A Trispeptide Circularly Organized through Inter-chain Hydrogen Bonds

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Three lipophilic amino acids attached to a C_3-symmetric base are shown by n.m.r. and i.r. spectroscopy and force-field calculations to exhibit a cage-like structure stabilized by inter-chain hydrogen bonds.

There is a growing interest in cage-like structures for the design of selective ion carriers, receptor sites, and enzyme models.[1] In this communication we report a representative of a new family of tripod molecules, composed of three lipophilic chains of amino acid residues attached to a common anchor. Hydrogen bonds (H-bonds) between non-identical amides of adjacent chains create a chiral organized structure.

The compound, trispeptide (**1**), was prepared by coupling reactions between tris-1,3,5-(aminomethyl)benzene and protected L-leucine, using the active ester method commonly employed in peptide synthesis.† The monopeptide analogue (**2**) was also prepared for comparative studies.

The conformational properties of trispeptide (**1**) are derived from the combined results of ^{1}H-n.m.r. and i.r. spectroscopy and empirical force field (EFF) calculations.

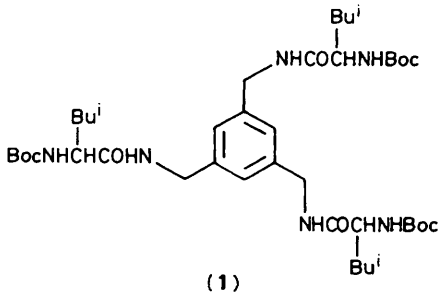
The ^{1}H-n.m.r. spectrum of trispeptide (**1**) (Table 1) shows a single set of signals in both polar and apolar solvents, indicating C_3 symmetric or rapidly interconverting conformations. The diastereotopic benzylic methylene protons are observed by ^{1}H n.m.r. spectroscopy to be non-equivalent in $CDCl_3$ solution. They exhibit a widely split AB pattern and different coupling constants with neighbouring NH protons, (J_{NHCH} 5.5, < 1.0 Hz), which indicate conformational constraints. Although the pattern shifts somewhat with concentration, it is retained down to 5×10^{-4} M, and is therefore not due to aggregation. It collapses, however, in polar solvents. In contrast, the benzylic protons of the monopeptide analogue (**2**) give rise to a single signal even in apolar solvents. The chemical shift of the $C_\alpha H$ proton of the monopeptide (**2**) differs by 0.3 p.p.m. from that of the trispeptide (**1**) in $CDCl_3$, while in polar solvents the difference is only 0.03 p.p.m. All these indicate that the conformational constraints are, at least in part, due to polar interactions between the chains.

† *N*-Boc-L-leucine *N*-hydroxysuccinimide active ester was treated with tris-1,3,5-(aminomethyl)benzene to afford (**1**) in 58% isolated yield. M.p. 230 °C (decomp.), $[\alpha]_D^{32}$ + 24° (*c* 0.7, $CHCl_3$). The mono derivative (**2**) was obtained in 93% isolated yield by replacing the triamine by benzylamine. M.p. 76—79 °C, $[\alpha]_D^{32}$ −28° (*c* 0.6, $CHCl_3$).

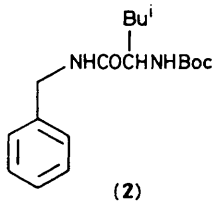
Table 1. ^{1}H N.m.r. chemical shift values for trispeptide (**1**) and monopeptide (**2**) in various solvents.[a]

	Trispeptide (**1**)			Monopeptide (**2**)		
Proton	$CDCl_3$	CD_3OD	[$^2H_6$]-DMSO	$CDCl_3$	CD_3OD	[$^2H_6$]-DMSO
CH_2NH	4.32[b] (4.24)[c]	4.34	4.20	4.43	4.37	4.26
	4.00[b] (3.97)					
$C_\alpha H$	4.43[b] (4.46)	4.12	3.99	4.12	4.09	3.97
$PhCH_2NH$	7.64 (7.85)		8.25	6.41		8.31
BocNH	5.43 (5.58)		6.84	4.82		6.89

[a] N.m.r. spectra were recorded at 270 MHz, 5×10^{-3} M, 298 K, unless otherwise stated. [b] 293 K. [c] Values in parentheses are for a 2×10^{-2} M solution.



(1)



(2)

Boc = t-butoxycarbonyl

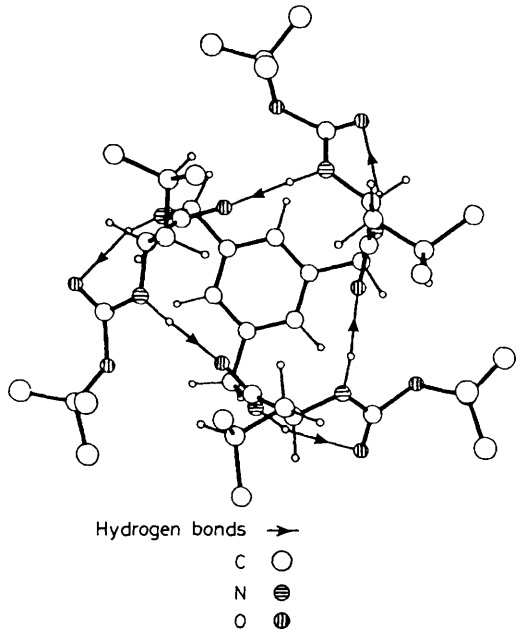


Figure 1. The calculated lowest energy conformation of trispeptide (**1**).

The i.r. spectrum shows two stretching frequencies for the NH bonds of the trispeptide (**1**) in dilute $CHCl_3$ solutions (5×10^{-4} M), corresponding to two types of NH bonds: one, at 3436 cm^{-1}, indicates a free NH, the other, at 3354 cm^{-1}, indicates a H-bonded NH. The monopeptide analogue (**2**) shows a strong band at 3437 cm^{-1} with only a tiny shoulder at 3350 cm^{-1}, indicating essentially free NH. This suggests that the H-bond in the trispeptide (**1**) is between the chains. The n.m.r. temperature coefficients $d\delta/dT$ of the chemical shifts of the benzylic and Boc amide protons of trispeptide (**1**) (measured in $CDCl_3$ at 5×10^{-3} M) are significantly larger (−0.0170 and −0.0086 p.p.m. K^{-1}, respectively) than those of the monopeptide (**2**) (−0.0026 and −0.0023 p.p.m. K^{-1}, respectively) indicating thermal lability of the H-bonds in (**1**).[2]

The NH–ND exchange rate of the Boc NH protons in $CDCl_3$ solution of (**1**) is half that of the benzyl NH proton. Small increments of [$^2H_6$]dimethyl sulphoxide (DMSO) cause a smaller shift of the Boc NH than of the benzyl NH signal. These data suggest that inter-chain H-bonds are formed, with the Boc NH as a donor. The acceptor must be the benzylamide carbonyl since H-bonds among amides of the same kind in tripod molecules are geometrically unfavoured.[3]

Empirical force field (EFF) calculations[3,4] suggest the preferred equilibrium conformation of trispeptide (**1**) shown in Figure 1. Its energy is lower by *ca.* 4 kcal mol^{-1} (1 kcal = 4.184 kJ) than that of the next local minimum, and by more than 5 kcal mol^{-1} than all other local minima. It contains the inter-chain H-bonds observed in chloroform. It also contains intra-chain H-bonds that were not observed. These H-bonds are distorted according to the EEF calculation (NH ··· O 151°, H ··· O=C 98°) and therefore weaker. Since our EFF calculation ignores solvent effects and since polar solvents reduce the strength of H-bonds, prediction of such H-bonds might be confirmed in purely apolar solvents.[4b] Indeed, the stretching frequencies of the two types of NH bonds in a CCl_4 solution (*ca.* 1×10^{-4} M) are 3351 and 3288 cm^{-1}, indicating two H-bonds of different strength, as predicted by the EFF calculation.

When forming inter-chain H-bonds in trispeptide (**1**), NH may bond to O=C on either the right or the left. These alternatives are diastereoisomeric, because the chains are chiral. The calculations predict that the most stable conformation is counter-clockwise, as seen in Figure 1, while the next most stable conformation, 4 kcal mol^{-1} more strained, is clockwise.

This type of organization through inter-chain H bonds is not a mere curiosity. It has been observed in other tripod structures derived from tris(2-aminoethyl)amine and 1,1,1-tris(hydroxymethyl)ethane.

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