

Design and synthesis of self-assembling peptides

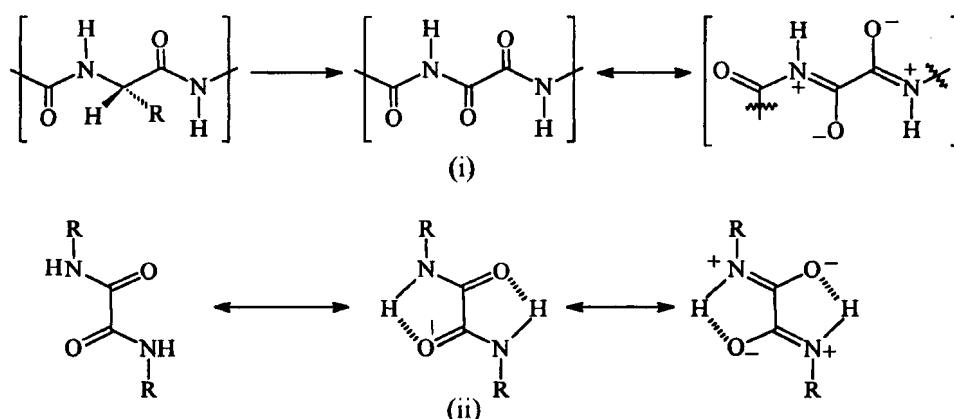
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ABSTRACT: Bis-acyl $[\text{CO}-(\text{CH}_2)_n\text{-CO}; n = 0, 1, 2, \dots, 8]$ units have been identified as suitable molecular inserts which when placed at the core of symmetric retro or normal peptides show a strong tendency to generate highly regular and extensively hydrogen-bonded supramolecular assemblies of relevance in protein design and in developing novel peptide based materials

In recent years, several, suitably crafted aromatic molecules have been used to create exotic self-assemblies¹ which may have relevance in understanding molecular recognition processes in biological chemistry or in material science. To our surprise, although peptides should represent an ideal choice for the study of self-assemblies because of their involvement in information transfer systems, these have been rarely used for this purpose². The flexible nature of linear peptides and the possibility of a multitude of conformations poses one of the major obstacles in their use as self-assembling systems.

Our recent demonstration^{3,4} of the transformation of the α -carbon of serine and threonine residues to a carbonyl (CO) function in assembled peptides, resulting in the generation of an extended, planar, bispeptide ($-\text{CONH-CO-CO-NH}-$) segment (i) in peptide backbone, coupled, with the finding from the X-ray crystallographic studies⁵ of a range of core oxalo retro-bispeptides that the core oxalamido ($-\text{NHCOCONH}-$) unit is locked in a consecutive C_5 type of intramolecular hydrogen bonding creating a hydrogen bonded bicyclo [3.3.0] octane motif (ii), suggested, that this may be exploited for evolving a design strategy for the control of molecular orientation in linear peptides during hydrogen bond mediated association with the ultimate aim of assembling peptides in a predictable manner.



Promise of this approach is illustrated here with self-assemblies of peptides containing core oxalamido ($-\text{NHCOCONH}-$), CO extended oxalamido ($-\text{CONHCO-CO-NH}-$) and polymethylene bridged ($-\text{NHCO}-(\text{CH}_2)_n\text{-CONH}-$) oxalamido units in the backbone.

The self-assembly patterns observed in a variety of core oxalo retropeptides clearly show the role of core $-\text{NHCOCONH}-$ unit in generating highly regular yet side-chain dependent hydrogen-bonded network.⁶

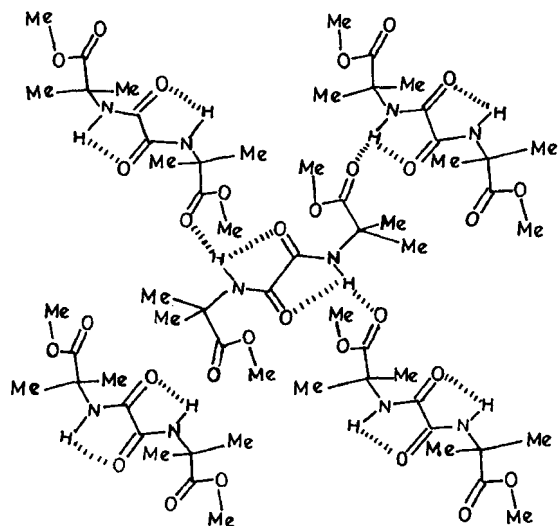


Fig. 1. Self-assembly of **1**. Each molecule is hydrogen bonded to four neighbours forming a pentameric motif.

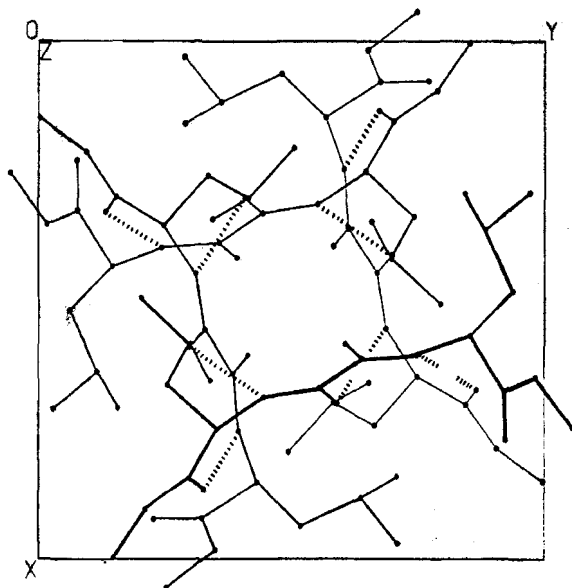
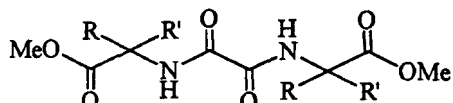


Fig. 2. Four fold helix formed by four molecules of **2**. View is down the c axis.

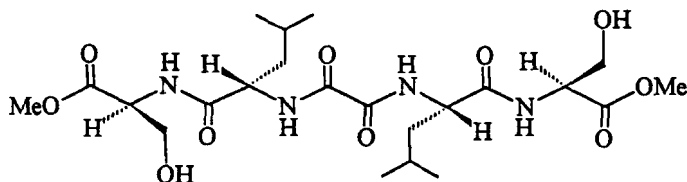
This is reflected in MeO-Aib-COCO-Aib-OMe (**1**)⁷ by a regular array of $\text{NH}\cdots\text{O}=\text{C}$ hydrogen bonds with proximally aligned ester carbonyls leading to an extended hydrogen-bonded sheet (Fig. 1); in MeO-Leu-COCO-Leu-OMe (**2**) where each half of the molecule forms an antiparallel β -sheet assembly with separate adjacent molecules which in turn form an extended helix with four-fold screw repetition (Fig. 2); and in chain extended MeO-Ser-Leu-COCO-Leu-Ser-OMe (**3**) molecules, which assemble into extended ribbons by laterally displaced half-units held together by a β -sheet type hydrogen bonds. The outer surface of the helix in (**2**) is totally hydrophobic.

It was interesting to note that the bicyclo [3.3.0] octane motif [arising from the consecutive φ C_5 type hydrogen bonding of perfectly trans -NHCOC(=O)NH- unit] is retained in the self-assemblies of peptides (**1**)-(3).



1: MeO-Aib-COCO-Aib-OMe; $\text{R} = \text{R}' = \text{Me}$

2: MeO-Leu-COCO-Leu-OMe; $\text{R} = \text{H}$; $\text{R}' = \text{CH}_2\text{CHMe}_2$



3: MeO-Ser-Leu-COCO-Leu-Ser-OMe

The notion that the pentameric motif observed in the self-assembly of **1** is a characteristic motif of core oxalo unit in Aib containing peptides was strongly supported by its recurrence in the self-assemblies of HO-Aib-COCO-Aib-OH⁸ and its copper complex⁹.

It was interesting to find that the backbone modified tripeptide Bz-Aib-NHCOCO-Aib-OMe (**4**) containing the carbonyl extended oxalamido unit (-CO-NHCOC(=O)NH-), arising from the C^α oxidation of Bz-Aib-Ser-Aib-OMe⁴, retains the consecutive pseudo C_5 intramolecular hydrogen bonding typical of core oxalo retrobispeptides (*vide supra*). Tripeptide (**4**) self-assembles (Fig. 3) in the solid state into highly ordered two dimensional array through MeOH mediated intermolecular stacking of dimeric 'disk' modules formed by an antiparallel β -sheet type arrangement of tripeptide molecules¹⁰. This is one of the rare, solvent mediated, organization of secondary structural elements.

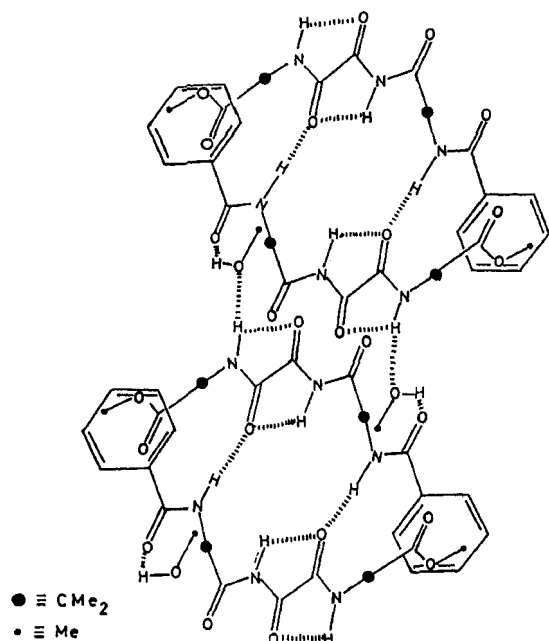


Fig.3. Supramolecular assembly (in4) of disk modules bridged by pairs of intervening CH_2OH molecules

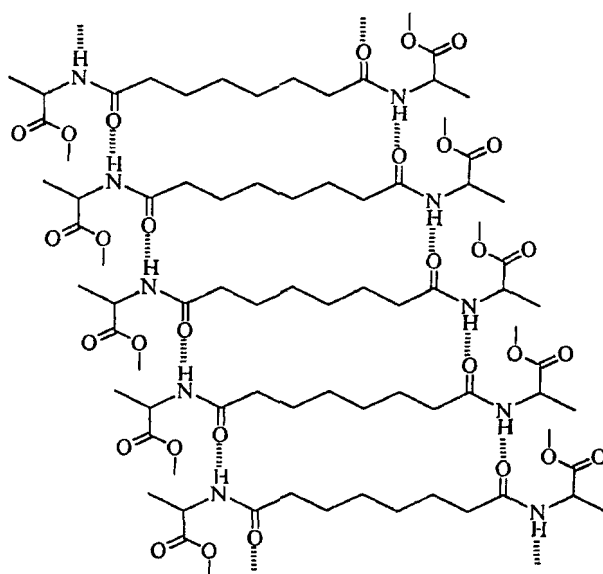
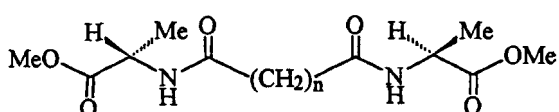


Fig.4. Self-assembly of 5e (continuous β -sheet)

To further illustrate the role of hydrogen-bonded bicyclo [3.3.0] octane motif in directing the self-assemblies of core oxalo-peptides, the φ C_5 hydrogen-bonding was disrupted by introducing polymethylene bridges between $-\text{COCO}-$ unit. Thus, a host of bisacyl Ala retropeptides 5a-f ($n = 0, 2, 3, 4, 6, 8$) were synthesized. Both, adipoyl and suberoyl linked molecules show an almost identical and completely extended conformation with Ala side chains symmetrically disposed at the ends. Figure 4 shows the hydrogen bonding pattern of suberoyl linked analogue in crystal lattice. Both adipoyl and suberoyl analogues show a highly ordered β -sheet type secondary structure formed by stacking of multiple parallel strands, linked by pairs of $\text{NH}\cdots\text{O}=\text{C}$ intermolecular hydrogen bonds.¹¹



- 5 a: $n = 0$, MeO-Ala-Oxalyl-Ala-OMe;
 b: $n = 2$, MeO-Ala-Succinoyl-Ala-OMe;
 c: $n = 3$, MeO-Ala-Glutaroyl-Ala-OMe;
 d: $n = 4$, MeO-Ala-Adipoyl-Ala-OMe;
 e: $n = 6$, MeO-Ala-Suberoyl-Ala-OMe;
 f: $n = 8$, MeO-Ala-Sebacoyl-Ala-OMe.

Thus, in summary, the present work has shown that suitably crafted, conformationally rigid, molecular inserts at the core of peptide backbone can be utilized to control the orientation of peptide molecules in the solid state, to create highly organized self-assemblies.

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