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Tetrahedron: *Asymmetry* 16 (2005) 2025–2029

A catalytic asymmetric synthesis of 5,5-dimethylproline

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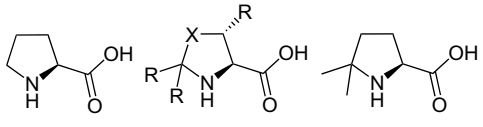
Received 8 April 2005; accepted 2 May 2005

Abstract—The methyl ester derivative of commercially available *N*-acetyl-allylglycine readily undergoes cross-metathesis with 2-methylbut-2-ene and ruthenium–alkylidene catalyst to afford the prenylglycine derivative. Acid-catalysed cyclisation then affords 5,5-dimethylproline in near quantitative yield and enantioselectivity.

1. Introduction

In folded proteins only 0.03% of Xaa$_{i-1}$-nonPro$_i$ bonds are in the *cis*-conformation.[1] The inclusion of the cyclic amino acid proline **1**, however, increases the prevalence of *cis*-configured Xaa$_{i-1}$-Pro$_i$ bonds to 5.2%.[2] Despite its low occurrence, the *cis*-peptide bond is an important structural feature in a multitude of naturally occurring peptides and proteins where it can induce a turn in the peptide backbone, decrease intermolecular aggregation and ultimately influence the protein folding and stability.[3] Attempts to constrain peptide bonds into the *cis*-conformation have involved replacement of constituent proline residues with nonproteinaceous analogues. For example, reversible pseudoproline (ΨPro) residues **2** derived from cysteine, serine and threonine have been used to increase the percentage of Xaa$_{i-1}$-ΨPro$_i$ bonds in the *cis*-conformation (Fig. 1).[4] Although the pseudoproline derivatives possess varying stability, their acid sensitivity does not facilitate long term incorporation into peptides.[5] A structurally similar analogue, 5,5-dimethylproline (dmP) **3**, locks the imidic Xaa$_{i-1}$-dmP$_i$ bond exclusively in the *cis*-conformation.[5,6] The irreversible formation, stability and conformational properties of this nonproteinaceous amino acid render it useful in peptidomimetic studies. For example, replacement of a proline residue with 5,5-dimethylproline in ribonuclease A was recently shown to accelerate protein folding and enhance conformational stability.[7] To date, nontrivial syntheses of this unnatural chiral amino acid have limited its use in peptidomimetics. Current routes involve the formation of the racemate of **3** followed by chemical resolution.[5,6,8] Herein, we report a convenient catalytic asymmetric synthesis of 5,5-dimethylproline **3** from commercially available starting materials.



1 **2** **3**

Figure 1. Proline **1** and pseudoprolines **2** and **3**.

2. Results and discussion

Our interest in asymmetric hydrogenation firstly led us to investigate the synthesis of a prenylglycine derivative **4** as a precursor to 5,5-dimethylproline **3**. Rh(I)–Et–DuPHOS catalysed hydrogenation of dienamide precursor **5** proceeded in a highly regioselective and stereoselective manner (>99% ee) to afford the prenylglycine derivative **4** in excellent yield (98%). Acid catalysed hydrolysis and cyclisation of amido ester **4** gave 5,5-dimethylproline hydrochloride **6** in a single step (Scheme 1). A minor by-product, alcohol **7**, was readily removed via chromatography to give 5,5-dimethylproline **3** in 60% yield. No epimerisation was observed and the enantiomeric excess was determined to be 98% ee by specific rotation determination.

Although asymmetric hydrogenation enables expedient and equal access to both isomers of **4**, and hence

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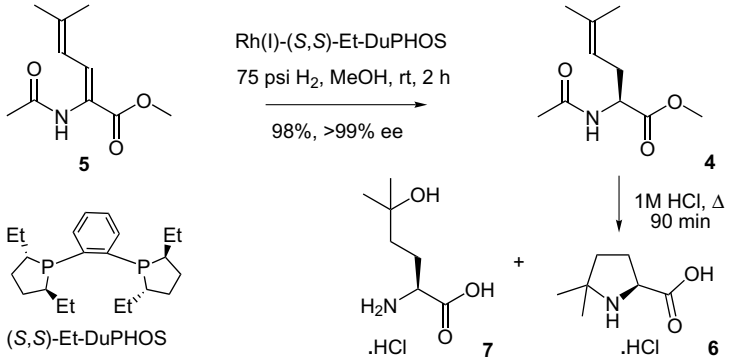
0957-4166/$ - see front matter © 2005 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2005.05.003



Scheme 1. Asymmetric hydrogenation route to pseudoproline **6**.

D- and L-dmP **3**, a four step synthesis of the starting dienamide **5** from acetamide and glyoxylic acid,[9] however, marred an otherwise useful synthesis. We therefore examined the use of a ruthenium-catalysed metathesis reaction to prepare the key prenylglycine intermediate **4**. Cross-metathesis of commercially available allylglycine derivatives **8** with isobutylene or 2-methylbut-2-ene using second generation Grubbs' catalyst gave the protected prenylglycines **4a**–**d** (Scheme 2).

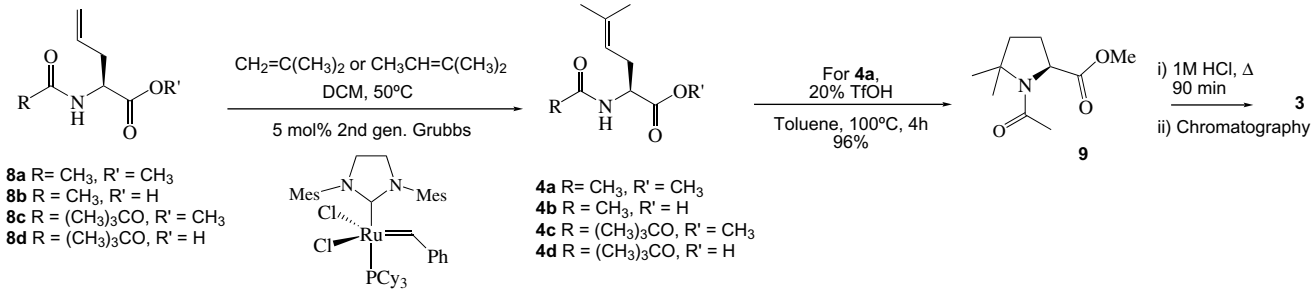
Metathesis of the *N*-acetyl-protected allylglycine methyl ester **8a** was complete after 4 h, however reaction of the corresponding free carboxylic acid derivative **8b** proceeded at a slower rate (3 days for completion). Complete conversion of **8b** to **4b** was achieved in 12 h using 2-methylbut-2-ene. Similarly, cross-metathesis of *N*-Boc-protected allylglycine methyl ester **8c** proceeded smoothly to give the prenylglycine derivative **4c** in quantitative yield after 5 h. Interestingly, cross-metathesis of the free acid **8d** with isobutylene failed to go to completion even after 3 days (58% conversion). The use of 2-methylbut-2ene, however led to **4d** with 95% conversion in 12 h. In our experiments, the generation of the prenyl group via cross-metathesis was found to be more expedient when the potential for Ru–methylidene intermediate formation was reduced.[10] Hence, the use of 2-methylbut-2-ene favours the catalytic cycling through the more stable ruthenium ethylidene species. Attempts to cross-metathesis the hydrochloride salt of allylglycine with isobutylene and 2-methylbut-2-ene were unsuccessful and gave only poor conversions (<25%) to the desired prenylglycine derivative.

The *N*-acetyl-protected analogues **4a** and **4b** were readily cyclised to 5,5-dimethylproline hydrochloride **6** under conditions previously described (1 M HCl, reflux, 90 min). In order to eliminate the formation of **7**, acid-catalysed cyclisation was also affected under nonaqueous conditions using triflic acid in toluene.[11] After 4 h, quantitative cyclisation to the *N*-acetyl protected 5,5-dimethylproline methyl ester **9** was achieved (Scheme 2). Acid catalysed hydrolysis of the protected dmP derivative **9** then afforded pure 5,5-dimethylproline **3** in 90% yield and 98% ee after chromatography.

Interestingly, exposure of Boc-protected prenylglycine methyl ester **4c** to the same cyclisation conditions failed to yield the desired proline analogue **6** and gave only a mixture of prenylglycine hydrochloride and alcohol **7**. This result suggests that the acid-catalysed transformation of **4** into **6** follows a mechanism involving initial amide protonation followed by cyclisation and hydrolysis of the *N*-acetyl protecting group.

3. Conclusion

In conclusion, we have developed an expeditious procedure for the synthesis of enantiomerically pure 5,5-dimethylproline **3** from commercially available starting materials. A ruthenium-catalysed cross-metathesis reaction of *N*-acetyl-protected allylglycine **8b**, or its methyl ester **8a**, with isobutylene or 2-methylbut-2-ene, followed by an acid catalysed cyclisation of the resultant chiral prenylglycine analogues afforded the cyclic amino acid **3** in 90% yield and 98% ee.



Scheme 2. Metathesis route to pseudoproline **3**.

4. Experimental

4.1. General experimental methods

Melting points were determined using a hot-stage melting point apparatus and are uncorrected. Infrared spectra were recorded on a FT-IR spectrophotometer as potassium bromide disks of solids (KBr) or as thin films of liquids (neat) between sodium chloride plates. Nuclear magnetic resonance spectra (^{1}H and ^{13}C NMR) were recorded on either 300 or 400 MHz spectrometers. Low resolution electrospray ionisation (ESI) were recorded in the positive mode (ESI^+) on a QMS-quadrupole mass spectrometer. Accurate mass measurements were obtained at high resolution with a FTMS and a 4.7 T superconducting magnet. The instrument was externally calibrated with FC5311. Analytical thin-layer chromatography (TLC) was performed on plastic slides coated with silica gel (Polygram SIL g/uv254) and preparative chromatography was performed on C18 reverse phase silica gel. Flash chromatography was performed using Merck silica gel 60 (Merck no. 9385), 0.063–0.200 mm (230–400 mesh). Solvents were purified according to standard procedures. Chloroform used for optical rotations was of analytical purity. Degassed methanol was used in all hydrogenation reactions. Degassed dichloromethane was used in metathesis reactions. Deuterated chloroform was used as supplied. Grubbs' catalyst refers to bis(tricyclohexylphosphine)benzylidene ruthenium dichloride.[12] Second generation Grubbs' catalyst refers to tricyclohexylphosphine[1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene][benzylidene]ruthenium dichloride.[13] Rh(I)–(*S*,*S*)-Et–DuPHOS refers to (+)-1,2-bis[(2*S*,5*S*)-2,5-diethylphospholano]benzene(1,5-cyclooctadiene)rhodium(I) tetrafluoroborate.[14] All ruthenium-catalysed metathesis reactions were performed using standard Schlenk techniques under an atmosphere of nitrogen or in an argon filled drybox. Solvents were dried and degassed using standard procedures. In all rhodium–phosphine hydrogenations, high purity (<10 ppm of oxygen) hydrogen and argon were used and purified by passage through a series of traps to remove water, oxygen and hydrocarbons. Isobutylene and 2-methylbut-2-ene were used as supplied. The enantiomeric excess of **4a** was determined via analytical gas chromatography (GC) using a chiral column Model C-024 (column: 0.25 mm × 50 cm, 50CP2/XE60-SVALSA-PEA) using helium as the carrier gas. Optical rotations were measured with a polarimeter (in a cell length of 1 dm) at a wavelength of 589 nm (sodium D line) at a temperature of 22 °C.

4.2. Preparation of dienamide

4.2.1. (2*Z*)-Methyl 2*N*-acetylamino-5-methylhexa-2,4-dienoate 5. Dienamide **5** was prepared according to modified literature procedures.[9] Tetramethylguanidine (7.0 mL, 55.8 mmol) was added to a solution of methyl 2*N*-acetylamino-2-(dimethoxyphosphinyl)acetate (10.08 g, 42.2 mmol) in distilled tetrahydrofuran (100 mL) at −78 °C. After 15 min, 3-methyl-2-butenal (5.0 mL, 51.8 mmol) was added and the mixture stirred for 2 h at −78 °C. The mixture was warmed to 25 °C using a warm water bath and stirred at this temperature for an additional 14 h. The reaction mixture was then diluted with dichloromethane (150 mL) and washed with dilute hydrochloric acid solution (1 M, 2 × 100 mL), copper sulfate solution (1 M, 2 × 100 mL), saturated sodium bicarbonate solution (2 × 100 mL) and saturated sodium chloride solution (1 × 100 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to give an off-white solid (7.50 g). Purification by flash chromatography (SiO_2, dichloromethane–ethyl acetate–light petroleum, 2:2:1) gave dienoate **5** (6.10 g, 73%) as a pale brown solid, mp 115–116 °C. t_R = 6.3 min (GC column 30QC5/BPX5, 150 °C for 1 min, 10 °C min^{-1} to 280 °C for 6 min). ν_{max} (neat): 3258m, 3009w, 2956w, 1729s, 1663s, 1610m, 1560w, 1522s, 1440m, 1374m, 1338m, 1286s, 1255s, 1208s, 1156m, 1123s, 1041w, 1016m, 687m, 896w, 868m, 768s, 716m, 658w, 603m, 581w, 603m, 561w cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.89 (s, 6H, $(CH_3)_2$), 2.13 (s, 3H, CH_3CO), 3.77 (s, 3H, OCH_3), 5.95 (d, *J* 11.8 Hz, 1H, H4), 6.97 (br s, 1H, NH), 7.34 (br d, *J* 11.8 Hz, 1H, H3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 19.3, 23.6 ($(CH_3)_2$), 27.1 (CH_3CO), 52.4 (OCH_3), 120.8 (C4), 121.1 (C5), 130.5 (C3), 147.2 (C2), 166.0, 168.0 (C1, CONH). HRMS (ESI^+, MeOH): *m*/*z* calcd for $C_{10}H_{15}NO_3Na$ $[(M+Na)^+]$ 220.0950. Found 220.0947.

4.3. General asymmetric hydrogenation procedure

In a drybox, a Fischer–Porter tube was charged with catalyst (1–3 mg), deoxygenated solvent (~5 mL) and substrate (28–108 mg). Three vacuum/argon cycles to purge the gas line of any oxygen followed by three vacuum/argon cycles of the vessel were carried out before the tube was pressurised with hydrogen to the specified pressure (psi). The reaction mixture was then stirred at room temperature for the specified period of time. The pressure in the vessel was then released, and the contents evaporated under reduced pressure. The crude product was passed through a short plug of silica (eluent = ethyl acetate) prior to spectroscopic and chromatographic analysis. The hydrogenation experiment is described using the following format: substrate, solvent, catalyst, hydrogen pressure, reaction time, isolated yield, enantiomeric excess (assigned configuration), retention time (GC conditions).

4.3.1. (2*S*)-Methyl 2*N*-acetylamino-5-methylhex-4-enoate 4a. (2*Z*)-Methyl 2*N*-acetylamino-5-methylhexa-2,4-dienoate **5** (74.0 mg, 0.38 mmol), methanol (5 mL), [(COD)Rh(*S*,*S*)-Et–DuPHOS]OTf (2 mg), 75 psi H_2, 2 h, 100% yield, 100% ee (2*S*)-**4a**, t_R = 24.2 min (GC chiral column Model C-024, 100 °C for 1 min, 5 °C min^{-1} to 210 °C for 7 min). $[\alpha]_D^{22} = +58.2$ (*c* 0.79, $CHCl_3$), mp 46–48 °C. ν_{max} (neat): 3288m, 2955w, 1746s, 1660s, 1538m, 1436m, 1377m, 1274w, 1210w, 1126w, 1030w, 736w cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.59 (s, 3H, H6a), 1.69 (d, *J* 0.9 Hz, 3H, H6b), 2.00 (s, 3H, CH_3CO), 2.39–2.60 (m, 2H, H3), 3.72 (s, 3H, OCH_3), 4.63 (dt, *J* 7.9, 5.6 Hz, 1H, H2), 4.99 (t, *J* 7.5 Hz, 1H, H4), 6.02 (br s, 1H, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.0, 26.0, $((CH_3)_2)$, 23.3 (CH_3CO), 30.8 (C3), 52.2,

52.4 (C2, OCH_3), 117.6 (C4), 136.6 (C5), 169.8, 172.8 (C1, CONH). HRMS (ESI^+, MeOH): *m/z* calcd for $C_{10}H_{17}NO_3Na$ [$(M+Na)^+$] 222.1106. Found 222.1105.

4.3.2. 5,5-Dimethylproline 3.[5,6,8] Hydrochloric acid (5 mL) was added to enamide **4a** (160 mg, 0.80 mmol) and the mixture heated at reflux for 90 min. The reaction mixture was then evaporated under reduced pressure to afford the crude hydrochloride **6** as a dark yellow oil. Purification by preparative reverse phase thin layer chromatography (ethyl acetate–methanol, 1:1) gave 5,5-dimethylproline **3** (68.8 mg, 60%) as a colourless oil. ν_{max} (neat): 3333bs, 2975s, 1737s, 1591w, 1453w, 1382m, 1248m, 1154w, 1125w, 1089s, 1048s, 926w, 880s, 805w, 736w cm^{-1}. ^1H NMR (300 MHz, CD_3OD): δ 1.45 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 1.89 (t, *J* 7.2 Hz, 2H, H4), 2.16–2.28 (m, 1H, H3a), 2.38–2.50 (m, 1H, H3b), 4.04 (dd, *J* 9.1, 6.4 Hz, 1H, H2). ^{13}C NMR (50 MHz, CD_3OD): δ 25.3, 25.6 ($(CH_3)_2$), 28.3 (C4), 38.4 (C3), 60.0 (C2), 67.3 (C5), 171.6 (COOH). HRMS (ESI^+, MeOH): *m/z* calcd for $C_7H_{14}NO_2$ [$(M+H)^+$] 144.1025. Found 144.1021. $[\alpha]_D^{22} = -49.7$ (*c* 1.01, H_2O) {lit.[5] $[\alpha]_D^{22} = -51.2$ (*c* 1, H_2O)}, 97% ee.

4.3.3. (2*S*)-*N*-Acetyl-5,5-dimethylproline methyl ester 9. The enamide **4a** was subjected to a modified procedure described by Schlummer et al.[11] Trifluoromethanesulfonic acid (12 μL, 0.14 mmol, 20 mol %) was added to a solution of enamide **4a** (120 mg, 0.60 mmol) in toluene (5 mL). The reaction mixture was heated at 100 °C for 4 h. The reaction mixture was then evaporated under reduced pressure to give an oil. The oil was diluted with dichloromethane (20 mL) and washed with saturated sodium bicarbonate solution (2 × 15 mL) and water (2 × 15 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure to give the fully protected dimethylproline derivative **9** (116 mg, 96%) as a pale yellow oil. ν_{max} (neat): 3479bw, 2979m, 1745s, 1644m, 1462w, 1372w, 1227s, 1168s, 1085w, 1031s, 738m, 703w, 639m cm^{-1}. ^1H NMR spectroscopic analysis confirmed the presence of *cis*- and *trans*-isomers in a ratio of 2.2: 1. ^1H NMR (300 MHz, $CDCl_3$): (Major isomer) δ 1.39 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 1.80–1.86 (m, 2H, H4), 1.89 (CH_3CO), 1.95–2.05 (m, 2H, H3), 3.73 (s, 3H, OCH_3), 4.36 (m, 1H, H2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.2, 26.6 ($(CH_3)_2$), 27.9 (C4), 28.0 (CH_3CO), 39.9 (C3), 52.5 (OCH_3), 62.7 (C2), 63.4 (C5), 169.3, 173.1 (COOMe, NCOMe). ^1H NMR (300 MHz, $CDCl_3$): (Minor isomer) δ 1.37 (s, 3H, CH_3), 1.54 (s, 3H, CH_3), 1.71–1.74 (m, 2H, H4), 2.13 (CH_3CO), 2.18–2.28 (m, 2H, H3), 3.68 (s, 3H, OCH_3), 4.53–4.56 (m, 1H, H2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8, 24.2 ($(CH_3)_2$), 25.3 (C4), 27.8 (CH_3CO), 42.3 (C3), 52.1 (OCH_3), 62.0 (C2), 60.8 (C5), 169.4, 173.2 (COOMe, NCOMe). HRMS (ESI^+, MeOH): *m/z* calcd for $C_{10}H_{17}NO_3Na$ [$(M+Na)^+$] 222.1106. Found 222.1105.

Hydrochloric acid (5 mL) was added to the fully protected dmP derivative **9** (91 mg, 0.46 mmol) and the mixture heated at reflux for 90 min. The reaction mixture was then evaporated under reduced pressure to afford the dimethylproline hydrochloride **6** as a yellow oil. ν_{max} (neat): 3333bs, 2975s, 1737s, 1591w, 1453w, 1382m, 1248m, 1154w, 1125w, 1089s, 1048s, 926w, 880s, 805w, 736w cm^{-1}. ^1H NMR (200 MHz, CD_3OD): δ 1.47 (s, 3H, CH_3), 1.50 (s, 3H, CH_3), 1.98 (t, *J* 7.3 Hz, 2H, H4), 2.20–2.39 (m, 1H, H3a), 2.46–2.65 (m, 1H, H3b), 4.52 (apparent t, *J* 8.5 Hz, 1H, H2). HRMS (ESI^+, MeOH): *m/z* calcd for $C_7H_{14}NO_2$ [$(M+H)^+$] 144.1025. Found 144.1021. $[\alpha]_D^{22} = -49.7$ (*c* 0.75, H_2O), 98% ee. Purification by preparative reverse phase thin layer chromatography (ethyl acetate–methanol, 1:1) gave 5,5-dimethylproline **3** (58.9 mg, 90%) as a colourless oil. Spectral data was consistent with that previously reported. $[\alpha]_D^{22} = -50.2$ (*c* 1.01, H_2O) {lit.[5] $[\alpha]_D^{22} = -51.2$ (*c* 1, H_2O)}, 98% ee.

4.4. General metathesis procedure

A Schlenk flask was charged with a catalyst (5–20 mol %), deoxygenated solvent (~5 mL) and substrate (10–60 mg). The reaction mixture was left to stir at 50 °C for a specified period of time. Metathesis reactions were terminated upon exposure to oxygen and volatile species removed under reduced pressure. The crude product was purified by flash chromatography. Metathesis experiments are described using the following format: substrate, solvent, catalyst, reaction time, reaction temperature, percent conversion. Chromatographic purification conditions (isolated yield).

4.4.1. (2*S*)-Methyl 2*N*-acetylamino-5-methylhex-4-enoate 4a. (2*S*)-Methyl 2*N*-acetylaminopent-4-enoate **8a** (30.3 mg, 0.18 mmol), dichloromethane (4 mL), second generation Grubbs' catalyst (7.5 mg, 0.01 mmol, 5 mol %), isobutylene (5 psi), 4 h, 50 °C, 100% conversion. Purification by flash chromatography (SiO_2, dichloromethane–light petroleum–ethyl acetate, 1:1:1) furnished prenylglycine derivative **4a** (29.9 mg, 85%) as a brown oil. t_R = 24.2 min (GC column 30QC5/BPX5, 150 °C for 1 min, 10 °C min^{-1} to 280 °C for 6 min). $[\alpha]_D^{22} = +58.1$ (*c* 1.50, $CHCl_3$). Spectral data was consistent with that previously reported.

4.4.2. (2*S*)-2*N*-Acetylamino-5-methylhex-4-enoic acid 4b.[15] **Method A**: (2*S*)-2*N*-Acetylaminopent-4-enoic acid **8b** (78.0 mg, 0.50 mmol), dichloromethane (7 mL), second generation Grubbs' catalyst (21.2 mg, 0.02 mmol, 5 mol %), isobutylene (5 psi), 72 h, 50 °C, 74% conversion into **4b**.

Method B: (2*S*)-2*N*-Acetylaminopent-4-enoic acid **8b** (78.0 mg, 0.50 mmol), dichloromethane (7 mL), second generation Grubbs' catalyst (21.2 mg, 0.02 mmol, 5 mol %), 2-methylbut-2-ene (1 mL), 12 h, 50 °C, 100% conversion. Trituration with diethyl ether afforded the prenylglycine derivative **4b** as a colourless solid, mp 107–110 °C. ν_{max} (neat): 3421bs, 3324w, 3055m, 2971w, 2932m, 1728s, 1671s, 1526m, 1438m, 1386m, 1266s, 1103w, 910w, 737s, 704m cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 1.55 (s, 3H, H6a), 1.64 (s, 3H, H6b), 1.96 (s, 3H, CH_3CO), 2.32–2.58 (m, 2H, H3), 4.51 (q, *J* 6.2 Hz, 1H, H2), 5.02 (t, *J* 6.8 Hz, 1H, H4), 6.48 (br d, *J* 7.3 Hz, 1H, NH), 8.34 (br s, 1H, OH).

^{13}C NMR (100 MHz, $CDCl_3$): δ 18.0, 23.0 (($CH_3)_2$), 25.9 (CH_3CO), 30.4 (C3), 52.7 (C2), 118.0 (C4), 136.2 (C5), 171.1, 174.9 (C1, CONH). HRMS (ESI$^+$, MeOH): *m/z* calcd for $C_9H_{15}NO_3Na$ [(M+Na)$^+$] 208.0950. Found 208.0931.

4.4.3. (2*S*)-Methyl 2*N*-*tert*-butoxycarbonylamino-5-methylhex-4-enoate 4c.[16] (2*S*)-Methyl 2*N*-*tert*-butoxycarbonylaminopent-4-enoate **8c** (182 mg, 0.80 mmol), dichloromethane (7 mL), second generation Grubbs' catalyst (31.0 mg, 0.04 mmol, 5 mol %), isobutylene (5 psi), 4 h, 50 °C, 100% conversion. The crude product was used in the subsequent acid catalysed cyclisation reaction. ν_{max} (neat): 3435w, 3057w, 2982s, 2934s, 2857w, 1810s, 1750s, 1715s, 1633w, 1499m, 1456w, 1396w, 1372s, 1310w, 1266s, 1218s, 1167m, 1120s, 1074s, 952w, 844m, 746m, 704m cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41 (s, 9H, $(CH_3)_3$), 1.58 (s, 3H, H6a), 1.68 (s, 3H, H6b), 2.34–2.54 (m, 2H, H3), 3.70 (s, 3H, OCH_3), 4.29–4.31 (m, 1H, H2), 4.99 (apparent t, *J* 7.4 Hz, 2H, H4, NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.8 (C6a), 25.9 (C6b), 28.3 (($CH_3)_3$ C), 31.0 (C3), 52.2 (OCH_3), 53.3 (C2), 85.1 (O*C*$(CH_3)_3$), 117.7 (C4), 136.2 (C5), 155.3 (CONH), 172.9 (C1). HRMS (ESI$^+$, MeOH): *m/z* calcd for $C_{13}H_{23}NO_4Na$ [(M+Na)$^+$] 280.1525. Found 280.1526.

4.4.4. (2*S*)-2*N*-*tert*-Butoxycarbonylamino-5-methylhex-4-enoic acid 4d.[17] **Method A**: (2*S*)-2*N*-*tert*-Butoxycarbonylaminopentanoic acid **8d** (65.1 mg, 0.30 mmol), dichloromethane (4 mL), second generation Grubbs' catalyst (13.2 mg, 0.02 mmol, 5 mol %), isobutylene (5 psi), 42 h, 50 °C, 58% conversion into **4d**.

Method B: (2*S*)-2*N*-*tert*-Butoxycarbonylaminopentanoic acid **8d** (65.1 mg, 0.30 mmol), dichloromethane (4 mL), second generation Grubbs' catalyst (13.2 mg, 0.02 mmol, 5 mol %), 2-methylbut-2-ene (1 mL), 12 h, 50 °C, 95% conversion into **4d**.

ν_{max} (neat): 3434m, 3057m, 2982s, 2956m, 1716s, 1643m, 1502s, 1439s, 1393w, 1368s, 1265s, 1222w, 1163m, 1104w, 1060m, 1024m, 995w, 926m, 896w, 889w, 733s, 705w cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44 (s, 9H, $(CH_3)_3$), 1.62 (s, 3H, H6a), 1.72 (s, 3H, H6b), 2.44–2.58 (m, 2H, H3), 4.31–4.33 (m, 1H, H2), 5.00 (br d *J* 4.8 Hz, 1H, NH), 5.07 (t, *J* 7.3 Hz, 1H, H4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 18.1, 26.0 (($CH_3)_2$), 28.5 (($CH_3)_3$ C), 30.7 (C3), 53.4 (C2), 61.5 (($CH_3)_3C$), 117.6 (C4), 136.8 (C5), 155.8 (CONH), 176.2 (COOH). HRMS (ESI$^+$, MeOH): *m/z* calcd for $C_{12}H_{21}NO_4Na$ [(M+Na)$^+$] 266.1368. Found 266.1368.

Acknowledgements

The authors gratefully acknowledge the financial assistance of the Australian Research Council and the provision of an Australian Postgraduate Research Award (to J.E.).

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