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The synthesis and crystal structure of alpha-keto tetrazole-based dipeptide mimics

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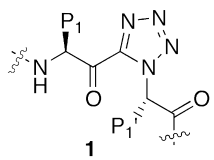
Abstract—Here we report the synthesis of a *cis*-dipeptide mimic *N*-Boc-Phe-[$COCN_4$]-Gly-OBn, **7**, containing the non-hydrolysable α-keto tetrazole isostere and an unusual 2,5-disubstituted α-keto tetrazole-based peptidomimetic, **8**. The incorporation of the novel *cis*-amide bond isostere was achieved via direct alkylation of a precursor five-substituted (1*H*)-tetrazole. The assignment of the resulting 1,5- and 2,5-tetrazoyl regiomers was based on the first reported X-ray structure analysis of an α-keto tetrazole, compound **8**.

We have embarked on a program to develop a range of novel conformationally restricted amide bond isosteres that incorporate a 1,5-disubstituted tetrazole.[1] The tetrazole ring has been shown to be an excellent mimic of the *cis*-amide bond, and as such, this structural motif can be used to pre-organise the amide bonds of peptides, enzyme substrates and inhibitors into a *cis*-conformation.[2] We have now extended this work with the design and preparation of a novel conformationally restricted α-keto tetrazole isostere [$COCN_4$], **1**, that combines the conformational restriction of a 1,5-disubstituted tetrazole ring with the potency of a non-hydrolysable α-keto amide isostere [COCONH], **2**. The α-keto amide functionality (e.g. **2**) has found widespread application as an isosteric replacement in reversible inhibitors of the proteinase family of enzymes. Amino acid derived α-keto amides have been incorporated into inhibitors of α-chymotrypsin,[3] calpain,[4] cathepsin B,[5] pepsin[6] and HIV protease.[7] The potency of the α-keto amide isostere has been attributed to formation of a stabilised tetrahedral adduct between the electrophilic carbonyl of the isostere and catalytic residues of the proteolytic enzyme.[8] As an extension of this work, some simple alpha-keto heterocycles have also been shown to inhibit serine proteases.[9] The α-keto tetrazole isostere, **1**, is an important addition to the amide bond analogues available to peptidomimetic chemists for incorporation into enzyme inhibitors and conformational probes.

By far the most common route used to prepare α-keto amide isosteres of type **2** is by oxidation of a precursor α-hydroxy amino acid. In our earlier work,[1] we synthesised a tetrazole-based dipeptide mimic containing an α-keto tetrazole isostere from a precursor dipeptide containing an N-terminal α-acetoxy β-amino acid. The amide linkage was then converted into the required tetrazole on reaction with phosphorous pentachloride and hydrazoic acid. The free hydroxyl group was then revealed and finally oxidised to the α-keto substituent. We now report a more expedient route to α-keto tetrazole-based dipeptide mimics.



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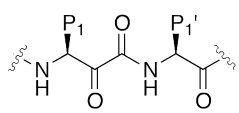

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It is known that five-substituted (1*H*)-tetrazoles can be directly alkylated to yield 1,5-disubstituted and 2,5-disubstituted tetrazoles.[10,11] However, difficulties are often encountered when assigning the regiochemistry of the resulting tetrazoyl products due to similar chemical shifts between the 1,5- and 2,5-regioisomers. We postulated that direct alkylation of a suitable (1*H*)-tetrazole could be used for the preparation of the α-keto tetrazole isostere, **1**. Using this approach we have successfully synthesised both the *cis*-dipeptide mimic *N*-Boc-Phe-[$COCN_4$]-Gly-OBn, (**7**), which contains an α-keto tetrazole isostere and the unusual 2,5-disubsti-

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tuted tetrazoyl peptidomimetic, **8**. Additionally, we have been able to unequivocally assign the regioisomers by NMR analysis and by solving a unique X-ray analysis of the 2,5-disubstitiuted tetrazoyl product, **8**.

The key steps in the synthesis of **7** and **8** involved adding a C-terminal glycine residue by direct alkylation of the tetrazole ring of **4** followed by oxidation of the free hydroxyl group (Scheme 1). Tetrazole **4** was prepared from the *N*-Boc-protected tetrazole, **3**, itself prepared in two steps according to the method of Satoh et al.[12] (49% overall yield) from commercially available *N*-Boc-phenylalanine. A catalytic hydrogenation of the *N*-Boc-protected tetrazole, **3**, resulted in removal of the benzyl methylether tetrazole protecting group, as required, but also in reduction of the α-carbonyl group (Scheme 1). Upon work-up, the reaction gave a mixture of epimeric α-hydroxy (1*H*)-tetrazoles, **4a** and **4b**, in 90% yield, which was used without further purification.[13] Alkylation of the α-hydroxy (1*H*)-tetrazoles, **4a** and **4b**, with benzyl bromoacetate in the presence of *N*,*N*-diisoproplyethylamine (DIPEA) gave the epimeric 1,5-disubstituted tetrazoyl products, **5a** and **5b**, and also the 2,5-disubstituted tetrazoyl products, **6a** and **6b**. The products were purified, but not separated, by flash column chromatography to give the regiomeric tetrazoles, **5** and **6**, as a mixture in 60% yield.[14] The α-hydroxy tetrazoles, **5** and **6** were then oxidised, as a mixture, with a solution of TEMPO,[15] to give the regiomeric α-keto tetrazoles, **7** and **8** (3:4 by ^{1}H NMR). The tetrazoles, **7** and **8**, were separated by flash column chromatography, the less polar 1,5-disubstituted tetrazole, **7**, eluting prior to the 2,5-disubstituted tetrazole, **8**. The tetrazoles were obtained in 30 and 40% yields, respectively.[16]

The structures of the regiomeric tetrazoles were assigned on the basis of ^{1}H and ^{13}C NMR, and an X-ray structure determination of **8**. Based on previous ^{1}H NMR studies of regiomeric tetrazoles,[17] it would be expected that the Gly-α-H_2 ^{1}H resonance for the 1,5-disubstituted tetrazole would appear further upfield than the Gly-α-H_2 signal of the 2,5-disubstituted tetrazole. ^{13}C NMR studies of 1- and 2-methyltetrazoles have shown that the methyl and CN_4 carbon atoms of the 1,5-disubstituted tetrazole are more shielded than the corresponding carbon atoms of the 2,5-disubstituted tetrazoles.[18] Based on these reported observations, and the structural determination of **8** (see discussion below), the tetrazole that was eluted first was designated the 1,5-disubstituted tetrazole, **7**, since it showed a Gly-α-H_2 ^{1}H resonance at δ 5.47, and Gly-α-CH_2 and CN_4 ^{13}C resonances at δ 50.11 and δ 148.09, respectively. The more polar tetrazole, **8**, had a Gly-α-H_2 ^{1}H resonance at δ 5.53, and Gly-α-CH_2 and CN_4 ^{13}C resonances at δ 53.76, and δ 161.15, respectively. More evidence for the stereochemical assignment of the tetrazoyl regiomers, **7** and **8**, was obtained by a heteronuclear multiple bond correlation (HMBC) experiment. Compounds **7** and **8** show similar HMBC correlations, however, only the 1,5-disubstituted tetrazole, **7**, showed a correlation between the Gly-α-H_2 protons and the CN_4 carbon centre.

Single crystals of the 2,5-disubstituted tetrazole, **8**, were grown from methanol by slow evaporation. The structure of **8** was confirmed by an X-ray structure determination at 173(2) K and was satisfactorily refined (Fig. 1).[19] To our knowledge this is the first structural deter-

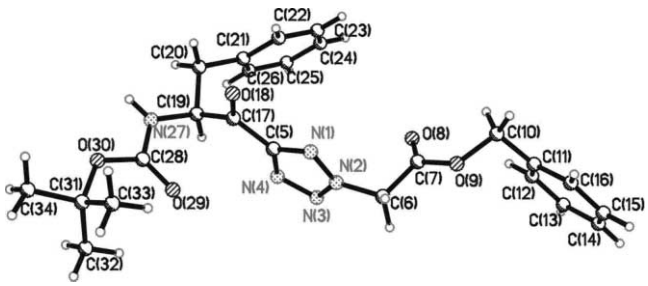
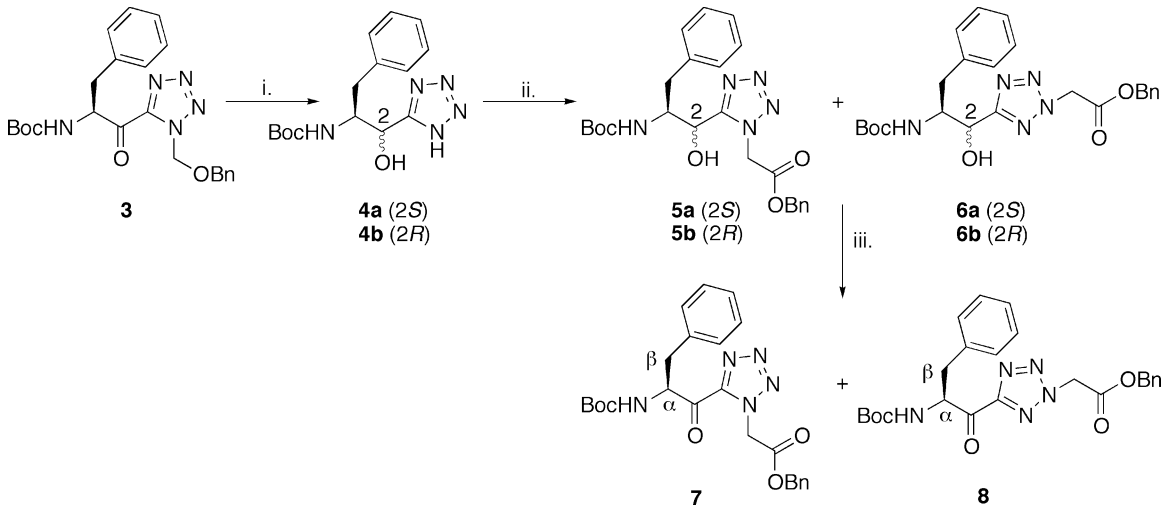


Figure 1. Crystal structure of the 2,5-disubstituted tetrazole, *N*-Boc-Phe-[$COCN_4$]-Gly-OBn, **8**.



Scheme 1. (i) H_2, 10% Pd/C, MeOH, rt, 24 h; (ii) DIPEA, $BrCH_2COOBn$, CH_2Cl_2, rt, 24 h; (iii) TEMPO, KBr, NaOCl, CH_2Cl_2/H_2O, 0°C, 30 min.

mination of an α-keto tetrazole-based peptidomimetic and is the principle supporting data for the assignment of the regiochemistry of the tetrazole products. In the structure of **8** it is apparent that the tetrazole ring is essentially planar with the ring torsion angles, C5–N1–N2–N3, N1–N2–N3–N4, N2–N3–N4–C5, N3–N4–C5–N1, N4–C5–N1–N2 being −0.3(4), 0.0(4), 0.3(4), −0.6(4) and 0.5(4)°, respectively. The tetrazole ring of **8** showed a mean deviation from the plane of 0.002 Å. The bond lengths of the tetrazole ring are all similar, with the C5–N1, N1–N2, N2–N3, N3–N4, N4–C5, being, 1.345(4), 1.325(4), 1.351(3), 1.327(3), and 1.352(4) Å, respectively. The α-keto carbonyl bond sits in the same plane as the tetrazole ring, with the O18–C17–C5–N4 and O18–C17–C5–N4 torsion angles being −178.1(3) and −0.1 (5)°, respectively. The core isostere [C17···C6] is essentially planar, with a mean deviation from the plane of 0.013 Å. The C17–C5 and N2–C6 bonds are offset by −10°. The phenylalanine ring is essentially planar and sits in a flagpole position over the tetrazole heterocycle.

In this letter we have presented a short route to some new conformationally restricted peptidomimetics, the design of which is based on novel α-keto 1,5- and 2,5-disubstituted tetrazole [$COCN_4$] amide bond isosteres. We have also unequivocally assigned structures to the resulting 1,5- and 2,5-disubstituted tetrazole isomers, a problem that is often encountered in tetrazole-based chemistry, using a combination of NMR and X-ray crystallography.

Acknowledgements

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13. **4a** and **4b**: ^{1}H NMR (300 MHz, CD_3OD, for the mixture) δ 1.23, 1.26 (9H, s, Boc-$(CH_3)_3$), 2.90 (2H, AB system, $\delta_A=2.76$, $\delta_B=3.03$, dd, $J=6.9$, 13.2 Hz, Phe-β-H_2), 4.07, 4.12 (1H, m, Phe-α-H), 5.01 (1H, d, $J=6.6$ Hz, NH), 5.08, 5.13 (1H, bs, Phe-α-H), 7.17–7.28 (5H, m, ArH). ^{13}C NMR (75 MHz, CD_3OD, for the mixture) δ 28.36, 28.82 (Boc-$(CH_3)_3$), 38.37, 39.52 (Phe-β-CH_2), 57.77, 59.42 (Phe-α-CH), 67.22, 69.01 (Phe-CHOH), 80.50, 80.55 (Boc-$C(CH_3)_3$), 127.46, 127.67, 129.43, 129.63, 130.50 (ArCH), 139.38, 139.39 (ArC), 157.51 (CN_4), 159.99, 160.02 (Boc-CO). ES MS 320.1721 $C_{15}H_{22}N_5O_3$ (MH^+) requires m/z 320.1723.
14. **5a**, **5b**, **6a**, and **6b**: A solution of 1*H*-tetrazole as a mixture of **4a** and **4b** (1.0 equiv., 110 mg, 0.35 mmol) in dry CH_2Cl_2 (5 mL) was prepared in a flame-dried flask under Ar. DIPEA (3.0 equiv., 130 mg, 1.04 mmol, 175 μL) was added dropwise to the stirred solution at rt. After 5 min benzyl bromoacetate (2.0 equiv., 160 mg, 0.70 mmol, 110 μL) was added dropwise and the reaction was stirred for 24 h. The reaction was diluted with ethyl acetate (20 mL), washed with 10% aqueous HCl (2×10 mL), 1 M aqueous NaOH (2×10 mL), saturated aqueous NaCl (10 mL), dried ($MgSO_4$), filtered and evaporated. The crude reaction product was purified by flash column chromatography (40% ethyl acetate/petroleum ether) to give α-hydroxy tetrazoles, **5a**, **5b**, **6a** and **6b** (127 mg, 77%), which were not separated. Selected ^{1}H NMR (300 MHz, $CDCl_3$, for the mixture) δ 1.32 (9H, bs, Boc-$(CH_3)_3$), 3.04 (2H, m, Phe-β-H_2), 4.20 (1H, m, Phe-α-H), 4.96 (1H, d, $J=6.6$ Hz, NH), 5.06 (1H, d, $J=3.0$ Hz, Phe-CHOH), 5.14 (1H, d, $J=5.1$ Hz, Phe-CHOH), 5.20 (2H, bs, Bn-H_2), 5.41 (2H, bs, Gly-α-H_2), 7.17–7.36 (10H, m, ArH). TLC (analytical, 40% ethyl acetate/petroleum ether) $R_f=0.17$.
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16. **7**: (41 mg, 30%), eluted first as a clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (9H, s, Boc-$(CH_3)_3$), 3.22 (2H, AB system, $\delta_A=3.07$, $\delta_B=3.37$, dd, $J=8.1$, 14.1 Hz, Phe-β-H_2), 5.12 (1H, d, $J=6.6$ Hz, NH), 5.21 (2H, d, $J=4.8$ Hz, Bn-H_2), 5.42 (1H, m, Phe-α-H), 5.47 (2H, d, $J=3.0$ Hz, Gly-α-H_2), 7.13–7.38 (10H, m, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.97 (Boc-$(CH_3)_3$), 37.02 (Phe-β-CH_2), 50.11 (Gly-α-CH_2), 59.48 (Phe-α-CH) 68.14 (Bn-CH_2), 80.25 (Boc-$C(CH_3)_3$), 124.03, 127.06, 128.38, 128.55, 128.68, 129.18 (ArCH), 134.14, 135.13 (ArC), 148.09

(CN_4), 154.00 (Boc-CO), 164.71 (Gly-CO), 189.05 (Phe-CO). TLC (analytical, 30% ethyl acetate/petroleum ether) $R_f = 0.39$; IR ($CDCl_3$, cm^{-1}), 3392, 3324, 3215, 1756, 1632, 1603, 1528, 1497, 1230; ES MS 466.2097 $C_{24}H_{28}N_5O_5$ (MH^+) requires m/z 466.2090. Further elution gave the 2,5-regioisomer, **8** (65 mg, 48%), as a white solid. Mp 120–121°C; $[\alpha]_D^{20}$ −18.3 ($c = 0.00010$, methanol). ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 (9H, s, Boc-$(CH_3)_3$), 3.19 (2H, AB system, $\delta_A = 3.06$, $\delta_B = 3.33$, dd, $J = 6.9$, 13.8 Hz, Phe-β-H_2), 5.24 (1H, d, $J = 6.4$ Hz, NH), 5.29 (2H, s, Bn-H_2), 5.53 (2H, s, Gly-α-H_2), 5.58 (1H, m, Phe-α-H), 7.05–7.34 (10H, m, ArH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.18 (Boc-$(CH_3)_3$), 37.93 (Phe-β-CH_2), 53.76 (Gly-α-CH_2), 58.70 (Phe-α-CH) 58.57 (Bn-CH_2), 80.11 (Boc-$C(CH_3)_3$), 127.05, 128.52, 128.60, 128.74, 128.95, 129.34 (ArCH), 134.01, 135.24 (ArC), 155.00 (Boc-CO), 161.15 (CN_4), 164.03 (Gly-CO), 189.32 (Phe-CO). TLC (analytical, 30% ethyl acetate/petroleum ether) $R_f = 0.29$; IR ($CDCl_3$, cm^{-1}), 3395, 3307, 3215, 1756, 1649, 1610, 1533, 1500; ES MS 466.2096 $C_{24}H_{28}N_5O_5$ (MH^+) requires m/z 466.2090.

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19. Crystallographic data for **8**: $C_{24}H_{27}N_5O_5$, M 465.51, mp 120–121°C, crystal dimensions 0.55×0.26×0.02 mm, orthorhombic, $a = 6.140$ (3), $b = 16.264$ (7), $c = 23.523$ (10) Å, $\alpha = 90$ (2), $\beta = 90$ (2), $\gamma = 90$ (2)°, $V = 2349.0$ (17) $Å^3$, space group $P2(1)2(1)2(1)$, $Z = 4$, $F(000) = 984$, $D_{calcd} = 1.316$ mg/m^3, absorption coefficient 0.096 mm^{-1}, θ range for data collection 2.14–26.45, index ranges $-7 \le h \le 7$, $-20 \le k \le 18$, $-29 \le l \le 29$, data/restraints/parameters 4837/0/307, goodness of fit on F^2 was 0.794, final R indices [$I > 2\sigma(I)$] $R_1 = 0.0448$, w$R_2 = 0.0714$, R indices (all data) $R_1 = 0.1525$, w$R_2 = 0.0904$, largest difference peak and hole 0.174 and −0.355 e $Å^{-3}$. A unique data set was measured at 173(2) K within $2\theta_{max} = 57°$ limit (ϖ scans). Of the 30663 reflections obtained, 4837 were unique ($R_{int} = 0.1593$) and were used in the full-matrix least-squares refinement. The structure was solved by direct methods. Hydrogen atoms were fixed in idealised positions. All non-hydrogen atoms were refined with anisotropic atomic displacement parameters. Neutral scattering factors and anomalous dispersion corrections for non-hydrogen atoms were taken from Ibers and Hamilton. Crystallographic data for the structure in this paper have been deposited with the Cambridge Crystallographic Data Centre; deposition number CCDC 157463.