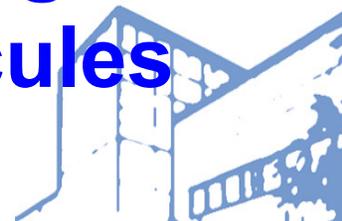


Solid-Phase a Useful Tool for Organic Synthesis of both Small Molecules and Biomolecules



Fernando Albericio



**ISCHIA ADVANCED
SCHOOL OF ORGANIC CHEMISTRY
Ischia Porto
September 16-21, 2006**

Bruce Merrifield

The Nobel Prize in Chemistry 1984



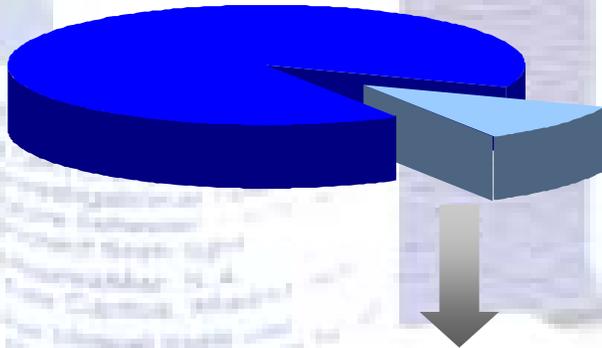
I WONDER IF TRYING TO SYNTHESIZE
THE *POLYSATURATED* POLYPEPTIDE
CYCUTRINE TURNS OUT TO BE A STUPID
WASTE OF TIME.

YOU SHOULD HAVE THOUGHT
ABOUT THAT 20 YEARS
AGO...MAN

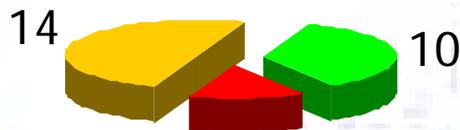


Peptide pharmaceuticals: the market

Total drug sales (world):
 $\sim 280 \times 10^9$ \$



Peptides / proteins:
 28×10^9 \$ (10.5%)



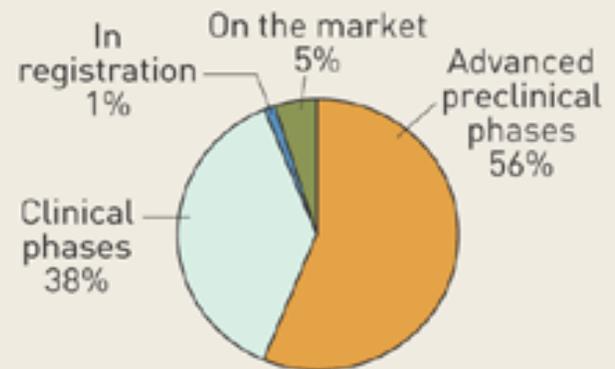
■ recombinant ■ synthetic ■ mAbs

Therapeutic areas

- Allergy, asthma
- Analgesia
- Antimicrobials
- Antivirals
- Baldness
- Ca^{2+} metabolism
- Cancer
- CNS
- Diagnostics

DEVELOPMENT

No lack of hopefuls in the global peptide drug pipeline



2004 peptide drugs/candidates ≈ 720

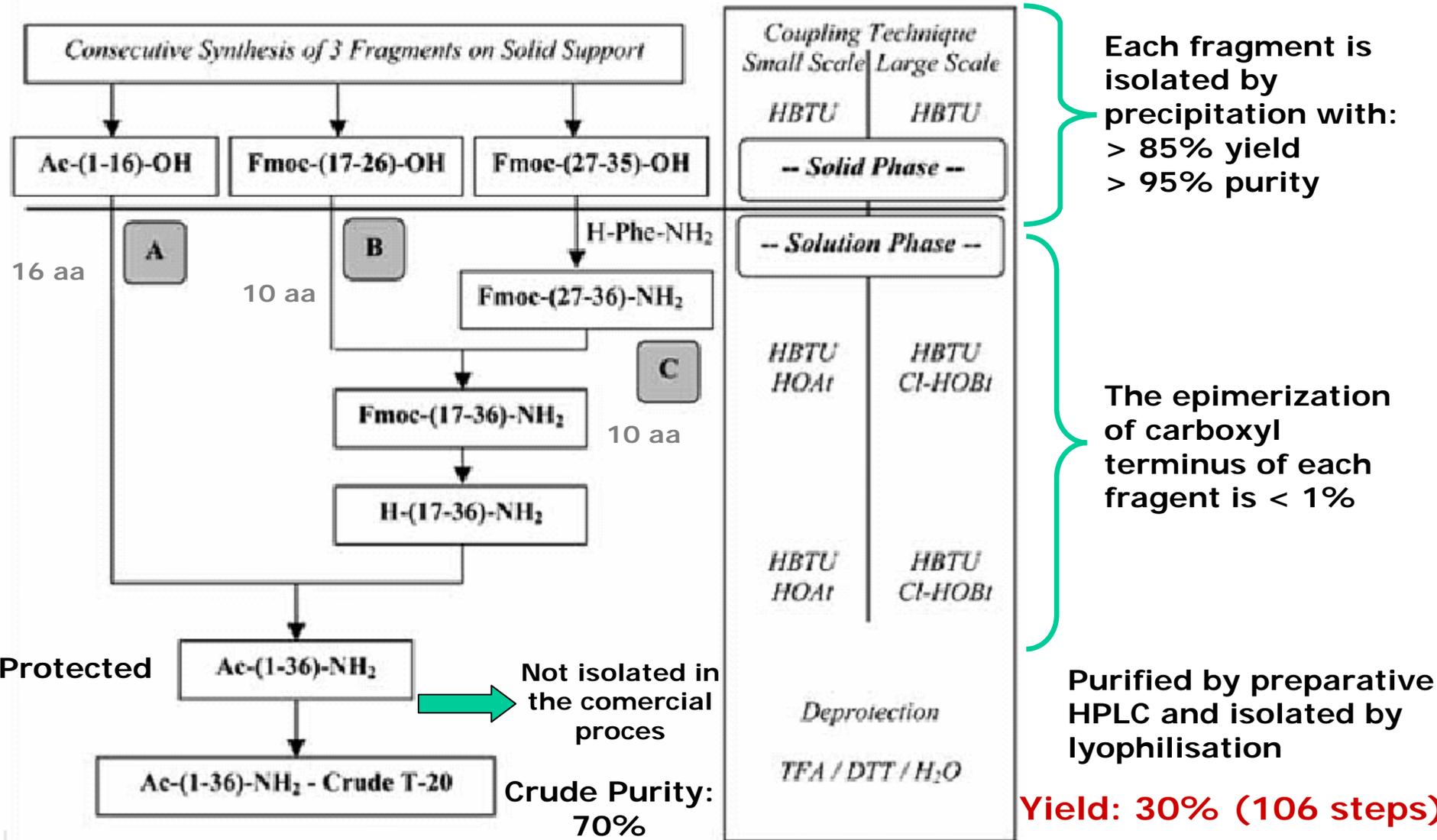
SOURCE: Frost & Sullivan

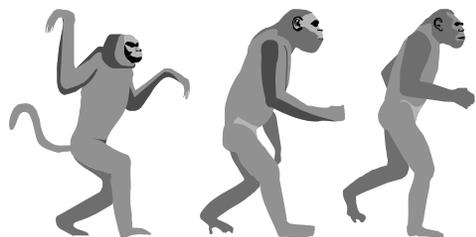
Production Peptide Drugs Today

PEPTIDES	Length	Quantites	Status	Strategy
ACTH (1-24)	24	50-100Kg	Market	Solution Phase
Autosiban	9	50-100Kg	Market	Solution Phase
Zinconotide	25	1-5Kg	Market	Solid Phase
Lanreotide	8	100-200Kg	Market	Solid Phase
Cyclosporin	11	100 Tons	Market	Biotechnology
Fuzeon T-20	36	3-5 tons	Market	Hybrid Method
Insulin	51	Multi tone	Market	Biotechnology
LH-RH analogues	10	150-200 Kg	Market	Solution or Solid Phase
Oxytocin	9	50-100Kg	Market	Solution Phase

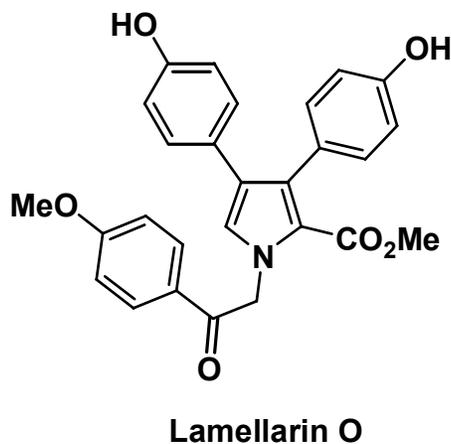
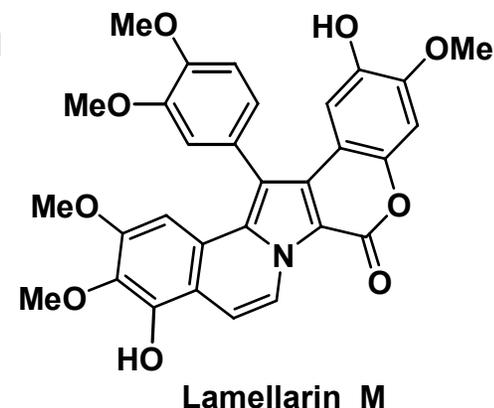
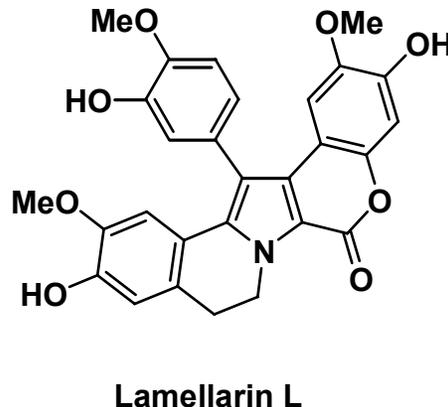
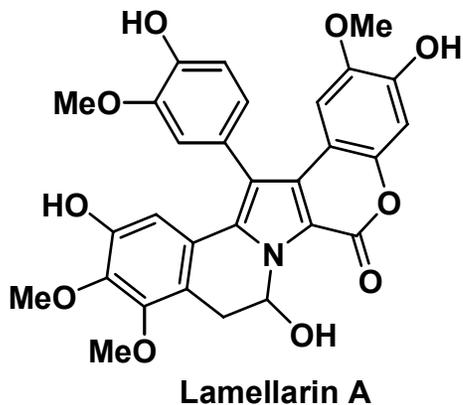
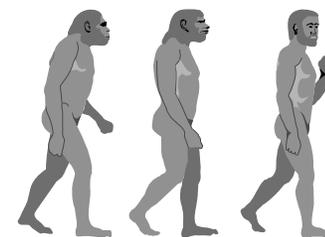
Example of the Hybrid Approach: Fuzeon T20

Ac-Tyr-Thr-Ser-Leu-Ile-His-Ser-Leu-Ile-Glu-Glu-Ser-Gln-Asn-Gln-Gln-Glu-Lys-Asn-Glu-Gln-Glu-Leu-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-NH₂





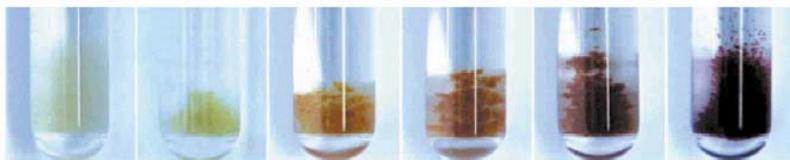
Reviewing the History of Lamellarins



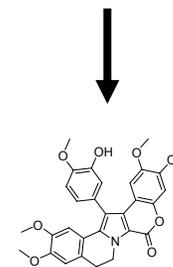
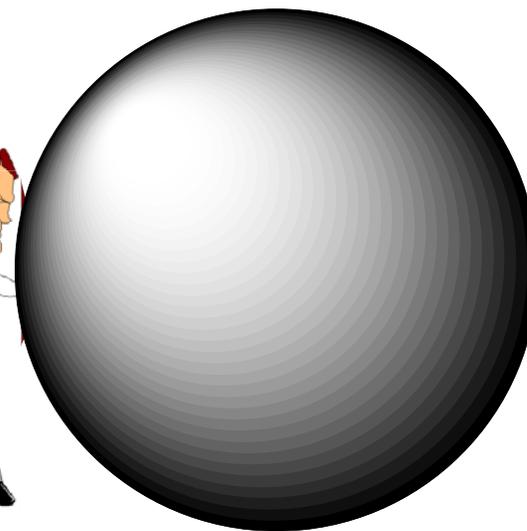
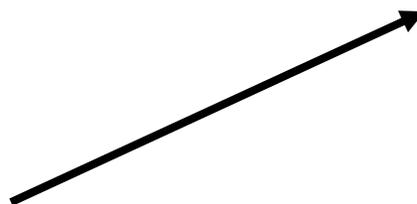
ACTIVITY

- Inhibition of cell division
- Inhibition of HIV-1 integrasa
- Cytotoxic against P388 and A549
- Immunomodulatory activity
- Selectivity toward melanoma cell lines
- Effective in the treatment of multidrugs resistant tumors

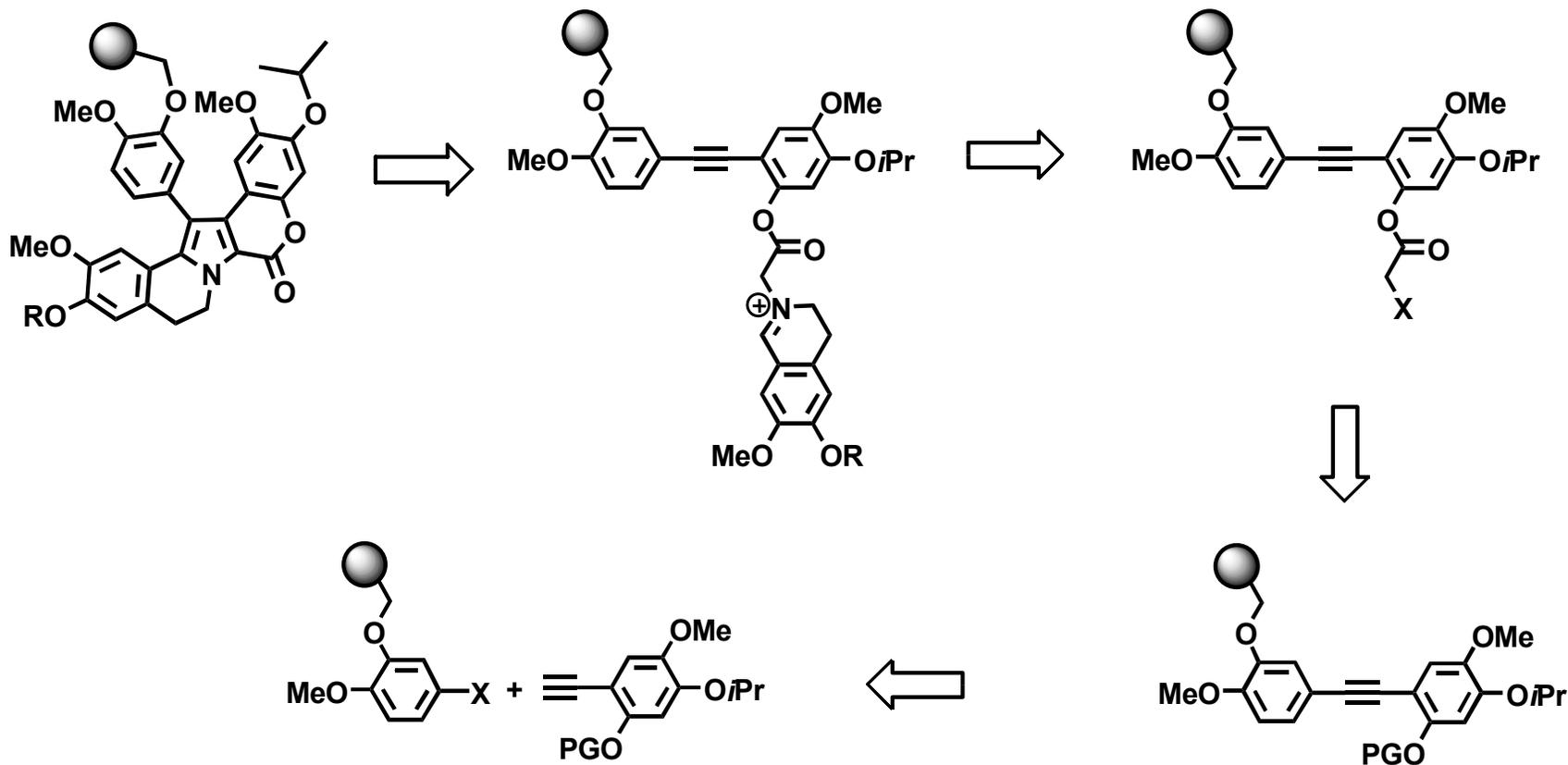
The Bead, a Black Box?



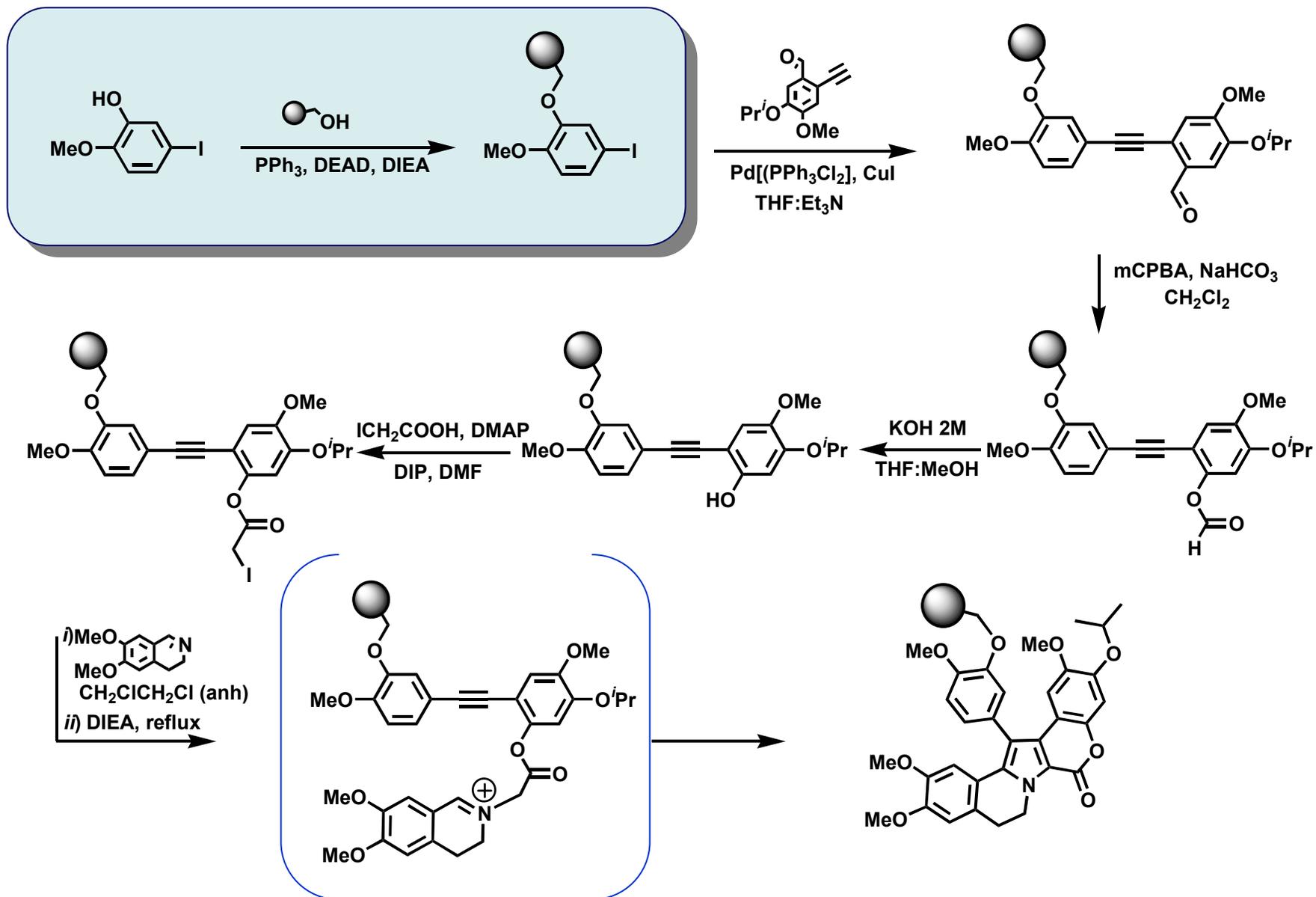
%CHO: 0% 1% 6% 30% 54% 98%



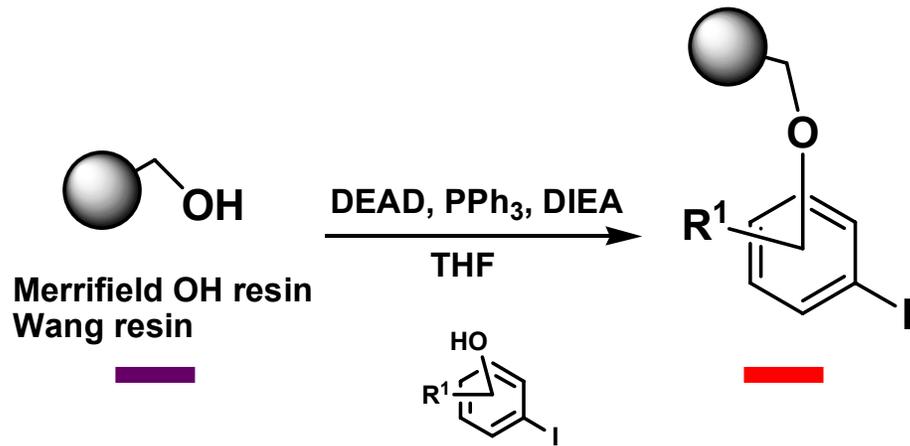
Lamellarins Solid-Phase Synthesis: Retrosynthetic Analysis



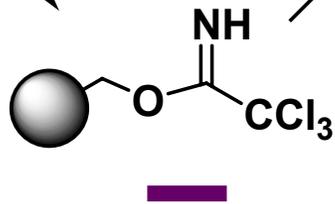
Lamellarins Solid-Phase Synthesis



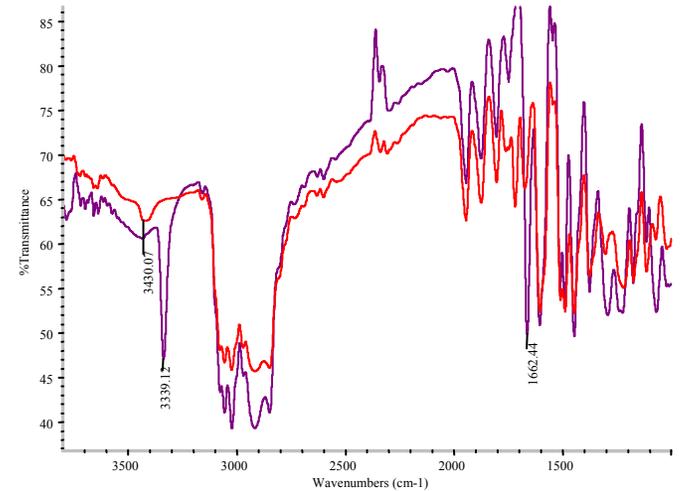
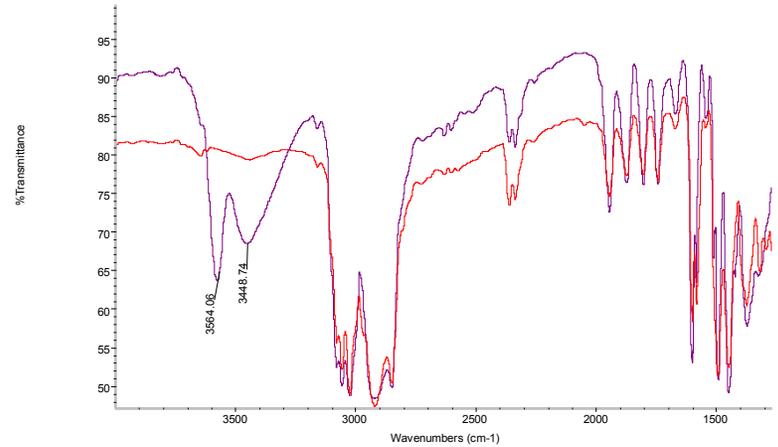
Anchorage into the resin: IR



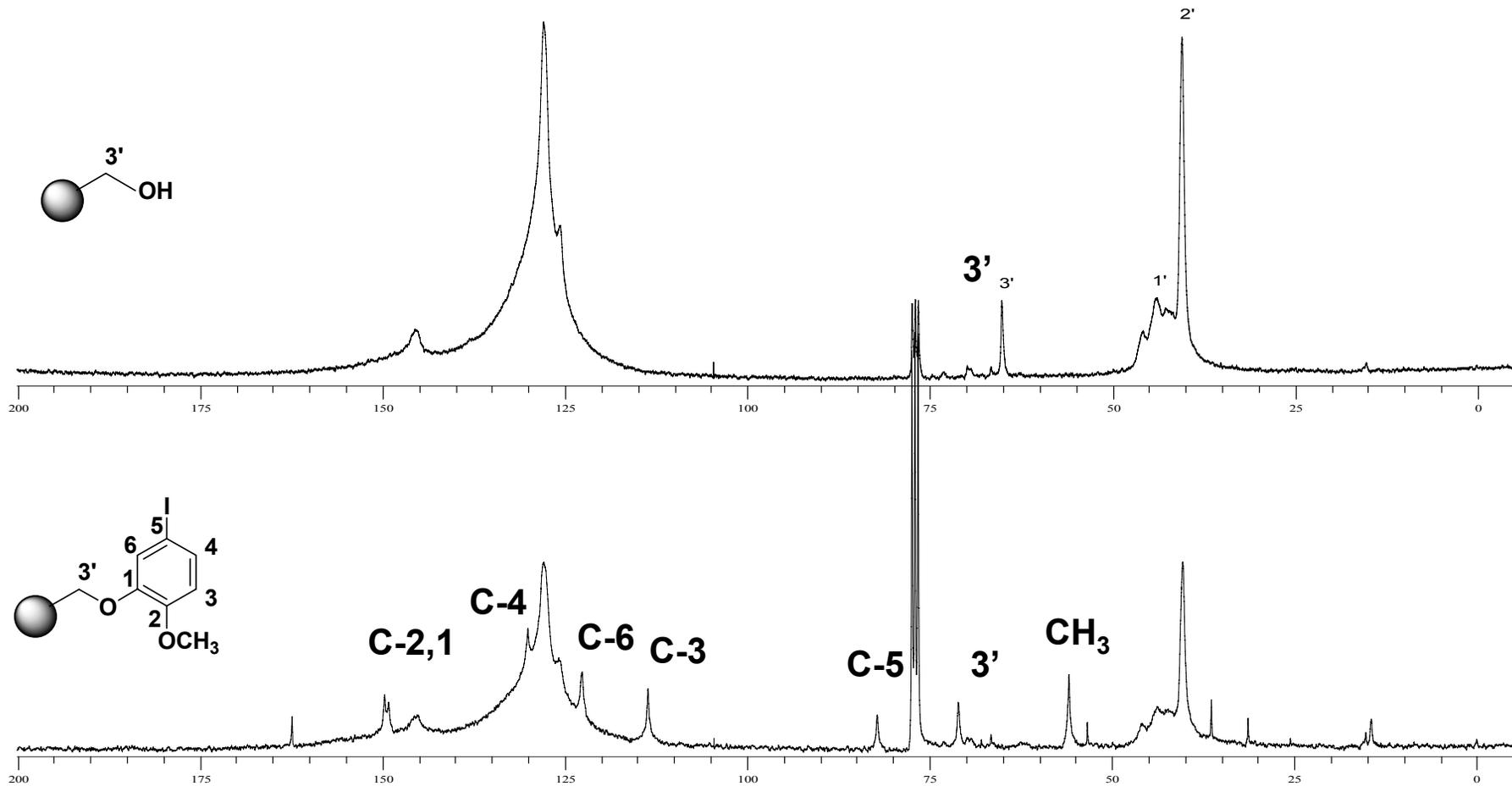
Cl₃CCN
DBU (0° C, 40 min)
DCM



BF₃.Et₂O, THF

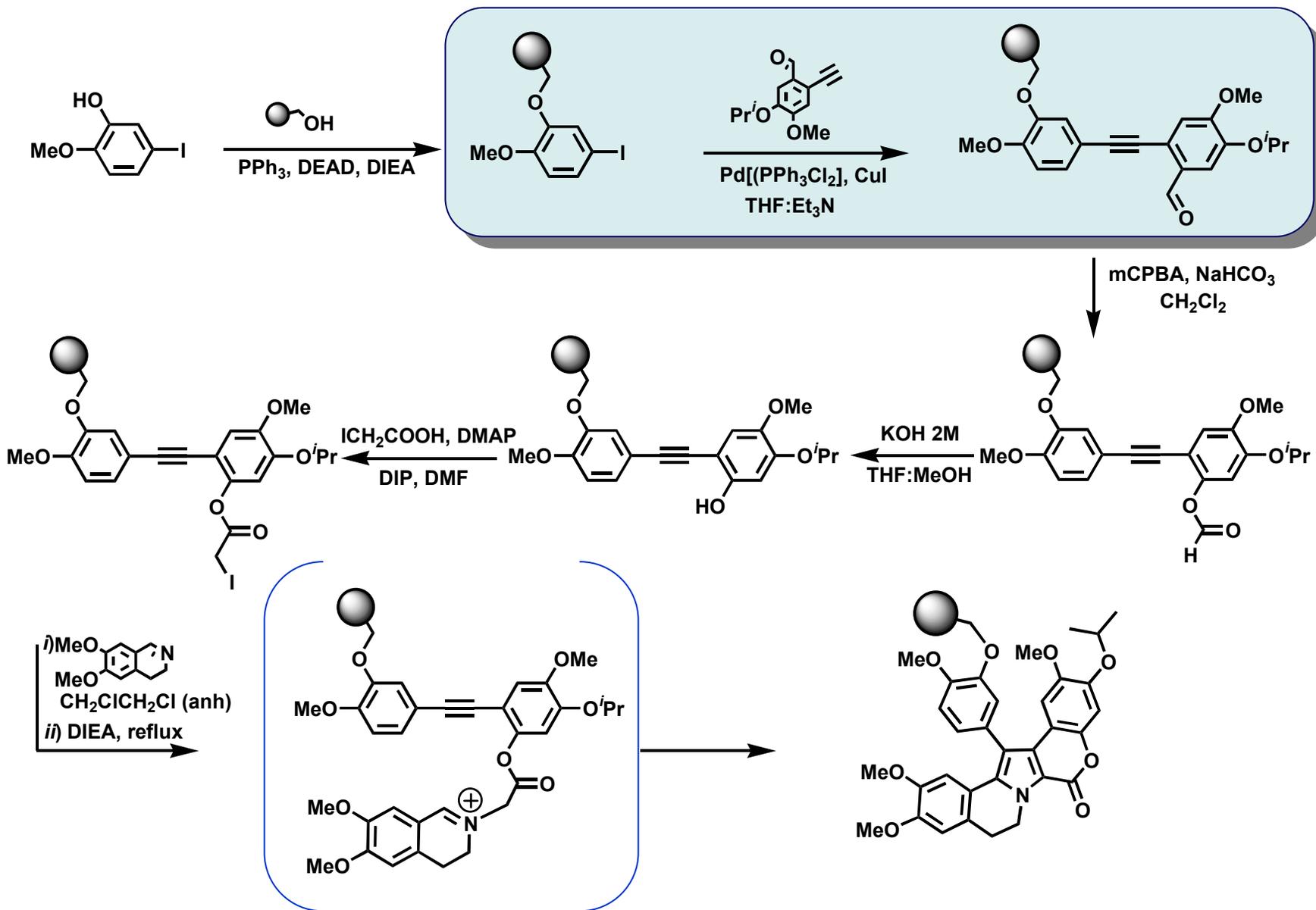


Anchorage into the resin: Gel-phase ^{13}C NMR

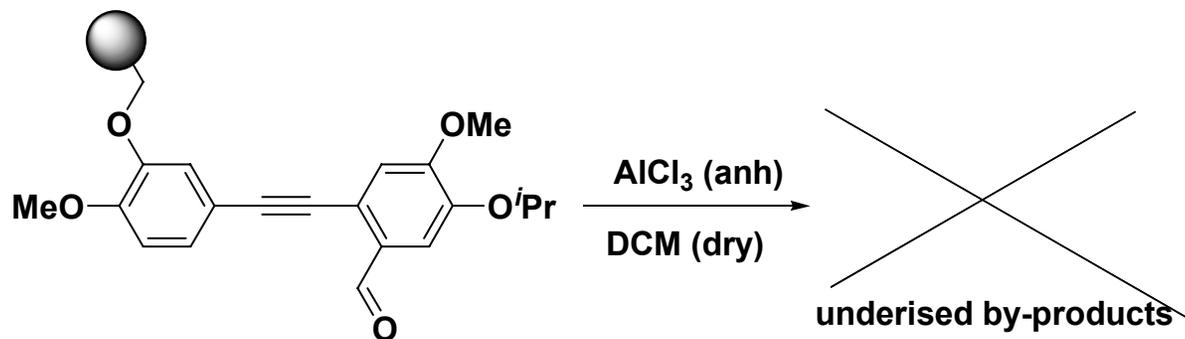
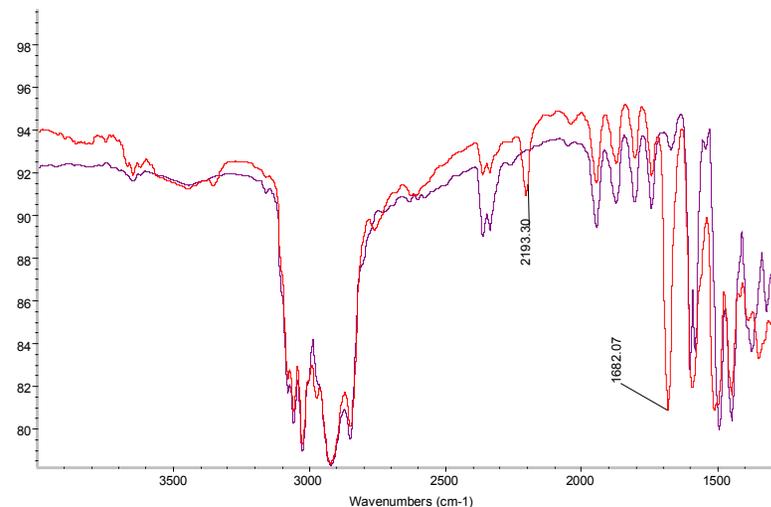
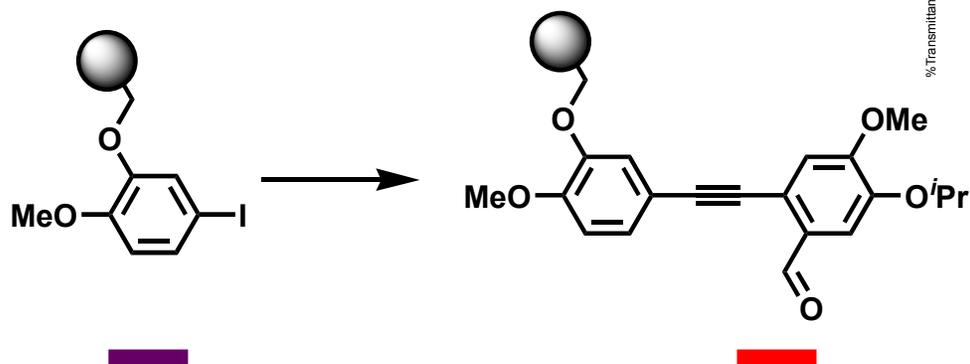


10 mg of resin

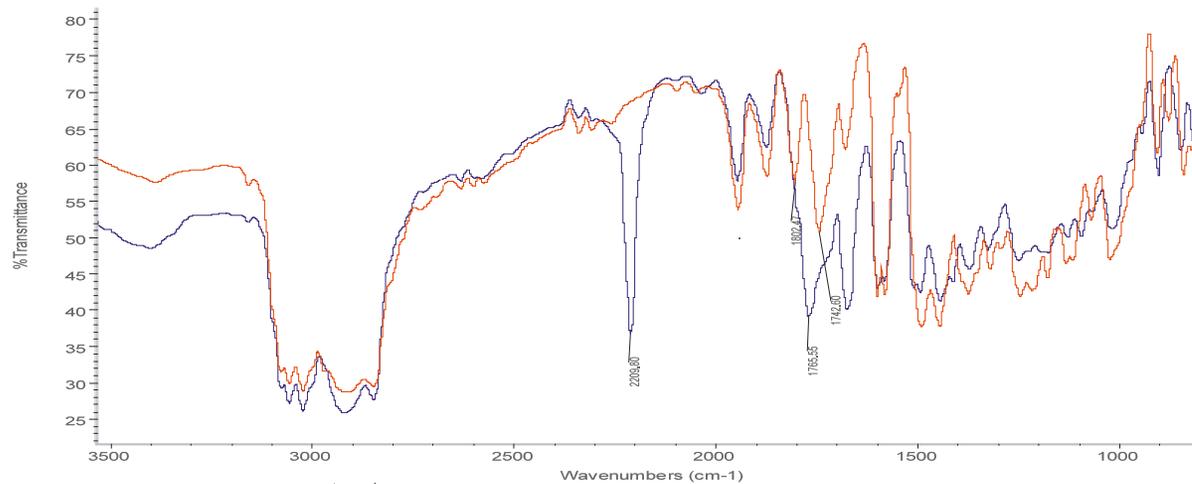
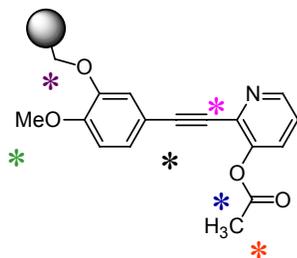
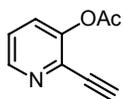
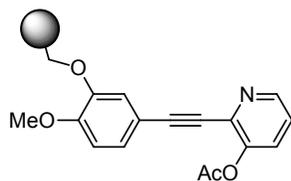
Lamellarins SPS: Sonogashira Reaction



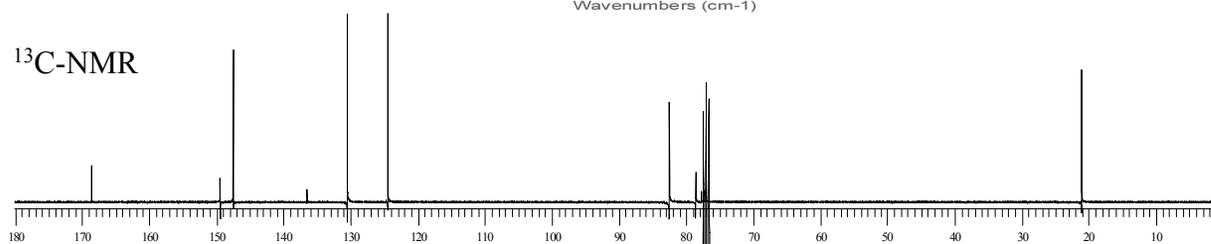
Sonogashira Cross-Coupling Reaction



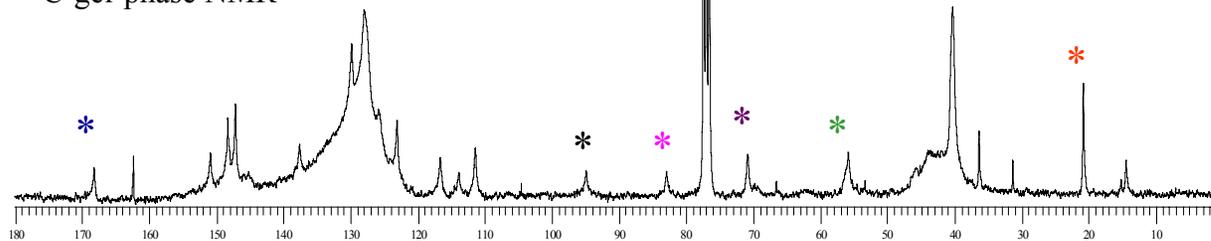
Sonogashira Cross-Coupling Reaction



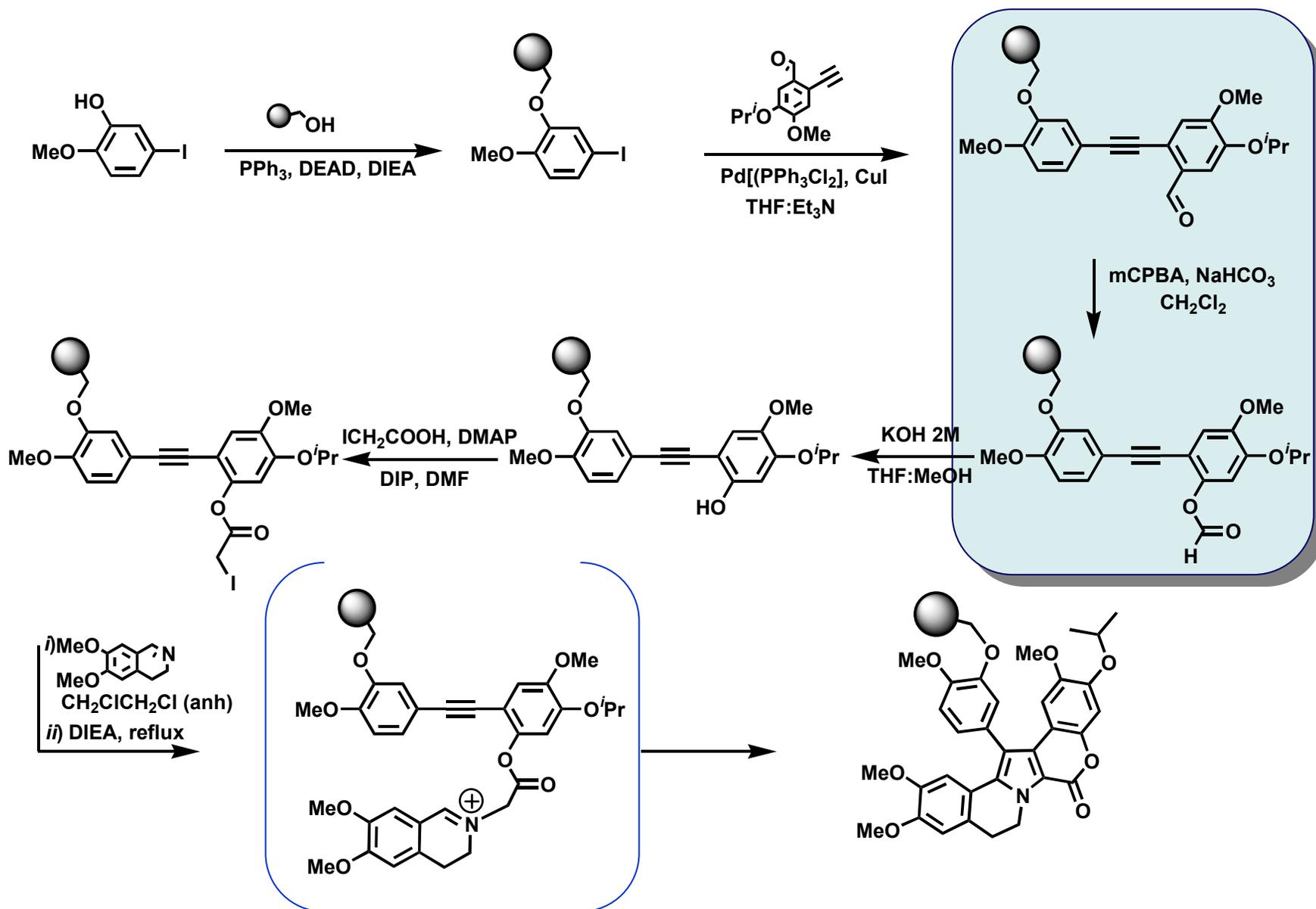
¹³C-NMR



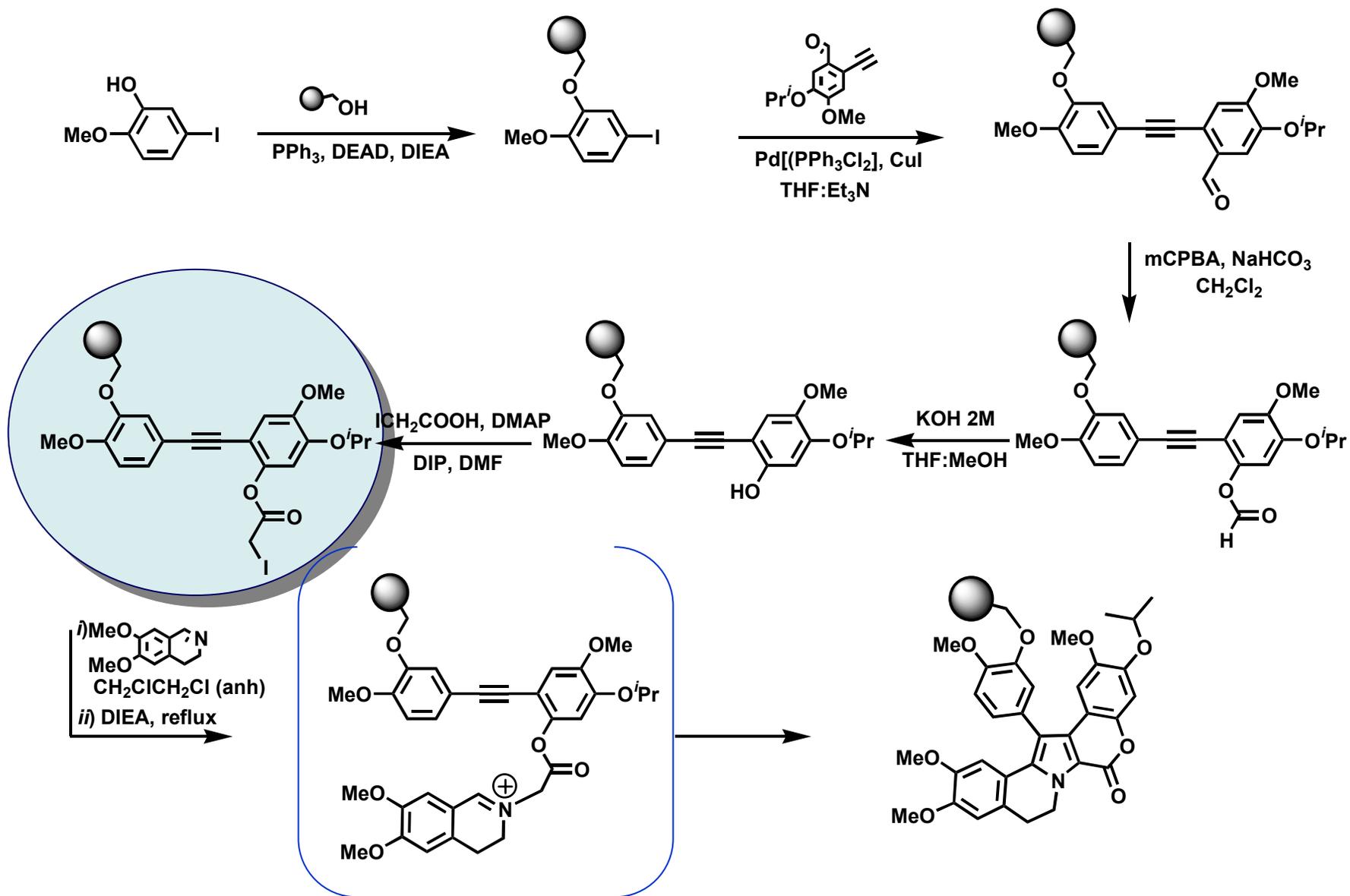
¹³C-gel-phase NMR



Lamellarins SPS: Baeyer-Villiger Reaction

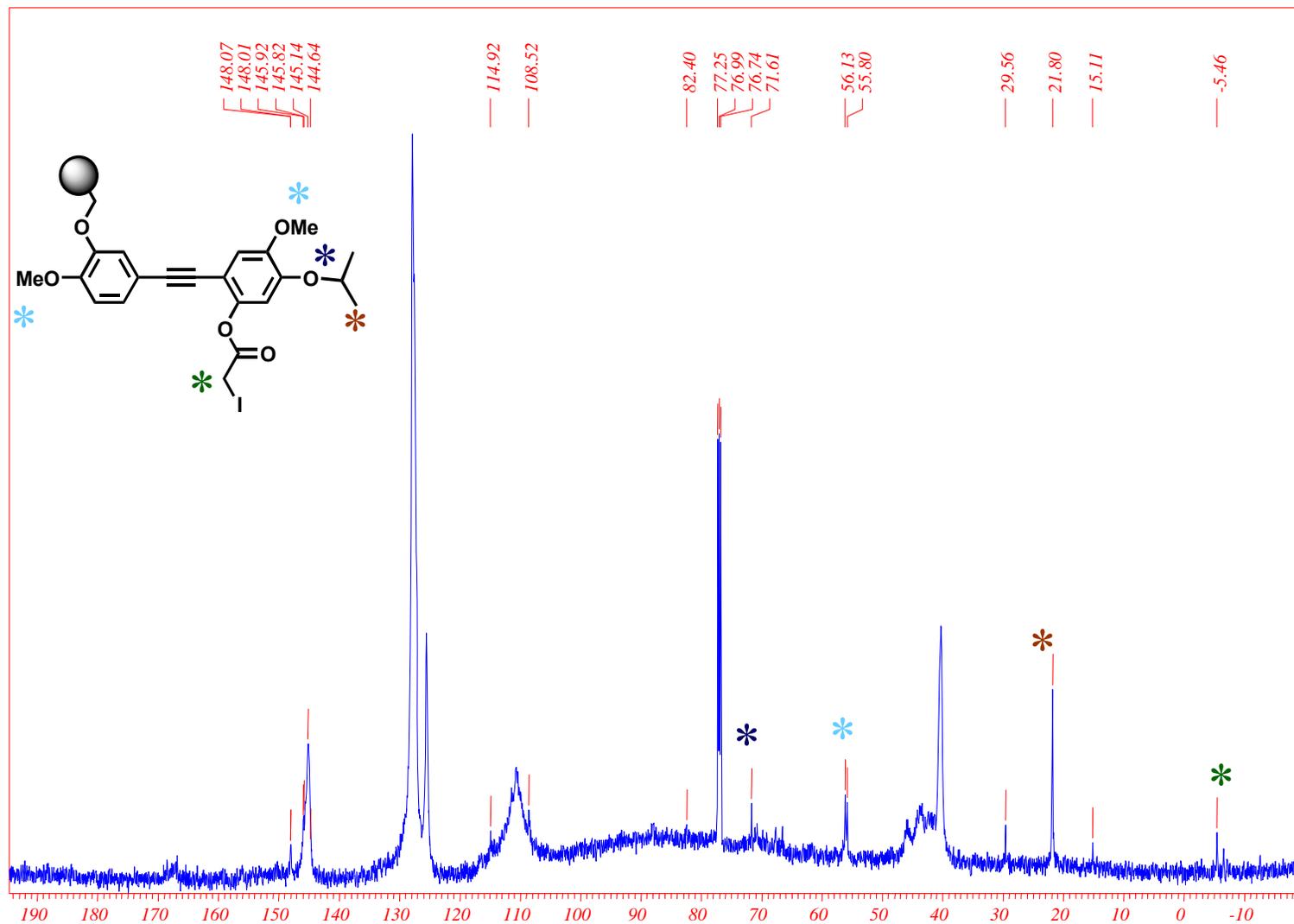


Lamellarins SPS: Hydrolysis and Acylation

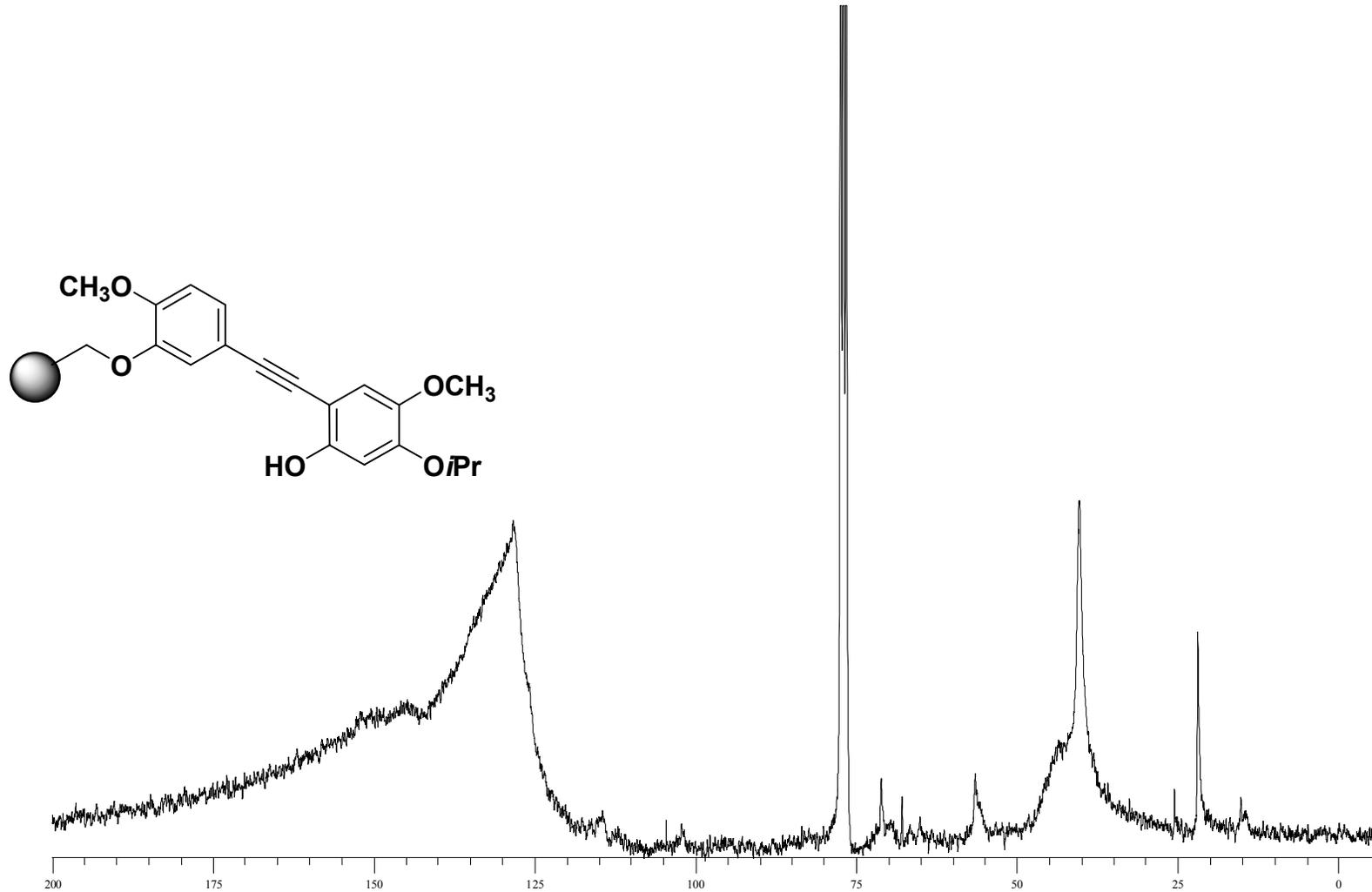


Control of Solid-Phase Reactions: ^{13}C MAS-NMR

C13-HRMAS/ Bruker DMX500 CDCl3/ 25C PCironiData: 11/07/02 /50 uL amb espaiadorgir 5000 Hz

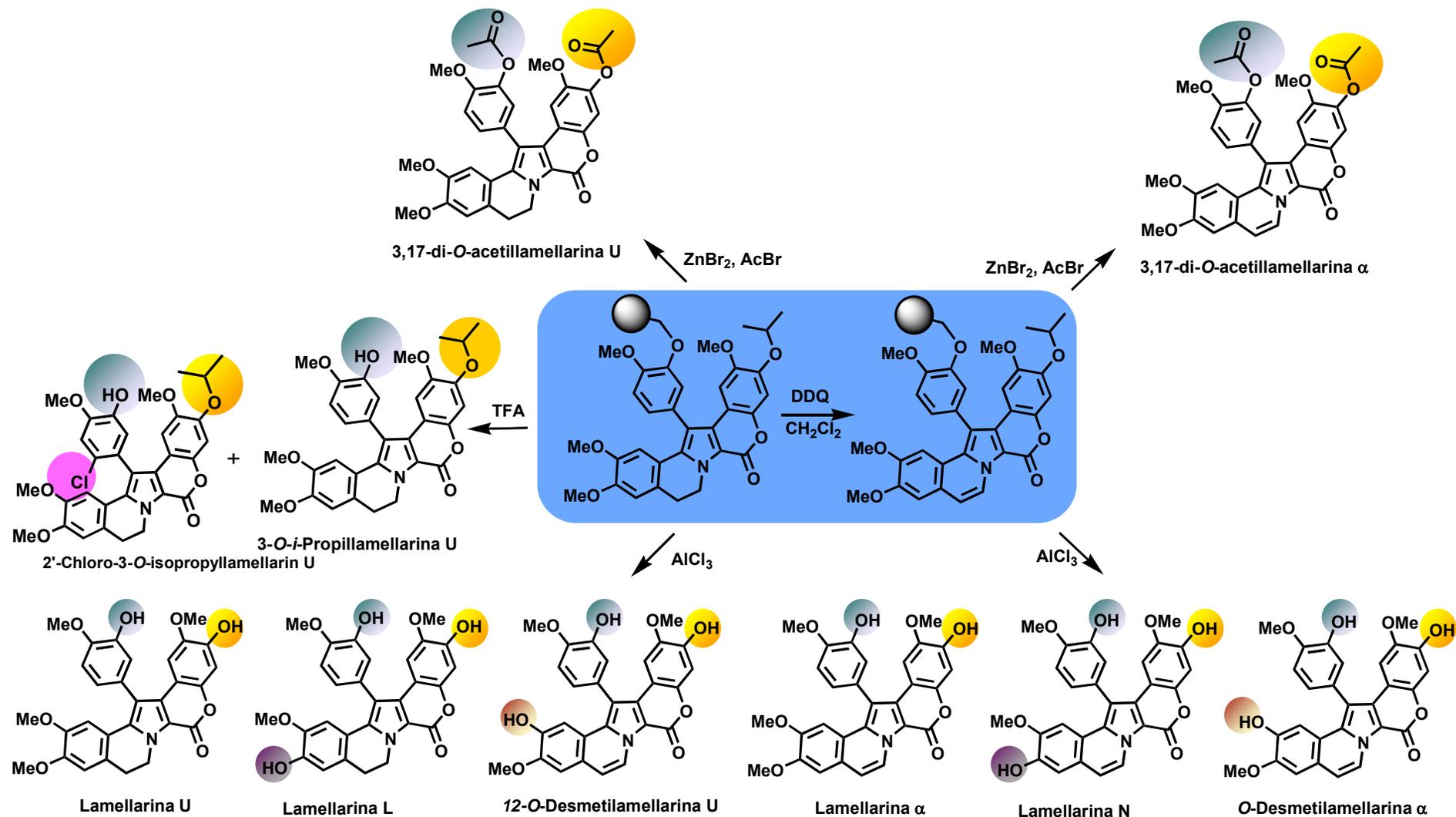


"Aging" of the Resin: Gel-phase ^{13}C NMR



Resin more rigid and less amenable to swelling

Oxidation and Cleavage: Cleavage Conditions Gives Diversity

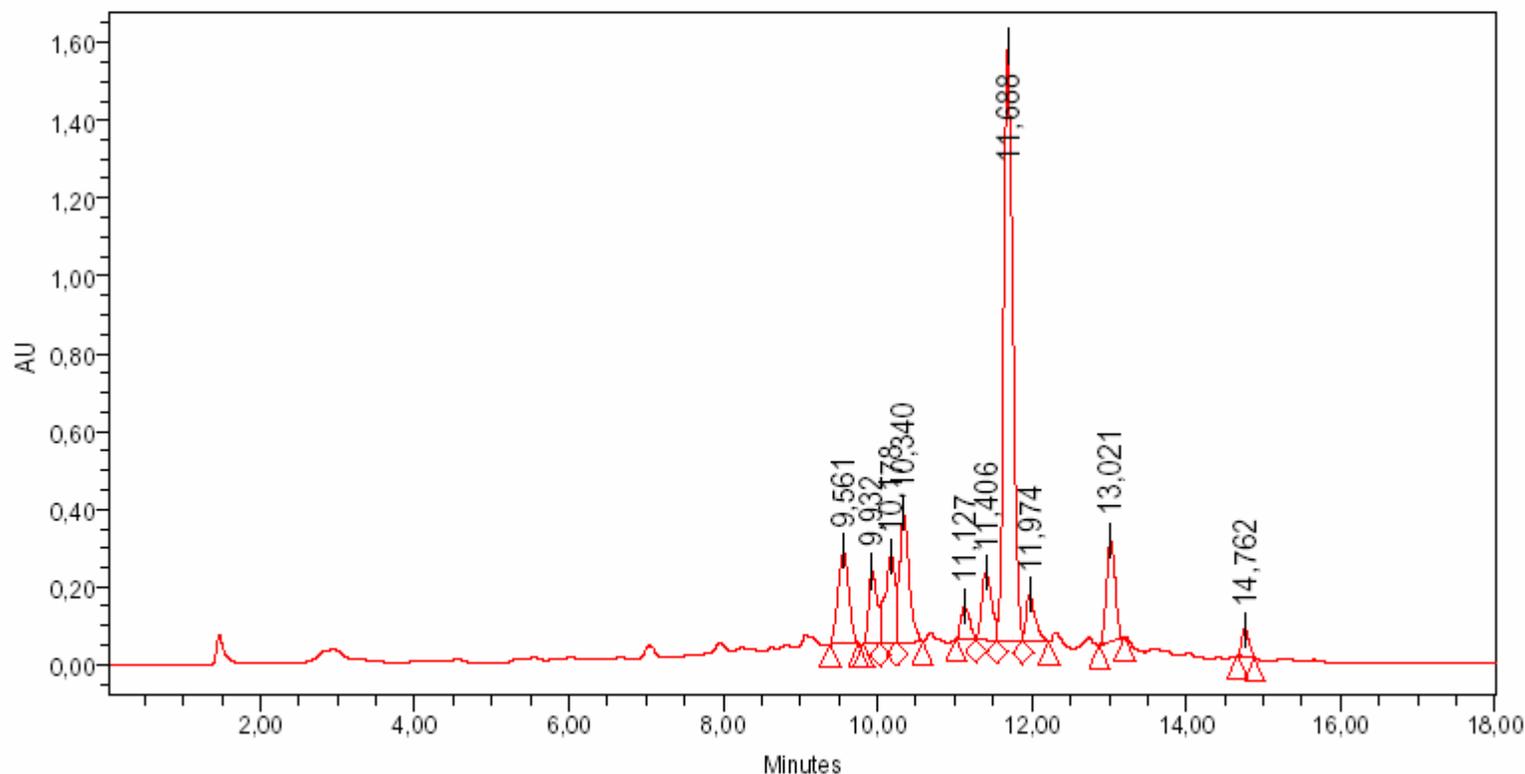


Lamellarins SPS: HPLC

Sample Name: R126
Sample Type: Unknown
Vial: 1
Injection #: 1
Injection Volume: 20,00 ul
Run Time: 18,0 Minutes
Sample Set Name: pablo290702

Acquired By: System
Date Acquired: 29/07/2002 11:51:19
Acq. Method Set: G30 100 AcCN en 15 min sin TFA
Date Processed: 13/02/2003 19:31:14
Processing Method: Default
Channel Name: WvIn Ch4
Proc. Chnl. Descr.: PDA 315,0 nm

Auto-Scaled Chromatogram



Solid-Phase Peptide Synthesis

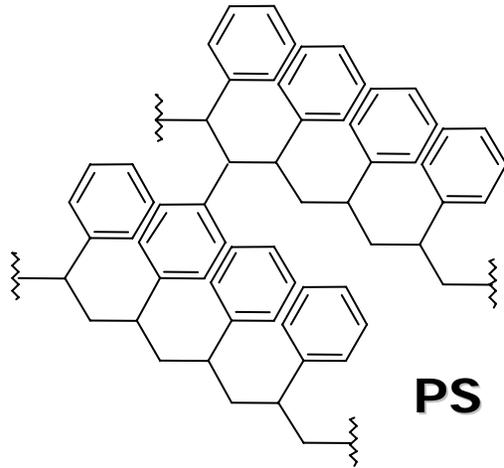
is a proper combination of

solid supports

**protecting groups
(handles/linkers)**

and coupling reagents

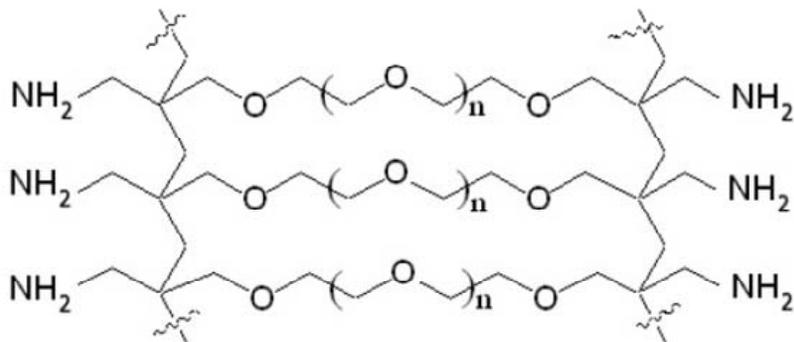
Solid Supports



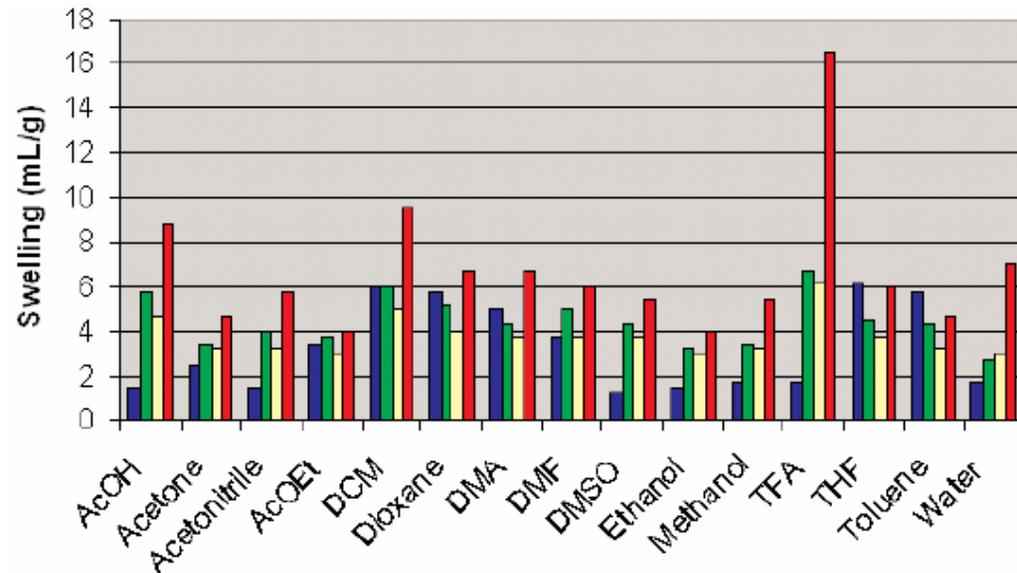
PEG-PS/TentaGel



CLEAR



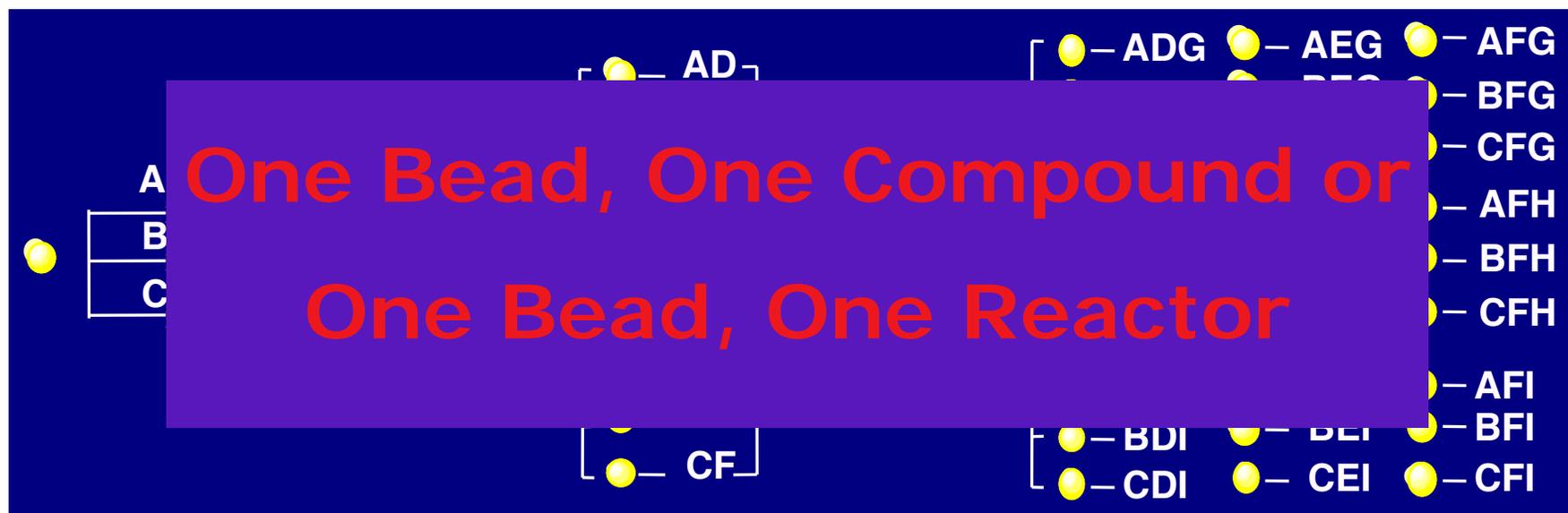
ChemMatrix®



■ PS ■ "PEG-PS"
■ CLEAR ■ ChemMatrix

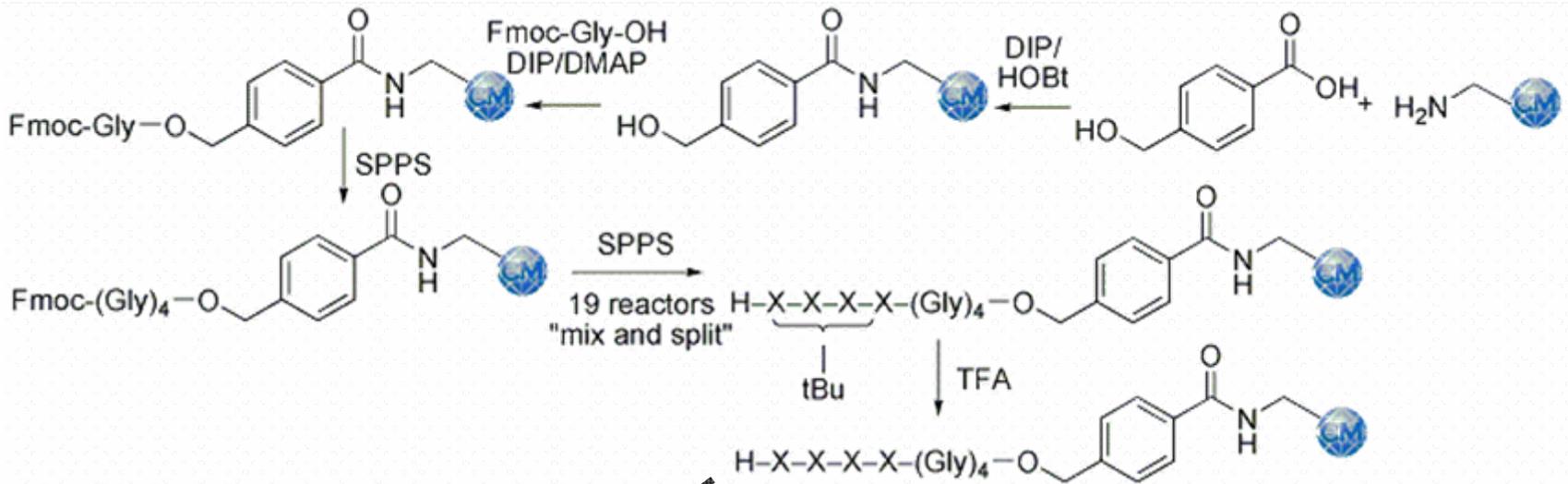
Library of Mixtures: Simultaneous Synthesis

Mix and Split (One bead, one compound)

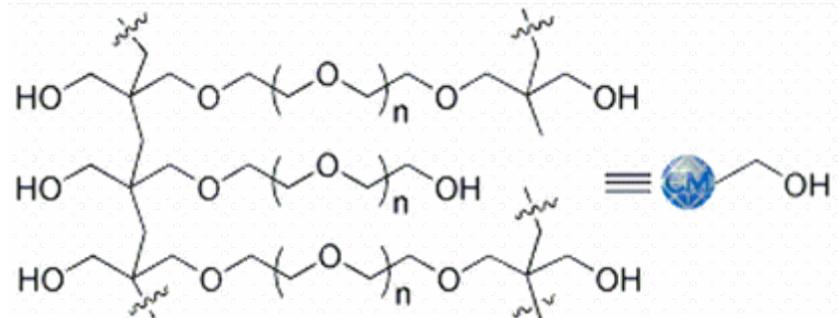


Affinity Chromatography for Monoclonal Antibodies Against Granucocyte Macrophage-Colony Stimulating Factor (GM-CSF)

Library of 130.321 (19^4) tetrapeptides



Solid-phase Screening

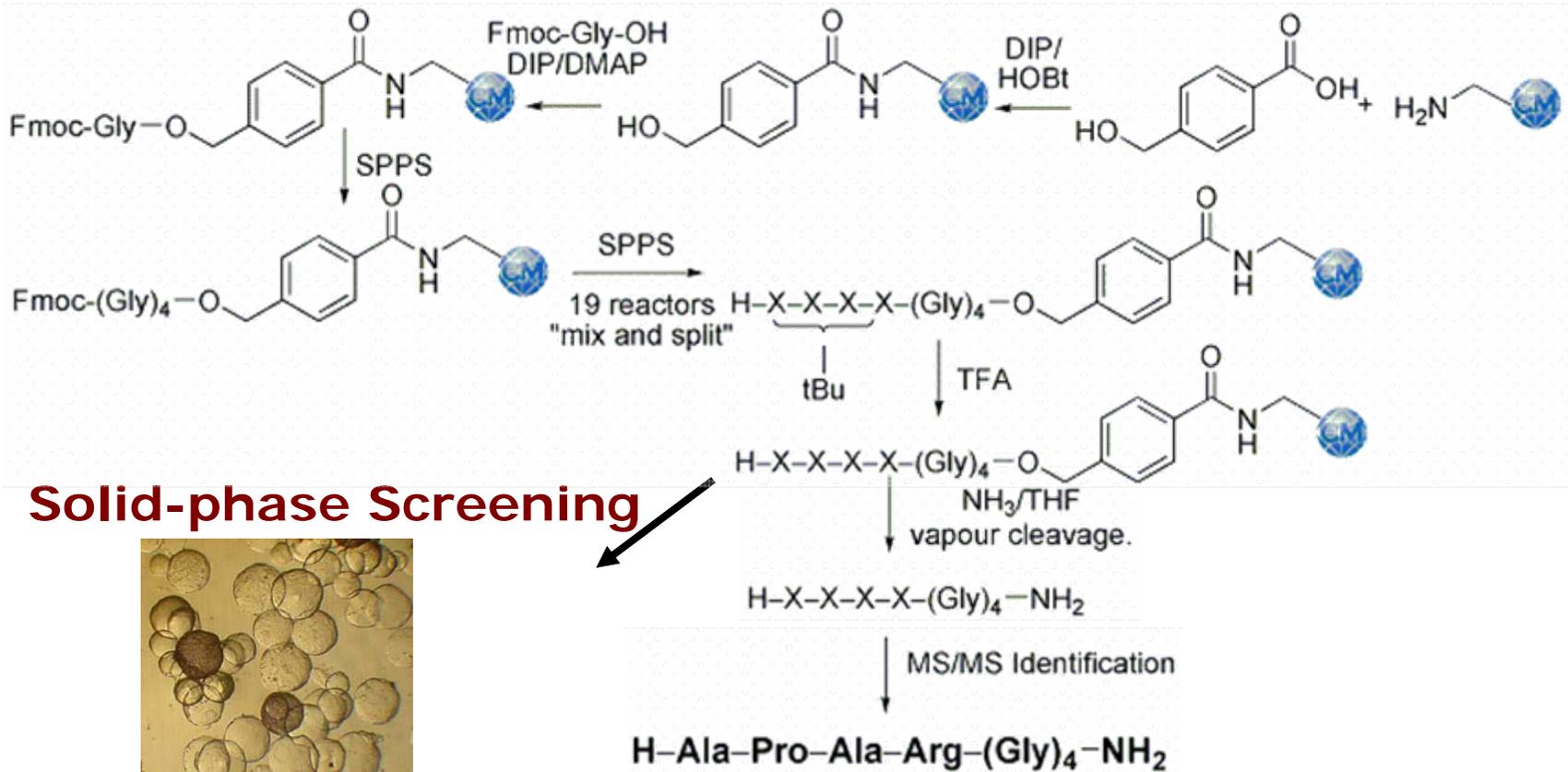


Solid-phase Screening (Immunoaffinity)

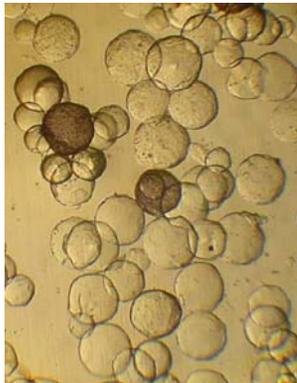


Affinity Chromatography for Monoclonal Antibodies Against Granucocyte Macrophage-Colony Stimulating Factor (GM-CSF)

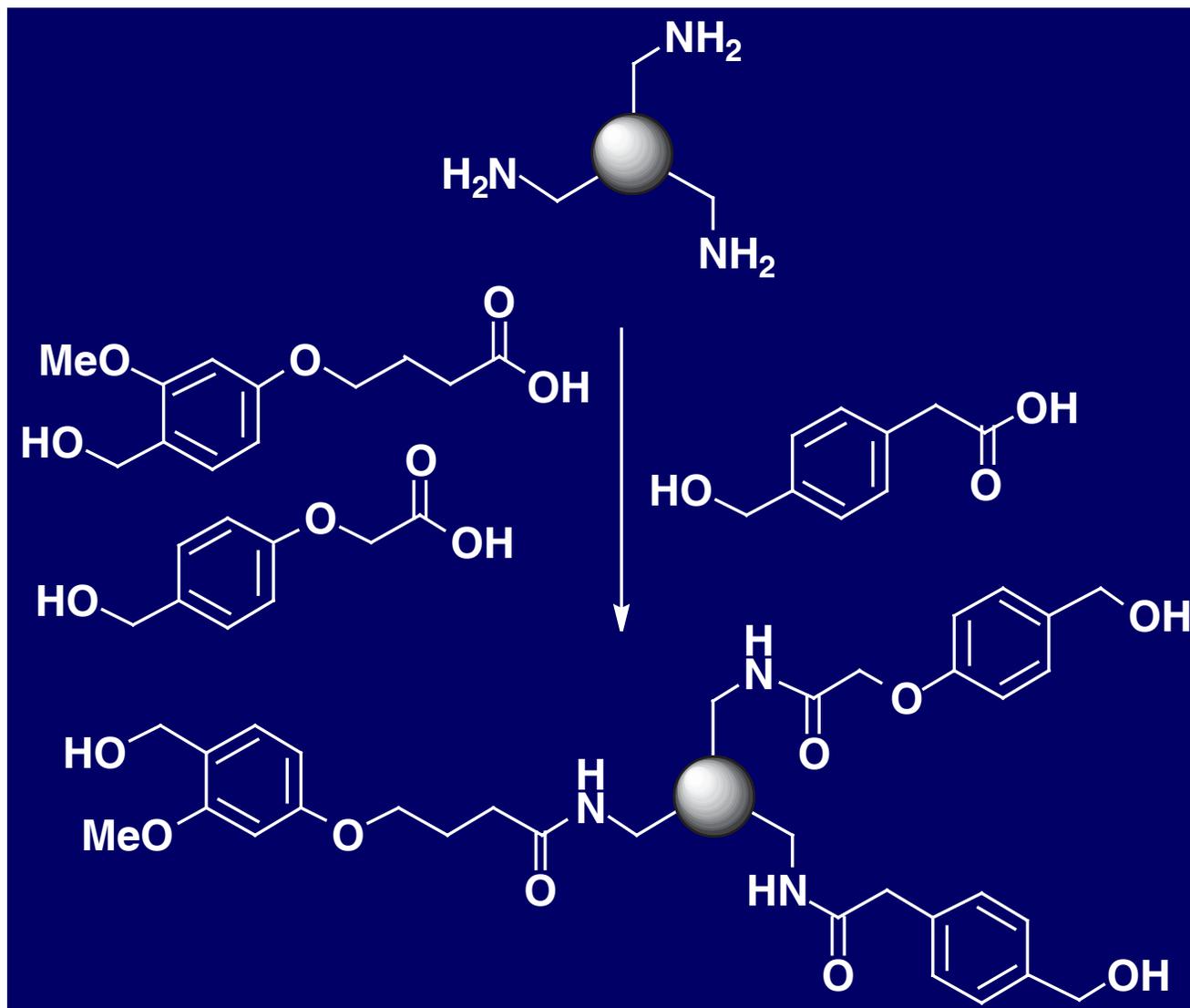
Library of 130.321 (19⁴) tetrapeptides



Solid-phase Screening



Staged Released

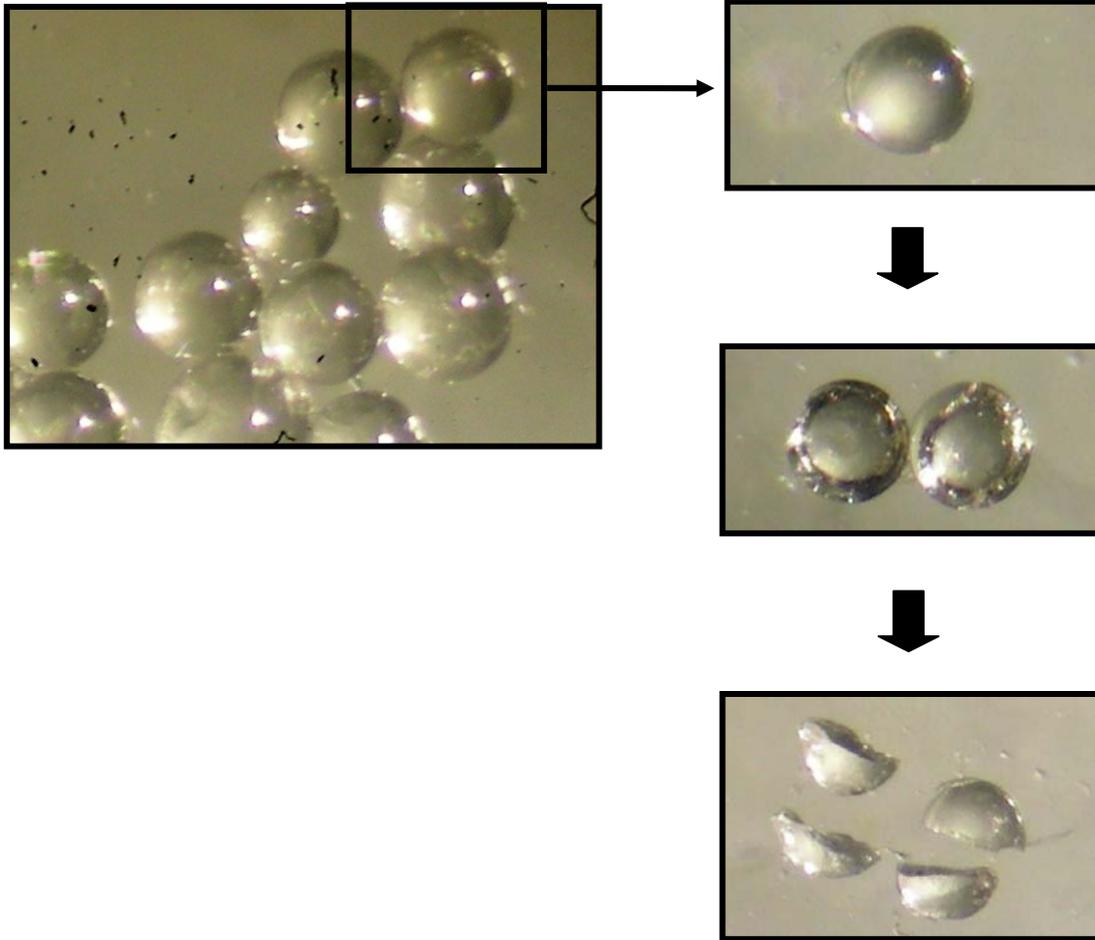


1 % TFA

TFA

NH₃

ChemMatrix Bead Portion Released



β -Amyloid 1-42

Formation of amyloid plaques is thought to contribute to the degradation of the neurons in the brain and the subsequent symptoms of **Alzheimer's** disease.

Synthesis attempts:

Synthesis in solution

Solid-Phase using different resins: polystyrene, Tentagel, PEG-PS and Pepsyn K

Several strategies have been studied to overcome the difficulties of this synthesis: introduction of an oxidized Met-35, the use of DMSO as a coupling co-solvent, the use of DBU, the introduction of Hmb backbone amide protection.

Recently, the use of an O–N intramolecular acyl migration reaction of the corresponding O-acyl isopeptide*.

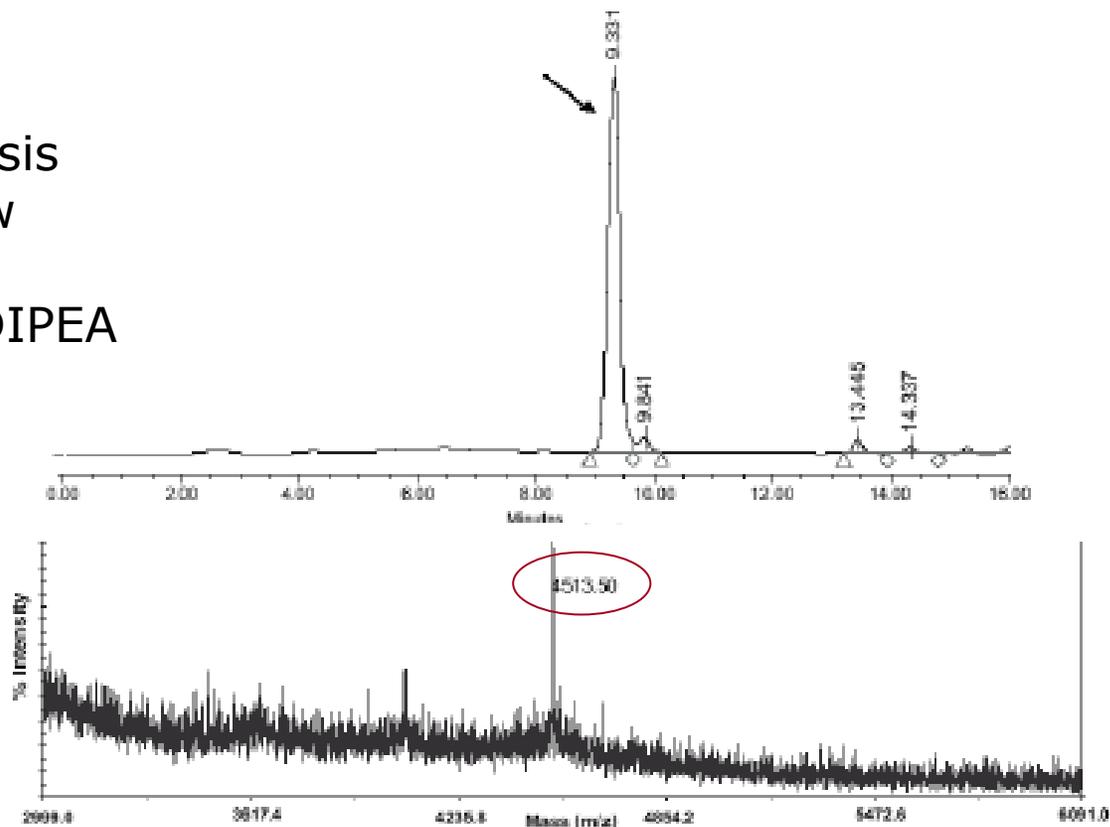
*Y. Sohma, Y. Kiso. et al. *J. Peptide Sci.* **2005** vol 11 441–451

Synthesis of β -Amyloid 1-42

ChemMatrix allows synthesis
without the need for new
strategies or methods:

Automatically, HBTU/HOBt/DIPEA

Major peak
91% purity



Coupling Reagents

EDC

HOBt

CI-HOBT

PyAOP

CDI

TCTU

TCT

HOAt

dipcdi

PyClock

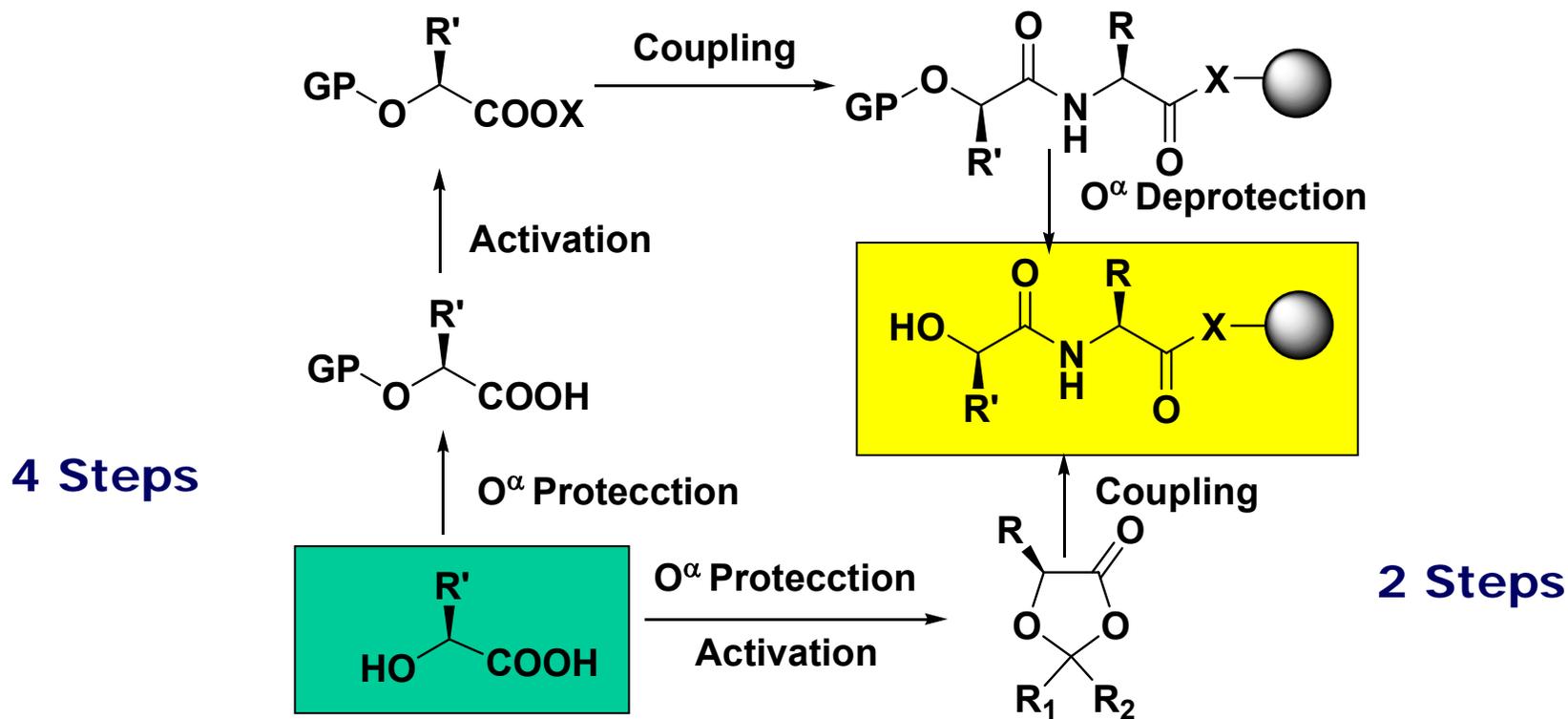
TMUCI CI

HOSu

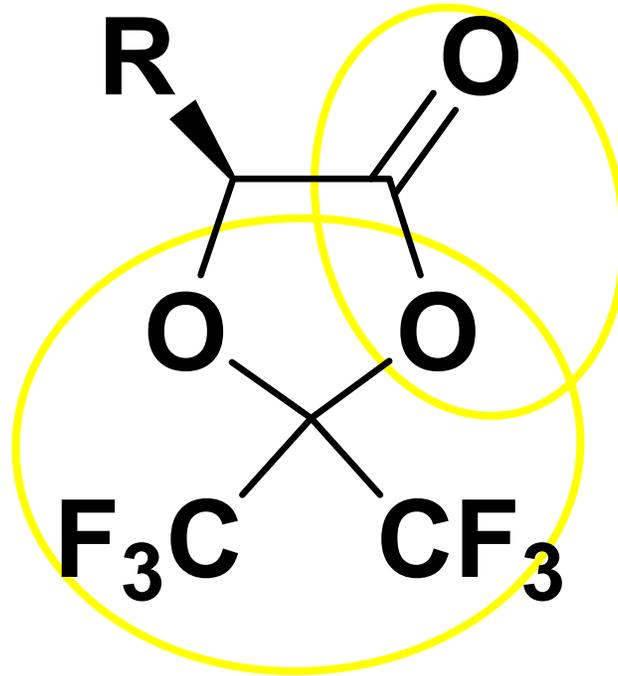
HATU

Couplin Methods: Solid-Phase Synthesis of Depsipeptides

SPS Strategies

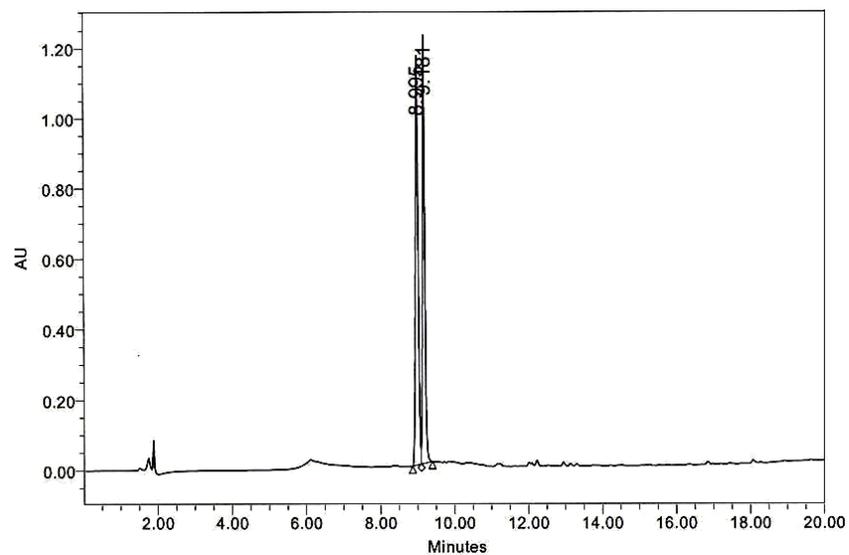
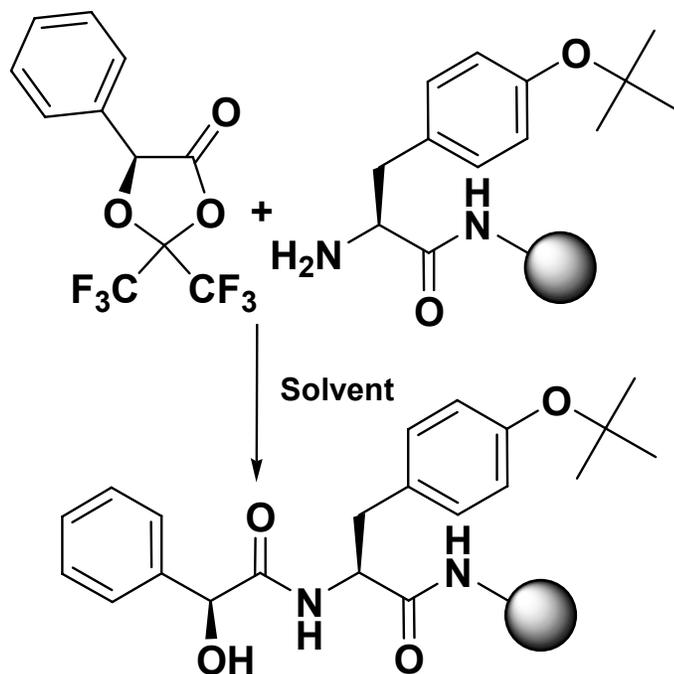
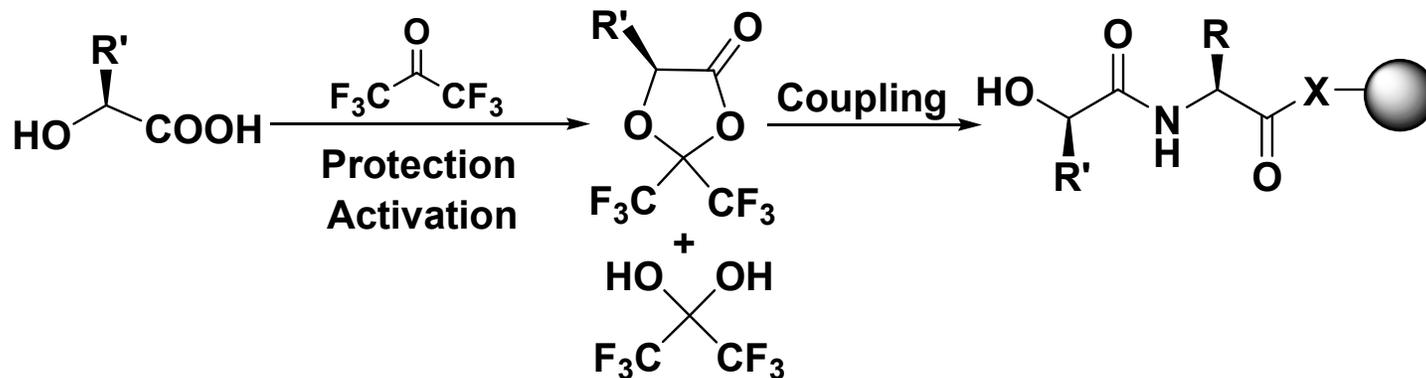


HFA-Hydroxyacids



- Activation of carboxyl group
- Protection of hydroxyl group

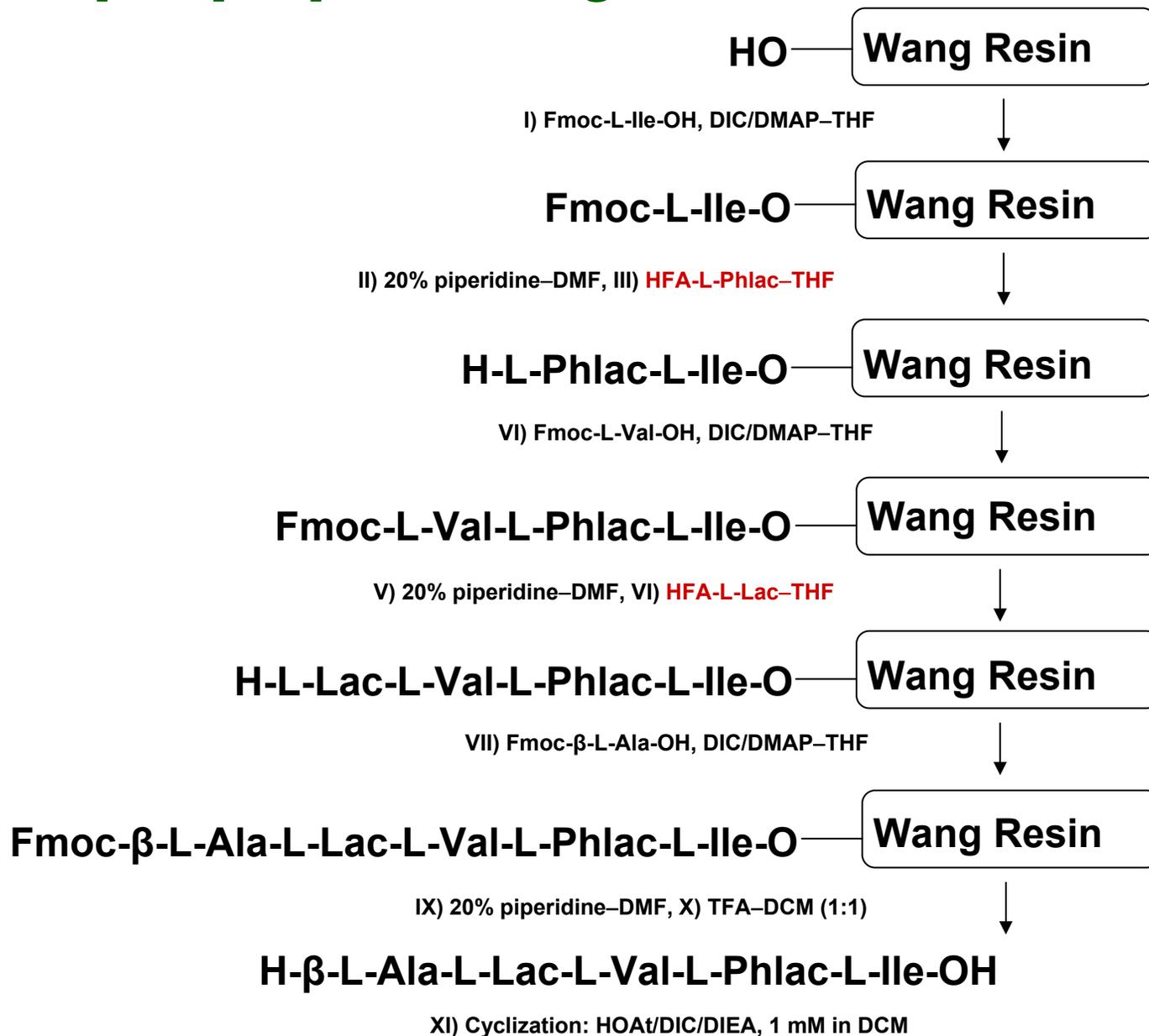
SPS of Depsipeptides with HFA



Peptide Synthesis: Chossing the Solvent

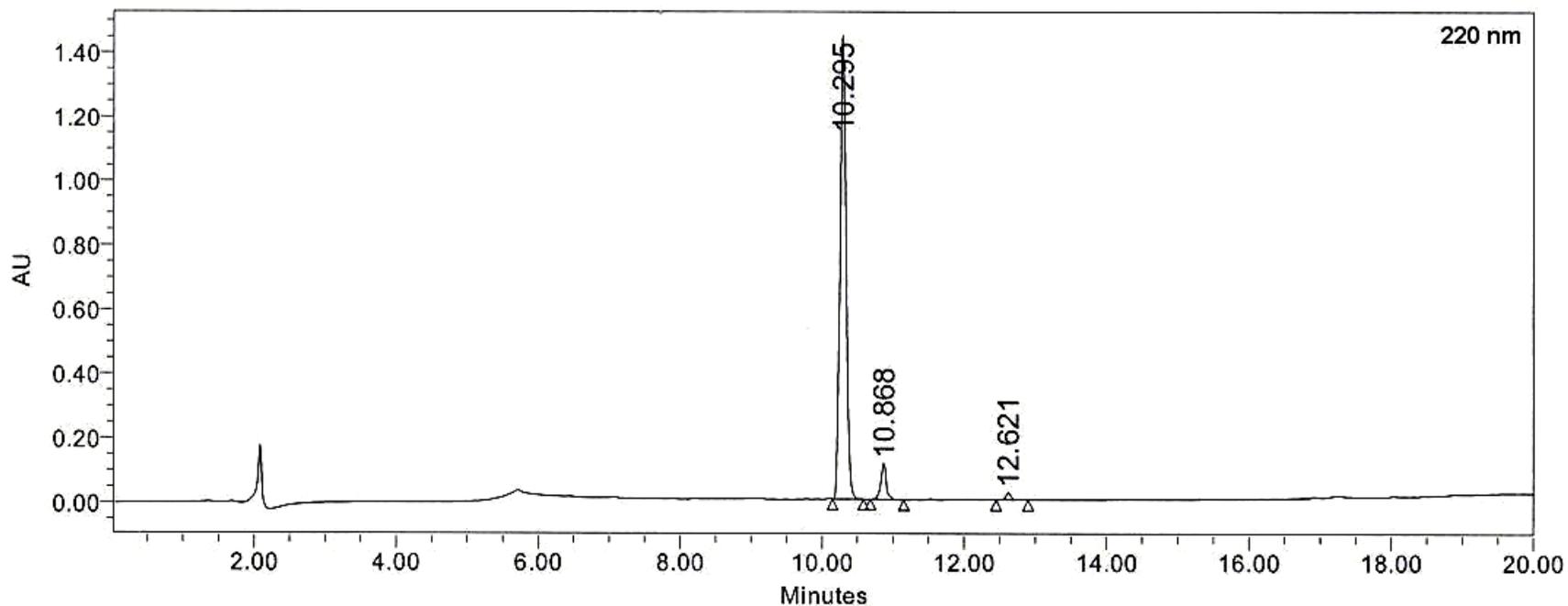
Solvent	V _{reaction} (h)	Racemization
DMSO	4 ^{1/2}	<18%
DMF	24	<11%
THF	5	<1%
CHCl ₃	50	<1%
DCM	28	<1%
Toluene	48	<1%
Hexane	No reaction	

Depsipeptide Synthesis



Depsipeptide Synthesis

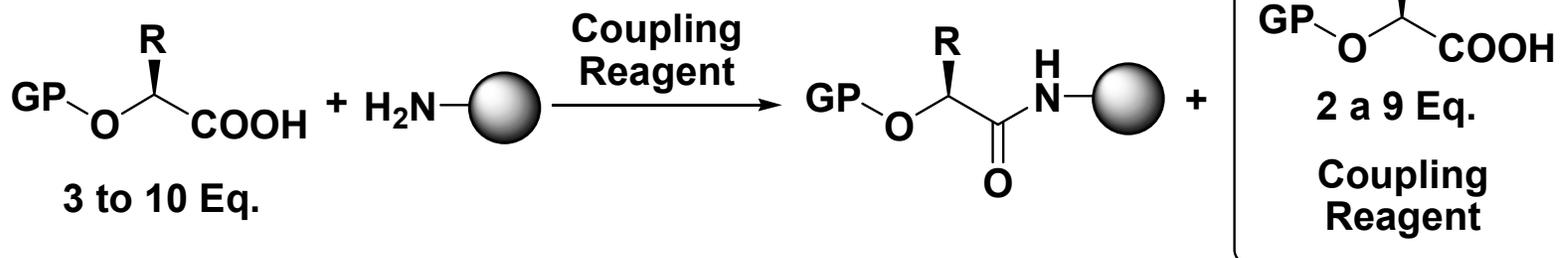
β -L-Ala-L-Lac-L-Val-L-Phlac-L-Ile



For Nomenclature: Spengler, Jiménez, Burger, Giralt, Albericio. *J. Peptide Res.*, 65, 550 (2005)

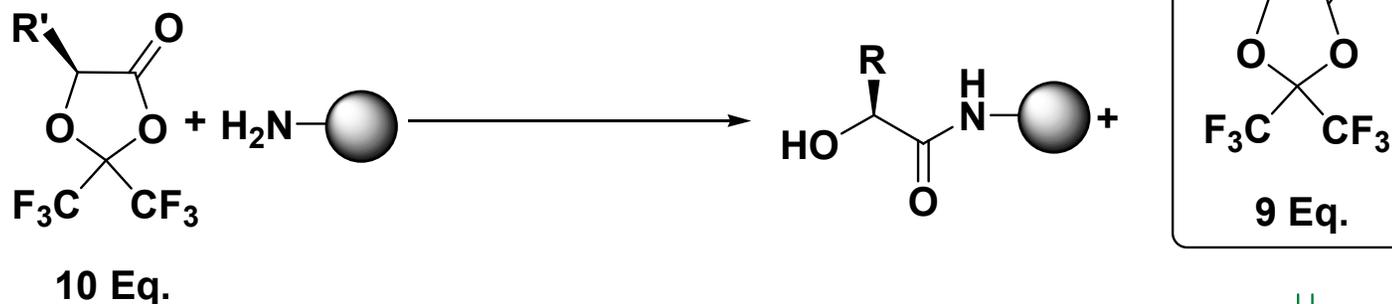
Reuse of HFA-Hydroxyacids

SPPS



To Waste

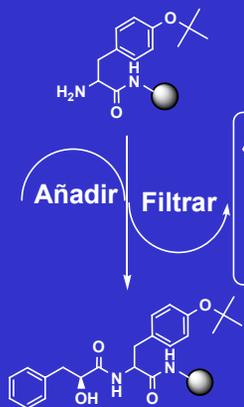
HFA-HAs



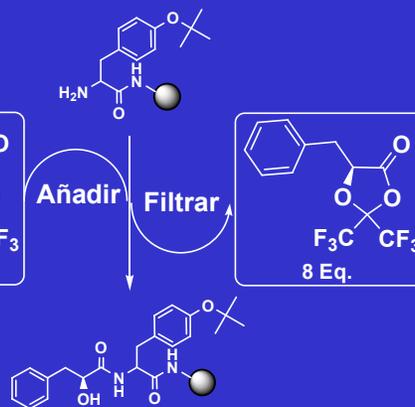
Filter and Reuse

Reuse of HFA-Hydroxyacids

1° Acoplamiento

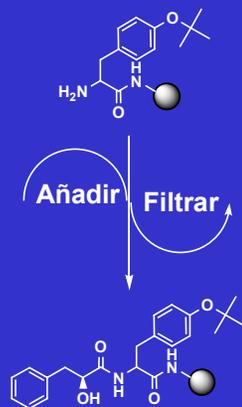


2° Acoplamiento

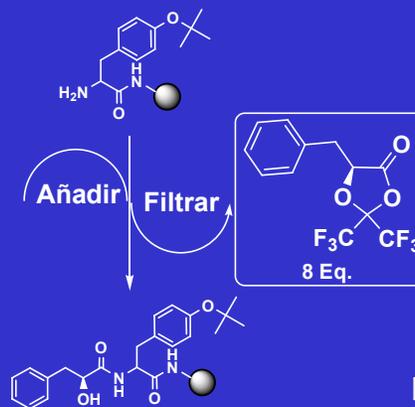


Reuse of HFA-Hydroxyacids

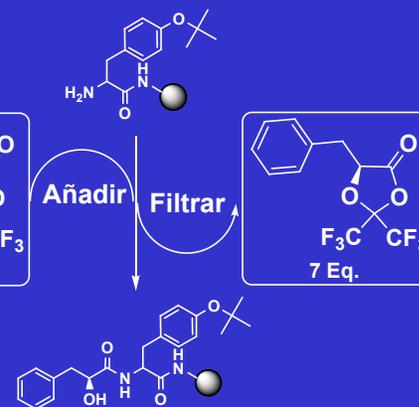
1° Acoplamiento



2° Acoplamiento

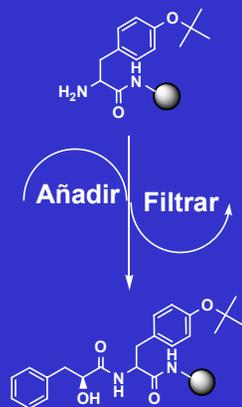


3° Acoplamiento

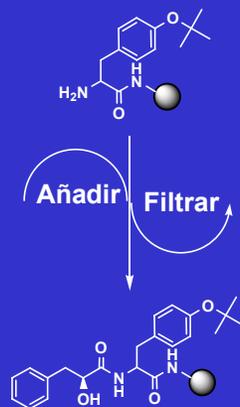


Reuse of HFA-Hydroxyacids

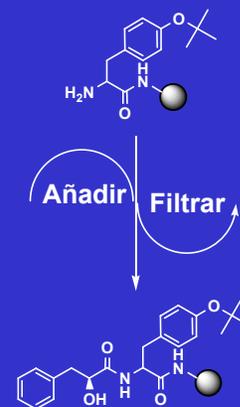
1° Acoplamiento



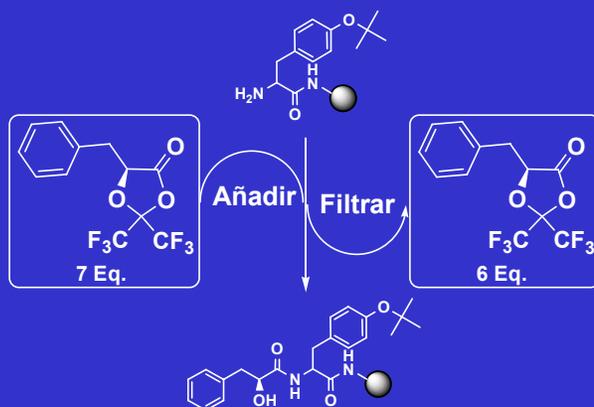
2° Acoplamiento



3° Acoplamiento

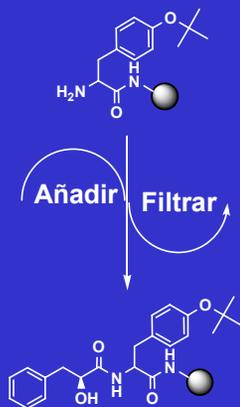


4° Acoplamiento

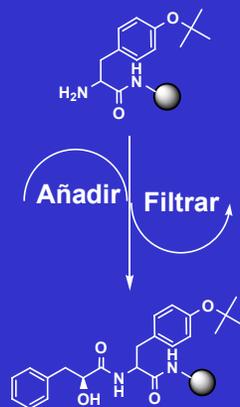


Reuse of HFA-Hydroxyacids

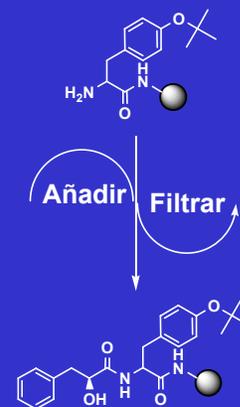
1° Acoplamiento



2° Acoplamiento



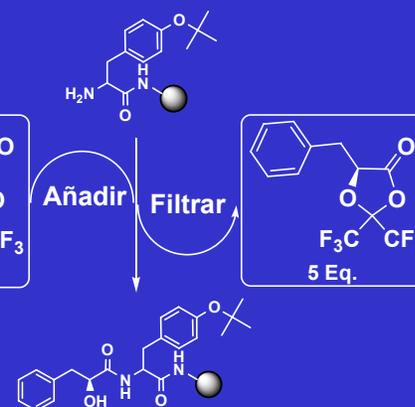
3° Acoplamiento



4° Acoplamiento



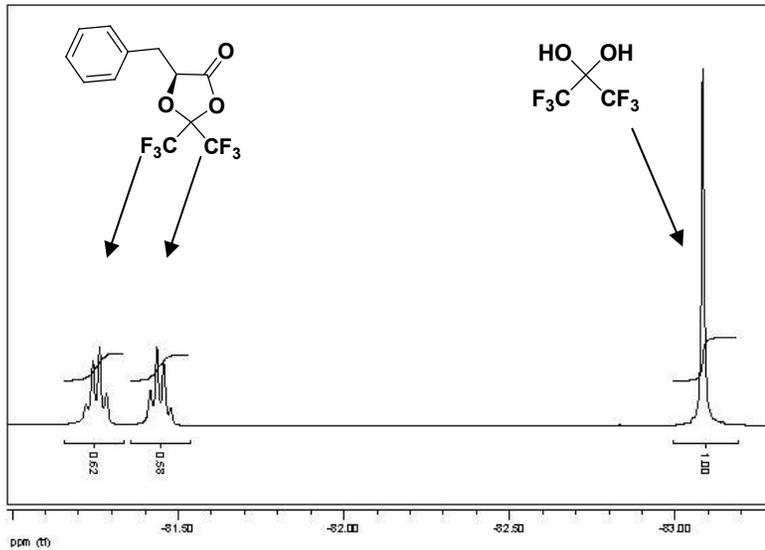
5° Acoplamiento



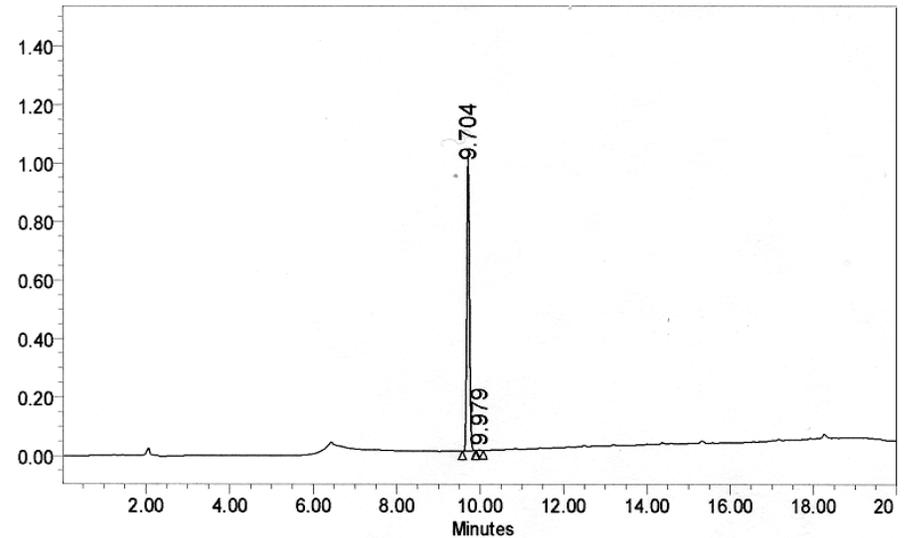
Reuse of HFA-Hydroxyacids

After 5 couplings

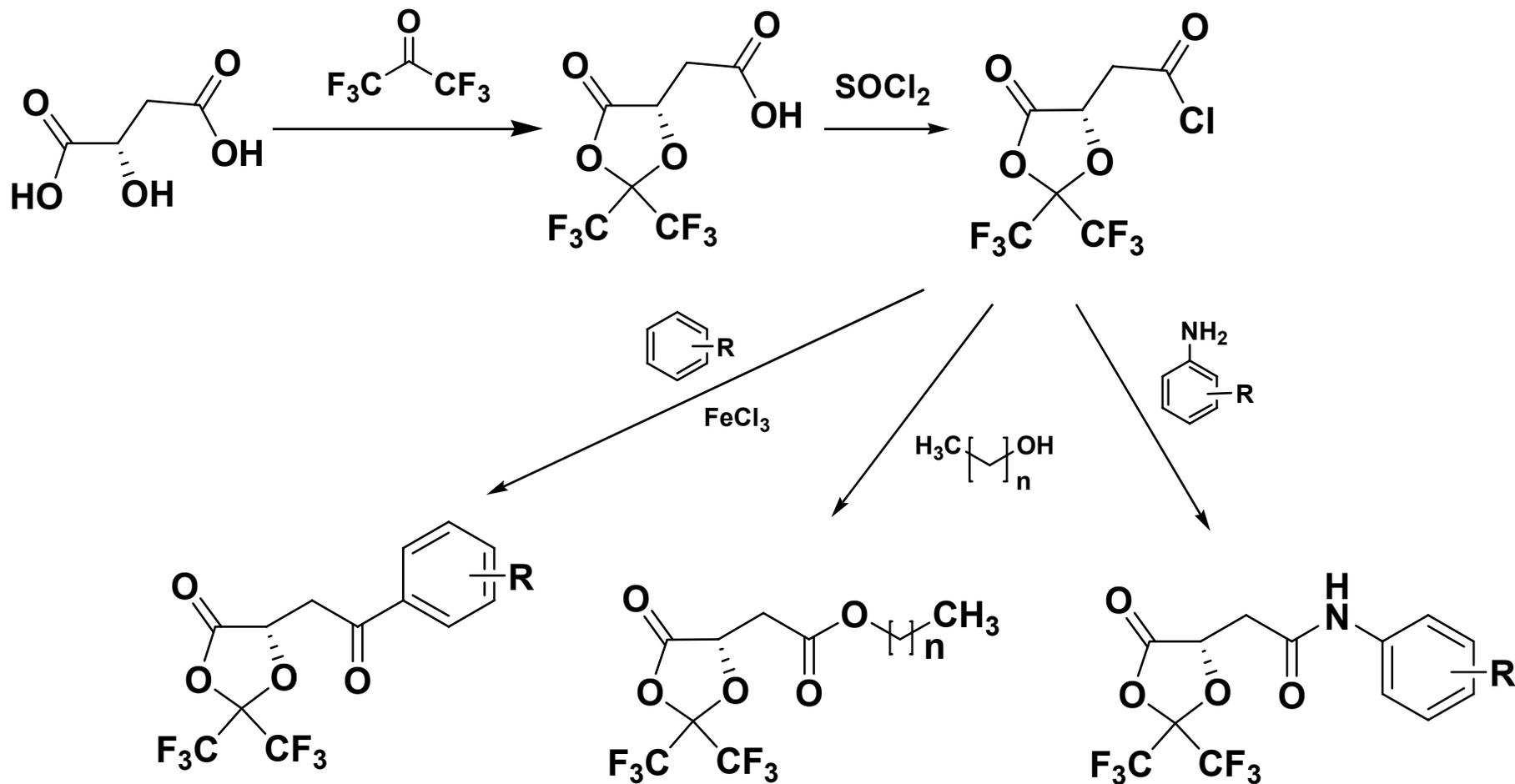
^{19}F -RMN



HPLC

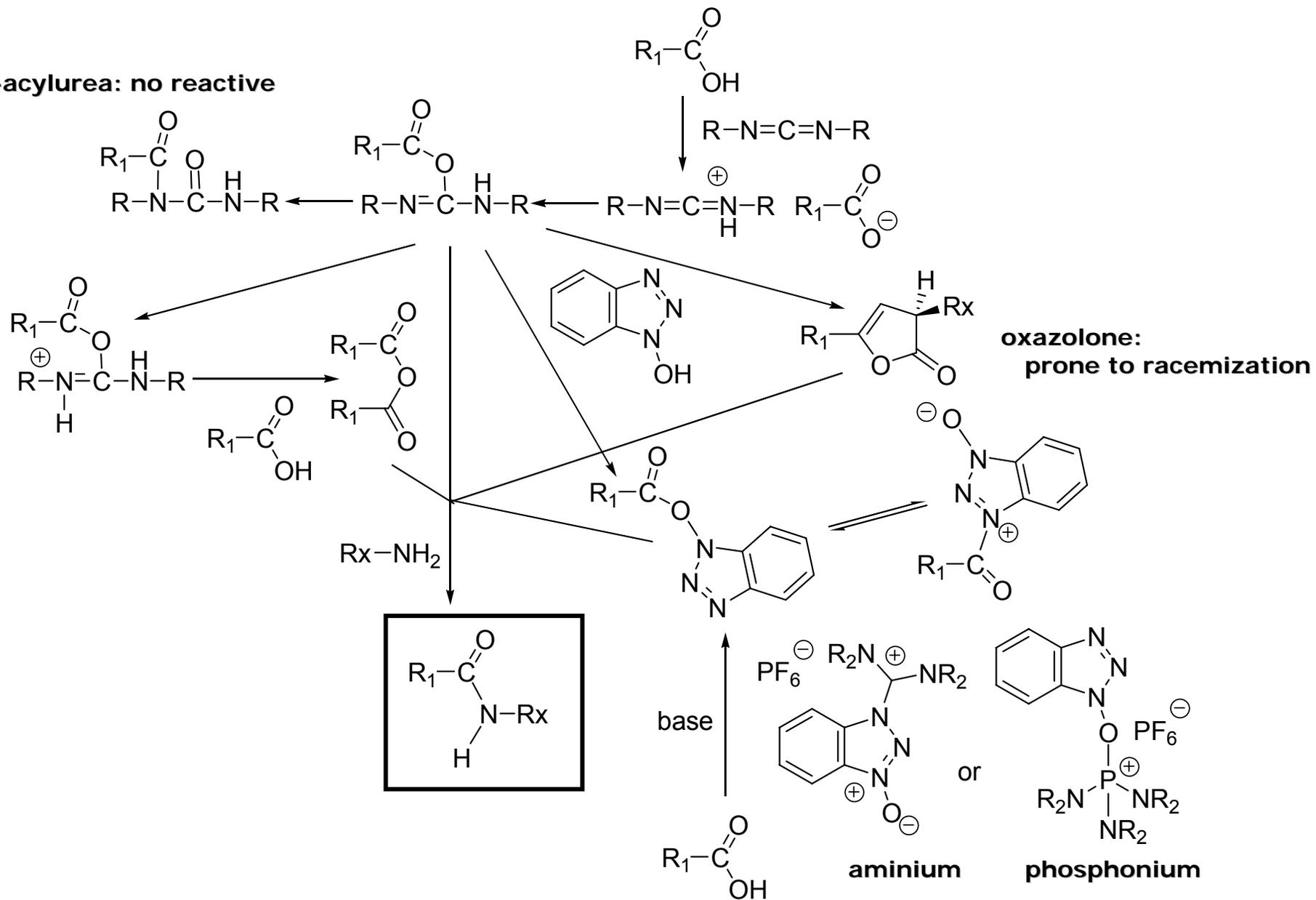


Synthesis of Non-natural HAs

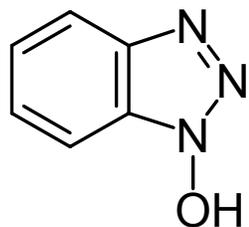


Coupling Chemistries: Carbodiimide Coupling and Aminium Salts

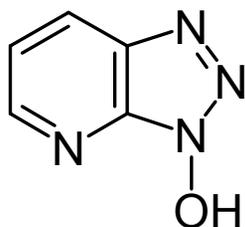
N-acylurea: no reactive



Coupling Chemistries: HOBt vs HOAt

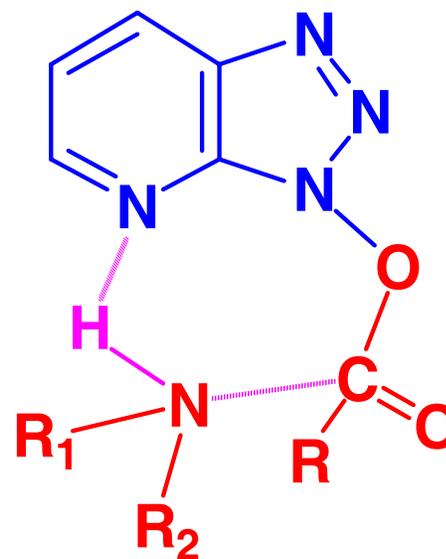


HOBt



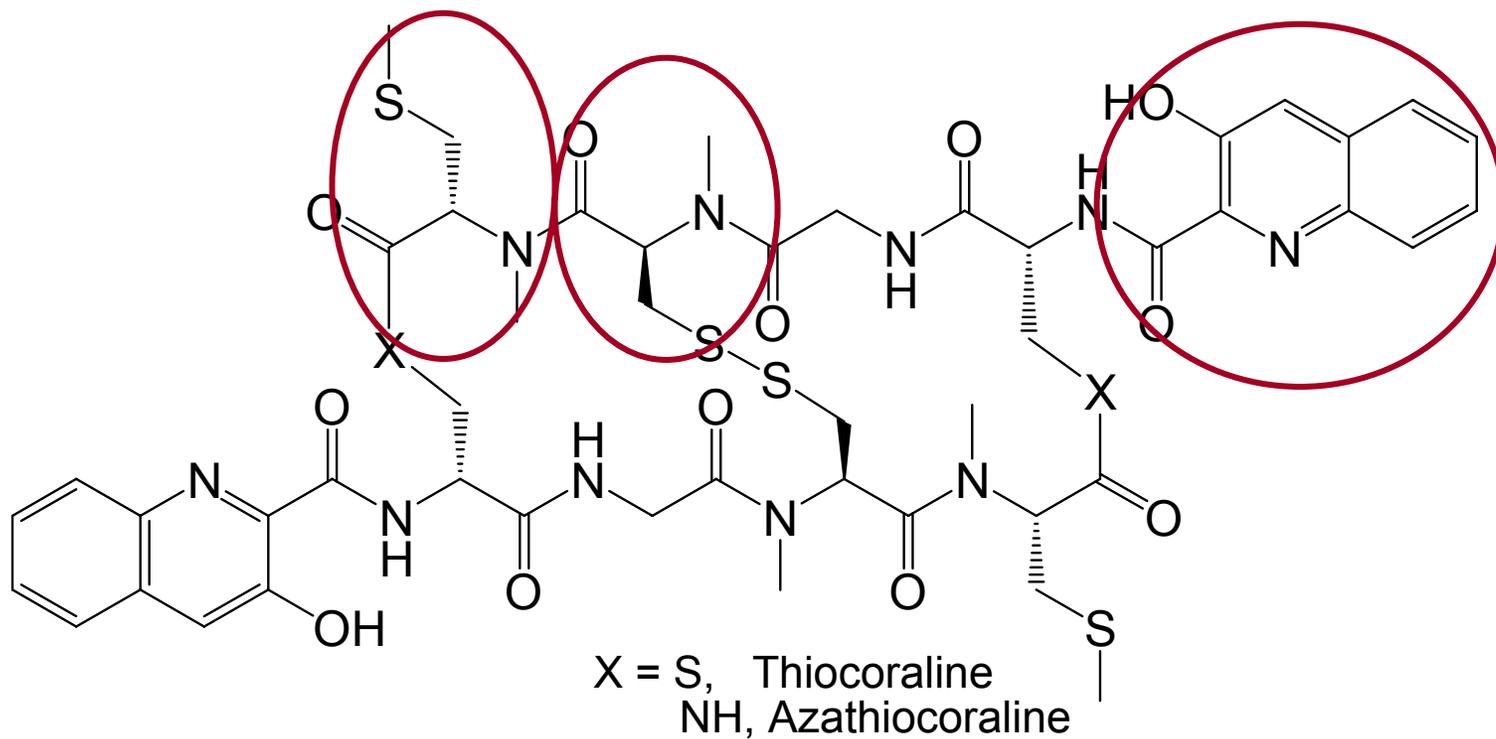
HOAt

Mechanism



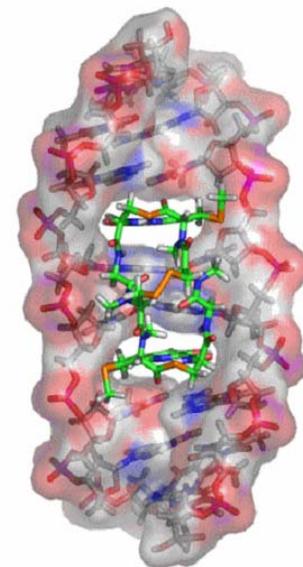
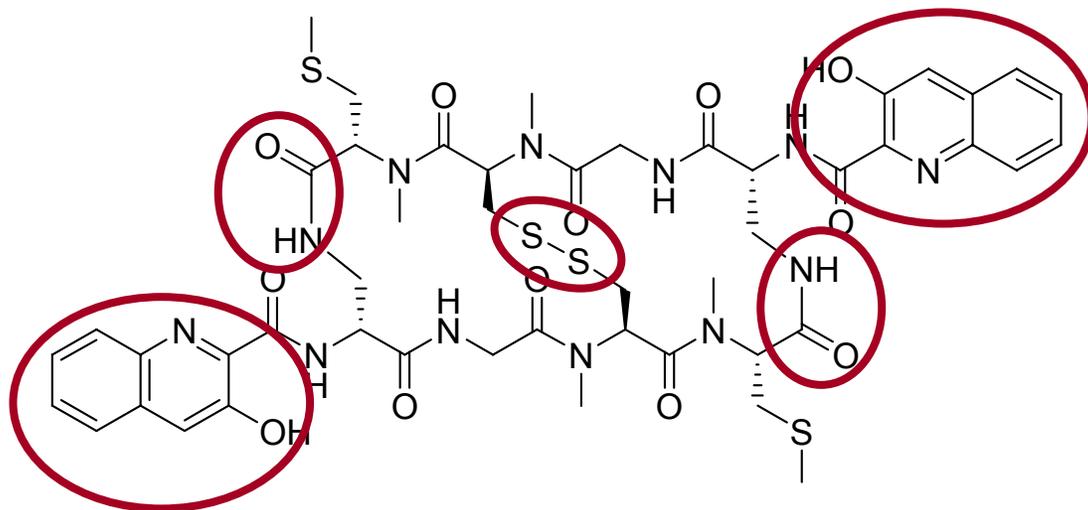
	pK_a	
	H ₂ O	DMSO
HOBt	4.60	9.3
7-HOAt	3.47	8.7
4-HOAt	3.14	8.1

Thiocoraline Family



Azathiocoraline

Azathiocoraline Solid-Phase Synthetic Strategy

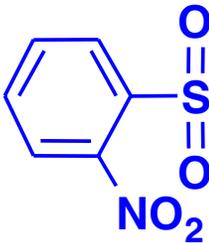


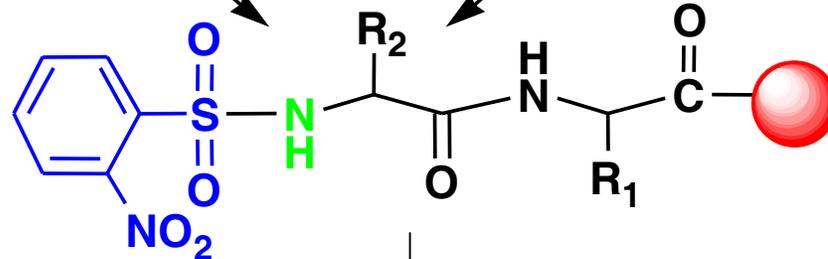
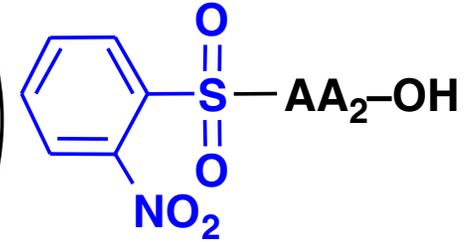
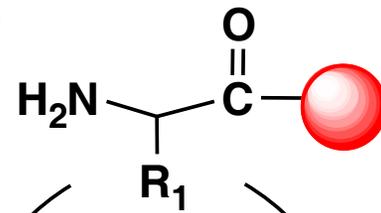
- Solid-Phase Peptide Chain Elongation 
- Disulfide Bridge (S-Acm) Formation on Solid-Phase
- If possible, Macrolactamization on Solid-Phase
- Incorporation of the Heterocycle in the last step

SPS of *N*-Methyl Amino Acids: *o*-Nosyl (*o*-NBS)

(i) Fmoc-AA₂-OH

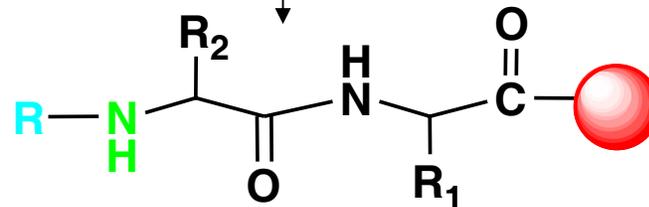
(ii) piperidine-DMF (1:4)

(iii) -Cl, TMP



(i) R-L, DBU

(ii) thiophenol/BSH-DBU-DMF

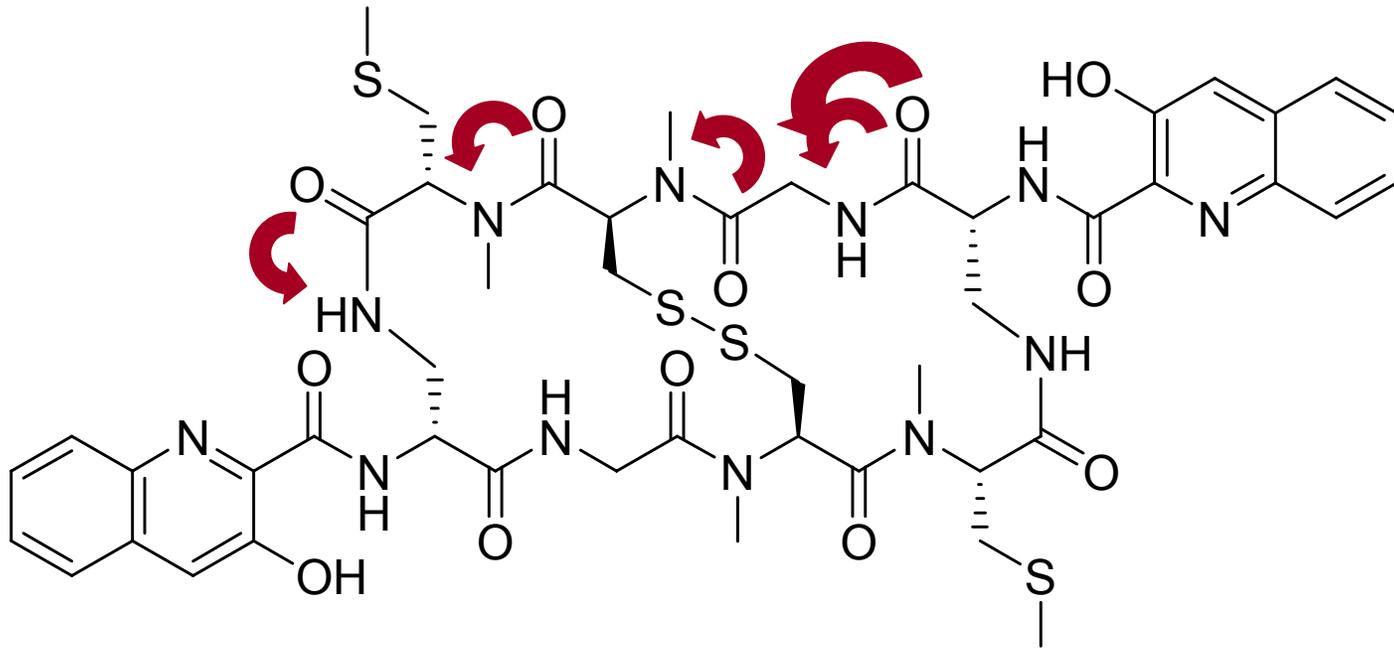


- Fukuyama *et al.* TL36, 6373 (1997)

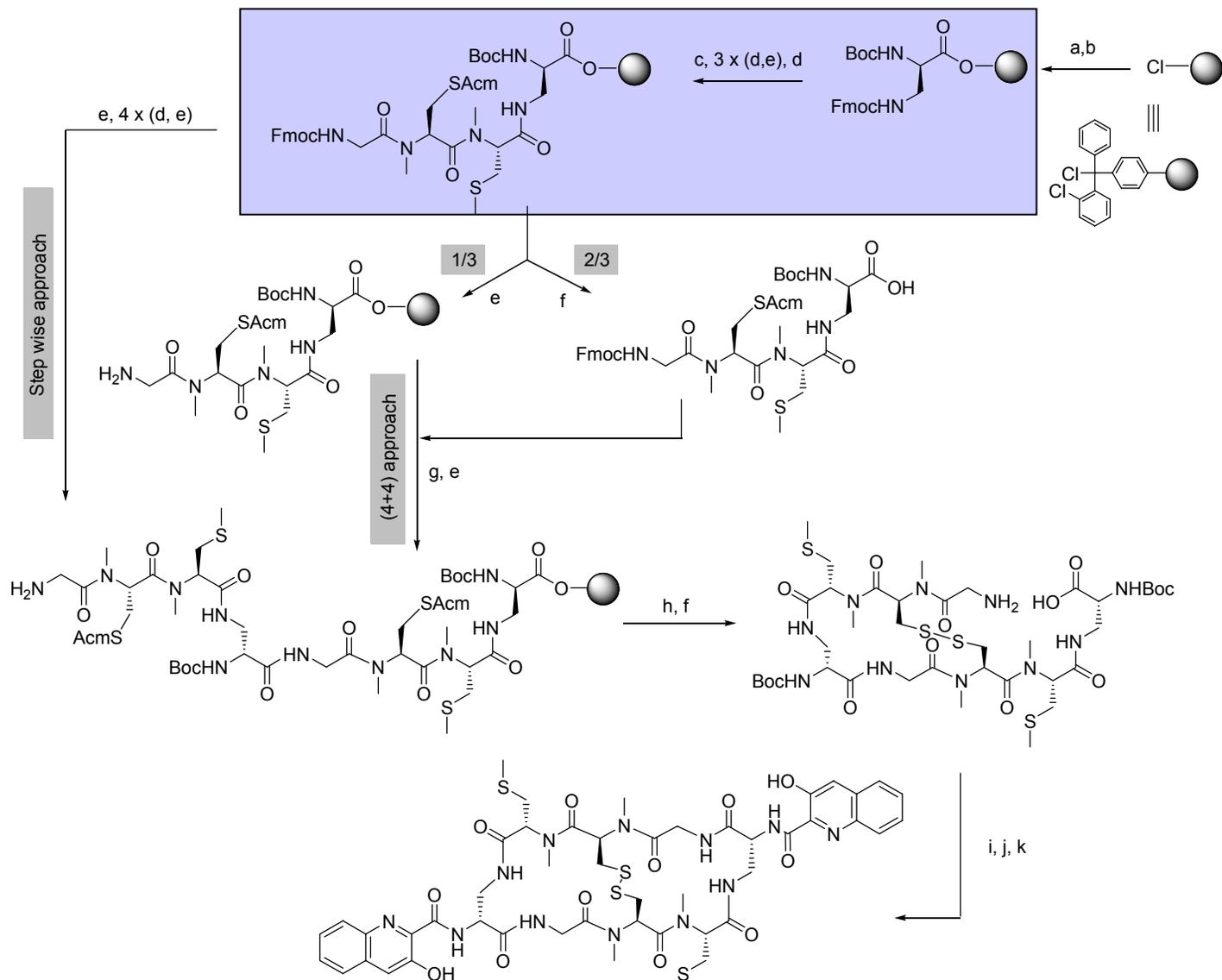
- Miller, Scanlan, JACS 119, 2301

(1997), and 120, 2690 (1998)

Choose of the Cyclization Point

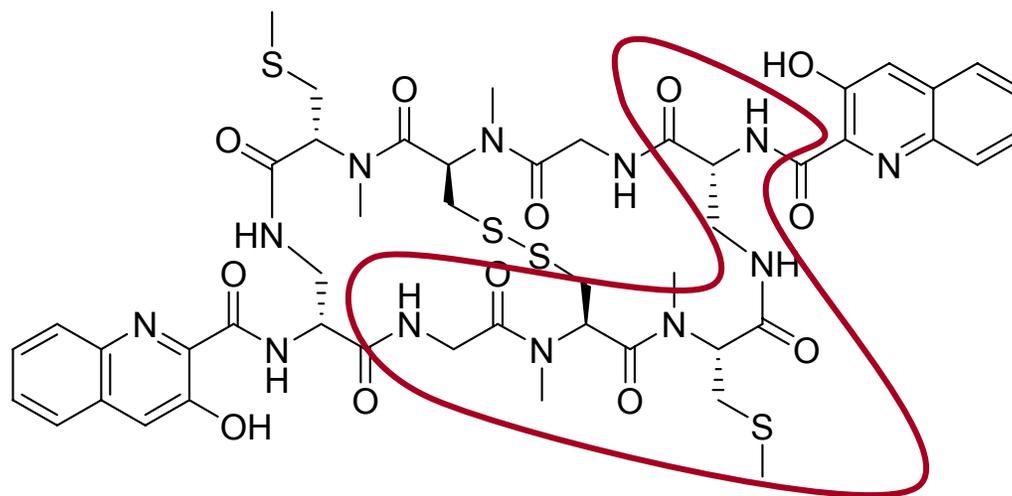
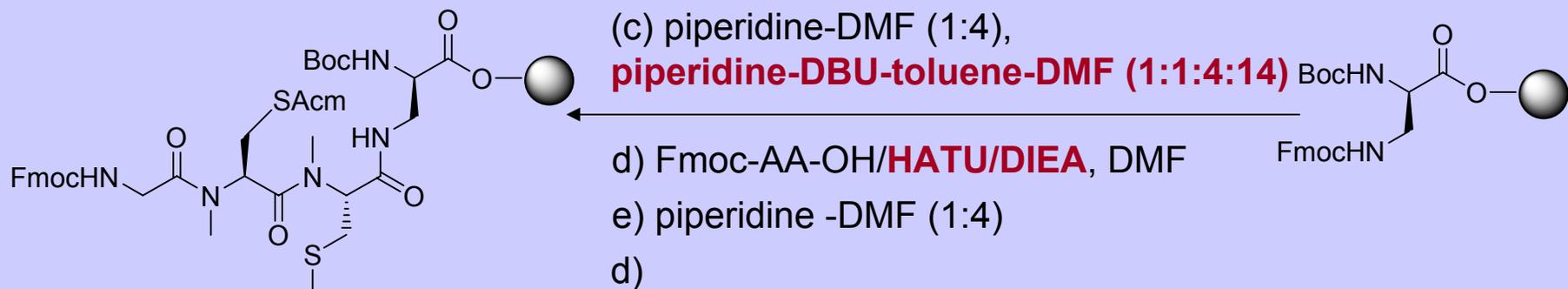


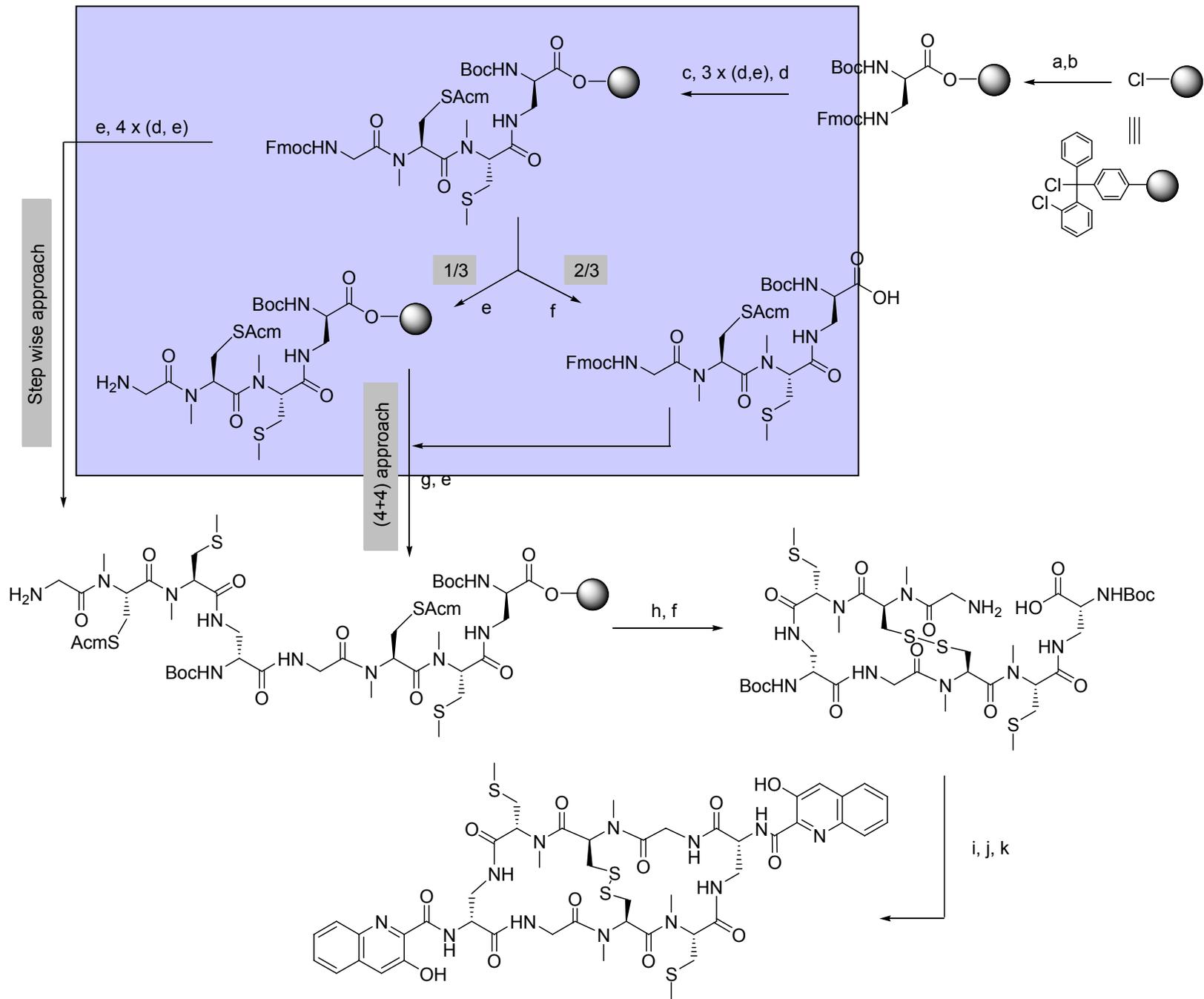
N-Methyl Amino Acid as Amino Component ↪ Hindered
MeCys as Acid Component ↪ Racemization



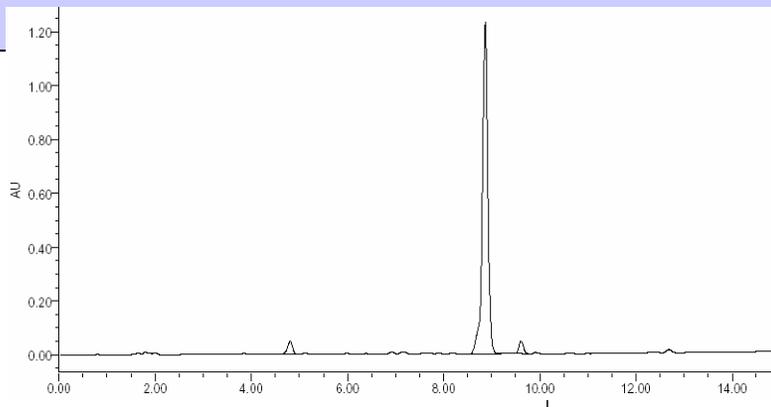
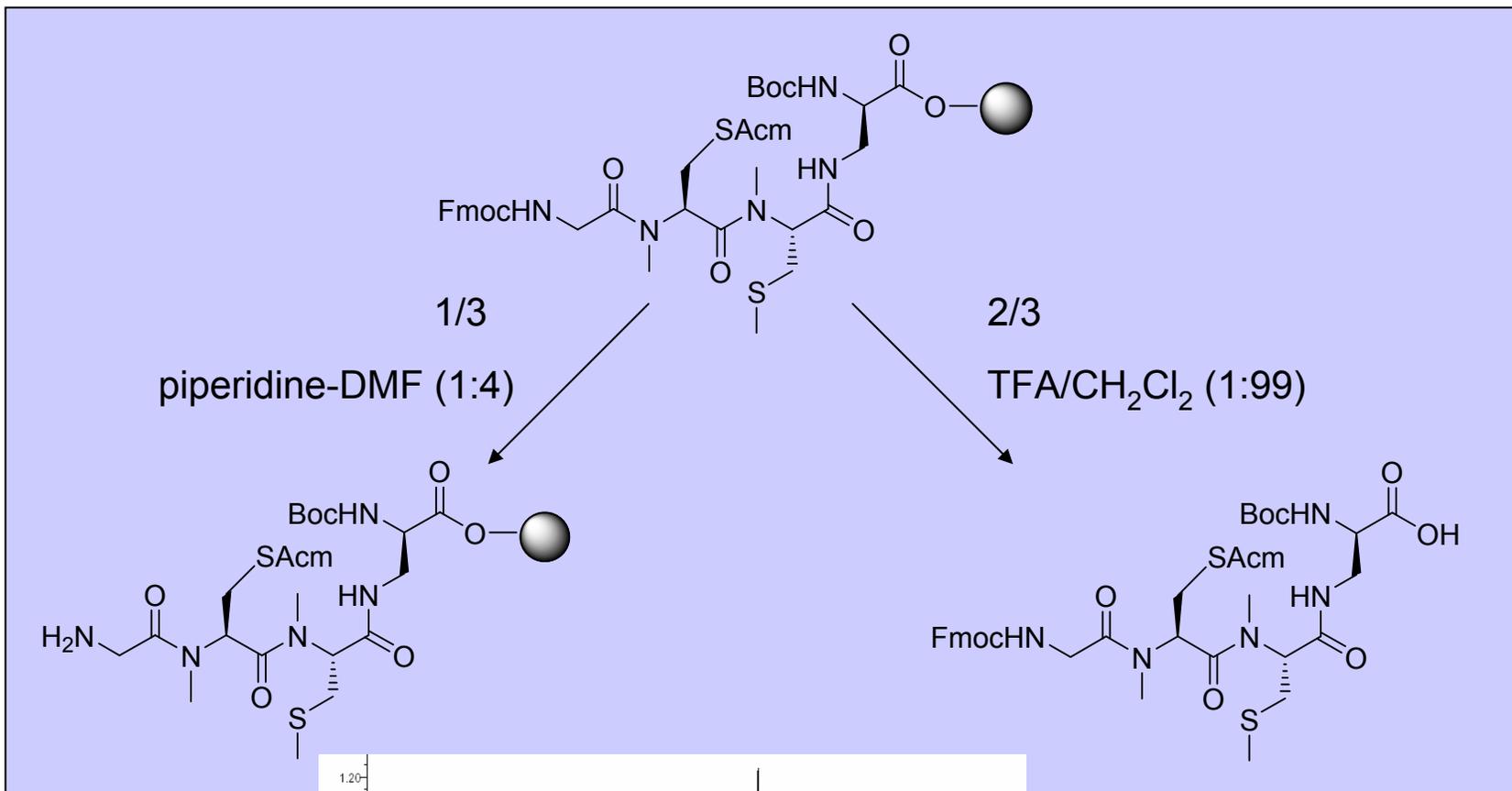
Scheme 1. a) Boc-D-Dap(Fmoc)-OH, DIEA, CH_2Cl_2 ; b) MeOH; c) piperidine-DMF (1:4), piperidine-DBU-toluene-DMF (1:1:4:14); d) Fmoc-AA-OH/HATU/DIEA, DMF; e) piperidine -DMF (1:4); f) TFA/ CH_2Cl_2 (1:99); g) PyAOP/DIEA, CH_2Cl_2 ; h) I_2 , DMF; i) EDC·HCl/HOAt/DIEA, CH_2Cl_2 (1mM); j) TFA- H_2O (19:1); k) 3-hydroxyquinaldic acid/EDC·HCl/HOSu/DIEA, CH_2Cl_2 .

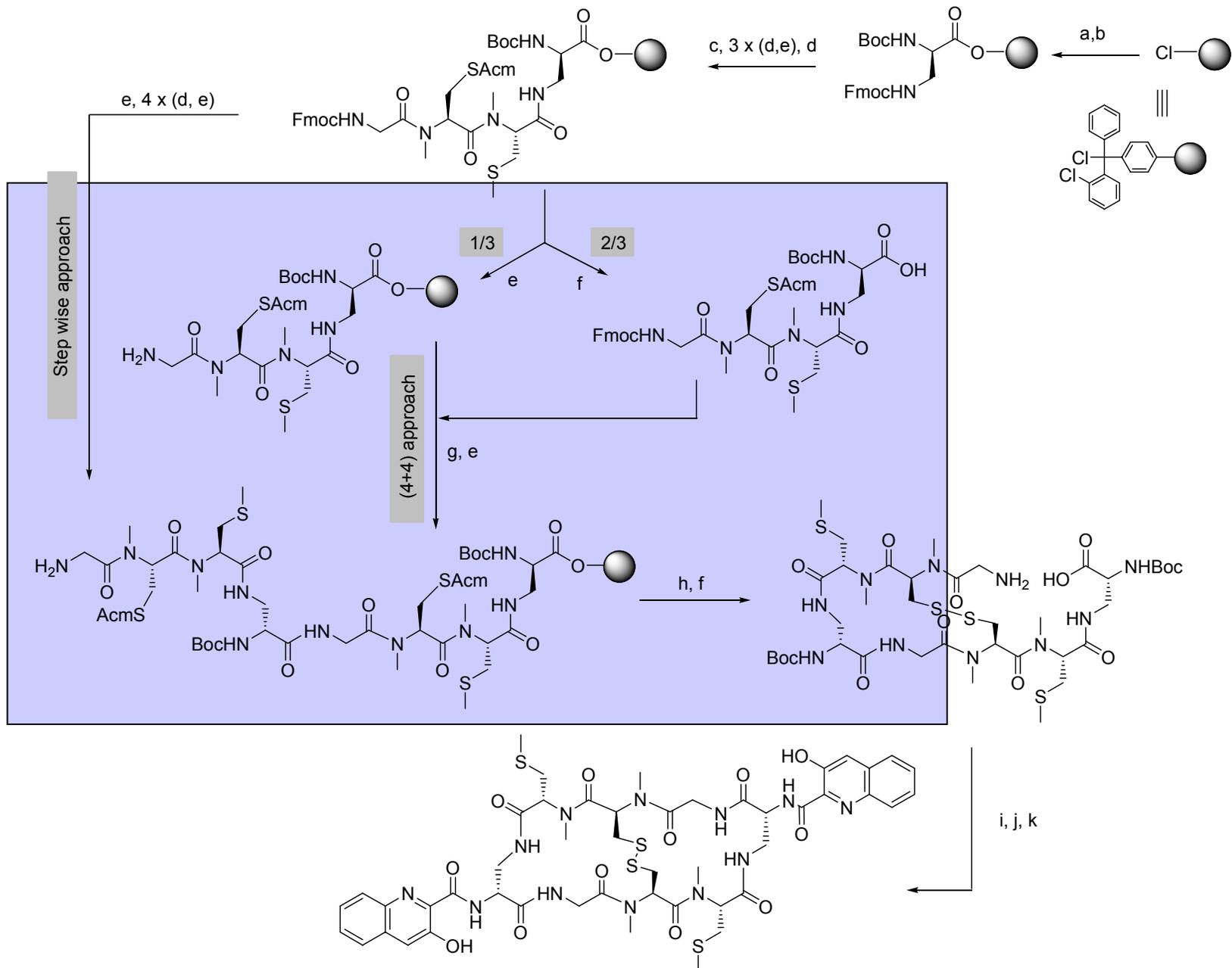
Azathiocoraline: Peptide Elongation



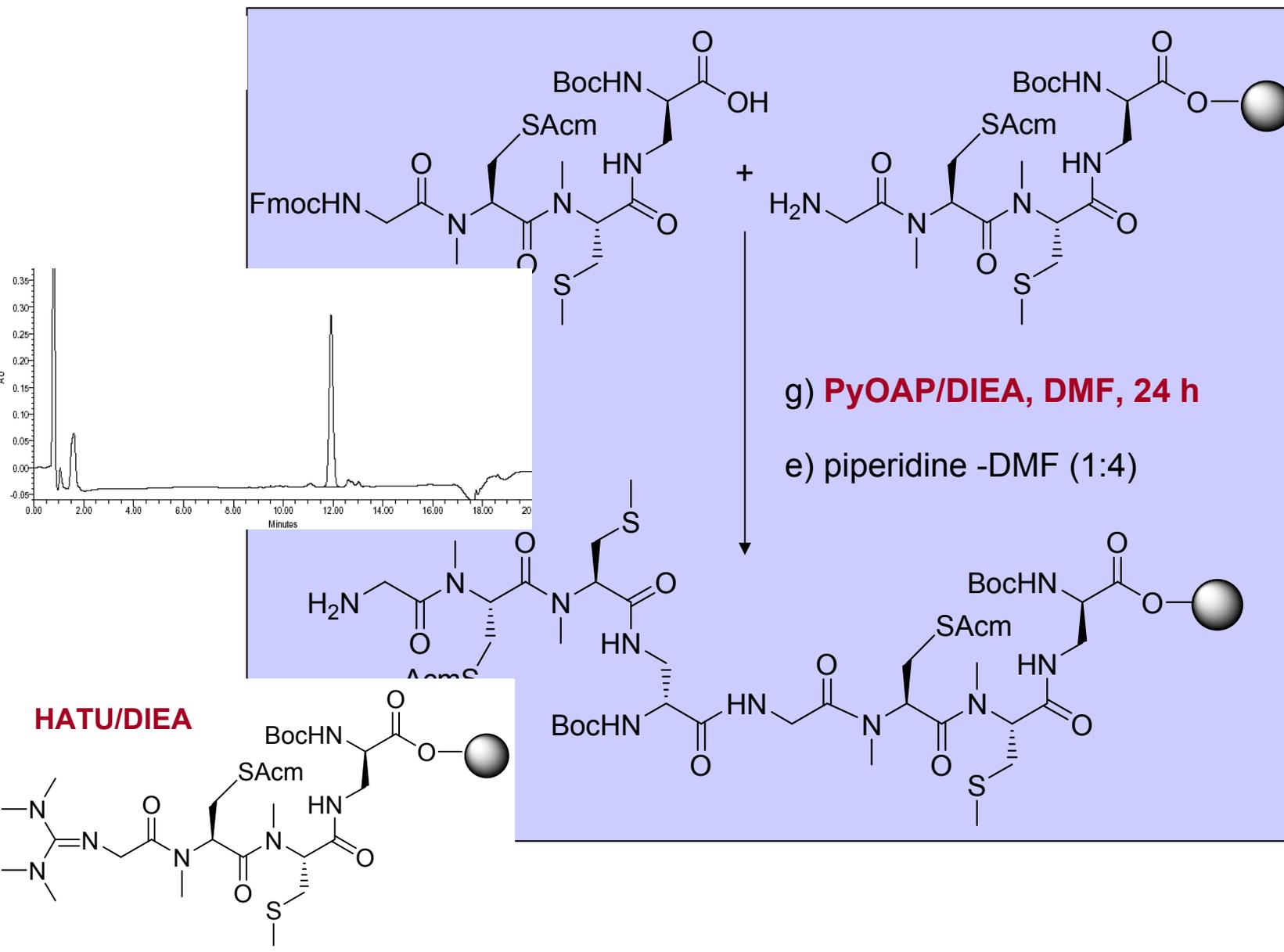


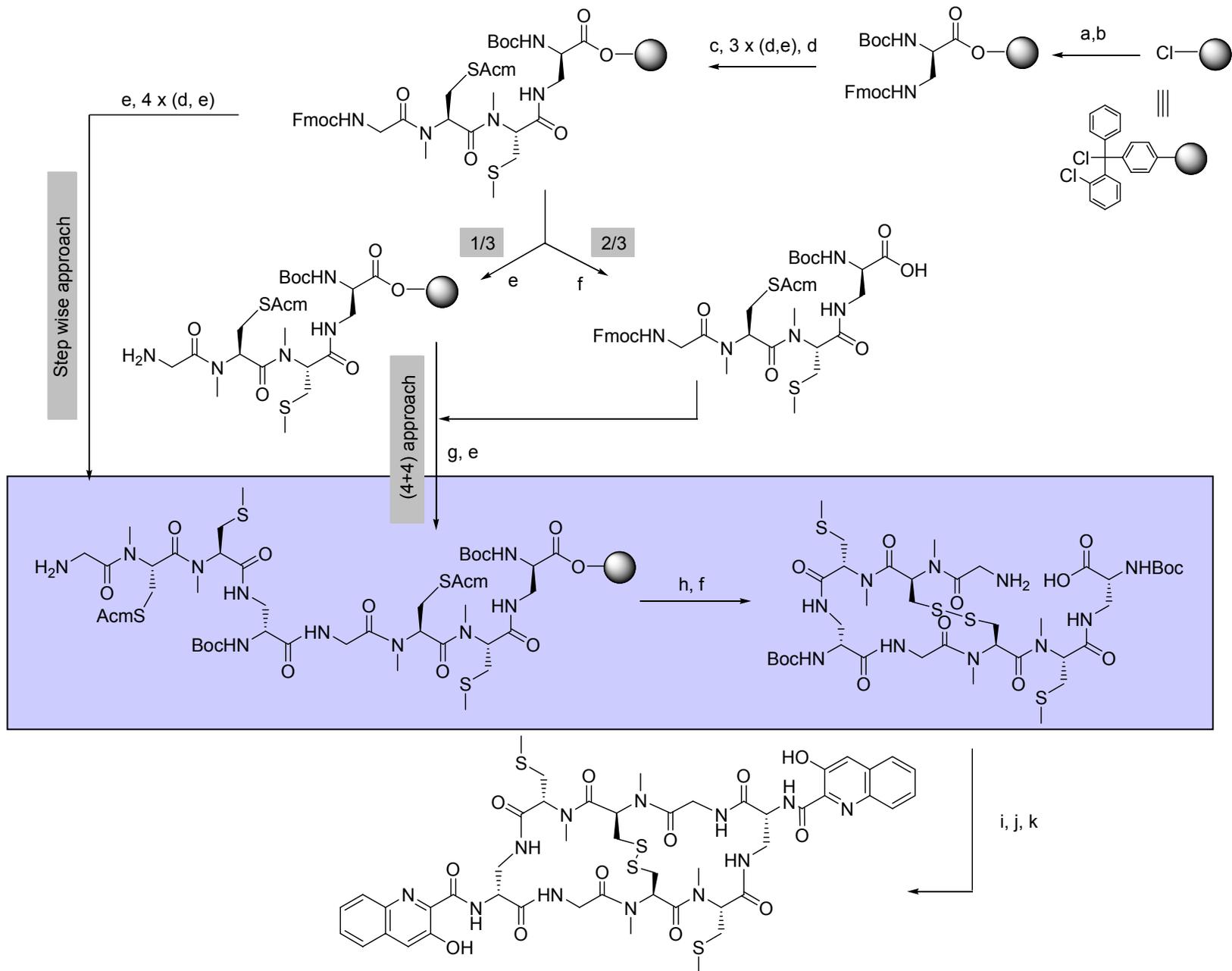
Azathiocoraline: Cleavage, Convergent Approach



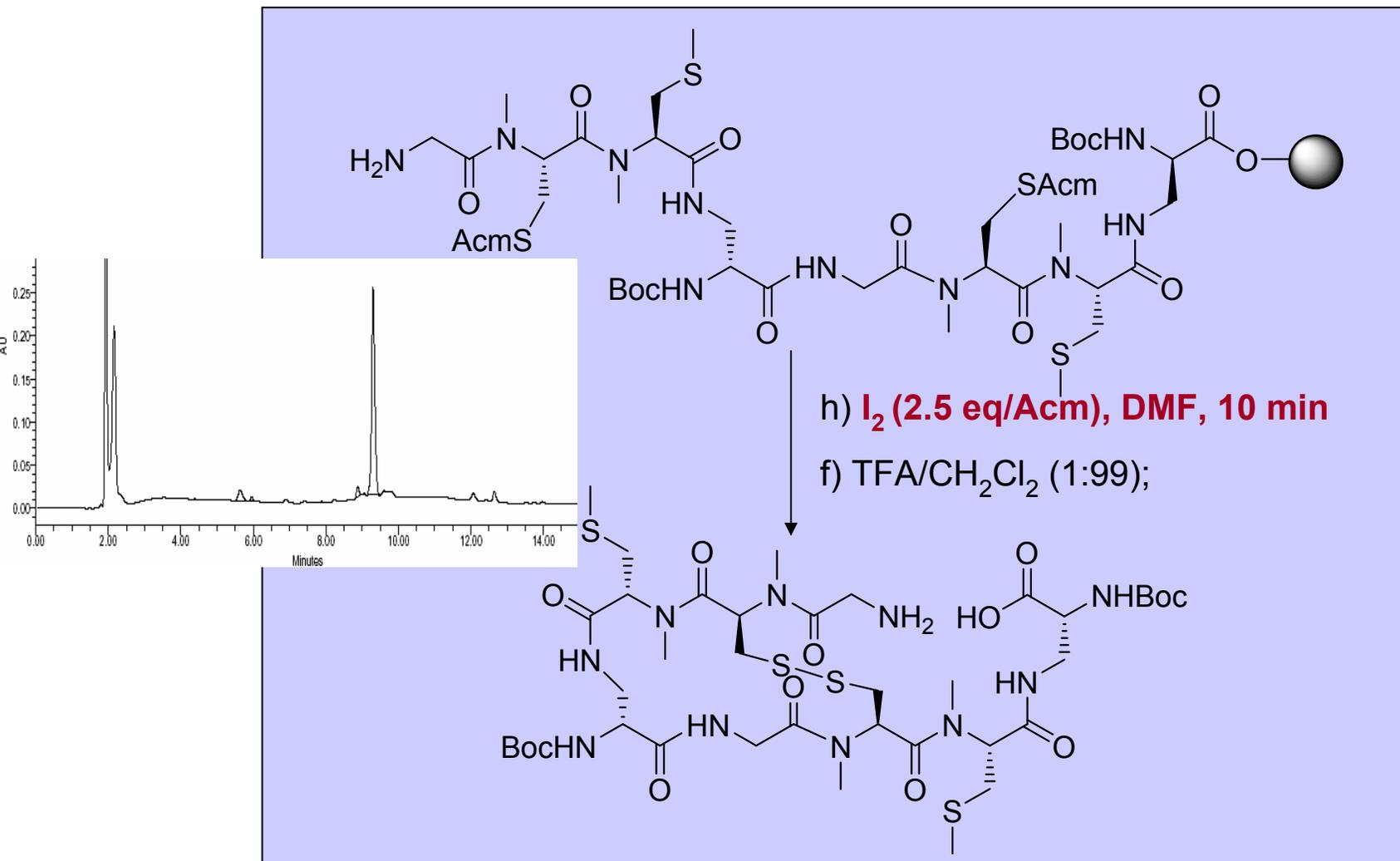


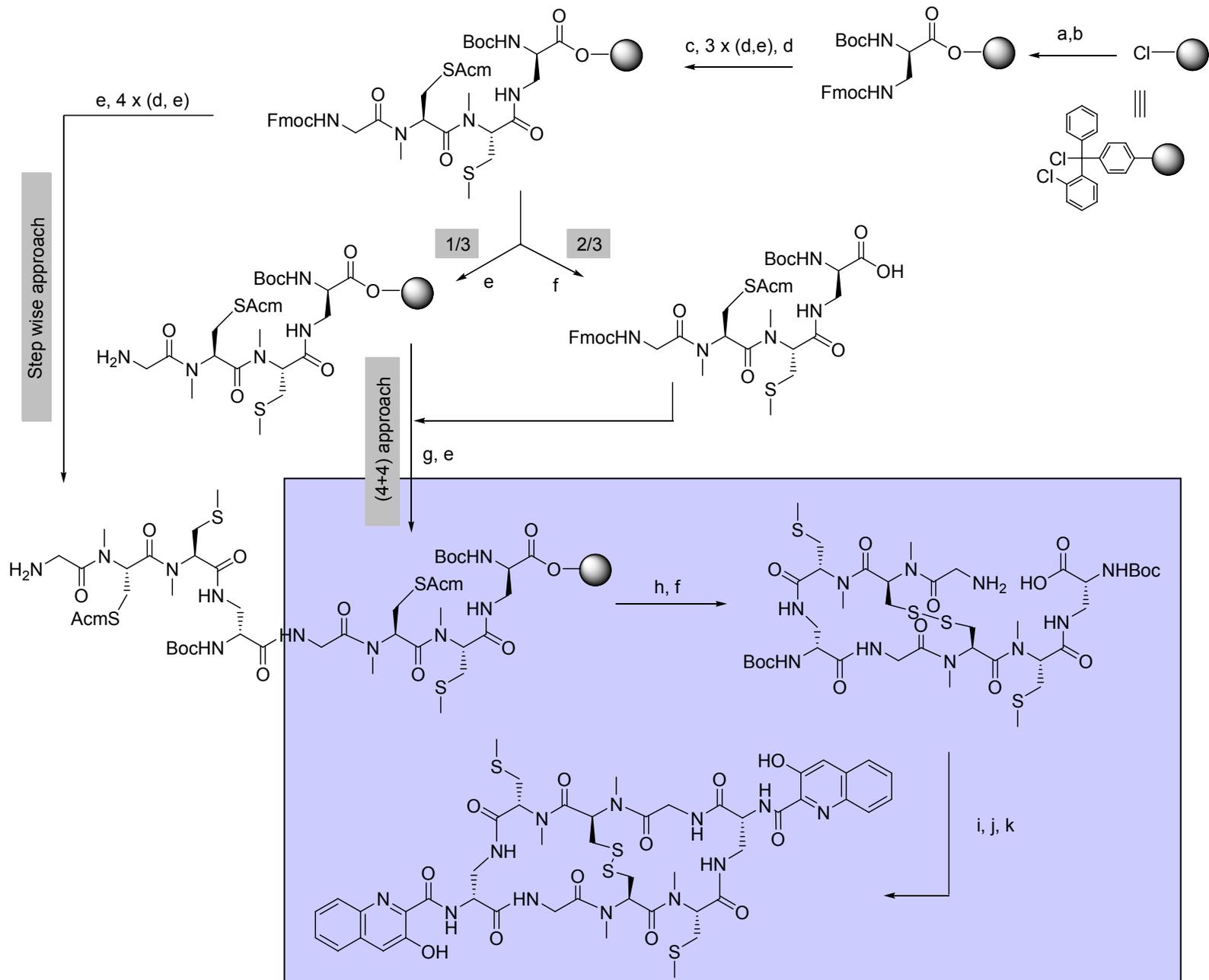
Azathiocoraline: Coupling, Convergent Approach



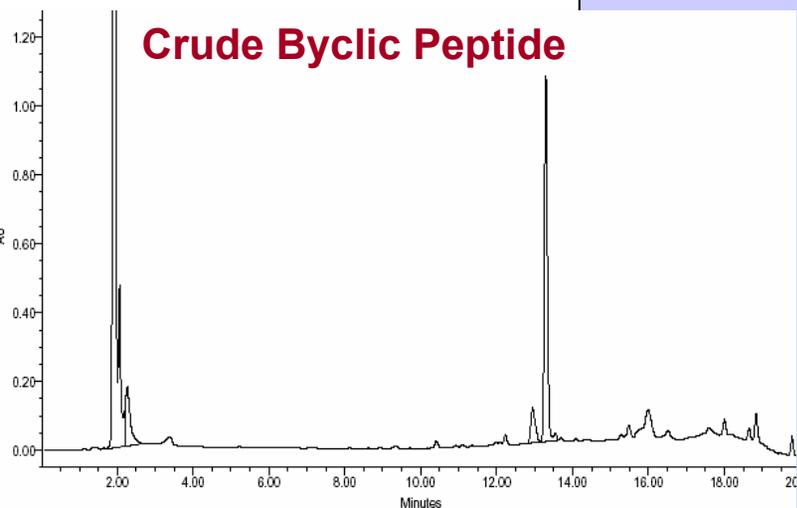


Azathiocoraline: On resin disulfide formation





Azathiocoraline: Macrolactamization

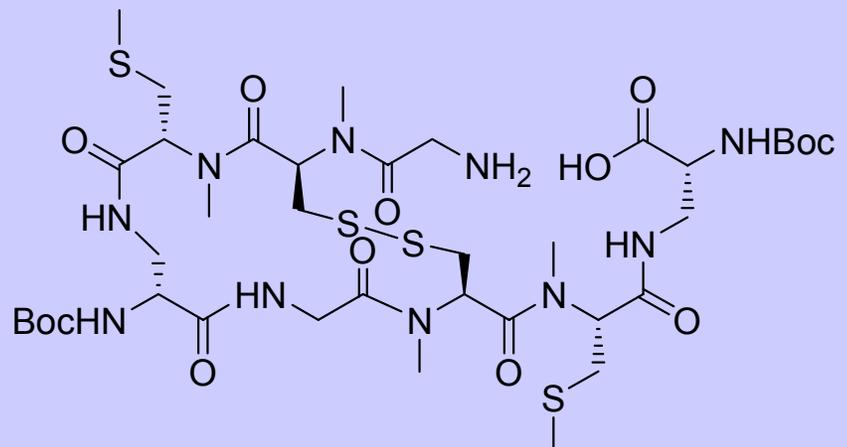


Other conditions

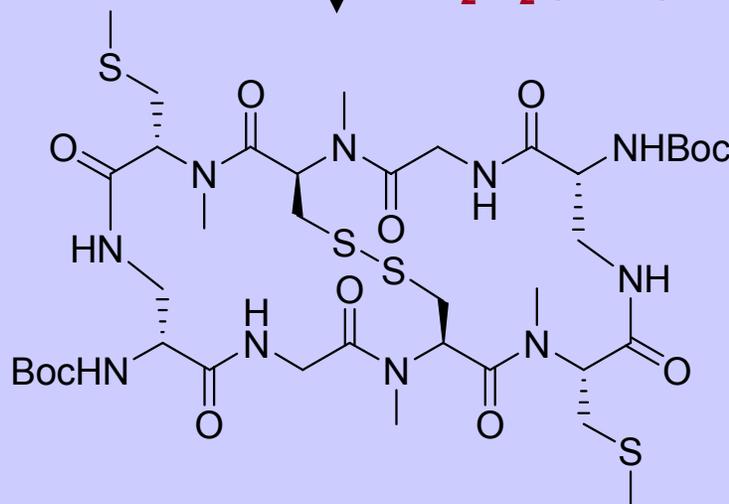
PyAOP/DIEA, it is fast but with byproduct formation

DIPCDI/HOBt, it is slow (> 3 d)

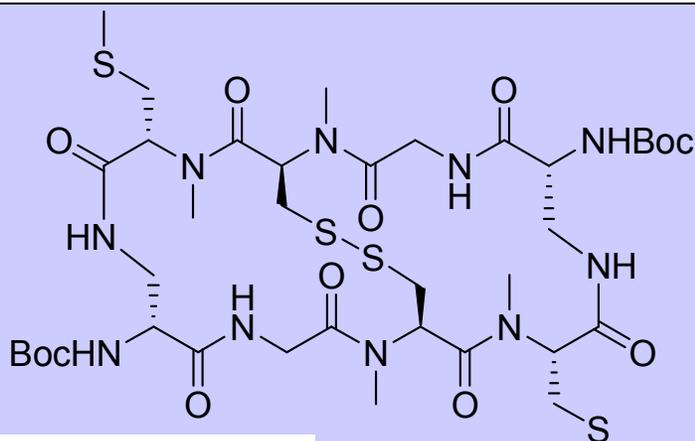
DIPCDI/HOAt (2 h), but purification is required



**i) EDC-HCl/HOAt/DIEA,
CH₂Cl₂ (1mM), 2 h**



Azathiocoraline: Incorporation of 3-Hydroxyquinaldic Acid



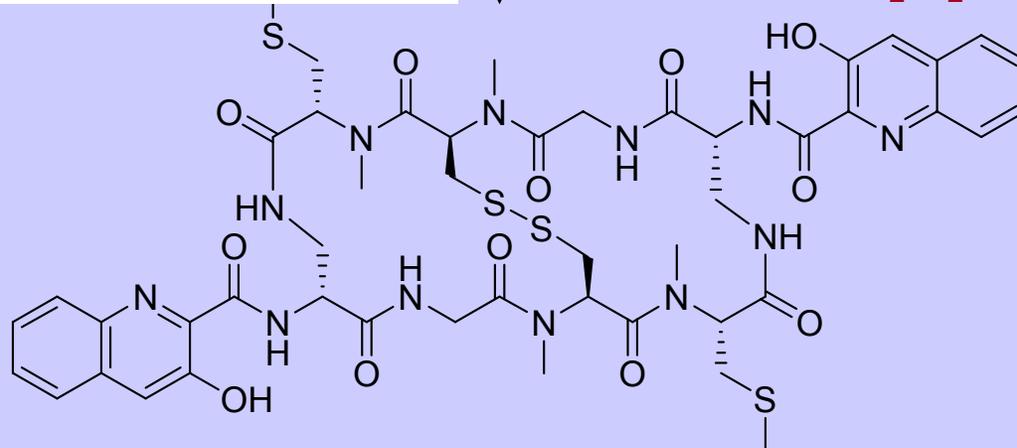
Other conditions

EDC·HCl/HOAt over incorporation

EDC·HCl/HOBt less over incorporation

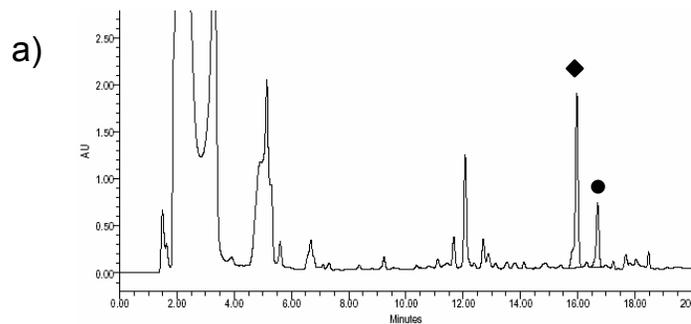
j) TFA-H₂O (19:1);

k) 3-hydroxyquinaldic acid/**EDC·HCl/**
HOSu/DIEA, CH₂Cl₂

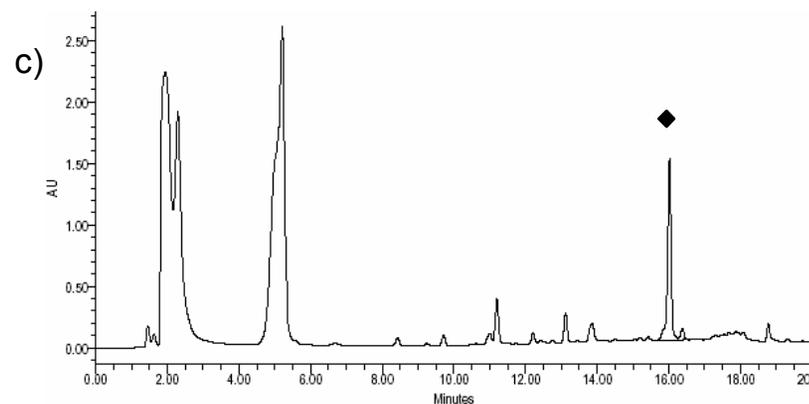


Azathiocoraline: Final Product

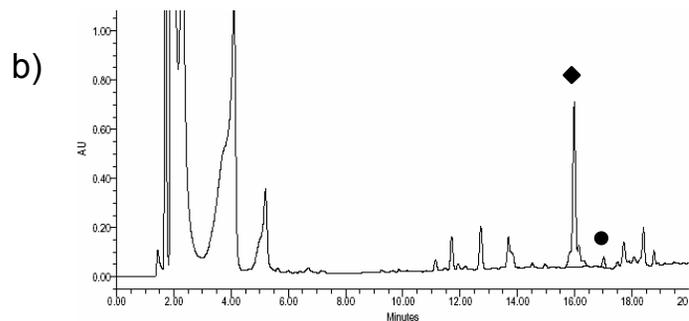
EDC·HCl / HOAt



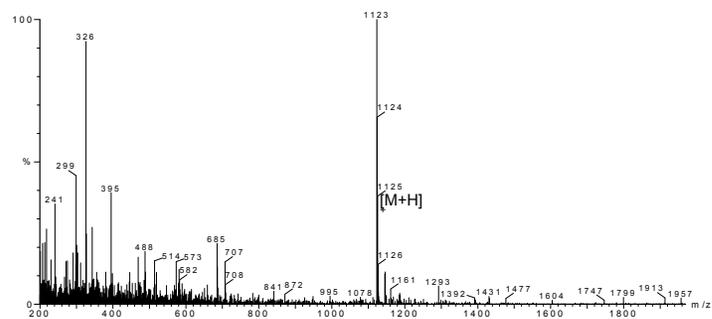
EDC·HCl / HOSu

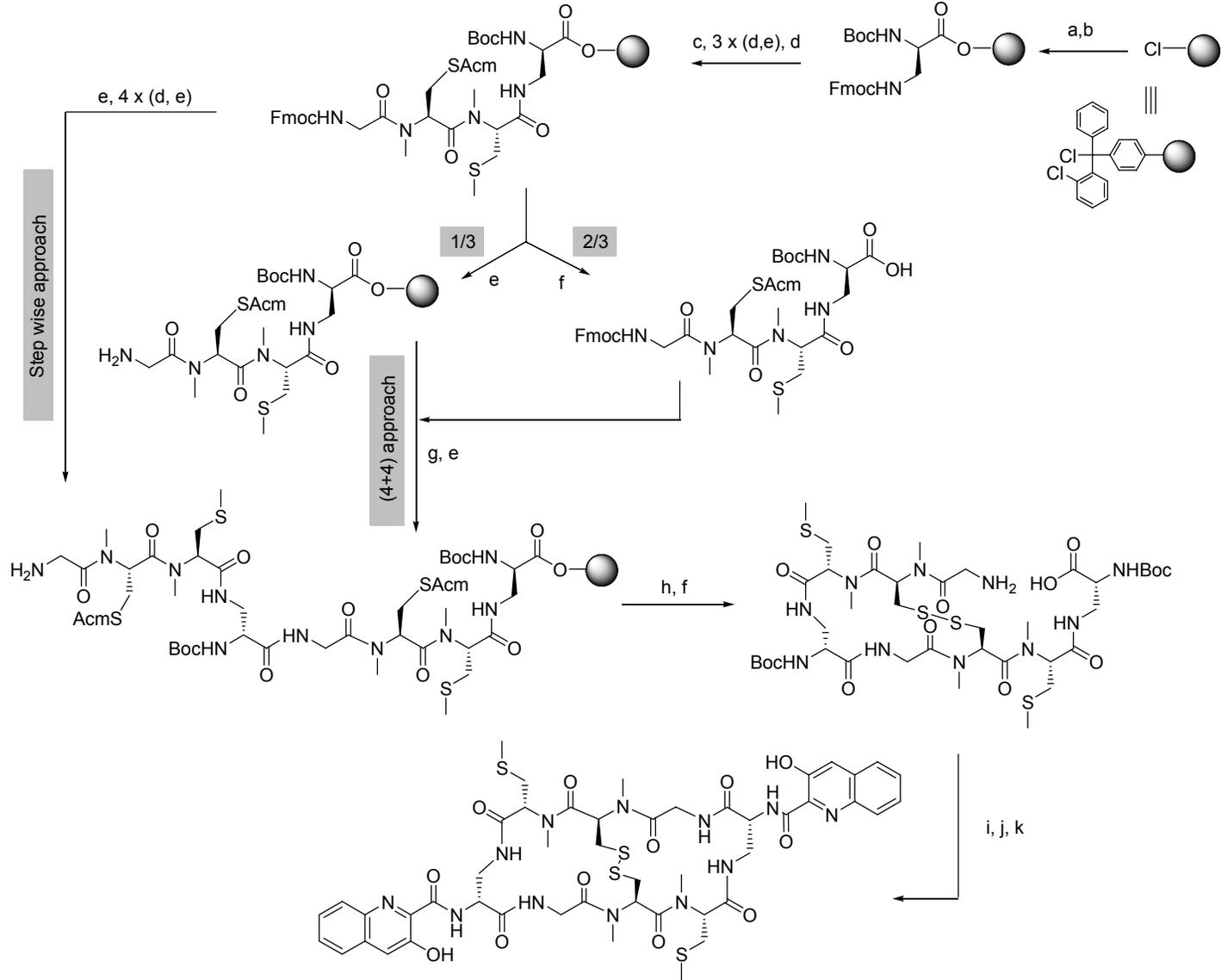


EDC·HCl / HOBt



- ◆ Azathiocoraline
- Azathiocoraline + 3HQA

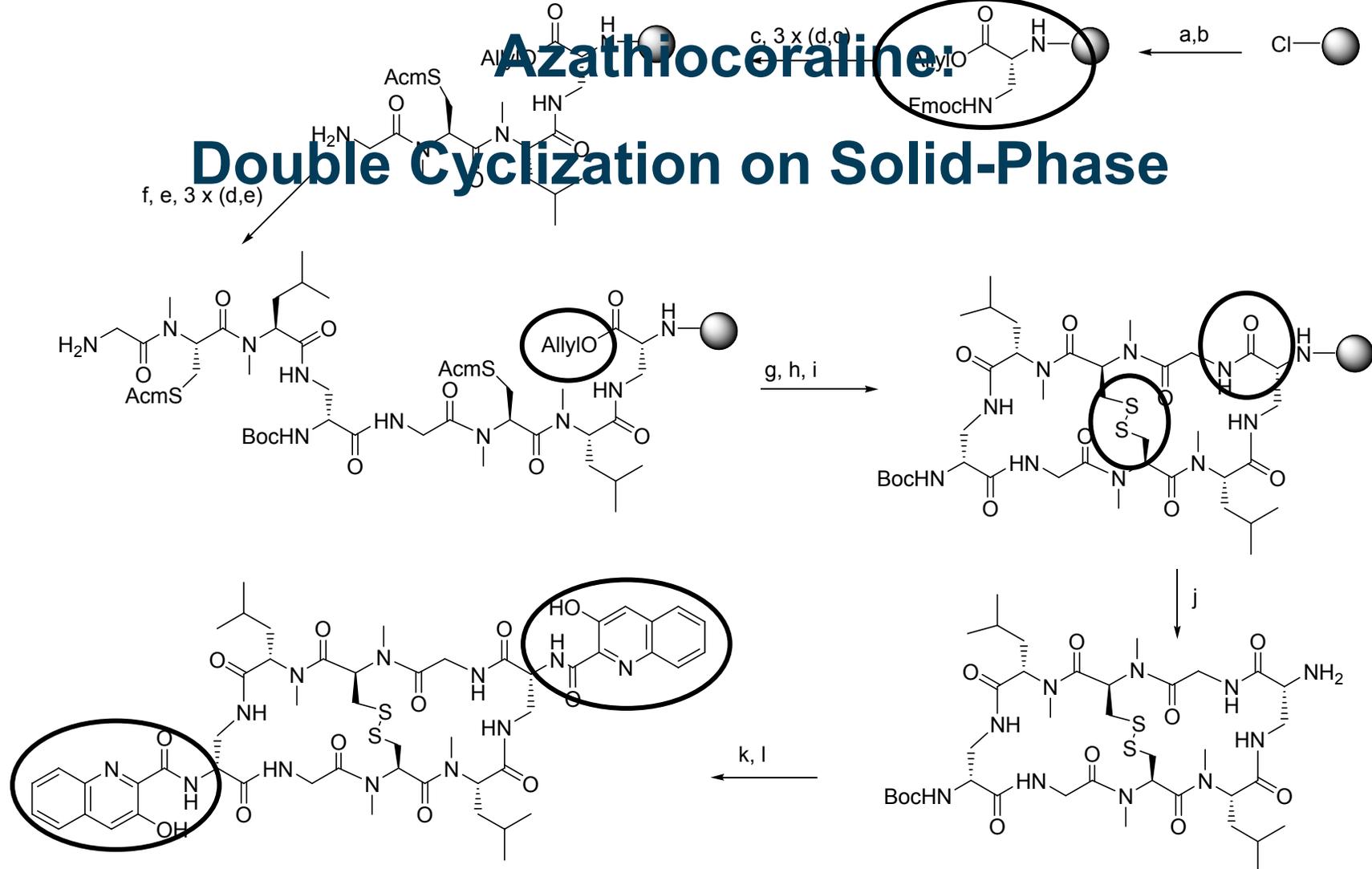




Scheme 1. a) Boc-D-Dap(Fmoc)-OH, DIEA, CH₂Cl₂; b) MeOH; c) piperidine-DMF (1:4), piperidine-DBU-toluene-DMF (1:1:4:14); d) Fmoc-AA-OH/**HATU**/DIEA, DMF; e) piperidine-DMF (1:4); f) TFA/CH₂Cl₂ (1:99); g) **PyAOP**/DIEA, CH₂Cl₂; h) I₂, DMF; i) **EDC·HCl/HOAt**/DIEA, CH₂Cl₂ (1mM); j) TFA-H₂O (19:1); k) 3-hydroxyquinaldic acid/**EDC·HCl/HOSu**/DIEA, CH₂Cl₂.

Azathiocoraline

Double Cyclization on Solid-Phase



Scheme 2. a) H-D-Dap(Fmoc)-OAllyl, DIEA, CH₂Cl₂; b) MeOH; c) piperidine-DMF (1:4), piperidine-DBU/toluene/DMF (1:1:4:14); d) Fmoc-AA-OH/HATU/DIEA, DMF; e) piperidine-DMF (1:4); f) Boc-D-Dap(Fmoc)-OH/HATU/DIEA, DMF; g) [Pd(PPh₃)₄], PhSiH₃, CH₂Cl₂; h) I₂, DMF; i) DIPCDI/HOAt, DMF; j) TFA-CH₂Cl₂ (1:99); k) TFA-H₂O (19:1); l) 2-hydroxyquinaldic acid/EDC·HCl/HOSu/DIEA, CH₂Cl₂

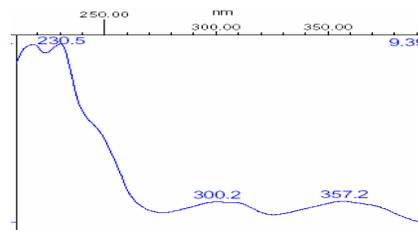
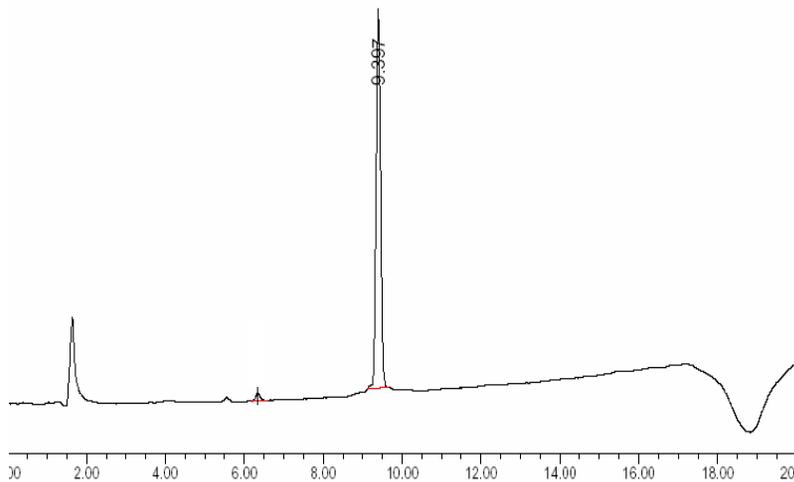
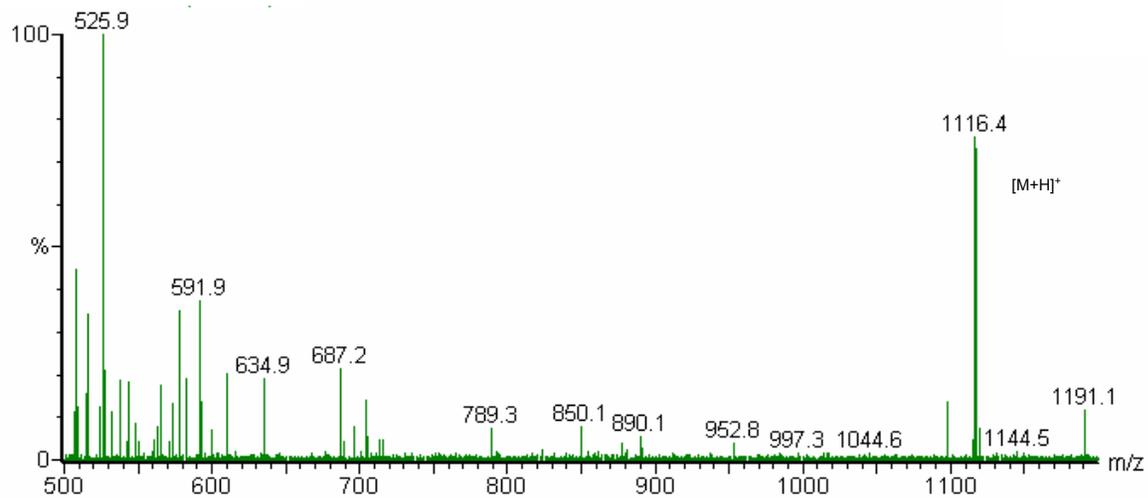


Figure 2. HPLC chromatograms of purified Azathiocoraline. Reverse-phase C18 columns was used for the analysis with elution by linear gradient over 15 min of 0.036% TFA in CH₃CN and 0.045% TFA in H₂O from 5:5 to 9:1.



Life doesn't exist without barriers ...

**But therapy requires that we able
to breach those barriers**

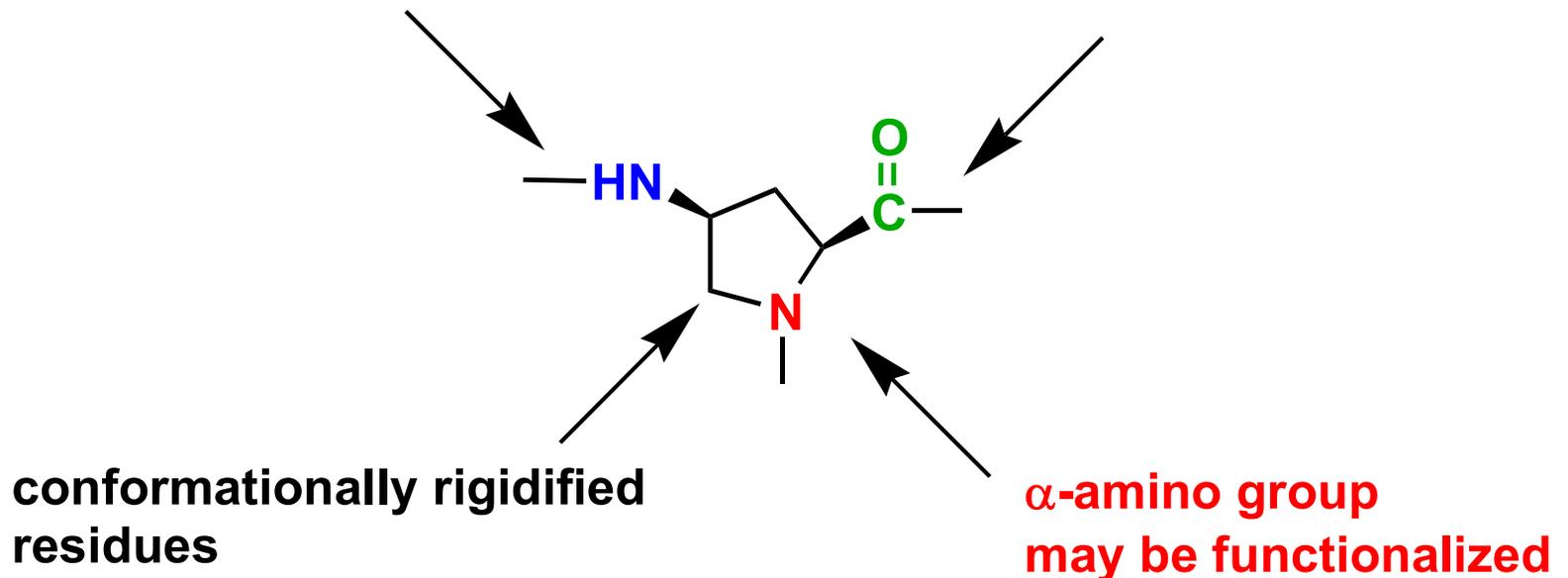
Paul Wenders, Stanford University

γ -Peptides as Foldamers

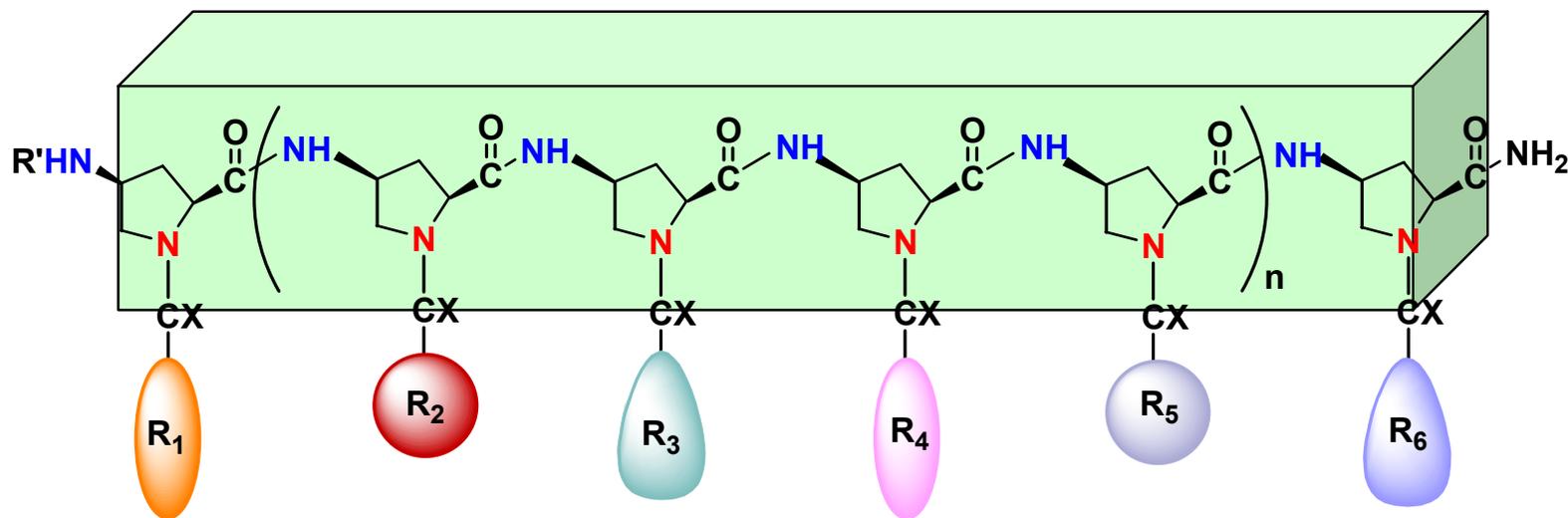
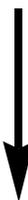
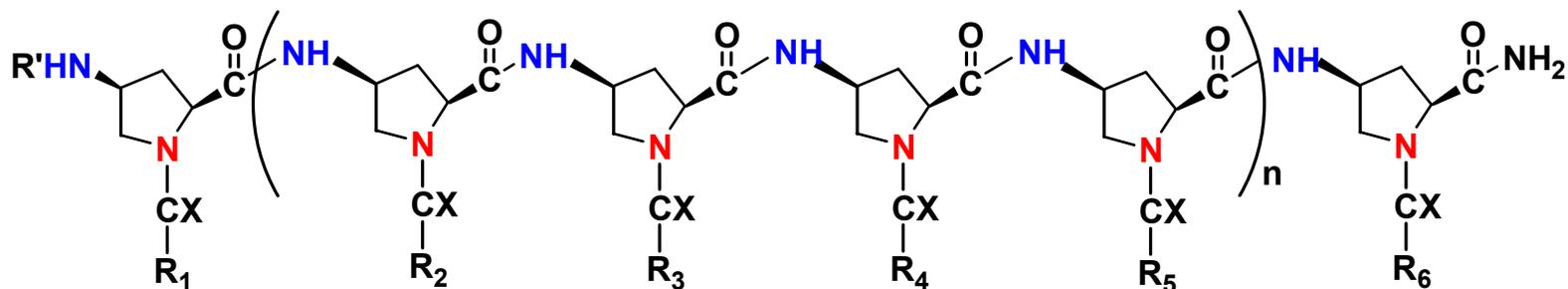
New Oligomers (γ -Peptides) Based in Amp with Well Defined Secondary Structure

γ -amino group participates in the peptide backbone

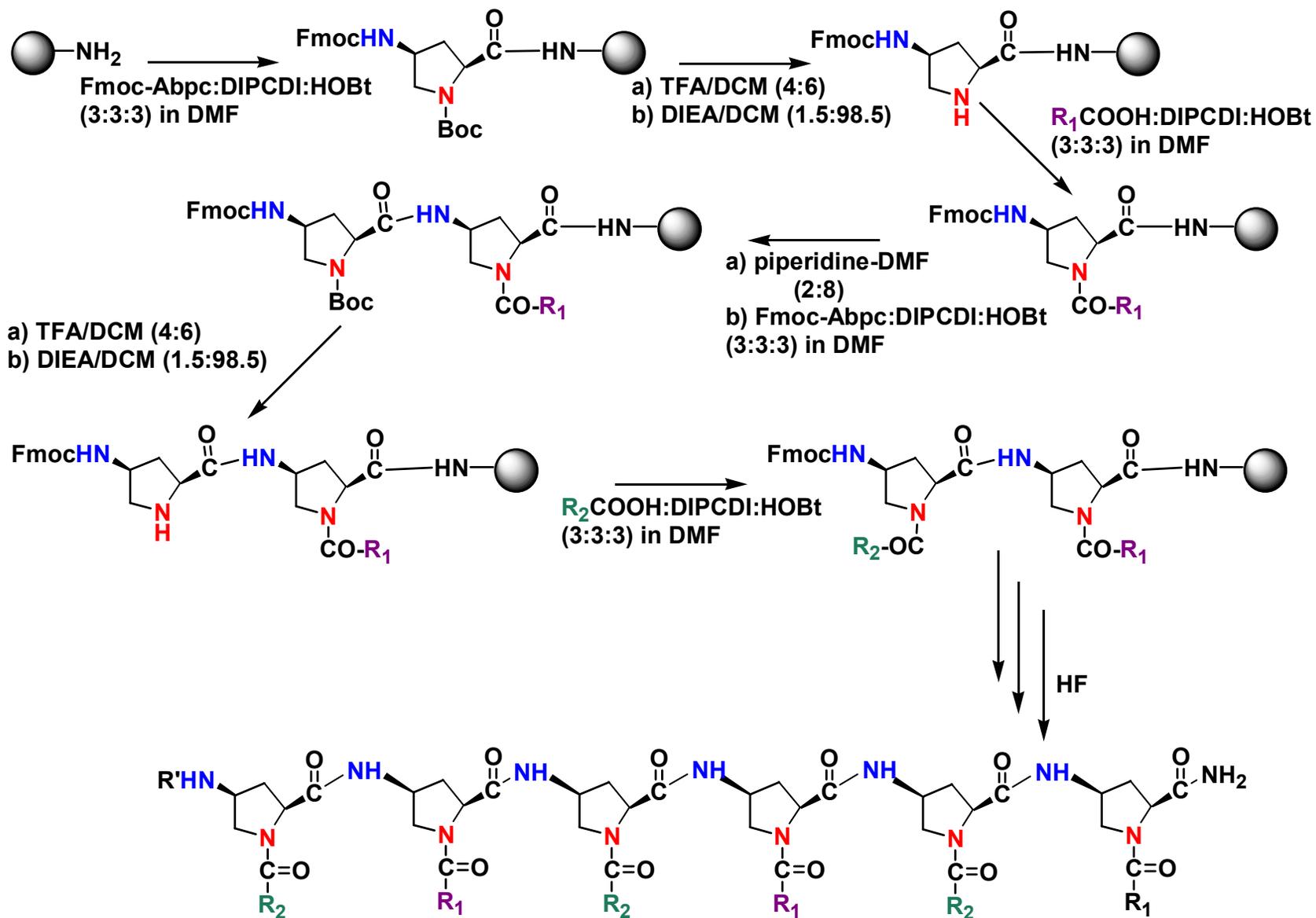
α -carboxy group participates in the peptide backbone



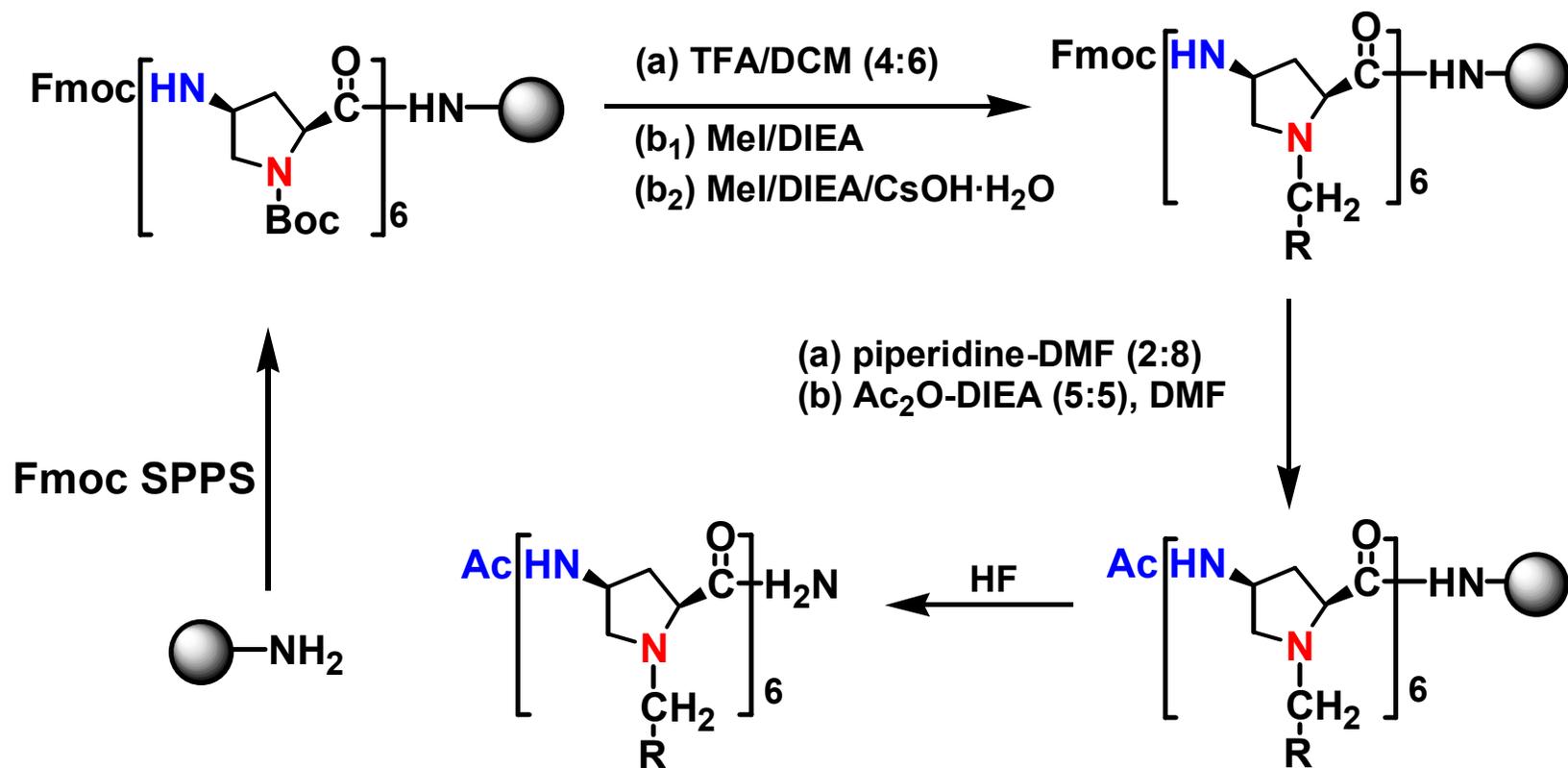
Foldamers Based in Amp



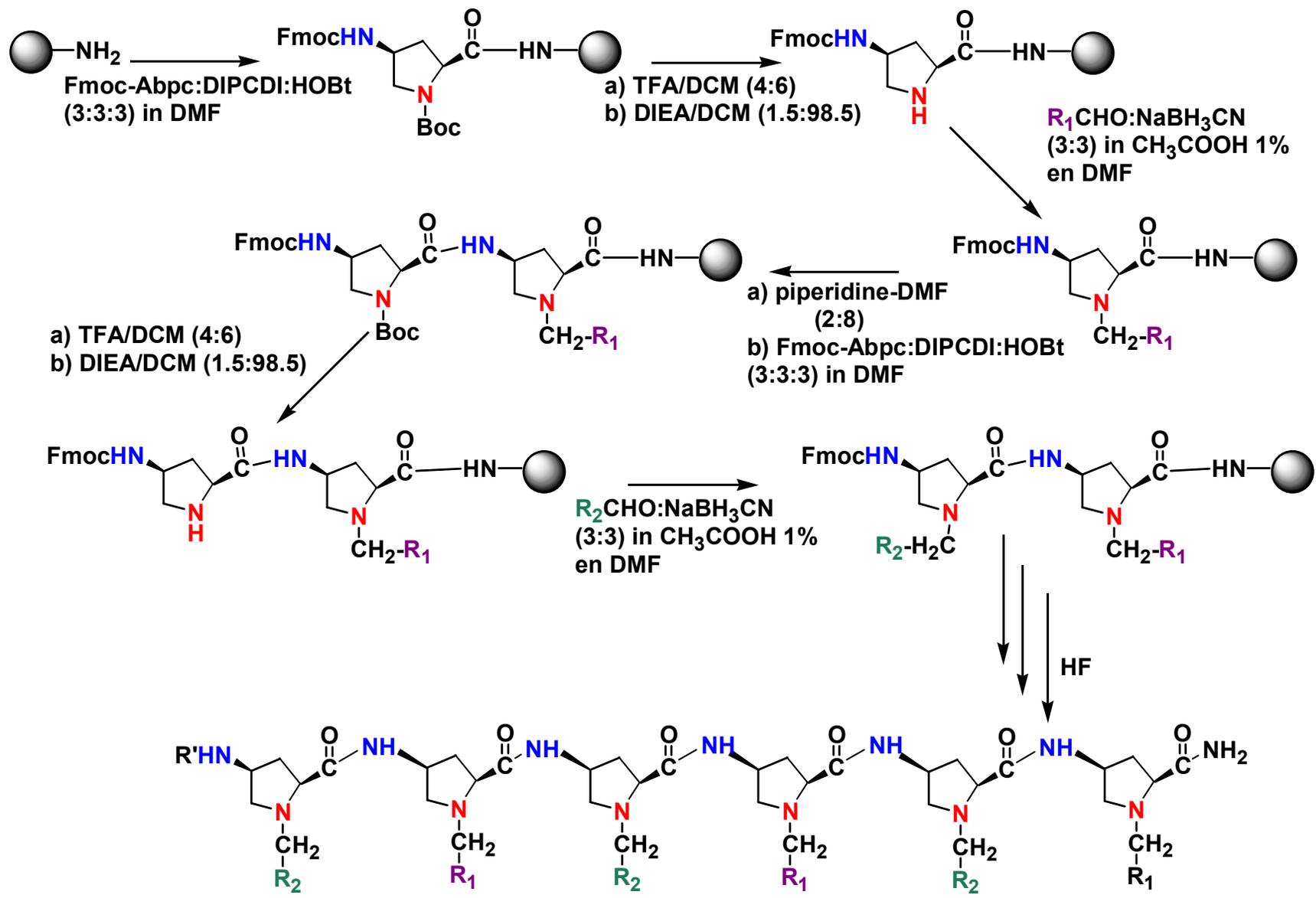
Synthesis of Side-Chain Amide γ -Peptides



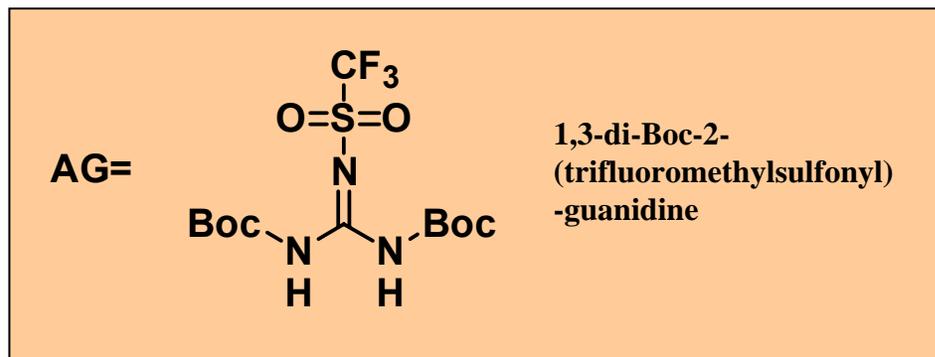
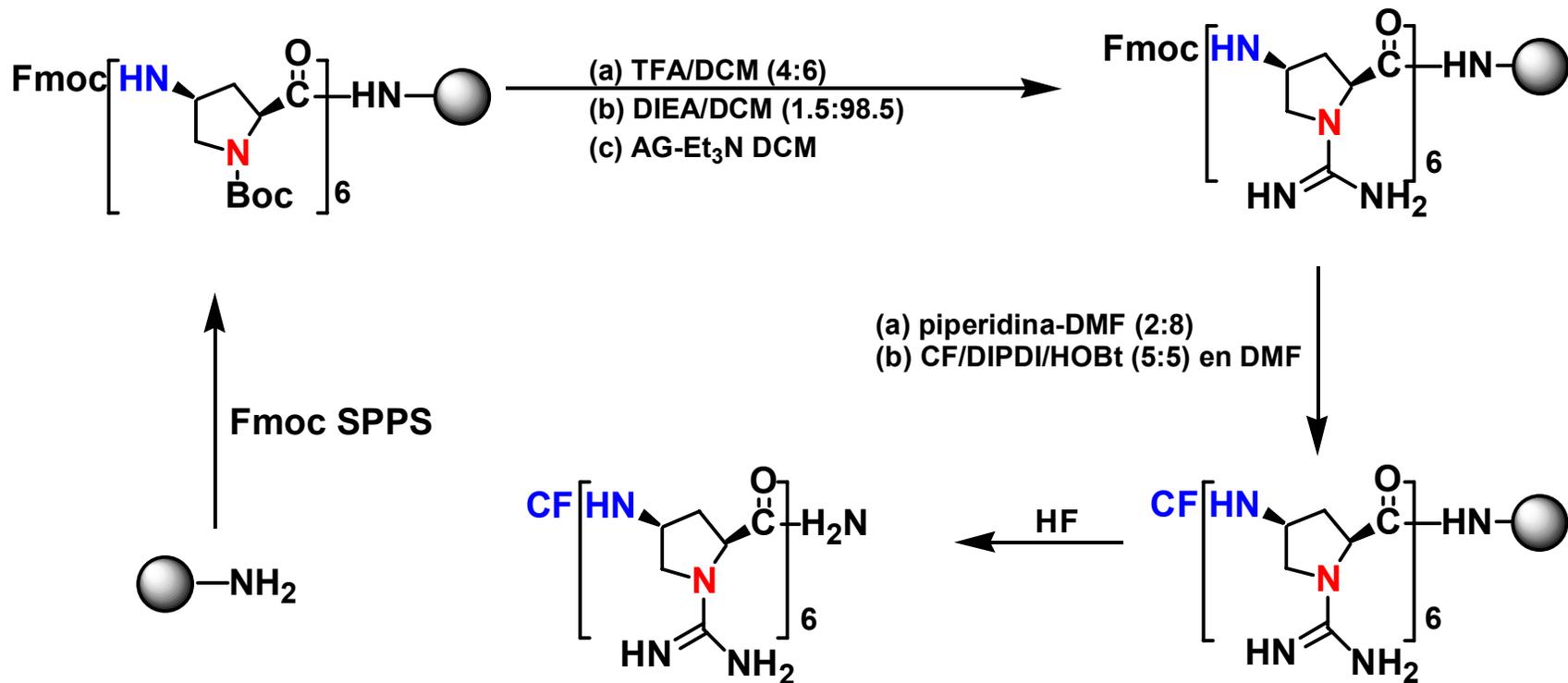
Synthesis of Side-Chain Amine γ -Peptides I



Synthesis of Side-Chain Amine γ -Peptides II

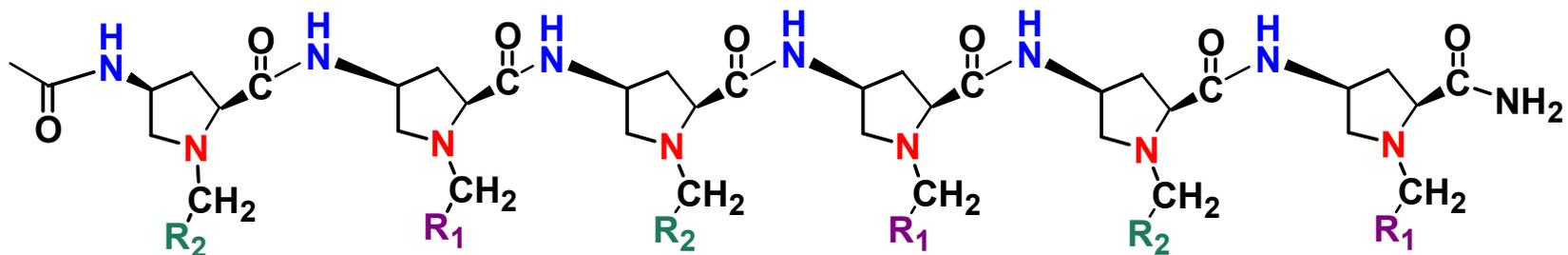


Synthesis of Side-Chain Guanilidated γ -Peptides

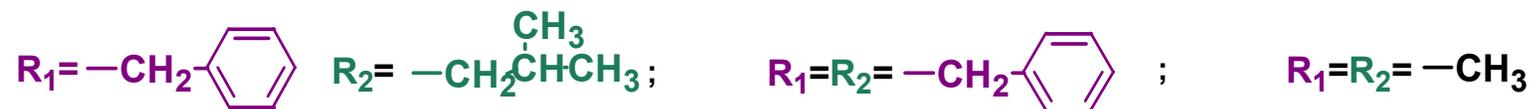
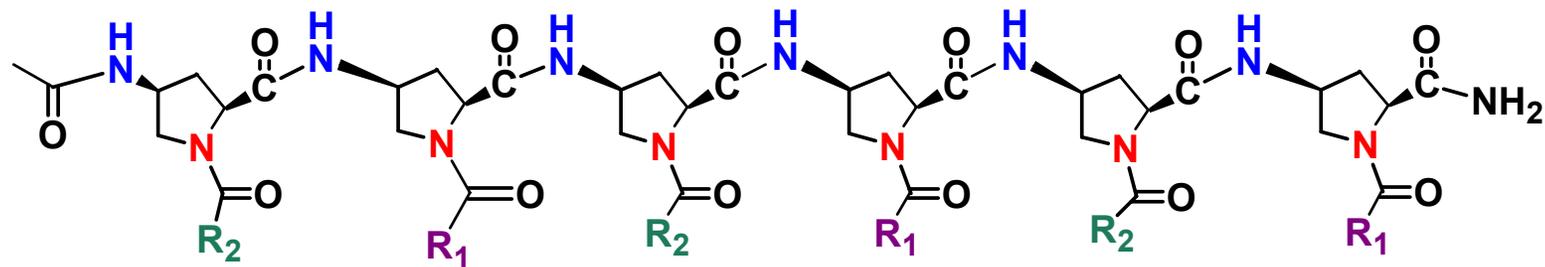


Amine and Amide γ -Peptides

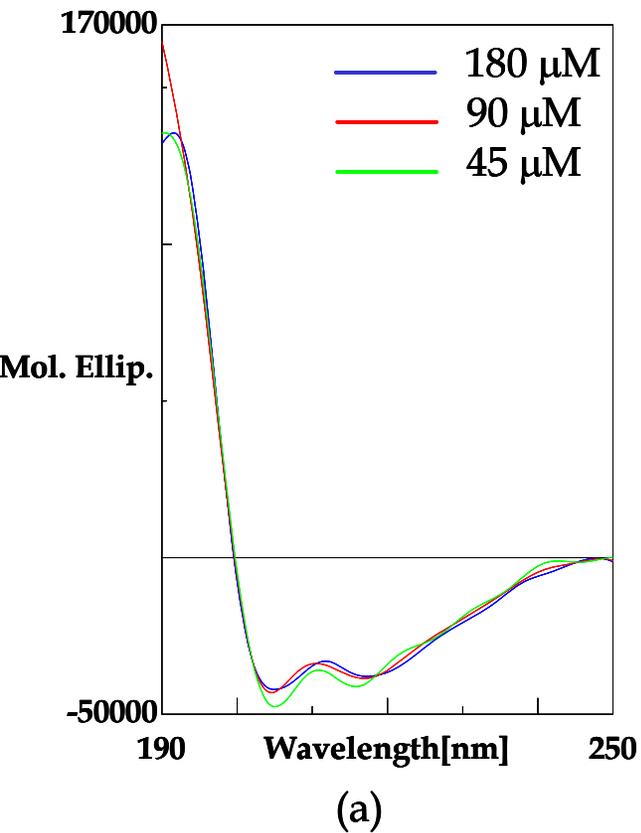
(a)



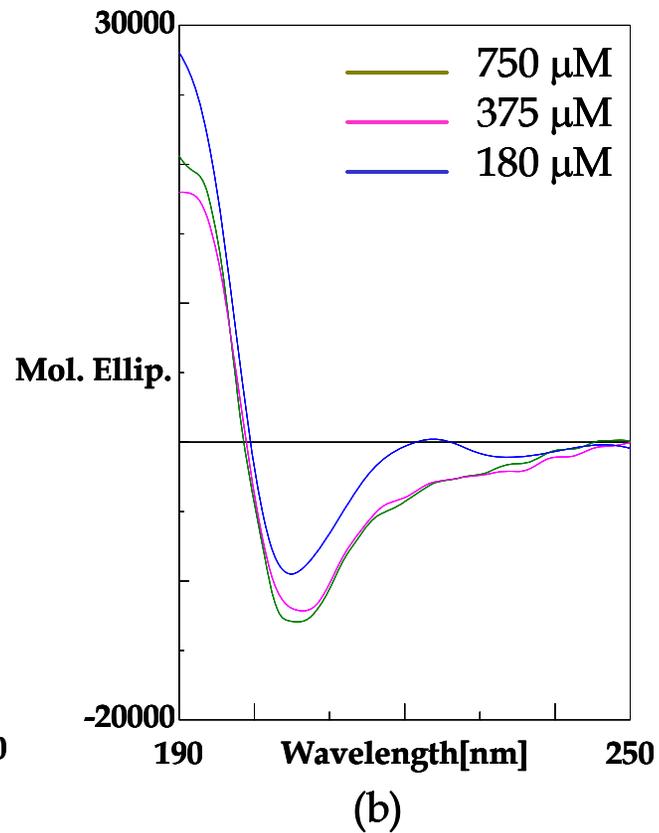
(b)



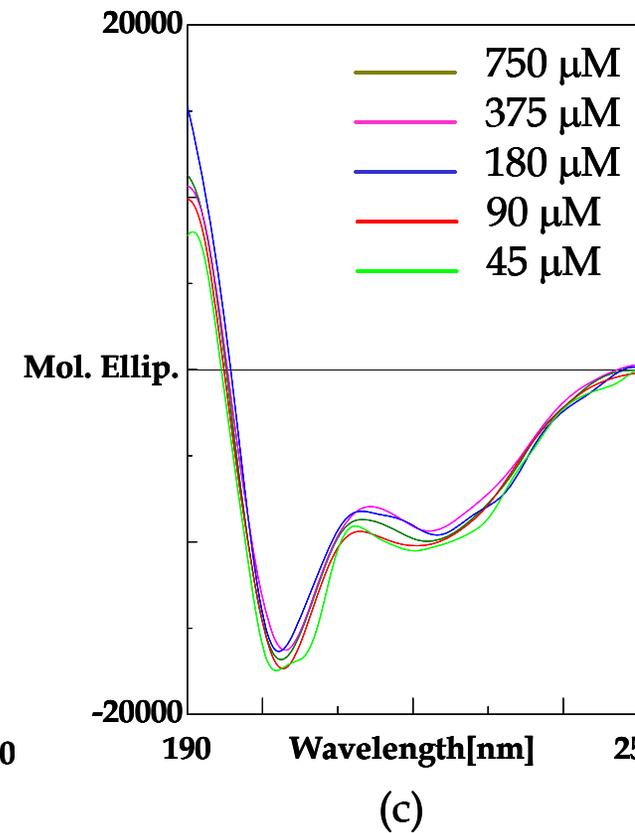
DC of $\text{Ac}-[\gamma\text{-Amp}(\text{N}^\alpha\text{-Ac})]_6\text{-NH}_2$



H_2O

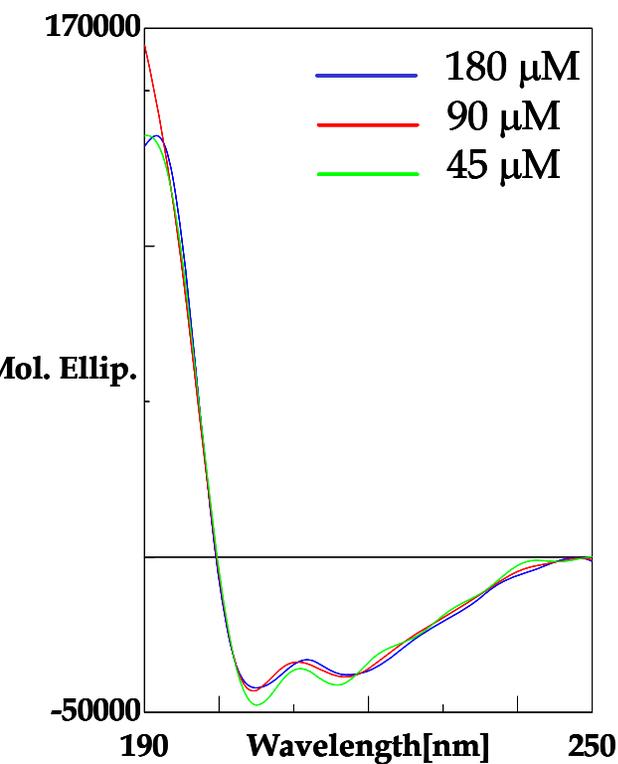


MeOH



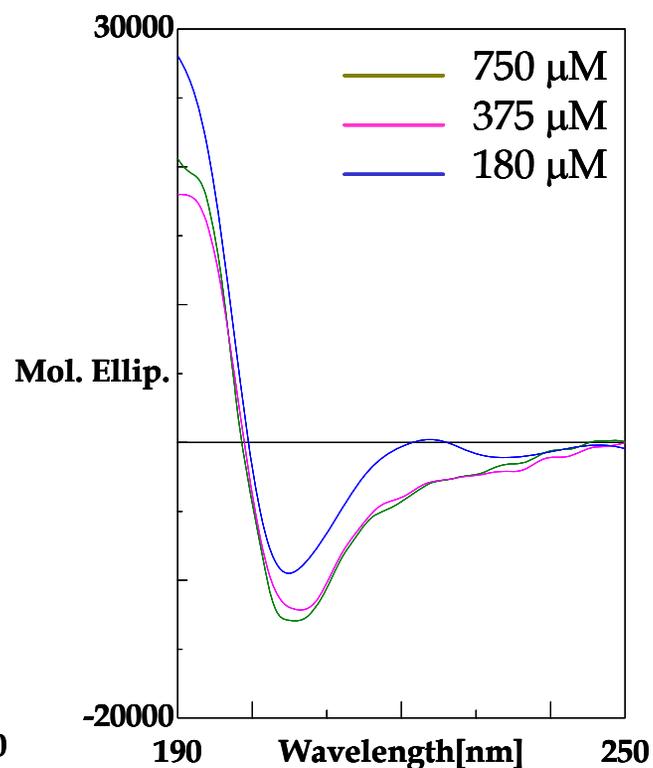
TFE

DC of
 $Ac-[\gamma - Amp(N^\alpha - N) - \gamma - Amp(N^\alpha - PhAc)]_3 - NH_2$



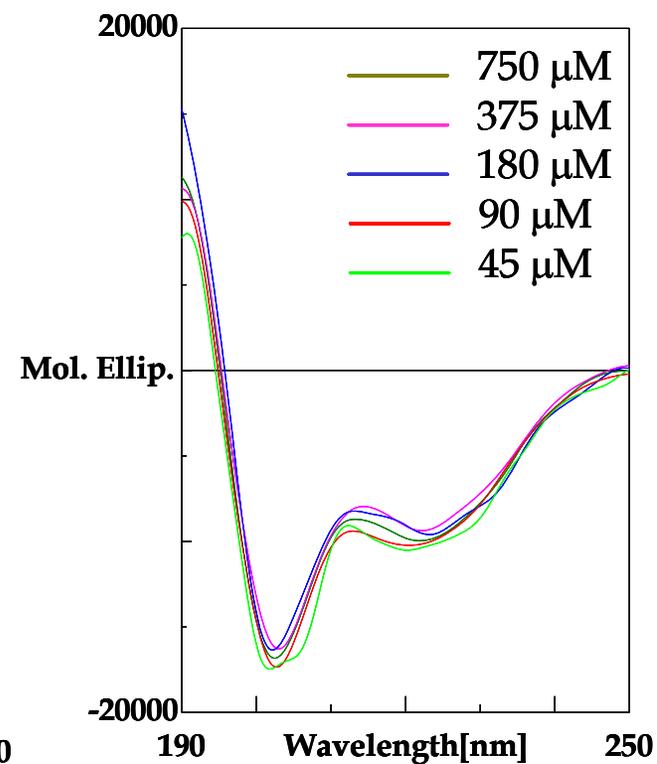
(a)

H₂O



(b)

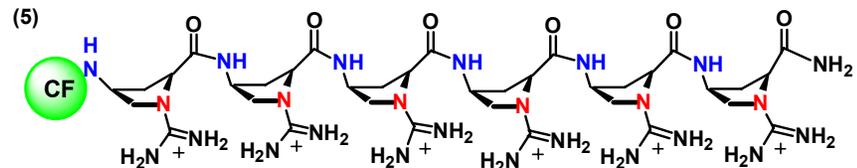
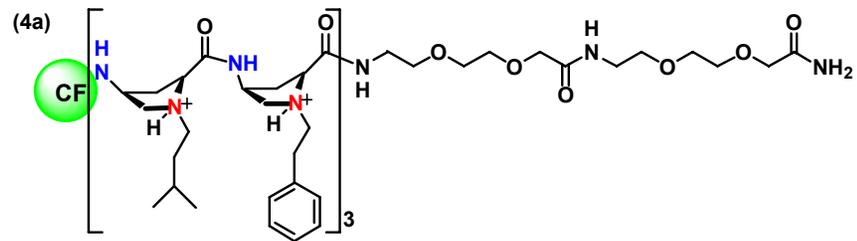
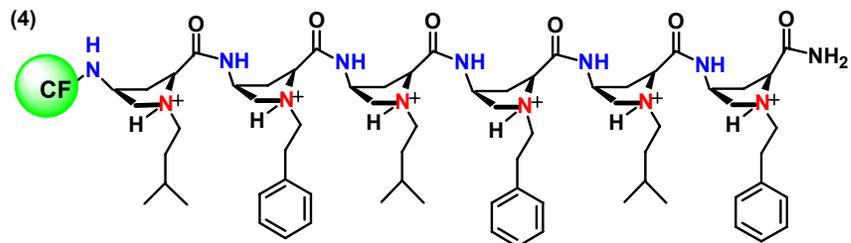
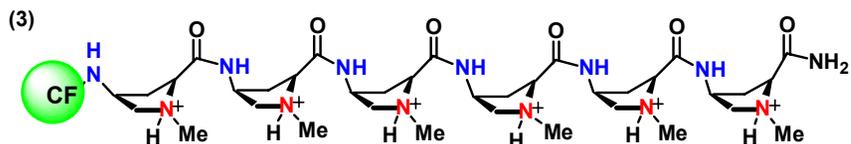
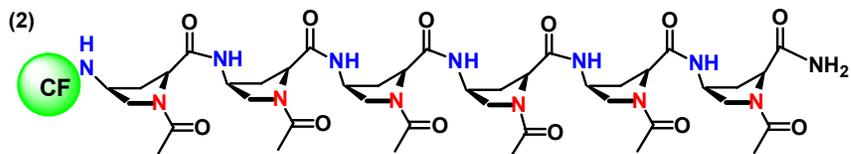
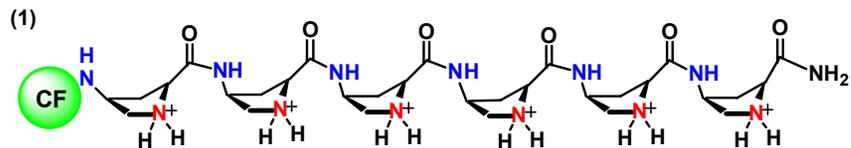
MeOH



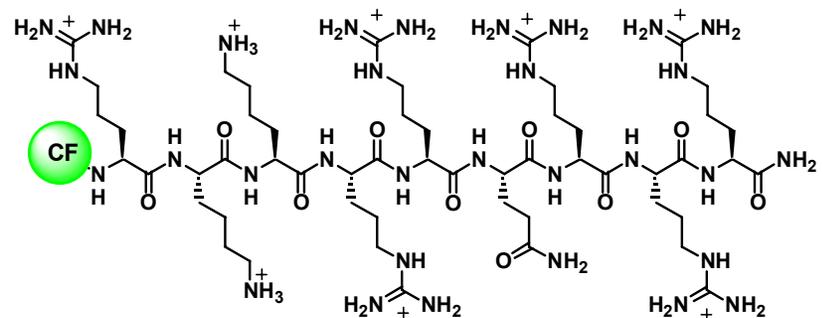
(c)

TFE

Solid-Phase Synthesis of γ -Peptides Labeled with CF

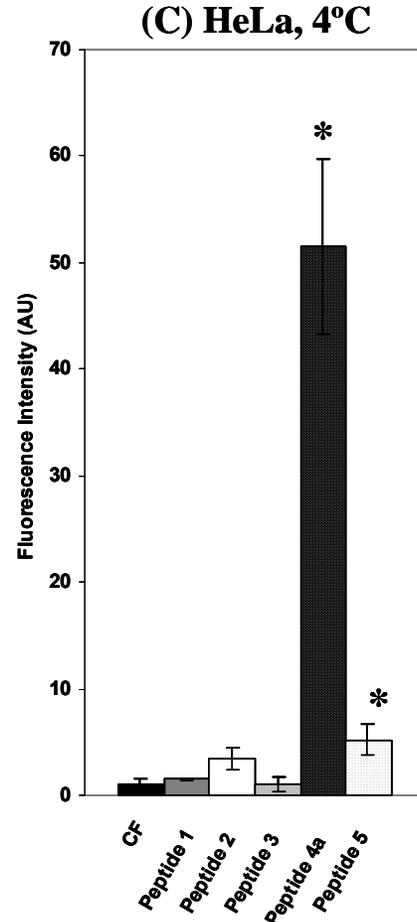
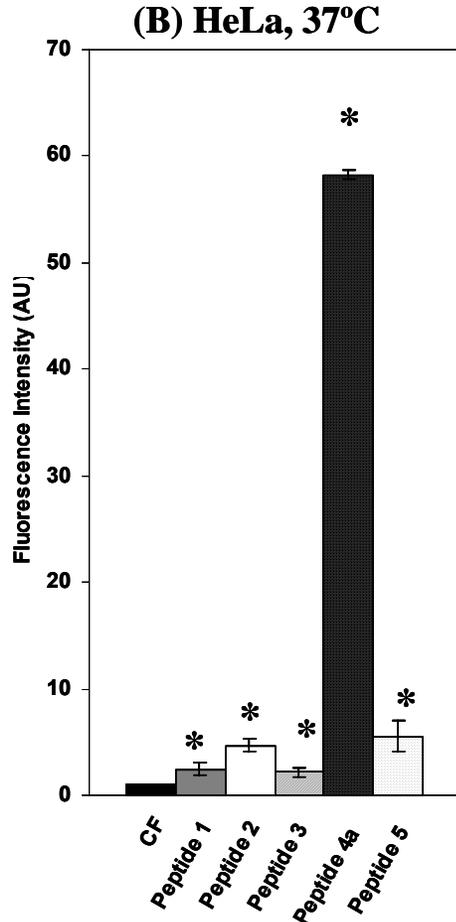
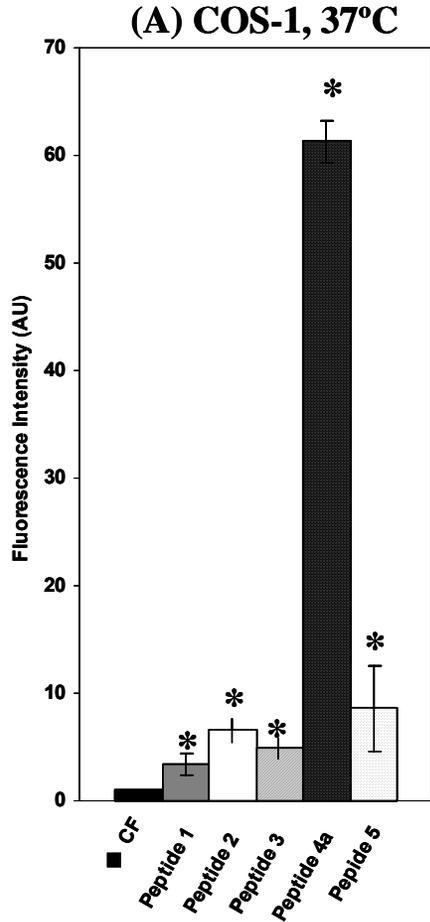


TAT [49-57] peptide



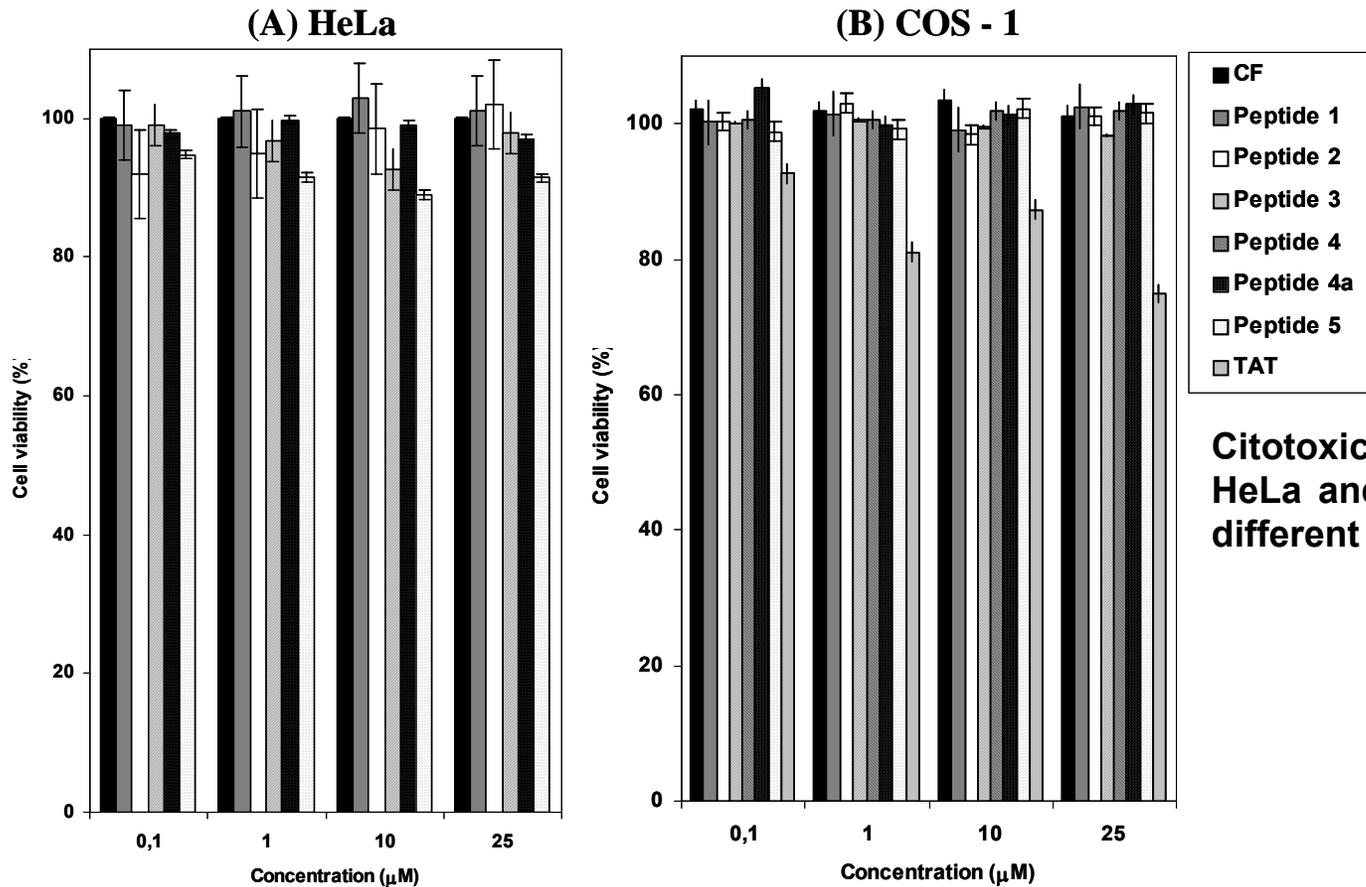
Labelling with CF: CF/DIPCDI/HOBt (5eq:5eq:5eq)

Cell Uptake by Fluorimetry

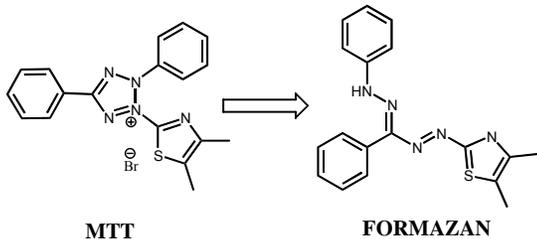


(A) COS-1 and (B) HeLa. Conditions: 10 μ M for 2h

MTT Essay: Cell Viability



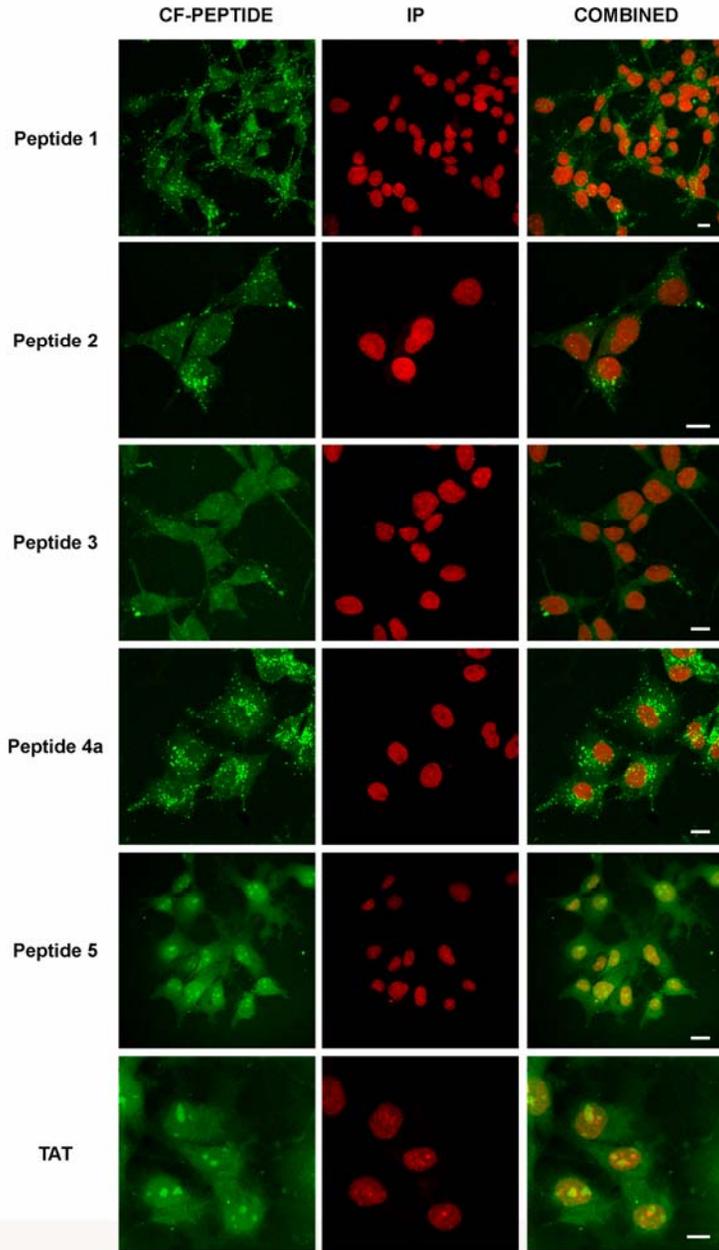
Citotoxicity Essay: γ -Peptids (A) HeLa and (B) COS-1 for 24 h at different concentrations.



It is detectable at 570 nm

Low TOXICITY!

Confocal Microscopy of γ -Peptides at 37°C in Fixed COS-1 Cells



Conditions:

10 μ M at 37°C, 2h

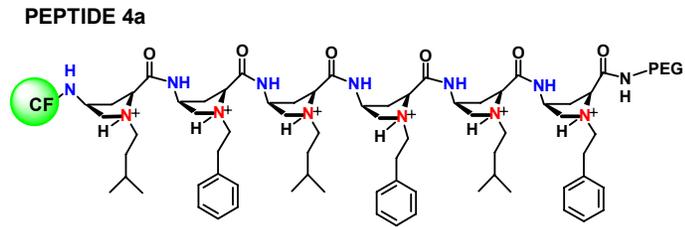
Fijación: 3% p-formadehído

Marcaje del núcleo: IP

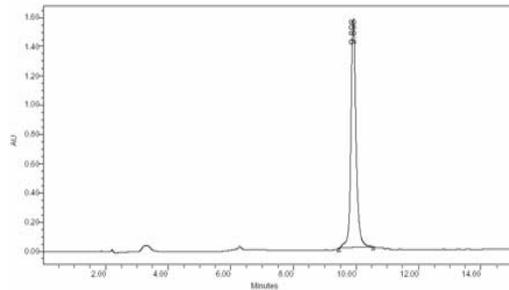
Scale bars 10 μ m

All Peptides show Cell Penetrating Capacity, specially 4a!!!

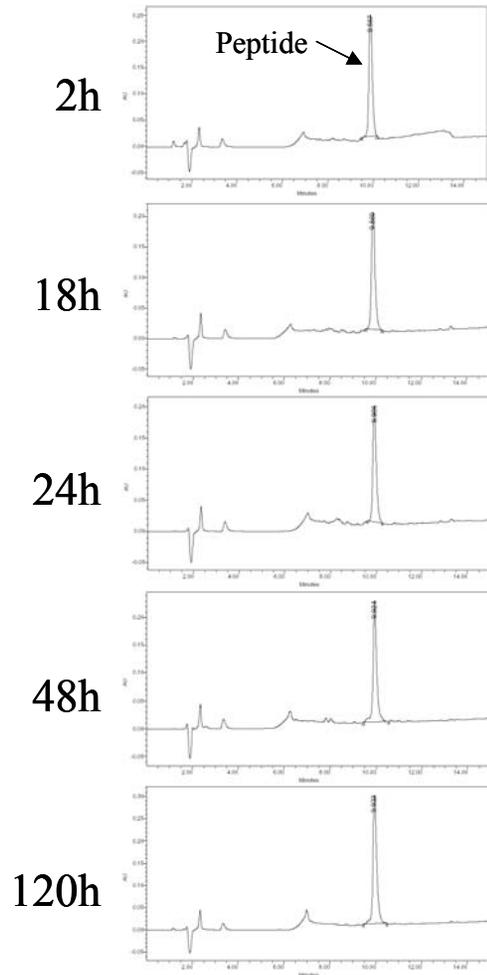
Enzymatic Stability of γ -Peptids



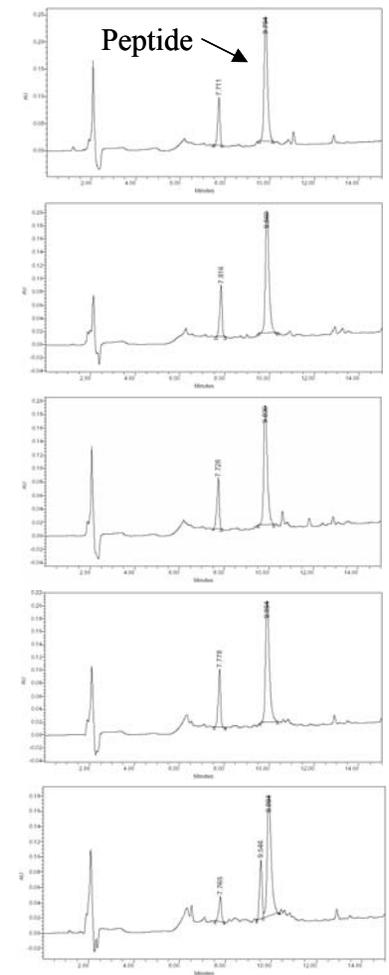
(A)



(B) Trypsin

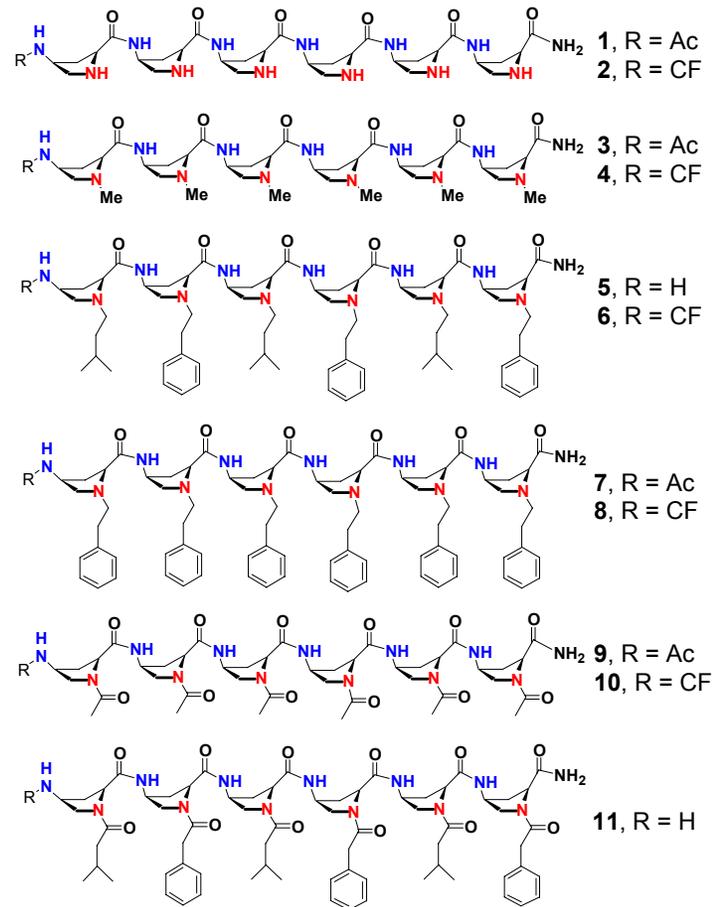
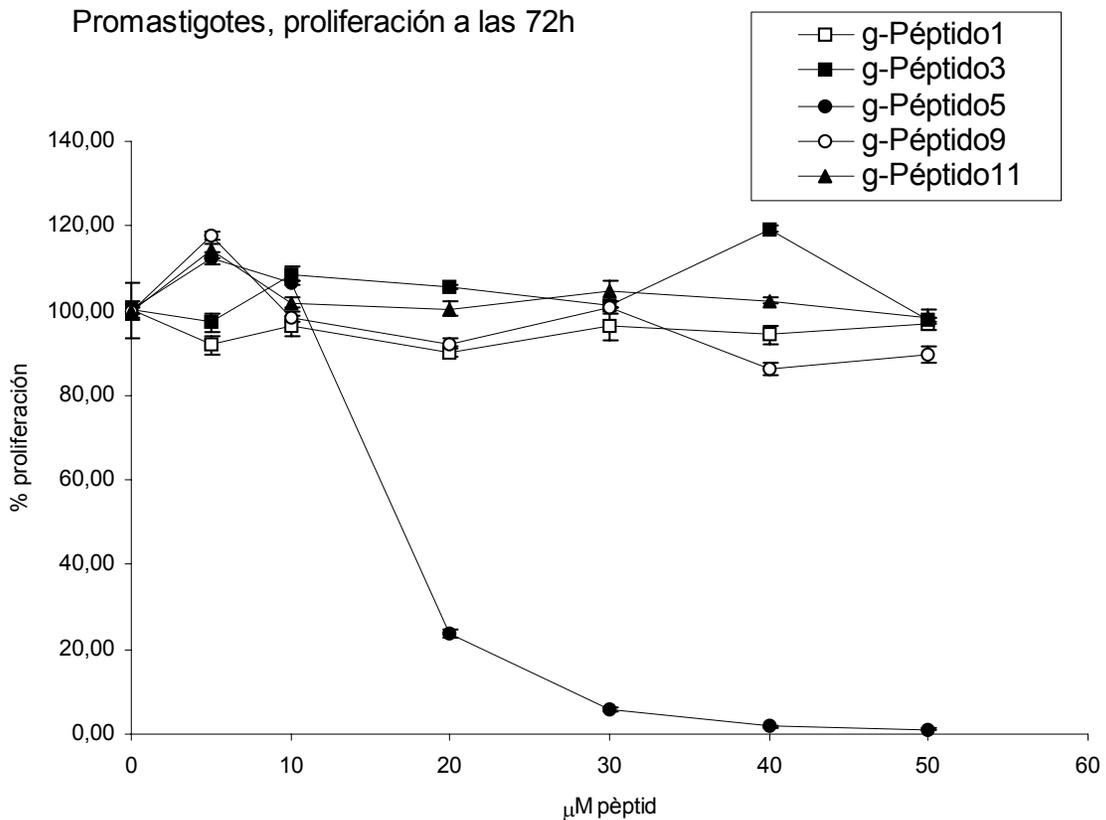


(C) Human serum



Leishmanicide Activity

Promastigotes, proliferación a las 72h



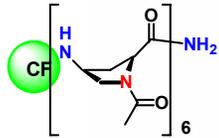
Acknowledgements



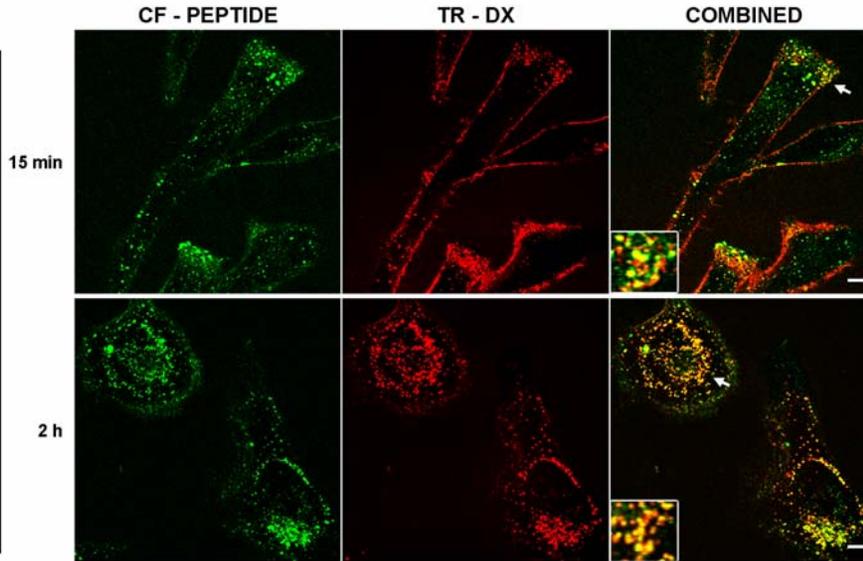
- Núria Bayó-Puxan
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- Ariadna Fernández
- Marc Vendrell
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- Prof. Klaus Burger
- Prof. Osvaldo Cascone
- Prof. Ernest Giralt
- Dr. Miriam Royo

Co-localización con DX-TR en células vivas

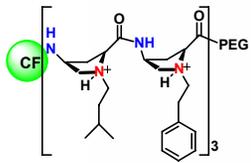
PEPTIDE 2



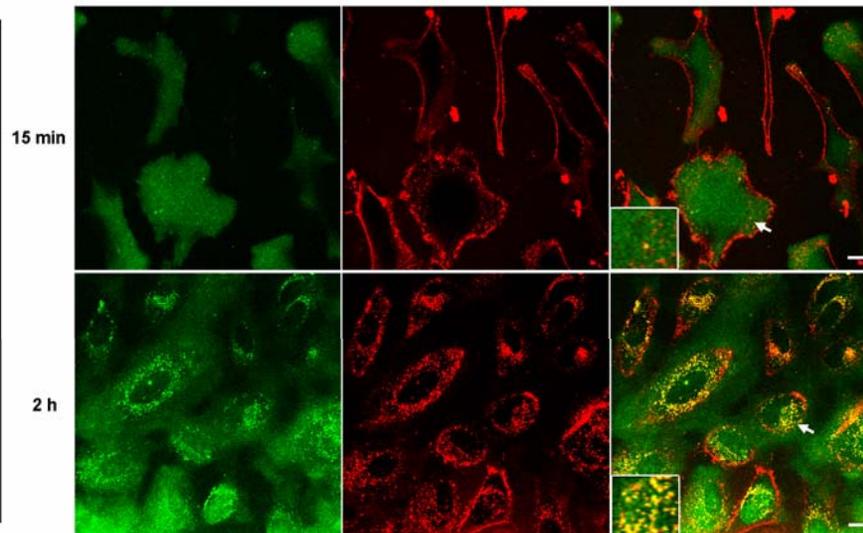
Peptide 2



PEPTIDE 4a



Peptide 4a



Condiciones

- CF-péptido 10 μ M, 37°C en células HeLa
- Marcador de fase fluida: Texas Red-Dextran (TR-DX)