3-(1-Aminoalkyl)pyrazole- and 4,5-Dihydropyrazole-5-carboxylic Acids as Peptide Bond Replacements

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Dedicated to Professor Alberto Brandi on the occasion of his 60th birthday

Abstract: Orthogonally protected 3-(1-aminoalkyl)pyrazole- and 4,5-dihydropyrazole-5-carboxylic acids are prepared by 1,3-dipolar cycloaddition of α-aminonitrile imines with electron-deficient alkenes; the pyrazole is incorporated into pseudotri- and tetrapeptides.

Key words: cycloaddition, dipole, heterocycles, amino acids, peptide mimics

Peptides mediate many biological processes of potential medicinal significance, however, the therapeutic use of peptides can be compromised by metabolic instability and bioavailability issues.[1] Amide bond modification and the introduction of conformational restraints into bioactive peptides are established strategies in the search for new receptor agonists or antagonists, or for new peptidase inhibitors.[2] There is thus an ongoing quest for peptide mimetics ('pseudopeptides') incorporating both replacement of important amide bonds and restriction of conformational freedom relative to the native peptide. One focus of this search is on molecules that enforce a reverse turn on a predominantly peptide chain.[3] As part of a programme for the incorporation of heterocycles into peptide mimetics as backbone constraints, we have previously reported cyclic amidines **1** (Figure 1) as amide bond replacements[4] and 3-(1-aminoalkyl)-4-carboxyisoxazoles **2** as pseudodipeptides that may be regarded as replacing a *cis*-amide bond.[5] Examples of **1** (n = 1) show a turn conformation by ^{1}H VT-NMR studies,[4a] whilst for **2** a turn conformation has been observed in the solid state.[6] We now report on the synthesis of orthogonally protected derivatives of pyrazoles **3** and their flexible incorporation into pseudopeptide segments, and of 4,5-dihydropyrazoles **4**. Pyrazoles have been shown to possess a range of interesting biological properties.[7]

The synthetic strategy (Scheme 1) is related to that employed in our assembly of isoxazole pseudopeptides[5] and used extensively in our work on cyclic tricarbonyl natural products.[8] It involves the dipolar cycloaddition of α-amino-1,3-dipoles, in this case nitrile imines, with suitable electron-deficient alkenes as dipolarophiles.[9,10] Synthesis of the dipole began with α-amino acids and followed two routes to the required hydrazonoyl chloride **5**, via a hydrazone or hydrazide; these routes were compared by the use of alanine as the starting amino acid.

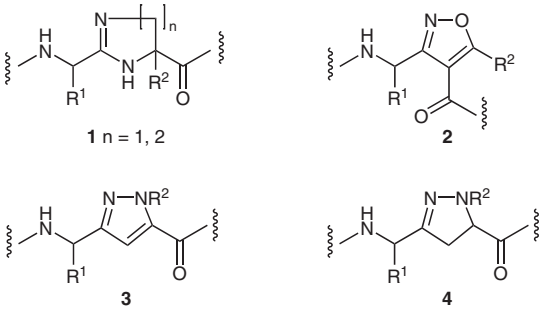
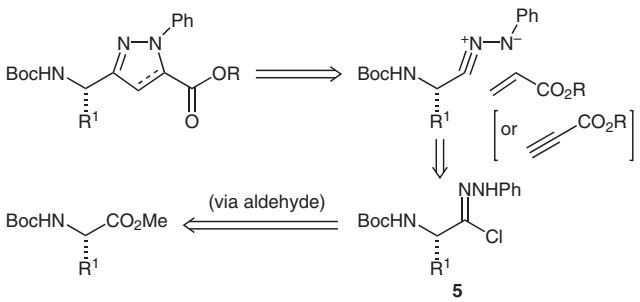


Figure 1



Scheme 1 Synthetic strategy for synthesis of (dihydro)pyrazoles

The preferred hydrazone route commenced by reduction of commercial *N*-*tert*-butoxycarbonyl-(*S*)-alanine methyl ester (DIBAL-H, toluene, –78 °C) to afford 2-(*tert*-butoxycarbonylamino)propanal (93%) which was not further purified, but converted directly into its phenylhydrazone **6** ($PhNHNH_2$·HCl, NaOAc, EtOH–H_2O, 70 °C; Scheme 2), isolated as a solid (81%) that was stable towards racemisation.[11] To form the nitrile imine, the hydrazone was C-chlorinated using NCS (EtOAc, 60 °C, 1 h) or the complex of NCS with dimethyl sulfide (DMS) (CH_2Cl_2, –78 °C to 20 °C over 3 h).[12] The hydrazonoyl chloride **5** was then treated without further purification under a range of basic conditions to form the nitrile imine in situ, in the presence of ethyl propenoate as a suitable dipolarophile. Using direct NCS chlorination, the pseudodipeptide dihydropyrazole **7** was isolated in 41% yield using $KHCO_3$ and a few drops of water (70 °C, 20 h) as the basic medium (method 1);[13,14] almost the same yield (40%) was obtained using Et_3N as the base (method 2).[15] Improvement to 57% yield of **7** was found by employing NCS·DMS fol-

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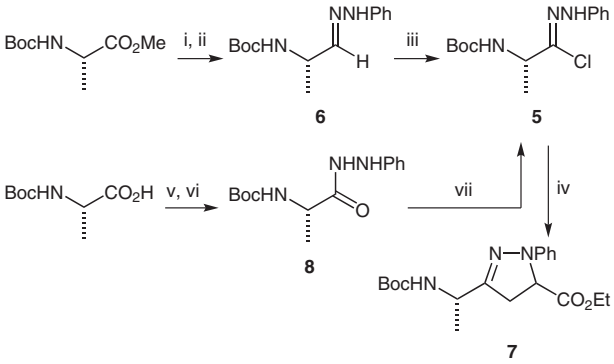
lowed by 0.1 M $NaHCO_3$ solution and tetrahexylammonium chloride as a phase-transfer agent (20 °C, 2 h; method 3).[16] In this case the product was removed by filtration and needed no further purification, whereas the other protocols required chromatography. An attempt using silver acetate as base (toluene, 20 °C, 20 h, method 4) after chlorination with NCS·DMS, led to dihydropyrazole **7** in only 25% yield.[17]



Scheme 2 Dihydropyrazole synthesis. *Reagents and conditions*: (i) DIBAL-H, toluene, –78 °C; (ii) $PhNHNH_2$·HCl, NaOAc, EtOH–H_2O, 70 °C; (iii) NCS, EtOAc, 60 °C, 1 h, or NCS·DMS, CH_2Cl_2, –78 °C to 20 °C over 3 h (see text); (iv) $H_2C{=}CHCO_2Et$; methods 1–4 (see text): method 1, $KHCO_3$ aq, 70 °C, 20 h; method 2, Et_3N; method 3, 0.1 M $NaHCO_3$ solution, $(C_6H_{13})_4NCl$, 20 °C, 2 h; method 4, AgOAc, toluene, 20 °C, 20 h; (v) HOBt, EDCI, DMF, 20 °C, 2 h; (vi) $PhNHNH_2$, DMF, 0 °C; (vii) CCl_4–Ph_3P, MeCN, 20 °C, 17 h.

The alternative hydrazide route to dipole precursor **5** began with *N*-*tert*-butoxycarbonyl-(*S*)-alanine which was activated [*N*-hydroxybenzotriazole (HOBt), EDCI, DMF, 20 °C, 2 h] and then added to phenylhydrazine (DMF, 0 °C) to afford the hydrazide **8** (56%; Scheme 2).[18] Formation of the hydrazonoyl chloride **5** was accomplished using CCl_4–Ph_3P (MeCN, 20 °C, 17 h).[19] Cycloaddition was completed using the crude chloride in EtOAc, according to method 1, to give dihydropyrazole **7** (31%). The hydrazide route proved less efficient and less technically convenient than the hydrazone approach, so that all subsequent cycloadditions used hydrazonyl chlorides prepared via the hydrazone. The *N*-benzyloxycarbonylalanine series failed to provide any cycloadduct in our hands, as did the use of *N*-*tert*-butoxy- and *N*-benzyloxycarbonyl hydrazones, or the more electron-rich dipolarophile, prop-2-en-1-ol.[20]

The dihydropyrazole **7** was shown to be a 1:1 mixture of two diastereomers, by chiral-phase HPLC. Unfortunately, the two epimers could not be routinely preparatively separated, although on one occasion a sample selectively crystallised to afford crystals of one diastereomer of sufficient quality for an X-ray diffraction study.[21] This confirmed the regiochemistry of the cycloaddition to be as predicted, affording the 5-ethoxycarbonyl derivative (Figure 2).

That the diastereomeric mixture was derived from C-5 and not from epimerization of the original alanine α-carbon centre, was demonstrated by using methyl propynoate

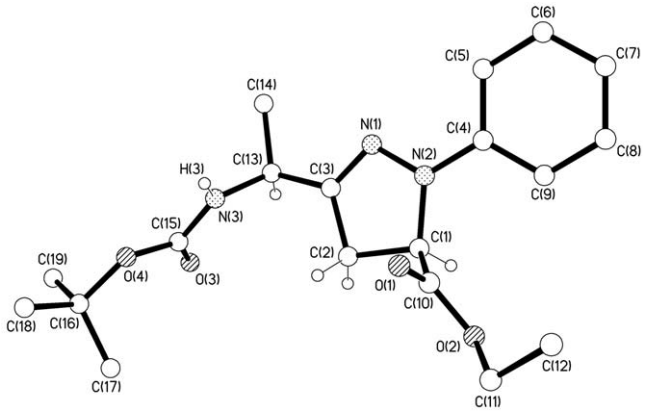
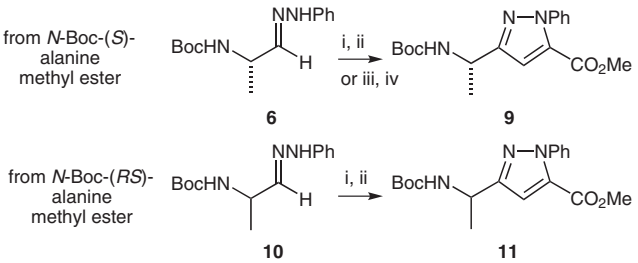


Figure 2 X-ray crystal structure of one diastereomer of dihydropyrazole **7** (crystallographic numbering)

as dipolarophile. Using NCS chlorination of **6** and method 1 gave a 25% yield of pyrazole **9**, improved to 40% using NCS·DMS and method 3 (Scheme 3). Chiral-phase HPLC of pyrazole **9** showed essentially one peak, indicating one enantiomer, that is, the amino acid chiral centre retaining its integrity. This conclusion was supported when the sequence was repeated with *N*-*tert*-butoxycarbonyl-(*RS*)-alanine methyl ester (Scheme 3). Thus DIBAL-H reduction (95%) and reaction with phenylhydrazine gave the racemic hydrazone **10** (88%) which underwent C-chlorination and cycloaddition with methyl propynoate using method 1 to give racemic pyrazole **11** (30%). Chiral-phase HPLC examination of **11** revealed two peaks in a 1:1 ratio, clearly showing that the two enantiomers could be distinguished by HPLC if present.



Scheme 3 Pyrazole synthesis. *Reagents and conditions*: (i) NCS, EtOAc, 60 °C, 1 h; (ii) $HC{\equiv}CCO_2Me$, method 1; (iii) NCS·DMS, CH_2Cl_2, –78 °C to 20 °C over 3 h; (iv) $HC{\equiv}CCO_2Me$, method 3 (see text and Scheme 2).

The scope of the cycloaddition to generate dihydropyrazoles was briefly explored (Figure 3). In unoptimised reactions using hydrazone **6**, NCS chlorination and dipole generation by method 1, *tert*-butyl propenoate as dipolarophile afforded dihydropyrazole **12** (27%; raised to 32% using method 3), *tert*-butyl 2-methylpropenoate gave **13** (25%), and dimethyl fumarate led to the 4,5-*trans*-diester mixture **14** (12%); the amide *N*,*N*-dimethylpropenamide gave the dihydropyrazole amide **15** (25%). All of **12**–**15** were still isolated as 1:1 diastereomer mixtures, despite the larger dipolarophile steric requirement. The use of propenamide dipolarophiles alerted us to the potential

for assembling a dihydropyrazole pseudotripeptide wherein the C-terminal residue formed part of the dipolarophile. Thus *N*-propenoyl-(*S*)-proline methyl ester was prepared and used as dipolarophile, giving cycloadduct **16** (28%); once again, no diastereomeric preference was observed.

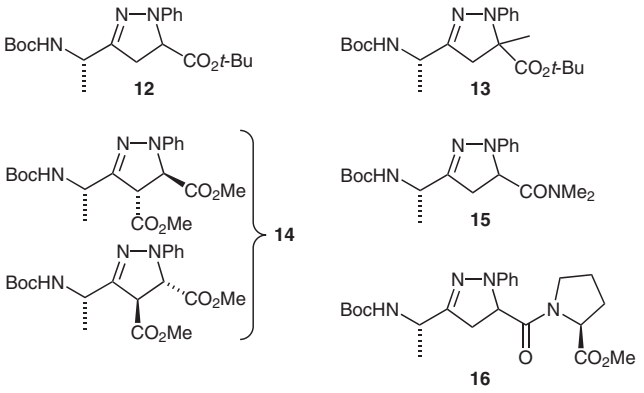


Figure 3

The cycloaddition sequence was repeated with valine as initial amino acid. Thus *N*-*tert*-butoxycarbonyl-(*S*)-valine methyl ester was reduced and converted into the solid phenylhydrazone **17** (DIBAL-H, toluene, –78 °C, 76%; $PhNHNH_2$·HCl, NaOAc, EtOH–H_2O, 70 °C, 96%). Cycloaddition via NCS chlorination and method 1, with ethyl propenoate as partner, afforded the dihydropyrazole **18** (20%; Figure 4). Glycine as initial amino acid via the same sequence gave only a low yield (10%) of cycloadduct **19**.

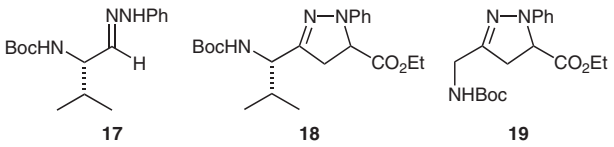
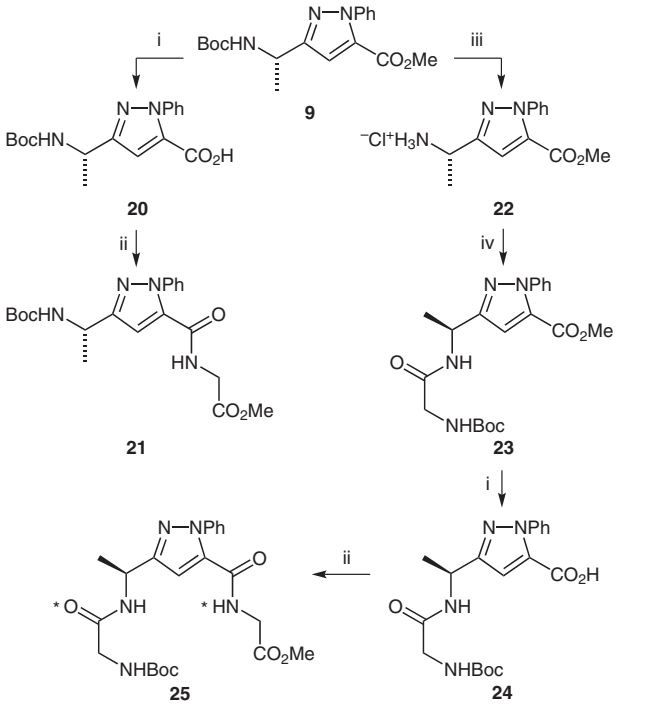


Figure 4

To demonstrate the incorporation of the novel heterocyclic amino acids into pseudopeptide sequences (Scheme 4), the (*S*)-pyrazole **9** was first treated with aqueous base (2 M NaOH aq, THF, 20 °C, 3 h) to afford the 5-carboxylic acid **20** (97%), which was coupled to glycine methyl ester (EDCI, Et_2O, 20 °C, 16 h) to give the pseudotripeptide **21** (46%). Alternatively, the N-terminus of **9** was unmasked to leave the amine salt **22** (TFA, CH_2Cl_2, 20 °C, 16 h; then 2 M HCl aq, 65%), which was coupled to *N*-*tert*-butoxycarbonylglycine (Et_3N; then EDCI, CH_2Cl_2, 20 °C, 19 h) to give another pseudotripeptide, dihydropyrazole **23** (46%). Successive coupling at both termini was achieved by hydrolysis of ester **23** (2 M NaOH aq, THF, 20 °C, 3 h) to give acid **24** (70%), and coupling to glycine methyl ester using the EDCI protocol employed above with acid **20**. This afforded the pseudotetrapeptide **25** (33%). Preliminary investigation of compound **25** by variable-temperature solution ^1H NMR spectroscopy[22] shows that an intramolecular H bond between centres marked *, as would be found if the molecule adopted a reverse turn conformation, is unlikely but cannot be discounted without further study.



Scheme 4 Synthesis of pseudopeptides from pyrazole **9**. *Reagents and conditions*: (i) 2 M NaOH aq, THF, 20 °C, 3 h; (ii) EDCI, Et_2O, 20 °C, 16; (iii) TFA, CH_2Cl_2, 20 °C, 16 h; then 2 M HCl aq; (iv) Et_3N; then EDCI, CH_2Cl_2, 20 °C, 19 h.

We have thus demonstrated the assembly of pyrazole and dihydropyrazole amino acids by nitrile imine cycloaddition, and the feasibility of their incorporation into pseudopeptides. Further investigations of the conformational properties of the pseudopeptides are under way.

Acknowledgment

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(14) **Typical Procedure for NCS Chlorination and Method 1 (*S*)-3-(1-*tert*-Butoxycarbonylaminoethyl)-2-phenyl-4,5-dihydro-1*H*-pyrazole-5-carboxylic Acid Ethyl Ester (7)**
To (*S*)-[1-methyl-2-(phenylhydrazono)ethyl]carbamic acid *tert*-butyl ester (**5**, 1.24 g, 4.72 mmol) in EtOAc (15 mL) at 60 °C was added NCS (0.71 g, 5.35 mmol, 1.1 equiv) and the mixture stirred for 1 h. Ethyl propenoate (0.918 g, 1.0 mL, 9.16 mmol, 1.9 equiv), $KHCO_3$ (2.41 g, 23.97 mmol, 5.1 equiv) and a few drops of H_2O were added and the mixture stirred at 70 °C for 20 h. The mixture was then filtered and the filtrate concentrated under reduced pressure to give a dark orange oil, purified by column chromatography on silica gel eluting with light PE–EtOAc (7:1, v/v) to yield the title compound **7** (0.72 g, 41%) in an inseparable 1:1 mixture of diastereomers, as an orange solid; mp 93–95 °C. IR ($CHCl_3$): ν_{max} = 3354 (NH), 1599 (C=N), 1708 (C=O), 1168 (CO), 750 (PhCH) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.16 (3 H, t, *J* = 7.2 Hz, CH_2CH_3), 1.35 (3 H, d, *J* = 7.0 Hz, CH_3CH), 1.38 [9 H, s, $C(CH_3)_3$], 2.99 (1 H, dd, *J* = 7.2, 17.6 Hz, 4-CHH), 3.24 (1 H, dd, *J* = 12.4, 17.6 Hz, 4-CHH), 4.14 (2 H, q, *J* = 7.2 Hz, CH_2CH_3), 4.43 (1 H, m, CH_3CH), 4.54, 4,57 (each 0.5 H, dd, *J* = 7.2, 12.4 Hz, $CHCO_2Et$, diastereomers 1 and 2), 5.00 (1 H, br s, NH), 6.78 (1 H, m, ArH), 6.93 (2 H, m, ArH), 7.18 (2 H, m, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.2, 21.1 (CH_3) 28.2 [$(CH_3)_3C$], 40.15 (4-CH_2), 46.1 (5-CH), 61.7 (OCH_2), 113.0 (PhCH), 113.0, 119.7, 119.8, 129.0 (4 × CH), 129.1 (2 × C), 145.3 (CN), 171.2, 171.5 (2 × CO). MS (EI): *m/z* = 362 [MH^+], 171 (12), 154 (24), 147 (19), 123 (21), 111 (28), 109 (35), 95 (54), 81 (54), 69 (85), 57 (100), 55 (99). HRMS (EI): *m/z* calcd for $C_{19}H_{27}N_3O_4$: 362.2074 [MH^+]; found 362.2073 [MH^+]. Anal. Calcd (%) for $C_{19}H_{27}N_3O_4$: C, 63.1; H, 7.5; N, 11.6. Found: C, 62.6; H, 7.2; N, 11.8.

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(21) **Crystal Data for 7**
$C_{19}H_{27}N_3O_4$, *M* = 361.44, monoclinic, *a* = 5.14990 (10), *b* = 11.4316 (4), *c* = 16.8254 (6) Å, β = 96.320 (2), *U* = 984.52 (5) $Å^3$, *T* = 120 (2) K, space group $P2_1$, graphite monochromated Mo Kα radiation, λ = 0.71073 Å, *Z* = 2, D_c = 1.219 g cm^{-3}, *F*(000) = 388, colourless, dimensions 0.36 × 0.09 × 0.04 mm^3, μ = 0.086 mm^{-1}, 3.02 < θ < 28.19°, 11290 reflections measured, 2363 unique reflections, R_{int} = 0.0376. The structure was solved by direct methods and refined on F^2. Friedel pairs were merged due to the lack of any significant anomalous scattering. *wR*2 = 0.0833 (all data, 244 parameters); *R*1 = 0.0351 [2223 data with $F^2 > 2\sigma(F^2)$]. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. 787832. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44 (1223)336033 or e-mail: deposit@ccdc.cam.ac.uk).

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