First synthesis of cyclotrihydrazino peptides

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Synthesis of novel cyclotrihydrazino peptides using an original cyclisation method at high dilution.

A growing number of peptides are regularly isolated from animal and human tissues. Most exhibit interesting biological effects as hormones or neuromediators. From this point of view they appear to possess high potential as therapeutic agents. However, among other reasons, their low bioavailability represents a strong limitation on their use in this field. To solve this problem peptide analogues have been synthesised and screened to produce active peptidomimetic compounds with enhanced enzyme resistance.[1] Among them, pseudo-peptides, in which the peptidic linkage is modified, are the object of great interest and have already lead to some concrete applications. For example 'Zoladex' is a powerful anticancer drug in the azapeptide class.[2]

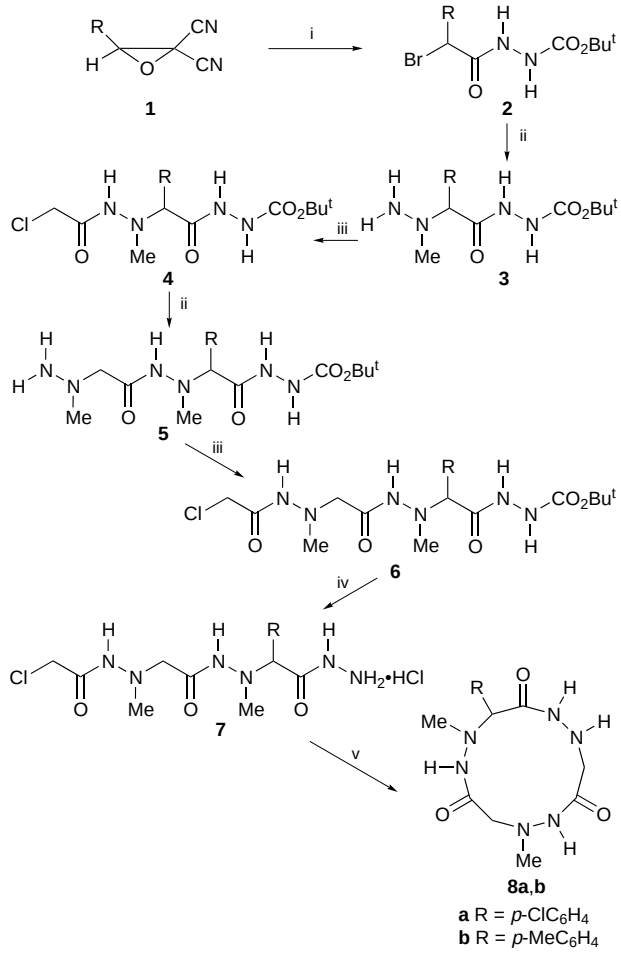
Peptidase action is also generally lower on cyclic peptides and some, like Bottromycin, have found good use as antibiotics.[3] However, obtaining cyclic derivatives remains a problematic aspect of peptidic synthesis; yields are generally low and various factors influence the success or failure of cyclisation, including the nature and the connections of amino acid residues, solvent effects, concentration and also the type of reaction involved to induce ring closure.[4,5]

Here we report preliminary results of the synthesis of, to the best of our knowledge, the first cyclohydrazino peptides. The linear precursors were prepared using improvements to a methodology that we have already described in a previous paper.[6] To achieve the ring closure we perfected an original strategy which supplements classical methods of peptide cyclisation such as azide activation,[7] activated esters[8] or coupling reagents.[9] Scheme 1 summarizes the synthesis. Reacting *gem*-dicyano epoxides[10] **1** with $Bu^tCO_2NHNH_2{\cdot}HBr$ leads to α-halo hydrazides **2**.[11] Substitution of the halogen by methylhydrazine gives **3** which is then acylated by chloroacetyl chloride to afford **4**. Reiteration of these two steps gives **6** which includes a terminal azaglycine ester linked to an aryl hydrazinosarcosine, itself connected to an *N*-chloroacylated hydrazinosarcosine.

We took advantage of the high lability of the halogen atom in α-halo hydrazides. Indeed, we have shown that such halogens are easily displaced by nucleophiles when the reaction takes place in the presence of an additional base.[12] After elimination of the Boc function we get the polyhalo compound **7** (with undetermined HCl stoichiometry); this was then treated in high dilution in MeCN with pyridine (5 equiv.). Cyclotrihydrazino peptide **8** precipitated slowly as an amorphous powder (20% yield after purification). Compound **8** was characterized by ^1H and ^{13}C NMR spectroscopy, and its monomeric structure was established by FAB mass spectroscopy (formation of the cyclodimer had to be ruled out). Microanalysis confirmed the high purity of the compound.† At the time we undertook this work, Gani used a similar method to synthesise 1,2,5-triazepine-3,6-diones.[13]

It has been shown by X-ray and model studies that hydrazino acid residues can stabilize the folded conformation of small pseudo-peptidic patterns by hydrogen bonding.[14] The success of our synthesis of pseudo-peptide **8** may arise from such a favourable conformation bringing nearer the two reactive ends (Fig. 1).

It is worth noting that despite the high solubility of **7** and the insolubility of **8** in water, this solvent failed to allow cyclisation, possibly because competitive hydrogen bonding with water



Scheme 1 *Reagents and conditions* (for R = *p*-ClC_6H_4): i, $Bu^tCO_2NHNH_2{\cdot}HBr$, MeCN, room temp., 8 h, 99%; ii, NH_2NHMe, MeCN, 0 °C, 2 h, 93%; iii, $ClCH_2COCl$, pyridine, CH_2Cl_2, 0 °C, 84%; iv, HCl (g), CH_2Cl_2, 5 min., room temp., 99%; v, pyridine, MeCN, room temp. 2 d, 20%

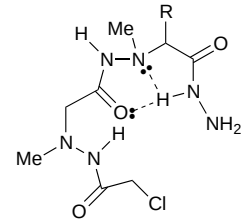


Fig. 1

destroys the favourable conformation for closure. This result emphasizes the crucial role played by the solvent in determining the precursor's geometry.

This preliminary work opens the way to the preparation of other original cyclopseudo-peptides of smaller or larger size, with different arrangements and various side chains. We also plan to investigate solvent effects in more detail; in particular we will examine the influence of adding ions to the reactions carried out in water.

Footnotes

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† *Selected data* for **8a**: 20%; mp > 260 °C; ^{1}H NMR (200 MHz, D_2O–CF_3CO_2H) 2.24 (s, 3 H), 2.58 (s, 3 H), 3.27 (m, 2 H), 3.36 (m, 2 H), 4.24 (s, 1 H), 7.37 (m, 4 H); ^{13}C NMR (200 MHz, D_2O–[$^2H_6$]DMSO) 43 (q, *J* 142 Hz), 44 (q, *J* 142 Hz), 53 (t, *J* 147 Hz), 59 (t, *J* 150 Hz), 70 (d, *J* 142 Hz), 128.5 (d, *J* 168 Hz), 129 (d, *J* 168 Hz), 132, 135, 166, 168, 172; ν_{max} (Nujol)/cm^{-1} 3290, 3240 br (NH), 1700, 1650, 1620 br (CO); HRMS (FAB) (MH^+) Calc. for $C_{14}H_{20}N_6O_3Cl$: 355.1284. Found: 355.1263. Calc.: C, 47.39; H, 5.40; N, 23.69; Cl, 9.90. Found: C, 47.45; H, 5.36; N, 23.66; Cl, 10.00%.

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