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3-(1-Aminoalkyl)isoxazole-4-carboxylic acids as peptide bond replacements

Raymond C. F. Jones,*,† Stephen J. Hollis and James N. Iley

Department of Chemistry, The Open University, Walton Hall, Milton Keynes MK7 6AA, UK

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Abstract

An orthogonally protected 3-(1-aminoalkyl)isoxazole-4-carboxylic acid has been prepared by 1,3-dipolar cycloaddition of a suitably protected α-aminonitrile oxide with an enaminoester dipolarophile; this protected amino acid has been deprotected and coupled independently at either the C- or N-terminus to produce pseudopeptide segments as peptide mimetics that contain a *cis*-amide bond replacement.

There is an ongoing search for peptide mimetics that incorporate both replacement of important amide bonds and restriction of conformational freedom relative to the native peptide.[1] Such 'pseudopeptides' have potential as agonists or antagonists at peptide receptors, or as inhibitors of peptidase enzymes. One focus of this search is on molecules that enforce a reverse turn on a predominantly peptide chain.[2] As part of a programme for the incorporation of heterocycles into peptide mimetics, that has previously reported on the use of cyclic amidines as amide bond replacements,[3] we proposed the 3-(1-aminoalkyl)-4-carboxyisoxazoles **1** as pseudodipeptides that may be regarded as replacing a *cis*-amide bond.[4] We now report on the synthesis of suitably orthogonally protected derivatives of isoxazoles **1**, and their flexible incorporation into pseudopeptide segments.

The synthetic strategy was based on 1,3-dipolar cycloaddition of a protected α-aminonitrile oxide **2** with an enaminoester dipolarophile **3** (Scheme 1).[5] Synthesis of the dipole began with *N*-*tert*-butyloxycarbonyl-(*S*)-alanine methyl ester **4**, which was reduced (diisobutylaluminium hydride, toluene, −78°C) to afford 2-(*tert*-butyloxycarbonylamino)propanal which was not generally purified, but converted directly to its oxime **5** ($NH_2OH{\cdot}HCl$, NaOAc, EtOH–H_2O, 60°C) (Scheme 2). Recrystallisation afforded a 2:1 mixture of oxime geometric isomers, *syn* and *anti* geometry not assigned, (63% from ester **4**) that proved stable towards racemisation.[6]

* Corresponding author. E-mail: r.c.f.jones@lboro.ac.uk

† Current address: Department of Chemistry, Loughborough University, Loughborough, Leics. LE11 3TU, UK.

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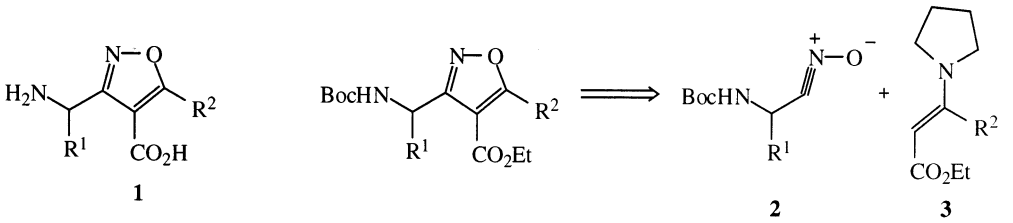
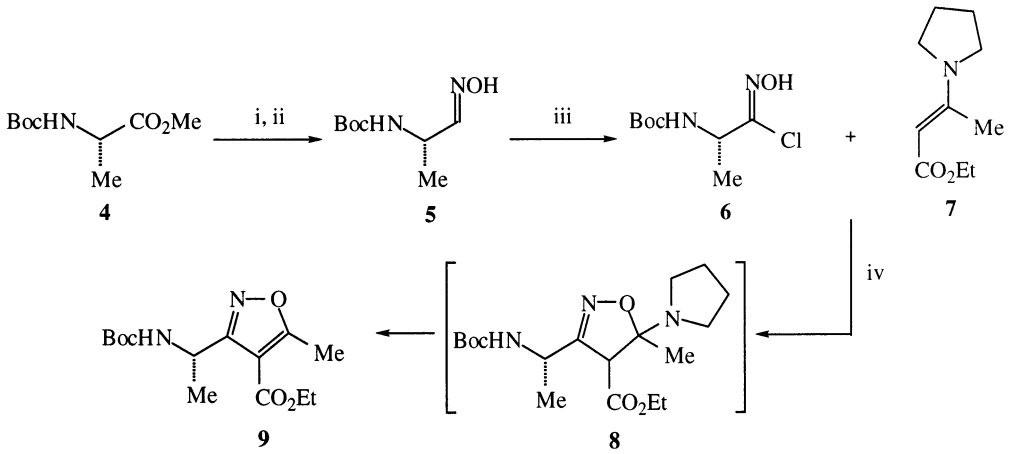
N-Chlorination could be accomplished with *N*-chlorosuccinimide ($CHCl_3$, reflux; one isomer converted in 2 h, the other required at least 16 h), but this conversion was performed much better with *tert*-butyl hypochlorite ($CHCl_3$, 0°C). When this conversion was complete (45 min as monitored by ^1H NMR spectroscopy), enaminoester **7** (2–3 mol equiv.; previously prepared from ethyl acetoacetate and pyrrolidine in toluene at reflux, Dean–Stark water removal) was added to the solution of crude imidoyl chloride **6**, and triethylamine was added dropwise ($CHCl_3$, reflux). Cycloaddition of the nitrile oxide generated in situ from **6**, with the enaminoester **7** afforded the required isoxazole **9**, $[\alpha]_D^{26}$ −29.8 ($c=1.13$, $CHCl_3$), as a single regioisomer (66% from oxime **5**), presumably via an intermediate **8** that aromatizes by spontaneous elimination of pyrrolidine.[7]



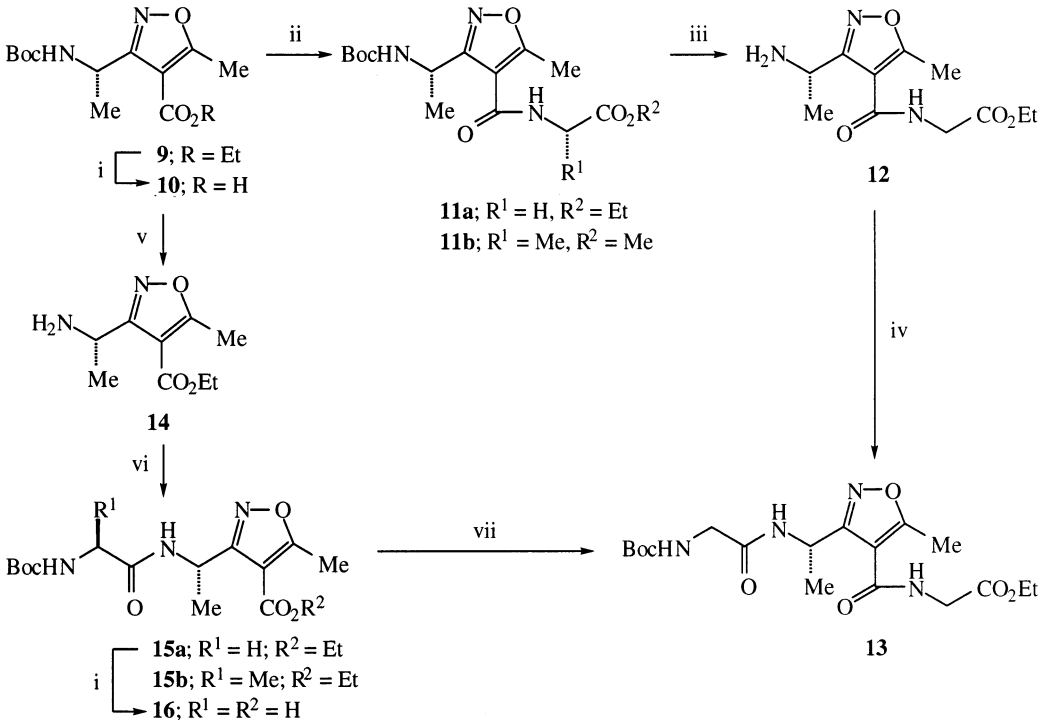
Scheme 1.



Scheme 2. Reagents: (i) *i*-Bu_2AlH, toluene, −78°C; (ii) $NH_2OH{\cdot}HCl$, NaOAc, EtOH–H_2O, 60°C; (iii) *t*-BuOCl, $CHCl_3$, 0°C; (iv) Et_3N, $CHCl_3$, reflux

Coupling of *N*-protected amino ester **9** with other amino acid residues could be achieved via either initial C- or N-terminal deprotection. For example, hydrolysis of ester **9** (LiOH, aq. EtOH–THF, 20°C, 16 h) afforded the isoxazole-4-carboxylic acid **10** (65%), $[\alpha]_D^{22}$ −22.1 ($c=1.08$, MeOH) (Scheme 3). This was coupled with glycine ethyl ester hydrochloride [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), Et_3N, CH_2Cl_2 or ether] to afford amide **11a** (39%), $[\alpha]_D^{22}$ −24.0 ($c=0.51$, MeOH), and with (*S*)-alanine methyl ester hydrochloride under these conditions to afford amide **11b** (24%), $[\alpha]_D^{25}$ +14.2 ($c=1.37$, $CHCl_3$). The latter compound was isolated as a single diastereoisomer (HPLC, ^{13}C and ^1H NMR spectra), verifying the chiral integrity of isoxazole **9** and carboxylic acid **10**. For comparison, when hydrolysis of ester **9** was effected with KOH in ethanol at reflux, coupling of this sample of acid **10** (97%) with

(*S*)-alanine methyl ester hydrochloride under the same conditions afforded amide **11b** but as a 5:1 mixture of two diastereoisomers. Subsequent unmasking of the N-terminus of pseudodipeptide **11a** (AcCl, EtOH) to give amine **12** as its hydrochloride salt (quantitative) was followed by coupling of the free amine **12** with *N*-*tert*-butyloxycarbonylglycine (EDCI) to give the pseudotetrapeptide segment **13** in low yield (10%), $[\alpha]_D^{28}$ +7.2 ($c=1.31$, $CHCl_3$).



Scheme 3. Reagents: (i) LiOH, aq. EtOH–THF; (ii) H-Gly-OEt·HCl (for **11a**) or H-Ala-OMe·HCl (for **11b**), EDCI, Et_3N, CH_2Cl_2; (iii) AcCl, EtOH; (iv) Boc-Gly-OH, EDCI; (v) TFA, CH_2Cl_2, then 2 M aq. HCl; (vi) Boc-Gly-OH (for **15a**) or Boc-Ala-OH (for **15b**), EDCI, Et_3N; (vii) H-Gly-OEt·HCl, EDCI, Et_3N

The orthogonal sequence could also be employed, with initial *N*-deprotection of isoxazole ester **9** (TFA–CH_2Cl_2; then 2 M aq. HCl) to afford the 3-(1-aminoalkyl)isoxazole **14** as its hydrochloride salt (94%; more easily handled than the trifluoroacetate salt), $[\alpha]_D^{25}$ −29.9 ($c=1.03$, MeOH). The amine salt **14** could be coupled to *N*-*tert*-butyloxycarbonylglycine or *N*-*tert*-butyloxycarbonyl-(*S*)-alanine (EDCI, Et_3N) to afford the pseudotripeptide amides **15a**, $[\alpha]_D^{27}$ −31.3 ($c=1.05$, $CHCl_3$), and **15b**, $[\alpha]_D^{25}$ −49.0 ($c=1.11$, $CHCl_3$), respectively (57, 74%); again, the latter compound was produced as a single diastereoisomer (HPLC, ^{13}C and ^1H NMR spectra), reinforcing the conclusion that isoxazole ester **9** had been formed without racemisation. Hydrolysis of the ester **15a** as before (LiOH, aq. EtOH–THF; 43%) was followed by coupling of the crude carboxylic acid **16** with glycine ethyl ester hydrochloride as usual, to again afford pseudopeptide segment **13** (27%) by this alternative sequence. The sometimes low yields obtained by coupling to the termini of amino acid residue **1** may be attributed to deactivation of the C-terminus, which is a vinylogous carbonate, and also to steric factors.

We have thus demonstrated the formation and incorporation into pseudopeptides of the isoxazole amino acid moiety **1**, as a *cis*-amide bond replacement. Preliminary molecular

modelling of these systems[8] indicates that they have a number of minimum energy conformations that place a turn in the peptide backbone.

Acknowledgements

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References

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8. Performed in SPARTAN 4.1 using the AM1 semi-empirical method on a Silicon Graphics Indy workstation.