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Self-assembling Urea-based Peptidomimetics: A Simple One-step Synthesis and Crystal Structure of Core β-Alanyl Ureylene Retro-bispeptides (MeO-A_{aa}-[NH-CO-NH]-CH_2-CH_2-CO-NH-A_{aa}-OMe; A_{aa} = amino acid A)

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Abstract: A novel family of urea-based peptidomimetics containing β-alanyl unit at the core has been synthesized by a simple one-step procedure. Solid state structures of some representative examples have shown self-assembly into highly ordered, extended hydrogen-bonded chains and ribbons with modest NLO activity. © 1997 Elsevier Science Ltd.

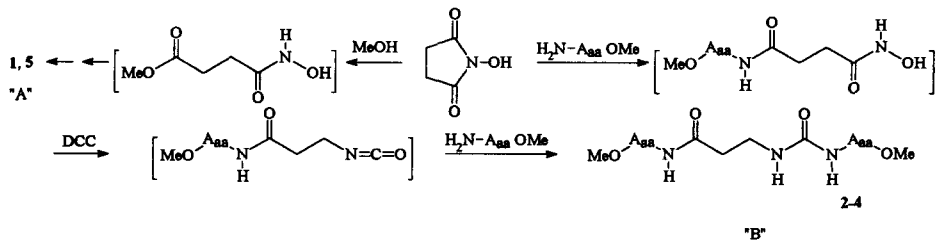
Modification of peptides by introducing conformational constraints in the backbone is an area of intense current interest.[1] Research studies with constrained peptides have assumed a new dimension in recent years with an ever increasing number of reports describing preformed peptides, pseudopeptides and non-peptide chains to mimic or induce particular conformational features.[2] These modifications provide appropriately altered peptide substrates with subtle and necessary conformational changes to fine-tune the binding properties of natural peptide analogs and thus, may have direct relevance in drug design.[3] In addition, the backbone modifications can also be used to improve enzymatic stability and to modulate solubility and *in vivo* transport properties of peptide analogs.[4]

In recent years ureido (-NH-CO-NH-) unit has gained considerable importance[5] in the design of enzyme inhibitors,[6] as a switching point in crafting retro-inverso peptidomimetics[7] and as a self-complementary bi-directional hydrogen bonding motif in supramolecular chemistry.[8] In this communication we report a one-step synthesis, characterization and self-assembling properties of a novel family of ureylene retro-bispeptides discovered in a fortuitous manner during our endeavours related to the design of glutamic acid-based peptide dendrimers.[9]

Thus, when N-protected glutamic acid was treated with excess of Glu-diOMe in the presence of N-hydroxy succinimide and DCC in dry CH_2Cl_2, two side products A and B were obtained. Spectral data established that A and B contained elements of Glu-diOMe and β-alanine in their structural framework. A series of control experiments traced the origin of β-alanine from N-OH succinimide and further showed that

while product A arose from an intermediate on the way to product B when methanol was present as a trace impurity, the product B was consistently obtained as the main product of the reaction. Thus, this observation led to a single-step, very simple and attractive route to β-alanine-containing ureylene retro-bispeptides, in modest yields.

In a typical procedure,[10] reaction of H_2N-A_{aa}-OMe (A_{aa} = amino acid A) with N-OH succinimide in the presence of DCC[11] in CH_2Cl_2 solvent under standard peptide formation conditions gave 25-30% (isolated) yields[12] of MeO-A_{aa}-[NH-CO-NH]-CH_2-CH_2-CO-NH-A_{aa}-OMe peptide. Scheme 1 presents a plausible route for the formation of ureylene peptides. Control experiments have shown that methanol when present in the reaction mixture competes with the amino acid free base leading to minor amounts of product A. Based on spectral and analytical data, structures 1 and 4 were assigned for side products A and B. Using the present procedure, a variety of ureylene peptides were synthesized (1-5, Scheme 1) and fully characterized.[13]



Entry	Core modified peptide [yield [a], %; mp, °C; $[\alpha]_D^{25}$, deg (c, solvent)]
1.	MeO_2C-CH_2-CH_2-CH (CO_2Me)-[NH-CO-NH]-CH_2-CH_2-CO_2Me (1) [~ 10; 99-100°C; +15.74 (2.56, $CHCl_3$)]
2.	MeO_2C-C(Me_2)-[NH-CO-NH]-CH_2-CH_2-CO-NH-C(Me_2)-CO_2Me (2) [30; 149-150°C]
3.	MeO_2C-CH-(CH_2Ph)-[NH-CO-NH]-CH_2-CH_2-CO-NH-CH-(CH_2Ph)-CO_2Me (3) [30; 159-160°C]
4.	MeO_2C-CH_2-CH_2-CH (CO_2Me)-[NH-CO-NH]-CH_2-CH_2-CO-NH-CH (CO_2Me)-CH_2- CH_2-CO_2Me (4) [25; 95-98 °C; +35.29 (2.963, $CHCl_3$)]
5.	MeO-Pro-CO-NH-CH_2-CH_2-CO_2Me (5) [72, syrup]

Scheme 1: Synthesis of Ureylene peptides

The ureylene peptides show strong tendency to self-assemble into highly organized hydrogen bonded α-networks of chains and sheets as demonstrated by the crystal structure of 1 which shows an extended backbone with central urea group engaged in continuous hydrogen bonds [N(1)....O(0) 2.982, H....O 2.27 A°; N1(s)....O(0) 2.889, H....O 2.06] (Figure 1a). The control by the ureido moiety to form an infinite self-assembly is further illustrated in the crystal structure of a larger ureylene peptide (2) crafted from one β-alanine and two Aib residues. The ureylene tripeptide 2 self-assembles (Figure 1b) to form continuous ribbons characterized by antiparallel stacking of tripeptide molecules with urea NH groups engaged in complementary hydrogen bonding [N(0)....O(0s) 3.097, H....O 2.29; N(1)....O(0s) 2.944, H....O 2.14; N(1s)....O(0) 2.909, H....O 2.10] on the same face. The augmentation of urea hydrogen bonding by amide bonds to generate sheet like structures may have significance in protein folding and in designing

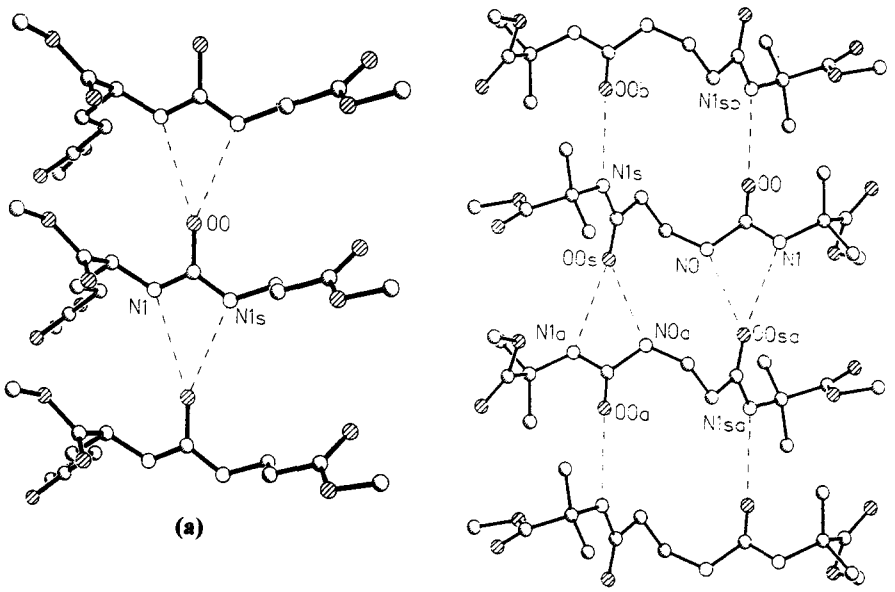


Figure 1

peptide-based materials. The ureylene peptides **1**, **3** and **4** show modest (~18% vs. urea) amount of non-linear optical activity.[14]

The simple one-step synthesis of ureylene peptides described here opens an avenue for identification of several other urea-based peptidomimetics with interesting self-assembling properties and potentially useful pharmaceutical properties. Facets of these are currently receiving our attention.

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10. To a well-stirred and ice-cooled mixture of dicyclohexylcarbodiimide and N-hydroxysuccinimide (1 mmol each) in dry CH_2Cl_2 (~20 ml), was added A_{aa}-OMe (free base generated in situ from 4 mmol each of methyl ester hydrochloride and triethylamine in dry CH_2Cl_2) and the reaction mixture left stirred at room temperature for 48 h. After normal peptide work up, the residue obtained was either directly crystallized from ethyl acetate/ hexane or purified through a small column of silica gel using ethyl acetate/ hexane as solvents to afford ureylene peptides in ~ 25-30 % yields.

11. DCC is also known to mediate Lossen rearrangement of hydroxamic acids under neutral conditions (Hoare, D. G.; Olson, A.; Koshland, Jr., D. E. *J. Am Chem. Soc.* **1968**, *90*, 1638; Bergman, J.; Lindstrom, J.-O.; Abrahamsson, J.; Hadler, E. *Tetrahedron Lett.* **1976**, 3615.

12. The modest yield may be attributed to the formation of some intractable polymeric material.

13. Selected spectral data for **1-5**.
1: IR (KBr) 3340, 2960, 1759, 1734, 1638, 1595, 1526, 1458, 1439, 1391 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ 1.66 (1H, m), 1.87 (1H, m), 2.14 (2H, m), 2.24 (2H, m), 3.15 (2H, m), 3.39, 3.41, 3.45 (3H, 3H, 3H, s, s, s), 4.17 (1H, m), 5.98 (2H, brs); ^{13}C NMR ($CDCl_3$) δ 27.88, 30.11, 34.49, 35.74, 51.77, 52.41, 157.31, 173.37, 173.51; FAB-MS m/z: 305 (100%) $(MH)^+$; **2:** ^1H NMR (90 MHz, $CDCl_3$) δ 1.44 (6H, s), 2.37 (2H, t, J = 5 Hz), 3.4 (2H, m), 3.71 (6H, s), 5.62 (2H, m), 7.2 (1H, s); FAB-MS m/z: 332 (100%) $(MH)^+$; **3:** IR (KBr) 3398, 3326, 3071, 3037, 2956, 1750, 1727, 1653, 1550, 1504 (sh), 1442 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 2.26 (2H, m), 2.55-3.13 (6H, m), 3.61, 3.62 (3H, 3H, s, s), 4.73 (2H, m), 5.42 (2H, m), 6.72 (1H, d, J =7.5 Hz),7.2 (10H, brs); FAB-MS m/z: 456 (100%) $(MH)^+$; **4:** IR (KBr) 3361, 3308, 2963, 1752 (br), 1651, 1567, 1544, 1443, 1385 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 1.90 (2H, m), 2.10 (2H, m), 2.36 (6H, m), 3.33 (1H, m), 3.45 (1H, m), 3.60, 3.61, 3.65, 3.68 (3H, 3H, 3H, 3H, s, s, s, s), 4.38 (1H, m), 4.51 (1H, m), 5.74 (2H, brs), 7.12 (1H, d, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 26.43, 27.17, 30.22, 30.26, 36.72, 37.29, 51.81, 51.90, 51.96, 52.49, 52.78, 52.82, 158.72, 172.88, 173.16, 173.24, 173.39, 174.05; FAB-MS m/z: 448 (100%) $(MH)^+$; **5:** ^1H NMR (90 MHz, $CDCl_3$) δ 2.04 (4H, m), 2.55 (2H, t, J=7.5 Hz), 3.44 (4H, m), 3.75, 3.79 (3H, 3H, s, s), 4.43 (1H, t), 5.41 (1H, t); FAB-MS m/z: 259 (100%) $(MH)^+$.

14. NLO activity was measured (powder method) by detection with a photomultiplier of 532 nm light generated from powder materials. Samples were held between two glass plates and set in an optical sphere. They were irradiated with a pulsed Nd: YAG laser at 1064 nm (11ns, 400 mJ/ pulse).

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