STRUCTURE ACTIVITY
RELATIONSHIPS OF BIOACTIVE
PEPTIDES

DEPEND ON

1) CHEMICAL STRUCTURE

2) MOLECULAR CONFORMATION

COMPLETE DESCRIPTION OF STRUCTURE ACTIVITY RELATIONSHIPS (SAR) of Bioactive Peptides is DIFFICULT because

1) Peptides are flexible molecules
2) It is not easy to single out the "bioactive" conformation
3) Direct studies of the peptide-receptor complex are complicate.

THE STUDIES SHOULD BE CARRIED OUT ON:

(1) THE NATIVE PEPTIDE
(2) ITS ANALOGS
(3) FRAGMENTS
(4) APPROPRIATE MODEL COMPOUNDS

Whenever possible direct investigation of the receptor-peptide complex wuold be desiderable

METHODOLOGIES TO BE USED IN STRUCTURE ACTIVITY STUDIES:

(1) Sequence Analysis
(2) Physico-Chemical Measurements in solution
(3) Solid-State Structure Determinations
(4) Theoretical Calculations
(5) Model Building

SEQUENCE ANALYSIS

1) Synthesis of analogs in which systematic changes in residues along the chain are introduced.

2) Deletion of one or more residue at both N- and C-termini.

3) Preparation of "ad hoc" constrained analogs.

TESTS OF BIOACTIVITY

in vivo and in vitro tests of biological activity should be available.

The screening should be carried out on analogs and fragments.

CONSTRAINED ANALOGS

(1) Introduction of constrained residues at preselected positions along the chain (to induce particular conformations).

(2) Cyclization of the peptide (or portion of it) by:

a) end-to-end link

b) main-chain to side chain link

c) side-chain to side-chain link

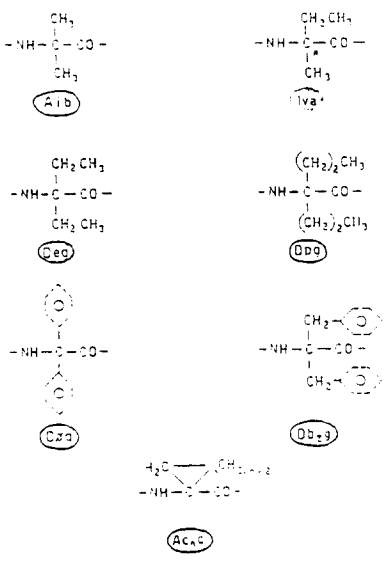
d) side-chain to end link

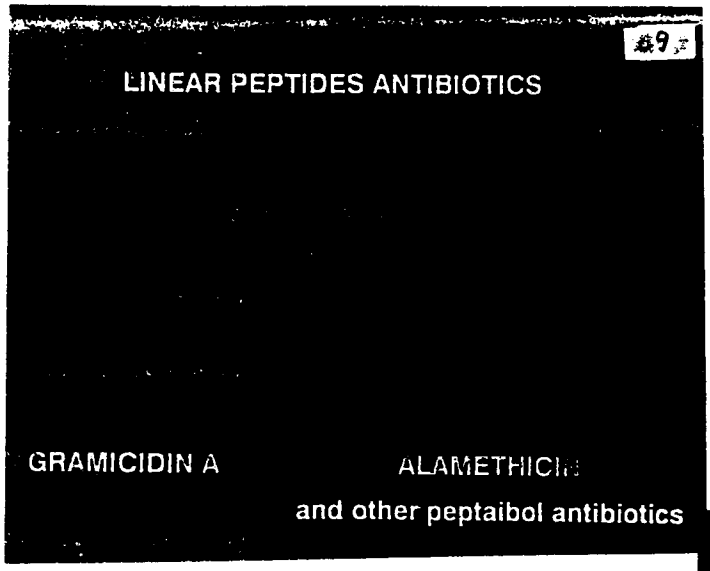
(3) "Peptido-mimetic" substitution of the NH-CO bond with a "surrogate":

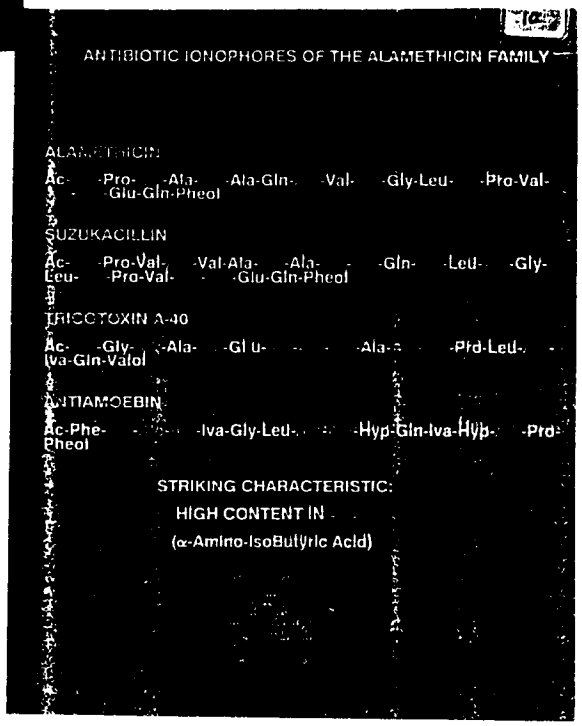
$-CH_2CO-$; $-NH-CH_2-$; $-CH_2-SO-$;

$-CH_2-CH_2-$;  etc.

α,α-dialkylated AA residues







CRYSTAL STRUCTURES OF
AIB-CONTAINING PEPTIDES
SOLVED AT THE *UNIV. OF NAPOLI*

—o—

$-(Aib)_2-$ incipient 3_{10}-helix

$-(Aib)_3-$ inc. 3_{10}-helix

$-(Aib)_4-$ 3_{10}-helix

$-(Aib)_5-$ 3_{10}-helix

$-(Aib)_7-$ 3_{10}-helix

$-(Aib)_8-$ 3_{10}-helix

$-(Aib)_{10}-$ 3_{10}-helix

3_{10}-helix
$\varphi = -30°$
$\psi = -60°$

α-helix
$\varphi = -45°$
$\psi = -55°$

$-Aib-L\text{-}Ala-Aib-$ incipient 3_{10}-helix

$-Aib-L\text{-}Pro-Aib-$ incipient 3_{10}-helix

$-(Aib)_3-L\text{-}Val$ 3_{10}-helix

$-(Aib)_3-L\text{-}Val-Gly-$ 3_{10}-helix

$-(Aib)_3-L\text{-}Val-Gly-L\text{-}Leu-(Aib)_2-$ 3_{10}-helix

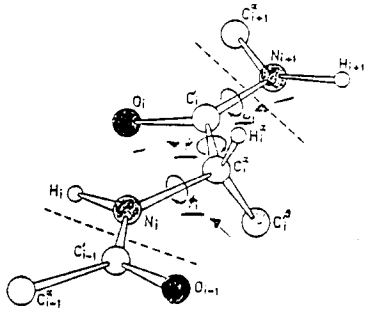
$-(Aib)_4-L\text{-}Leu-(Aib)_2-$ 3_{10}-helix

$-(Aib)_5-L\text{-}Leu-(Aib)_2-$ 3_{10}-helix

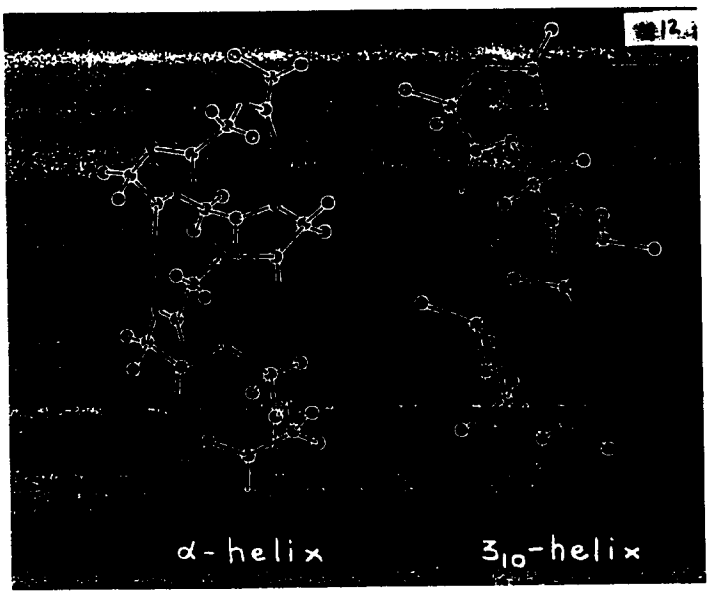
$-(Aib-L\text{-}Ala)_4-$ mixed $3_{10}-\alpha$-helix

$-(Aib-L\text{-}Ala)_5-$ mostly α-helix

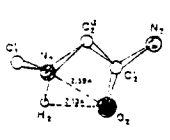
$-(Aib-L\text{-}Ala)_7-$ mostly α-helix



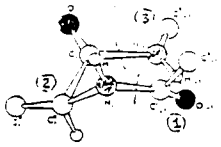
Definition of φ, ψ, ω.



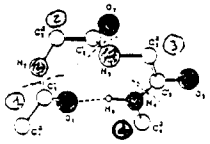
INTRAMOLECULARLY
HYDROGEN BONDED STRUCTURES



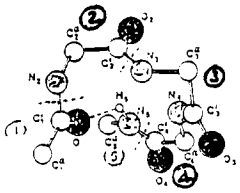
2→2
C_5



3→1
C_7



4→1
C_{10}



5→1
C_{13}

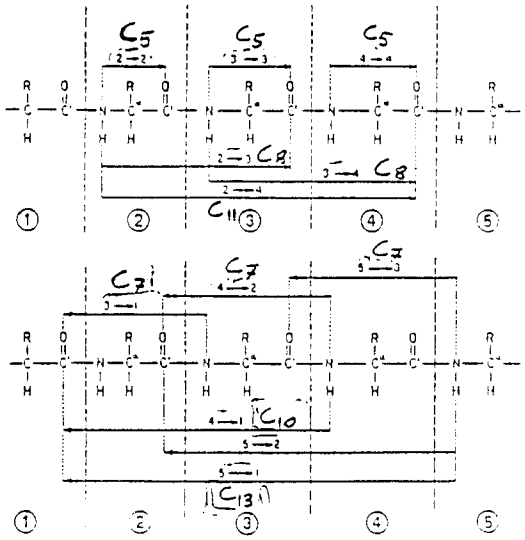


FIG. Possible intramolecular hydrogen bonds occurring in a system of four linked peptide residues.

HELICAL CONFORMATIONS IN A PEPTIDE

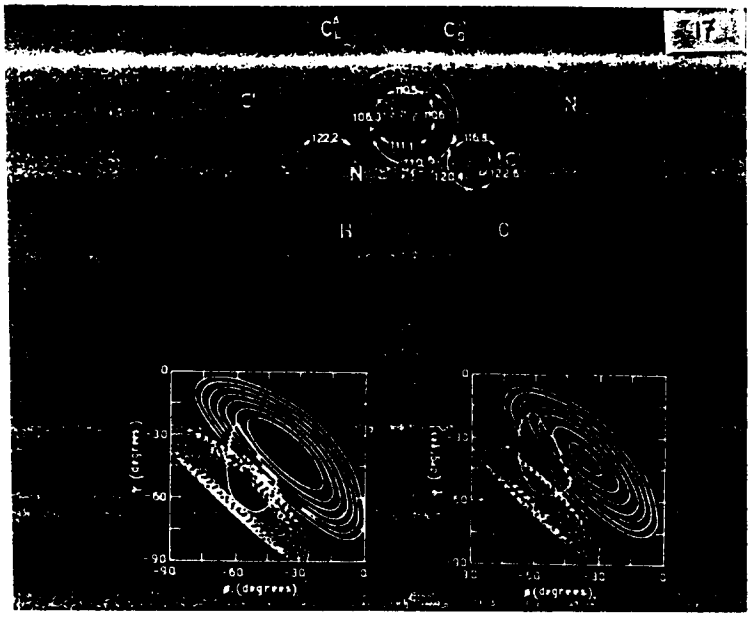
ARE GENERATED WHEN

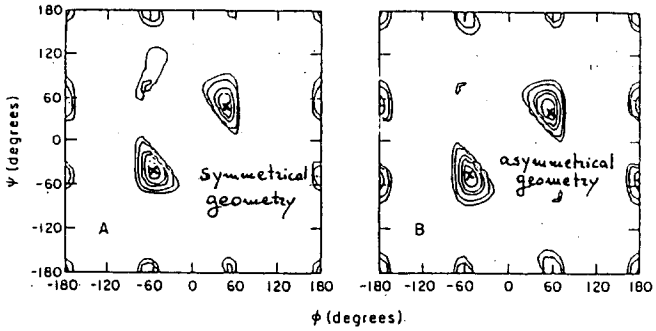
THE φ_i, ψ_i, ω_i CONFORMATIONAL ANGLES ASSUME THE SAME VALUES FOR EACH CONSECUTIVE RESIDUE

MAJOR STABILIZING FACTOR FOR HELICAL CONFORMATIONS IS THE HYDROGEN BOND FORMATION

Table II. Characteristic Parameters of Polypeptide Helica Structures.

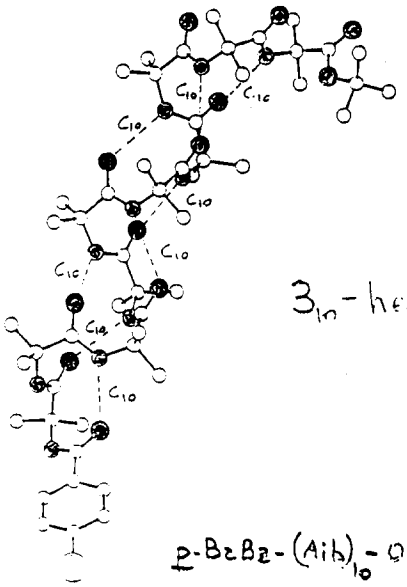
Parameters		2_1-helix	3_{10}-helix	α-helix
Symmetry		(2_1)	(3_1)	(18_5)
Residue Repeat (length per residue in A)		2.75	2.0	1.50
Type of Intramolecular H-bond		3→1	4→1	5→1
Number of Atom in Ring		7	10	13
Conformational Angles for Right-Handed Helices of L-Residues	φ	-80	-60	-55
	ψ	70	-30	-45
	ω	160	180	180
Designation according to Bragg		2_7	3_{10}	3.60_{13}





CONFORMATIONAL MAP
DERIVED FROM ENERGY MINIMIZATION
OF

$Ac-Aib-NHCH_3$



3_{10}-helix

p-BzBz-$(Aib)_{10}$-OtBu

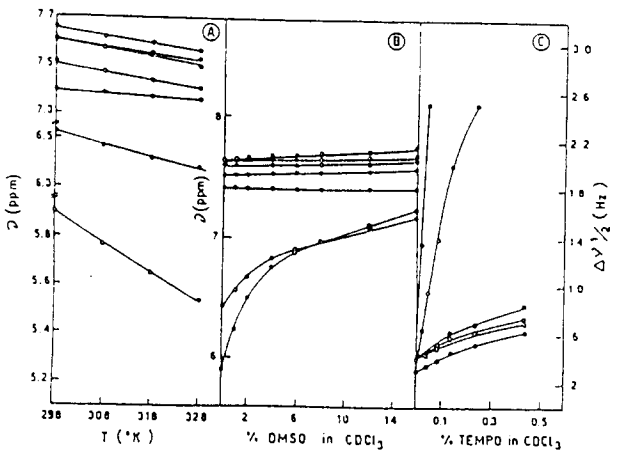
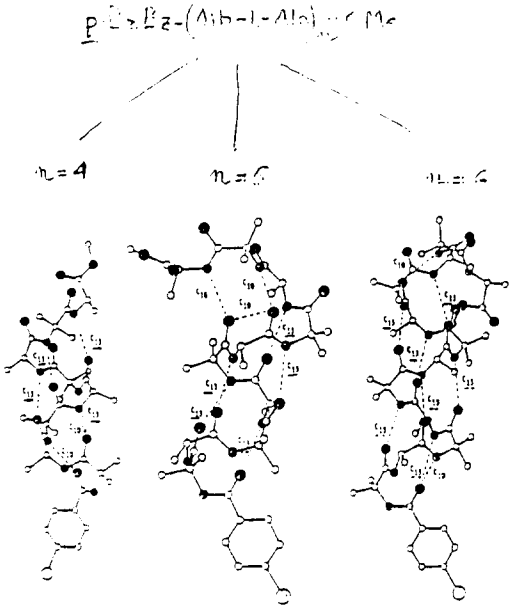


Fig. 3 - Plots of NH chemical shifts in the ^1H NMR spectra of Z(Aib)$_{10}$OtBu in $CDCl_3$ (C=15 mM) as a function of (A) increasing temperature and (B) increasing percentages of DMSO added to the $CDCl_3$ solution (v/v). (C) Plot of the bandwidth of the NH protons of the same peptide as a function of increasing percentages of TEMPO (w/v) in $CDCl_3$ (C=15 mM). Adapted from ref. 34.

In each plot, 2 protons are more "available" than the others.



n = 3
3_{10} - helix
(only C_{10})

RESULTS FROM THE SOLID STATE STRUCTURES AND SOLUTION CONFORMATIONAL ANALYSES OF ...-RICH OLIGOPEPTIDES:

- Aib residues tend to stabilize helices of the 3_{10} or α types
- Peptides smaller than 8 residues with at least 30% of Aib have 3_{10}-helical structures.
- Peptides longer than 8 residues (with less than 50% Aib tend to be α-helical)
- α-helical Aib-rich peptides of appropriate lenght show voltage-dependent pores formation

DIPOLE-DIPOLE INTERACTIONS WITHIN AGGREGATES OF α-HELICES PRODUCE THE PORES AND BY A GATING MECHANISM THE PASSAGE OF IONS

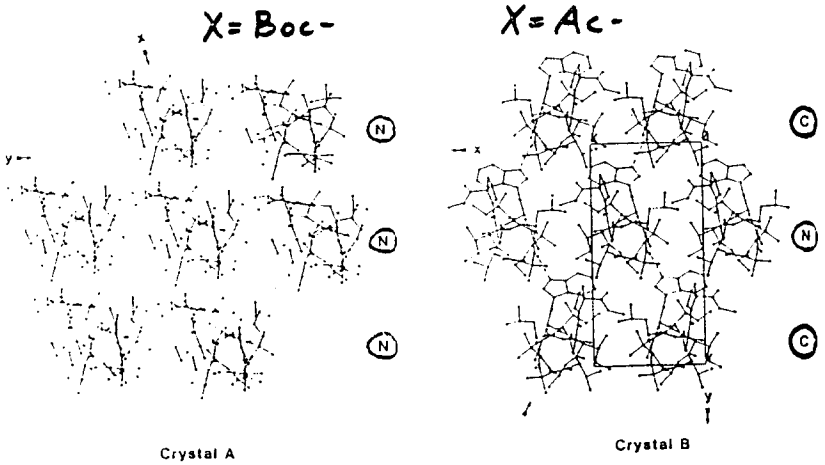
VOLTAGE-DEPENDENT PORES FORMATION MUST INVOLVE MOLECULAR AGGREGATES (neither the 3_{10} or the α-helix can accomodate ions)

ESSENTIAL FOR THE BIOLOGICAL FUNCTION IS THE ... OF ...

X-Trp-Ile-Ala-Aib-Ile-Val-Aib-Leu-Aib-Pro-OMe

X = Boc-

X = Ac-

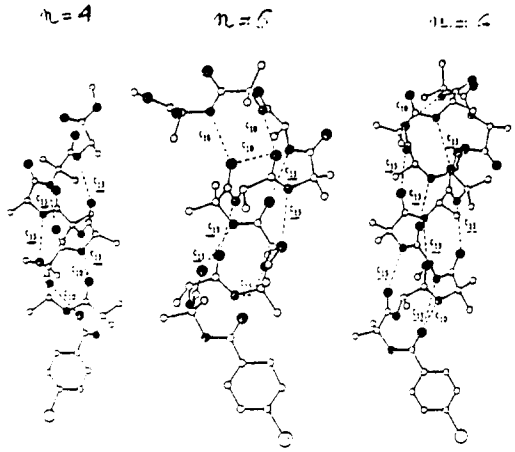


Crystal A

Crystal B

- Packing of Helices -

Boc-Z-(Aib-L-Ala)$_n$-OMe



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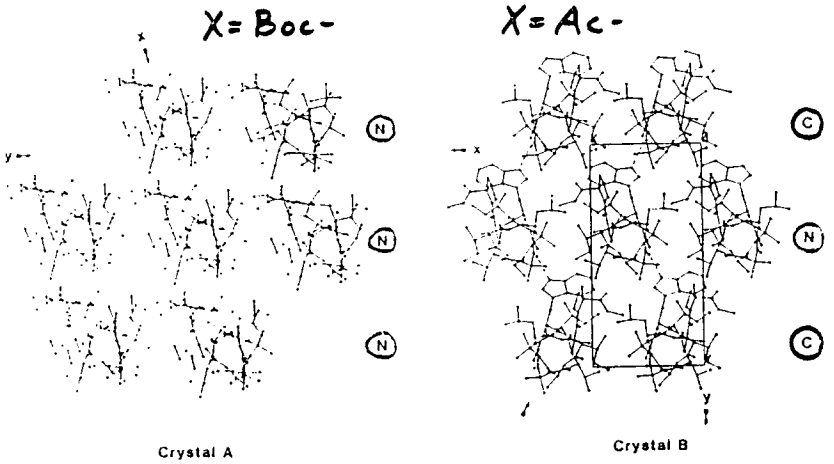


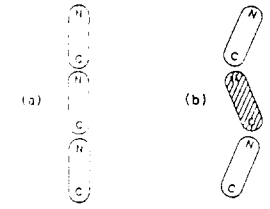
Crystal A

Crystal B

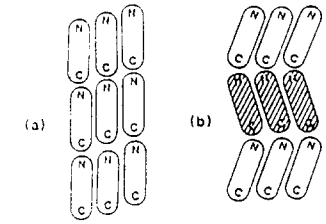
- Packing of Helices -

ELEMENTS OF AGGREGATION OF HELICES

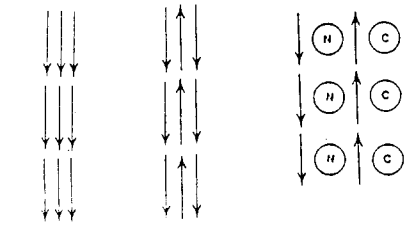
1. COLUMNS by head-to-tail hydrogen bonding, COMMON motif



2. SHEETS with PARALLEL packing, COMMON motif

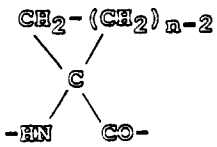


3. ASSEMBLY of SHEETS with PARALLEL packing, VARYING motifs



(a) Parallel (b) Antiparallel (c) Skewed

α-aminocycloalkylcarboxylic acids, Ac_nc,



n=3,4,5,6,...

behave conformationally as Aib residues, preferring helical conformations.

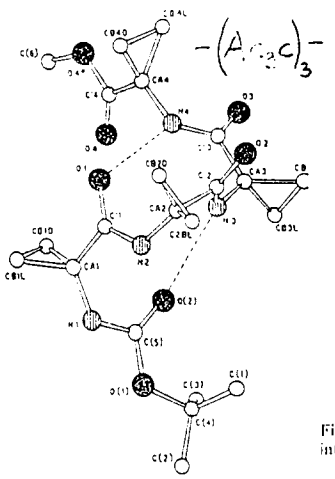


Figure 6 Molecular structure of t-Boc-(Ac$_5$c)$_4$-OMe (6) with numbering of the atoms. The two intramolecular H-bonds are indicated as broken lines

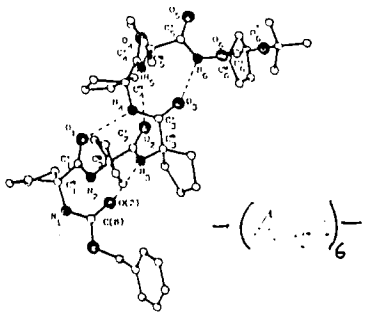


Figure 4 Molecular structure of Z(Ace^5)$_6$OtBu. The intramolecular H-bonds are indicated as broken lines

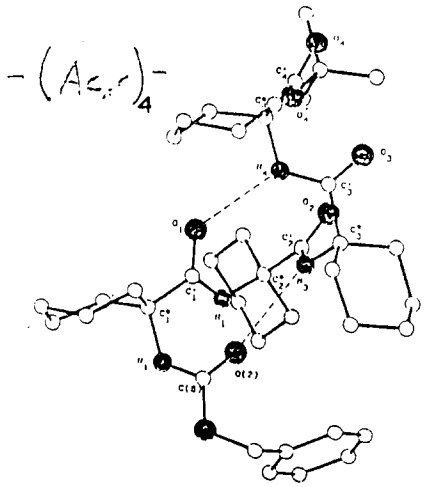
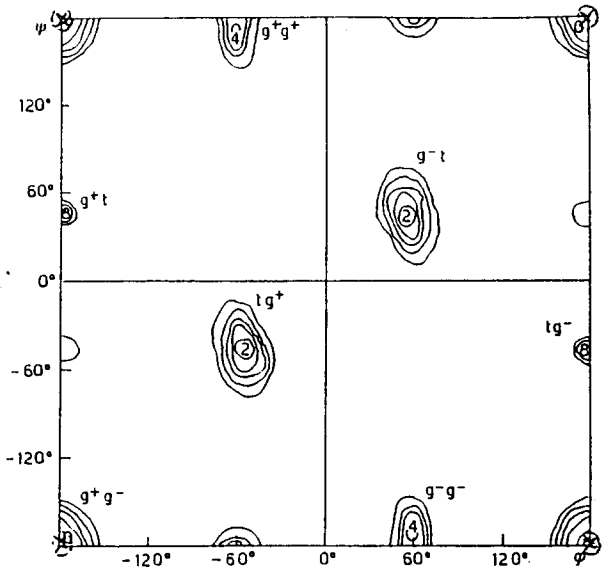
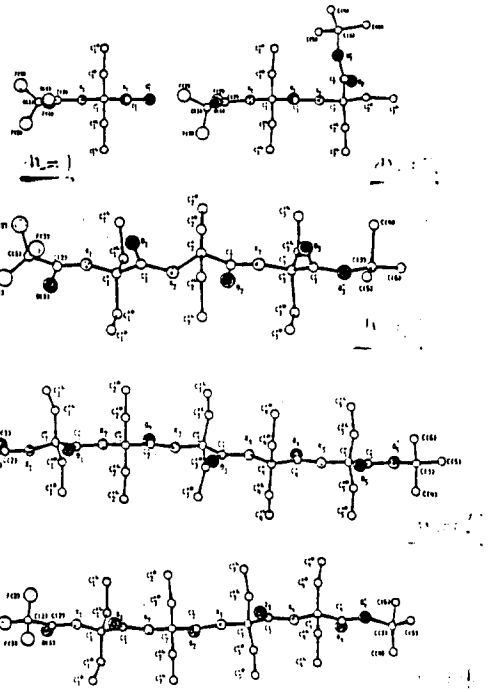


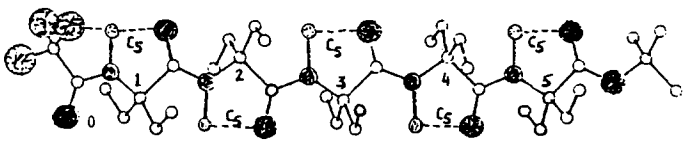
Figure 3. Molecular structure of Z(Ace^6)$_4$O-t-Bu (triclinic form). The intramolecular H bonds are represented as dashed lines



Ac-Deg-NHCH$_3$

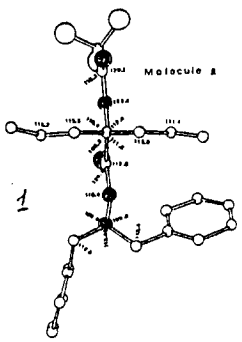
The structures of TFA-(Dpg)$_n$-OtBu





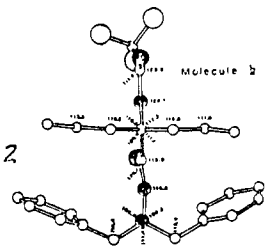
Tfa-(Deg)$_5$-OtBu

J. Am. Chem. Soc., Vol. 106, No. 26, 1984

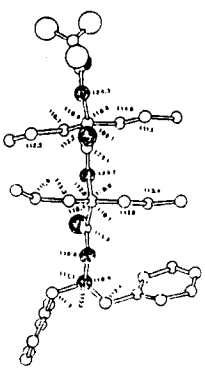


Molecule 1

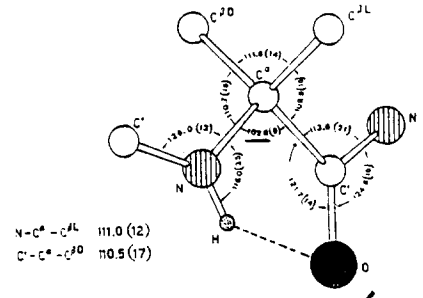
Tfa-Dpg-DBH



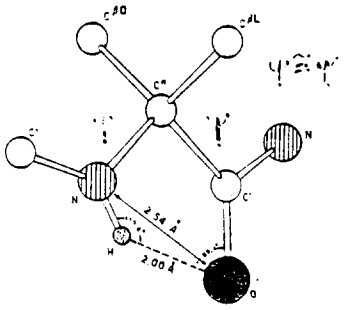
Molecule 2

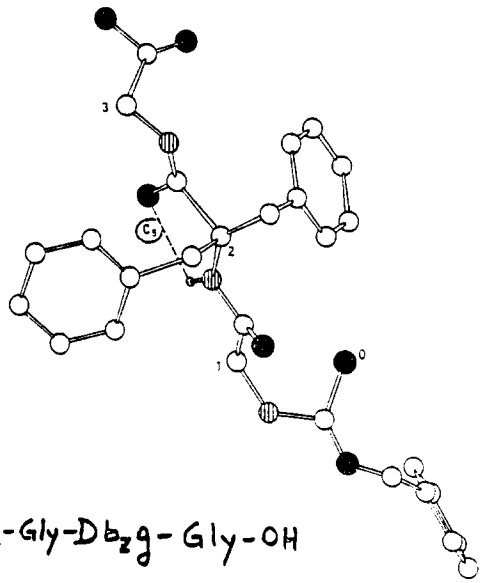


Tfa-(Dpg)$_2$-DBH



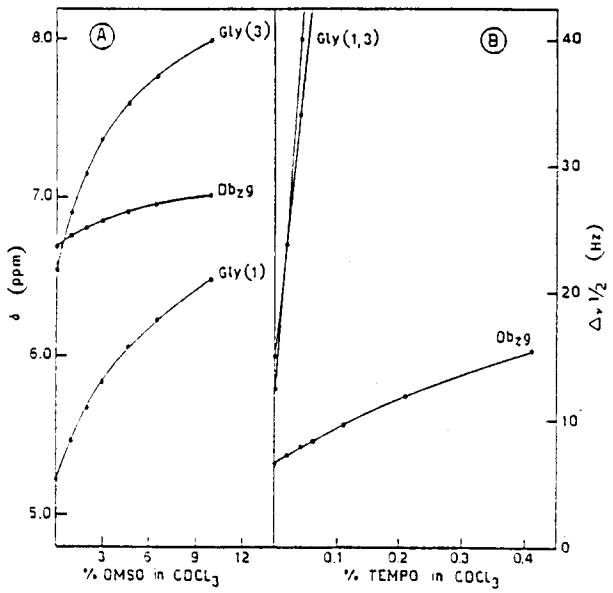
Geometry of the C_5 (2←2) fully extended conformation





Z-Gly-Dbzg-Gly-OH

Z-Gly-Db_zg-Gly-OH



CONCLUSIONS

α,α-Dialkylated residues tend to adopt specific conformations:

- **Aib**, **Ac_nc**, **Iva** favour helical structures;
- **Deg**, **Dpg**, **Dφg**, **Db_{zg}** favour fully extended structures.

TOXINS: ANTAGONISTS OF TOXINS

TO THE MUSHROOMS

Amatoxins

Cyclopeptides and Biologically Related Peptides

Amatoxins are components of AMANITA mushroom responsible of fatal intoxication after thei ingestion.

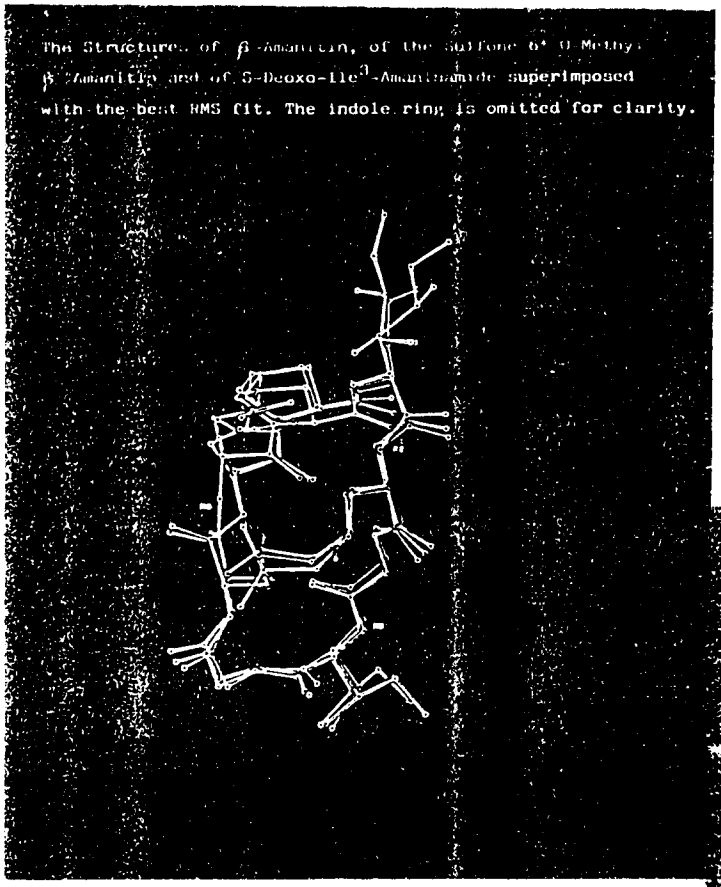
3

Amatoxins bind very tightly to eukaryotic RN polymerase B in liver and kidney forming a very compact ternary complex of enzyme, template DNA and nascent messenger RNA, in which translocation is no longer feasible.

	R^1	R^2	R^3	R^4	R^5
	CH_2OH	OH	NH_2	OH	OH
	CH_2OH	OH	OH	OH	OH
	CH_3	OH	NH_2	OH	OH
	CH_3	OH	OH	OH	OH
	CH_2OH	OH	OH	H	OH
	CH_2OH	OH	NH_2	H	OH
	CH_3		NH_2	OH	OH
	CH_3		OH	OH	OH
	CH_3		NH_2	OH	H

S-deoxo [γ(*R*) hydroxy- Ile^1]-amaninamide

	R1	R2	R3	X
OH(*R*)	H	H	S	



The Structure of β-Amanitin, of the Sulfone 6' O-Methyl-β-Amanitin and of 5-Deoxo-Ile^3-Amanitamide superimposed with the best RMS fit. The indole ring is omitted for clarity.

SOLID-STATE AND SOLUTION STUDIES INDICATE THAT

Amatoxins exhibit similar overall structure.

Their conformation is not influenced by crystal forces or solvent interactions.

The differences in biological activity are due to small differences in chemical nature and REM conformation.

The interaction with the RNA polymerase enzyme takes place on the concave side of the 24-membered macrocycle.

H-bonding, hydrophobic and polar interactions through OH of Hyp^2 Ile^3 side chain and Gly^4 Ile^5 facilitate the interactions with the RNA polymerase enzyme.

STRUCTURE ACTIVITY RELATIONSHIPS

(1) The sulfoxide oxigen can be removed resulting in an equally toxic thioether.

(2) Side chain $ 1 may be Asp or Asn resulting in toxic compound (Amanitin α or β).

(3) The -OH in γ-position of residue $ 3 is indispensable and activity is greatly reduced if the γ-carbon has the (R)-configuration.

CYTOPROTECTIVE PEPTIDES:

AA, SA, CLA

—PROTECT LIVER FROM LESIONS CAUSED BY CELL TOXINS (ethanol, DMSO, cysteamine, phalloidin).

— PRESENT 2 ADJACENT AROMATIC RESIDUES.

— CYTOPROTECTION OCCURS WHEN PEPTIDES ARE APPLIED BEFORE OR SIMULTANEOUSLY WITH TOXIN (phalloidins).

ANTAMANIDE, (AA)

Phe-Pro-Pro-Phe-Phe
| |
Phe-Ala-Pro-Pro-Val

SOMATOSTATIN, (SA)

H-Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp
| |
Cys-Ser-Thr-Phe-Thr-Lys

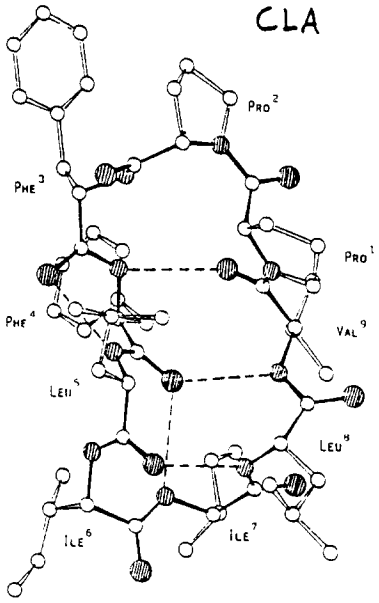
CYCLOLINOPEPTIDE A, (CLA)

Pro-Pro-Phe-Phe
Val |
Leu-Ile-Ile-Leu

These materials act as competitive antagonists (or protector) by inhibiting the system responsible for the transport of cholate from the blood into the bile (they are able to purify the blood via the bile).

Table 1. 50% Inhibition of cholate uptake in isolated hepatocytes by cyclopeptides (6).

No.	Peptide	Name	Concentration [μM]
SA 2	H—Ala—Gly—Cys—Lys—Asn—Phe—Phe—Trp (H) / H—Cys—Ser—Thr—Phe—Thr—Lys (H)	Somatostatin	(220)
	D-Trp—Phe—D-Trp (H) / Phe—Thr—Lys (H)	"Veber-peptide"	100
A 1	Phe—Pro—Pro—Phe—Phe (H) / Phe—Ala—Pro—Pro—Val (H)	Antamanide	(8)
CLA 4	Val: Pro—Pro—Phe—Phe (H) / Leu—Ile—Ile—Leu (H)	Cyclolinopeptide A	(3)
5	D-Pro—Phe—Phe (H) / Phe—Phe—Pro (H)	"PFF"	3
3	D-Pro—Phe—Thr (H) / Phe—Trp—Lys(Z) (H)	"008"	1.5



Structural Features of Cyclolinopeptide A in the solid state.

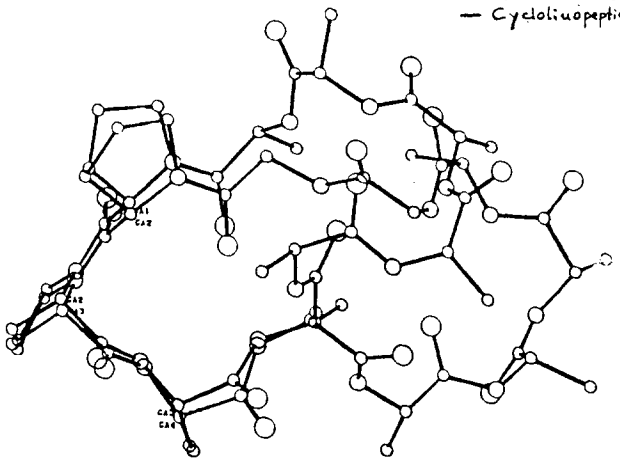
(1) The solid-state structure is stabilized by 3 intramolecular H-bonds of the N-H...O=C' type.

(2) One peptide unit (Pro^1-Pro^2) is in the cis conformation.

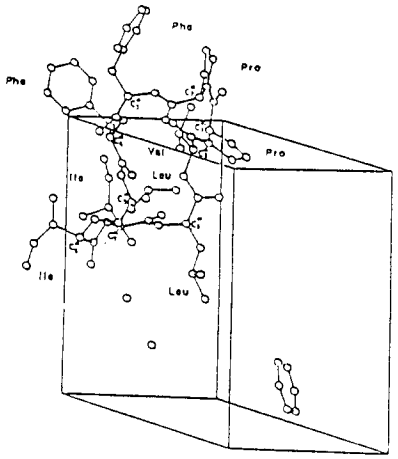
(3) Considerable conformational analogy with antamanide is present in the solid-state.

C_7	γ-turn	NH(Leu^5)....O=C (Phe^3)
C_{10}	β-turn(II)	NH(Ile^7)....O=C (Phe^4)
C_{10}	β-turn(I)	NH(Leu^8)....O=C (Leu^5)
C_{13}	α-turn	NH(Val^9)....O=C (Phe^4)
C_{16}		NH(Phe^4)....O=C (Val^9)

— Antamanide
— Cyclolinopeptide A



CLA Triclinic



Cyclolinopeptide A Polymorphs

Orthorhombic, $P2_12_12_1$, [illegible]

Di Blasio, Benedetti, Pavone, Pedone
R = 0.082 Biopolymers, 26, 2099 (1987)

Triclinic, $P1$, [illegible]

Neela, Manjula, Ramakumar, Balasubramanian, Viswamitra, Biopolymers, 29, 1499 (1990)
R = 0.082

Monoclinic, $P2_1$, solvated ($5H_2O$, DMF)

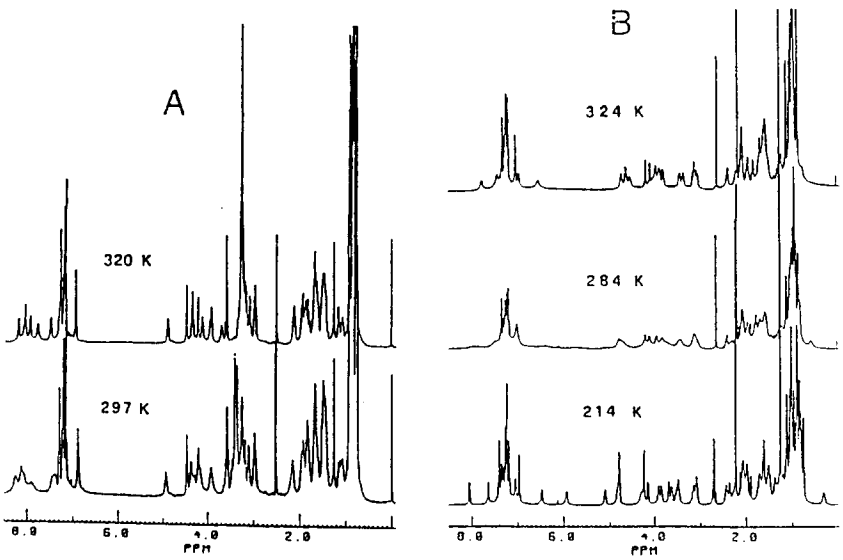
Neela, Manjula, Ramakumar, Viswamitra
R = 0.118 IUC Meeting, Bordeaux (1990)

The peptide folding and the twisting of the side chains are very similar.

The observed conformation is energetically favorable.

Other polymorphs are under investigation

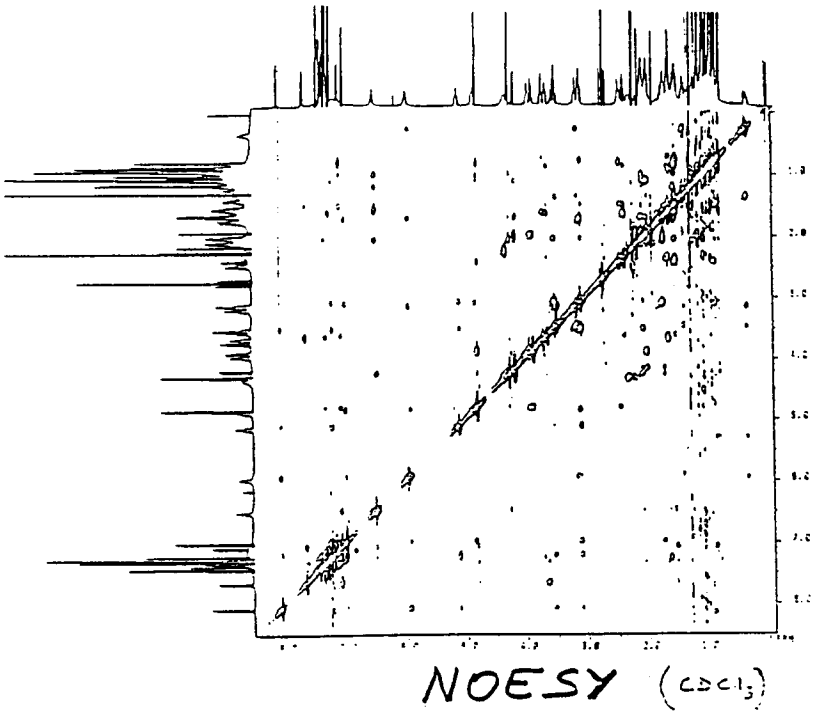
NMR - solution study of cyclolinopeptide A -



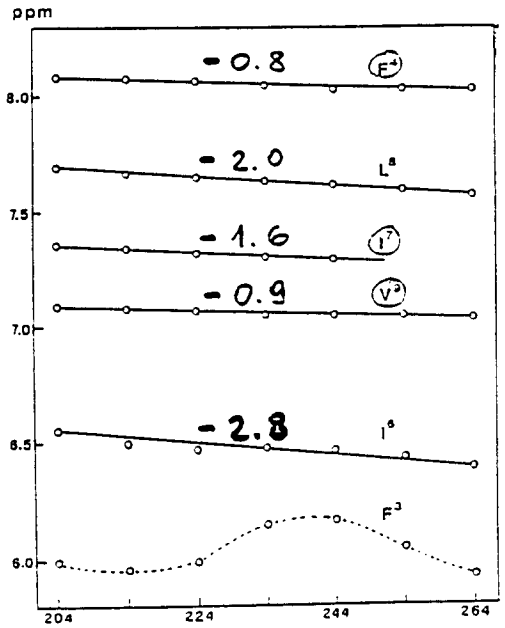
DMSO

$CDCl_3$

CLA



NOESY ($CDCl_3$)



^{1}Pro-^{2}Pro-^{3}Phe-^{4}Phe-^{5}Leu
^{6}Ile-^{7}Ile-^{8}Leu-^{9}Val

Solid-state : $\underline{F^4}, L^5, \underline{I^7}, L^8, \underline{V^9}$

Structural features of Cyclolinopeptide A in solution.

① Room temperature NMR spectra in several solvents (DMSO, H_2O, methanol, acetone, chloroform) indicate the presence of chemical exchange among several conformers.

② The solution conformation, at 214 K in $CDCl_3$, is consistent with the solid-state structure.

THE STRUCTURE OBSERVED FOR CLA IS THE "BIOACTIVE" STRUCTURE?

UNIVERSITY OF NAPOLI

Carlo PEDONE
Benedetto DI BLASIO
Vincenzo PAVONE
Angela LOMBARDI
Filomena ROSSI
Antonello SANTINI
Ottavia SPINIELLO

UNIVERSITY OF PADOVA

Claudio TONIOLO
Gian Maria BONORA
Marco CRISMA

UNIVERSITY OF POTENZA

Alfonso BAVOSO

UNIVERSITY OF ROMA

Giancarlo ZANOTTI

E. T. H. OF ZURICH

Gian Paolo LORENZI

UNIVERSITY OF CALIFORNIA, SAN DIEGO

Murray GOODMAN

The structure observed most probably is not in the active conformation.

Restricted and much less mobile analogs having the same structure in solution and in the solid state are much less active.