, 52.1, 37.7, 32.0; IR (neat) 3298, 1714, 1657 cm^{–1}; HRMS (FAB) calcd for C₂₆H₂₉N₂O₅ 449.2079 (M + H⁺), found 449.2057; Anal. Calcd C, 69.6; H, 6.3; N, 6.2. Found C, 69.7; H, 6.3; N, 6.1.

4.1.2. (S)-2-Benzyloxycarbonylamino-1-(4-benzyloxyphenyl)-hex-5-en-3-one (7). Allylmagnesium bromide (1 M solution in diethyl ether, 8.35 mL, 8.35 mmol) was added dropwise to a solution of Weinreb amide **6** (1.50 g, 3.34 mmol) in THF (32 mL) at –78 °C. After being stirred for 1.5 h, the reaction was quenched with satd aq NH₄Cl (20 mL) and the mixture was extracted with CH₂Cl₂ (3 × 20 mL). The combined organic layers were then washed with brine, dried over MgSO₄, filtered and concentrated. Flash chromatography (toluene/ethanol, 20:1 → 10:1) of the residue gave **7** as a white amorphous solid (1.35 g, 94%): [α]_D²⁰ = +9.6 (c 0.1, CHCl₃); ¹H NMR (400 MHz, CDCl₃) δ

7.32–7.43 (10H, m, Ph), 7.02 (2H, d, $J=8.2$ Hz, Ph_{Tyr}), 6.89 (2H, d, $J=8.2$ Hz, Ph_{Tyr}), 5.85 (1H, m, $\text{CH}=\text{CH}_2$), 5.34 (1H, d, $J=7.7$ Hz, NH), 5.02–5.20 (6H, m, CH_2Ph , CH_2Ph and $\text{CH}=\text{CH}_2$), 4.63 (1H, m, CHN), 3.19 (1H, ABX type dd, $J=7.2$, 17.3 Hz, $\text{CH}_2\text{CH}=\text{CH}_2$), 3.13 (1H, ABX type dd, $J=7.2$, 17.3 Hz, $\text{CH}_2\text{CH}=\text{CH}_2$), 3.03 (1H, ABX type dd, $J=6.8$, 14.2 Hz, Tyr β -H), 2.96 (1H, ABX type dd, $J=6.2$, 14.2 Hz, Tyr β -H); ^{13}C NMR (100 MHz, CDCl_3) δ 206.6, 157.9, 155.6, 136.9, 136.2, 130.2, 129.5, 128.5, 128.4, 128.1, 128.0, 127.9, 127.8, 127.4, 119.4, 115.1, 70.0, 66.9, 60.1, 45.5, 36.8; IR (neat) 3309, 1716, 1689 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{27}\text{H}_{28}\text{NO}_4$ 430.1979 ($\text{M}+\text{H}^+$), found 430.2024; Anal. Calcd C, 75.5; H, 6.3; N, 3.3. Found C, 75.5; H, 6.3; N, 3.3.

4.1.3. (4S,5S)-5-Allyl-4-(4-benzyloxybenzyl)-oxazolidin-2-one (10). K-Selectride (1 M solution in THF, 18.0 mL, 18.0 mmol) was added dropwise to a solution of allyl ketone **7** (5.15 g, 12.0 mmol) in THF (82 mL) at -78°C . After being stirred for 19 h, the reaction was quenched with H_2O (20 mL) and acidified with 10% citric acid to adjust the pH to ~ 4 . The reaction mixture was extracted with CH_2Cl_2 (3×30 mL). The combined organic layers were then washed with brine, dried over MgSO_4 , filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 10:1 \rightarrow 1:1.5) of the residue gave a mixture of **8** and **9** (4.93 g) and pure *anti*-oxazolidinone **10** (0.47 g). Then the mixture of **8** and **9** was stirred in a mixture of THF, MeOH and aq 7.5 M KOH (4:2:1, 140 mL) at room temperature for 5 h and poured into H_2O (130 mL). The phases were separated and the aqueous phase was extracted with EtOAc (4×50 mL). The combined organic phases were washed with brine and dried over MgSO_4 . Flash column chromatography (heptane/ethyl acetate 10:1 \rightarrow 1:1.5) of the residue gave *anti*-oxazolidinone **10** as a white amorphous solid (2.38 g in total, 72% for **7**): $[\alpha]_{\text{D}}^{20} = -63.2$ (c 0.8, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.32–7.44 (5H, m, Ph), 7.08 (2H, d, $J=8.3$ Hz, Ph_{Tyr}), 6.94 (2H, d, $J=8.3$ Hz, Ph_{Tyr}), 5.92 (1H, s, NH), 5.68 (1H, m, $\text{CH}=\text{CH}_2$), 5.11 (2H, m, $\text{CH}=\text{CH}_2$), 5.05 (2H, s, CH_2Ph), 4.32 (1H, q, $J=5.7$, 5.7, 5.7 Hz, CHO), 3.68 (1H, q, $J=6.3$, 6.3, 6.3 Hz, CHN), 2.78 (2H, m, Tyr β -H), 2.34 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$); ^{13}C NMR (100 MHz, CDCl_3) δ 158.9, 158.0, 137.0, 131.5, 130.3, 128.7, 128.3, 128.1, 127.6, 119.4, 115.5, 80.7, 70.1, 58.2, 40.7, 38.7; IR (neat) 3269, 1749, 1687, 1512 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{20}\text{H}_{22}\text{NO}_3$ 324.1679 ($\text{M}+\text{H}^+$), found 324.1609; Anal. Calcd C, 74.3; H, 6.6; N, 4.3. Found C, 74.5; H, 6.6; N, 4.3.

4.1.4. (2S,3S)-2-Amino-1-(4-benzyloxyphenyl)-hex-5-en-3-ol (11). *Anti*-oxazolidinone **10** (1.54 g, 4.76 mmol) was refluxed in a mixture of EtOH (35 mL) and aq KOH (1 M, 35 mL) for 6 h, after which the solvent was evaporated and the residue was partitioned between H_2O (15 mL) and CH_2Cl_2 (15 mL). The aqueous phase was extracted with CH_2Cl_2 (4×30 mL). The combined organic layers were then washed with brine, dried over MgSO_4 , filtered and concentrated. Flash column chromatography (toluene/ethanol, 10:1 \rightarrow 1:2) of the residue furnished amino alcohol **11** as a white amorphous solid (1.18 g, 84%): $[\alpha]_{\text{D}}^{20} = -9.9$ (c 1.1, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.33–7.45 (5H, m, Ph), 7.12 (2H, d, $J=8.4$ Hz, Ph_{Tyr}), 6.93 (2H, d, $J=8.4$ Hz, Ph_{Tyr}), 5.90 (1H, m, $\text{CH}=\text{CH}_2$), 5.14 (2H, m, $\text{CH}=\text{CH}_2$),

5.05 (2H, s, CH_2Ph), 3.48 (1H, m, CHOH), 2.88 (2H, m, CHNH_2 and Tyr β -H), 2.47 (1H, ABX type dd, $J=8.9, 12.9$ Hz, Tyr β -H), 2.33 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 2.06 (3H, br s, OH and NH_2); ^{13}C NMR (100 MHz, CDCl_3) δ 157.5, 137.0, 134.9, 131.1, 130.2, 128.6, 127.9, 127.5, 117.5, 115.0, 72.3, 70.0, 56.0, 39.9, 39.2; IR (neat) 3359, 3298, 1610, 1581, 1510 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{19}\text{H}_{24}\text{NO}_2$ 298.1779 ($\text{M}+\text{H}^+$), found 298.1798.

4.1.5. (2S,3S)-2-Azido-1-(4-benzyloxy-phenyl)-hex-5-en-3-ol (12). A solution of freshly prepared triflyl azide³¹ (14.8 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a solution of **11** (1.05 g, 3.53 mmol), DMAP (216 mg, 1.77 mmol) and CuSO_4 (28.2 mg, 0.177 mmol) in CH_2Cl_2 (10 mL) at room temperature. After being stirred for 2 h, the reaction mixture was washed with 10% citric acid ($2 \times$), satd aq NaHCO_3 ($2 \times$) and brine. The combined organic layers were dried over Na_2SO_4 , filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 10:1 \rightarrow 3:1) of the residue gave azido alcohol **12** as white amorphous solid (0.966 g, 85%): ^1H NMR (400 MHz, CDCl_3) δ 7.31–7.45 (5H, m, Ph), 7.18 (2H, d, $J=8.6$ Hz, Ph_{Tyr}), 6.95 (2H, d, $J=8.6$ Hz, Ph_{Tyr}), 5.79 (1H, m, $\text{CH}=\text{CH}_2$), 5.17 (2H, m, $\text{CH}=\text{CH}_2$), 5.06 (2H, s, CH_2Ph), 3.62 (1H, m, CHOH), 3.44 (1H, ddd, $J=3.3$, 6.4, 8.2 Hz, CHN_3), 2.99 (1H, ABX type dd, $J=6.4$, 13.8 Hz, Tyr β -H), 2.91 (1H, ABX type dd, $J=6.4$, 13.8 Hz, Tyr β -H), 2.36 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 1.82 (1H, d, $J=5.8$ Hz, OH); ^{13}C NMR (100 MHz, CDCl_3) δ 157.8, 137.0, 133.7, 130.3, 129.7, 128.6, 128.5, 127.9, 127.5, 127.5, 127.4, 118.7, 71.3, 70.1, 67.1, 39.1, 36.3; IR (neat) 3365, 3031, 2106, 1610, 1510 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{19}\text{H}_{21}\text{N}_3\text{O}_2$ 323.1679 (M^+), found 323.1631.

4.1.6. α -[(4S,5S)-5-Azido-6-(4-benzyloxyphenyl)-1,2-dihydroxy-hex-4-oxy]-acetic acid *tert*-butyl ester (14). *tert*-Butyl bromoacetate (0.72 mL, 4.89 mmol) was added dropwise to a vigorously stirred mixture of azido alcohol **12** (0.878 g, 2.71 mmol) and tetrabutyl ammonium hydrogen sulphate (257 mg) in benzene–aq 50% NaOH (1:1, 30 mL) at room temperature. After being stirred for 1.5 h, the mixture was separated and the aqueous phase was extracted with CH_2Cl_2 (3×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give crude O-alkylated azido alcohol **13**. Compound **13** was dissolved in a mixture of THF, acetone and water (1:1:1, 30 mL). OsO_4 (69 mg, 0.21 mmol) and NMO (0.586 g, 4.34 mmol) were added sequentially to the solution. After being stirred for 2.5 h the solution was acidified with 0.2 M aq HCl to adjust the pH to ~ 2 and then extracted with EtOAc (4×15 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 and concentrated. Flash column chromatography (heptane/ethyl acetate, 5:1 \rightarrow 1:3) of the residue gave azido diol **14** as a colourless oil (0.832 g, 65%): ^1H NMR (400 MHz, CDCl_3) δ for a 1:1 diastereomeric mixture 7.30–7.45 (5H, m, Ph), 7.15 (2H, d, $J=8.5$ Hz, Ph_{Tyr}), 6.94 (2H, d, $J=8.5$ Hz, Ph_{Tyr}), 5.06 (2H, s, CH_2Ph), 4.17–4.28 (1.5H, m, $\text{CH}_2\text{COO}t\text{Bu}$), 4.15 (0.5H, m, CHO), 4.05 (0.5H, d, $J=16.3$ Hz, $\text{CH}_2\text{COO}t\text{Bu}$), 3.95 (0.5H, m, CHO), 3.76 (0.5H, m, CHO), 3.64 (2.5H, m, CHN_3 , CHO and CH_2OH), 3.54 (1H m, CH_2OH), 2.92–3.02 (1H, m, Tyr β -H), 2.58–2.67 (1H, m, Tyr β -H), 1.59–1.83 (2H, m, CH_2CHO), 1.52 (4.5H, s, $t\text{Bu}$), 1.49 (4.5H, s, $t\text{Bu}$);

^{13}C NMR (100 MHz, CDCl_3) δ 171.1, 170.2, 157.8, 157.8, 137.0, 137.0, 130.2, 130.0, 129.9, 128.6, 127.9, 127.5, 115.1, 115.1, 82.9, 82.8, 78.4, 71.0, 70.1, 68.2, 67.7, 66.8, 66.7, 66.7, 66.0, 35.5, 35.0, 34.3, 33.8, 28.1; IR (neat) 3408, 2106, 1732, 1612, 1512 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{25}\text{H}_{33}\text{N}_3\text{O}_6\text{Na}$ 494.2198 ($\text{M} + \text{Na}^+$), found 494.2191.

4.1.7. (1S,2S)-2-Azido-3-(4-benzyloxyphenyl)-1-(2-oxoethyl)-propoxy]-acetic acid *tert*-butyl ester (3). Lead tetraacetate (0.237 g, 0.534 mmol) was added to a mixture of azido diol **14** (0.168 g, 0.356 mmol) and Na_2CO_3 (94.3 mg, 0.890 mmol) in benzene (10 mL) at 0 °C. After being stirred for 10 min, the cooling bath was removed and stirring was continued for 1 h. Then the reaction was quenched with 10 drops of ethylene glycol and stirring was continued for 5 min. Et_2O and satd aq NaHCO_3 were added and the phases were separated. The aqueous phase was extracted with Et_2O (4×10 mL), the combined organic phases were washed with brine, dried with Na_2SO_4 , filtered and concentrated. Flash column chromatography (heptane/ethyl acetate, 5:1 \rightarrow 3:1) of the residue gave azido aldehyde **3** as a colourless oil (0.138 g, 88%): $[\alpha]_{\text{D}}^{20} = -22.5$ (c 0.1, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 9.84 (1H, s, CHO), 7.31–7.45 (5H, m, Ph), 7.19 (2H, d, $J = 8.3$ Hz, Ph_{tyr}), 6.95 (2H, d, $J = 8.3$ Hz, Ph_{tyr}), 5.06 (2H, s, CH_2Ph), 4.11 (2H, s, $\text{CH}_2\text{COO}t\text{Bu}$), 4.02 (1H, m, CHN_3CHO), 3.60 (1H, dt, $J = 4.6$, 9.2 Hz, CHN_3), 3.06 (1H, ABX type dd, $J = 4.6$, 14.0 Hz, Tyr β -H), 2.80–2.95 (3H, m, Tyr β -H and CH_2CHO), 1.49 (9H, s, $t\text{Bu}$); ^{13}C NMR (100 MHz, CDCl_3) δ 200.1, 169.2, 157.7, 136.9, 130.2, 129.6, 128.6, 128.5, 127.9, 127.5, 115.0, 81.9, 67.0, 68.9, 65.8, 45.7, 35.3, 28.1; IR (neat) 2107, 1743, 1722 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{24}\text{H}_{28}\text{N}_3\text{O}_5$ ($\text{M} + \text{H}^+$) 438.2021, found 438.2030.

4.1.8. *O*-(*tert*-Butyldiphenylsilyl)-L-prolinol (15). *tert*-Butyldiphenylsilyl chloride (4.92 mL, 21.0 mmol) was added to a solution of L-prolinol (0.987 mL, 10.0 mmol) and imidazole (1.50 g, 22.0 mmol) in CH_2Cl_2 (100 mL) at 0 °C. After being stirred for 3 h the resulting precipitate was filtered off and the reaction was quenched by addition of satd aq NH_4Cl . The aqueous phase was extracted with CH_2Cl_2 (2×20 mL), the combined organic phases were dried with Na_2SO_4 , filtered and concentrated. Flash column chromatography (toluene/ethanol, 10:1 \rightarrow 3:1) of the residue gave **15** as a colourless oil (3.26 g, 96%): $[\alpha]_{\text{D}}^{20} = -5.9$ (c 0.5, CHCl_3); ^1H NMR (400 MHz, CDCl_3) δ 7.67 (4H, m, Ph), 7.35–7.44 (6H, m, Ph), 3.65 (1H, ABX type dd, $J = 4.9$, 10.0 Hz, CH_2OSi), 3.59 (1H, ABX type dd, $J = 6.1$, 10.0 Hz, CH_2OSi), 3.23 (1H, m, Pro α -H), 2.97 (1H, m, Pro δ -H), 2.85 (1H, m, Pro δ -H), 2.13 (1H, br s, NH), 1.69–1.82 (3H, m, Pro β -H and Pro γ -H), 1.48 (1H, m, Pro β -H), 1.06 (9H, s, $t\text{Bu}$); ^{13}C NMR (100 MHz, CDCl_3) δ 135.6, 135.6, 133.7, 129.6, 127.7, 127.7, 66.5, 59.9, 46.5, 27.5, 26.9, 25.4, 19.3; IR (neat) 2856, 1111 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{21}\text{H}_{30}\text{NOSi}$ 340.2079 ($\text{M} + \text{H}^+$), found 340.2086; Anal. Calcd C, 74.3; H, 8.6; N, 4.1. Found C, 73.9; H, 8.6; N, 4.1.

4.1.9. N^δ -(Allyloxycarbonyl)- N^α -(fluoren-9-ylmethoxycarbonyl)-L-ornithyl-*O*-(*tert*-butyldiphenylsilyl)-L-prolinol (16). Fmoc-Orn(Aloc)-OH (0.386 g, 0.881 mmol) was activated for 15 min with HATU (0.305 g, 0.801 mmol) in the presence of DIEA (0.308 mL, 1.76 mmol) in DMF (2 mL) at 0 °C. Then **15** (0.275 g, 0.801 mmol) in DMF

(2 mL) was added to the solution which was stirred for 3 h before being diluted with water (100 mL) and extracted with CH_2Cl_2 (5×10 mL). The combined organic layers were washed with aq 0.2 M HCl, H_2O , satd aq NaHCO_3 and brine, dried with Na_2SO_4 , filtered and concentrated. Flash column chromatography (toluene/ethanol, 20:1 \rightarrow 10:1) of the residue gave **16** as a white amorphous solid (0.502 g, 82%): $[\alpha]_{\text{D}}^{20} = -35.7$ (c 0.8, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , rotamer ratio 4:1) δ (major rotamer) 7.76 (2H, d, $J = 7.4$ Hz, Ph), 7.62 (6H, m, Ph), 7.29–7.43 (10H, m, Ph), 5.90 (1H, m, $\text{CH}=\text{CH}_2$), 5.75 (1H, d, $J = 8.5$ Hz, Orn α -NH), 5.28 (1H, d, $J = 17.1$ Hz, $\text{CH}=\text{CH}_2$), 5.19 (1H, d, $J = 17.1$ Hz, $\text{CH}=\text{CH}_2$), 4.75 (1H, br s, Orn δ -NH), 4.53 (3H, m, $\text{CH}_2\text{CH}=\text{CH}_2$ and Orn α -H), 4.37 (2H, m, Fmoc- CH_2), 4.26 (1H, m, Pro α -H), 4.21 (1H, t, $J = 6.9$ Hz, Fmoc-CH), 3.85 (1H, m, CH_2OSi), 3.72 (1H, m, CH_2OSi), 3.64 (1H, m, Pro δ -H), 3.48 (1H, m, Pro δ -H), 3.15 (2H, m, Orn δ -H), 2.08 (2H, m, Pro β -H and Pro γ -H), 1.93 (2H, m, Pro β -H and Pro γ -H), 1.73 (1H, m, Orn β -H), 1.56 (3H, m, Orn β -H and Orn γ -H), 1.06 (9H, s, $t\text{Bu}$); ^{13}C NMR (100 MHz, CDCl_3) δ 170.4, 156.2, 143.9, 143.8, 141.3, 135.5, 135.5, 133.4, 133.3, 132.9, 129.7, 127.7, 127.7, 127.7, 127.0, 125.2, 125.1, 119.9, 119.9, 117.5, 66.9, 65.4, 63.3, 58.6, 52.1, 47.6, 47.2, 40.7, 30.7, 26.9, 26.8, 25.5, 24.5, 19.2; IR (neat) 3307, 3278, 3970, 1716, 1633, 1525, 1448 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{45}\text{H}_{54}\text{N}_3\text{O}_6\text{Si}$ 760.3779 ($\text{M} + \text{H}^+$), found 760.3768; Anal. Calcd C, 71.1; H, 7.0; N, 5.5. Found C, 70.8; H, 7.0; N, 5.5.

4.1.10. N^δ -(Allyloxycarbonyl)-L-ornithyl-*O*-(*tert*-butyldiphenylsilyl)-L-prolinol (4). Compound **16** (3.35 g, 4.41 mmol) was stirred in morpholine (24 mL) at room temperature for 2 h. Then the precipitate was filtered off and the filtrate was co-evaporated with toluene. Flash column chromatography (toluene/ethanol, 10:1 \rightarrow 1:1) of the residue gave **4** as a colourless oil (2.22 g, 94%): $[\alpha]_{\text{D}}^{20} = -20.8$ (c 0.1, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , rotamer ratio 4:1) δ (major rotamer) 7.62 (4H, m, Ph), 7.34–7.44 (6H, m, Ph), 5.89 (2H, m, Orn δ -NH and $\text{CH}=\text{CH}_2$), 5.28 (2H, m, $\text{CH}=\text{CH}_2$), 4.53 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 4.24 (1H, m, Pro α -H), 3.82 (1H, ABX type dd, $J = 5.3$, 9.8 Hz, CH_2OSi), 3.72 (1H, ABX type dd, $J = 2.8$, 9.8 Hz, CH_2OSi), 3.53 (2H, m, Orn α -H and Pro δ -H), 3.41 (1H, m, Pro δ -H), 3.14 (2H, m, Orn δ -H), 2.07 (4H, m, Orn α -NH₂, Pro β -H and Pro γ -H), 1.81–1.95 (2H, m, Pro β -H and Pro γ -H), 1.58–1.71 (3H, m, Orn γ -H and Orn β -H), 1.49 (1H, m, Orn β -H), 1.04 (9H, s, $t\text{Bu}$); ^{13}C NMR (100 MHz, CDCl_3) δ 173.9, 156.2, 135.5, 135.4, 133.4, 132.9, 132.7, 129.9, 129.6, 128.2, 127.8, 127.6, 127.6, 117.4, 65.3, 63.4, 58.4, 52.6, 47.1, 40.6, 32.4, 26.8, 26.7, 26.3, 24.4, 19.2; IR (neat) 3298, 3070, 1712, 1631, 1531 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{30}\text{H}_{44}\text{N}_3\text{O}_4\text{Si}$ 538.3079 ($\text{M} + \text{H}^+$), found 538.3096.

4.1.11. Compound 17. $\text{NaBH}(\text{OAc})_3$ (101 mg, 0.478 mmol) was added to a solution of azido aldehyde **3** (132 mg, 0.30 mmol) and primary amine **4** (258 mg, 0.48 mmol) in dichloroethane (7.5 mL) at room temperature. After being stirred for 1 h the reaction was quenched with satd aq NaHCO_3 and extracted with CH_2Cl_2 (3×10 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 , filtered and concentrated. Flash column chromatography (toluene/ethanol, 20:1 \rightarrow 5:1) of the residue gave secondary amine **17** as a colourless oil (274 mg, 95%):

$[\alpha]_{\text{D}}^{20} = -31.1$ (*c* 2.5, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , rotamer ratio 4:1) δ (major rotamer) 7.62 (4H, m, Ph), 7.30–7.45 (11H, m, Ph), 7.18 (2H, d, $J=8.6$ Hz, Ph_{Tyr}), 6.93 (2H, d, $J=8.6$ Hz, Ph_{Tyr}), 5.90 (1H, m, $\text{CH}=\text{CH}_2$), 5.25–5.32 (2H, m, $\text{CH}=\text{CH}_2$ and Orn δ -NH), 5.18 (1H, m, $\text{CH}=\text{CH}_2$), 5.05 (2H, s, CH_2Ph), 4.54 (2H, dd, $J=5.5$, 13.0 Hz, $\text{CH}_2\text{CH}=\text{CH}_2$), 4.29 (1H, m, Pro α -H), 4.12 (1H, d, $J=16.1$ Hz, $\text{CH}_2\text{COO}t\text{Bu}$), 4.04 (1H, d, $J=16.1$ Hz, $\text{CH}_2\text{COO}t\text{Bu}$), 3.82 (1H, ABX type dd, $J=5.6$, 9.8 Hz, CH_2OSi), 3.73 (1H, dd, $J=2.8$, 9.8 Hz, CH_2OSi), 3.55 (2H, m, Pro δ -H and CHN_3CHO), 3.45 (2H, m, Pro δ -H and CHN_3), 3.25 (1H, m, Orn α -H), 3.12 (2H, m, Orn δ -H), 3.02 (1H, ABX type dd, $J=4.2$, 13.7 Hz, Tyr β -H), 2.77 (1H, ABX type dd, $J=9.3$, 14.0 Hz, Tyr β -H), 2.69 (1H, m, CH_2NH), 2.51 (1H, m, CH_2NH), 2.08 (2H, m, Pro β -H and Pro γ -H), 1.91 (2H, m, Pro β -H and Pro γ -H), 1.81 (2H, m, CH_2CHO), 1.59 (3H, m, Orn β -H and Orn γ -H), 1.49 (9H, s, $\text{COO}t\text{Bu}$), 1.46 (1H, m, Orn β -H), 1.05 (9H, s, OSiPh_2tBu); ^{13}C NMR (100 MHz, CDCl_3) δ 173.2, 169.3, 157.6, 156.2, 133.4, 133.0, 133.4, 133.3, 133.0, 130.2, 129.7, 128.5, 127.9, 127.8, 127.7, 127.6, 127.4, 117.4, 114.9, 81.6, 80.2, 70.0, 68.4, 65.3, 65.2, 63.3, 59.6, 58.3, 47.2, 44.6, 40.7, 35.4, 31.0, 28.1, 27.9, 26.8, 26.6, 24.4, 21.1 19.2; IR (neat) 3325, 3070, 2933, 2860, 2110, 1747, 1722, 1635, 1511 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{54}\text{H}_{73}\text{N}_6\text{O}_8\text{Si}$ 961.5279 ($\text{M}+\text{H}^+$), found 961.5258; Anal. Calcd C, 67.5; H, 7.6; N, 8.7. Found C, 67.4; H, 7.6; N, 8.7.

4.1.12. Compound 18. Fmoc-Leu-OH (**5**, 0.192 g, 0.543 mmol) was activated with diisopropylethylamine (DIEA, 0.19 mL, 1.09 mmol) and HATU (0.188 g, 0.494 mmol) at 0°C for 30 min in DMF (1.0 mL). Then a solution of **17** (0.475 g, 0.494 mmol) in DMF (2.0 mL) was added. After stirring for 24 h the solution was diluted with CH_2Cl_2 (30 mL) and then poured into H_2O (150 mL) and the aqueous phase was extracted with CH_2Cl_2 (5×10 mL). The combined organic layers were washed with 0.2 M aq HCl, H_2O , satd aq NaHCO_3 and brine. The organic layer was dried with Na_2SO_4 , filtered and concentrated. Flash column chromatography (toluene/ethanol, 30:1 \rightarrow 15:1 and heptane/ethyl acetate 10:1 \rightarrow 2:1) of the residue gave **18** as a white amorphous solid (333 mg, 52, 70% yield based on the recovered **17**): $[\alpha]_{\text{D}}^{20} = -54.2$ (*c* 0.3, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , rotamer ratio 4:1) δ (major rotamer) 7.75 (2H, d, $J=7.3$ Hz, Ph), 7.60 (6H, m, Ph), 7.29–7.44 (15H, m, Ph), 7.18 (2H, d, $J=8.1$ Hz, Ph_{Tyr}), 6.90 (2H, d, $J=8.1$ Hz, Ph_{Tyr}), 5.85 (1H, m, $\text{CH}=\text{CH}_2$), 5.33 (1H, d, $J=9.0$ Hz, Leu α -NH), 5.19–5.26 (2H, m, $\text{CH}=\text{CH}_2$ and Orn α -H), 5.13 (1H, d, $J=10.0$ Hz, $\text{CH}=\text{CH}_2$), 5.02 (2H, s, CH_2Ph), 4.90 (1H, m, Orn δ -NH), 4.67 (1H, m, Leu α -H), 4.48 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 4.31–4.43 (2H, m, Fmoc- CHCH_2), 4.21 (2H, m, Fmoc- CHCH_2 and Pro α -H), 4.11 (1H, d, $J=16.3$ Hz, $\text{CH}_2\text{COO}t\text{Bu}$), 4.02 (1H, d, $J=16.3$ Hz, $\text{CH}_2\text{COO}t\text{Bu}$), 3.87 (1H, ABX type dd, $J=5.0$, 10.0 Hz, CH_2OSi), 3.72 (1H, ABX type dd, $J=2.8$, 10.1 Hz, CH_2OSi), 3.65 (1H, m, CH_2N), 3.56 (1H, m, CHN_3), 3.49 (2H, m, Pro δ -H), 3.43 (2H, m, CH_2N and CHO), 3.07–3.17 (3H, m, Orn δ -H and Tyr β -H), 2.81 (1H, ABX type dd, $J=9.6$, 14.0 Hz, Tyr β -H), 1.85–2.10 (6H, m, Pro β -H, Pro γ -H, CH_2CHO and Orn β -H), 1.58–1.82 (4H, m, Pro γ -H, Orn β -H, Leu β -H, Leu γ -H), 1.45 (9H, s, $\text{COO}t\text{Bu}$), 1.28–1.36 (3H, m, Orn γ -H and Leu β -H), 1.05 (9H, s, $\text{OSi}t\text{Bu}$), 0.98 (6H, d, $J=6.3$ Hz, Leu γ -H); ^{13}C NMR (100 MHz, CDCl_3)

δ 173.8, 169.3, 168.6, 157.7, 156.2, 156.1, 143.8, 143.7, 141.3, 137.0, 135.7, 135.5, 135.5, 133.4, 133.0, 130.3, 130.1, 129.7, 129.7, 128.5, 127.9, 127.7, 127.5, 127.0, 125.2, 125.1, 120.0, 119.9, 117.4, 115.0, 81.6, 79.3, 70.0, 67.7, 67.0, 65.3, 64.7, 63.2, 58.7, 54.6, 49.9, 47.3, 47.1, 42.3, 40.5, 40.4, 34.9, 31.7, 28.1, 27.2, 27.0, 26.9, 26.2, 24.6, 24.4, 23.5, 21.3, 19.3; IR (neat) 3301, 2108, 1720, 1635, 1511 cm^{-1} ; ESMS calcd for $\text{C}_{75}\text{H}_{94}\text{N}_7\text{O}_{11}\text{Si}$ 1297.6 ($\text{M}+\text{H}^+$), found 1297.4.

4.1.13. Compound 19. Compound **18** (0.240 mg, 0.185 mmol) was stirred in formic acid (6.0 mL) for 19 h, after which the solution was poured into H_2O (15 mL) and extracted with EtOAc (5×5 mL). The combined organic layers were washed with brine, dried with Na_2SO_4 , filtered and concentrated. Flash column chromatography (toluene/ethanol, 30:1 \rightarrow 10:1) of the residue gave **19** as a white amorphous solid (124 mg, 65%): $[\alpha]_{\text{D}}^{20} = -53.5$ (*c* 0.2, CHCl_3); ^1H NMR (400 MHz, CDCl_3 , rotamer ratio 4:1) δ (major rotamer) 8.05 (1H, s, HCOO), 7.75 (2H, d, $J=7.5$ Hz, Ph), 7.56 (2H, d, $J=7.5$ Hz, Ph), 7.28–7.43 (9H, m, Ph), 7.15 (2H, d, $J=8.2$ Hz, Ph_{Tyr}), 6.91 (2H, d, $J=8.2$ Hz, Ph_{Tyr}), 5.88 (1H, m, $\text{CH}=\text{CH}_2$), 5.54 (1H, d, $J=9.1$ Hz, Leu-NH), 5.12–5.29 (3H, m, $\text{CH}=\text{CH}_2$ and Orn α -H), 5.01 (2H, s, CH_2Ph), 5.00 (1H, m, Orn δ -NH), 4.68 (1H, m, Leu α -H), 4.47–4.58 (2H, m, $\text{CH}_2\text{CH}=\text{CH}_2$), 4.16–4.43 (8H, m, CH_2COOH , Fmoc- CHCH_2 , Pro α -H, and CH_2OCHO), 3.80 (1H, m, CH_2N), 3.45–3.60 (5H, m, CHO, CHN_3 , Pro δ -H and CH_2N), 3.17 (2H, m, Orn δ -H), 3.01 (1H, ABX type dd, $J=3.2$, 13.4 Hz, Tyr β -H), 2.74 (1H, ABX type dd, $J=9.6$, 13.4 Hz, Tyr β -H), 1.96–2.06 (3H, m, Pro β -H, Pro γ -H and CH_2CHO), 1.82–1.92 (4H, m, Pro β -H, Pro γ -H, CH_2CHO and Orn β -H), 1.61–1.76 (3H, m, Orn β -H, Leu β -H), 1.41–1.51 (2H, m, Orn γ -H), 1.33 (1H, m, Leu γ -H), 1.01 (6H, d, $J=5.3$ Hz, $\text{CH}(\text{CH}_3)_2$); ^{13}C NMR (100 MHz, CDCl_3) δ 173.9, 171.1, 169.4, 160.8, 157.8, 156.2, 143.6, 141.2, 136.9, 132.8, 130.2, 129.3, 128.5, 127.9, 127.7, 127.5, 127.0, 125.1, 125.0, 120.0, 119.9, 117.6, 115.1, 79.6, 69.4, 67.9, 67.1, 65.5, 64.9, 62.9, 56.0, 54.1, 49.6, 47.2, 47.0, 42.4, 40.5, 40.2, 35.1, 32.2, 27.3, 26.9, 26.1, 24.6, 24.2, 23.4, 21.6; IR (neat) 3309, 2107, 1720, 1635, 1511 cm^{-1} ; HRMS (FAB) calcd for $\text{C}_{56}\text{H}_{67}\text{N}_7\text{O}_{12}\text{Na}$ 1052.4698 ($\text{M}+\text{Na}^+$), found 1052.4734; Anal. Calcd C, 65.2; H, 6.6; N, 9.5. Found C, 64.8; H, 6.6; N, 9.4.

4.1.14. Compound 21. Compound **19** (120 mg, 0.116 mmol) was activated by treatment with DIC (18.0 μL , 0.116 mmol) in ethyl acetate (0.8 mL) at 0°C for 10 min, after which pentafluorophenol (32.2 mg, 0.175 mmol) was added. After stirring for 1 h the solvent was evaporated and the residue was purified by flash column chromatography (heptane/ethyl acetate, 5:1 \rightarrow 1:1) to give the pentafluorophenyl ester corresponding to **19** as a white amorphous solid (115 mg). The pentafluorophenyl ester (115 mg) in dioxane (25 mL) was then added dropwise to a solution of DBU (43.9 mg, 0.288 mmol) in refluxing dioxane (25 mL) during 3 h. After adding all of the ester, the solution was allowed to reflux for a further 30 min and was then concentrated. Flash column chromatography (heptane/ethyl acetate, 2:1 then toluene/ethanol 25:1 \rightarrow 5:1) of the residue gave **20** (39 mg) and **21** (5 mg) as white solids, as well as a mixture of **20** and **21** (7 mg). Then aq LiOH solution (1 M, 67.0 μL , 67.0 μmol) was added to a solution of **20** (39 mg) and the mixture of **20**

and **21** (7 mg) in THF, MeOH and H₂O (3:1:1, 2.0 mL) at 0 °C. After being stirred for 30 min the cooling bath was removed and the reaction was stirred for a further 3 h. The solution was acidified with aq HCl (1 M) (5 drops), then poured into EtOAc and H₂O (4:1, 10 mL). The phases were separated and the aqueous phase was extracted with EtOAc (3 × 4 mL). The combined organic phases were then washed with brine, dried over Na₂SO₄, filtered and concentrated. Flash column chromatography (toluene/EtOH, 25:1 → 10:1) of the residue gave **21** as a white amorphous solid (40 mg in total, 51% from **19**): $[\alpha]_D^{20} = -69.7$ (c 0.3, CHCl₃); ¹H NMR (400 MHz, CDCl₃, rotamer ratio 4:1) δ (major rotamer) 7.33–7.45 (5H, m, Ph), 7.14 (2H, d, $J = 8.3$ Hz, Ph_{Tyr}), 6.95 (2H, d, $J = 8.4$ Hz, Ph_{Tyr}), 6.50 (1H, d, $J = 8.4$ Hz, Leu NH), 5.90 (1H, m, CH=CH₂), 5.15–5.35 (3H, m, Orn α -H and CH=CH₂), 5.06 (2H, s, CH₂Ph), 4.93 (1H, m, Orn δ -NH), 4.71 (1H, m, Leu α -H), 4.54 (2H, m, CH₂CH=CH₂), 4.41 (1H, d, $J = 15.3$ Hz, OCH₂CON), 3.91 (1H, m, Pro α -H), 3.78 (1H, m, bridge CH₂N), 3.70 (1H, m, CH₂OH), 3.55 (1H, m, CH₂OH and Pro δ -H), 3.53 (1H, d, $J = 15.4$ Hz, OCH₂CON), 3.43 (3H, m, Pro δ -H, bridge CH₂N and CHN₃), 3.35 (1H, m, CHO), 3.21 (2H, m, Orn δ -H), 3.01 (1H, ABX type dd, $J = 7.1$, 13.8 Hz, Tyr β -H), 2.93 (1H, ABX type dd, $J = 7.5$, 13.9 Hz, Tyr β -H), 1.90–2.02 (4H, m, bridge CH₂, Orn β -H, Pro β -H and OH), 1.66–1.85 (5H, m, Pro γ -H, Leu γ -H and Leu β -H), 1.52–1.63 (3H, m, Orn β -H, bridge CH₂ and Pro β -H), 1.45 (2H, m, Orn γ -H), 1.03 (3H, t, $J = 5.7$ Hz, Leu δ -CH₃), 0.98 (3H, t, $J = 5.6$ Hz, Leu δ -CH₃); ¹³C NMR (100 MHz, CDCl₃) δ 171.8, 171.2, 170.8, 158.0, 156.3, 136.8, 132.9, 130.3, 128.6, 128.6, 128.0, 127.5, 117.6, 115.2, 80.8, 70.2, 70.1, 66.5, 65.5, 65.0, 62.2, 54.6, 54.3, 47.9, 41.0, 40.5, 38.0, 36.2, 31.8, 28.3, 25.9, 25.9, 25.0, 24.2, 23.0, 21.2; IR (neat) 3334, 2106, 1701, 1679, 1624, 1512 cm⁻¹; HRMS (FAB) calcd for C₄₀H₅₅N₇O₈Na 784.3998 (M + Na⁺), found 784.3990.

4.1.15. Compound 2. Freshly prepared Jones reagent³⁹ (1 M, 0.257 mL, 0.257 mmol) was added to a solution of **21** (56.0 mg, 73.5 μ mol) in acetone (1.8 mL) at 0 °C. After being stirred for 10 min the solution was allowed to reach room temperature and stirred for a further 3.5 h. Then the reaction was quenched with isopropanol (5 drops) and the solution was concentrated. The residue was partitioned between CH₂Cl₂ and H₂O (4:1, 5 mL). The aqueous layer was extracted with CH₂Cl₂ (4 × 5 mL) and the combined organic layers were dried with Na₂SO₄, filtered and concentrated. Flash column chromatography (toluene/ethanol, 25:1 → 5:1) of the residue gave **2** as a white amorphous solid (40 mg, 70%): $[\alpha]_D^{20} = -51.4$ (c 0.3, CHCl₃); ¹H NMR (400 MHz, CD₃OD, rotamer ratio 3:1) δ (major rotamer) δ 7.33–7.47 (5H, m, Ph), 7.28 (2H, d, $J = 8.6$ Hz, Ph_{Tyr}), 7.01 (2H, d, $J = 8.6$ Hz, Ph_{Tyr}), 5.96 (1H, m, CH=CH₂), 5.43 (1H, t, $J = 7.4$ Hz, Orn α -H), 5.17–5.35 (2H, m, CH=CH₂), 5.12 (2H, s, CH₂Ph), 4.72 (1H, m, Leu α -H), 4.55 (2H, m, CH₂CH=CH₂), 4.46 (1H, m, OCH₂CON), 4.32 (1H, m, Pro α -H), 4.06 (1H, m, bridge CH₂N), 3.65–3.73 (3H, m, OCH₂CON, Pro δ -H and CHO), 3.55 (1H, m, Pro δ -H), 3.46 (1H, m, CHN₃), 3.35 (1H, m, bridge CH₂N), 3.17 (2H, m, Orn δ -H), 2.96 (1H, ABX type dd, $J = 4.0$, 14.3 Hz, Tyr β -H), 2.70 (1H, ABX type dd, $J = 9.2$, 14.3 Hz, Tyr β -H), 2.25 (1H, m, Pro β -H), 1.80–2.08 (7H, m, Pro β -H, Pro γ -H, Orn β -H, Leu β -H and bridge CH₂, Leu γ -H), 1.62–1.73 (3H, m, Orn β -H, Leu β -H, bridge CH₂), 1.46 (2H, m, Orn

γ -H), 1.06 (3H, t, $J = 6.5$ Hz, Leu δ -CH₃), 1.00 (3H, t, $J = 6.5$ Hz, Leu δ -CH₃); ¹³C NMR (100 MHz, CD₃OD) δ 173.9, 172.8, 169.4, 157.9, 157.4, 137.3, 133.1, 130.4, 130.2, 130.1, 129.7, 129.6, 128.2, 127.6, 127.3, 127.3, 116.4, 115.0, 114.9, 114.7, 82.3, 71.3, 70.9, 66.9, 66.2, 62.5, 56.0, 55.5, 48.4, 41.3, 41.2, 39.1, 37.1, 31.7, 30.5, 26.6, 26.5, 25.8, 25.5, 23.3, 21.3; IR (neat) 3325, 2106, 1714, 1624, 1512 cm⁻¹; HRMS (FAB) calcd for C₄₀H₅₄N₇O₉ 776.3979 (M + H⁺), found 776.3999.

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