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Synthesis and Hydrolysis of Dipeptide Isosteres containing a Proline Analogue with Latent Reactivity

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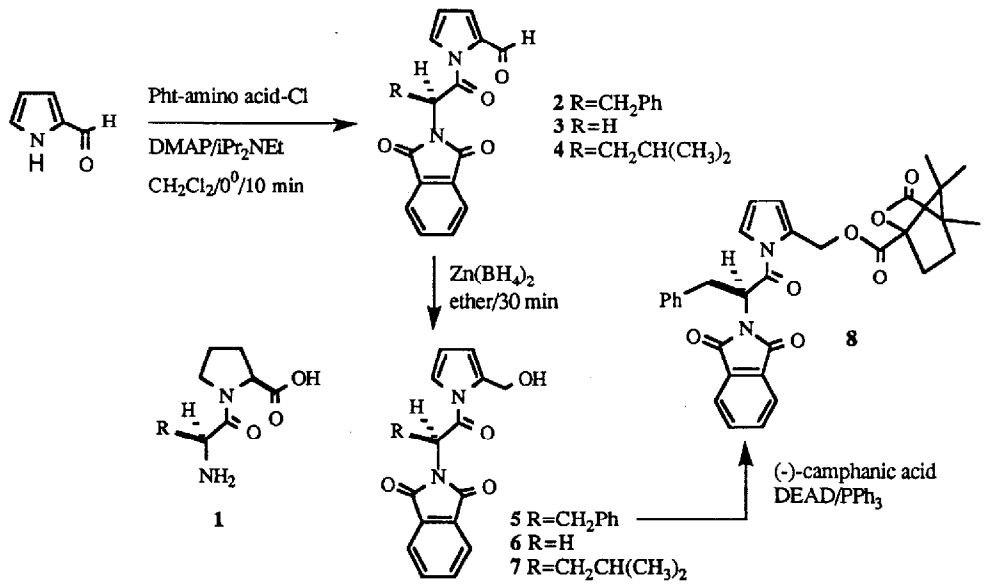
Key Words: Dipeptide isosteres; N-Acyl hydroxymethylpyrroles; Hydrolysis; Latent reactivity; Azafulvene

Abstract: *The N-acyl hydroxymethylpyrrole amino acid analogues 5-7 and 9 were synthesized via a mild DMAP catalysed acylation of pyrrole-2-carboxaldehyde with an acid chloride followed by formyl group reduciion with $Zn(BH_4)_2$. The N-acyl substituted hydroxymethylpyrroles are activated on hydrolysis to give an electrophilic species that can be trapped by an external nucleophile.*

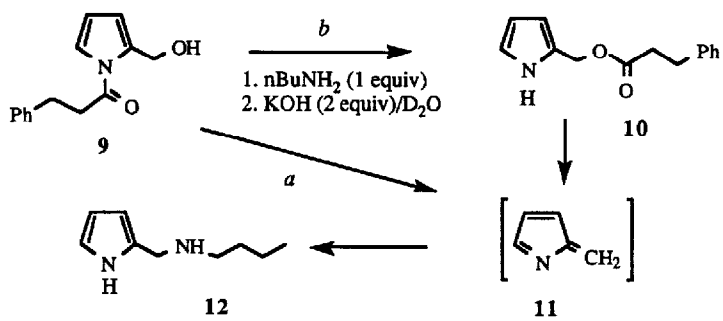
The design and synthesis of amino acid analogues with latent reactivity that is specifically released at the active site of a target protease, to give covalent inactivation, continues to attract considerable interest[1]. Our aim is to develop new classes of compounds possessing latent reactivity with an application to the inhibiton of proteases including the few that cleavage on the amino side of proline. These unusual enzymes include manganese-dependent prolidase[2] and a number of virally encoded endopeptidases that process the gene products gag and pol in HIV-1[3] and other retroviruses. In this paper we report initial studies on the synthesis of **5-7** and **9**, latent reactive amino acid analogues that also represent isosteres of the X-Pro dipeptide **1**. Analogue **9** is shown to be activated on hydrolysis to give an electrophilic species, probably the azafulvene **11**, that can be trapped by an external nucleophile. The activation proceeds in part via an initial *N*- to *O*-acyl transfer with probable release of the same azafulvene, **11**.

Methodology is given for the synthesis of **5** (Scheme 1). Pyrrole-2-carboxaldehyde was *N*-acylated with *N*-phthaloyl-L-phenylalanine acid chloride[4] in ice cooled CH_2Cl_2 containing 0.1 equivalents of DMAP and an equivalent of diisopropylethylamine to give **2**[5]. The common literature[6] preparation of *N*-acyl derivatives of pyrrole-2-carboxaldehyde via an initial acylation of potassium pyrrole followed by Vilsmeier-Haack formylation was unsuccessful. The *N*-phthaloyl protecting group proved to be the most efficient *N*-protection for the *N*-acylation reaction. Reduction of **2** with 1.1 equivalents of $Zn(BH_4)_2$ in ether gave **5**[7] (60%). An alternative reduction using $NaBH_4$ in CH_3OH/CH_2Cl_2 gave problems with competing acetal formation. The enantiomeric excess (95%) of **5** was determined by analysis of the ^1H NMR spectrum of the camphanate **8**[8], prepared from **5** using (-)-camphanic acid under Mitsunobu conditions[9]. D-Phenylalanine provided the isomeric camphanate as a reference. An analogous *N*-acylation of pyrrole-2-carboxaldehyde with the acid chlorides derived from hydrocinnamic acid and *N*-phthaloyl protected L-leucine and glycine followed by reduction with $Zn(BH_4)_2$ gave **9** (85%), **6** (35%) and **7** (60%), respectively.



Scheme 1

A hypothetical mechanism for the inactivation of a protease by the *N*-acylpyrroles **5-7** and **9** requires the release of latent reactivity on deacylation to give an electrophilic species that is subsequently trapped by a nucleophile. The release of latent reactivity from **9** is demonstrated by a simple hydrolysis study (Scheme 2) designed to mimic an enzyme catalysed cleavage[10]. An equivalent of $nBuNH_2$ (external nucleophile)



Scheme 2

and CH_2Cl_2 (internal standard) were added to a solution of **9** (10mg) in CD_3CN. Two equivalents of KOH (20µl, 4.4 M in D_2O) were added and ^1H NMR spectra were recorded over 90 min (Figure 1). The reaction course was followed by monitoring the integral of the pyrrole-CH_2-X resonance for starting material **9** (δ 4.62), a reaction intermediate identified as the *O*-acylpyrrole **10**[11] (δ 5.07) and the final substitution product, the aminomethylpyrrole **12**[12] (δ 3.62). The observed formation of the aminomethylpyrrole **12** from **9** occurs by

either of two pathways, hydrolysis, azafulvene formation and nucleophilic substitution (pathway *a*, Scheme 2), or indirectly via an initial rapid *N*- to *O*-acyl transfer (pathway *b*, Scheme 2).

The deacylation of **9**, either by the *N*- to *O*-acyl transfer, or a simple hydrolysis, increases electron density on the pyrrole nitrogen. This promotes the formation of the azafulvene **11** and thus activates the 2-pyrrylmethyl position to nucleophilic substitution. The general observation of enhanced reactivity of 2-pyrrylmethyl derivatives with nucleophiles has been explained by the ready formation of a resonance stabilized azafulvene, *eg* **11**, and although there is no direct evidence the reaction is considered to have a high S_N1 character[13]. Importantly, the hydroxymethylpyrrole **9** did not react with $nBuNH_2$ over 48 hrs in the absence of KOH. *N*-Acylation therefore inhibits azafulvene formation and as a consequence nucleophilic substitution. An electron withdrawing substituent on the pyrrole nitrogen of a hydroxymethylpyrrole has been shown to suppress azafulvene formation[14]. A more detailed analysis of the hydrolysis of N-acylpyrroles and work on extending the amino acid sequence of **5-7** in the N and C directions will be reported elsewhere.

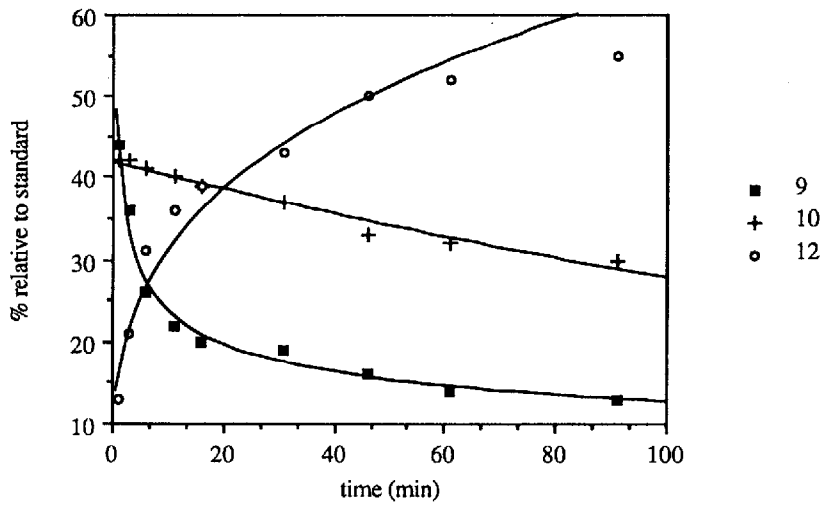


Figure 1. Hydrolysis of **9** with KOH in the presence of $nBuNH_2$

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4. Prepared from *N*-phthaloyl-L-phenylalanine with oxalyl chloride/DMF.
5. Data for **2**: $[\alpha]^{D}_{25}$ -210° (*c*1, MeOH). FTIR (film) 3167, 1781, 1716, 1660 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$) δ 3.53 (2H, A & B part ABX, CH_2); 5.77 (1H, X part ABX, *J*=9.2 & 6.4 Hz, -CH); 6.19 (1H, t, *J*=3.4 Hz, H4); 7.07 (1H, dd, *J*=3.4 & 2.3 Hz, H3); 7.08-7.11 (5H, m, Ph); 7.18 (1H, dd, *J*=3.4 & 2.3 Hz, H5); 7.63 (2H, dd, *J*=5.5 & 2.6 Hz, Ar-Pht); 7.69 (2H, dd, *J*=5.5 & 2.6 Hz, Ar-Pht); 10.10 (1H, s, CHO); ^{13}C NMR δ 34.9, 54.2, 113.3, 122.9, 123.8, 125.7, 127.2, 128.7, 129.2, 131.0,

134.5, 135.6, 166.9, 167.8, 181.6; HRMS (M) Calcd 372.11100, Found 372.11100; MS (EI) 372 (8%), 250 (72%), 225 (25%), 129 (16%), 95 (100%). The acid chloride derived from D-phenylalanine gave the formylpyrrole: $[\alpha]^{D}_{25}$ +196° (*c*1, MeOH).

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7. Purified by radial chromatography (EtOAc/ petroleum ether). Data for **5**: $[\alpha]^{D}_{25}$ -195° (*c*1, MeOH); FTIR (film) 3478, 1772, 1714, 1614, 1494, 1455 cm^{-1}; ^{1}H NMR (300MHz, $CDCl_3$) δ 3.52 (2H, d, *J*=7.8 Hz, CH_2-Ph); 4.59 (2H, bs, CH_2-OH); 5.59 (1H, t, *J*=7.8 Hz, CH); 6.05 (1H, t, *J*=3.4 Hz, H4); 6.14 (1H, dd, *J*=3.4 & 1.4 Hz, H3); 6.93 (1H, dd, *J*=3.4 & 1.5 Hz, H5); 7.09-7.19 (5H, m, Ph); 7.63 (2H, dd, *J*=5.6 & 3.0 Hz, Ar-Pht); 7.70 (2H, dd, *J*=5.6 & 3.0 Hz, Ar-Pht); ^{13}C NMR δ 35.0, 53.9, 57.6, 113.1, 114.9, 120.2, 123.8, 127.2, 128.7, 129.2, 131.1, 134.3, 134.5, 135.9, 167.0, 168.4; HRMS (M) Calcd: 374.12665, Found 374.12665; MS (EI) 374 (14%), 278 (11%), 250 (100%). The formylpyrrole derived from D-phenylalanine gave the corresponding hydroxymethylpyrrole: $[\alpha]^{D}_{25}$ +180° (*c*1, MeOH).
8. Data for **8**: $[\alpha]^{D}_{25}$ -90° (c1, MeOH); ^{1}H NMR (300MHz, $CDCl_3$) δ 0.89 (3H, s, camph-CH_3); 0.99 (3H, s, camph-CH_3); 1.04 (3H, s, camph-CH_3); 1.65 (1H, m, CH_2-camph); 1.95 (2H, m, CH_2-camph); 2.40 (1H, m, CH_2-camph); 3.50 (2H, m, CH_2-Ph); 5.38 (1H, dd, *J*=13.2 & 0.8 Hz, CH_2-O camph); 5.45 (1H, dd, *J*=13.2 & 0.9 Hz, CH_2-O camph); 5.57 (1H, dd, *J*=9.3 & 6.2 Hz, CH); 6.09 (1H, t, *J*=3.4 Hz, H4); 6.28 (1H, ddd, *J*=3.5 & 0.9 & 0.8 Hz, H3); 7.02 (1H, dd, *J*=3.5 & 2.0 Hz, H5); 7.20-7.40 (5H, m, Ph); 7.65-7.75 (4H, m, Ar-Pht). Data for D-phenylalanine derived camphanate: $[\alpha]^{D}_{25}$ +114° (*c*1, MeOH); ^{1}H NMR (300MHz, $CDCl_3$) δ 0.90 (3H, s, camph-CH_3); 0.97 (3H, s, camph-CH_3); 1.04 (3H, s, camph-CH_3); 1.65 (1H, m, CH_2-camph); 1.90 (2H, m, CH_2-camph); 2.40 (1H, m, CH_2-camph); 3.50 (2H, m, CH_2-Ph); 5.37 (1H, dd, *J*=13.2 & 0.8 Hz, CH_2-O-camph); 5.45 (1H, dd, *J*=13.2 & 0.9 Hz, CH_2-O-camph); 5.57 (1H, dd, *J*=9.3 & 6.2 Hz, CH); 6.09 (1H, t, *J*=3.4 Hz, H4); 6.28 (1H, ddd, *J*=3.5 & 0.9 & 0.8 Hz, H3); 7.02 (1H, dd, *J*=3.5 & 2.0 Hz, H5); 7.20-7.40 (5H, m, Ph); 7.65-7.75 (4H, m, Ar-Pht).
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11. Data for **10**: ^{1}H NMR (300MHz, CD_3CN) δ 2.66 (2H, t, *J* =7.9 Hz, CH_2-Ph); 2.95 (2H, t, *J*=7.5 Hz, CH_2-CO); 5.07 (2H, s, pyrrole-CH_2); 6.10 (1H, t, *J*=2.2 Hz, H4); 6.18 (1H, dd, *J*=2.5 & 1.5 Hz, H3); 6.80 (1H, dd, *J*=2.3 & 1.5 Hz, H5) ^{13}C NMR (CD_3CN) δ 29.7, 34.7, 58.0, 106.9, 108.9, 118.2, 125.2, 125.4, 127.6, 127.7, 140.1, 172.3.
12. Data for **12**: ^{1}H NMR (300MHz, CD_3CN) δ 0.87 (3H, t, *J*=7.3 Hz, CH_3); 1.26-1.42 (4H, m, CH_2-CH_2); 2.90 (2H, bt, *J*=7.4 Hz, NH-CH_2); 3.62 (2H, s, pyrrole-CH_2); 6.03 (1H, m, H3); 6.06 (1H, t, *J*=2.9 Hz, H4); 6.76 (1H, m, H5); ^{13}C NMR (CD_3CN) δ 13.6, 20.5, 35.8, 50.5, 59.1, 109.8, 119.2, 121.8, 125.9; HRMS (M) Calcd 152.13134, Found 152.13134; MS (EI) 152 (13%), 150 (29%), 104 (30%), 91 (74%) 80 (100%).
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