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An Imidazoline Pseudodipeptide Suitable for Solid Phase Peptide Synthesis

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Abstract: This paper describes the synthesis of two diastereoisomers of an imidazoline dipeptide mimetic (a 4,5-dihydroimidazole-4-carboxylic acid), suitably protected for incorporation into solid phase peptide synthesis (SPPS) using the Fmoc protocol, from a phenylalanine-derived thioimidate and an α,β-diaminopropanoic acid ester, followed by protecting group manipulation. Copyright © 2000 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: imidazoline; pseudodipeptide; Fmoc SPPS

The modification of key amide bonds is an important strategy in the search for new agents to act at peptide receptors, or for new inhibitors of peptidase enzymes [1,2]. We have developed cyclic amidines, as in the imidazolines (4,5-dihydroimidazoles) **1** and tetrahydropyrimidines **2** (Figure 1), as amide bond replacements with distinct acid, base and hydrolysis behaviour [3,4]. Units **1** and **2** of the pseudodipeptide have been incorporated into small peptides by either a convergent approach [5], wherein the heterocycle is formed as the final step by the junction of two modified peptide fragments, or by a divergent approach [3,6,7], wherein unit **1** or **2** of the pseudodipeptide is assembled first, suitably orthogonally protected, and then coupled selectively at either the *N*- or the *C*-terminus.

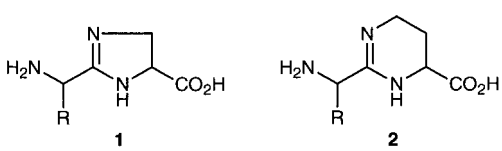

Figure 1 Cyclic amidine pseudodipeptides.

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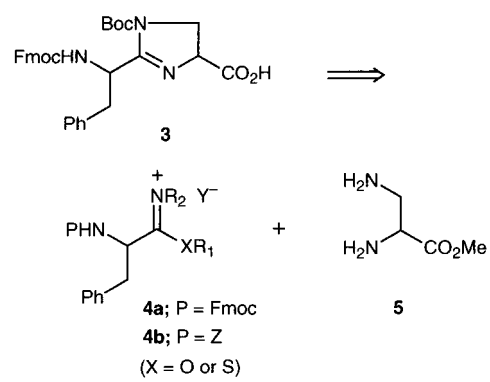

Scheme 1 Retrosynthetic analysis of pseudodipeptide **3**.

For a pseudodipeptide unit such as **1** or **2** to be easily incorporated into a number of peptidomimetics, the divergent 'building block' approach is more appropriate. Furthermore, for rapid evaluation in a range of targets, the pseudodipeptide should ideally be suitable for utilization in automated solid phase peptide synthesis (SPPS). We report here on a protocol for the adaptation of the cyclic amidine pseudodipeptide moiety **1** into substrates suitable for the Fmoc protocol of SPPS [8].

As a test target, we selected orthogonally protected pseudodipeptide **3** (Scheme 1), a Phe-Gly mimetic (Phe-ψ-[imidazoline]Gly-OH) for evaluation as an isostere in insect kinins, for which a

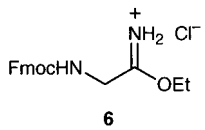

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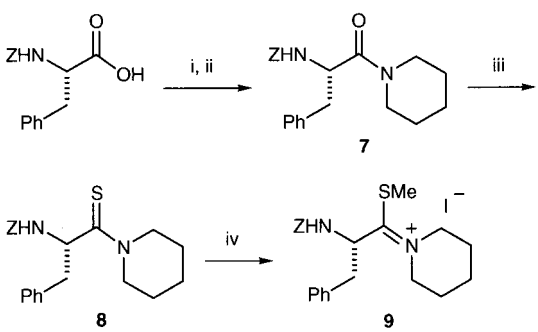
Figure 2 A model Fmoc-imidate.

C-terminal pentapeptide Phe-Phe-Pro-Trp-Gly-NH_2 is known to retain the biological activity of insect neuropeptides [9] (R.J. Nachman, pers. comm.). Previous experience suggested the need to protect the amidine functional group [3,10]; Boc protection was selected for this 'side-chain' masking, with the requisite *N*-terminal Fmoc protection.

Our usual retrosynthetic analysis (Scheme 1) suggests an Fmoc-protected imidate **4a** as one component and a diaminopropanoate ester **5** as the other. Both should be of known configuration, with the *S*-enantiomers preferred, to mimic as closely as possible the natural amino acids. We first examined the compatibility of the Fmoc group with this sequence for imidazoline formation. Thus, Fmoc-aminoacetonitrile was prepared from aminoacetonitrile hydrogensulphate (FmocCl, aq. Na_2CO_3; 88%) and treated with ethanolic hydrogen chloride to afford the model Fmoc-imidate **6** (Figure 2). Unfortunately, when **6** was treated with diaminopropanoate esters, the Fmoc group was unstable to the basic functionality present.

We, therefore, elected to complete the ring synthesis with the Z protecting group at the *N*-terminus, as this had previously been shown to survive heterocycle assembly and to be easily removed under neutral conditions [3,4]. A suitable imidate **9**, of type **4b**, was constructed according to Scheme 2 [3]. Benzyloxycarbonyl-*S*-Phe was converted to the pentafluorophenyl ester (pentafluorophenol, DCC);



Reagents: i, pentafluorophenol, DCC, CH_2Cl_2; ii, piperidine, CH_2Cl_2; iii, Lawesson's reagent, toluene, reflux; iv, MeI.

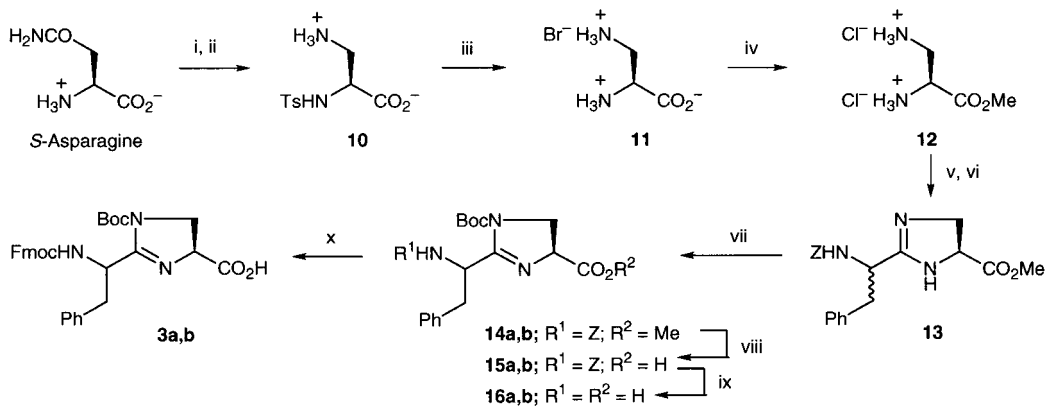
Scheme 2 Synthesis of imidate **9**.

this was not isolated but was treated directly with excess piperidine in CH_2Cl_2 to form the amide **7** (80%), $[\alpha]_D^{20} + 7.0$ ($c = 2.0$, EtOH). Reaction with Lawesson's reagent (toluene, reflux) readily yielded the corresponding thioamide **8** (80%), $[\alpha]_D^{20} + 29.0$ ($c = 1.0$, EtOH), and *S*-alkylation of this thioamide was accomplished in neat iodomethane at reflux [11]. The thioimidate salt **9** so produced was not stored but was prepared as required and used directly, in order to minimize loss through hydrolysis, etc.

The necessary carboxyl-protected diamine fragment of type **5** was successfully prepared as salt **12**, according to Scheme 3. The key transformation of *S*-Asn to *S*-2,3-diaminopropanoic acid was completed in three steps, as follows [5,12]. Tosyl-*S*-Asn was prepared (toluene-p-sulphonyl chloride, MgO–H_2O; 92%), because this protecting group is reported to be superior to others at suppressing racemization in the subsequent steps [13]. This sulphonamide underwent Hofmann rearrangement (Br_2, aq. NaOH) to give N^{α}-tosyl-2,3-diaminopropanoic acid **10** (58%), $[\alpha]_D^{20} + 18.0$ ($c = 2.0$, 5 M HCl), followed by reductive detosylation using HBr–acetic acid (30% w/w, 75°C; phenol as the Br_2 scavenger) to afford 2,3-diaminopropanoic acid as the crude monohydrobromide salt **11** (88%), $[\alpha]_D^{20} +$ 17.5 ($c = 2.0$, 1 M HCl). *C*-Terminus protection as the methyl ester (AcCl–MeOH, reflux) afforded the stable crystalline dihydrochloride **12** (59%), $[\alpha]_D^{20} +$ 7.5 ($c = 2.0$, H_2O). This reaction also served to purify the crude diaminoacid salt **11**. Attempts to prepare the corresponding benzyl and *tert*-butyl esters were not successful.

Treatment of the diamine salt **12** with a solution of ammonia in $CHCl_3$ (20°C) liberated the free diaminoester **5** (96%), which was condensed with the thioimidate salt **9** (MeOH, reflux) to afford the 2-imidazoline pseudopeptide Z-Phe-ψ-[imidazoline]Gly-OMe **13** in good yield (83%) (Scheme 3) as an inseparable mixture of diastereoisomers (indicated, *inter alia*, by two methyl ester singlets, δ_H 3.71 and 3.75, in the ^1H NMR spectrum). We presume that epimerization of the *S*-Phe-derived stereocentre occurs after condensation with the diaminoacid, by means of a proton-transfer mechanism such as that shown in Figure 3; such proton exchange in imidazolines is well precedented [14].

Protecting group manipulation to yield the target Fmoc-acid **3** was initiated by conversion of the cyclic amidine **13** to the *tert*-butyl carbamate (Boc_2O, $NaHCO_3$, THF–H_2O; 67%).[1] Two close-running components were observed on TLC analysis, separated by column chromatography, and



Reagents: i, TsCl, $MgO–H_2O$; ii, Br_2, aq. NaOH, 0°C; iii, HBr-AcOH, phenol, 70°C; iv, AcCl in MeOH, reflux; v, NH_3-$CHCl_3$ solution, 20°C; vi, **9**, MeOH, reflux; vii, Boc_2O, aq. $NaHCO_3$, THF: separate diastereoisomers; viii, aq. LiOH, THF; ix, H_2, $Pd(OH)_2$-C, MeOH; x, FmocCl, aq. Na_2CO_3, 0°C.

Scheme 3 Synthesis of pseudodipeptide **3**.

identified as diastereoisomers **14a** and **14b** (approximately 1:1) epimeric at the Phe-derived stereocentre; isomer **14a** m.p. 117°C, $[\alpha]_D^{21}$ –24.0 ($c = 1.0$, $CHCl_3$); isomer **14b** m.p. 97°C, $[\alpha]_D^{21}$ + 14.0 ($c = 2.0$, $CHCl_3$).[2] Only one carbamate regioisomer was produced, which we depict as N-1 on steric grounds, although further evidence is required before the definitive location is known.

The separated diastereoisomers (absolute configurations at the Phe stereocentre as yet unknown) were separately hydrolysed to the acids **15a** (m.p. 90–91°C) and **15b** (m.p. 93–94°C), respectively (0.1 M aq. LiOH–THF, 25°C; 99%), and deprotected at the *N*-terminus by hydrogenolysis (H_2, $Pd(OH)_2$–C, MeOH; 93%) to produce acids **16a** and **16b**, respectively. The side-chain protected pseudodipeptide amino acids **16a** (m.p. 136°C) and **16b** (m.p. 109–110°C) are obviously now available for a variety of *N*-protection protocols.

Our targets, **3a** and **3b**, were achieved by the addition of Fmoc separately to **16a** and **16b**, respectively (FmocCl, aq. Na_2CO_3, 0°C; 36%), isomer **3a** m.p. 126–127°C, $[\alpha]_D^{18}$ –24.4 ($c = 0.4$, MeOH); isomer **3b** m.p. 149–150°C, $[\alpha]_D^{17}$ –13.3 ($c = 0.27$, MeOH).[3]

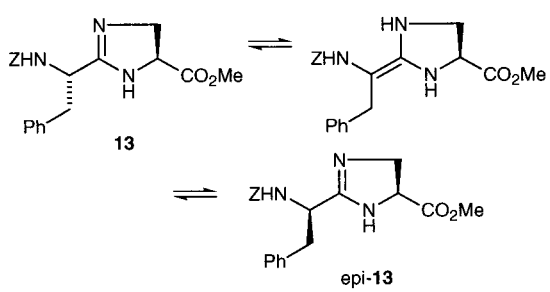


Figure 3 Epimerization of the pseudodipeptide.

We have thus demonstrated a protocol for manipulation of the imidazoline pseudodipeptides to yield components suitable for SPPS. The molecules **3a** and **3b** are currently undergoing evaluation for incorporation into insect neuropeptide mimics.

Acknowledgements

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NOTES

1. All new compounds have spectral data (IR, UV, NMR, MS) in accordance with the assigned structure and satisfactory combustion analysis or HRMS data.
2. No further epimerization takes place at the Phe-derived stereocentre, while the imidazolines are *N*-protected as carbamates; cf. [3]. We have also shown [3] that single diastereoisomers of the imidazolines may be isolated as salts following TFA deprotection.
3. Selected data for **3a**: δ_H[400 MHz; $(CD_3)_2CO$] 1.27–1.40 (9H, m, CMe_3), 2.91–3.30 (2H, m, NCH_2CH), 3.20–3.30 (2H, m, $PhCH_2CH$), 3.80 (1H, m, $CHCH_2O$), 4.10 (1H, m, NCH_2CH), 4.15 (2H, m, $CHCH_2O$), 4.20 (1H, m, $PhCH_2CH$), 4.25 (NH), 7.05–7.40 (9H, m, Ar-H) and 7.60–7.85 (4H, m, Ar-H); δ_C[100 MHz; $(CD_3)_2SO$] 174.4 (CO_2H), 171.3 (C=N), 157.5 and 156.8 (OCON), 144.9, 142.1 and 136.9 (Ar-C), 130.5, 129.3, 128.6, 127.9, 127.2, 126.2 and 120.9 (Ar-CH), 79.3 (CMe_3), 67.2 ($CHCH_2O$), 58.8 ($PhCH_2CH$), 54.0 (NCH_2CH), 47.8

(CH*C*H_2O), 39.9 (Ph*C*H_2CH), 38.3 (NCH_2CH) and 28.5 (C*Me*$_3$). Selected data for **3b**: δ_H[400 MHz; $(CD_3)_2CO$] 1.28–1.40 (9H, m, CMe_3), 2.95–3.25 (2H, m, NCH_2CH), 3.40–3.60 (2H, m, PhCH_2CH), 3.84 (1H, m, C*H*CH_2O), 4.13 (1H, m, NCH_2C*H*), 4.26 (2H, m, C*H*CH_2O), 4.42 (1H, m, PhCH_2C*H*), 4.50 (NH), 7.05–7.40 (9H, m, Ar-H) and 7.50–7.90 (4H, m, Ar-H); δ_C[100 MHz, $(CD_3)_2SO$] 173.4 (CO_2H), 171.9 (C=N), 157.3 and 157.0 (OCON), 144.3, 142.2 and 136.7 (Ar-C), 130.6, 129.5, 128.6, 128.1, 127.4, 126.4 and 120.9 (Ar-CH), 79.5 (*C*Me_3), 67.5 (*C*HCH_2O), 59.0 (PhCH_2*C*H), 54.0 (NCH_2*C*H), 48.0 (CH*C*H_2O), 40.0 (Ph*C*H_2CH), 38.8 (NCH_2CH) and 28.7 (C*Me*$_3$); ν_{max}/cm^{-1}($CHCl_3$) 3428, 3068, 3037, 2982, 2932, 1724, 1510, 1451, 1369, 1335 and 1238; *m*/*z* (FAB) 538 (M^+–OH), 474, 456, 179 (100%) and 105.

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