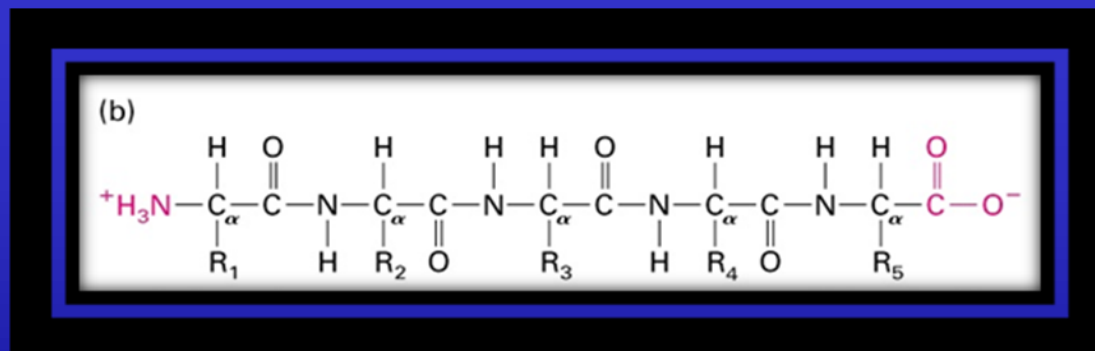
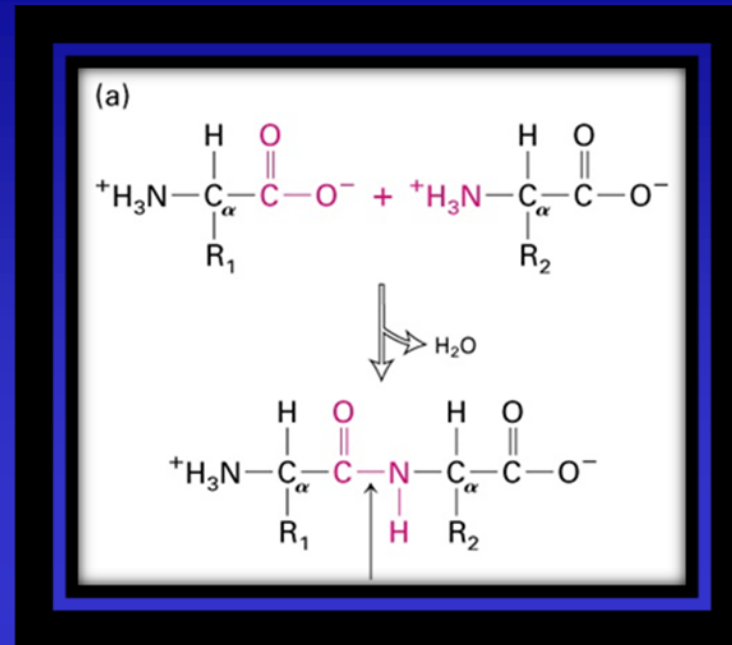




UNIVERSIDADE FEDERAL DE SÃO PAULO
DEPARTAMENTO DE BIOFÍSICA

Peptídeos e polímeros: exemplos de interação ciências básica-aplicada

CLÓVIS R. NAKAIE



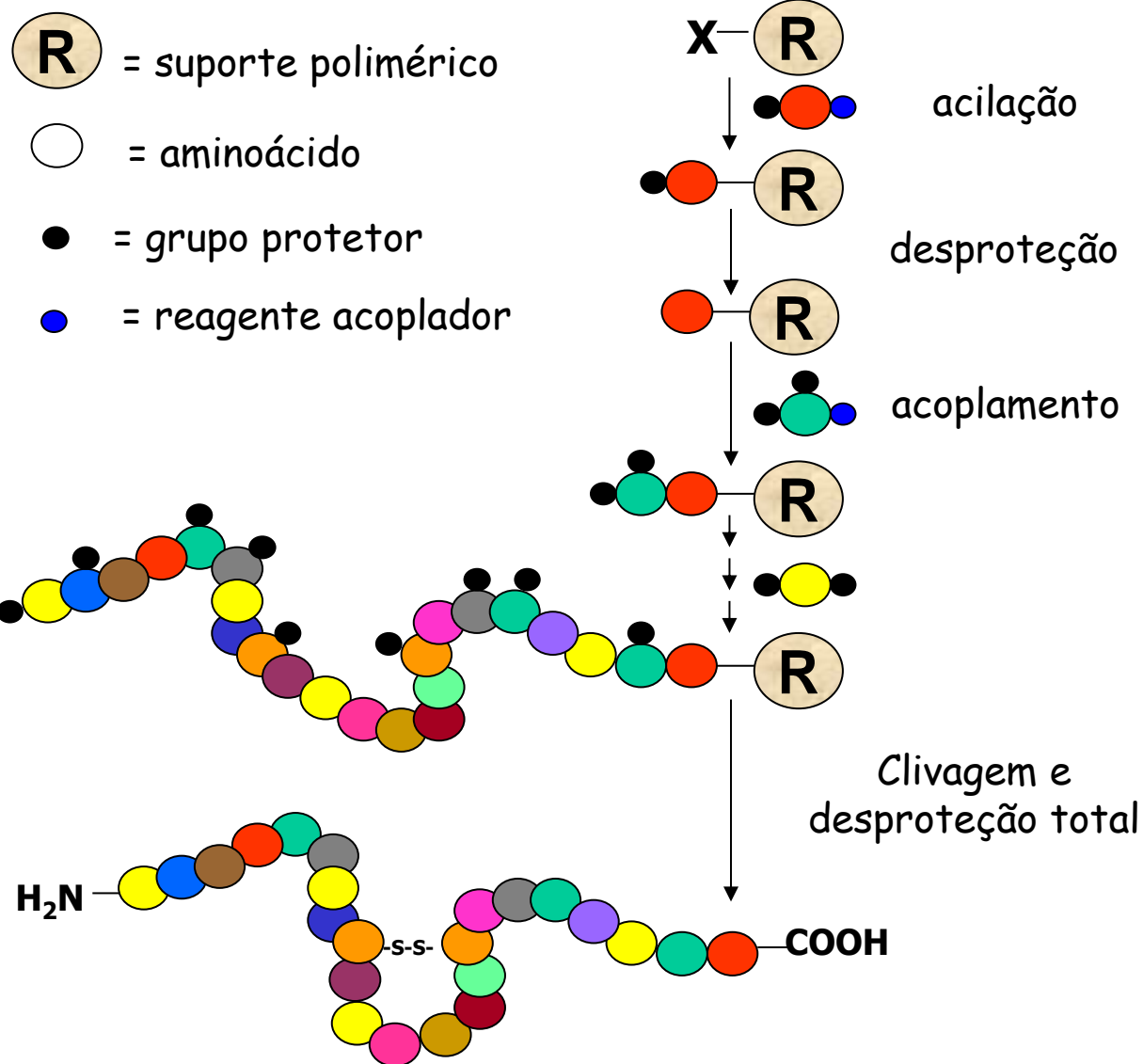
Representação da formação de uma ligação peptídica e estrutura de um pentapeptídeo

Peptídeos Biologicamente Ativos

- 1- Combinação de aproximadamente 50 aminoácidos (aa).
- 2- Alta atividade biológica (ativo em doses de picograma)
- 3- Isolamento: Baixas concentrações nas fontes naturais
Síntese química: Complexa e cara.

Peptídeo	Processo biológico/doença
1. Tireoliberina TRH (n=3 aa)	Doença da tiróide
2. Angiotensina II (n=8 aa)	Hipertensão arterial
3. Bradicinina (n=9 aa)	Hipotensão, inflamação, asma
4. Ocitocina (n=9 aa)	Parto e aleitamento
5. Desmopressina (n=9 aa)	Diabetes insipidus
6. MSH (n=13 aa)	Coloração da pele
7. Gastrina (n=17 aa)	Secreção gástrica
8. Leptina (n=49 aa)	Obesidade
9. GHRF (n=44 aa)	Crescimento
10. Insulina (n=51 aa)	Diabetes mellitus
11. Inibidor de aspartil-protease (n=3-6 aa)	AIDS (coquetel)

Síntese peptídica



Metodologia da SPFS

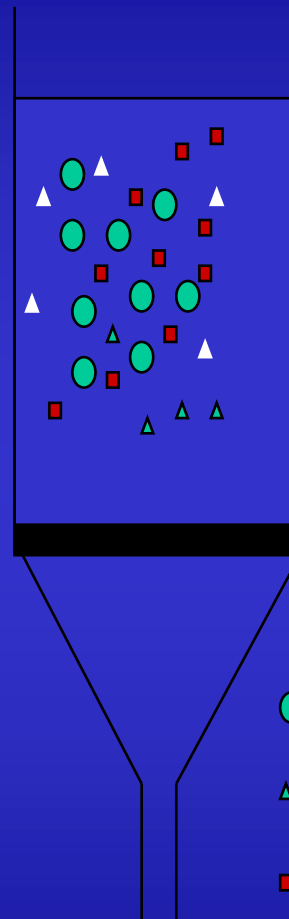
Experimento pioneiro:

J. Am. Chem. Soc., 1963, 85, 2149.

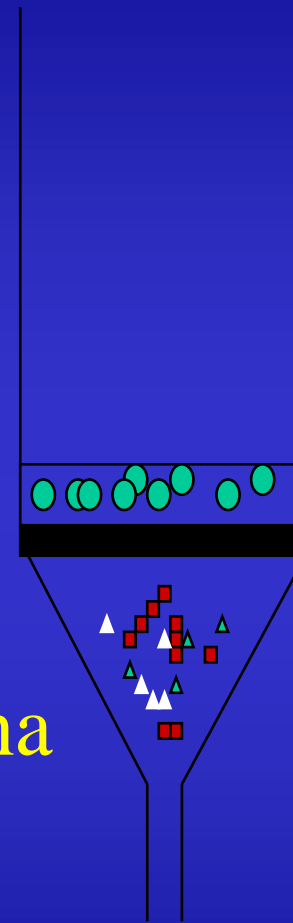
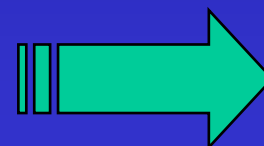
>4800 citações!



R. Bruce Merrifield
Prêmio Nobel, 1984

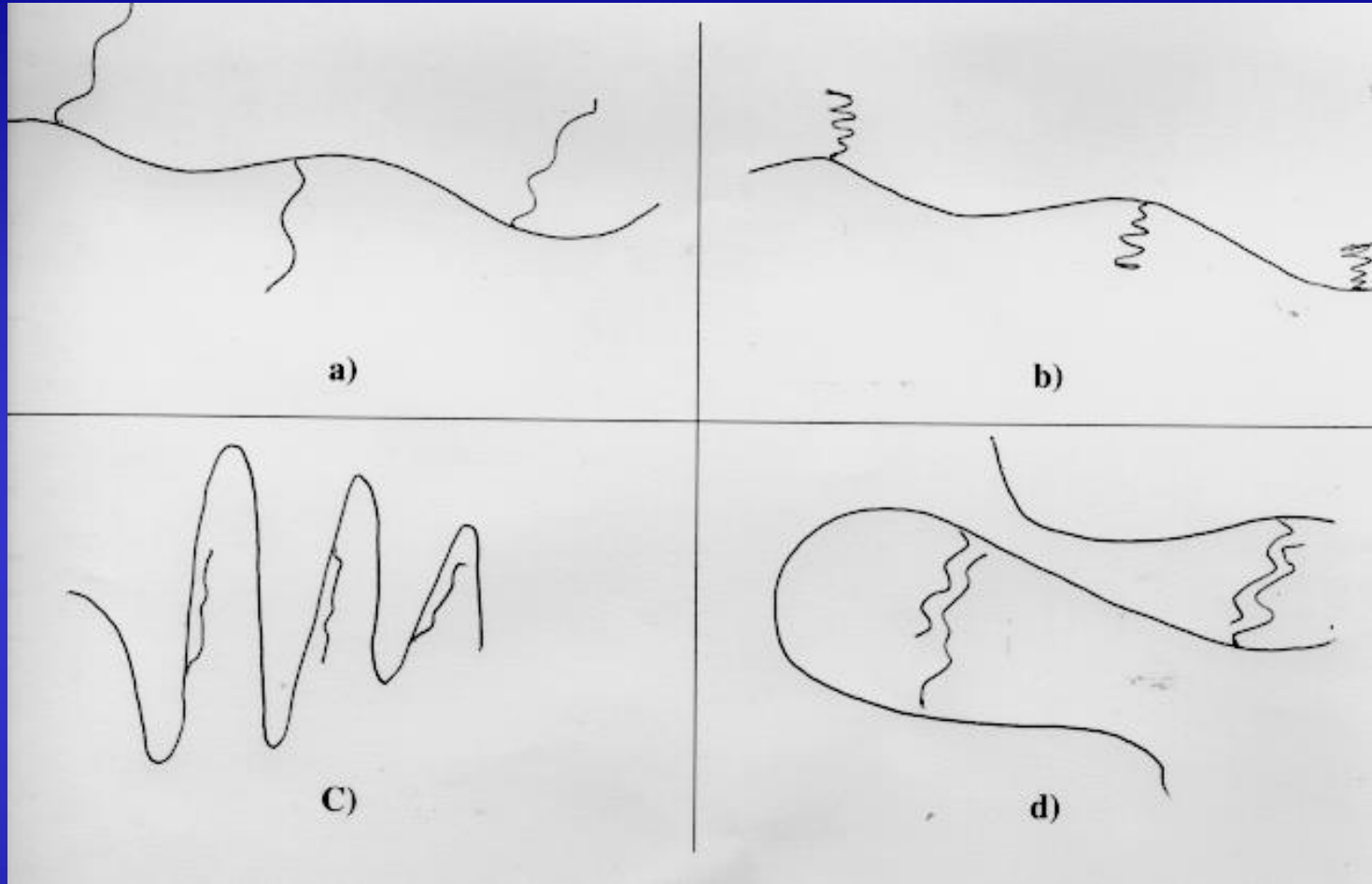


Vácuo



- Peptidil-resina
- △ Reagentes
- solventes
- △ subprodutos

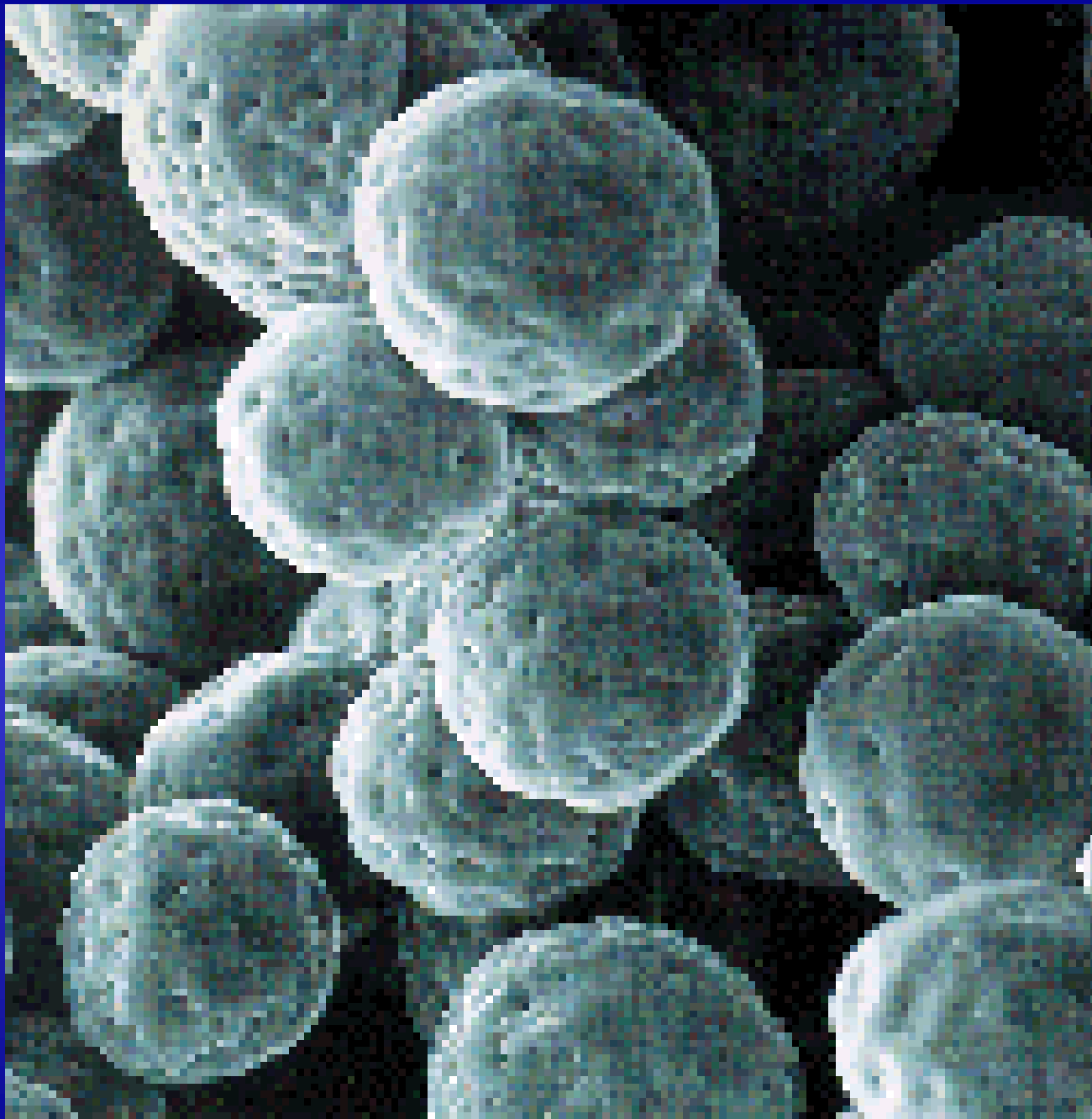




Peptide-polymer structures

Table: Calculated Product Yields of Peptides of Various Chain Lengths for Different Constant Yields per Cycle

Polypeptide, protein	Amino acid residues	Number of cycles	Yield per step			
			95%	98%	99%	99,9%
Angiotensin II	8	7	69,8	86,8	93,2	99,3
Oxytocin, vasopressin, bradykinin	9	8	66,3	85,1	92,3	99,2
Antamanide, gramicidin	10	9	63,0	83,4	91,4	99,1
α -Melanotropin	13	12	54,0	78,5	88,6	98,8
Gramicidin A	15	14	48,8	75,4	86,9	98,6
Insulin A-Chain	21	20	35,8	66,8	81,8	98,0
Insulin B-Chain	30	29	22,6	55,7	74,7	97,1
Staphylococcal nuclease-P	42	41	12,2	43,7	66,2	96,0
Ferredoxin	55	54	6,3	33,6	58,1	94,7
Bovine trypsin inhibitor	58	57	5,4	31,6	56,4	94,5
Cytochrome c	104	103	0,5	12,4	35,5	90,2
Ribonuclease A	124	123	0,2	8,3	29,1	88,4
Human growth hormone	190	189	0,006	2,2	15	82,8



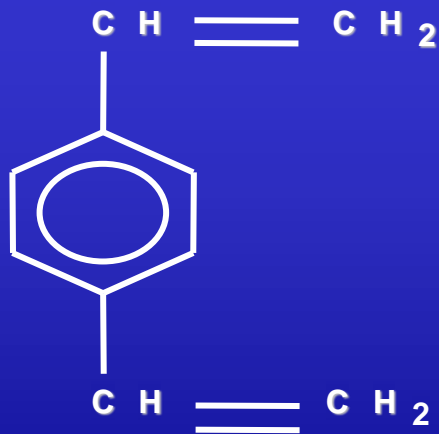
Características da Resina Ideal

- a) Conter **sítios ativos** onde deve ocorrer a ligação do aminoácido carboxi-terminal, através de ligação estável covalente;
- b) Possuir estrutura que possibilite a **rápida difusão dos reagentes** até os sítios de reação e que facilite a remoção de subprodutos, através de lavagens por filtração;
- c) Não introduzir **impedimentos estéricos** significativos nas reações químicas que ocorrem em seu interior;
- d) Não apresentar nenhum **outro sítio** reativo que possa induzir reações ou interações indesejadas;
- e) Ser **insolúvel** em todos os solventes utilizados;
- f) Apresentar completa **estabilidade** química e física.

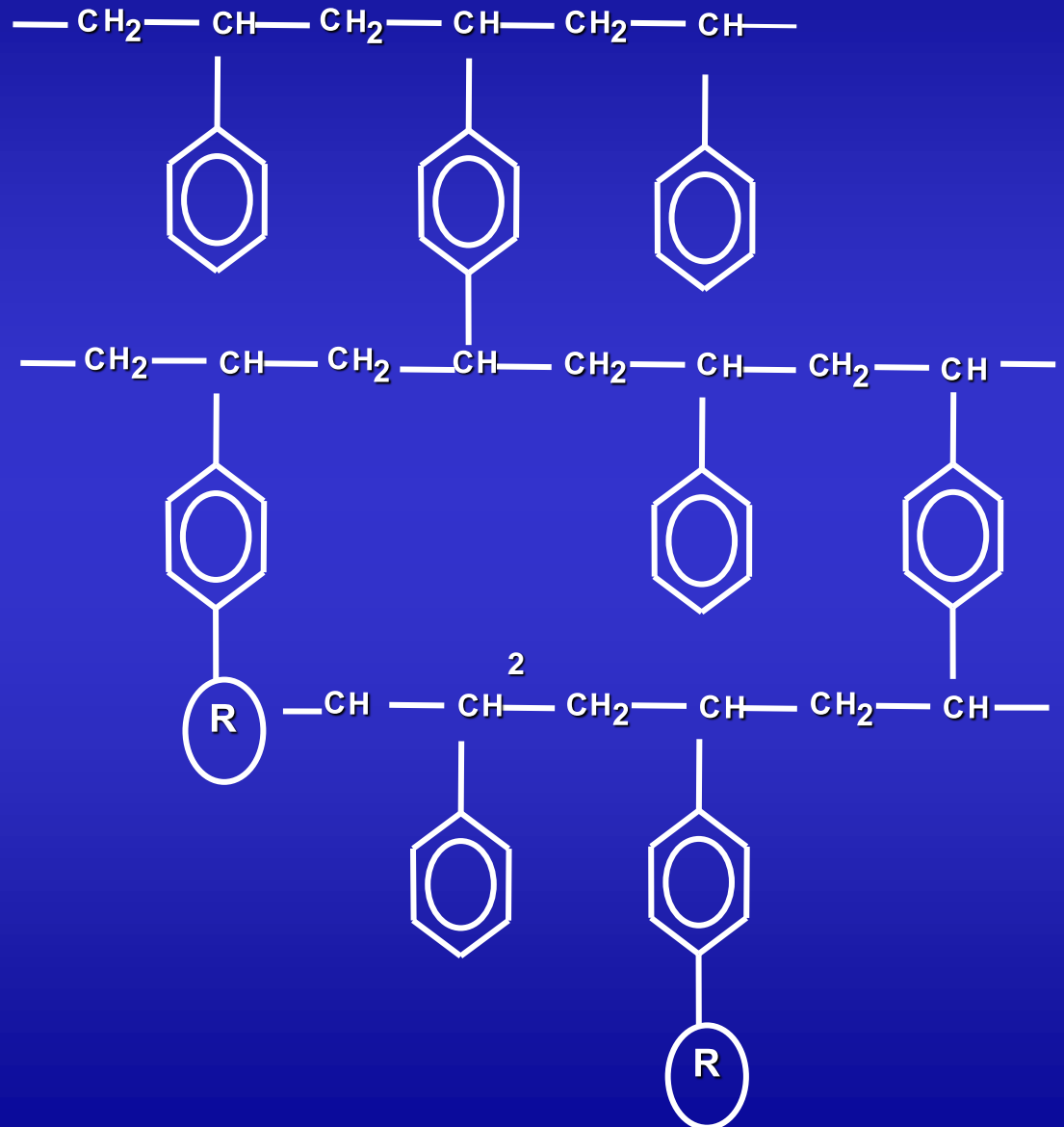
A resina mais tradicional: PS-DVB



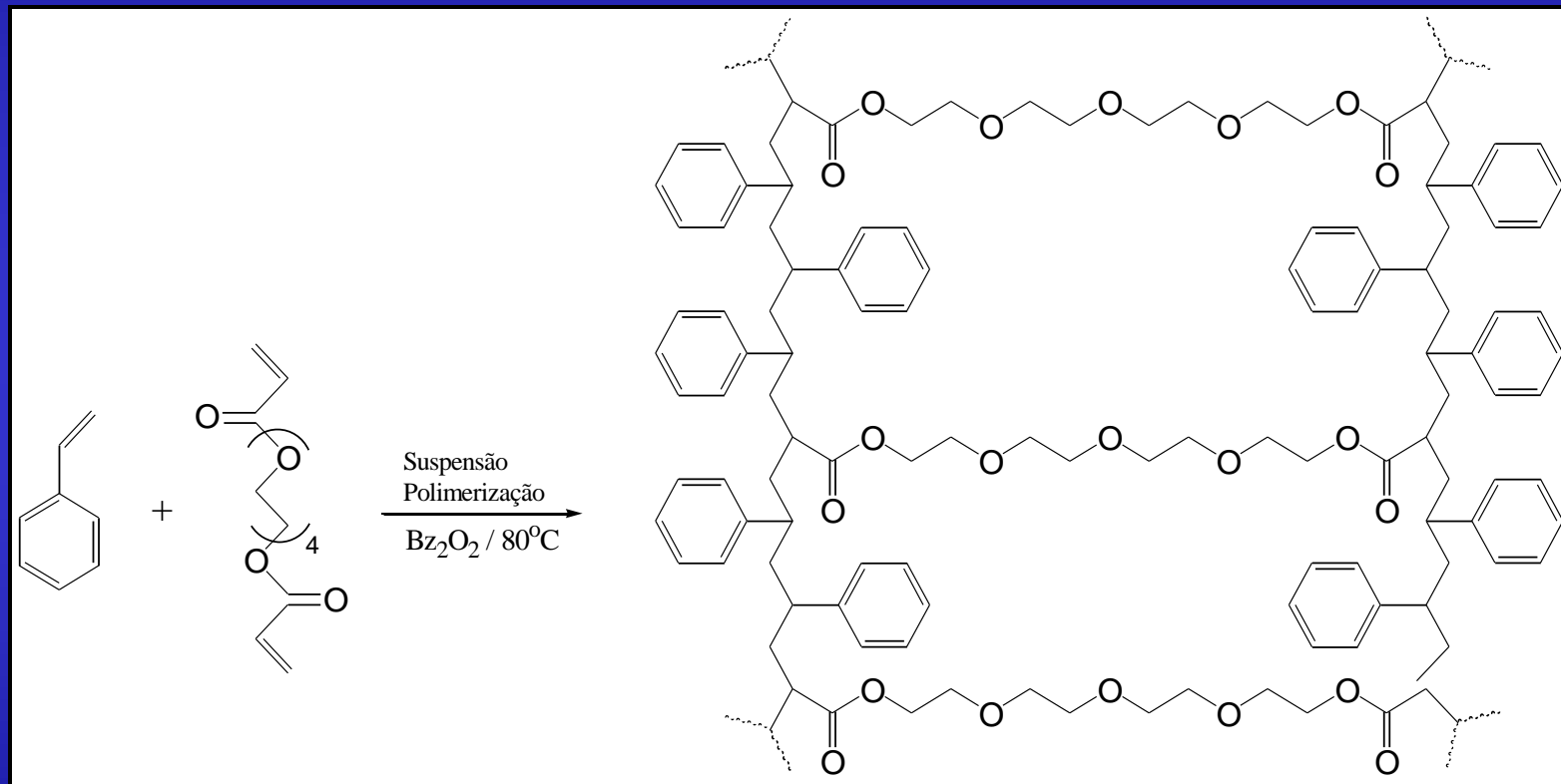
Estireno



Divinilbenzeno



Síntese do Copolímero de Estireno e Diacrilato de Tetraetenoglicol (EDTG)



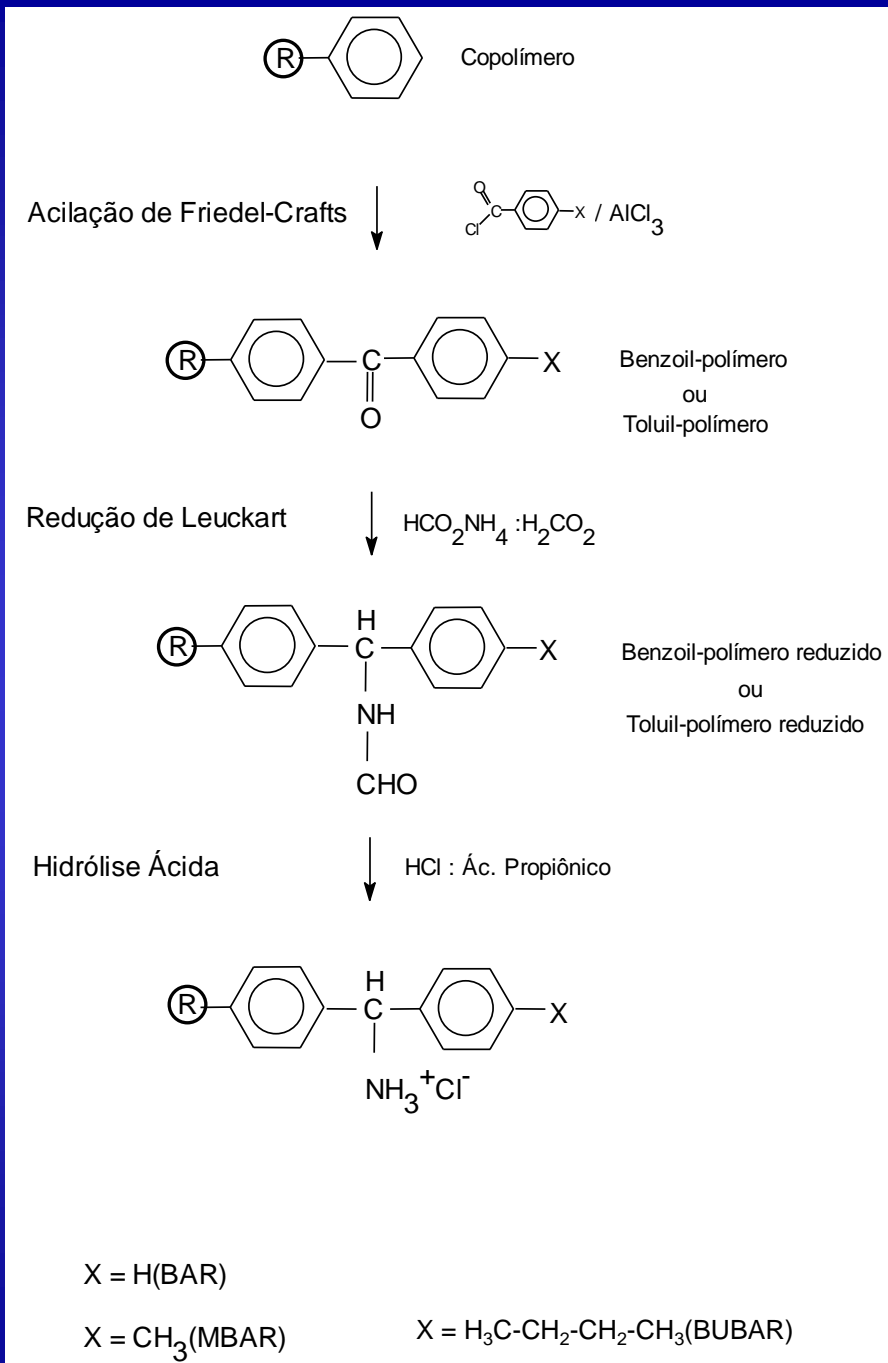
RESULTADOS

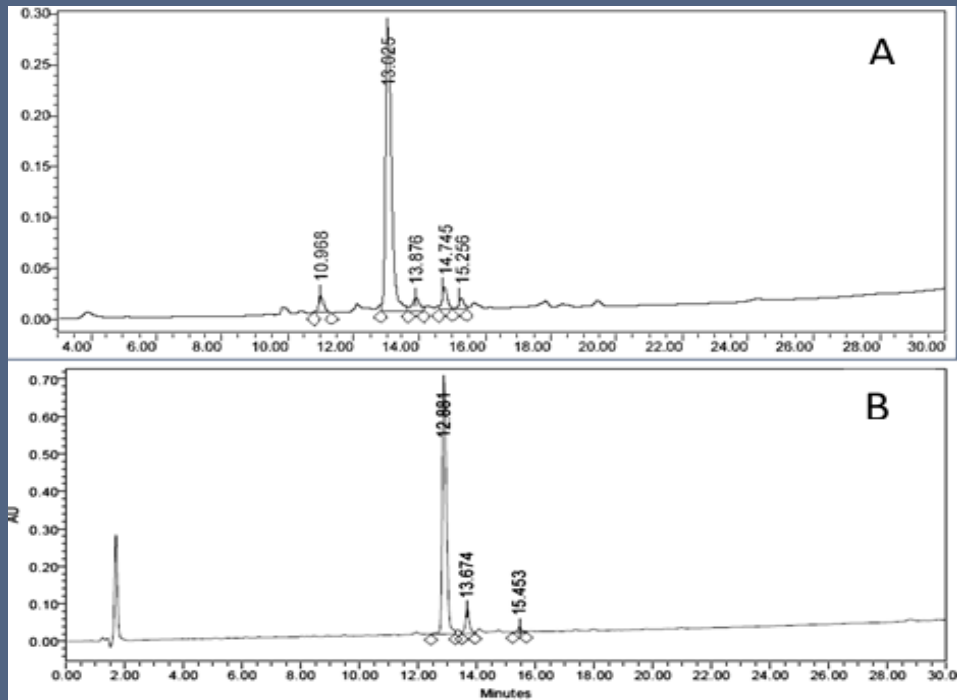
1. Síntese de Resinas

•BAR;

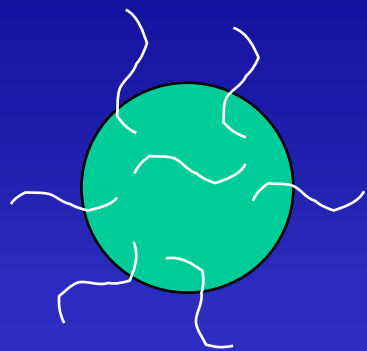
•MBAR;

•BUBAR.



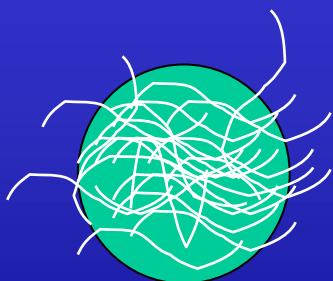
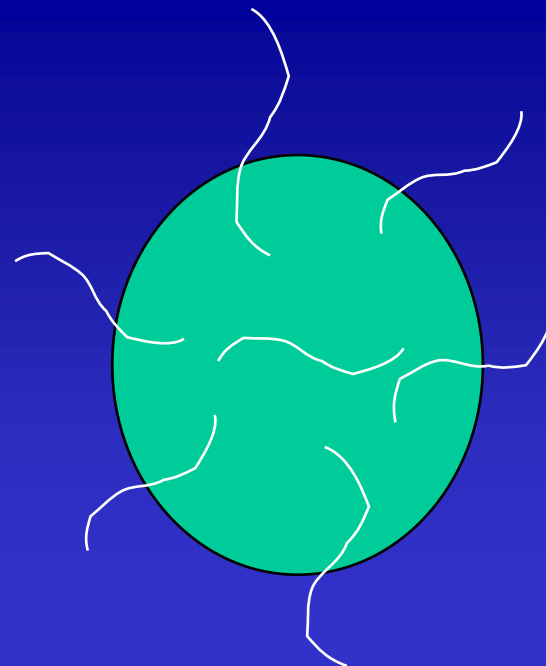


Perfis cromatográficos em HPLC dos peptídeos-modelo brutos (A) AngII-NH₂ e (B) Gly8-AngII-NH₂ clivados em HF anidro a 0°C 2 horas, sintetizados na BUBAR.



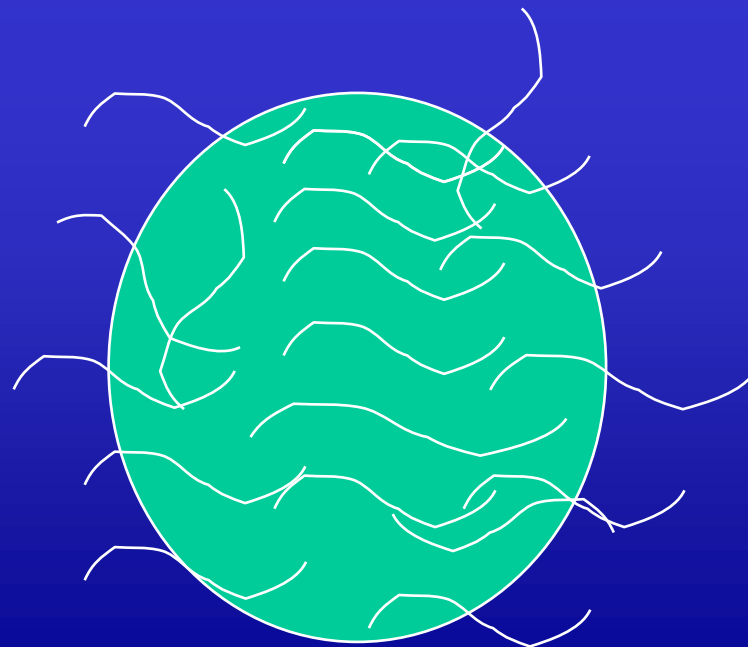
**baixo grau de
substituição**

solvatação



**alto grau de
substituição**

solvatação



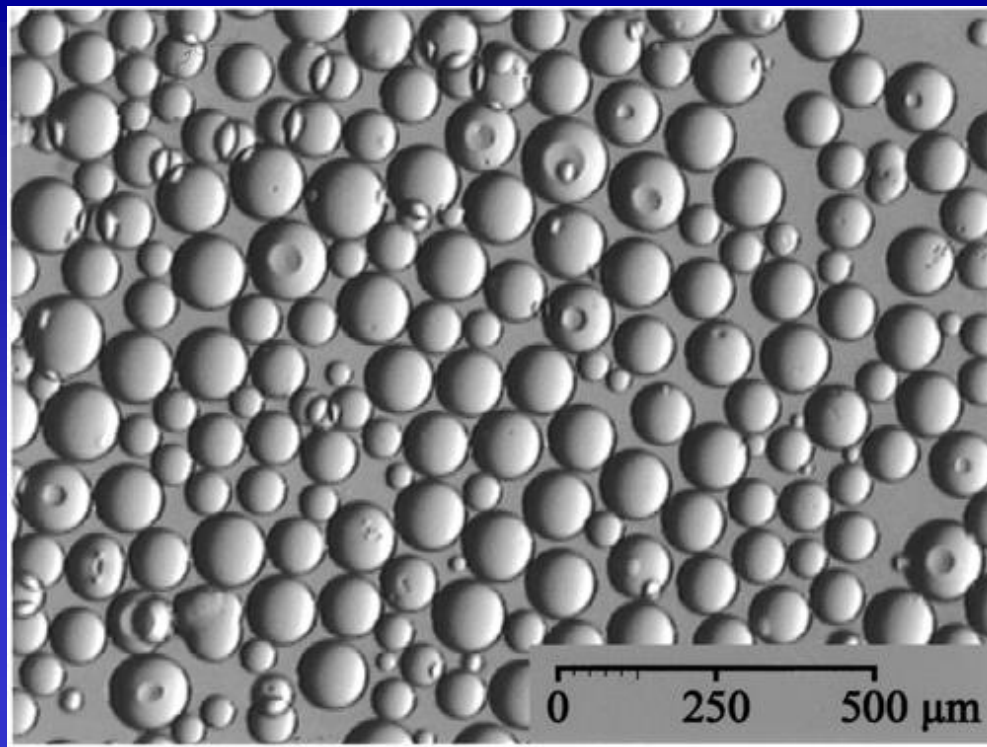
THEORETICAL YIELD OF SYNTHESIS WITH LOW AND HIGHLY SUBSTITUTED RESINS

Resin (mmol/g)	Weight of peptide (per 1g of starting resin)	
	MW = 1000 (8 aa)	MW = 4000 (35 aa)
0.3 mmol/g	0.3 g (PC = 30%)	1.2 g (PC = 60%)
3.0 mmol/g	3.0 g (PC = 80%)	12.0 g (PC = 95%)

•PC = peptide content

Solvation Studies of Polymers

a) Microscopic Measurement of Bead Sizes

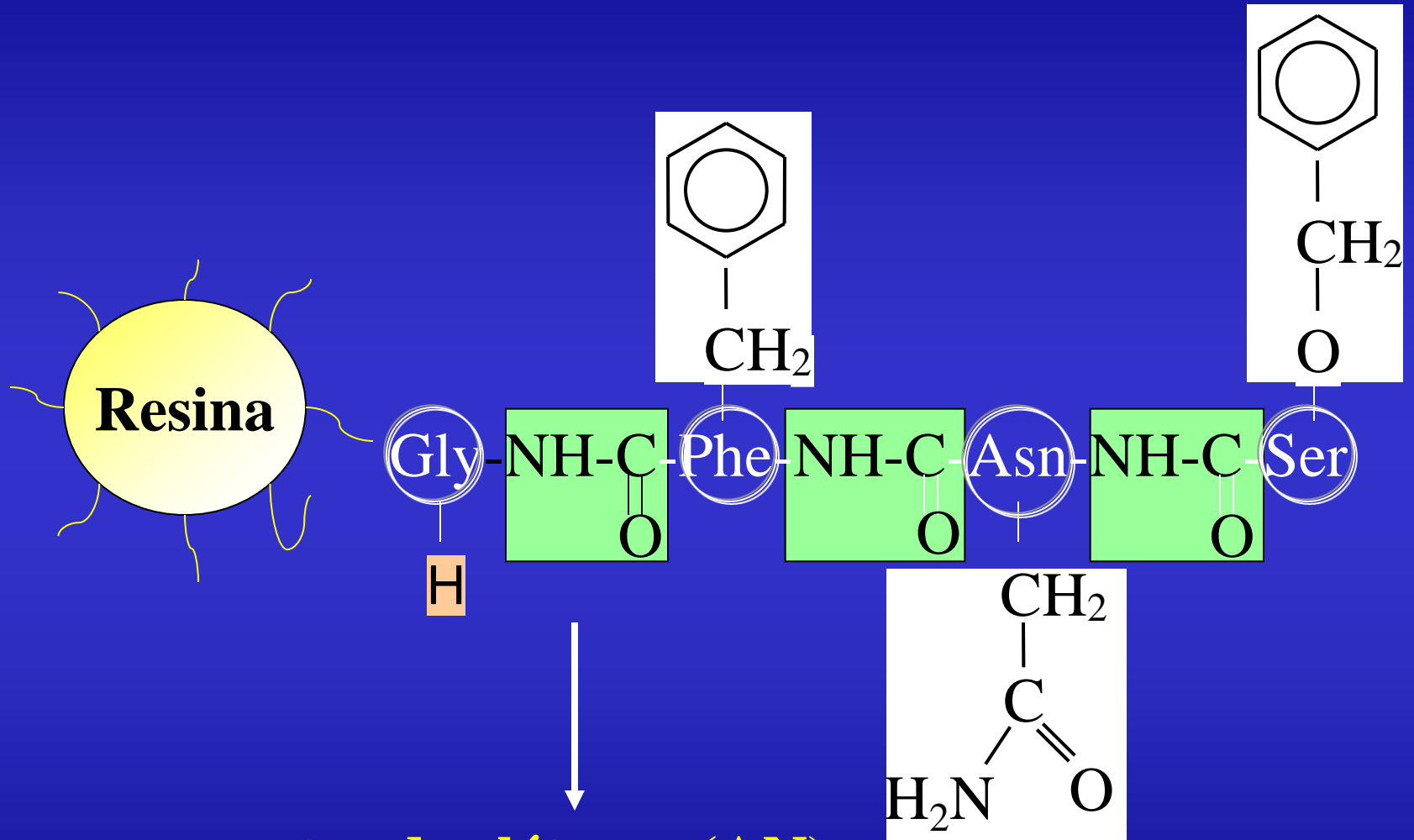


Microscopia óptica dos grãos de resina

Resinas	Solvente DCM			
	Diâm. grãos secos ^a (μm)	Diâm. grãos solvatados ^a (μm)	Volume do solvente por grão ^b ($10^5 \mu\text{m}^3$)	Solvente dentro do grão ^c (%)
AEDTG 0,7 mmol/g	45	75	1,7	78

^aMédias geométricas; ^bvolume grão solvatado - volume grão seco; ^cporcentagem do volume do grão solvatado ocupado pelo solvente

Peptidil-Resina

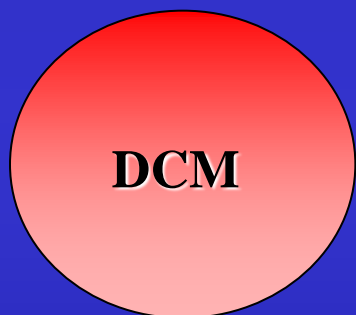
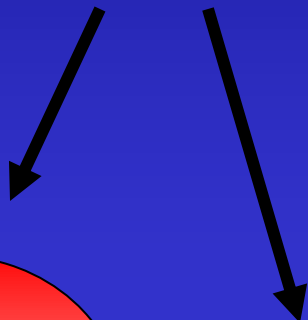


N-H → receptor de elétrons (AN)

C=O → doador de elétrons (DN)

BAR

Seco



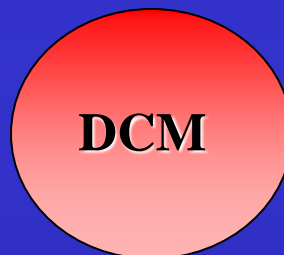
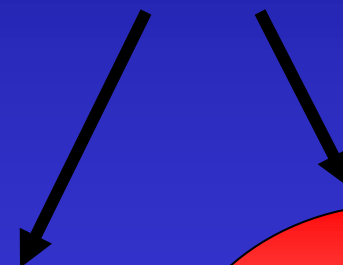
85% solvente



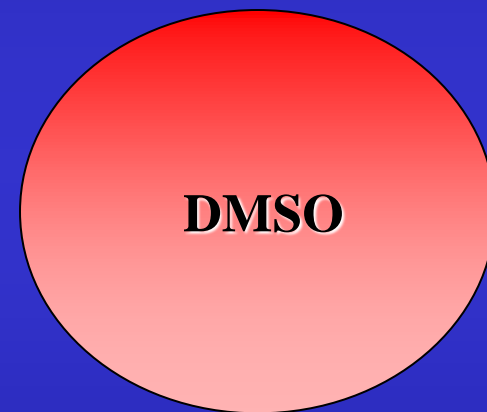
33% solvente

(NANP)₄-BAR

Seco



42% solvente



85% solvente

Solvatação

Inchamento da BAR e da (NANP)₄-BAR de 1,4 mmol/g
(68% de conteúdo peptídico)

CORRELAÇÃO ENTRE
SOLVATAÇÃO DE POLÍMEROS E
POLARIDADE DO SOLVENTE

Parâmetros do solvente e grau de inchamento de resinas

	Solvente	ϵ	AN	DN	(AN+DN)	Resina ^a (% inchamento)			
						1	2	3	4
1	Tolueno	2,4	3.3	0.1	3.4	87	64	26	40
2	DCM	8,9	20.4	1.0	21.4	84	79	46	52
3	Clorofórmio	4,7	23.1	4.0	27.1	83	83	53	64
4	NMP	33,0	13.3	27.3	40.6	67	75	70	64
5	DMF	36,7	16.0	26.6	42.6	70	70	75	57
6	DMSO	46,7	19.3	29.8	49.1	51	71	76	65
7	TFE	26,7	53.5	0.0	53.5	28	77	63	60
8	EtOH	24,3	37.1	32.0	69.1	19	53	38	40
9	MeOH	32,6	41.3	30.0	71.3	17	59	45	41
10	Formamida	109,5	39.8	24.0	63.8	23	61	61	46
11	50% TFE/Tolueno	14,6	28.4	0.1	28.5	71	82	62	64
12	20% TFE/DCM	12,5	27.0	0.8	27.5	72	78	70	60
13	50% TFE/DCM	17,8	36.9	0.5	37.5	56	80	73	58
14	80% TFE/DCM	23,1	46.9	0.2	47.4	42	80	75	65
15	20% DMSO/NMP	35,7	14.5	27.8	42.3	73	71	65	61
16	50% DMSO/THF	27,1	13.7	24.9	38.6	65	68	62	55
17	65% NMP/THF	24,1	11.5	24.8	36.1	79	75	68	66
18	50% DCM/DMF	22,8	18.2	13.8	32.0	70	76	66	61
19	50% DCM/DMSO	27,8	19.9	15.4	35.3	68	69	68	65
20	50% MeOH/DMSO	39,7	30.3	29.9	60.2	25	66	72	56
21	50% TFE/DMF	31,7	34.8	13.3	48.1	27	69	29	47
22	50% TFE/DMSO	36,7	36.4	14.9	51.3	28	70	31	47
23	10% TEA/DCM	8,3	18.5	6.6	25.1	76	81	60	62
24	10% TEA/DMF	33,3	14.5	30.0	44.5	66	78	69	65
25	10% TEA/DMSO	42,3	17.5	32.9	50.4	47	72	71	64
26	20% PIP/DCM	8,3	16.3	8.8	25.1	78	76	55	nd
27	20% PIP/DMF	30,5	12.8	29.3	42.1	73	75	66	nd
28	20% PIP/DMSO	38,5	15.4	31.8	47.2	62	71	70	nd
29	Acetonitrila (ACN)	36,0	18.9	14.1	33.0	32	65	24	36
30	Acetona (ACE)	20,7	12.5	17.0	29.5	48	63	21	40
31	2-Propanol (iPrOH)	18,3	33.5	36.0	69.5	14	46	10	37

^a [(volume solvatado – volume seco) / volume solvatado] x 100 utilizando os seguintes valores para as medidas dos diâmetros dos grãos secos: Resinas: **1**=50 μm **2**=114 μm , **3**=87 μm , **4**=94 μm , nd = não determinado.

Resinas:

1. BAR

2. PAC-PEG-PS

3. (NANP)₃-Nle-BAR

4. VHHQKLVFFAEDV-BAR

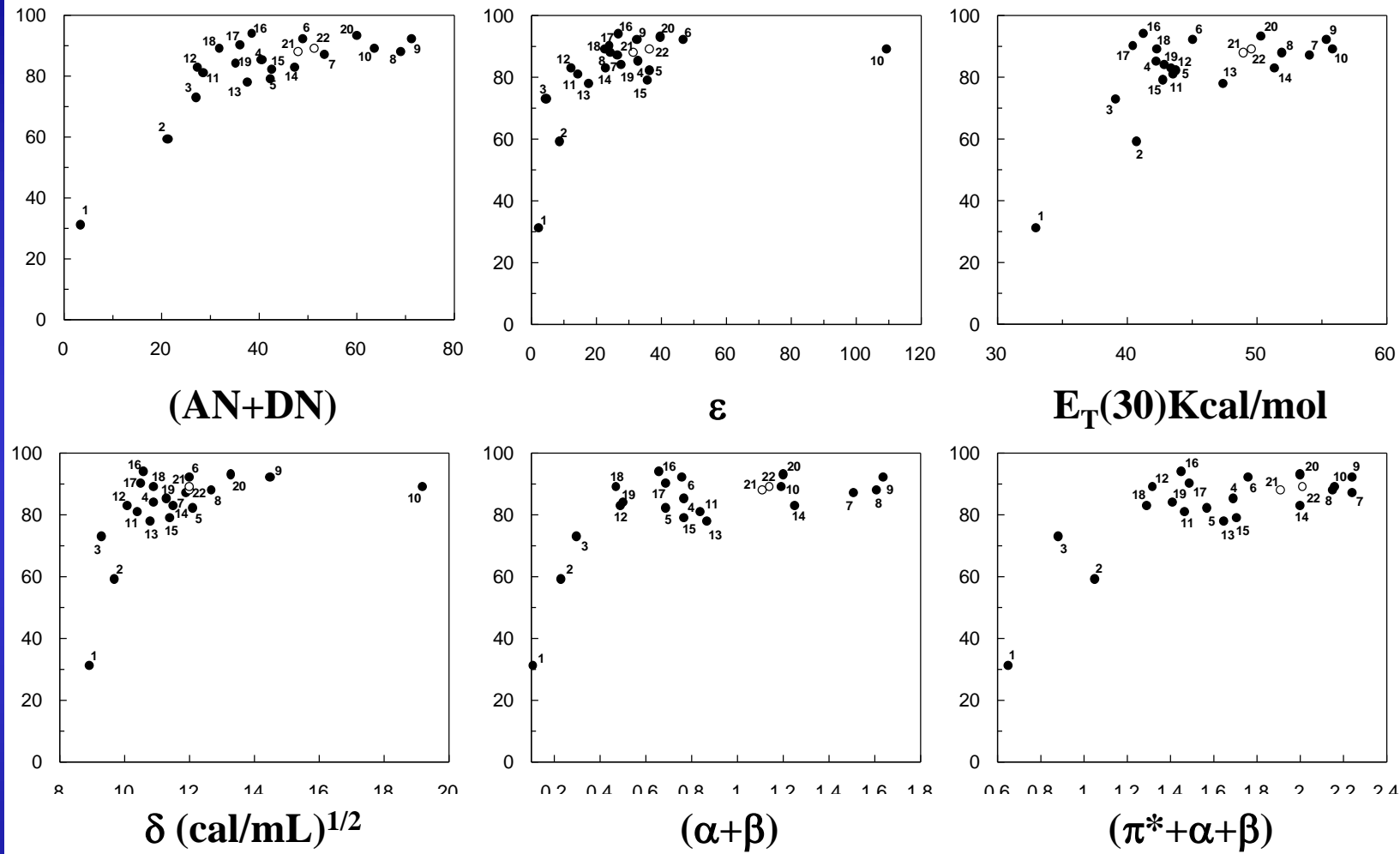
(fragmento 12-24 do peptídeo β -amiloicde - 42 aa)

Alguns Parâmetros de Solvente Utilizados

- a) Constante dielétrica (ϵ);
- b) Parâmetro $E_T(30)$ - Dimroth-Reichardt
- c) Parâmetro de Solubilidade - Hildebrand (δ);
- d) Parâmetro π^* - Kamlet-Taft
- e) Parâmetros α e β (capacidade doadora e receptora protônica, respectivamente) – Kamlet-Taft
- f) Parâmetros AN e DN (receptor e doador eletrônico, respectivamente) – Gutmann.
- g) Parâmetro de polaridade testado : (AN+DN)

Resina BAR-NH₃⁺

Volume de solvente dentro do grão (%)



Inchamento da resina (1), BAR-NH₃⁺, 2.4 mmol/g em função dos parâmetros de polaridade (AN+DN), ϵ , $E_T(30)$, δ , $(\alpha+\beta)$ e $(\pi^*+\alpha+\beta)$ de solventes.

Escala do solvente (AN+DN).

Solvente	(AN+DN)	Solvente	(AN+DN)
n-Hexano	0.0	N-Metil-pirrolidinona (NMP)	40.6
n-Heptano	0.0	Dimetilacetamida	41.4
Tolueno	3.4	N,N-dimetilformamida (DMF)	42.6
Benzeno	8.3	Dietilacetamida	45.8
Tetracloroeto de carbono	8.6	Piridina	47.3
1,1-Dicloroetano	16.2	Dimetilsulfóxido (DMSO)	49.1
1,2-Dicloroetano	16.7	Hexametilfosforamida	49.4
Nitrobenzeno	19.2	Hexametilfosforamida	49.4
Dicloroetilenocarbonato	19.9	1,1,1-Trifluoroetanol (TFE)	53.5
Diclorometano (DCM)	21.4	2-Feniletanol	56.8
Dietiléter	23.1	N-Metilformamida	59.1
Nitrometano	23.2	Dietilamina	59.4
Dioxano	24.6	Álcool benzílico	59.8
Acetato etílico	26.4	Etilamina	60.3
Acetato metílico	27.0	Trietilamina (TEA)	62.4
Clorofórmio	27.1	Formamida	63.8
Benzonitrila	27.4	t-Butanol	65.1
Tetrahidrofurano (THF)	28.0	1-Butanol	65.8
Acetona	29.5	Etanol (EtOH)	69.1
Dimetoxietano	30.2	2-Propanol	69.5
Acetonitrila	33.0	Metanol (MeOH)	71.3
Propileno CO ₃	33.4	Água	72.8
Tributilfosfato	33.6	Ácido acético	72.9
Sulfolano	34.0	Diaminoetano	75.9
Sulfano tetrametileno	34.0	Etilenodiamino	75.9
4-Butirolactona	35.3	Ácido fórmico	102.6
Tetrametiluréia	38.8	Ácido trifluoracético (TFA)	105.0
Trimetilfosfato	39.3	Ácido trifluormetanosulfônico	129.1
Piperidina (PIP)	40.0		

Cilli et al. J.Org.Chem. (1996), 81, 8992

Malavolta et al. Tetrahedron (2002),58, 4383

Oliveira et al. Eur. J. Org. Chem. (2002), 3686

Malavolta e Nakaie.Tetrahedron (2004) 60, 9417

SOLVATION STUDIES OF POLYMERS

b) Electron Paramagnetic Resonance Spectroscopy

Ressonância Paramagnética Eletrônica (RPE)

- Definição: método espectroscópico que detecta compostos que possuem **elétron desemparelhado**.
- Inúmeros compostos possuem elétrons desemparelhados: ex: **radicais livres, íons de transição metálica, etc.**
- Compostos com elétrons desemparelhados são geralmente muito **instáveis** mas participam de processos cruciais como na fotossíntese, oxidações, catálises e reações de polimerização.

ESTUDOS ESPECTROSCÓPICOS

ESPECTRO ELETROMAGNÉTICO

Técnicas:

Rotacional
NMR
EPR
Raman
e Vibracional
Absorção ótica
Fluorescência
Espalhamento de luz
Dicroísmo Circular
Visível e UV
Difração
de Raios-X

Comprimento de onda (m)



Ondas largas

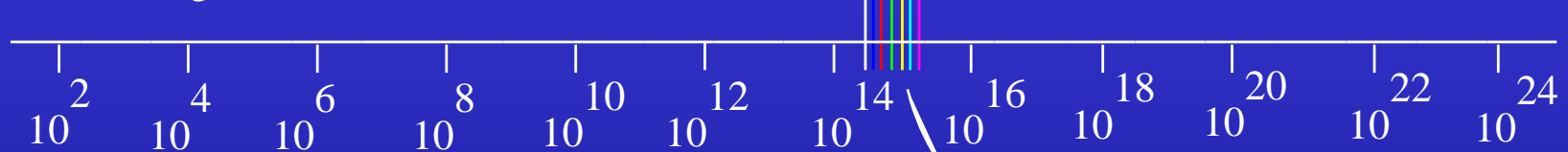
Ondas de rádio

Infravermelho

Ultravioleta

Raios-X

Raios Gama



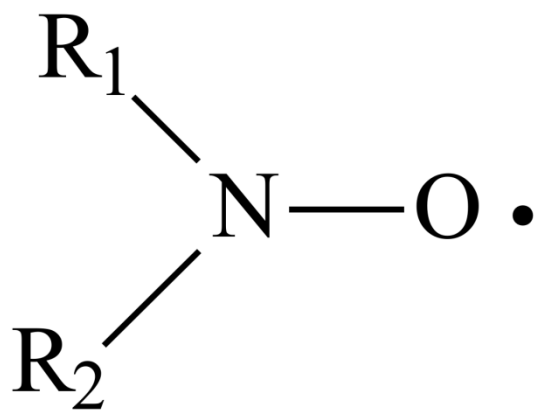
Frequência (Hz)

**Espectro Visível
(400 a 700nm)**

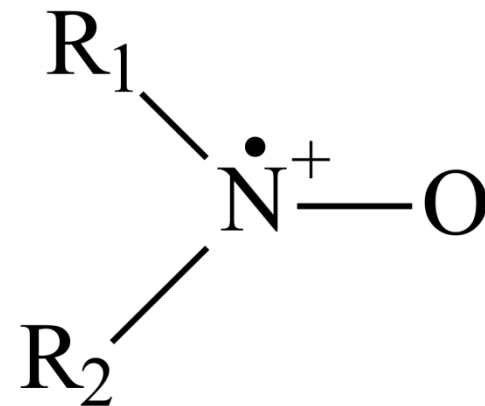
ESTUDOS ESPECTROSCÓPICOS

As técnicas espectroscópicas obtêm informação do sistema estudado analisando a radiação que é transmitida ou espalhada.



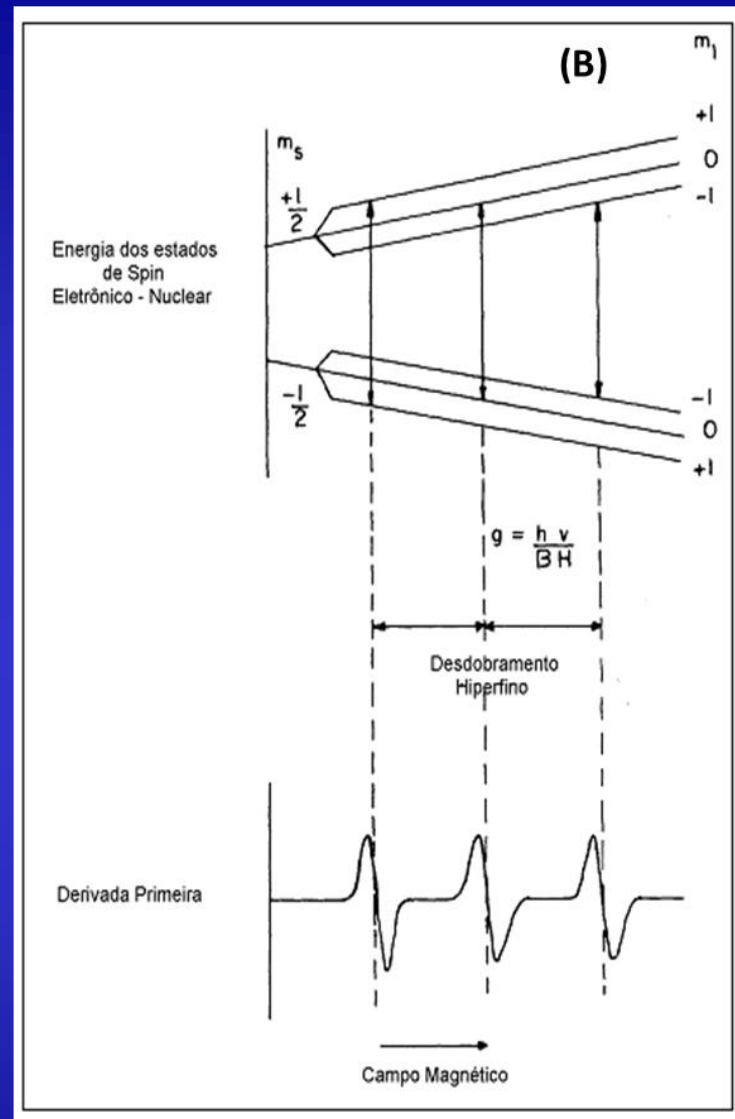
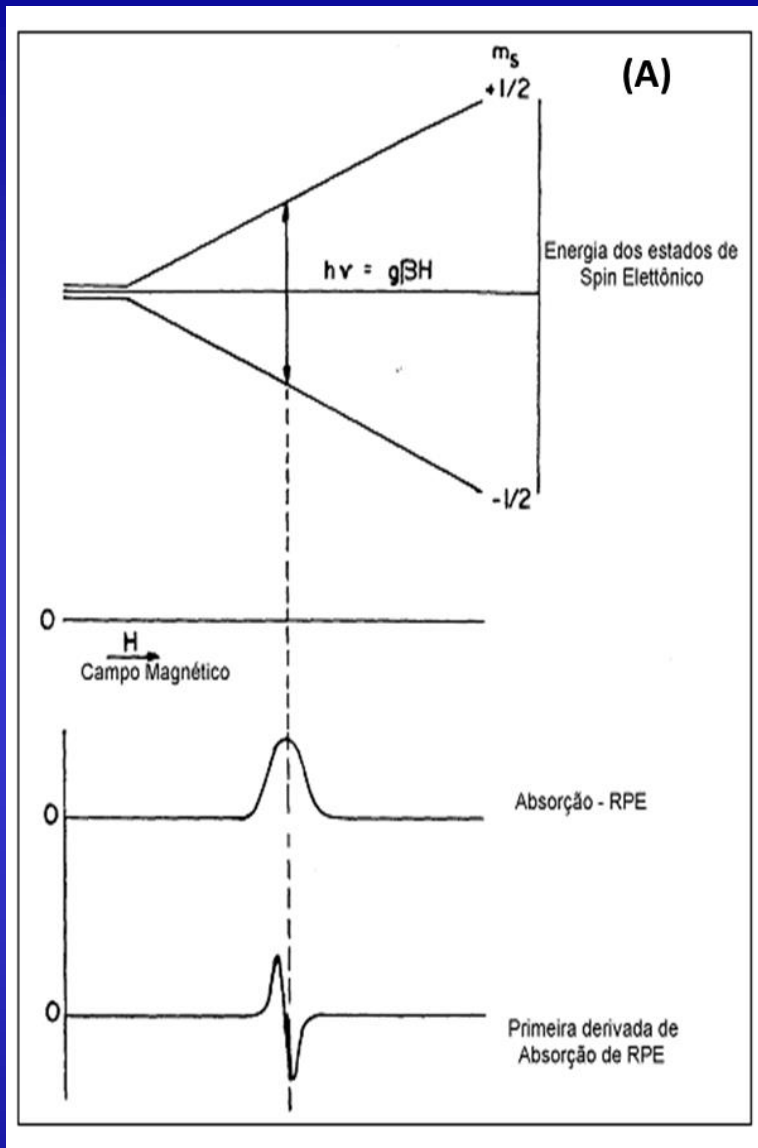


(I)

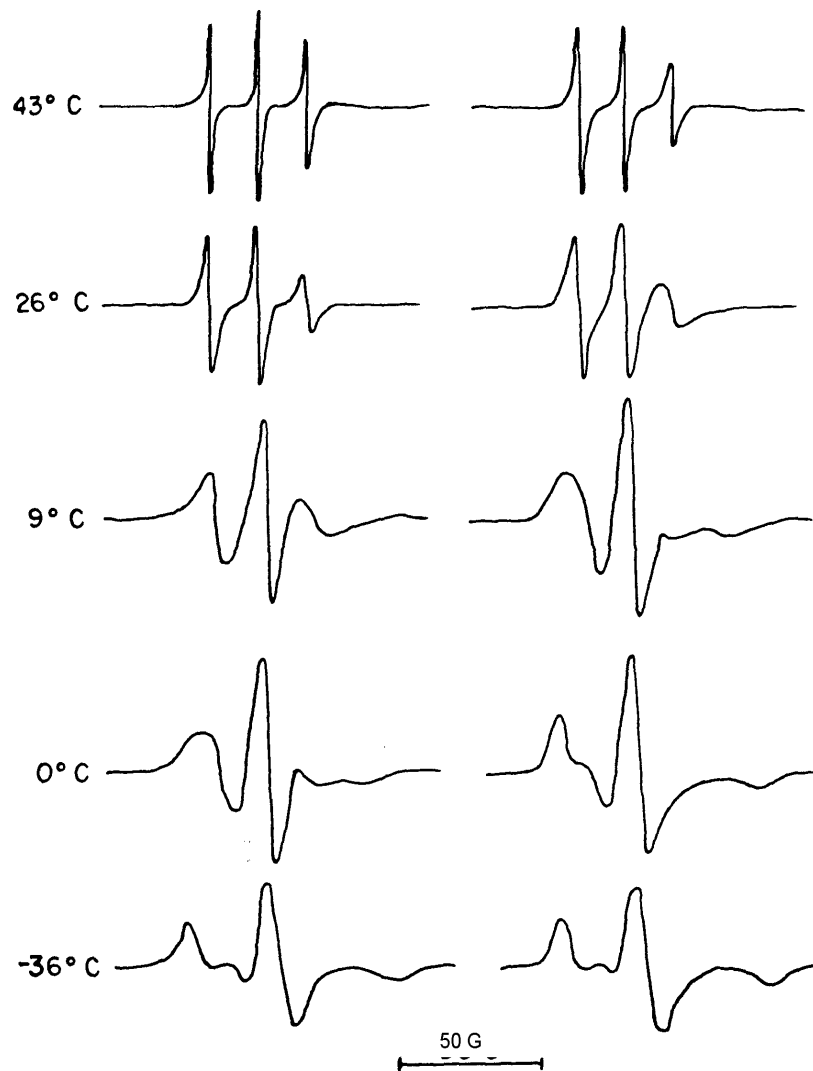
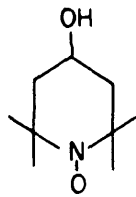
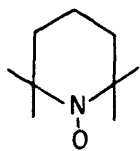


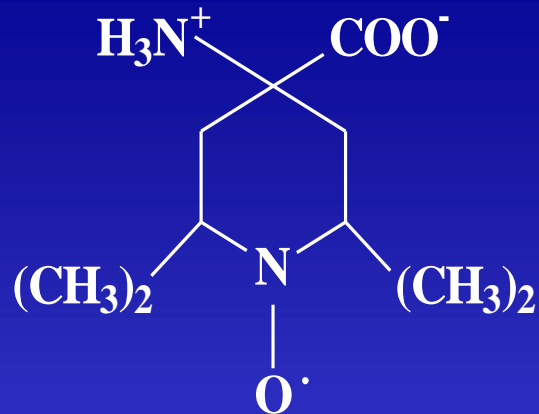
(II)

Formas canônicas do radical nitróxido.



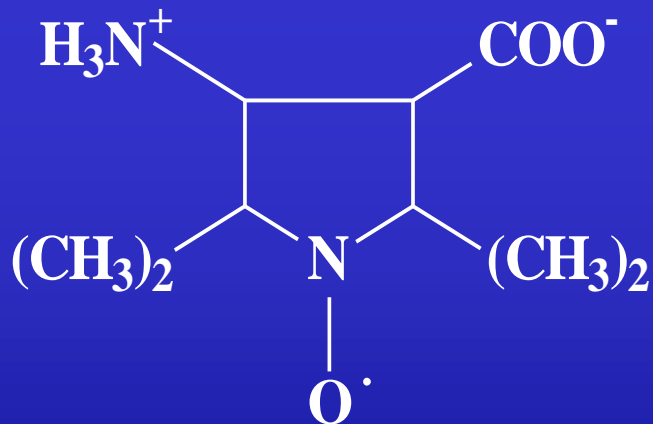
(A) Desdobramento dos níveis do spin eletrônico em presença de um campo magnético; (B) Desdobramento dos níveis de energia do spin eletrônico para um átomo com spin nuclear $I = 1$.





TOAC

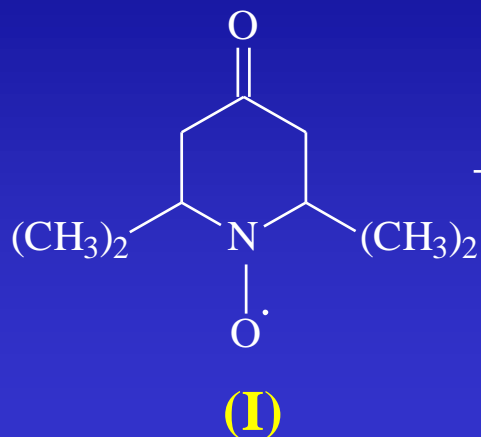
2,2,6,6-tetramethylpiperidine-1-oxyl-4-amine-4-carboxylic acid



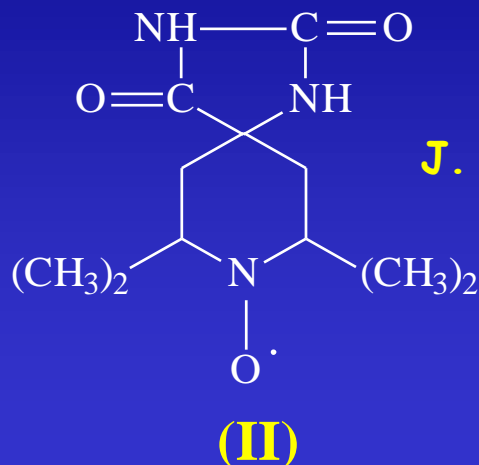
POAC

2,2,5,5-tetramethylpyrrolidine-1-oxyl-3-amine-4-carboxylic acid

Síntese do Fmoc-TOAC

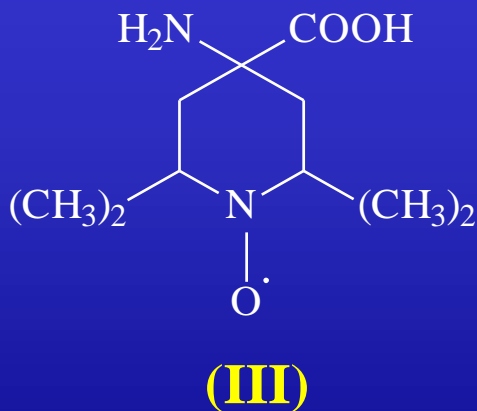


4-oxo-Tempo (**TEMPO**)

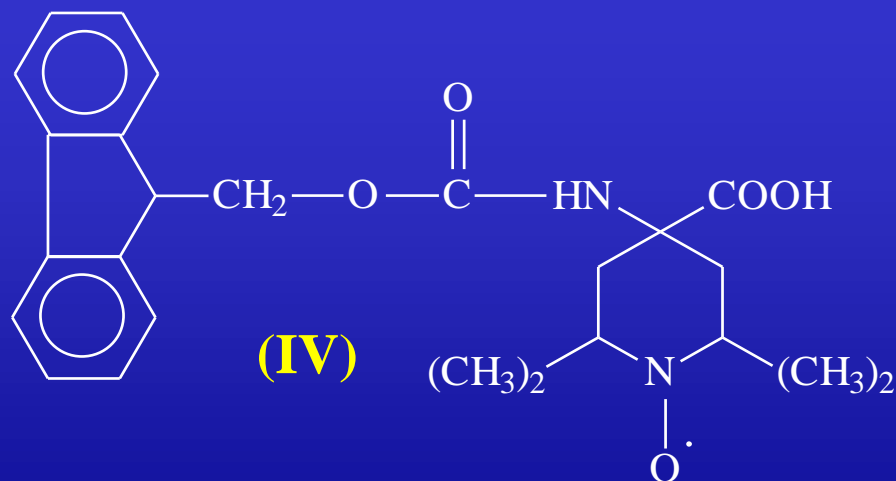


Hidantoína-5,4'-(2',2',6',6'-tetrametil-piperidina-N-óxido (**ESPIRO**))

Marchetto, Schreier & Nakaie
J. Am. Chem. Soc. (1993) 115, 11042



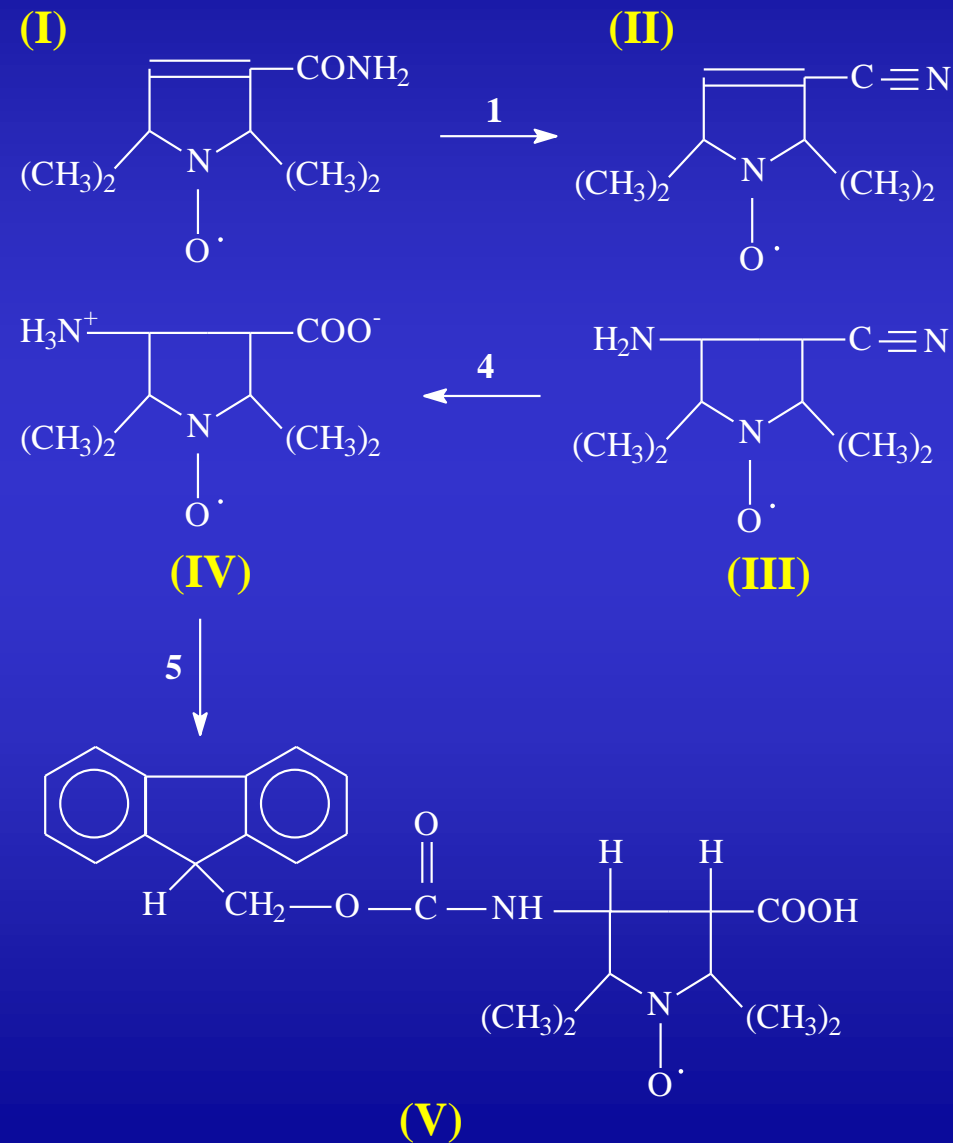
Ácido 2,2,6,6-tetrametilpiperidina-N-óxido
4-amino-4-carboxílico (**TOAC**)



Ácido N^α-(9-fluorenilmetiloxicarbonil)-2,2,6,6-tetrametilpi-
peridina-N-óxido-4-carboxílico (**Fmoc-TOAC**)

Síntese do Fmoc-POAC

Chem. Pharm. Bull.(2001)49, 1027



I) 2,2,5,5-tetrametil-3-carbamidopirrolidina-1-oxil

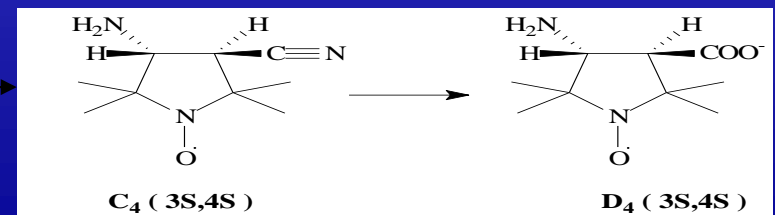
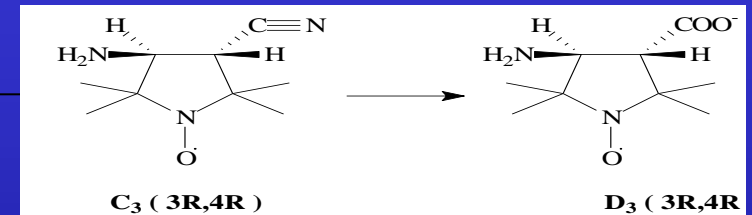
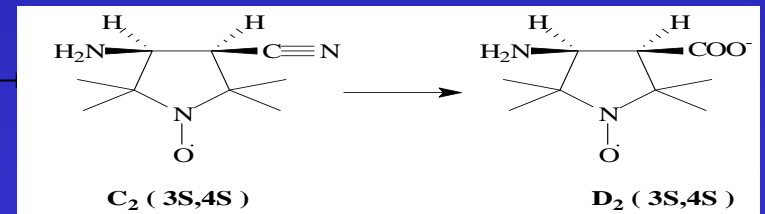
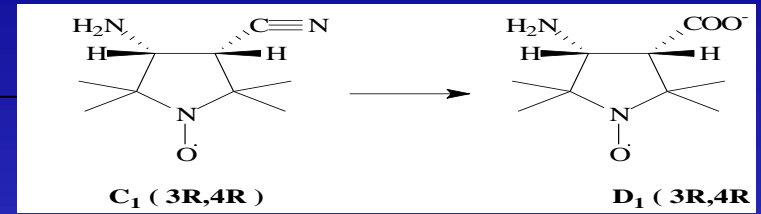
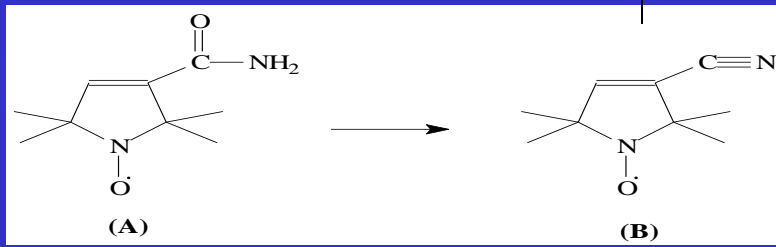
II) 2,2,5,5-tetrametil-3-cianopirrolidina-1-oxil

III) 2,2,5,5-tetrametil-3-ciano-4-amino-pirrolidina-1-oxil

IV) Ácido 2,2,5,5-tetrametilpirrolidina-N-oxil-3-amino-4-carboxílico

V) Ácido (9-fluorenilmetoxicarbonil)-2,2,5,5-tetrametilpirrolidina-N-oxil-3-amino-4-carboxílico

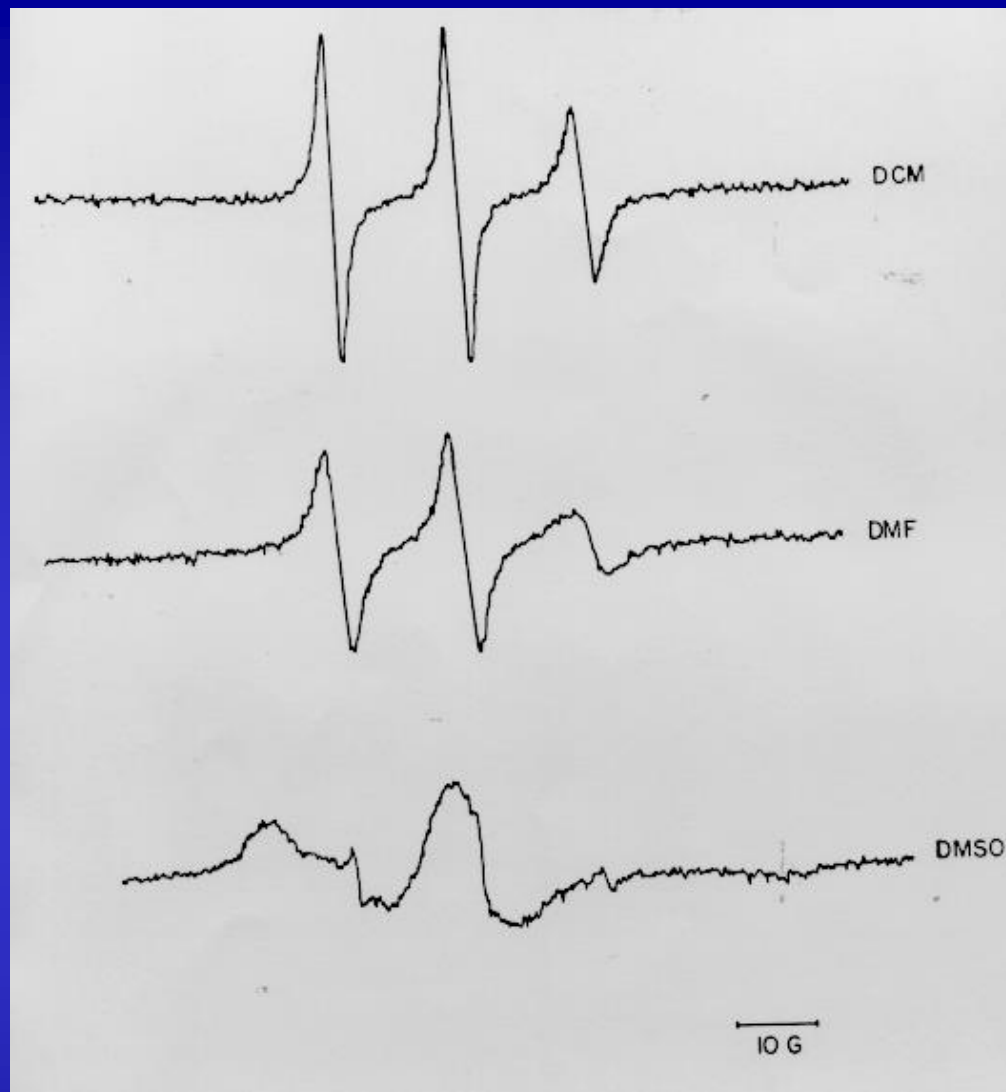
Síntese do POAC e a sua estereoquímica



Toronto Research Chemicals, Inc. Spin Labels

<u>Cat. #</u>	<u>Chemical</u>	<u>Size / Price US\$</u>
087400	1-Oxyl-2,2,5,5-Tetramethylpyrroline-3-Carboxylate N-Hydroxysuccinimide Ester NHS mw 281.29 $C_{13}H_{17}N_2O_5$	OXYL-1- 10 mg \$45.00 100 mg \$360.00
	A highly amine reactive spin-label.	
087500	(1-Oxyl-2,2,5,5-Tetramethyl-β-3-Pyrroline-3-Methyl) Methanethiosulfonate MTSL mw 264.30 $C_{10}H_{16}NO_3S_2$	10 mg \$45.00 100 mg \$360.00
	A highly reactive thiol-specific spin-label. A specific conformational probe of thiol site structure by virtue of its minimal rotational freedom and distance from the covalent disulfide linkage to the macromolecule under study. Berliner, L.J., et al.: Analytical Biochemistry, 119, 450 (1982)	
087505	(1-Oxyl-2,2,5,5-Tetramethyl-β-3-Pyrroline-3-Methyl) Methanethiosulfonate-d15 MTSL-d15 mw 279.48 $C_{10}H_3D_{15}NO_3S_2$	10 mg \$650.00 100 mg \$5200.00
	A thiol reactive spin label compound used to probe the conformation and dynamics of thiolated proteins.	
087510	(1-Oxyl-2,2,5,5-Tetramethyl-β-3-Pyrroline-3-Methyl) Methanethiosulfonate-15N-D15 MTSL-15N-d15 mw 280.49 $C_{10}H_3D_{15}NO_3S_2$	5 mg \$650.00 50 mg \$5200.00
	A thiol reactive spin label compound used to probe the conformation and dynamics of thiolated proteins.	
087600	(1-Oxyl-2,2,5,5-Tetramethylpyrroline-3-Yl)carbamidoethyl Methanethiosulfonate MTS-4-Oxyl mw 321.43 $C_{12}H_{21}N_2O_4S_2$	10 mg \$81.00 100 mg \$648.00
	A highly reactive thiol-specific spin-label. A specific conformational probe of thiol site structure by virtue of its minimal rotational freedom and distance from the covalent disulfide linkage to the macromolecule under study.	
T30450	2,2,6,6-Tetramethylpiperidine-N-Oxyl-4-Amino-4-Carboxylic Acid TOAC mw 215.27 $C_{10}H_{19}N_2O_5$	10 mg \$45.00 100 mg \$360.00

A stable free radical spin label.
Marchetto, R. et al.: J.A.C.S., 115, 11042 (1993)



RPE spectra of Boc-TOAC-BHAR (0.25 mmol/g) in DCM, DMF and DMSO

VOLUME 70, NUMBER 12

JOC *Articles*

JUNE 10, 2005

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Determination of Site–Site Distance and Site Concentration within Polymer Beads: A Combined Swelling-Electron Paramagnetic Resonance Study

Reinaldo Marchetto,[†] Eduardo M. Cilli,[†] Guita N. Jubilut,[‡] Shirley Schreier,[§] and
Clovis R. Nakaie^{*,‡}

Department of Biochemistry and Technological Chemistry, Institute of Chemistry, UNESP, Araraquara, São Paulo 14800-900, Brazil, Department of Biophysics, Universidade Federal de São Paulo, Rua 3 de Maio 100, CEP 04044-020 São Paulo, Brazil, and Department of Biochemistry, Institute of Chemistry, Universidade de São Paulo, CP 26077, 05513-970 São Paulo, SP, Brazil

clovis.biof@epm.br

Received September 20, 2004

TABLE 1. Swelling Parameters of Differently Labeled Boc-TOAC-BHAR in DCM

	sample									
	col 1	col 2	col 3	col 4	col 5	col 6	col 7	col 8	col 9	col 10
BHAR ^a (mmol/g)	diam dry bead (μm)	diam swollen bead (μm)	vol solvent/ bead ($10^5 \mu\text{m}^3$)	vol dry sample/g copol ^b (mL)	wt dry sample/g copol (g)	vol dry sample/g sample (mL)	no. of beads/g sample (10^7)	no. of sites/bead (10^{12})	site-site distance (\AA)	site concn (mM)
0.003 ^c	57	98	4.0	1.8	1.17	1.54	1.59	0.1	169.8	0.4
0.019 ^c	57	98	4.0	1.8	1.18	1.53	1.58	0.7	88.9	2.9
0.035 ^c	57	98	4.0	1.8	1.18	1.53	1.58	1.3	72.2	5.4
0.050 ^c	57	98	4.0	1.8	1.18	1.53	1.58	1.9	63.7	7.9
0.065 ^d	57	98	4.0	1.8	1.19	1.51	1.56	2.5	58.1	10.4
0.134 ^d	57	98	4.0	1.8	1.22	1.48	1.53	5.2	45.5	21.7
0.646 ^e	57	98	4.0	1.8	1.45	1.24	1.28	30.3	25.3	126.3
0.988 ^f	58	99	4.1	1.9	1.66	1.14	1.12	52.9	21.2	215.0

^a Degree of Boc-TOAC-OH labeling. ^b Copolymer of styrene-1% divinylbenzene: $d = 0.99 \text{ g/mL}$; average diameter of dry beads = $47 \mu\text{m}$. ^c Obtained from 0.050 mmol/g of BHAR. ^d Obtained from 0.14 mmol/g of BHAR. ^e Obtained from 0.80 mmol/g of BHAR. ^f Obtained from 1.40 mmol/g of BHAR.

TABLE 4. Correlation between Yield^a of Boc-Pro-OH Coupling to BHAR (1.40 mmol/g) and Site Concentration and Site-Site Distance Values

solvent	site concn (M)	site-site distance (Å)	coupling (%)
DCM	0.21	21.7	90
DMF	0.55	17.0	67
DMSO	1.76	14.2	25

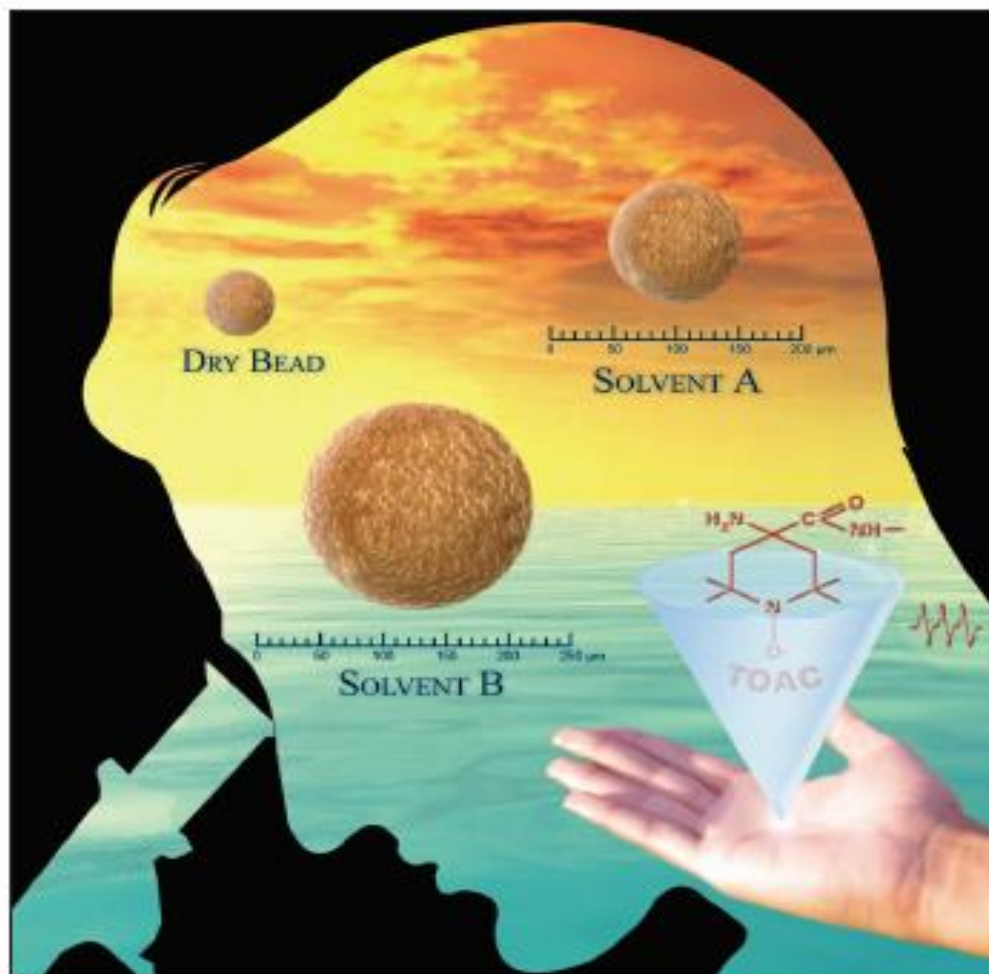
^a Yield of Boc-Pro-OH coupling after 30 min, at 25 °C with PSA method in equimolar conditions (1 mM of reactants).

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The Journal of Organic Chemistry
VOLUME 70 NUMBER 12 JUNE 10, 2005

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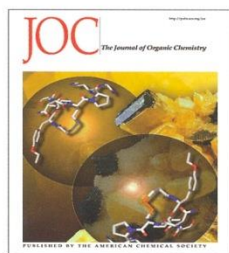
PUBLISHED BY THE AMERICAN CHEMICAL SOCIETY

The Journal of Organic Chemistry

2006 Calendar



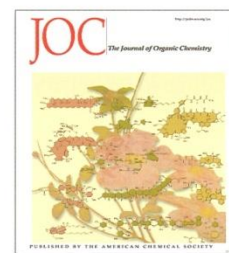
January



February



March



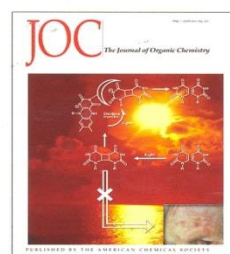
April



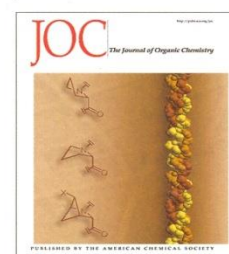
May



June



July



August



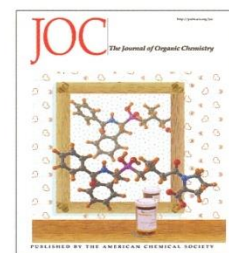
September



October



November



December



January 2007

On the Cover:

Determination of the Absolute Configuration of Flexible Molecules by ab Initio ORD Calculations: A Case Study with Cytotoxazones and Isocytoxazones

EGIDIO GIORGIO, MARIN ROJE, KATSUNORI TANAKA, ZDENKO HAMERSAK, VITOMIR SUNJIC, KOJI NAKANISHI, CARLO ROSINI, AND NINA BEROVA
J. Org. Chem.; 2005; 70(17) pp 6557 - 6563; (Article) DOI: 10.1021/jo048023+

(+)-cis-Isocytoxazone, a structural isomer of the natural cytokine modulator (-)-cis-cytoxazone, exemplifies a flexible molecule. Ab initio calculations of its optical rotations (OR) by Density Functional Theory at four wavelengths (589, 546, 435, and 405 nm) on 10 optimized conformations within 2 kcal/mol led to Boltzmann average OR values in excellent agreement with the experimental OR data in three different solvents. See Berova and co-workers, p 6557.



A NEW SPIN: PEPTIDE PROBES

Unnatural amino acid TOAC, a novel spin label used in EPR studies, helps illuminate peptide structure

Elizabeth K. Wilson
C&EN West Coast News Bureau

In this golden age of biotechnology, proteins and their abbreviated siblings, peptides, are under remarkable scrutiny. And as with proteins, the folding and structure of peptides are of immense interest to biochemists. They want to know precisely what drives a peptide to adopt a certain structure and how peptides interact with other peptides, proteins, and cell membranes—this knowledge will generate innumerable and invaluable leads for therapeutic drug design and other biological applications.

Scientists, of course, have numerous tools at their fingertips with which to study peptides and proteins, including nuclear magnetic resonance spectroscopy and X-ray crystallography. And thanks to recent advances in technology, electron paramagnetic resonance (EPR) spectroscopy, sometimes also referred to as electron spin resonance (ESR), has moved alongside these tried-and-true methods.

EPR has a number of powerful advantages. Like NMR, it measures the energy required to flip the spin of a particle in the presence of an electromagnetic field, except that the particle that has its spin flipped is an unpaired electron rather than a nucleus. Pairs of electrons interact much more strongly than nuclei, and so the EPR technique is more sensitive over longer distances. EPR experiments can be done in solution and in solids, and one can obtain spectra in real time at a resolution of 1 millisecond.

Although any chemical entity with an unpaired electron (such as a radical) can give rise to an EPR spectrum, the source of the unpaired electron used in EPR investigations often is a so-called



From left, São Paulo spin label researchers Schreier, Nakaie, and Paiva.

spin label—a moiety such as a nitroxide or a metal that chemists affix to a molecule.

Nitroxide spin labels typically used in peptide and protein studies are attached through flexible chains. For example, a common flexibly linked spin label consists of a disulfide group with methylene groups on either side, attached to a cysteine residue. But because these chains flop around, the resulting EPR spectra tend to be muddy, making it difficult to obtain accurate information.

But now a unique spin label that's particularly well suited for peptides is making a splash with some biochemists. The label is a little-known unnatural amino acid dubbed TOAC (2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid). TOAC has the advantage of being rigid and so yields extremely accurate information about the twists and turns of a peptide's backbone. It is also a strong helix stabilizer—that is, its presence in a peptide chain helps form α - and 3_10 helices, two common secondary structures in polypeptides.

The molecule actually has been around since the 1960s. In the early 1980s, then-graduate student Clovis R. Nakaie, guided by biophysics professor Antonio C. M. Paiva at the Federal Uni-

versity of São Paulo, in Brazil, and biochemistry professor Shirley Schreier at the University of São Paulo, figured out how to attach a TOAC derivative to the ends of a peptide.

But inserting TOAC site-specifically into the middle of a peptide was a more difficult problem. Unlike other spin labels, TOAC is a complete amino acid in and of itself, and so it can't be simply added to a peptide side chain. In order to insert TOAC, it must be worked into the peptide synthesis itself.

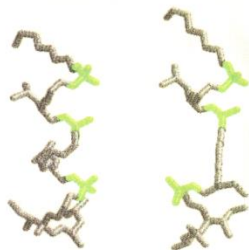
Unfortunately, the solid-phase technique used to synthesize peptides, in which amino acids are sequentially added onto a strand attached to a resin, employed treatments with trifluoroacetic acid (TFA) that irreversibly neutralized the vital nitroxide group.

In 1993, Nakaie, who is now a biophysics professor at the Federal University of São Paulo, and graduate student Reinaldo Marchet found a solution that involved, in part, using hy-

drofluoric acid instead of TFA during the synthesis, which preserved the nitroxide.

Several groups now are finding TOAC very useful for peptide EPR studies, and they say interest in the label is growing.

"This is a really wonderful reporter of



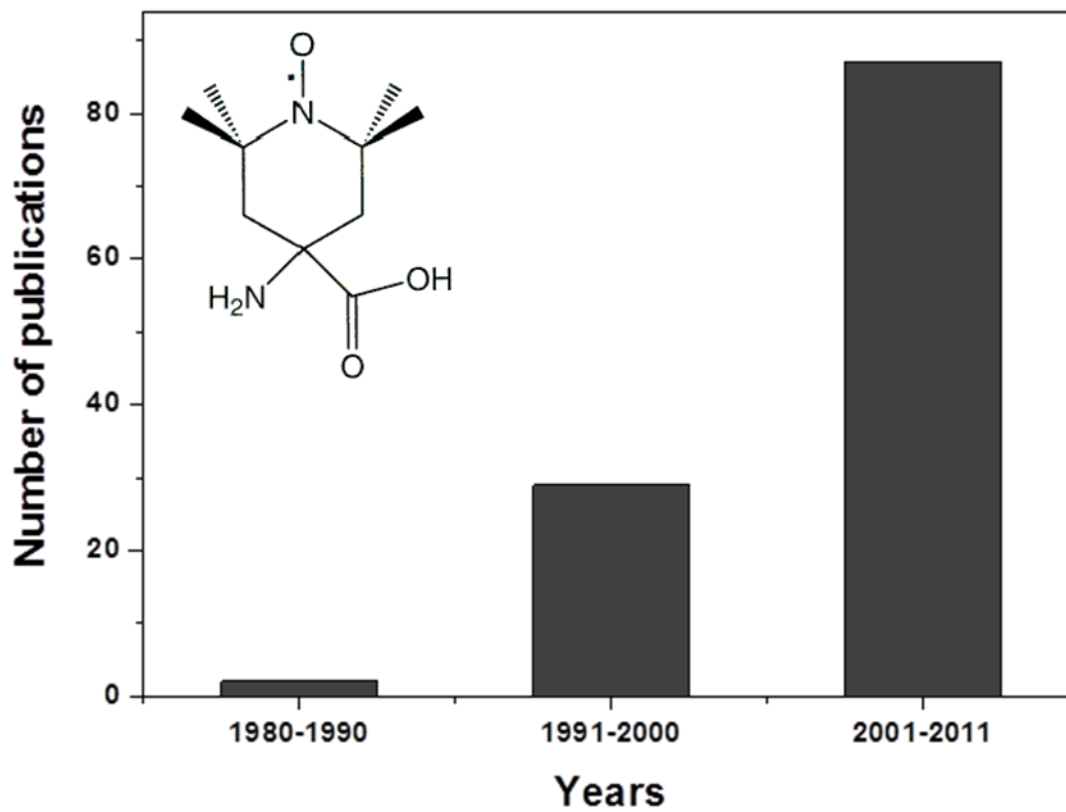
Two conformers of the fungal peptide trichogin. ESR measurements using TOAC spin labels show that the predominantly α -helical conformer at left and the mixed helical and unfolded conformer at right exist in nearly equal proportions. TOAC labels are green and the remaining backbone and side chain atoms are gray.

Courtesy of Glenn Millhauser and Claudio Toniolo



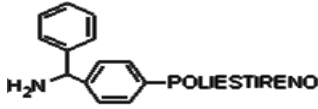
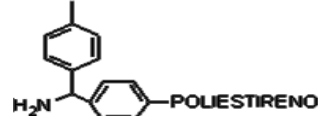
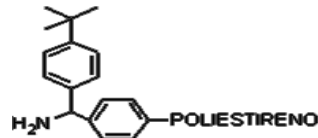
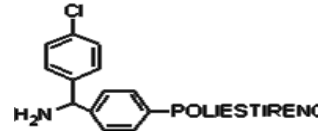
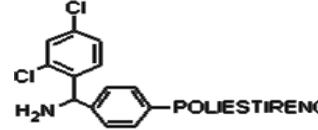
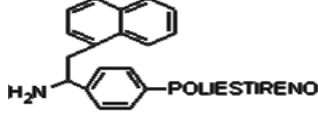
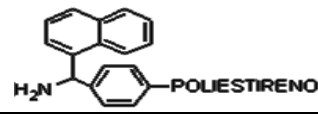
Chemical & Engineering News

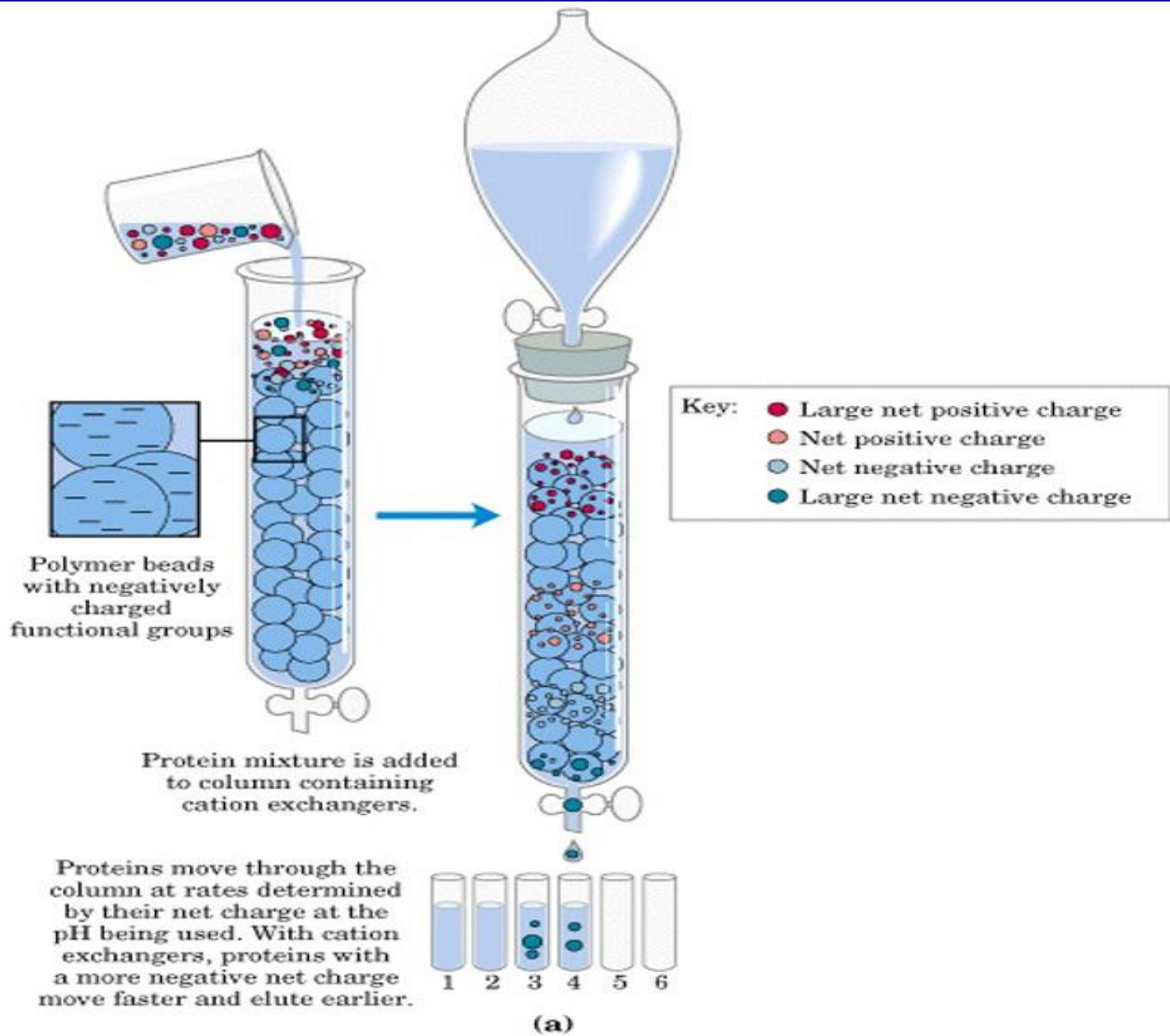
E. K. Wilson (2000) : 78, 54-59.



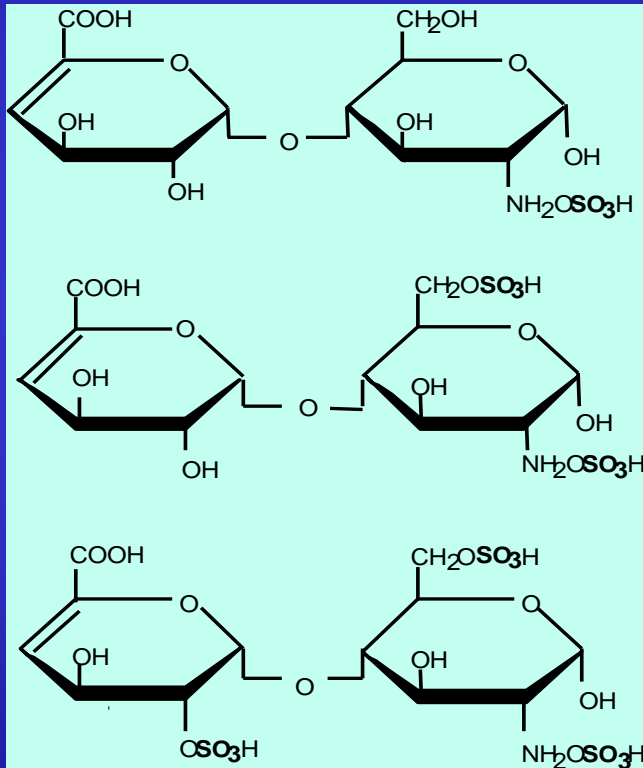
**DESENVOLVIMENTO DE NOVAS RESINAS
PARA A SÍNTESE PEPTÍDICA E
CROMATOLOGRAFIA LÍQUIDA
EM COLUNA**

RESINAS

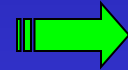
Resina	Abreviatura	Estrutura
Benzidrilamino	BAR	
4-metil-benzidrilamino	MBAR	
4-butil-benzidrilamino	BUBAR	
4-cloro-benzidrilamino	CIBAR	
2,4-dicloro-benzidrilamino	diCIBAR	
1- Naphthyl, Acetyl - benzidrilamino	NAFIR	
1- Naphthoyl - benzifrilamino	NAFOR	



DISSACARÍDEOS

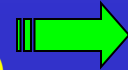


Dissacarídeo Insaturado
N-sulfatado - (Δ U-GlcNS)



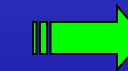
a
(-2)

Dissacarídeo Insaturado
N-sulfatado, 6-sulfato - (Δ U-GlcNS,6N)



b
(-3)

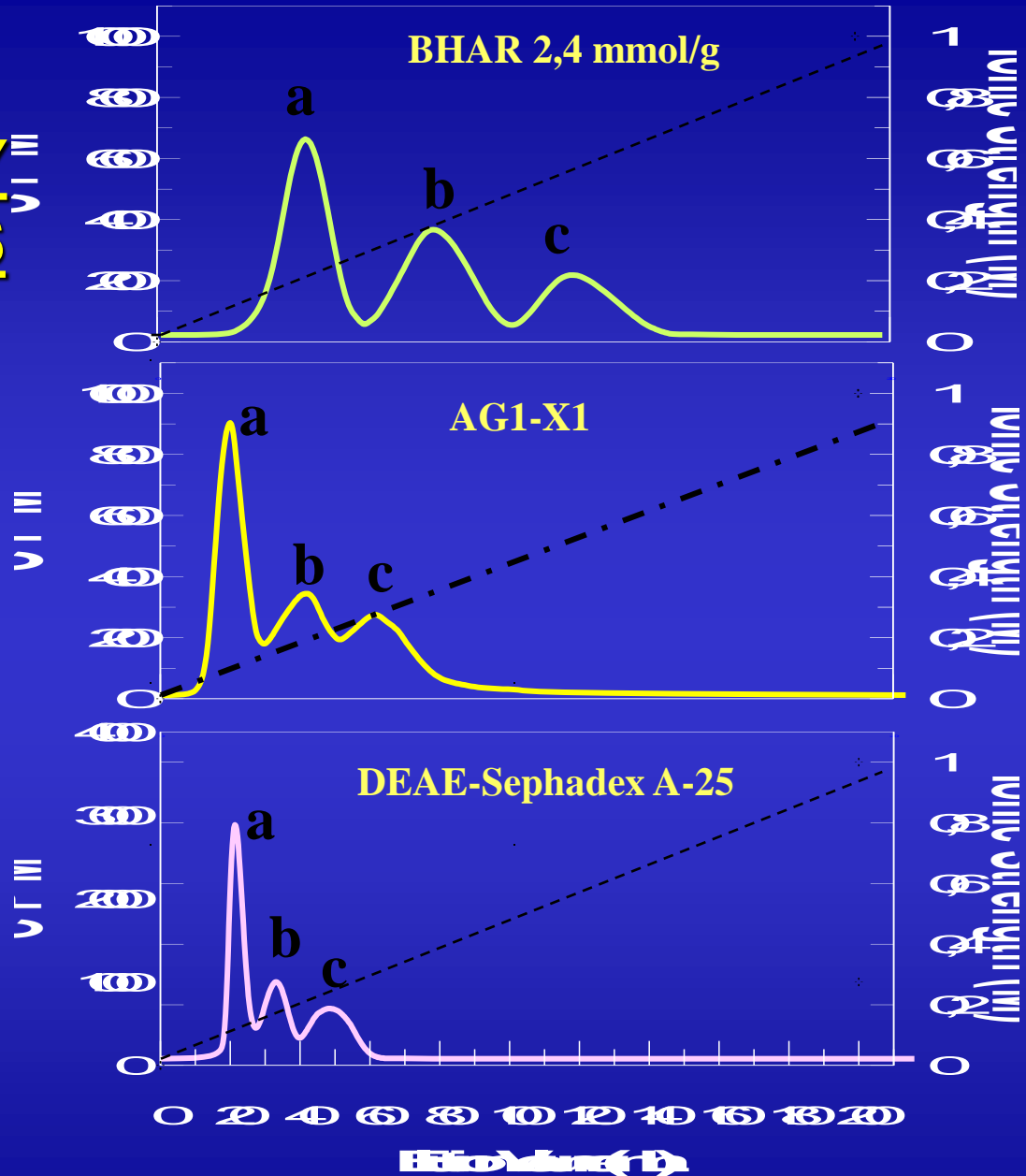
Dissacarídeo Insaturado
Trissulfatado - (Δ U,2S-GlcNS,6N)

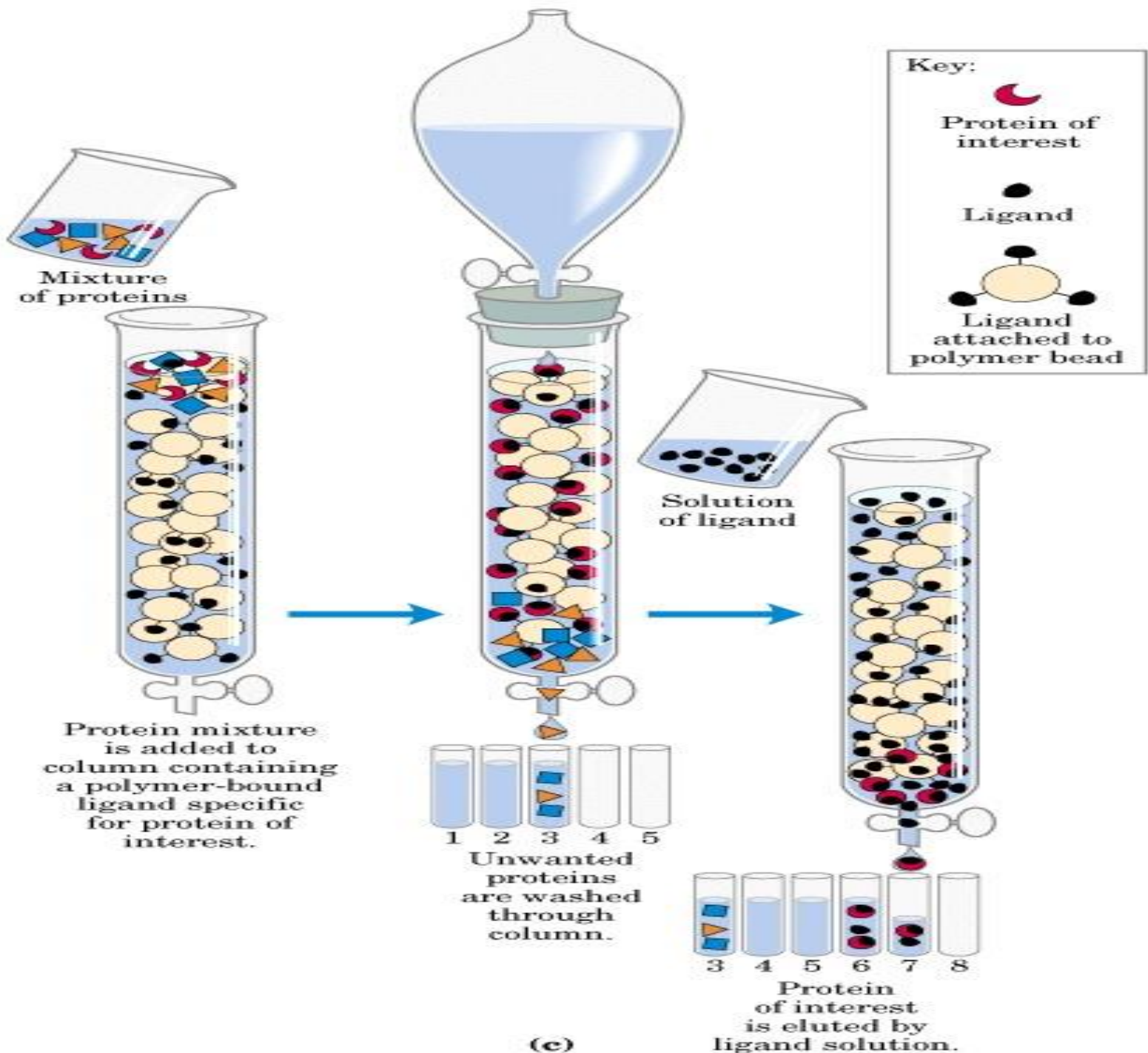


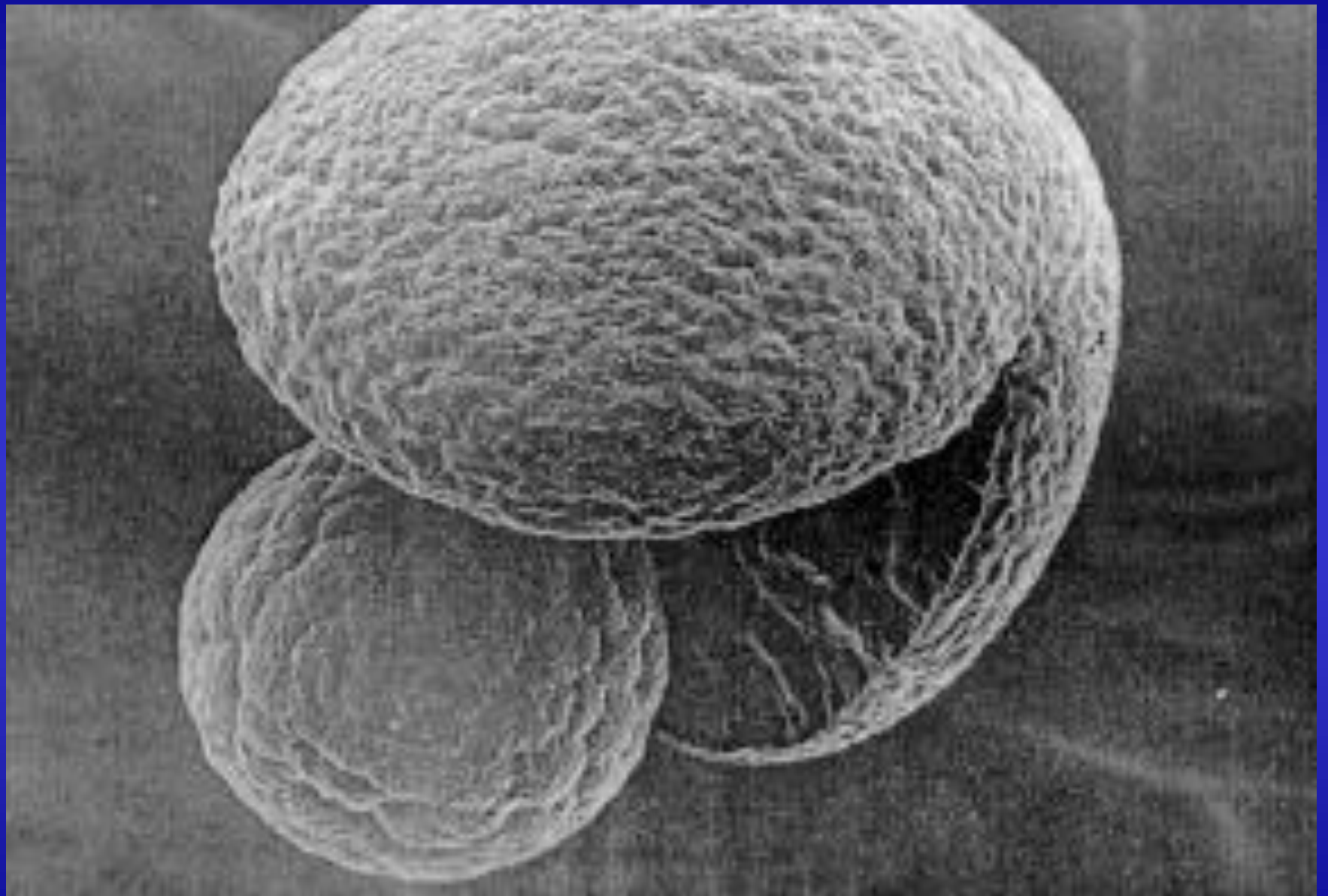
c
(-4)

ANION EXCHANGE CHROMATOGRAPHY OF DISACCHARIDES

Anion exchange chromatography of radiolabeled disaccharides {-2; [³⁵S]-ΔU-GlcNS}^a, {-3; [³⁵S]-ΔU-GlcNS, 6S}^b and {-4; [³⁵S]-ΔU,2S-GlcNS, 6S}^c in linear NaCl-gradient from 0.0 to 2.0 M (100 mL each) in 0.05 M ammonium acetate solution pH 5.0. Volume of resins : 1.4 cm³.





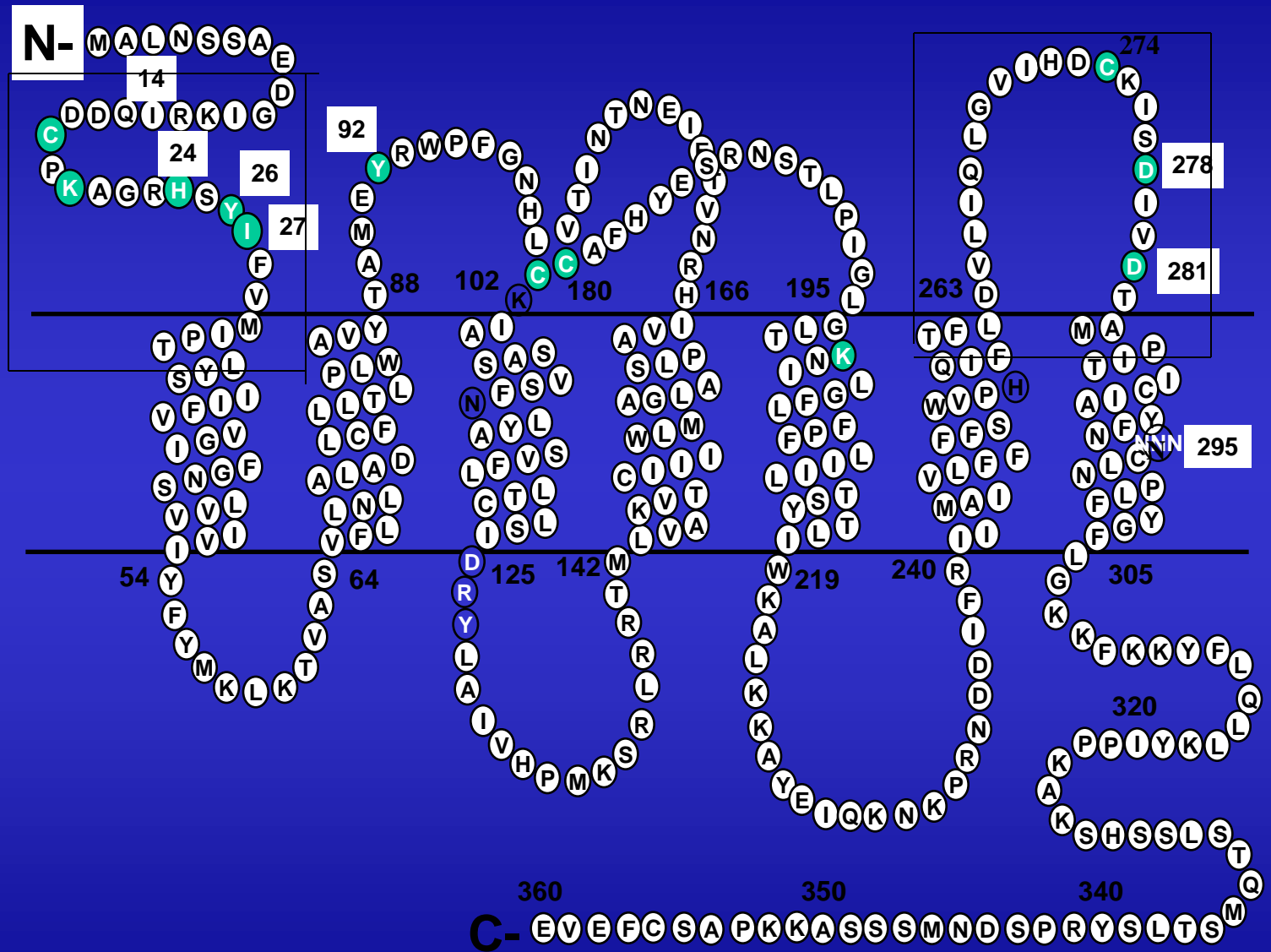


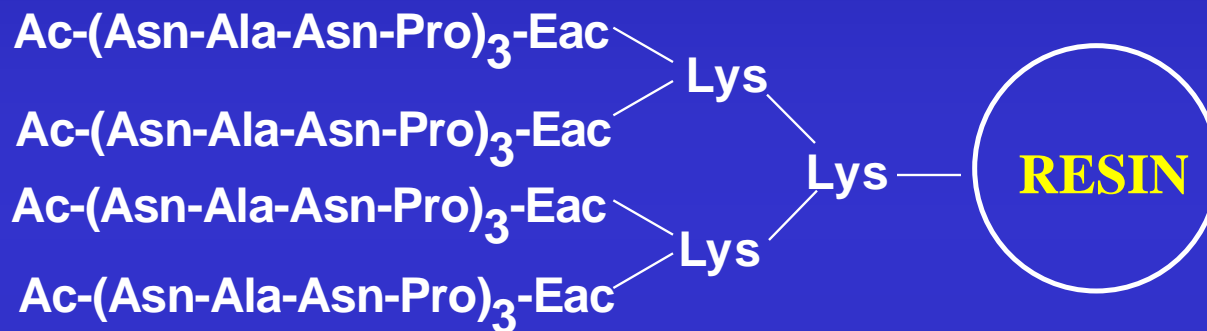


UNIVERSIDADE FEDERAL DE SÃO PAULO
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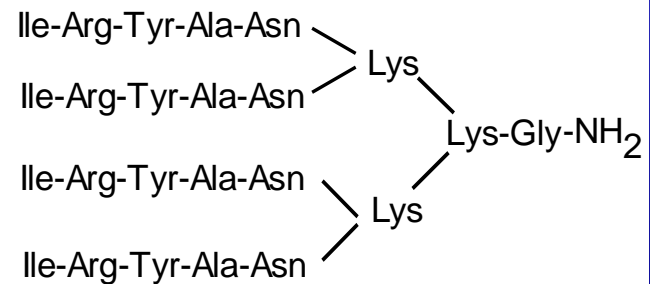
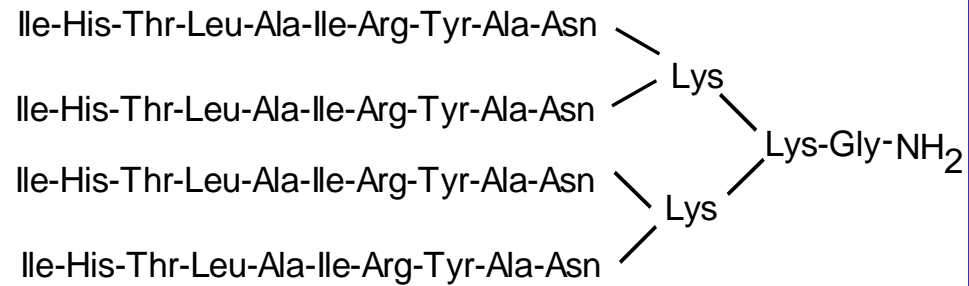
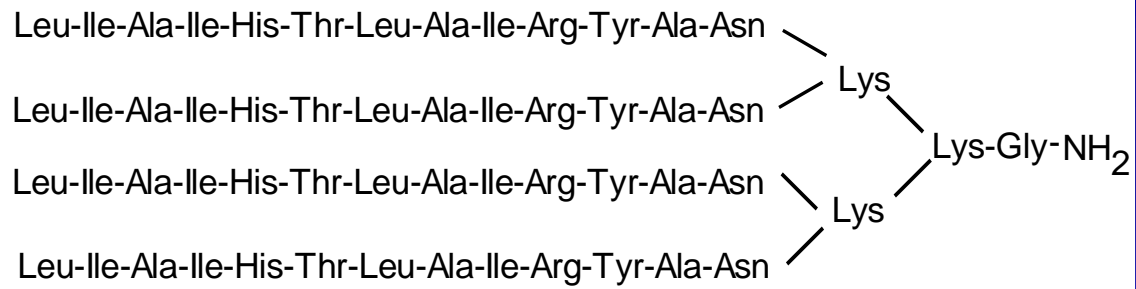
PEPTÍDEOS

Receptor AT₁

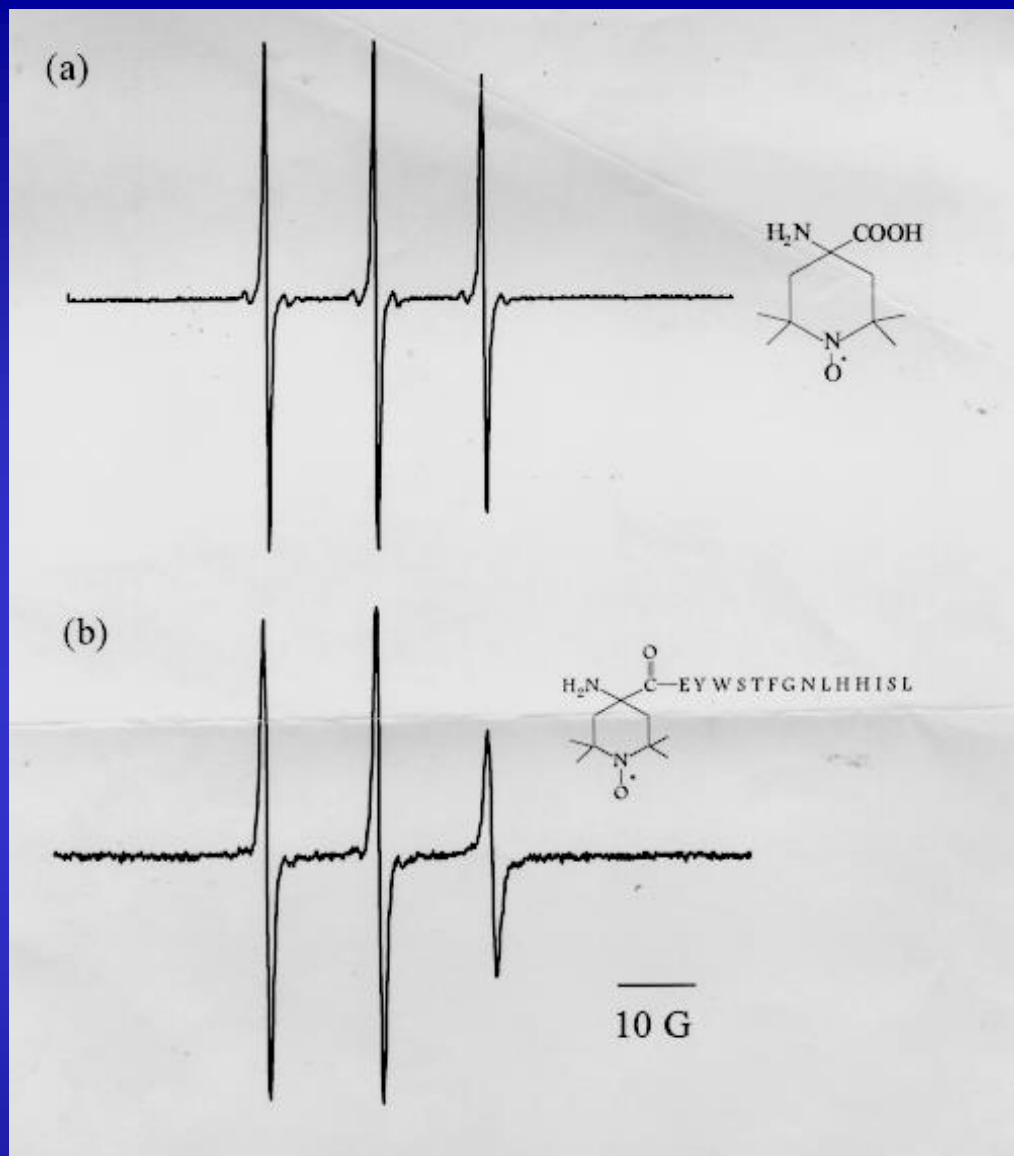




**Chemical structure of $\{[(\text{acetyl-NANP})_3\text{-Eac}]_4\text{-(Lys)}_2\text{-Lys-DEAE-}$
(MacroPrep or Sephadex A50) used for affinity chromatography.**



Peptídeos MAP do *Paracoccidioides brasiliensis*



EPR spectra of (a) Toac and (b) Toac-peptide in aqueous solution, at 22°C

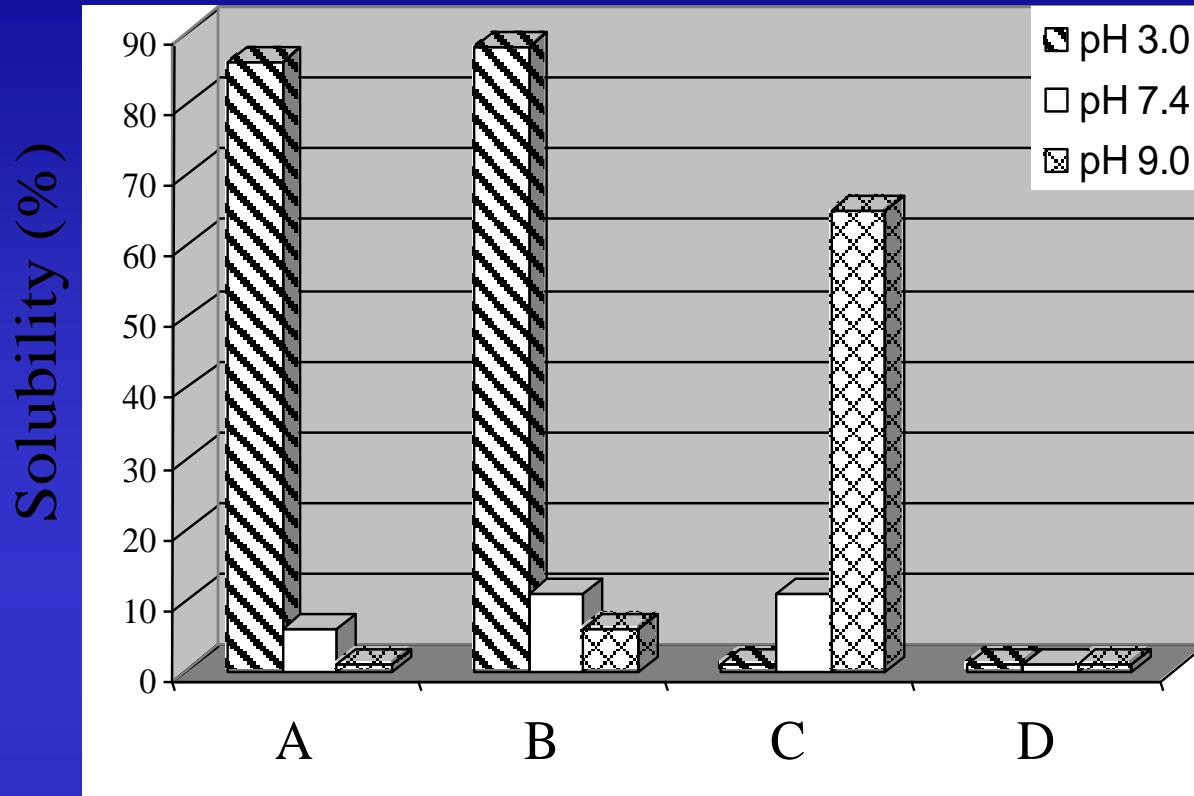
Atividade biológica de análogos da Angiotensina II contendo Toac e Pip

<u>Análogo</u>	<u>Aorta de coelho</u>	<u>Útero de rata</u>	<u>Íleo de cobaia</u>
Toac ⁰ -AII	3%	18%	2,3%
Toac ¹ -AII	1%	62%	14,2%
Toac ³ -AII	NR	NR	NR
Toac ⁷ -AII	NR	NR	NR
Pip ⁷ -AII	0,4%	0,9%	NR

NR : atividade biológica menor que 0,01%

Pip: Ácido pipecólico

**EFEITO DO SOLVENTE NA
SOLUBILIZAÇÃO DE PEPTÍDEOS**



Solubility degree of (1-42) β -amyloid [A], (1-21) β -amyloid [B], TM-32 [C] and VVLGAAIV-amide [D] peptides as a function of the pH of the medium.

Conclusões principais deste tópico

1. Como regra geral para a solubilização de peptídeos – mesmo os mais insolúveis - os melhores solventes são os que apresentam grande diferença entre os valores de AN e DN. Por esta razão, os solventes HFIP (alto AN) e DMSO (alto DN) são os melhores solubilizantes na classe dos solventes simples.

2. No caso dos solventes mistos, a adição da água (alto AN) deve ser avaliada com cautela. O seu efeito é, por exemplo, prejudicial na solubilização de peptídeos agregantes, se misturada com DMSO (alto DN). Por outro lado, a sua adição ao HFIP ou TFE (alto AN) parece não prejudicar o efeito solubilizante dos mesmos.

- Malavolta, L. e Nakaie, C. R. [*Tetrahedron* (2004) 60, 9417]
- Malavolta, L, Pinto, M.R.S., Cuvero, J.H., Nakaie, C.R. [*Protein Science* (2006) 15, 1476]

Amyloid β -Protein Fragment

H-4854

(D-Asp1)-Amyloid b-Protein (1-42)

H-D-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH

Catalog Number Unit Price per Unit QTY

H-4854.0500 0.5 mg - USD 345.00

H-4854.1000 1 mg - USD 540.00

C203H311N55O60S

Mr :4514.1

Longterm storage temperature: - 15 ° C

Bulk Quantity

CUSTOM PEPTIDES

Price list

in EUROS

AA	Immunograde			> 80%			> 95%		> 97%	
	5 mg	25 mg	50 mg	2 mg	10 mg	25 mg	2 mg	10 mg	5-10 mg	
6	178	223	290	218	305	396	333	488	808	
7	187	239	311	231	328	425	347	517	823	
8	197	256	332	245	351	456	361	546	838	
9	206	273	354	258	374	489	375	575	854	
10	216	290	375	272	396	523	389	604	869	
11	227	308	399	287	421	556	403	637	899	
12	239	326	427	302	447	590	417	671	930	
13	250	345	457	317	474	624	431	704	960	
14	261	363	488	332	502	657	445	738	991	
15	272	381	518	347	529	691	459	771	1 037	
16	285	406	549	362	556	724	477	805	1 082	
17	298	434	579	379	584	758	496	838	1 128	
18	311	466	610	396	611	791	515	872	1 174	
19	323	499	640	413	639	825	535	906	1 220	
20	336	531	671	429	666	858	554	939	1 296	
21	352	563	704	449	697	896	577	977	1 372	
22	368	595	738	475	730	935	605	1 015	1 448	
23	384	627	771	503	764	973	636	1 053	1 555	
24	400	659	805	535	797	1 011	671	1 092	1 662	
25	416	691	838	567	831	1 049	707	1 130	1 768	
Delivered with*	HPLC MS			HPLC AAA or MS NPC determination (option)			HPLC AAA or MS NPC determ.		HPLC AAA + MS NPC determ.	
Delivery	2 / 3 weeks			3 / 4 weeks			3 / 4 weeks		3 / 5 weeks	

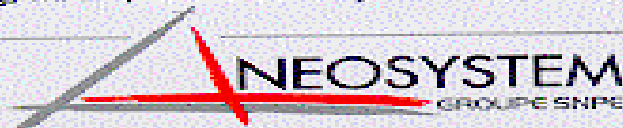
*Additional analyses upon request.
Prices are per peptide. Postage and insurance not included.
Do not hesitate to contact us for smaller or larger quantities.

AAA : Amino Acid Analysis
NPC : Net Peptide Content
MS : Mass Spectrum

ADDITIONAL CUSTOM SERVICES

Acetylation	Free	Amidation	Free	Biotinylation	23 €
Phosphorylation	61 €	Conjugation	168 €	Cyclisation	274 €

Other modifications upon request : amino acid spacers, non standard amino acids, N- or C-terminal labelling, MAP's, amide bond cyclisation, pseudopeptides...



September 2001

Estudos básicos e aplicações práticas

Peptídeos-

a) QUÍMICO

- Aprimoramento do método de síntese;
- Síntese de sequências difíceis ou não usuais;
- Com elevado teor peptídico no grão;
- Com aminoácidos não naturais;
- Com uso de marcadores de spin do tipo aminoácido (Toac, Poac)

b) FÍSICO-QUÍMICA/BIOFÍSICA/FARMACOLOGIA

- Estudo de interação peptídeo-solvente
- teoria de solubilização, via AN e DN;
- Estudos espectroscópicos de conformação (RPE, CD, NMR, etc);
- Propriedades farmacológicas e modelagem molecular
- Estudos de interação em sistemas membrana-miméticos;
- Efeito estrutural de irradiação gama;

c) APLICACÕES

- Autonomia na produção de diferentes tipos de peptídeos;
 - Autonomia na obtenção de matérias primas como copolímeros de partida, resinas, derivados de aminoácidos, etc
- Introdução de derivados dos marcadores de spin (Toac e Poac e peptídeos ativos com estes marcadores (α -MSH e derivado); Patentes.
- Peptídeo para *Diabetes insipidus* (desde 2004, Governo de SP);
 - Inibidores para doenças neurodegenerativas (em andamento).

Polímeros (Resinas)

a) QUÍMICO

- Síntese do copolímero de partida;
- Síntese de resinas com alto teor de sítios reativos;
- Síntese de resinas com tamanho e teor de intercruzamento alterados;

b) FÍSICO-QUÍMICA/BIOFÍSICA

- Estudos por microscopia de grãos e por RPE, NMR, FTIR;
- Regras de solvatação de polímeros
- Proposição de uma nova escala de polaridade de solventes.

c) APLICACÕES

- Síntese de resinas inéditas para uso em síntese peptídica
- Síntese de resinas inéditas para cromatografias em coluna (troca-iônica, afinidade, etc).
- Uso de resinas comerciais de troca iônica para síntese peptídica e cromatografia de afinidade;
- Centro de análise de medicamentos genéricos (ensaios de biodisponibilidade -NUBEC) – desde 2003.



Prof. Dr. Antônio Cechelli de Mattos Paiva.