

A Heterocyclic Peptide Nanotube

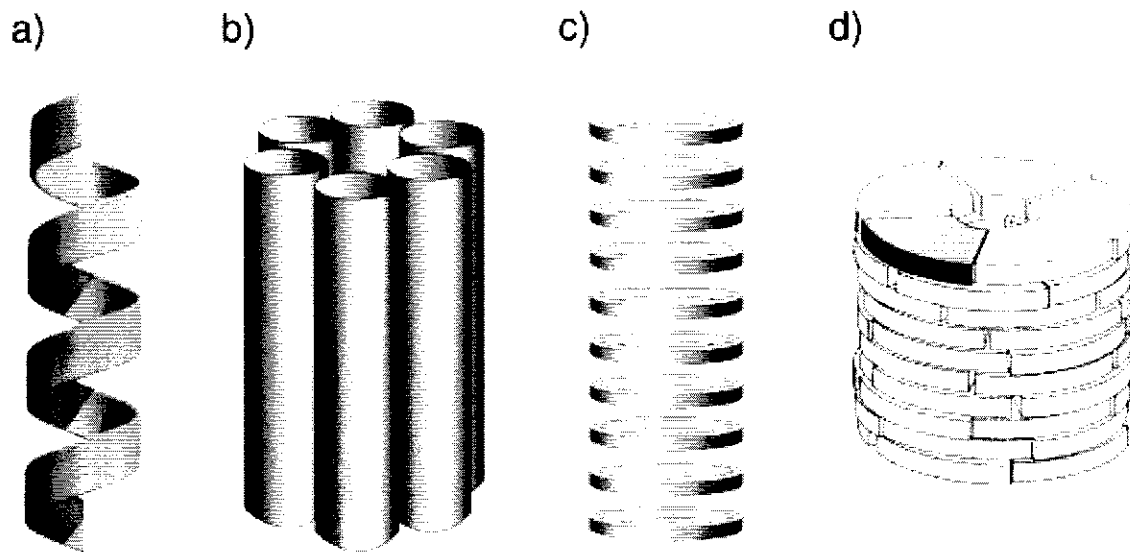
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Motifs of Tubular Assembly



Four possible strategies for the molecular assembly of tubular materials:

- a) Helical molecules can be coiled to form hollow, folded structures,
- b) rod-like molecules can be assembled in a barrel-stave fashion to form molecular bundles,
- c) macrocycles can be stacked to form continuous tubes, and
- d) sector or wedge-shaped molecules can assemble into discs that subsequently stack to form continuous cylinders, similar to macrocycles.

Dennis T. Bong, Thomas D. Clark, Juan R. Granja, M. Reza Ghadiri
Angew. Chem., Int. Ed., 2001, *40*, 988-1011.

Nanotubes based on cyclic peptides:

1972: First proposed by Hassal et al. who predicted that cyclic tetrapeptides composed of alternating α - and β -amino acids would stack through backbone-backbone hydrogen-bonding to form hollow cylindrical structures.

1974: De Santis and co-workers recognized the possibility of forming analogous cylindrical structures by ring-stacking of *cyclic D, L--peptides*. (*Macromolecules*, 1974, 7, 52-58)

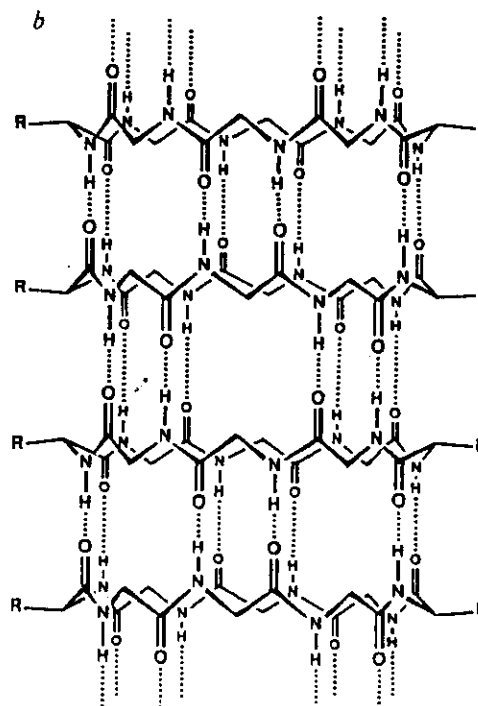
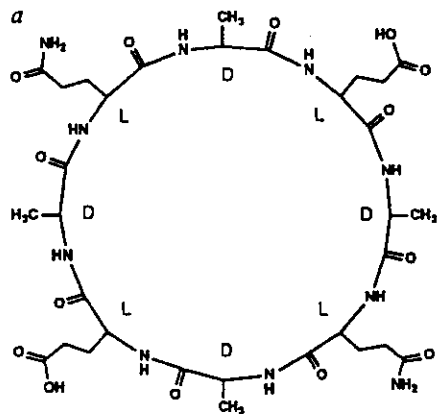
1975: X-ray crystallographic work partially validated these predictions by Hassal, although only two of four expected intersubunit hydrogen-bonds were observed. (*Acta Crystallogr.* 1975, *B* 31, 555-560)

1993: First compelling evidence for formation of hollow tubular structures by self-assembly of cyclic D,L—peptides by electron microscopy, electron diffraction, FT-IR, and molecular modeling studies by Ghadiri. (*Nature* 1993, 366, 324-327)

Advantages of using peptide-based macrocycles as nanotube subunits:

- 1. Sequential condensation and deprotection of suitably protected amino acids provide a facile route for synthesis of the assembling subunits.**
- 2. The number and sequence of amino acids used defines the internal diameter and outside surface properties of the resulting tubular assembly.**

First Compelling Sample of Cyclic Peptide Nanotubes

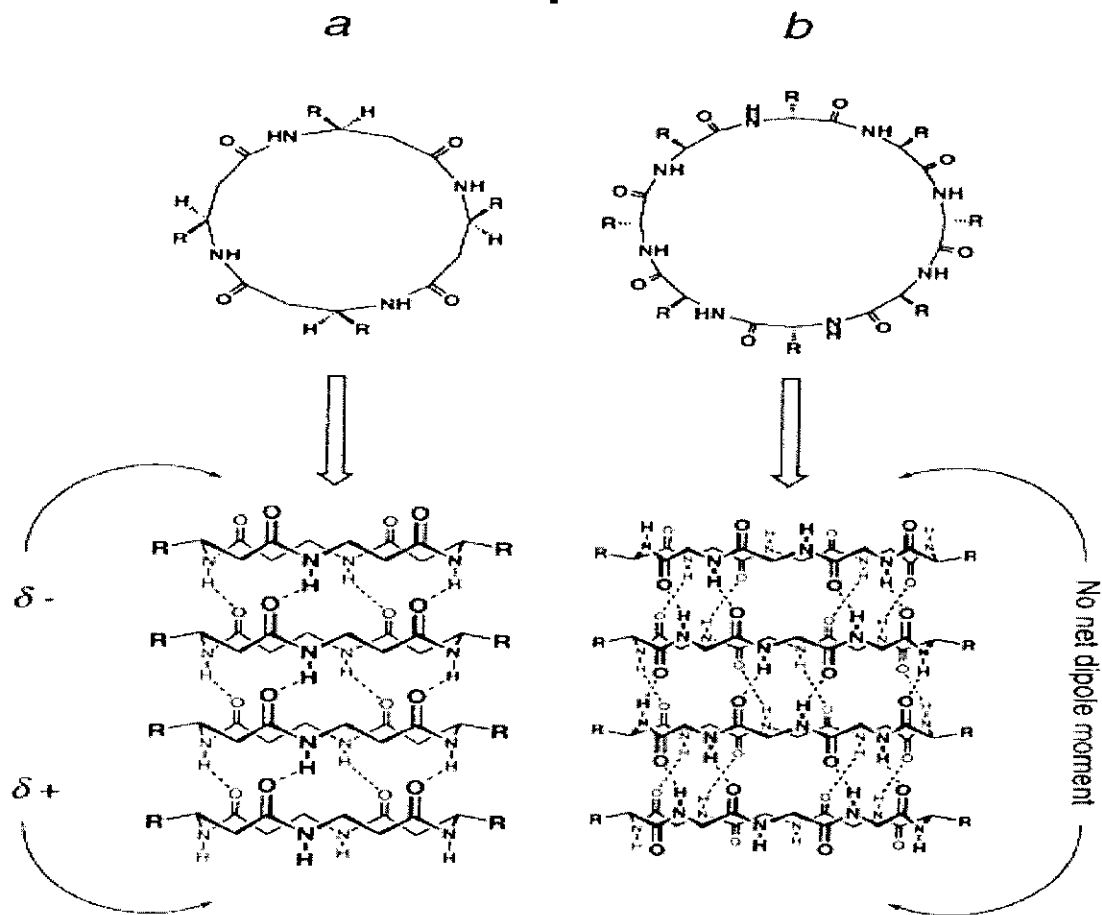


Design Principles:

1. with an even number of amino acids
2. have alternating D- and L- α -amino acids
3. antiparallel fashion of packing with backbone-backbone intermolecular hydrogen bonding

Ghadiri, M. R.; Granja, J. R.; Milligan, R. A.; McRee, D. E.; Khazanovich, N. *Nature* 1993, 366, 324-327.

Cyclic Peptide Nanotubes with β^3 -Amino Acids

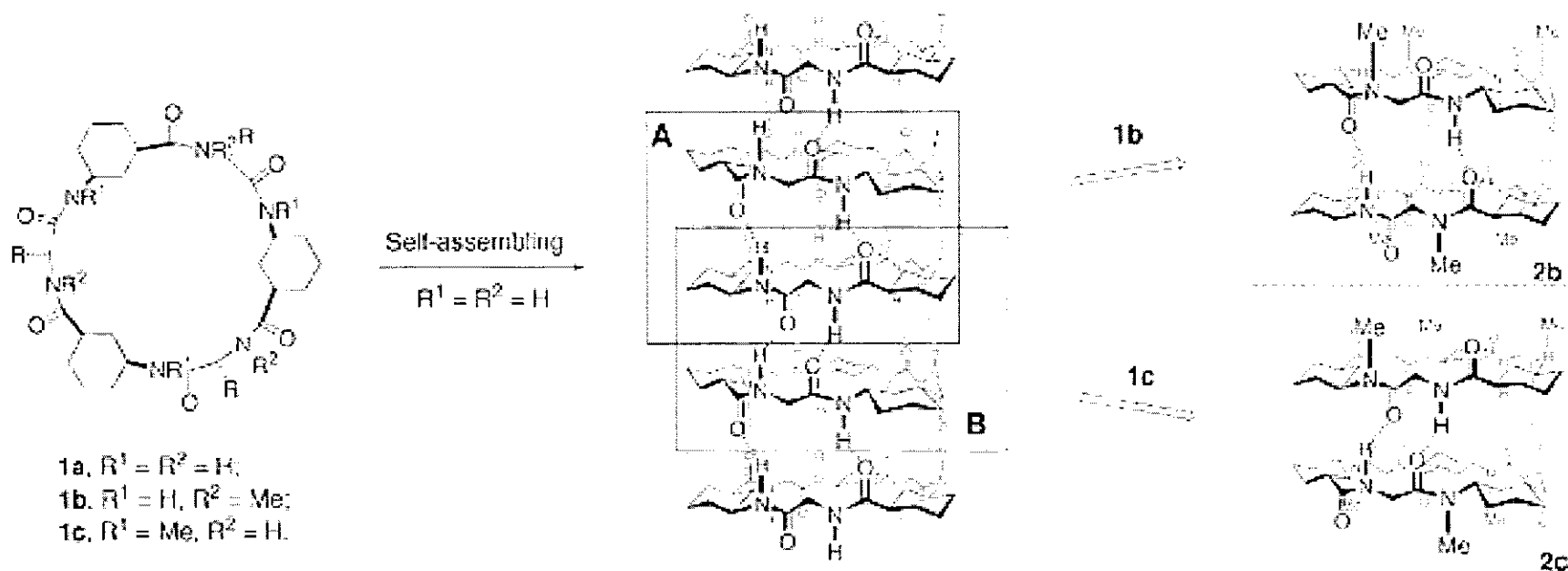


Thomas D. Clark, Lukas K. Buehler, and M. Reza Ghadiri *J. Am. Chem. Soc.*, 1998, 120, 651-656.

Cyclic Peptide Nanotubes with Alternating α , β -Amino Acids

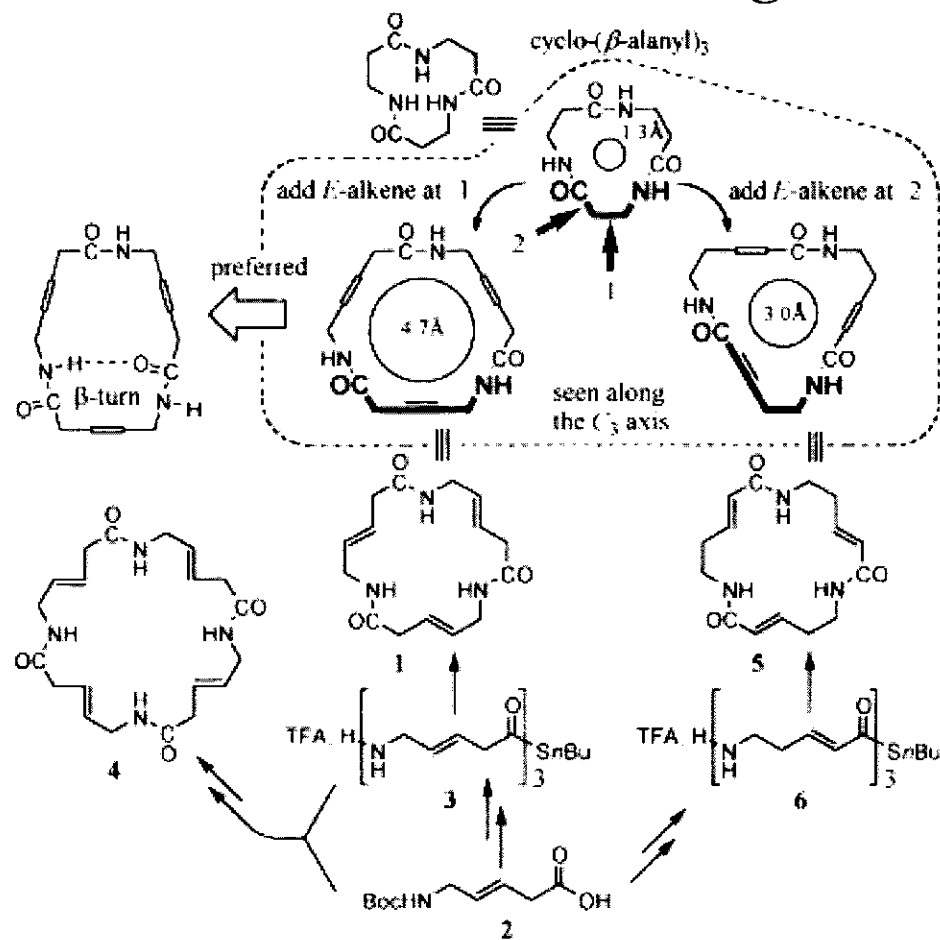
Karle, I. L.; Handa, B. K.; Hassall, C. H. *Acta Crystallogr., Sect. B* 1975, 31, 555-560.

Cyclic Peptide Nanotubes with Alternating α , γ -Amino Acids



Manuel Amorin, Luis Castedo, and Juan R. Granja *J. Am. Chem. Soc.*, 2003, 125, 2844-2845.

Cyclic Peptide Nanotubes with Alternating δ -Amino Acids



David Gauthier, Pierre Baillargeon, Marc Drouin, Yves L. Dory
 Angew. Chem., Int. Ed., 2001, 40, 4635-4638.

Cyclic Peptide Nanotubes with Alternating α , ϵ -Amino Acids

Design Reason:

Peptide nanotubes possessing hydrophobic internal characteristics have not yet been explored (solubility).

Design Principle:

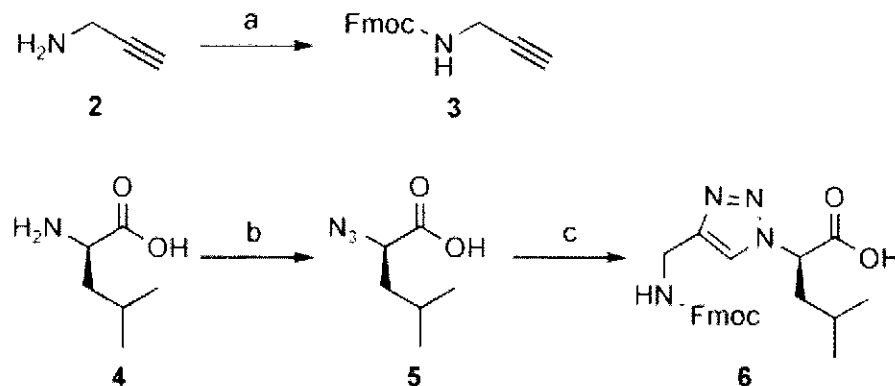
Modification of the interior properties requires peptide main-chain replacements with hydrophobic moieties that do not adversely effect the conformational requirements of the subunit for self-assembly via intermolecular hydrogen bonding.

Novel Design:

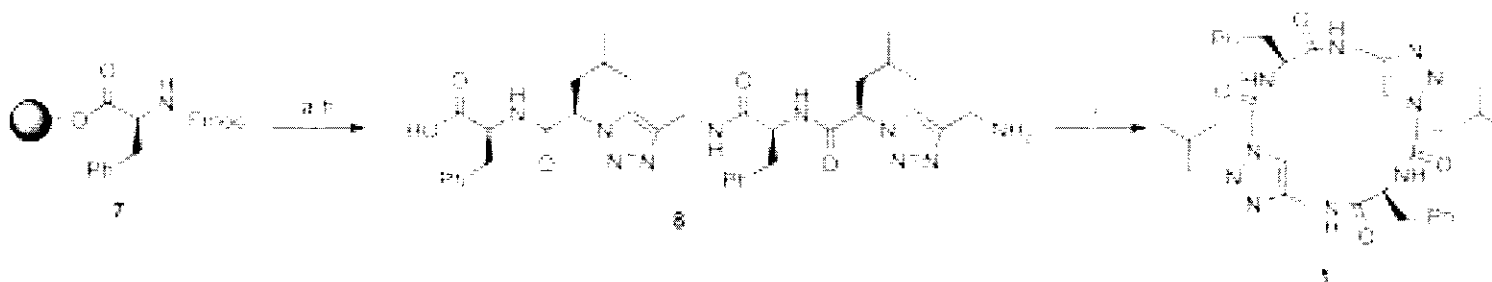
Utility of heterocyclic peptide backbone modifications in the design of a new form of self-assembling peptide nanotube that combines the structural aspects and capacity for outside surface functionalization of previously described cyclic D,L--peptide nanotubes with heterocyclic alterations introduced to modify the physical properties of the nanotube interior.

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J. Am. Chem. Soc., 2003, 125, 9372 –9376.

Synthesis of the Novel 1,4-Disubstituted-1,2,3-Triazole-amino Acid and Subunit



Scheme 1 (a) Fmoc-*N*-hydroxysuccinimide (71%); (b) (F₃CSO₂)₂O, NaN₃, then CuSO₄, K₂CO₃ (84%); (c) 3, CuI, diisopropylethylamine, 2,6-lutidine (97%).



Scheme 2 (a) 20% piperidine/DMF; (b) 6, DIC, HOBt; (c) 20% piperidine/DMF; (d) Fmoc-Phe-OH, HBTU, DIEA; (e) 20% piperidine/DMF; (f) 6, DIC, HOBt; (g) 20% piperidine/DMF; (h) 5% TFA/DCM; (i) PyBOP, HOAT, DIEA.

NMR and MS Study

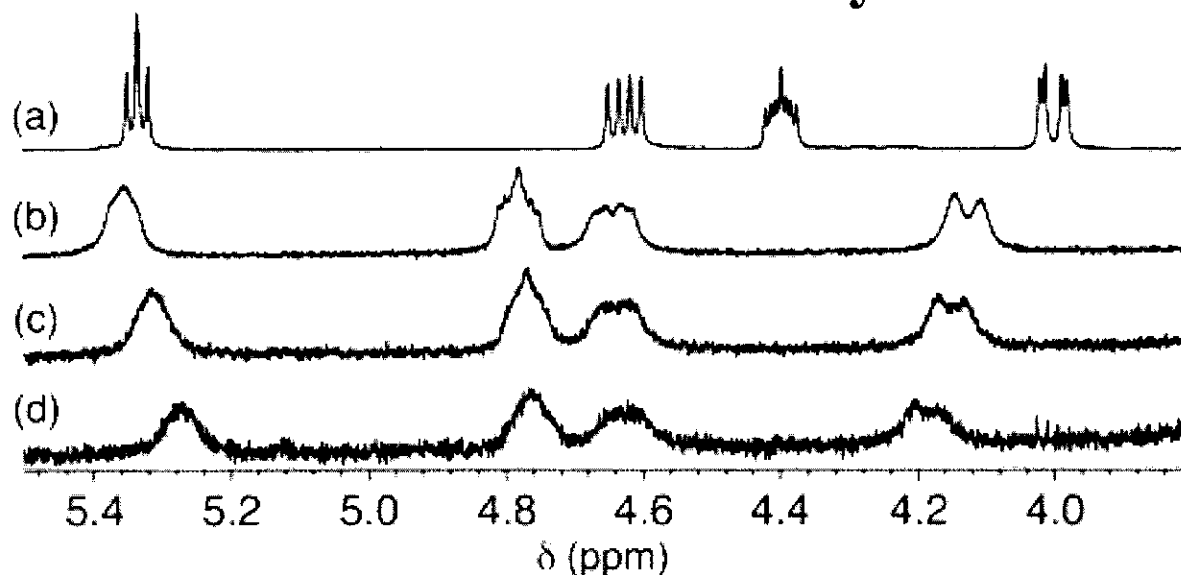
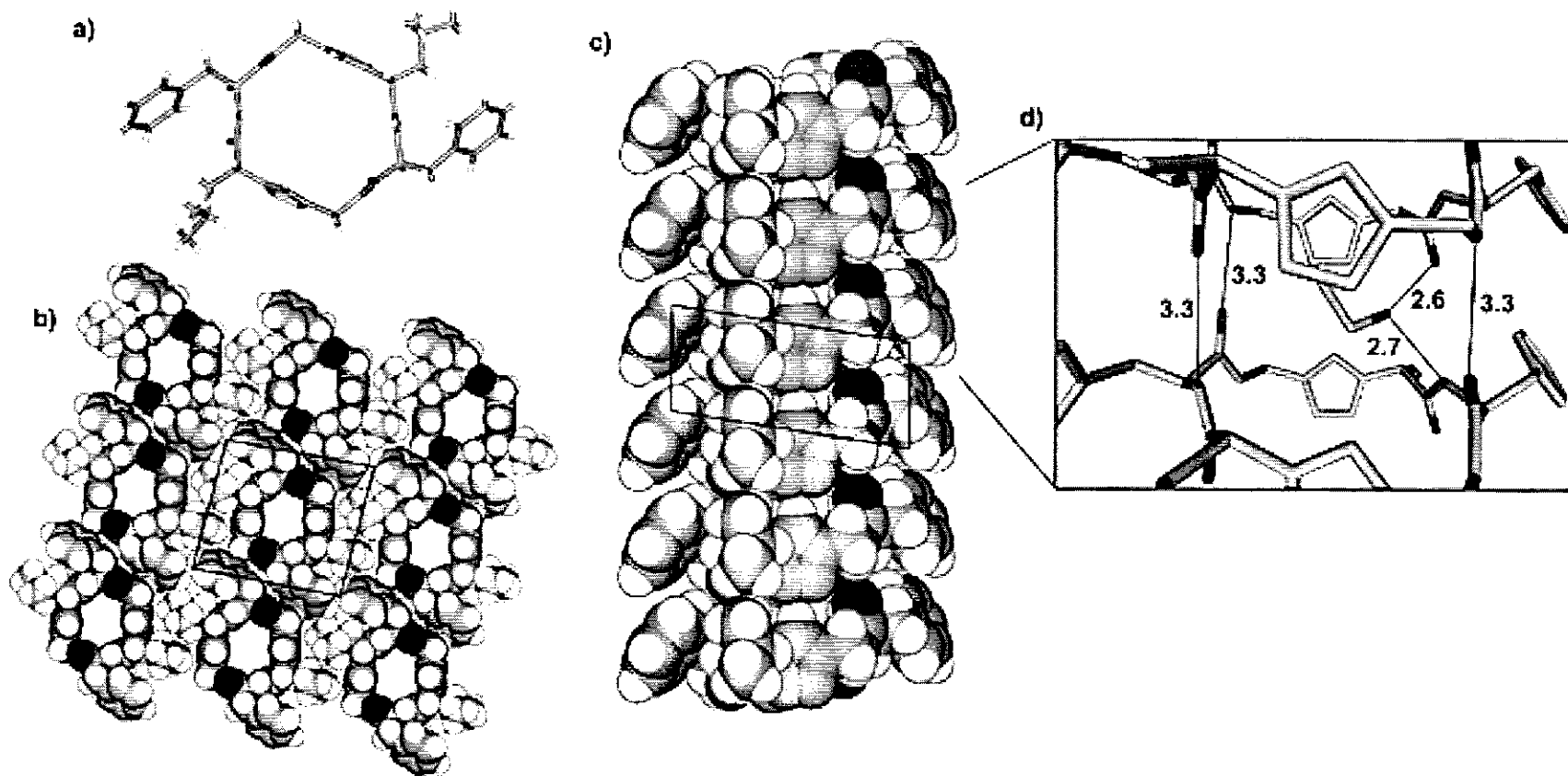


Figure 1 Selected region of ^1H NMR spectrum of **1** (a) in DMSO, (b) 1.0 mM in CDCl_3 , (c) 0.50 mM in CDCl_3 , and (d) 0.25 mM in CDCl_3 . The peaks in DMSO show sharp splitting, while the signals in CDCl_3 are broad and show concentration-dependent chemical shifts.

Infusion of a 0.5 mM solution of 1 in 9:1 acetonitrile:water gave rise to strong signals corresponding to monomeric :

$[1+\text{H}]^+$ (calcd = 683.4, obsd = 683.7), $[1+\text{Na}]^+$ (calcd = 705.4, obsd = 705.7) as well as noncovalent dimers $[1\cdot 1+\text{H}]^+$ (calcd = 1365.8, obsd = 1365.5) and $[1\cdot 1+\text{Na}]^+$ (calcd = 1387.7, obsd = 1387.2)

X-Ray Study



Conclusion

- 1. Design, synthesis, and characterization of a new class of peptide-based macrocycle incorporating 1,2,3-triazole -amino acids in the backbone.**
- 2. The synthesis is modular and straightforward with the protected triazole - amino acid readily prepared from the corresponding free amino acid.**
- 3. In the solid state, these molecules form a solvent-filled nanotube held together by an extended network of intermolecular amide backbone hydrogen bonds.**
- 4. NMR and mass spectrometry studies support similar behavior in solution and the gas phase.**