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5,5-Dimethylproline dipeptides: an acid-stable class of pseudoproline

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ABSTRACT

Commercially available Fmoc-protected L-amino acids were employed and coupled to L-allylglycine. Cross metathesis with 2-methyl-2-butene using second generation Grubbs' catalyst gave L-prenylglycine-containing dipeptides. Treatment with trifluoromethanesulfonic acid resulted in cyclisation and subsequent formation of acid-stable 5,5-dimethyl-L-proline dipeptides for direct insertion into linear peptide sequences.

1. Introduction

The cyclic nature of proline (Pro, **1**) offers it a unique set of conformational properties and its inclusion in peptide sequences can often promote *cisoidal* amide geometry at Xaa–Pro junctions.[1] Significantly, *cis–trans* isomerisation of this peptide bond is essential for regulating many important biological processes, including protein folding, recognition and signal transduction.[1–3] In fact, many biologically active peptides are activated by Pro isomerisation. Interconversion at a single point, not only triggers neurotransmitter-gated ion channels,[4] but also stabilises the bioactive cis conformation of δ-conotoxin EVIA,[2] somatostatin,[5] the gp120 V3 loop of HIV-1,[6] BBI proteins,[7] RNase A[8] and also the opioid peptides morphiceptin and endomorphin-2.[9]

Structural analogues of Pro **1**, such as pseudoprolines (ΨPro) **2** and **3**, can be used to examine the folding pathways and bioactive conformation of prolyl-containing therapeutic targets (Fig. 1).[10,11] These residues are readily prepared via the *cyclo*-condensation of amino acids serine, threonine and cysteine with aldehydes, ketones and acetals.[12,13] The nature and degree of ΨPro ring-substitution is known to affect the position of *cis–trans* equilibrium: Disubstituted ΨPro-containing peptides strongly favour a *cis*-configured amide bond, whereas unsubstituted rings generate a higher preference for the *trans* conformation.[3] In addition to influencing the conformation of an imidic Xaa–Pro bond, disubstitution also reduces the chemical stability of the oxa- **2** and thiozolidine **3** cores. These *cis*-promoting rings are readily cleaved under mild experimental conditions using dilute acid, and will readily revert back to parent acyclic residues.[12]

Structurally related 5,5-dimethyl-L-proline (dmP, **4**), on the other hand, acts in a similar fashion but its carbocyclic framework renders it completely stable to regular resin cleavage conditions.[14] Incorporation of this moiety into peptide sequences constrains the Xaa–Pro amide bond into a *cisoid*-arrangement and can be used to permanently lock a bioactive *cis*-prolyl conformation and potentially enhance activity.[1,15] We have recently reported an enantioselective synthesis of dmP **4** from commercially available

Abbreviations: Second gen Grubbs' cat., tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene](benzylidene)ruthenium(II) dichloride or second generation Grubbs' catalyst; Ac, acetyl; Agl, (2*S*)-2-amino-4-pentenoic acid or L-allylglycine; Ala, (2*S*)-2-aminopropanoic acid or L-alanine; Bn, benzyl; CM, cross metathesis; DMF, *N*,*N*-dimethylformamide; dmP, (2*S*)-5,5-dimethylpyrrolidine-2-carboxylic acid or 5,5-dimethyl-L-proline; Et, ethyl; Fmoc, *N*-(9*H*-fluoren-9-ylmethoxycarbonyl); Fmoc-OSu, *N*-(9*H*-fluoren-9-ylmethoxycarbonyl) pyrrolidine-2,5-dione; h, hour; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; min, minutes; Me, methyl; NMM, *N*-methylmorpholine; NMR, nuclear magnetic resonance; Phe, (2*S*)-2-amino-3-phenylpropanoic acid or L-phenylalanine; Pre, (2*S*)-2-amino-5-methyl-4-hexenoic acid or L-prenylglyine; Pro, (2*S*)-pyrrolidine-2-carboxylic acid or L-proline; ΨPro, pseudoproline; RP-HPLC, reverse phase high performance liquid chromatography; rt, room temperature; Ser, (2*S*)-2-amino-3-hydroxypropanoic acid or L-serine; SiO_2, silica gel; SPPS, solid phase peptide synthesis; ^{t}Bu, *tert*-butyl; TFA, trifluoroacetic acid; TfOH, trifluromethanesulfonic acid; TIPS, triisopropylsilane; TLC, thin layer chromatography; Val, (2*S*)-2-amino-3-methylbutanoic acid or L-valine; Xaa, unspecified amino acid in three letter code.

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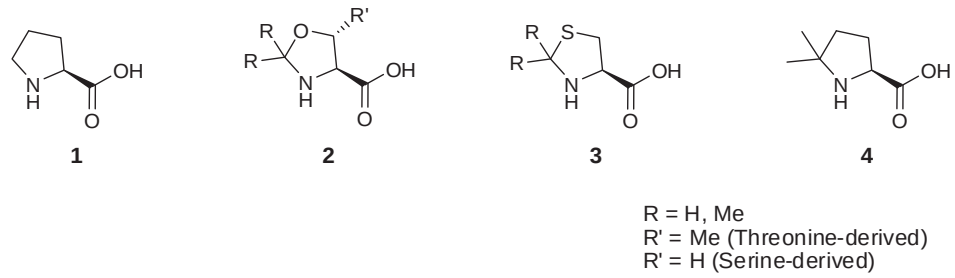


Figure 1. Proline **1** and structurally related pseudoprolines **2**–**4**.

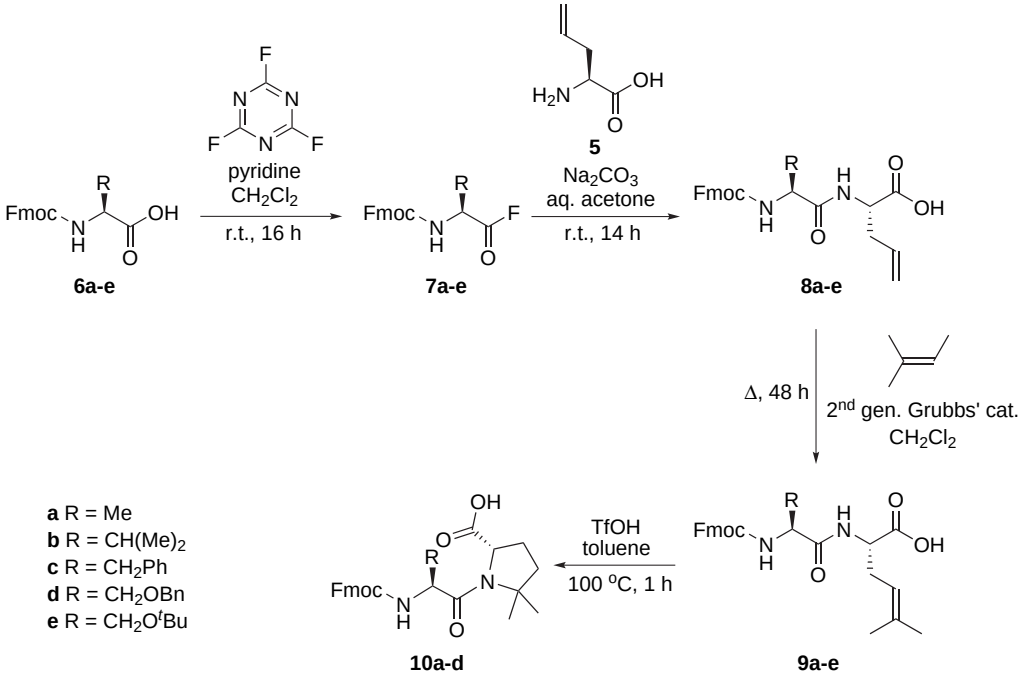
L-allylglycine (Agl, **5**).[16] While insertion of this residue into peptide primary sequences is unproblematic, the subsequent amino acid coupling can be troublesome due to the steric congestion imposed by ΨPro ring-substitution. Similarly, the structurally related disubstituted oxa- and thiozolidine analogues **2** and **3** are also difficult to acylate once incorporated into a peptide sequence and these ΨPro analogues are best inserted as preformed dipeptide units.[12,17] This communication describes the high yielding and enantioselective synthesis of acid stable dmP-containing dipeptide units, which are suitable for direct insertion into peptide primary sequences.

2. Results and discussion

Synthesis commenced with commercially available Fmoc-protected amino acids. The versatility of this methodology was assessed by selecting *N*-terminal residues bearing sidechain functionality of varying character: Linear (Fmoc-L-Ala-OH, **6a**), branched (Fmoc-L-Val-OH, **6b**), aromatic (Fmoc-L-Phe-OH, **6c**) and a sidechain bearing a bulky protecting group (Fmoc-L-Ser(Bn)-OH, **6d** and Fmoc-L-Ser(^{t}Bu)-OH, **6e**). Each *N*-terminal residue **6a**–**e** was *C*-activated as an acid fluoride, through the use of cyanuric fluoride and pyridine (Scheme 1). Reactions proceeded with quantitative yield to the expected products **7a**–**e**.

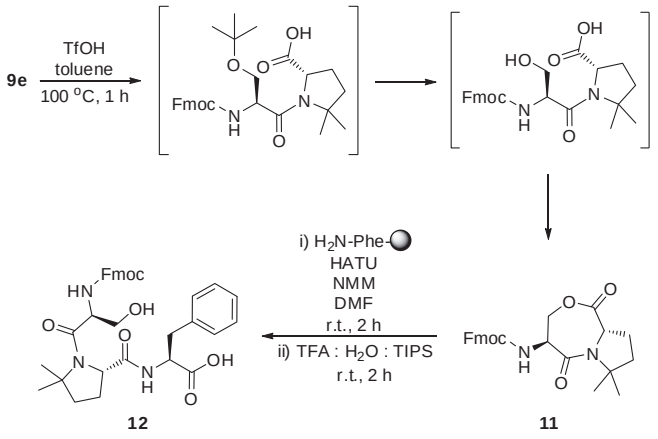
Formation of dipeptides **8a**–**e** was achieved via the coupling of Agl **5** to each activated amino acid under basic conditions (Scheme 1). Again, reactions proceeded with high conversion and each dipeptide unit was isolated in 74–98% yield after column chromatography. Subsequent cross metathesis (CM) of dipeptides **8a**–**e** and 2-methyl-2-butene, in the presence of either 5 mol % second generation Grubbs' catalyst or second generation Hoveyda-Grubbs' catalyst, gave prenylglycine derivatives **9a**–**e** in 48 h (Scheme 1). Proton NMR analysis of the crude reaction mixtures showed near quantitative conversion to the required products. In all five cases, trace amounts of the corresponding crotylglycine derivative accompanied the major product. Each dmP precursor **9a**–**e** was isolated in 72–90% yield after column chromatography.

Acid-catalysed cyclisation to give *N*-Fmoc-protected dmP dipeptides **10a**–**d** was affected under non-aqueous conditions on treatment with 20 mol % trifluoromethanesulfonic acid in toluene at 100 °C (Scheme 1). Anhydrous conditions were employed to prevent the formation of undesired by-products arising from hydration of the prenylglycine sidechain.[16] Quantitative cyclisation was achieved in 1 h and target dipeptides **10a**–**c** and **10d** were isolated in 74–86% and 48% yields respectively, after column chromatography. ^{1}H and ^{13}C NMR spectra of the dipeptides showed only a single set of resonances in all four cases. These were verified to be conformers with *cisoidal* amide geometry at the Xaa–Ψprolyl junction, on the basis of typical αH_{i-1}–αH_i and αH_{i-1}–βH_i cross-



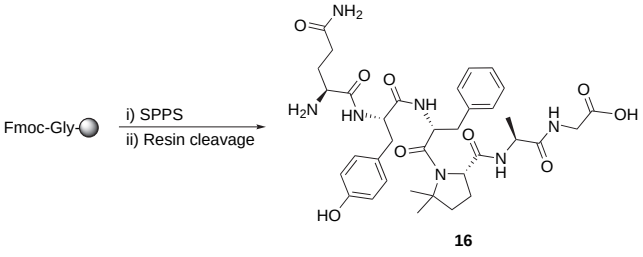
Scheme 1.

peaks observed in 2D NOESY experiments.[18] Chemoselective cyclisation of **9e** without concomitant *tert*-butyl deprotection of the serine sidechain, however, proved to be unsuccessful. Interestingly, under the reaction conditions used to generate analogues **10a**–**d**, dipeptide **9e** was found to undergo tandem cyclisation, deprotection and esterfication to afford pyrrolo–oxazepine **11** in 72% yield. Conveniently, this bicycle was readily incorporated into linear peptide sequence **12** via standard SPPS protocols (Scheme 2).



Scheme 2.

Although chromatography was used to purify each of the ΨPro dipetides, product mixtures **10a**–**d** were routinely employed without purification. Exploiting the crude purity of **10c**, standard SPPS was used to construct a linear Phe-dmP-containing hexapeptide **16** onto Fmoc-Gly-Wang resin with the aid of HATU–NMM activation and Fmoc-protected amino acids. Each intermediate was carried through without purification and characterisation until the desired hexapeptide had been constructed (Scheme 3). After sequence completion, a small aliquot of the resin-tethered peptide was subjected to *N*-Fmoc deprotection and TFA-mediated cleavage. Mass spectral analysis of the resultant solid gave a molecular ion peak at *m*/*z* 710.2, consistent with the structure of sidechain deprotected linear peptide **16**. The crude peptide was found to be of 85% purity by analytical RP-HPLC.



Scheme 3.

Interestingly, the preparation of dmP-containing ΨPro dipeptides **10a**–**d** proved to be more straightforward than the singular Fmoc-dmP-OH **13** unit. In our hands, two separate approaches were unsuccessful (Scheme 4). Attempts to directly cyclise Fmoc-protected prenylglycine **14**, with 20 mol% trifluoromethanesulfonic acid in toluene at 100 °C, lead to complete decomposition of the starting material. Furthermore, direct Fmoc-protection of dmP residue **15** (Scheme 4) was also unsuccessful and gave only low yields of the protected target **13**. The latter result was not surprising given the inefficient coupling of this residue with less sterically demanding amino acid residues during peptide synthesis. Indeed Scheraga et al.[8] have exploited the reduced reactivity of **15** by coupling the unprotected and activated residue onto resin in high yield. Subsequent acylation reactions, however, proved unpredictable and required several long-duration coupling attempts to achieve peptides with only modest yield and purity when compared to dipeptide cassettes.

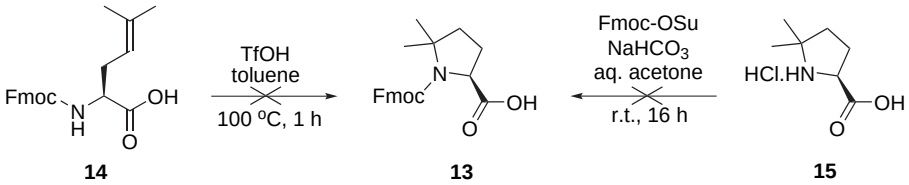
2.1. Conclusion

The methodology reported herein provides an expedient and generic synthesis of enantiomerically pure dmP-containing dipeptides from commercially available starting materials. Each transformation was high yielding and crude ΨPro dipeptides were of sufficient purity to allow direct and high yielding insertion into peptide sequences. For the generation of Xaa–dmP dipeptides possessing reactive sidechain functionality (which can interfere with SPPS), the use of a non-acid labile protecting group on the Xaa residue is recommended. The dmP-based pseudoproline dipeptides developed herein can be directly incorporated into linear sequences to give high purity peptide products with stable, *cis*-configured, Xaa–Ψprolyl amide bonds.

3. Experimental procedures

3.1. Instrumentation

Melting points (mp) were determined using a Reichert hot-stage melting point apparatus and are uncorrected. Infrared spectra (IR) spectra were recorded on a Perkin–Elmer 1600 series Fourier Transform infrared spectrophotometer as potassium bromide discs of solids (KBr) or as thin films of liquid (neat) between sodium chloride plates. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Bruker AV200, DPX300 or DRX400 spectrometers operating at 200, 300 or 400 MHz, respectively, as solutions in deuterated solvents as specified. Carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra were recorded on Bruker AV200, DPX300 or DRX400 spectrometers operating at 50, 75 or 100 MHz, respectively, as solutions in deuterated solvents as specified. Assignments were determined from *J*-Modulated Spin Echo experiments showing quaternary and methylene signals in the opposite phase to those of methine and methyl resonances. Correlation spectroscopy (COSY) was used to correlate chemical shifts of protons coupled to one another. Heteronuclear Multiple Quantum Correlation (HMQC) spectroscopy was used to correlate directly bonded ^{13}C–^{1}H nuclei. Heteronuclear Multiple Bond Correlation (HMBC) spectroscopy was used to determine long range ^{13}C–^{1}H connectivity. All two-dimensional (2D)-NMR experiments were recorded on Bruker DPX300 or DRX400 spectrometers. Low resolution electrospray ionisation (ESI) mass spectra were recorded



Scheme 4.

on a Micromass Platform Electrospray mass spectrometer (QMS-quadrupole mass electroscopy) as solutions in specified solvents. Spectra were recorded in the positive mode (ESI^+). High resolution electrospray mass spectra (HRMS) were recorded on a Bruker BioApex 47e Fourier Transform mass spectrometer (4.7 T magnet) fitted with an analytical electrospray source. The mass spectrometer was calibrated with an internal standard solution of sodium iodide in MeOH. Analytical thin layer chromatography (TLC) was performed on plastic plates coated with 0.25 mm of silica gel (Polygram SIL G/UV_{254}). Flash chromatography was carried out using Merck silica gel 60, 0.063–0.200 mm (70–230 mesh). The compounds were visualized under 254 nm ultraviolet irradiation. Eluent mixtures are expressed as volume to volume ratios. Analytical reverse phase high performance liquid chromatography (RP-HPLC) was performed on an Agilent 1200 series instrument equipped with photodiode array (PDA) detection (controlled by ChemStation software) and an automated injector (100 μL loop volume). Analytical separations were performed on a Vydac C18 (4.6×250 mm, 5 μm) analytical column at flow rates of 1.5 mL min^{-1}. Preparative RP-HPLC was performed on an Agilent 1200 series instrument equipped with multivariable wavelength (MVW) detection (controlled by ChemStation software) and an Agilent unit injector (2 mL loop volume). Preparative separations were performed on a Vydac C18 (22×250 mm, 10 μm) preparative column at flow rates of 10 mL min^{-1}. The solvent system used throughout this study was buffer A: 0.1% aqueous TFA; buffer B: 0.1% TFA in MeCN. Linear gradients of 0.1% TFA in MeCN (buffer B) were employed as specified.

3.2. Peptide materials and procedures

Solid phase peptide synthesis (SPPS) was performed in polypropylene Terumo syringes (5 or 10 mL) fitted with a porous (20 μm) polyethylene filter. Resin wash and filtering steps were aided by the use of a Visprep™ SPE DL 24-port model vacuum manifold as supplied by Supelco. Coupling reactions and cleavage mixtures were shaken on a KS125 basic KA elliptical shaker supplied by Labortechnik. Cleaved peptides were collected by centrifugation on a TMC-1 mini centrifuge supplied by Thermoline. *N*,*N*-Dimethylformamide (DMF) was supplied by Auspep and stored over 4 Å molecular sieves. CH_2Cl_2 was dried over calcium chloride ($CaCl_2$) and distilled from CaH_2 prior to use.

3.2.1. Manual SPPS procedure. Manual SPPS was carried out using fritted plastic syringes, which allowed filtration of solution without the loss of resin. The tap fitted syringes were attached to a vacuum tank and all washings were removed in vacuo. This involved soaking the resin in the required solvent for a reported period of time followed by evacuation, which allowed for removal of excess reagents before subsequent coupling reactions. In a fritted syringe, the resin was swollen with CH_2Cl_2 (7 mL, 3×1 min, 1×60 min) and DMF (7 mL, 3×1 min, 1×30 min). Prior to the first coupling, Fmoc-Gly-Wang resin was subjected to Fmoc-deprotection in the presence of 20% piperidine in DMF (7 mL, 1×1 min, 2×10 min) and further washed with DMF (7 mL, 5×1 min) to ensure traces of excess reagents had been removed. Amino acid pre-activation was achieved by addition of NMM (6 equiv) to a solution of the desired protected amino acid, Fmoc-L-Xaa-OH (3 equiv) and HATU (2 equiv) in DMF (3 mL). The mixture was sonicated for ~1 min and the resulting solution then added to the resin-tethered amino acid and gently shaken on an elliptical shaker. After the reported period of time, the peptidyl-resin was washed with DMF (7 mL, 3×1 min) to ensure excess reagents were removed. Kaiser tests were performed to monitor coupling success and any incomplete coupling reactions were repeated with extended reaction times. On negative test results for the presence of free amines, the resin-peptide was deprotected with 20%v/v piperidine in DMF (7 mL, 1×1 min, 2×10 min) and further washed with DMF (7 mL, 5×1 min) to remove traces of base prior to coupling the next amino acid. The above procedure was repeated until the desired peptide sequence was constructed. Once complete, the resin was washed with DMF (7 mL, 3×1 min), CH_2Cl_2 (7 mL, 3×1 min), MeOH (7 mL, 3×1 min), CH_2Cl_2 (7 mL, 3×1 min), MeOH (7 mL, 3×1 min) and left to dry in vacuo for 1 h. A small aliquot of resin-tethered peptide was then exposed to a TFA cleavage solution to allow for chromatographic and mass spectroscopic analysis.

3.2.2. TFA cleavage procedure. A small aliquot of resin-bound peptide (approx. 3 mg) was suspended in a cleavage solution (1 mL; 95:2:2:1; TFA–TIPS–water–thioanisol) and shaken gently for 2 h. The mixture was filtered through a fritted syringe and the beads rinsed with TFA (1×0.2 mL). The filtrate was concentrated under a constant stream of air and the resultant oil was induced to precipitate in ice-cold diethyl ether (1 mL). Cleaved peptides were collected by centrifugation (3×5 min) and dried for analysis by analytical RP-HPLC and mass spectrometry. For full scale resin cleavages, the resin was exposed to the cleavage solution (10 mL, 4 h) before being rinsed with TFA (3×3 mL). The filtrate was then concentrated under a constant stream of air and the resultant oil was induced to precipitate in ice-cold diethyl ether (35 mL). Collection by centrifugation proceeded over 5×6 min spin times.

3.3. Catalytic materials and procedures

Tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydro-imidazol-2-ylidene](benzylidene)ruthenium(II) dichloride (second generation Grubbs' catalyst) and (1,3-Bis-(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(o-isopropoxyphenylmethylene)ruthenium(II) (second generation Hoveyda-Grubbs' catalyst) were used as supplied by Aldrich and stored under argon in a dry box. High purity (<10 ppm oxygen) argon was supplied by BOC gases and additional purification was achieved by passage through water, oxygen and hydrocarbon traps. 2-Methyl-2-butene was used as supplied by Aldrich and degassed prior to use. The CH_2Cl_2 used in all metal-catalysed metathesis reactions was degassed with high purity argon prior to use. Toluene was stored over Na wire and distilled from Na prior to use. Trifluoromethanesulfonic acid was supplied by Aldrich and was distilled from phosphorus pentoxide prior to use.

3.3.1. Cross metathesis procedure. A Fischer–Porter tube was loaded with substrate, deoxygenated solvent, catalyst and 2-methyl-2-butene. The system was sealed, submerged in a hot water bath and the reaction mixture stirred at reflux for 48 h. Metathesis reactions were terminated by exposure to oxygen and volatile species removed under reduced pressure. Reaction mixtures could be purified by column chromatography, however, metathesis products were generally employed in subsequent reactions without purification. Metathesis experiments are described using the following format: substrate (mg), solvent (mL), catalyst (mg), reacting olefin (mL), reaction temperature (°C), reaction time (h), percent conversion (%). Where applicable, chromatographic purification conditions (isolated yield, %) are also listed.

3.4. Fmoc-L-Ala-L-dmP-OH, 10a

3.4.1. Fmoc-L-Ala-F, ***7a****.* The acid fluoride **7a** was prepared according to a modified procedure described by Carpino et al.[19] Cyanuric fluoride (0.17 g, 1.28 mmol) and pyridine (0.05 g, 0.64 mmol) were added to a stirred solution of Fmoc-L-Ala-OH **6a** (0.20 g, 0.64 mmol) in dry CH_2Cl_2 (15 mL). The colourless solution was stirred at room temperature for 15 h, resulting in the formation of a white

precipitate. This suspension was dissolved by addition of water (15 mL) and the phases were separated. The aqueous layer was further extracted with CH_2Cl_2 (2×10 mL) and the combined organic extract then washed with a saturated NaCl solution, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the acid fluoride **7a** as a colourless solid (0.20 g, 100%), mp 110–112 °C (lit.[19] 111–112 °C). ν_{max} (KBr): 3329s, 3049w, 2992w, 2983w, 1845s, 1689s, 1537s, 1450m, 1380w, 1334m, 1276s, 1188w, 1153w, 1084m, 1065m, 1020m, 936w, 804w, 760w, 740m, 697w, 625w, 550w, 514w, 491w, 428w cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.54 (d, *J* 7.1 Hz, 3H, CH_3), 4.23 (t, *J* 6.9 Hz, 1H, H9′), 4.40–4.65 (m, 3H, CH_2OCO, C*H*CH_3), 5.24 (br d, *J* 8.7 Hz, 1H, NH), 7.32 (td, *J* 7.4, 1.2 Hz, 2H, H2′, 7′), 7.41 (tt, *J* 7.4, 0.6 Hz, 2H, H3′, 6′), 7.59 (br d, *J* 7.4, 2H, H1′, 8′), 7.77 (d, *J* 7.4 Hz, 2H, H4′, 5′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 17.3 (CH_3), 47.3 (C9′), 48.7 (d, $^2J_{CF}$ 62.9 Hz, *C*HCH$_3$), 67.6 (CH_2OCO), 120.2 (C2′, 7′), 125.2 (C3′, 6′), 127.4 (C1′, 8′), 128.0 (C4′, 5′), 141.6 (C8a′, 9a′), 143.8 (C4a′, 4b′), 155.8 (OCONH), 163.3 (d, $^1J_{CF}$ 371.0 Hz, COF). Mass spectrum (ESI$^+$, MeOH): The acid fluoride **7b** readily converted to the corresponding methyl ester during analysis: *m*/*z* calcd for $C_{19}H_{19}NNaO_4$ (M+Na)$^+$ 348.1, found 348.3.

3.4.2. Fmoc-L-Ala-L-Agl-OH, ***8a****.* The dipeptide **8a** was prepared according to a procedure described by Mutter et al.[12] A solution of Agl **5** (77 mg, 0.67 mmol) in 10% w/v Na_2CO_3 (1.5 mL) was added to a stirred solution of Fmoc-L-Ala-F **7a** (0.19 g, 0.61 mmol) in acetone (12 mL). The resultant white suspension was stirred at room temperature and monitored by TLC (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05). After 4 h, the acetone was evaporated in vacuo and water (20 mL) was added. The resultant aqueous phase was diluted with CH_2Cl_2 (30 mL), cooled in an ice bath and carefully acidified to pH 2 with 1 M HCl. The phases were separated and the aqueous layer further extracted with CH_2Cl_2 (2×30 mL). The combined organic extract was washed with a saturated NaCl solution (1×40 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a pale yellow solid (0.21 g, 82%). The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.26) and lyophilised to afford the desired dipeptide **8a** as a colourless solid (0.18 g, 74%), mp 130–133 °C. ν_{max} (KBr): 3456w, 3302m, 3068w, 2937w, 2417w, 1691s, 1654s, 1543m, 1450m, 1255m, 1087w, 1040w, 930w, 740m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.33 (d, *J* 7.1 Hz, 3H, CHC*H*$_3$), 2.48 (m, 1H, C*H*aHbCH=), 2.60 (m, 1H, CHa*H*bCH=), 4.12–4.22 (m, 2H, H9′, C*H*CH$_3$), 4.29–4.40 (m, 2H, CH_2OCO), 4.43 (m, 1H, C*H*COOH), 5.04 (d, *J* 10.0 Hz, 1H, =C*H*aHb), 5.10 (d, *J* 17.0 Hz, 1H, =CHa*H*b), 5.76 (m, 1H, C*H*=CH$_2$), 7.30 (td, *J* 7.5, 0.9 Hz, 2H, H2′, 7′), 7.37 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.65 (br t, *J* 7.5 Hz, 2H, H1′, 8′), 7.78 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (100 MHz, MeOD): δ 18.2 (CHCH$_3$), 37.0 (*C*H$_2$CH=), 48.4 (C9′), 51.8 (*C*HCH$_3$), 53.5 (*C*HCOOH), 68.0 (CH_2OCO), 118.7 (=CH$_2$), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 128.2 (C1′, 8′), 128.8 (C4′, 5′), 134.5 (*C*H=CH$_2$), 142.6 (C8′a, 9′a), 145.2, 145.4 (C4′a, 4′b), 158.2 (OCONH), 174.6 (CONH), 175.3 (COOH). HRMS (ESI$^+$, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{23}H_{24}N_2NaO_5$ (M+Na)$^+$ 431.1583, found 431.1572.

3.4.3. Fmoc-L-Ala-L-Pre-OH, ***9a****.* Fmoc-L-Ala-L-Agl-OH **8a** was subjected to the general CM procedure outlined in Section 3.3.1.: Fmoc-L-Ala-L-Agl-OH **8a** (0.18 g, 0.45 mmol), CH_2Cl_2 (5 mL), second generation Grubbs′ catalyst (19 mg, 23 μmol), 2-methyl-2-butene (1 mL), Δ, 48 h, 95%. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.29) to afford the desired dipeptide **9a** as a colourless solid (0.16 g, 84%), mp >300 °C. ν_{max} (film): 3420m, 3320m, 3098w, 2934m, 2250w, 1950w, 1715s, 1668s, 1652s, 1538m, 1450m, 1260m, 1079m, 910w, 801w, 760w, 739w cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.28 (d, *J* 6.7 Hz, 3H, CHC*H*$_3$), 1.59 (s, 3H, CH_3C=), 1.65 (s, 3H, CH_3C=), 2.43 (m, 1H, C*H*aHbCH=), 2.56 (m, 1H, CHa*H*bCH=), 4.17 (m, 1H, C*H*CH$_3$), 4.22 (br t, *J* 6.7 Hz, 1H, H9′), 4.28–4.43 (m, 3H, CH_2OCO, C*H*COOH), 5.12 (br s, 1H, CH=), 7.31 (t, *J* 7.5 Hz, 2H, H2′, 7′), 7.38 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.67 (br t, *J* 7.5 Hz, 2H, H1′, 8′), 7.79 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (75 MHz, MeOD): δ 18.0 (*C*H$_3$C=), 18.9 (CH*C*H$_3$), 25.9 (*C*H$_3$C=), 30.5 (CH*C*H$_2$CH=), 47.2 (C9′), 50.6 (*C*HCH$_3$), 52.5 (*C*HCOOH), 67.4 (CH_2OCO), 117.6 (CH=), 120.1 (C2′, 7′), 125.2 (C3′, 6′), 127.2 (C1′, 8′), 127.8 (C4′, 5′), 136.7 (C=), 141.4 (C8′a, 9′a), 143.9 (C4′a, 4′b), 156.2 (OCONH), 172.6 (CONH), 174.6 (COOH). HRMS (ESI$^+$, CH_2Cl_2: MeOH): *m*/*z* calcd for $C_{25}H_{28}N_2NaO_5$ (M+Na)$^+$ 459.1896, found 459.1901.

3.4.4. Fmoc-L-Ala-L-dmP-OH, ***10a****.* Fmoc-L-Ala-L-Pre-OH **9a** was subjected to a modified procedure described by Hartwig and Schlummer.[20] Trifluoromethanesulfonic acid (3.5 μL, 40 μmol) was added to a suspension of enamine **9a** (86 mg, 0.20 mmol) in toluene (15 mL). The reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature and concentrated under reduced pressure to give a dark brown oil. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.33) to afford the desired dmP-containing dipeptide **10a** as a colourless solid (64 mg, 74%), mp 64–66 °C. ν_{max} (KBr): 3583m, 3413br s, 3322br s, 3064m, 2973m, 2934m, 2364w, 2258w, 2188w, 2124w, 2080w, 1954w, 1722s, 1715s, 1668s, 1612w, 1538s, 1532s, 1520s, 1478m, 1451s, 1376m, 1320m, 1266s, 1155m, 1118m, 1085s, 1034m, 972w, 951w, 940w, 908w, 877w, 859w, 760m, 738s, 703m, 666m, 621m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.15 (br s, 3H, CHC*H*$_3$), 1.34 (s, 3H, CCH$_3$), 1.36 (s, 3H, CCH$_3$), 1.43–1.54 (m, 2H, C*H*$_2$C(CH$_3$)$_2$), 1.76 (m, 1H, C*H*aHbCHCOOH), 1.96 (m, 1H, CHa*H*bCHCOOH), 4.15–4.26 (m, 2H, C*H*CH$_3$, H9′), 4.29–4.42 (m, 3H, CH_2OCO, C*H*COOH), 7.30 (t, *J* 7.5 Hz, 2H, H2′, 7′), 7.38 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.65 (br t, *J* 7.5 Hz, 2H, H1′, 8′), 7.78 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (100 MHz, MeOD): δ 18.2 (CH*C*H$_3$), 27.5 (CH*C*H$_2$CH$_2$), 29.0 (CCH$_3$), 29.3 (*C*CH$_3$), 40.3 (*C*H$_2$C(CH$_3$)$_2$), 48.4 (C9′), 51.8 (CHCOOH), 54.1 (*C*HCH$_3$), 68.0 (CH_2OCO), 70.8 (*C*(CH$_3$)$_2$), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 128.2 (C1′, 8′), 128.8 (C4′, 5′), 142.6 (C8′a, 9′a), 145.2, 145.4 (C4′a, 4′b), 158.2 (OCONH), 174.0 (CONH), 175.7 (COOH). HRMS (ESI$^+$, MeOH): *m*/*z* calcd for $C_{25}H_{29}N_2O_5$ (M+H)$^+$ 437.2076, found 437.2075; calcd for $C_{25}H_{28}N_2NaO_5$ (M+Na)$^+$ 459.1896, found 459.1895. $[\alpha]_D^{22}$ −29.5 (*c* 0.6, MeOH).

3.5. Fmoc-L-Val-L-dmP-OH, 10b

3.5.1. Fmoc-L-Val-F, ***7b****.* The acid fluoride **7b** was prepared according to a modified procedure described by Carpino et al.[19] Cyanuric fluoride (0.16 g, 1.18 mmol) and pyridine (0.05 g, 0.59 mmol) were added to a stirred solution of Fmoc-L-Val-OH **6b** (0.20 g, 0.59 mmol) in dry CH_2Cl_2 (15 mL). The colourless solution was stirred at room temperature for 15 h, resulting in the formation of a white precipitate. This suspension was dissolved by addition of water (10 mL) and the phases were separated. The aqueous layer was further extracted with CH_2Cl_2 (2×10 mL) and the combined organic extract then washed with a saturated NaCl solution, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the acid fluoride **7b** as a colourless solid (0.20 g, 100%), mp 115–117 °C (lit.[19] 113–114 °C). ν_{max} (KBr): 3314m, 3059w, 2969w, 1844s, 1690s, 1538s, 1451m, 1313m, 1283m, 1158w, 1094m, 1037w, 1006w, 980w, 944w, 897w, 872w, 761m, 741m, 644w, 542w, 429w cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.00 (d, *J* 6.8 Hz, 3H, C*H*$_3$CH), 1.05 (d, *J* 6.7 Hz, 3H, C*H*$_3$CH), 2.26 (m, 1H, C*H*(CH$_3$)$_2$), 4.24 (t, *J* 6.7 Hz, 1H, H9′), 4.41–4.61 (m, 3H, CH_2OCO, C*H*COF), 5.14 (br d, *J* 8.1 Hz, 1H, NH), 7.33 (tt, *J* 7.4, 1.0 Hz, 2H, H2′, 7′), 7.41 (t, *J* 7.4 Hz, 2H, H3′, 6′), 7.59 (br d, *J* 7.4, 2H, H1′, 8′), 7.77 (d, *J* 7.4 Hz, 2H, H4′, 5′). ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.7 (CH*C*H$_3$), 18.8 (CH*C*H$_3$), 30.5 (*C*HCH$_3$), 47.2 (C9′), 58.3

(d, $^2J_{CF}$ 57.4 Hz, CHCOF), 67.4 (CH_2OCO), 120.1 (C2′, 7′), 125.0 (C3′, 6′), 127.2 (C1′, 8′), 127.9 (C4′, 5′), 141.4 (C8′a, 9′a), 143.7, 143.8 (C4′a, 4′b), 156.2 (OCONH), 162.4 (d, $^1J_{CF}$ 372.3 Hz COF). Mass spectrum (ESI^+, MeOH): The acid fluoride **7b** readily converted to the corresponding methyl ester during analysis: *m/z* calcd for $C_{21}H_{24}NO_4$ $(M+H)^+$ 354.2, found 354.3; $C_{21}H_{23}NNaO_4$ $(M+Na)^+$ 376.2, found 376.3.

3.5.2. Fmoc-L-Val-L-Agl-OH, ***8b***. The dipeptide **8b** was prepared according to a procedure described by Mutter et al.[12] A solution of Agl **5** (74 mg, 0.64 mmol) in 10% w/v Na_2CO_3 (1.5 mL) was added to a stirred solution of Fmoc-L-Val-F **7b** (0.20 g, 0.59 mmol) in acetone (12 mL). The resultant white suspension was stirred at room temperature and monitored by TLC (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05). After 3 h, the acetone was evaporated in vacuo and water (20 mL) was added. The resultant aqueous phase was diluted with CH_2Cl_2 (20 mL), cooled in an ice bath and carefully acidified to pH 2 with 1 M HCl. The phases were separated and the aqueous layer further extracted with CH_2Cl_2 (2×20 mL). The combined organic extract was washed with a saturated NaCl solution (1×30 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a colourless solid (0.26 g, 100%). The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.49) to afford the desired dipeptide **8b** as a colourless solid (0.25 g, 98%), mp 184–186 °C. ν_{max} (KBr): 3412m, 3299m, 3068w, 2963m, 2415w, 1720s, 1688s, 1654s, 1544s, 1440m, 1260m, 1103m, 1030m, 924w, 800w, 739m, 427w cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 0.93 (d, *J* 6.8 Hz, 3H, CH_3), 0.96 (d, *J* 6.7 Hz, 3H, CH_3), 2.07 (m, 1H, C*H*$(CH_3)_2$), 2.47 (m, 1H, C*H*aHbCH=), 2.60 (m, 1H, CHa*H*bCH=), 3.98 (m, 1H, C*H*CH$(CH_3)_2$), 4.21 (t, *J* 6.8 Hz, 1H, H9′), 4.32–4.42 (m, 2H, CH_2OCO), 4.45 (m, 1H, C*H*COOH), 5.03 (d, *J* 10.1 Hz, 1H, =C*H*aHb), 5.10 (d, *J* 17.0 Hz, 1H, =CHa*H*b), 5.78 (m, 1H, CH=), 7.12 (br d, *J* 8.7 Hz, 1H, NH), 7.29 (dt, *J* 7.5, 1.0 Hz, 2H, H2′, 7′), 7.37 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.65 (br t, *J* 7.5 Hz, 2H, H1′, 8′), 7.78 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (50 MHz, MeOD): δ 18.7 (CH*C*H_3), 19.9 (CH*C*H_3), 32.2 (*C*H$(CH_3)_2$), 37.2 (*C*H_2CH=), 48.6 (H9′), 53.8 (*C*HCH$(CH_3)_2$), 62.2 (*CH*COOH), 68.1 (CH_2OCO), 118.8 (=CH_2), 121.1 (C2′, 7′), 126.4 (C3′, 6′), 128.3 (C1′, 8′), 128.9 (C4′, 5′), 134.6 (CH=), 142.8 (C8′a, 9′a), 145.4, 145.5 (C4′a, 4′b), 158.7 (OCONH), 174.1 (CONH), 174.8 (COOH). HRMS (ESI^+, CH_2Cl_2–MeOH): *m/z* calcd for $C_{25}H_{29}N_2O_5$ $(M+H)^+$ 437.2076, found 437.2070.

3.5.3. Fmoc-L-Val-L-Pre-OH, ***9b***. Fmoc-L-Val-L-Agl-OH **8b** was subjected to the general CM procedure outlined in Section 3.3.1: Fmoc-L-Val-L-Agl-OH **8b** (0.25 g, 0.57 mmol), CH_2Cl_2 (5 mL), second generation Grubbs′ catalyst (24 mg, 29 μmol), 2-methyl-2-butene (1 mL), Δ, 48 h, 100%. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.33) to afford the desired dipeptide **9b** as a colourless solid (0.24 g, 90%), mp 96–99 °C. ν_{max} (KBr): 3498m, 3415s, 3065w, 2963m, 1736m, 1685s, 1544m, 1450m, 1257m, 1101w, 1033m, 803w, 740w, 621w cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 0.93 (d, *J* 6.8 Hz, 3H, CHCH_3), 0.95 (d, *J* 7.1 Hz, 3H, CHCH_3), 1.59 (s, 3H, =CCH_3), 1.63 (s, 3H, =CCH_3), 2.10 (m, 1H, C*H*$(CH_3)_2$), 2.43 (m, 1H, C*H*aHbCH=), 2.56 (m, 1H, CHa*H*bCH=), 3.96 (m, 1H, C*H*CH$(CH_3)_2$), 4.23 (t, *J* 6.8 Hz, 1H, H9′), 4.30–4.44 (m, 2H, CH_2OCO, C*H*COOH), 5.12 (m, 1H, CH=), 7.30 (t, *J* 7.5 Hz, 2H, H2′, 7′), 7.38 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.66 (br d, *J* 7.5 Hz, 2H, H1′, 8′), 7.79 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (50 MHz, MeOD): δ 18.0 (=CCH_3), 18.5 (CH*C*H_3), 19.7 (CH*C*H_3), 26.0 (=*C*CH$_3$), 31.5 (*C*H_2CH=), 32.0 (*C*H$(CH_3)_2$), 48.4 (H9′), 54.0 (*C*HCH$(CH_3)_2$), 62.0 (*C*HCOOH), 68.0 (CH_2OCO), 119.9 (CH=), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 128.2 (C1′, 8′), 128.8 (C4′, 5′), 136.2, (=C), 142.6 (C8′a, 9′a), 145.1, 145.3 (C4′a, 4′b), 158.5 (OCONH), 173.8 (CONH), 174.9 (COOH). HRMS (ESI^+, CH_2Cl_2–MeOH): *m/z* calcd for $C_{27}H_{33}N_2O$ $(M+H)^+$ 465.2389, found 465.2398; $C_{27}H_{32}N_2NaO_5$ $(M+Na)^+$ 487.2209, found 487.2215.

3.5.4. Fmoc-L-Val-L-dmP-OH, ***10b***. Fmoc-L-Val-L-Pre-OH **9b** was subjected to a modified procedure described by Hartwig and Schlummer.[20] Trifluoromethanesulfonic acid (2.5 μL, 28 μmol) was added to a suspension of enamine **9b** (65 mg, 0.14 mmol) in toluene (15 mL). The reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature and concentrated under reduced pressure to yield a dark brown oil. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.43) to afford the desired dmP-containing dipeptide **10b** as a colourless solid (56 mg, 86%), mp 82–86 °C. ν_{max} (film): 3410bm, 3290s, 3063m, 2961m, 2934m, 2872w, 2856w, 1971w, 1952w, 1916w, 1731s, 1689s, 1646s, 1614w, 1538s, 1478w, 1463w, 1450m, 1390m, 1373m, 1320w, 1293w, 1266m, 1249s, 1235m, 1117s, 1082w, 1032m, 1009w, 935w, 895w, 879w, 855w, 826w, 797w, 758m, 739s, 702m, 666m, 644w, 620m cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 0.87–1.01 (m, 6H, CH(C*H*$_3$)$_2$), 1.44 (s, 3H, CCH_3), 1.47 (s, 3H, CCH_3), 1.83–2.01 (m, 3H, C*H*aHbCHCOOH, CH_2C), 2.17 (m, 1H, C*H*$(CH_3)_2$), 2.42 (m, 1H, CHa*H*bCHCOOH), 4.04 (m, 1H, C*H*CH$(CH_3)_2$), 4.23 (t, *J* 6.8 Hz, 1H, H9′), 4.30 (m, 1H, C*H*COOH), 4.37–4.99 (m, 2H, CH_2OCO), 5.37 (m, 1H, NH), 7.31 (t, *J* 7.4, 2H, H2′, 7′), 7.40 (t, *J* 7.4 Hz, 2H, H3′, 6′), 7.59 (d, *J* 7.4 Hz, 2H, H1′, 8′), 7.76 (d, *J* 7.4 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.6 (CH*C*H_3), 19.1 (CH*C*H_3), 24.5 (CH*C*H_2), 28.0 (*C*CH_3), 29.9 (C*C*H_3), 31.1 (*C*H$(CH_3)_2$), 34.1 (*C*H_2C$(CH_3)_2$), 47.2 (C9′), 49.8 (*C*HCOOH), 60.3 (*C*HCH$(CH_3)_2$), 67.1 (CH_2OCO), 84.2 (*C*$(CH_3)_2$), 120.0 (C2′, 7′), 125.1 (C3′, 6′), 127.1 (C1′, 8′), 127.7 (C4′, 5′), 141.3 (C8′a, 9′a), 143.8 (C4′a, 4′b), 156.4 (OCONH), 170.5 (CONH), 171.4 (COOH). HRMS (ESI^+, MeOH): *m/z* calcd for $C_{27}H_{32}N_2NaO_5$ $(M+Na)^+$ 487.2209, found 487.2215. $[\alpha]_D^{22}$ −16.2 (*c* 0.2, MeOH).

3.6. Fmoc-L-Phe-L-dmP-OH, 10c

3.6.1. Fmoc-L-Phe-F, ***7c***. The acid fluoride **7c** was prepared according to a modified procedure described by Carpino et al.[19] Cyanuric fluoride (0.14 g, 1.03 mmol) and pyridine (0.04 g, 0.52 mmol) were added to a stirred suspension of Fmoc-L-Phe-OH **6c** (0.20 g, 0.52 mmol) in dry CH_2Cl_2 (15 mL). The colourless solution was stirred at room temperature for 15 h, resulting in the formation of a white precipitate. This suspension was dissolved by addition of water (15 mL) and the phases were separated. The aqueous layer was further extracted with CH_2Cl_2 (2×15 mL) and the combined organic extract then washed with a saturated NaCl solution, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the acid fluoride **7c** as a colourless glassy solid (0.20 g, 100%), mp 118–120 °C (lit.[19] 118–120 °C). ν_{max} (KBr): 3319m, 3031w, 2965w, 1845s, 1701s, 1543s, 1448m, 1264s, 1106m, 1084m, 1044m, 934w, 804w, 739s, 699m, 537w, 425w cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 3.09–3.28 (m, 2H, CH_2Ph), 4.21 (t, *J* 6.6 Hz, 1H, H9′), 4.33–4.52 (m, 2H, CH_2OCO), 4.96 (m, 1H, C*H*COF), 5.08 (br d, *J* 7.9 Hz, 1H, NH), 7.14 (m, 1H, H4″), 7.24–7.37 (m, 5H, H2′, 7′, 2″, 3″, 5″, 6″), 7.37–7.45 (m, 2H, H3′, 6′), 7.51–7.59 (m, 2H, H1′, 8′), 7.77 (d, *J* 7.5 Hz, 2H, H4′, 5′). ^{13}C NMR (100 MHz, $CDCl_3$): δ 37.0 (*C*H_2Ph), 47.3 (C9′), 53.9 (d, $^2J_{CF}$ 59.9 Hz, *C*HCOF), 67.5 (CH_2OCO), 120.1 (C2′, 7′), 125.0, 125.1 (C3′, 6′), 127.2 (C1′, 8′), 127.8 (C4″), 127.9 (C4′, 5′), 129.1 (C2″, 6″), 129.3 (C3″ 5″), 134.4 (C1″), 141.4 (C8′a, 9′a), 143.6, 143.7 (C4′a, 4′b), 155.6 (OCONH), 161.9 (d, $^1J_{CF}$ 369.4 Hz, COF). Mass spectrum (ESI^+, MeOH): The acid fluoride **7c** readily converted into its corresponding methyl ester during analysis: *m/z* calcd for $C_{25}H_{24}NO_4$ $(M+H)^+$ 402.2, found 402.3; $C_{25}H_{23}NNaO_4$ $(M+Na)^+$ 424.2, found 424.2.

3.6.2. Fmoc-L-Phe-L-Agl-OH, ***8c***. The dipeptide **8c** was prepared according to a procedure described by Mutter et al.[12] A solution of Agl **5** (65 mg, 0.56 mmol) in 10% w/v Na_2CO_3 (1.5 mL) was added to

a stirred solution of Fmoc-L-Phe-F **7c** (0.20 g, 0.51 mmol) in acetone (15 mL). The resultant white suspension was stirred at room temperature and monitored by TLC (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05). After 4 h, the acetone was evaporated in vacuo and water (20 mL) was added. The resultant aqueous phase was diluted with CH_2Cl_2 (30 mL), cooled in an ice bath and carefully acidified to pH 2 with 1 M HCl. The phases were separated and the aqueous layer further extracted with CH_2Cl_2 (2×30 mL). The combined organic extract was washed with a saturated NaCl solution (1×30 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give the titled compound as a colourless solid (0.21 g, 86%). The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.48) to afford the desired dipeptide **8c** as a colourless solid (0.19 g, 76%), mp 158–160 °C. ν_{max} (KBr): 3415s, 3356m, 3086w, 2927w, 2470w, 1688s, 1653s, 1543m, 1436m, 1258m, 1084w, 1034w, 924w, 739m, 700w, 494w, 424w cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 2.48 (m, 1H, C*H*aHbCH=), 2.61 (m, 1H, CHa*H*bCH=), 2.90 (dd, *J* 13.6, 9.9 Hz, 1H, C*H*aHbPh), 3.18 (dd, *J* 13.9, 4.6 Hz, 1H, CHa*H*bPh), 4.10 (t, *J* 7.0 Hz; 1H, H9′), 4.14–4.31 (m, 2H, CH_2OCO), 4.41–4.49 (m, 2H, C*H*CH_2Ph, C*H*COOH), 5.04 (d, *J* 10.1 Hz, 1H, =C*H*aHb), 5.11 (d, *J* 17.0 Hz, 1H, =CHa*H*b), 5.77 (m, 1H, CH=), 7.17 (m, 1H, H4″), 7.21–7.30 (m, 6H, H2′, 7′, 2″, 3″, 5″, 6″), 7.36 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.54 (dd, *J* 7.1, 3.5 Hz, 2H, H1′, 8′), 7.76 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (100 MHz, MeOD): δ 37.3 (*C*H_2CH=), 39.2 (*C*H_2Ph), 48.5 (C9′), 53.8 (*C*HCH$_2$Ph), 57.8 (*C*HCOOH), 68.2 (CH_2OCO), 118.9 (=CH_2), 121.0 (C2′, 7′), 126.3, 126.4 (C3′, 6′), 127.8 (C1′, 8′), 128.3 (C4″), 128.9 (C4′, 5′), 129.6 (C2″, 6″), 130.5 (C3″, 5″), 134.5 (CH=), 138.8 (C1″), 142.7 (C8′a, 9′a), 145.3 (C4′a, 4′b), 158.3 (OCONH), 174.0 (CONH) 175.4 (COOH). HRMS (ESI^+, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{29}H_{29}N_2O_5$ $(M+H)^+$ 485.2076, found 485.2078.

3.6.3. Fmoc-L-Phe-L-Pre-OH, ***9c***. Fmoc-L-Phe-L-Agl-OH **8c** was subjected to the general CM procedure outlined in Section 3.3.1: Fmoc-L-Phe-L-Agl-OH **8c** (0.19 g, 0.39 mmol), CH_2Cl_2 (5 mL), second generation Grubbs′ catalyst (17 mg, 20 μmol), 2-methyl-2-butene (1 mL), Δ, 48 h, 95%. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.45) to afford the desired dipeptide **9c** as a colourless solid (0.18 g, 90%), mp 79–82 °C. ν_{max} (film): 3406m, 3307m, 3056w, 2983w, 1949m, 1732s, 1715s, 1651s, 1538m, 1505m, 1452m, 1416w, 1260s, 1084m, 910w, 801m, 760w, 740m, 701w, 647w cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.53 (s, 3H, =CC*H*$_3$), 1.62 (s, 3H, =CC*H*$_3$), 2.46 (m, 1H, C*H*aHbCH=), 2.56 (m, 1H, CHa*H*bCH=), 2.95–3.15 (m, 2H, C*H*$_2$Ph), 4.14 (t, *J* 6.3 Hz, 1H, H9′), 4.24 (m, 1H, C*H*CH$_2$Ph), 4.30–4.44 (m, 2H, CH_2OCO), 4.53 (m, 1H, C*H*COOH), 4.99 (m, 1H, CH=), 5.91 (br s, 1H, NH), 7.10–7.33 (m, 7H, H2′, 7′, 2″, 3″, 4″, 5″, 6″), 7.38 (t, *J* 7.3 Hz, 2H, H3′, 6′), 7.45–7.60 (m, 2H, H1′, 8′), 7.74 (d, *J* 7.3 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (75 MHz, MeOD): δ 17.9 (=CC*H*$_3$), 26.2 (=CC*H*$_3$), 29.8 (*C*H_2CH=), 38.8 (*C*H_2Ph), 47.2 (C9′), 52.5 (*C*HCH$_2$Ph), 55.9 (*C*HCOOH), 67.3 (CH_2OCO), 117.6 (CH=), 120.0 (C2′, 7′), 125.1 (C3′, 6′), 125.2 (C4″), 127.1 (C2″, 6″), 127.2 (C1′, 8′), 127.8 (C4′, 5′), 128.7 (C3″, 5″), 129.5 (C1″), 136.4 (=C), 141.4 (C8′a, 9′a), 143.8 (C4′a, 4′b), 156.1 (OCONH), 171.0 (CONH), 174.0 (COOH). HRMS (ESI^+, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{31}H_{32}N_2NaO_5$ $(M+Na)^+$ 535.2209, found 535.2213.

3.6.4. Fmoc-L-Phe-L-dmP-OH, ***10c***. Fmoc-L-Phe-L-Pre-OH **9c** was subjected to a modified procedure described by Hartwig and Schlummer.[20] Trifluoromethanesulfonic acid (1.7 μL, 20 μmol) was added to suspension of enamine **9c** (50 mg, 97 μmol) in toluene (15 mL). The resultant reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature and concentrated under reduced pressure to give a dark brown oil. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.41) to afford the desired dmP-containing dipeptide **10c** as a colourless solid (39 mg, 78%), mp 85–88 °C. ν_{max} (KBr): 3411bm, 3312br s, 3063m, 2981m, 2930m, 2857w, 1954w, 1919w, 1881w, 1715s, 1682s, 1668s, 1606w, 1538s, 1520s, 1505s, 1478w, 1451m, 1391m, 137m, 1321m, 1266s, 1153w, 1120s, 1082m, 1045m, 936m, 896w, 877w, 858w, 759s, 738s, 702s, 666m, 621m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.44 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 1.59–2.18 (m, 4H, C*H*$_2$CHCOOH, $CH_2C(CH_3)_2$), 2.89 (dd, *J* 13.9, 9.8 Hz, 1H, C*H*aHbPh), 3.18 (dd, *J* 13.9, 4.8 Hz, 1H, CHa*H*bPh), 4.11 (t, *J* 6.7 Hz; 1H, H9′), 4.18 (m, 1H, C*H*CH_2Ph), 4.26–4.36 (m, 2H, CH_2OCO), 4.41 (m, 1H, C*H*COOH), 7.19 (m, 1H, H4″), 7.21–7.31 (m, 6H, H2′, 7′, 2″, 3″, 5″, 6″), 7.37 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.56 (dd, *J* 6.8, 3.5 Hz, 2H, H1′, 8′), 7.77 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (100 MHz, MeOD): δ 25.0 ($CHCH_2CH_2$), 27.5 (CH_3), 30.2 (CH_3), 34.7 ($CH_2C(CH_3)_2$), 39.1 (CH_2Ph), 48.3 (C9′), 50.2 (*C*HCOOH), 57.7 ($CHCH_2$Ph), 68.0 (CH_2OCO), 85.2 (*C*$(CH_3)_2$), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 127.7 (C4″), 128.2 (C1′, 8′), 128.7 (C4′, 5′), 129.4 (C2″, 6″), 130.4 (C3″ 5″), 138.6 (C1″), 142.6 (C8′a, 9′a), 145.2 (C4′a, 4′b), 158.2 (OCONH), 172.4 (CONH), 173.9 (COOH). HRMS (ESI^+, MeOH): *m*/*z* calcd for $C_{31}H_{32}N_2NaO_5$ $(M+Na)^+$ 535.2209, found 535.2200. $[\alpha]_D^{22}$ −17.0 (*c* 0.1, MeOH).

3.6.5. A Phe–dmP containing hexapeptide, Gln-Tyr-Phe-dmP-Ala-Gly, ***16***. Synthesis of a phenylalanine–dmP containing hexapeptide **16** was performed according to the manual SPPS procedure outlined in Section 3.2.1 with Fmoc-Gly-Wang resin (96 mg, 50 μmol), NMM (33 μL, 0.60 mmol), the C-terminal amino acid Fmoc-L-Ala-OH (47 mg, 0.15 mmol) and HATU (73 mg, 0.20 mmol) in DMF (3 mL). The mixture was shaken gently for 17 h. At the end of the reaction period, the resin-peptide was washed with DMF (5 mL, 3×1 min), deprotected with 20% v/v piperidine in DMF (5 mL, 1×1 min, 2×10 min), and washed again with DMF (5 mL, 5×1 min). The above procedure was repeated until the desired peptide sequence was constructed. After sequence completion, the resin was transferred into a fritted syringe, washed with DMF (3×1 min), CH_2Cl_2 (3×1 min) and MeOH (3×1 min), then left to dry in vacuo for ~½ h. A small aliquot of resin-bound peptide **16**, was subjected to the cleavage procedure outlined in Section 3.2.2 for mass spectroscopic analysis. Mass spectrum (ESI^+, MeCN–H_2O:HCOOH): *m*/*z* calcd for $C_{50}H_{58}N_7O_{11}$ $(M+H)^+$ 932.4, found 932.2; $C_{50}H_{57}N_7NaO_{11}$ $(M+Na)^+$ 954.4, found 954.1. The resin-bound peptide was then re-swollen with CH_2Cl_2 (3×1 min, 1×60 min) and DMF (3×1 min, 1×30 min), subjected to Fmoc-deprotection in the presence of 20% v/v piperidine in DMF (1×1 min, 2×10 min.) and again washed with DMF (3×1 min), CH_2Cl_2 (3×1 min) and MeOH (3×1 min) before being left to dry in vacuo for ~½ h. The resin-bound peptide **16** was the subjected to TFA-mediated cleavage for mass spectroscopic and RP-HPLC analysis. This supported formation of the desired peptide **16** in 85% purity. Mass spectrum (ESI^+, MeCN–H_2O–HCOOH): *m*/*z* calcd for $C_{35}H_{48}N_7O_9$ $(M+H)^+$ 710.4, found 710.2. RP-HPLC (Agilent: Vydac C18 analytical column, 0→30% buffer B over 30 min): t_R 23.5 min.

3.7. Fmoc-L-Ser(OBn)-L-dmP-OH 10d

3.7.1. Fmoc-L-Ser(OBn)-F, ***7d***. The acid fluoride **7d** was prepared according to a modified procedure described by Carpino et al.[19] Cyanuric fluoride (0.16 g, 1.20 mmol) and pyridine (47.4 mg, 1.20 mmol) were added to a stirred solution of Fmoc-L-Ser(OBn)-OH **6d** (0.25 g, 0.60 mmol) in dry CH_2Cl_2 (15 mL). The colourless solution was stirred at room temperature for 6 h, resulting in the formation of a pale yellow precipitate. This suspension was dissolved by addition of water (15 mL) and the phases were separated. The aqueous layer was further extracted with CH_2Cl_2 (2×10 mL) and the combined organic extract then washed with a saturated NaCl

solution, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the acid fluoride **7d** as an orange oil (0.25 g, 100%). ν_{max} (KBr): 3419br s, 3334br s, 3066m, 3039m, 2951m, 2875m, 1852s, 1715s, 1515s, 1451s, 1405w, 1362w, 1337m, 1290m, 1250m, 1104m, 1080m, 1062m, 1040m, 939w, 916w, 759m, 740s, 689m, 666m, 621m cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 3.73 (dd, *J* 9.2, 1.9 Hz, 1H, CHC*H*aHbO), 3.90 (dd, *J* 9.2, 1.8 Hz, 1H, CHCHa*H*bO), 4.24 (t, *J* 6.8, 1H, H9′), 4.33–4.55 (m, 2H, CH_2OCO), 4.57 (br s, 2H, CH_2Ph), 4.72 (m, 1H, C*H*COF), 5.69 (br d, *J* 8.5 Hz, 1H, NH), 7.20–7.50 (m, 9H, H2′, 3′, 6′, 7′, 2″, 3″, 4″, 5″, 6″), 7.61 (d, *J* 7.0 Hz, 2H, H1′, 8′), 7.70 (d, *J* 7.3 Hz, 2H, H4′, 5′). ^{13}C NMR (200 MHz, $CDCl_3$): δ 47.2 (C9′), 53.8 (d, $^2J_{CF}$ 61.5 Hz, *C*HCH$_2$O), 67.6 (CH_2OCO), 68.7 (CHCH_2O), 73.8 (*C*H$_2$Ph), 120.2 (C2′, 7′), 125.2 (C3′, 6′), 127.3 (C1′, 8′), 127.9 (C4′, 5′, 2″, 6″), 128.4 (C4″), 128.8 (C3″, 5″), 136.8 (C1″), 141.5 (C8′a, 9′a), 143.7, 143.8 (C4′a, 4′b), 156.0 (OCONH), 161.0 (d, $^1J_{CF}$ 370.2 Hz, COF). Mass spectrum (ESI$^+$, MeOH): The acid fluoride **7d** readily converted to the corresponding methyl ester during analysis: *m*/*z* calcd for $C_{26}H_{25}NNaO_5$ $(M+Na)^+$ 454.2, found 454.2.

3.7.2. Fmoc-L-Ser(OBn)-L-Agl-OH, ***8d****.* The dipeptide **8d** was prepared according to a procedure described by Mutter et al.[12] A solution of Agl **5** (60 mg, 0.52 mmol) in 10% w/v Na_2CO_3 (4 mL) was added to a stirred solution of Fmoc-L-Ser(OBn)-F **7d** (0.20 g, 0.48 mmol) in acetone (25 mL). The resultant white suspension was stirred at room temperature and monitored by TLC (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05). After 14 h, the acetone was evaporated in vacuo and water (20 mL) was added. The resultant aqueous phase was diluted with CH_2Cl_2 (30 mL), cooled in an ice bath and carefully acidified to pH 2 with 1 M HCl. The phases were separated and the aqueous layer further extracted with CH_2Cl_2 (2×20 mL). The combined organic extract was washed with saturated NaCl solution (1×30 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a yellow oil (0.25 g, 97%). The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.48) and lyophilised to afford the desired dipeptide **8a** as a colourless solid (0.21 g, 86%), mp 110–112 °C. ν_{max} (KBr): 3327br s, 3067m, 2926m, 2857m, 1719s, 1664s, 1528s, 1451m, 1409w, 1364w, 1330w, 1247m, 1105m, 924w, 759m, 740m, 698w, 666m cm^{-1}. ^1H NMR (300 MHz, MeOD): δ 2.47 (m, 1H, C*H*aHbCH=), 2.62 (m, 1H, CHa*H*bCH=), 3.73 (d, *J* 4.9 Hz, 2H, CH_2OCO), 4.22 (t, *J* 6.8, 1H, H9′), 4.37 (d, *J* 6.8 Hz, 2H, CH_2OCO), 4.42 (m, 1H, C*H*CHaHbO), 4.49 (m, 1H, C*H*COOH), 4.53 (s, 2H, CH_2Ph), 5.02 (d, *J* 10.5 Hz, 1H, =C*H*aHb), 5.09 (d, *J* 17.1 Hz, 1H, =CHa*H*b), 5.75 (m, 1H, CH=), 7.21–7.34 (m, 7H, H2′, 7′, 2″, 3″, 4″, 5″, 6″), 7.38 (tt, *J* 7.5, 0.6 Hz, H3′, 6′), 7.58–7.67 (m, 2H, H1′, 8′), 7.78 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (75 MHz, MeOD): δ 37.0 (*C*H$_2$CH=), 48.4 (C9′), 53.5 (*C*HCH$_2$O), 56.3 (*C*HCOOH), 68.2 (CH_2OCO), 71.0 (CHCH_2O), 74.2 (*C*H$_2$Ph), 118.9 (=CH$_2$), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 128.1 (C1′, 8′), 128.7 (2″, 6″), 128.8 (C4′, 5′), 128.9 (C4″), 129.4 (C3″, 5″), 134.1 (CH=), 139.2 (C1″), 142.6 (C8′a, 9′a), 145.2, 145.3 (C4′a, 4′b), 158.4 (OCONH), 172.2 (CONH), 174.2 (COOH). HRMS (ESI$^+$, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{30}H_{31}N_2O_6$ $(M+H)^+$ 515.2182, found 515.2171.

3.7.3. Fmoc-L-Ser(OBn)-L-Pre-OH, ***9d****.* Fmoc-L-Ser(OBn)-L-Agl-OH **9d** was subjected to the general CM procedure outlined previously: Fmoc-L-Ser(OBn)-L-Agl-OH **9d** (0.20 mg, 0.39 mmol), CH_2Cl_2 (5 mL), second generation Hoveyda–Grubbs′ catalyst (12 mg, 19 μmol), 2-methyl-2-butene (1 mL), Δ, 40 h, 95%. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.46) to afford the desired dipeptide **9d** as a pale brown solid (0.15 g, 72%), mp 55–57 °C. ν_{max} (KBr): 3410br s, 3344br s, 3065m, 2972m, 2931m, 2873m, 2617w, 1952w, 1715s, 1668s, 1519s, 1452s, 1415w, 1378w, 1361w, 1324w, 1266s, 1105s, 1044m, 968w, 951w, 910w, 896w, 858w, 820w, 759m, 738s, 700m, 665m, 621m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.57 (s, 3H, =CCH$_3$), 1.62 (s, 3H, =CCH$_3$), 2.45 (m, 1H, C*H*aHbCH=), 2.55 (m, 1H, CHa*H*bCH=), 3.72 (br s, 2H, CHC*H*a*H*bO), 4.17 (t, *J* 6.4 Hz, 1H, H9′), 4.33 (d, *J* 6.4 Hz, 2H, CH_2OCO), 4.39–4.49 (m, 2H, C*H*CH$_2$O, C*H*COOH), 4.51 (s, 2H, CH_2Ph), 5.09 (t, *J* 5.4 Hz, 1H, CH=), 7.19–7.32 (m, 7H, H2′, 7′, 2″, 3″, 4″, 5″, 6″), 7.35 (t, *J* 7.5 Hz, H3′, 6′), 7.58–7.67 (m, 2H, H1′, 8′), 7.75 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (50 MHz, MeOD): δ 18.0 (=CCH$_3$), 26.2 (=CCH$_3$), 31.3 (CHCH$_2$CH=), 48.3 (C9′), 53.9 (*C*HCH$_2$OCH$_2$), 56.3 (*C*HCOOH), 68.2 (CH_2OCO), 72.4 (CHCH$_2$O), 74.2 (CH_2Ph), 119.4 (CH=), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 128.2 (C1′, 8′), 128.6 (2″, 6″), 128.7 (C4″), 128.8 (C4′, 5′), 129.3 (C3″, 5″), 136.5 (=C), 139.2 (C1″), 142.5 (C8′a, 9′a), 145.1, 145.2 (C4′a, 4′b), 158.3 (OCONH), 172.1 (CONH), 174.5 (COOH). HRMS (ESI$^+$, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{32}H_{35}N_2O_6$ $(M+H)^+$ 543.2495, found 543.2485.

The CM reaction was also conducted with 5 mol% second generation Grubbs' catalyst. A 60% conversion to the required prenylglycine derivative **9d** was obtained.

3.7.4. Fmoc-L-Ser(OBn)-L-dmP-OH, ***10d****.* Fmoc-L-Ser(OBn)-L-Pre-OH **9d** was subjected to a modified procedure described by Hartwig and Schlummer.[20] Trifluoromethanesulfonic acid (3.6 mg, 24 μmol) was added to a suspension of enamine **9d** (65 mg, 0.12 mmol) in toluene (10 mL). The reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature and concentrated under reduced pressure to give a dark brown oil. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.42) to afford the desired dmP-containing dipeptide **10d** as a colourless solid (31 mg, 48%), mp 64–66 °C. ν_{max} (KBr): 3432br s, 3065m, 2965m, 2928m, 1709s, 1638s, 1543m, 1508w, 1500m, 1477w, 1451, 1380w, 1364w, 1320w, 1224m, 1215m, 1178w, 1106m, 1079w, 1044w, 909w, 758m, 739m, 698m, 666m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.30 (s, 3H, CCH$_3$), 1.54 (s, 3H, CCH$_3$), 1.72 (m, 1H, CHC*H*aHbCH$_2$), 1.82 (m, 1H, CHCHa*H*bCH$_2$), 1.98–2.11 (m, 2H, C*H*$_2$C(CH$_3$)$_2$), 3.51–3.69 (m, 2H, CHC*H*$_2$O), 4.22 (t, *J* 7.5 Hz, 1H, H9′), 4.27–3.39 (m, 2H, CH$_2$O), 4.46 (m, 1H, C*H*COOH), 4.51 (m, 1H, C*H*CH$_2$O), 4.60 (s, 2H, CH_2Ph), 7.21 (m, 7H, H2′, 7′, 2″, 3″, 4″, 5″, 6″), 7.38 (t, *J* 7.5 Hz, H3′, 6′), 7.58–7.71 (m, 2H, H1′, 8′), 7.78 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (75 MHz, MeOD): δ 25.0 (CCH$_3$), 27.0 (CCH$_3$), 28.4 (CH$_2$C(CH$_3$)$_2$), 40.7 (CH$_2$CHCOOH), 48.3 (C9′), 54.0 (CHCH$_2$O), 56.3 (CHCOOH), 65.3 (*C*(CH$_2$)$_3$), 68.2 (CH_2OCO), 72.4 (CHCH$_2$O), 74.1 (*C*H$_2$Ph), 120.9 (C2′, 7′), 126.3 (C3′, 6′), 128.2 (C1′, 8′), 128.7 (2″, 6″), 128.8 (C4″), 129.3 (C4′, 5′), 129.4 (C3″, 5″), 139.3 (C1″), 142.6 (C8′a, 9′a), 145.2, 145.4 (C4′a, 4′b), 158.4 (OCONH), 171.1 (CONH) (COOH not observed). HRMS (ESI$^+$, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{32}H_{35}N_2O_6$ $(M+H)^+$ 543.2495, found 543.2490. $[\alpha]_D^{22}$ −8.2 (*c* 0.6, MeOH).

3.8. (4*S*,9a*S*)-Hexahydro-4-((9*H*-fluoren-9-ylmethoxycarbonyl)amino)-7,7-dimethyl-1*H*,5*H*-pyrrolo[2,1-*c*][1,4]oxazepine-1,5-dione, 11

3.8.1. Fmoc-L-Ser(^{t}*Bu)-F,* ***7e****.* The acid fluoride **7e** was prepared according to a modified procedure described by Carpino et al.[19] Cyanuric fluoride (0.14 g, 1.04 mmol) and pyridine (0.04 g, 0.52 mmol) were added to a stirred solution of Fmoc-L-Ser(^{t}Bu)-OH **6e** (0.20 g, 0.52 mmol) in dry CH_2Cl_2 (15 mL). The pale yellow solution was stirred at room temperature for 15 h, resulting in the formation of a white precipitate. This suspension was dissolved by addition of water (15 mL) and the phases were separated. The aqueous layer was further extracted with CH_2Cl_2 (2×10 mL) and the combined organic extract then washed with a saturated NaCl solution, dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford the acid fluoride **7e** as a colourless oil (0.21 g, 98%). ν_{max} (film): 3440m, 3318m, 3067m, 2976s, 2886m, 1854s, 1782s,

1715s, 1609w, 1515s, 1505s, 1478m, 1451s, 1394m, 1366m, 1338m, 1286m, 1253m, 1194s, 1098s, 1059s, 983w, 939w, 905w, 833w, 760m, 740s, 622m cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.20 (s, 9H, $C(CH_3)_3$), 3.63 (dd, *J* 9.2, 2.4 Hz, 1H, C*H*aHbO), 3.90 (dd, *J* 9.2, 2.7 Hz, 1H, CHa*H*bO), 4.26 (t, *J* 4.0, 1H, H9′), 4.38–4.51 (m, 2H, CH_2OCO), 4.71 (m, 1H, C*H*CH_2O), 5.63 (br d, *J* 8.9 Hz, 1H, NH), 7.33 (td, *J* 7.5, 1.1 Hz, 2H, H2′, 7′), 7.41 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.58–7.63 (m, 2H, H1′, 8′), 7.77 (d, *J* 7.5 Hz, 2H, H4′, 5′). ^{13}C NMR (50 MHz, $CDCl_3$): δ 27.3 ($C(CH_3)_3$), 47.3 (C9′), 54.1 (d, $^2J_{CF}$ 61.3 Hz, *C*HCH_2), 61.4 (CH*C*H_2O), 67.7 (CH_2OCO), 74.3 (*C*$(CH_3)_3$), 120.2 (C2′, 7′), 125.2 (C3′, 6′), 127.3 (C1′, 8′), 127.9 (C4′, 5′), 141.5 (C8′a, 9′a), 143.8, 143.9 (C4′a, 4′b), 156.1 (OCONH), 161.2 (d, $^1J_{CF}$ 369.5 Hz, COF). Mass spectrum (ESI^+, MeOH): The acid fluoride **7e** readily converted to the corresponding methyl ester during analysis: *m*/*z* calcd for $C_{23}H_{28}NO_5$ $(M+H)^+$ 398.2, found 398.4; $C_{23}H_{27}NNaO_5$ $(M+Na)^+$ 420.2, found 420.4.

*3.8.2. Fmoc-L-Ser(^{t}Bu)-L-Agl-OH, **8e**.* The dipeptide **8e** was prepared according to a procedure described by Mutter et al.[12] A solution of Agl **5** (68 mg, 0.59 mmol) in 10% w/v Na_2CO_3 (1.5 mL) was added to a stirred solution of Fmoc-L-Ser(^{t}Bu)-F **7e** (0.21 g, 0.54 mmol) in acetone (12 mL). The resultant white suspension was stirred at room temperature and monitored by TLC (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.48). After 4 h the acetone was evaporated in vacuo and water (20 mL) was added. The resultant aqueous phase was diluted with CH_2Cl_2 (20 mL), cooled in an ice bath and carefully acidified to pH 2 with 1 M HCl. The phases were separated and the aqueous layer was further extracted with CH_2Cl_2 (2×20 mL). The combined organic extract was washed with a saturated NaCl solution (1×30 mL), dried ($MgSO_4$), filtered and concentrated under reduced pressure to give a pale yellow solid (0.25 g, 95%). The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05) to afford the desired dipeptide **8e** as a colourless solid (0.21 g, 82%), mp 103–105 °C. ν_{max} (KBr): 3454m, 3098w, 2975m, 1732s, 1668s, 1652s, 1538m, 1506m, 1451m, 1366m, 1335w, 1194m, 1086m, 922w, 876w, 759m, 740m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.17 (s, 9H, $C(CH_3)_3$), 2.49 (m, 1H, C*H*aHbCH=), 2.61 (m, 1H, CHa*H*bCH=), 3.53–3.68 (m, 2H, C*H*$_2$OC$(CH_3)_3$), 4.18 (t, *J* 6.6, 1H, H9′), 4.29 (m, 1H, C*H*CH_2O), 4.42 (d, *J* 6.6 Hz, CH_2OCO), 4.51 (t, *J* 5.4 Hz, 1H, C*H*COOH), 5.05 (d, *J* 10.2 Hz, 1H, =C*H*aHb), 5.11 (d, *J* 17.1 Hz, 1H, =CHa*H*b), 5.77 (m, 1H, CH=), 7.27 (td, *J* 7.5, 1.0 Hz, 2H, H2′, 7′), 7.35 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.62 (br t, *J* 7.5 Hz, 2H, H1′, 8′), 7.74 (d, *J* 7.5 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (75 MHz, MeOD): δ 27.6 ($C(CH_3)_3$), 37.3 (*C*H_2CH=), 48.6 (C9′), 53.7 (*C*HCH_2O), 56.8 (*C*HCOOH), 63.2 (*C*H_2OC$(CH_3)_3$), 68.3 (CH_2OCO), 75.0 (*C*$(CH_3)_3$), 119.0 (=CH_2), 121.0 (C2′, 7′), 126.3 (C3′, 6′), 128.3 (C1′, 8′), 128.9 (C4′, 5′), 134.3 (CH=), 142.6 (C8′a, 9′a), 145.2, 145.4 (C4′a, 4′b), 158.4 (OCONH), 172.6 (CONH), 174.4 (COOH). HRMS (ESI^+, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{27}H_{32}N_2NaO_6$ $(M+Na)^+$ 503.2158, found 503.2159.

*3.8.3. Fmoc-L-Ser(^{t}Bu)-L-Pre-OH, **9e**.* Fmoc-L-Ser(^{t}Bu)-L-Agl-OH **8e** was subjected to the general CM procedure outlined in Section 3.3.1: Fmoc-L-Ser(^{t}Bu)-L-Agl-OH **8e** (0.20 g, 0.42 mmol), CH_2Cl_2 (5 mL), second generation Grubbs′ catalyst (18 mg, 21 μmol), 2-methyl-2-butene (1 mL), Δ, 48 h, 96%. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.49) to afford the desired dipeptide **9e** as a colourless solid (0.18 g, 86%), mp 68–71 °C. ν_{max} (film): 3459m, 3317m, 3086w, 2973m, 1947w, 1732s, 1681s, 1651s, 1538s, 1520m, 1506s, 1452m, 1366m, 1326w, 1260m, 1088m, 911w, 801w, 760m, 740m cm^{-1}. ^1H NMR (400 MHz, MeOD): δ 1.22 (s, 9H, $C(CH_3)_3$), 1.61 (s, 3H, =CCH_3), 1.67 (s, 3H, =CCH_3), 2.46 (m, 1H, C*H*aHbCH=), 2.57 (m, 1H, CHa*H*bCH=), 3.53–3.68 (m, 2H, $CH_2OC(CH_3)_3$), 4.18–4.30 (m, 2H, H9′, C*H*CH_2O), 4.37 (d, *J* 6.4 Hz, 2H, CH_2OCO), 4.45 (br t, *J* 6.0 Hz, 1H, C*H*COOH), 5.12 (br t, *J* 7.0 Hz, 1H, CH=), 7.30 (td, *J* 7.4, 0.9 Hz, 2H, H2′, 7′), 7.39 (t, *J* 7.4 Hz, 2H, H3′, 6′), 7.60–7.69 (m, 2H, H1′, 8′), 7.78 (d, *J* 7.4 Hz, 2H, H4′, 5′) (NH and OH not observed). ^{13}C NMR (75 MHz, MeOD): δ 18.0 (=CCH_3), 25.9 (=CCH_3), 27.7 (C$(CH_3)_3$), 31.4 (*C*H_2CH=), 48.4 (C9′), 52.8 (*C*HCH$_2$O), 56.6 (*C*HCOOH), 63.1 (CHCH_2O), 68.1 (CH_2OCO), 74.9 (*C*$(CH_3)_3$), 119.5 (CH=), 120.9 (C2′, 7′), 126.2 (C3′, 6′), 128.2 (C1′, 8′), 128.8 (C4′, 5′), 136.5 (=C), 142.6 (C8′a, 9′a), 145.2, 145.3 (C4′a, 4′b), 158.3 (OCONH), 172.4 (CONH), 174.5 (COOH). HRMS (ESI^+, CH_2Cl_2–MeOH): *m*/*z* calcd for $C_{29}H_{37}N_2NaO_6$ $(M+Na)^+$ 531.2471, found 531.2463.

*3.8.4. (4S,9aS)-Hexahydro-4-((9H-fluoren-9-ylmethoxycarbonyl) amino)-7,7-dimethyl-1H,5H-pyrrolo[2,1-c][1,4]oxazepine-1,5-dione, **11**.* Fmoc-L-Ser (^{t}βu)-L-Pre-OH **9e** was subjected to a modified procedure described by Hartwig and Schlummer.[20] Trifluoromethanesulfonic acid (1.7 μL, 20 μmol) was added to a suspension of enamine **9e** (50 mg, 0.10 mmol) in toluene (5 mL). The reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature, diluted with water (15 mL) and stirred for a further 30 min. The phases were separated and the aqueous layer further extracted with CH_2Cl_2 (3×10 mL). The combined organic extract was dried ($MgSO_4$), filtered and concentrated under reduced pressure to afford a dark brown oil. The crude reaction mixture was purified via column chromatography (SiO_2, light petroleum–EtOAc–MeOH–AcOH; 1:1:0.1:0.05, R_f 0.50) to afford bicyclic dipeptide **11** as a colourless solid (31 mg, 72%), mp 84–88 °C. ν_{max} (KBr): 3363 br s, 3067m, 2968m, 2928m, 1748s, 1668s, 1652s, 1536w, 1478w, 1451m, 1416m, 1366w, 1318w, 1264s, 1185w, 1106w, 1055w, 971w, 858w, 785w, 760m, 738s, 702w, 666m, 620m cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 3H, CH_3), 1.58 (s, 3H, CH_3), 2.13 (m, 1H, CHC*H*aHbCH$_2$), 2.25 (m, 1H, CHCHa*H*bCH$_2$), 4.19 (dd, *J* 10.6, 6.8 Hz, 1H, C*H*COOH), 2.78–2.93 (m, 2H, CH_2C), 4.25 (t, *J* 7.6 Hz, 1H, H9′), 4.28 (m, 1H, C*H*CH_2OC), 4.38 (dd, *J* 11.6, 8.4 Hz, 1H, C*H*aHbOCO), 4.46 (d, *J* 7.2 Hz, 2H, CH_2O), 4.80 (dd, *J* 11.6, 3.3 Hz, 1H, CHa*H*bOCO), 6.46 (br s, 1H, NH), 7.32 (td, *J* 7.5, 1.0 Hz, 2H, H2′, 7′), 7.41 (t, *J* 7.5 Hz, 2H, H3′, 6′), 7.60 (d, *J* 7.5 Hz, 2H, H1′, 8′). 7.78 (d, *J* 7.5 Hz, 2H, H4′, 5′) (OH not observed). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.3 (CH_3), 25.8 (CH_2CHCOOH), 26.8 (CH_3), 39.8 (CH_2C), 46.7 (C9′), 54.8 (*C*HCH$_2$O), 58.8 (*C*HCOOH), 64.1 (*C*$(CH_3)_2$), 66.6 (CH*C*H_2O), 70.5 (CH_2OCO), 120.3 (C2′, 7′), 125.3 (C3′, 6′), 127.4 (C1′, 8′), 128.1 (C4′, 5′), 141.5 (C8′a, 9′a), 143.3 (C4′a, 4′b), 155.2 (OCONH), 162.7 ($COOCH_2$), 170.0 (CONH). HRMS (ESI^+, MeOH): *m*/*z* calcd for $C_{25}H_{29}N_2O_6$ $(M+H)^+$ 435.1920, found 435.1912; $C_{25}H_{28}N_2NaO_6$ $(M+Na)^+$ 457.1739, found 457.1735. $[\alpha]_D^{22}$ −37.3 (*c* 0.5, MeOH).

*3.8.5. A Ser–dmP containing tripeptide, Fmoc-Ser-dmP-Phe, **12**.* Synthesis of a serine–dmP containing tripeptide **12** was performed according to the manual SPPS procedure outlined in Section 3.2.1 with Fmoc-Phe-Wang resin (5.0 mg, 12 μmol), NMM (2.7 μL, 25 μmol), **11** (11 mg, 25 μmol) and HATU (9.4 mg, 25 μmol) in DMF (3 mL). The mixture was shaken gently for 24 h. At the end of the reaction period, the resin-peptide was washed with DMF (3×1 min), CH_2Cl_2 (3×1 min) and MeOH (3×1 min), then left to dry in vacuo for ~½ h. A small aliquot of resin-bound peptide **12** was subjected to the cleavage procedure outlined in Section 3.2.2 for mass spectroscopic analysis. This supported formation of the desired peptide **12**. Mass spectrum (ESI^+, MeCN–H_2O–HCOOH): *m*/*z* calcd for $C_{38}H_{46}N_3O_7$ $(M+H)^+$ 656.3, found 656.2.

3.9. Fmoc-L-dmP-OH, 13

Method A: Fmoc-L-Pre-OH **14** was subjected to a modified procedure described by Hartwig and Schlummer.[20] Trifluoromethanesulfonic acid (2.6 μL, 27 μmol) was added to a suspension of enamine **14** (50 mg, 0.14 mmol) in toluene (5 mL). The resultant reaction mixture was stirred at 100 °C for 1 h, cooled to room temperature and concentrated under reduced pressure to give a dark brown oil. Isolation of **14** via column chromatography (SiO_2,

light petroleum–EtOAc–MeOH–AcOH; 1:1:0.05:0.05) was unsuccessful. *Method B*: The dmP derivative **13** was prepared according to a procedure described by Paquet.[21] Fmoc-OSu (24 mg, 70 μmol) was added to a stirred solution of L-dmP **4** (10 mg, 70 μmol) and $NaHCO_3$ (29 mg, 0.35 mmol) in water (5 mL) and acetone (5 mL). The resultant white suspension was stirred at room temperature and after 24 h TLC analysis (SiO_2; light petroleum–EtOAc–MeOH–AcOH; 1:1:0.05:0.05) showed only trace amounts of the titled compound **13**. The reaction mixture was stirred at room temperature for a further 6 days. No further conversion to **13** was observed.

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Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.05.068.

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