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Advances on Cys Protecting Groups

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Iris Biotech

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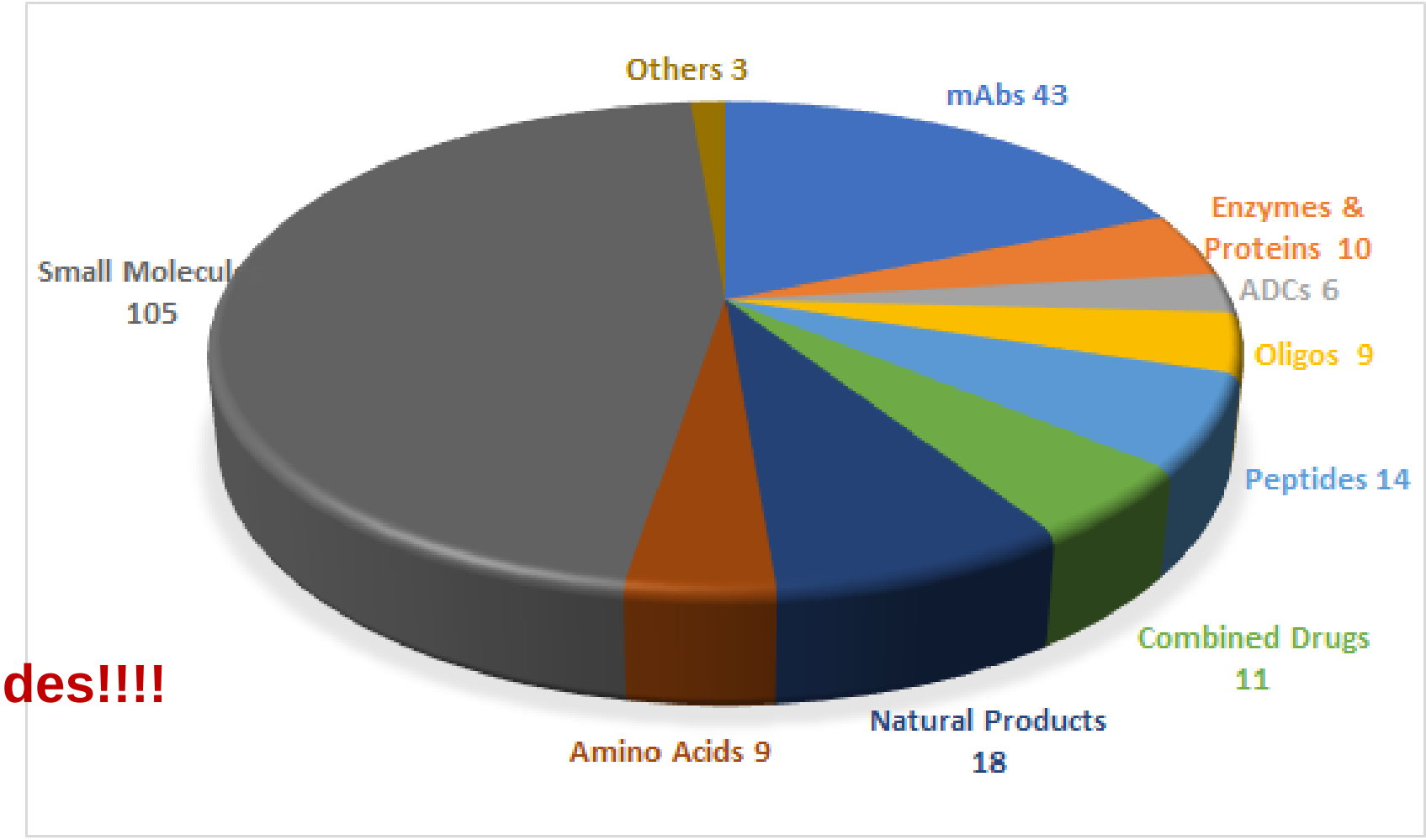
2016-2020 FDA Drugs Classified Chemically

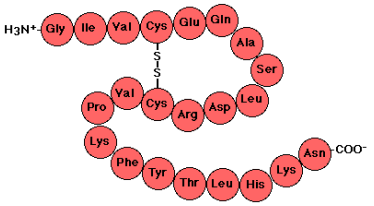
228 New Drugs



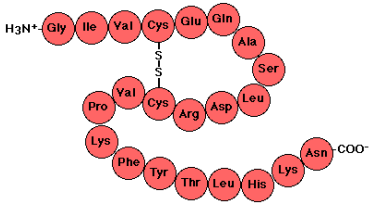
2021:
8 new peptides!!!!

Peptide Synthesis

Peptide Synthesis is a right combination of Coupling Reagents and Protecting Groups

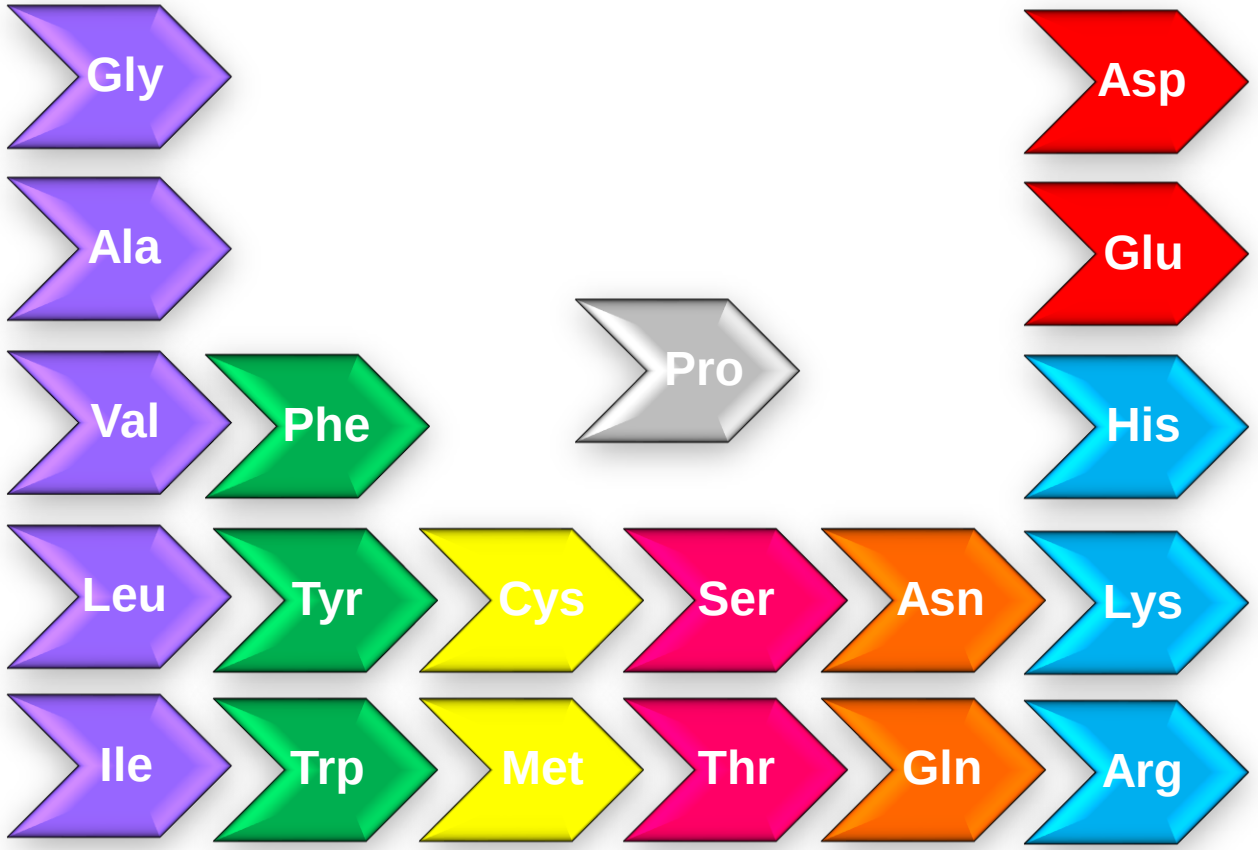


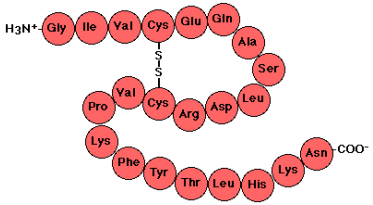
Coupling Reagents: A Letter Soup?



Amino Acids

Classification by side-chain chemical properties





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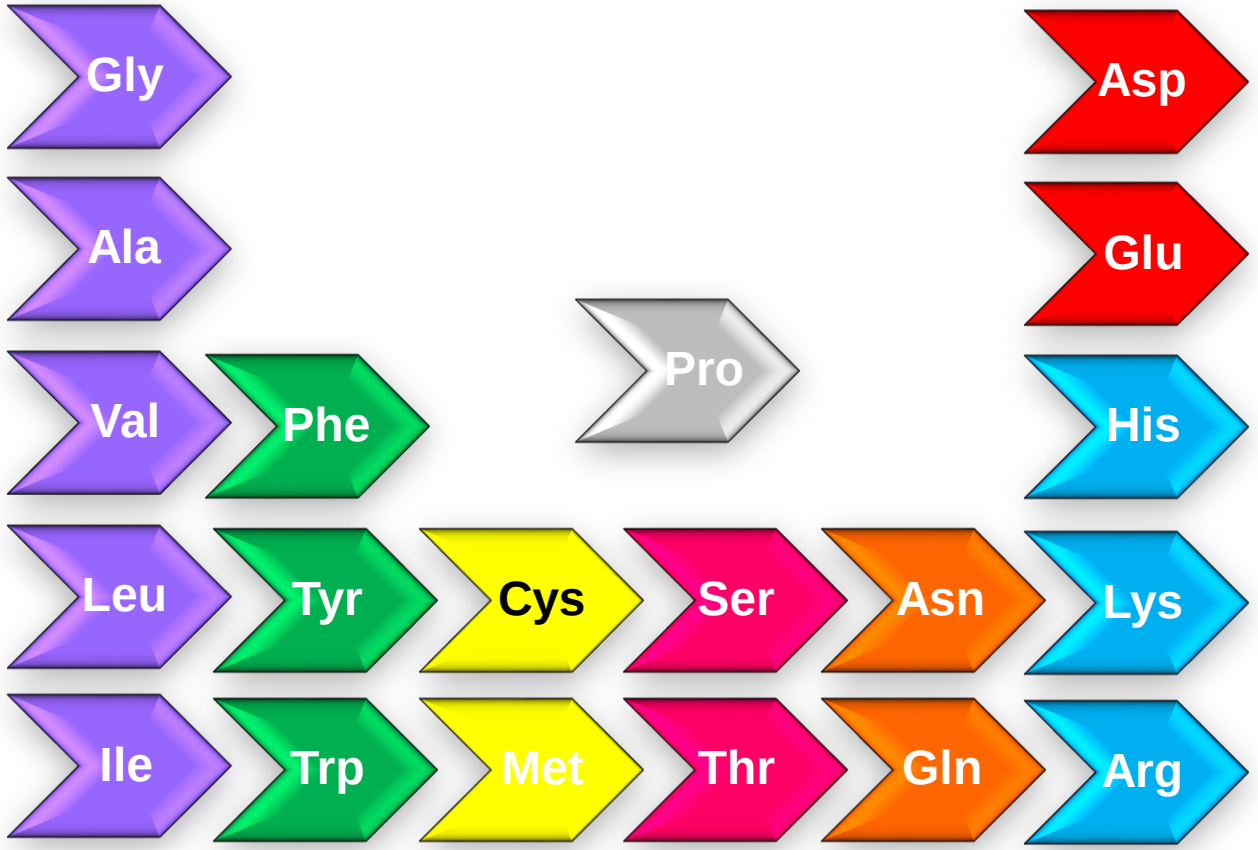
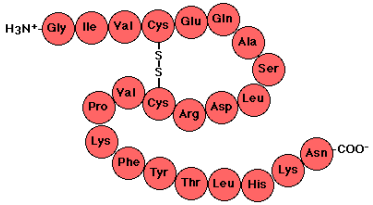
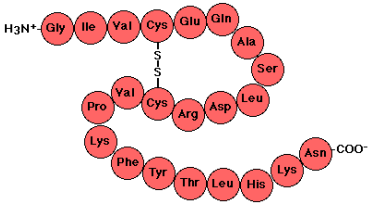




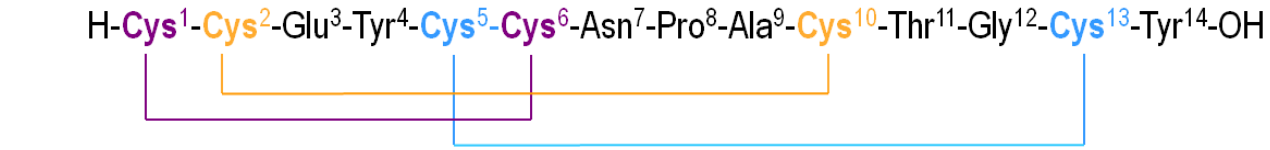
Cys Protecting Groups: So Many Flavours!

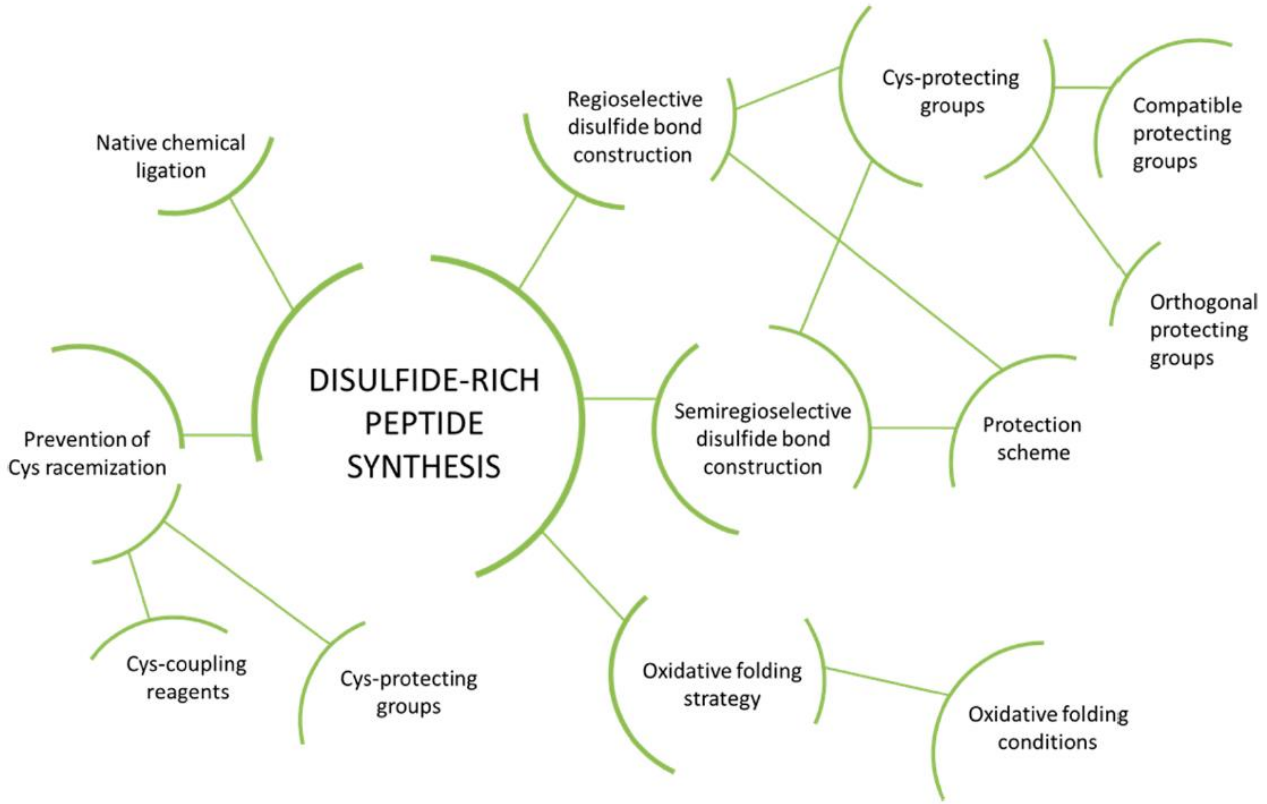
… and so delicious!



Cys, a Key and Multifaceted Amino Acid

Disulfide bridges





M. Góngora-Benítez, J. Tulla-Puche, F. Albericio. Chem. Rev., 114, 901-926 (2014).

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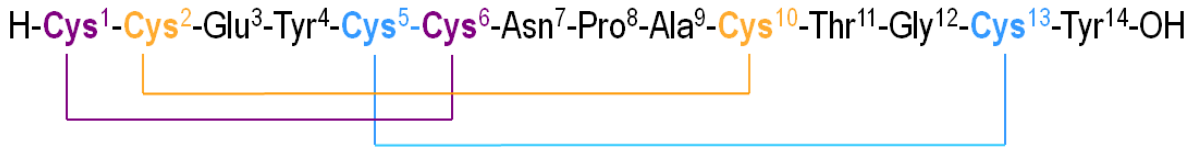
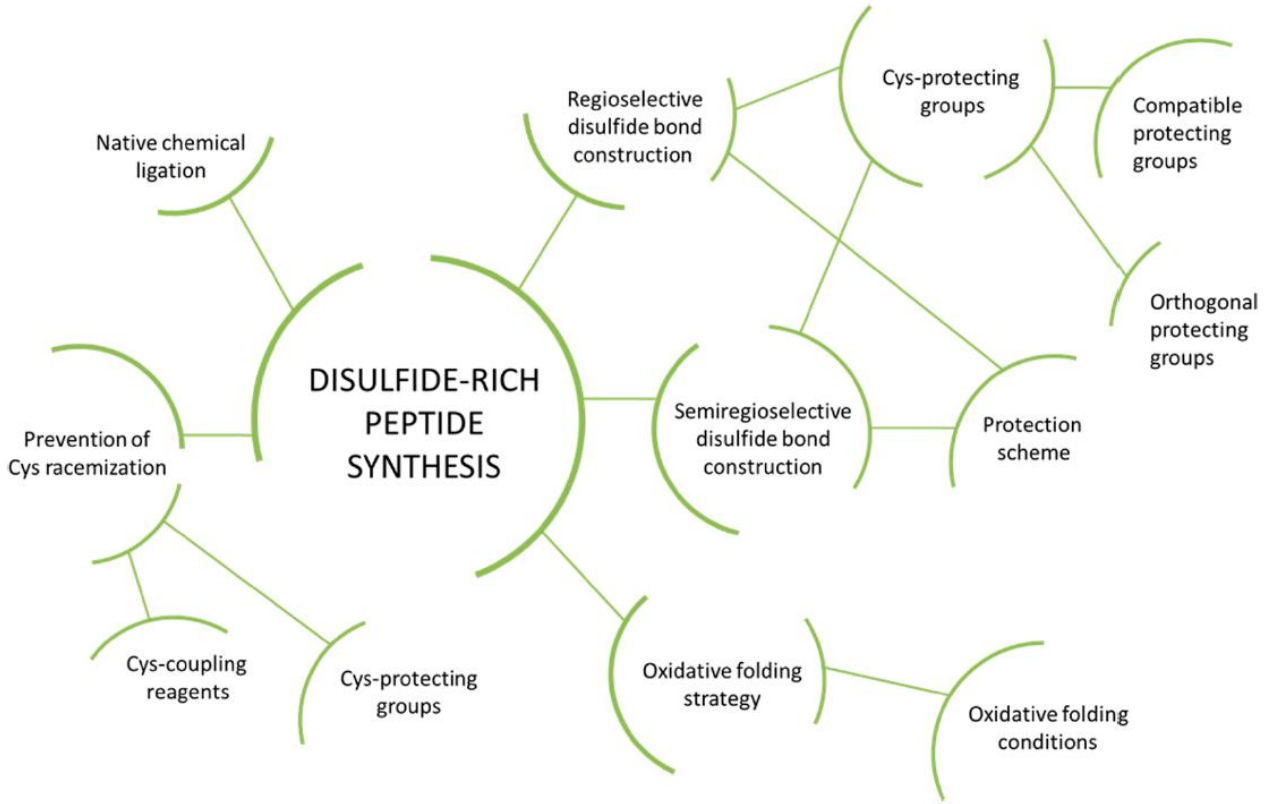


Nisin peptides (thioethers)

Chemical Ligation



Bioconjugation

Thiolactone peptides

MultiAntigenic Peptides (MAP)

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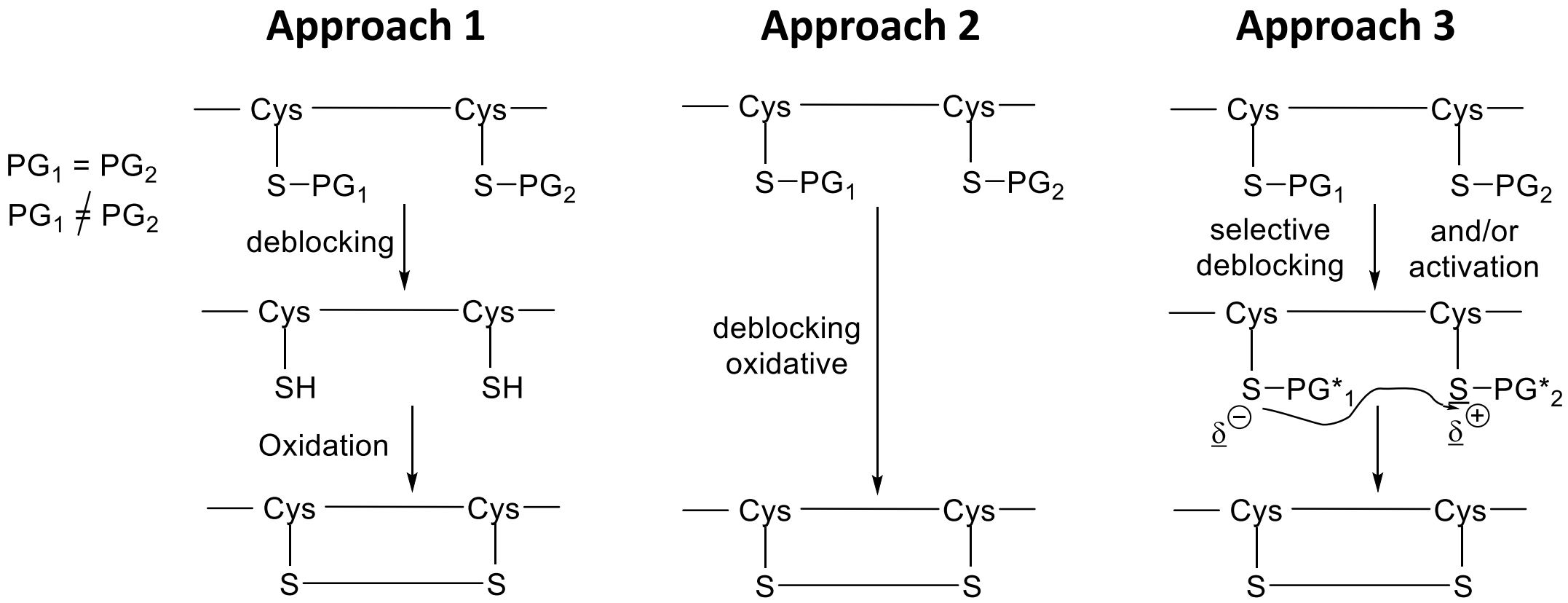
Cys, a Key and Multifaceted Amino Acid



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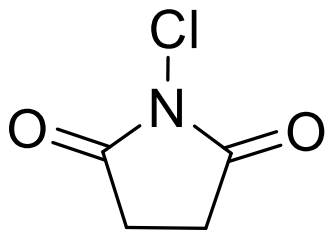
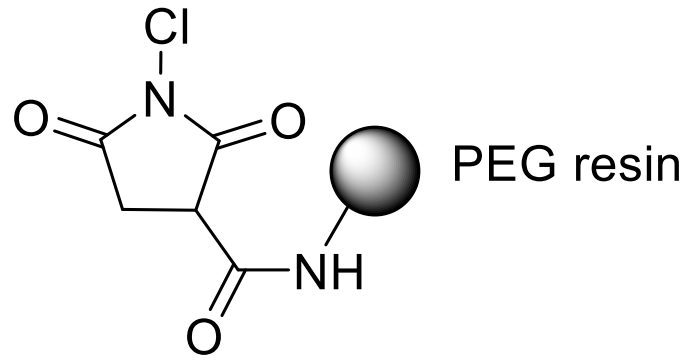
NCS, an Excellent Reagent for Disulfide Formation

- I_2 is very often used for oxidation of free thiols and deprotection/oxidation of Acm
- I_2 is difficult to handle and very often lead to overoxidation

N-chlorosuccinimide (NCS)





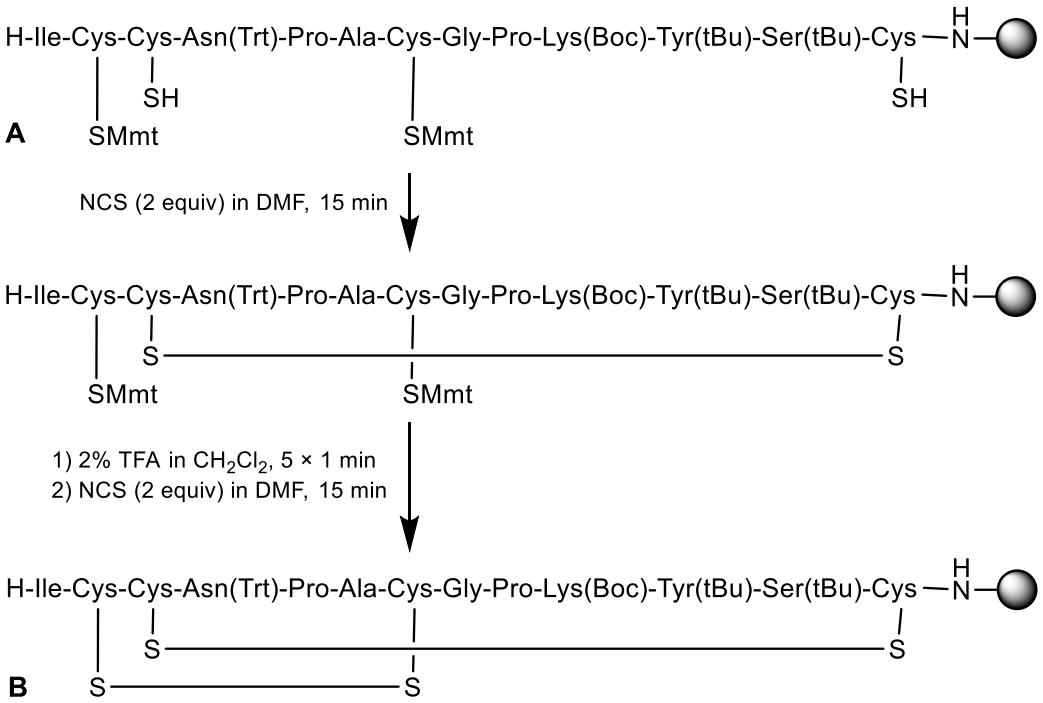
- NCS is a good alternative to I_2
- NCS is a solid soluble in a broad range of solvents: H_2O, H_2O-ACN, DMF
- NCS can oxidize the peptide in solution or while is still anchored on the solid support
- NCS supported resin can oxidize peptides in solution
- Oxidation takes 15 min using 1-2 equiv of NCS

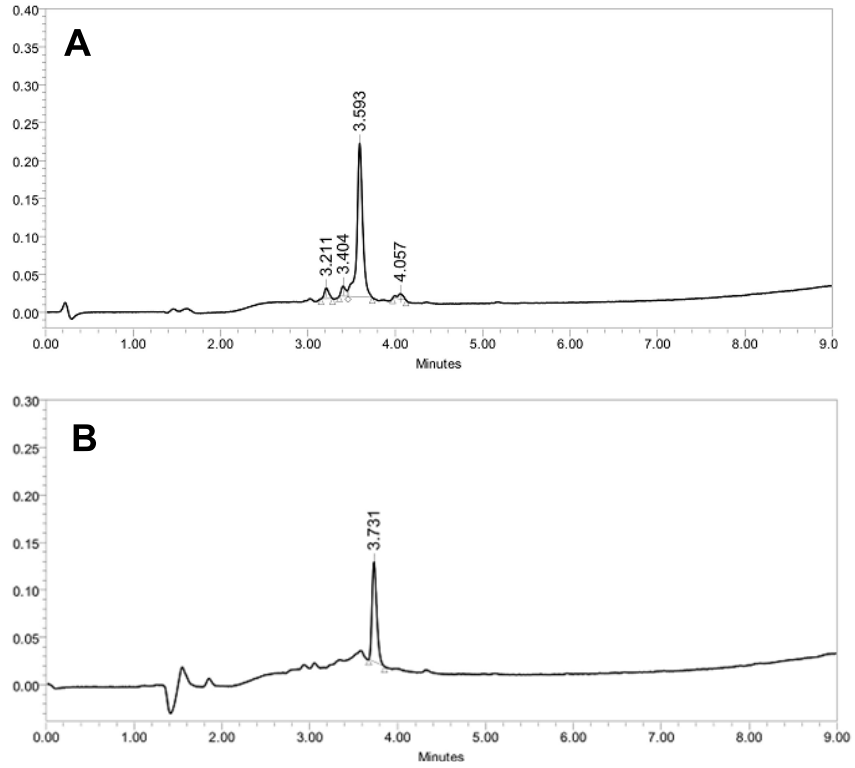
T.M. Postma, F. Albericio. Org. Lett., 15, 616 (2013); RSC Advances, 3, 14277 (2013); ACS Comb. Sci., 16, 43 (2014)

NCS for Regioselective Solid-Phase Disulfide Formation

- NCS does not oxidize Trt protecting group
- NCS allows regioselective disulfide formation

Si-Conotoxin





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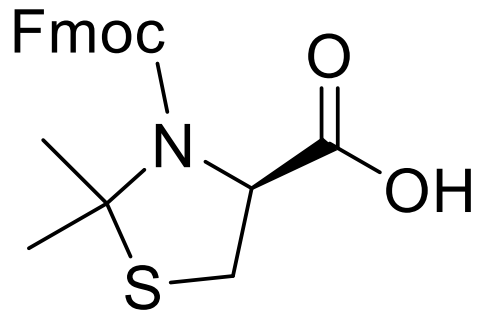
Cys diMe PseudoProline: A Dual Concept

Cys diMe PseudoProline
[Fmoc-Cys Me2 ψPro-OH; Fmoc-Thz(Me2)-OH]



- Favors Synthetic Strategies
  - Minimizes aggregation
  - Enhances cyclization
- Building Block
  - Restricts conformation

M. Pelay-Gimeno, A. Meli, J. Tulla-Puche, F. Albericio. J. Med. Chem., 56, 9780 (2013)
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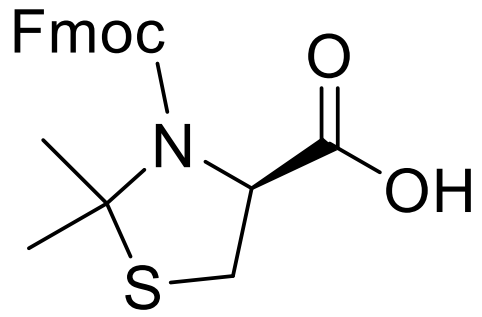

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- Removal is sequence dependent
  - In linear sequences, it could be removed with TFA-TIS-H_2O (95:2.5:2.5) for several hours
  - In cyclic sequences is more stable and could require TFMSA or HF

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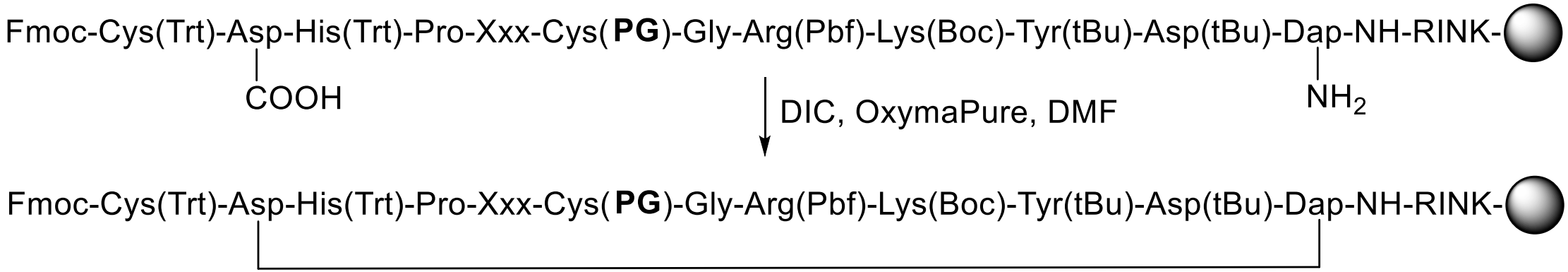
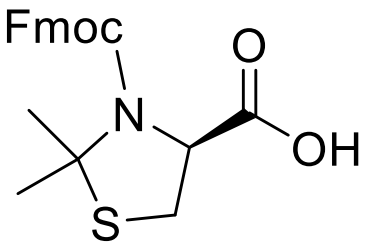
Cys diMe PseudoPro: Enhancing Cyclization





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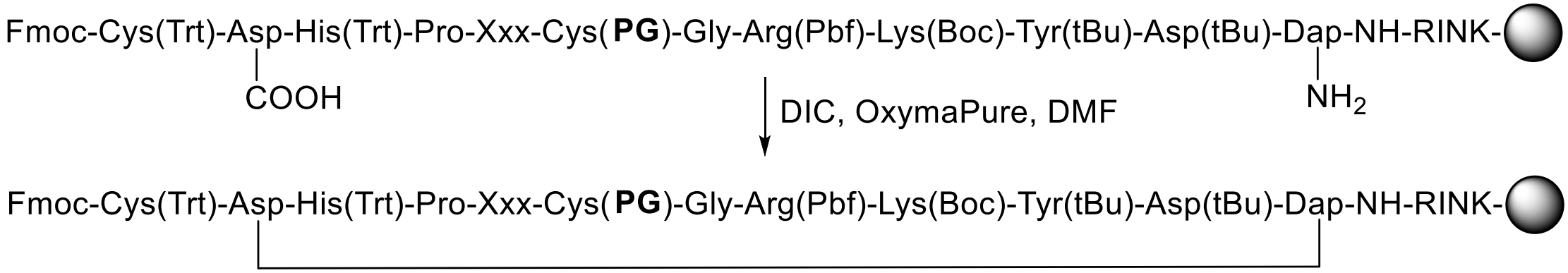

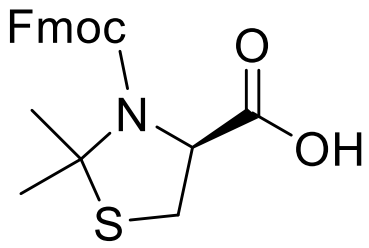

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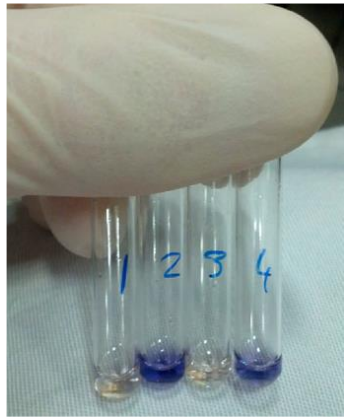

1 : Xxx = Ser(tBu); PG = Me2 ψPro

2 : Xxx = Ser(tBu); PG = Trt

3 : Xxx = Ala; PG = Me2 ψPro

4 : Xxx = Ala; PG = Trt

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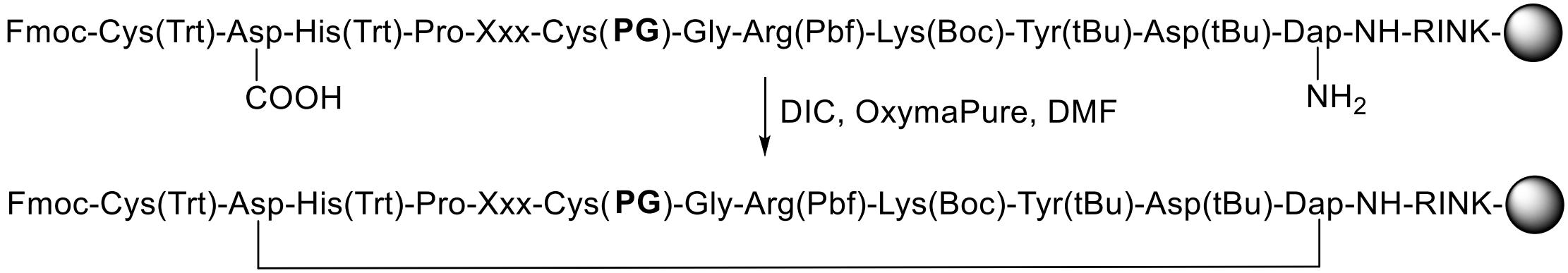
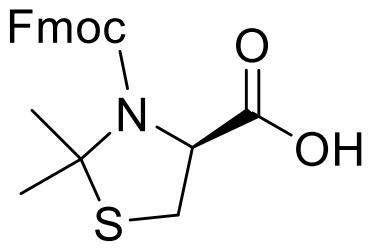
Cys diMe PseudoPro: Enhancing Cyclization





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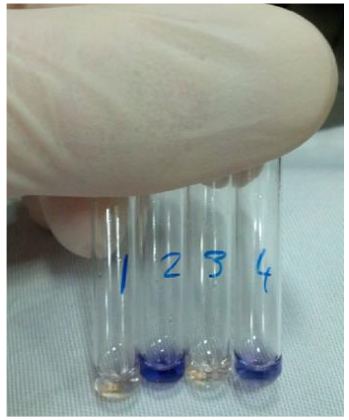

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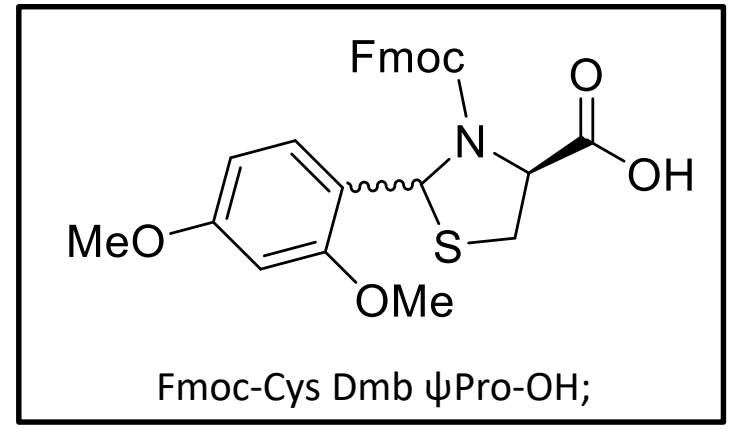
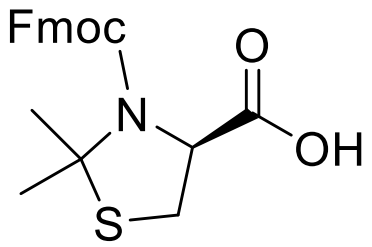
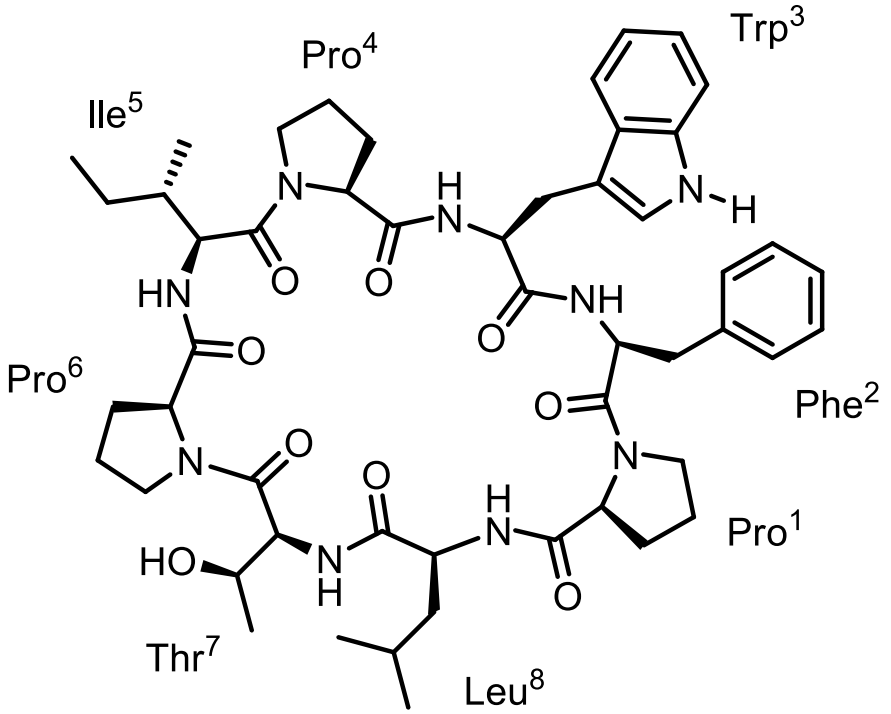


Fmoc-Cys Dmb ψPro-OH;

Cys diMe PseudoPro: Restricting Conformation



Fmoc-Cys Me2 ψPro-OH;
Fmoc-Thz(Me2)-OH



Phakellistatin 19

M. Pelay-Gimeno, A. Meli, J. Tulla-Puche, F. Albericio. J. Med. Chem., 56, 9780 (2013)

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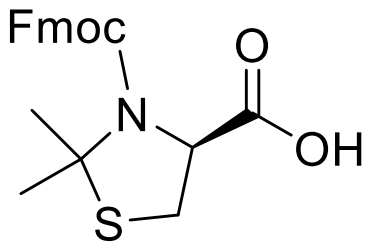

Fmoc-Cys Me2 ψPro-OH;
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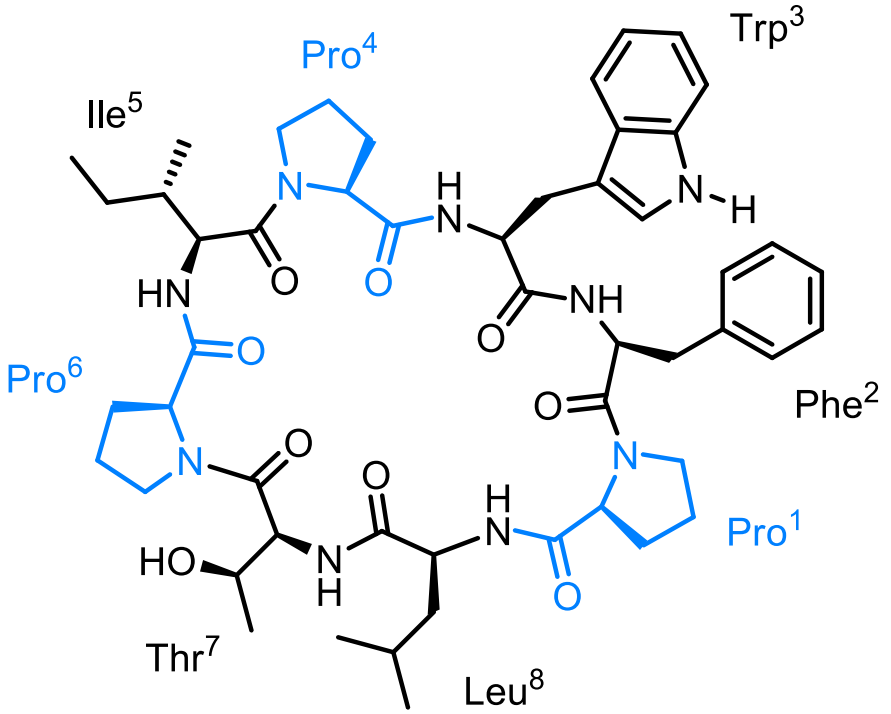

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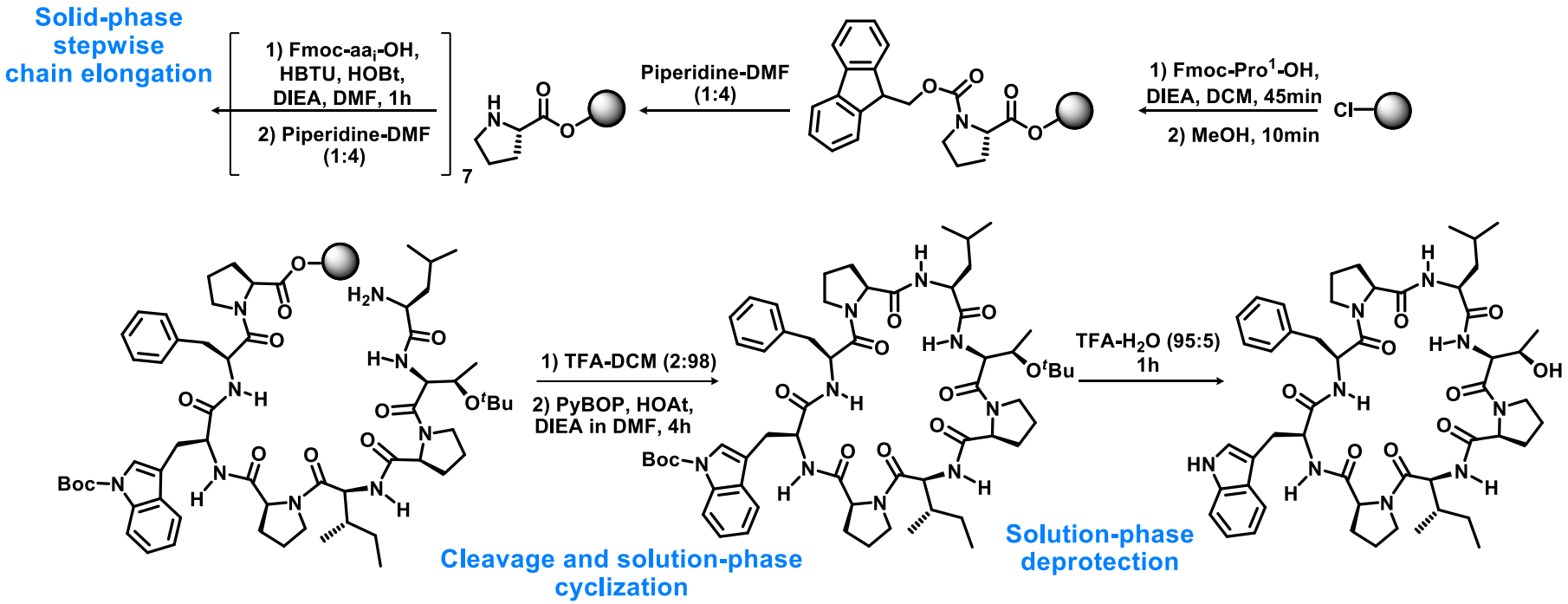
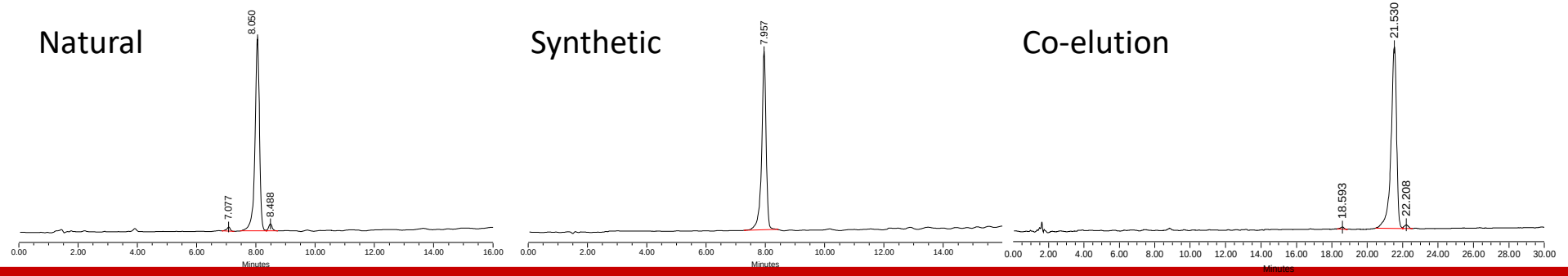
Cys diMe PseudoPro: Restricting Conformation

Synthesis



Chemical validation

Gradient: 35 to 55 % ACN over 30 min



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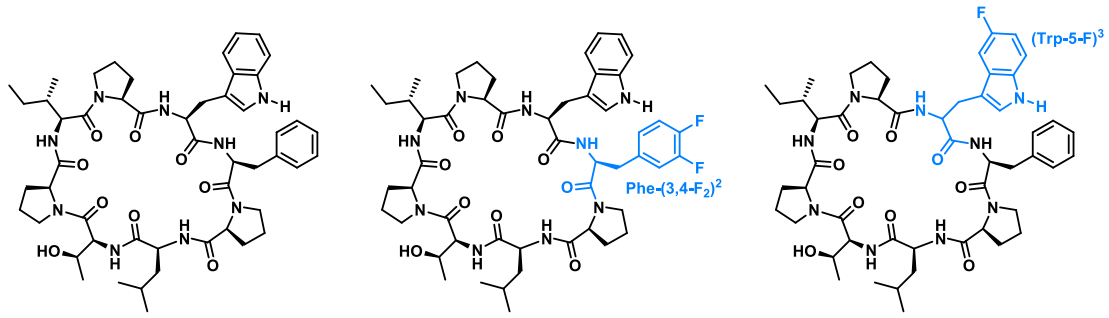
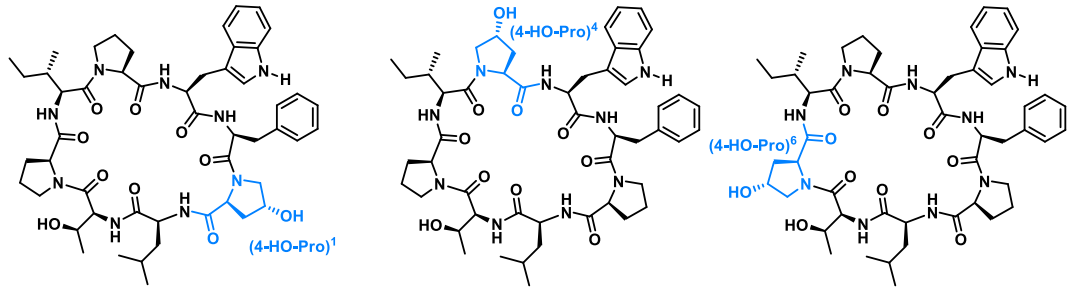
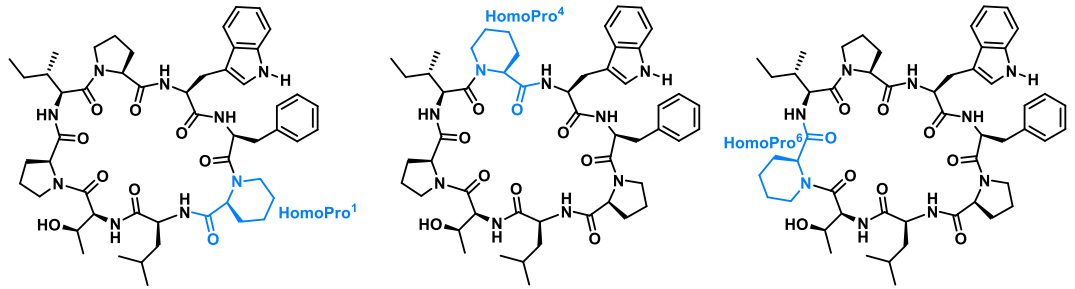
Biological Evaluation of Pakellistatin 19 and analogs



Phakellistatin 19 | 2-Phe-(3,4-F_2)-Phakellistatin 19 | 3-Trp-(5-F)-Phakellistatin 19



1-(4-HO-Pro)-Phakellistatin 19 | 4-(4-HO-Pro)-Phakellistatin 19 | 6-(4-HO-Pro)-Phakellistatin 19

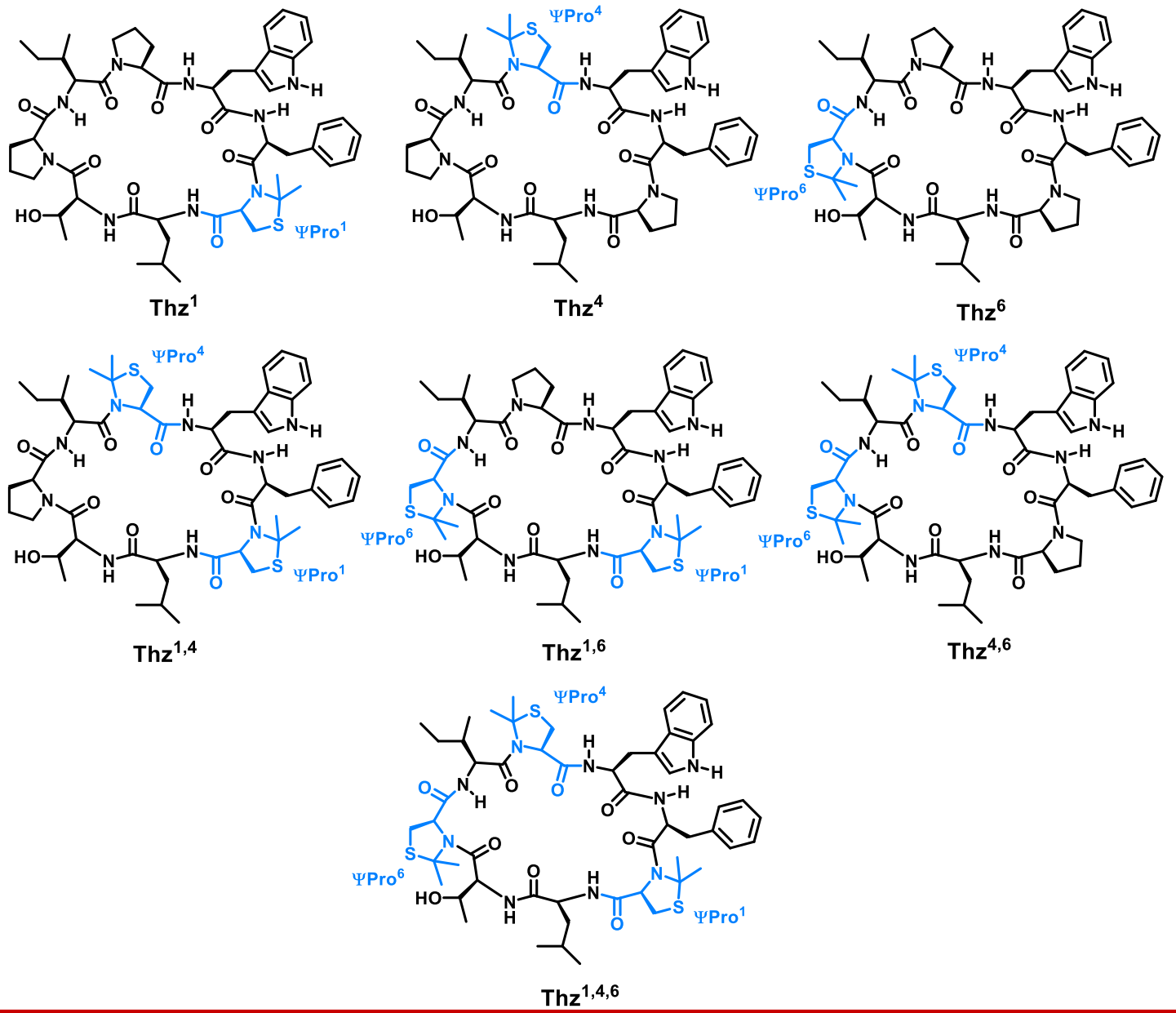


1-HomoPro-Phakellistatin 19 | 4-HomoPro-Phakellistatin 19 | 6-HomoPro-Phakellistatin 19

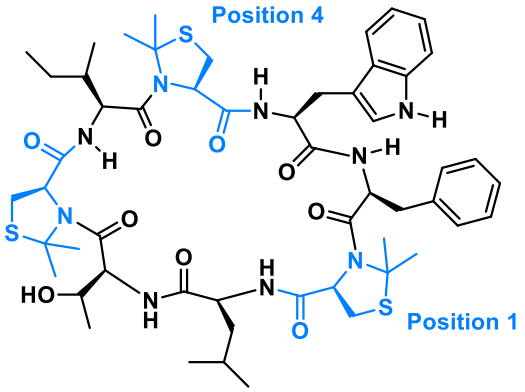
Peptide	GI_{50} (M) MDA-MB-231[1]	GI_{50} (M) A549[2]	GI_{50} (M) HT29[3]
Natural *Phakellistatin* 19	5.15E-07	4.62E-07	4.41E-07
Synthetic *Phakellistatin* 19	n.d.	n.d.	n.d.

Pharma Mar

Enhancing the *cis* isomerism: Pro replacement by Cys diMe PseudoPro



Enhancing the *cis* isomerism: Pro replacement by Cys diMe PseudoPro



Pro^{6} has a key structural role with direct influence on the bioactivity

Biological evaluation

Compound		A549		HT-29		MDA-MB-231	
		mg/mL	μM	mg/mL	μM	mg/mL	μM
Thz^{1}	GI_{50}	>1.0E+01	>10	5.7E+00	5.71	6.1E+00	6.11
	TGI	>1.0E+01	>10	>1.0E+01	>10	>1.0E+01	>10
	LC_{50}	>1.0E+01	>10	>1.0E+01	>10	>1.0E+01	>10
Thz^{4}	GI_{50}	>1.0E+01	>10	>1.0E+01	>10	>1.0E+01	>10
	TGI	>1.0E+01	>10	>1.0E+01	>10	>1.0E+01	>10
	LC_{50}	>1.0E+01	>10	>1.0E+01	>10	>1.0E+01	>10
Thz^{6}	GI_{50}	>1.0E+01	>10	4.0E+00	4.01	2.7E+00	2.70
	TGI	>1.0E+01	>10	4.2E+00	4.21	4.1E+00	4.11
	LC_{50}	>1.0E+01	>10	4.6E+00	4.61	6.0E+00	6.01
Thz^{1,4}	GI_{50}	>1.0E+01	>9.58	3.3E+00	3.16	5.9E+00	5.65
	TGI	>1.0E+01	>9.58	6.9E+00	6.61	>1.0E+01	>9.58
	LC_{50}	>1.0E+01	>9.58	>1.0E+01	>9.58	>1.0E+01	>9.58
Thz^{1,6}	GI_{50}	3.6E+00	3.45	1.9E+00	1.82	1.8E+00	1.72
	TGI	8.2E+00	7.85	2.3E+00	2.20	2.1E+00	2.01
	LC_{50}	>1.0E+01	>9.58	2.8E+00	2.68	2.4E+00	2.30
Thz^{4,6}	GI_{50}	3.8E+00	3.64	1.9E+00	1.82	2.3E+00	2.20
	TGI	>1.0E+01	>9.58	2.2E+00	2.11	3.5E+00	3.35
	LC_{50}	>1.0E+01	>9.58	2.5E+00	2.39	5.3E+00	5.08
Thz^{1,4,6}	GI_{50}	1.6E+00	1.47	1.5E+00	1.38	1.8E+00	1.65
	TGI	1.8E+00	1.65	1.9E+00	1.74	2.1E+00	1.93
	LC_{50}	2.0E+00	1.83	2.3E+00	2.11	2.4E+00	2.20

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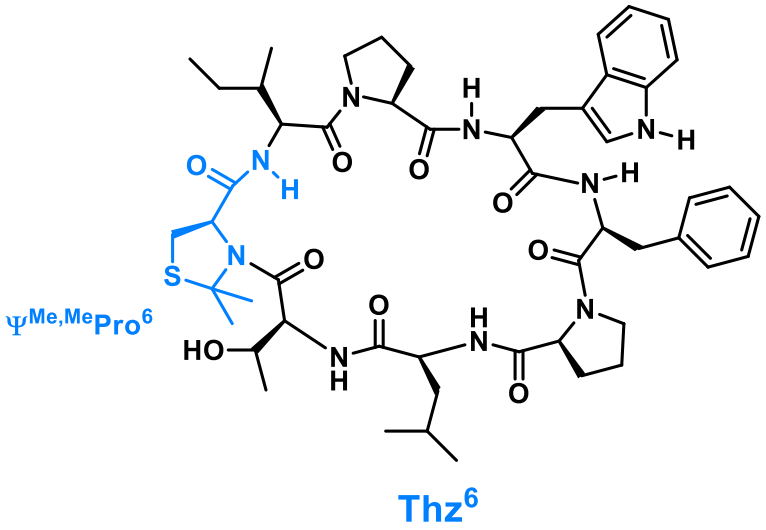
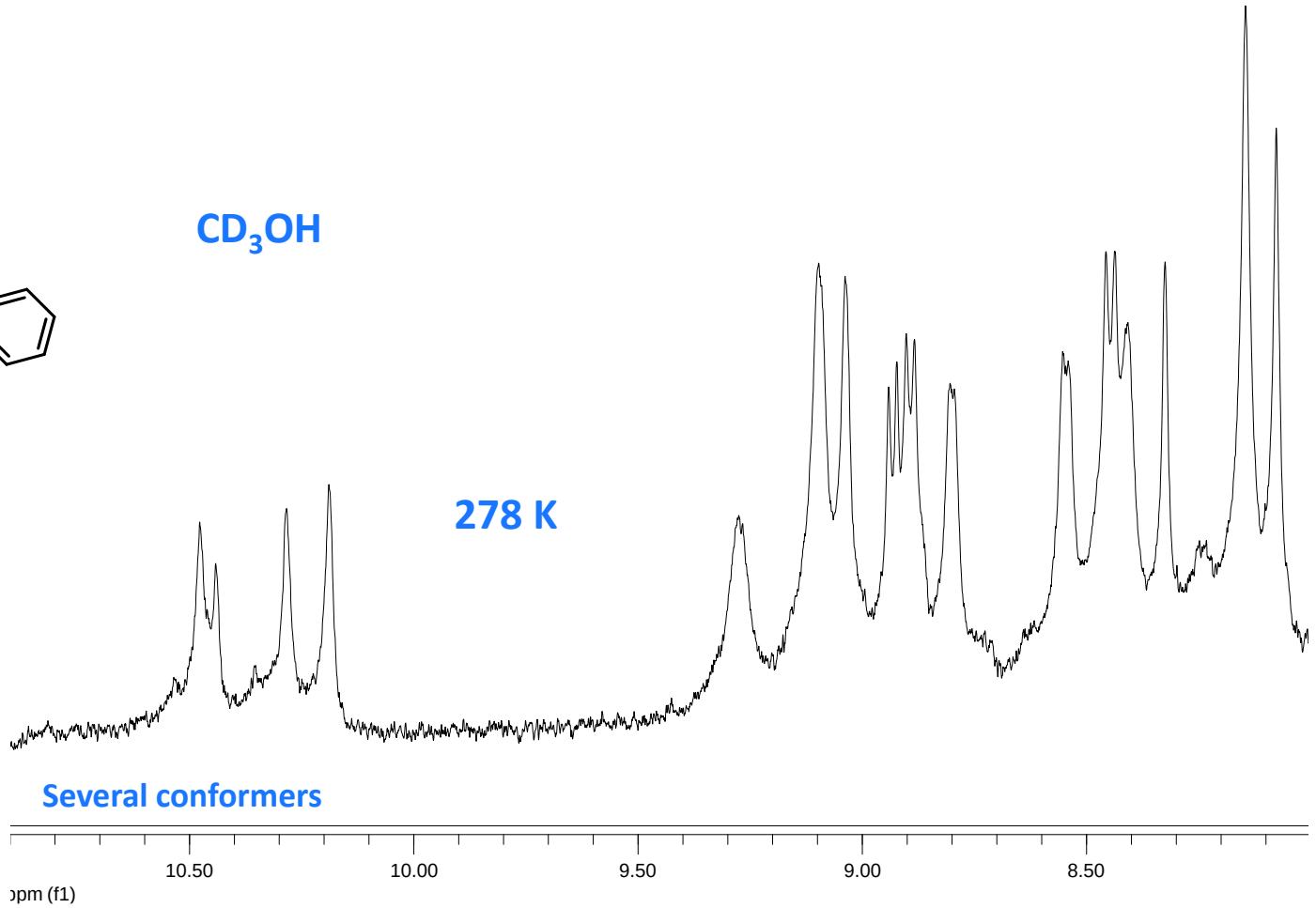
Enhancing the *cis* isomerism: Pro replacement by Cys diMe PseudoPro

NMR study





M. Pelay-Gimeno, A. Meli, J. Tulla-Puche, F. Albericio. J. Med. Chem., 56, 9780 (2013)

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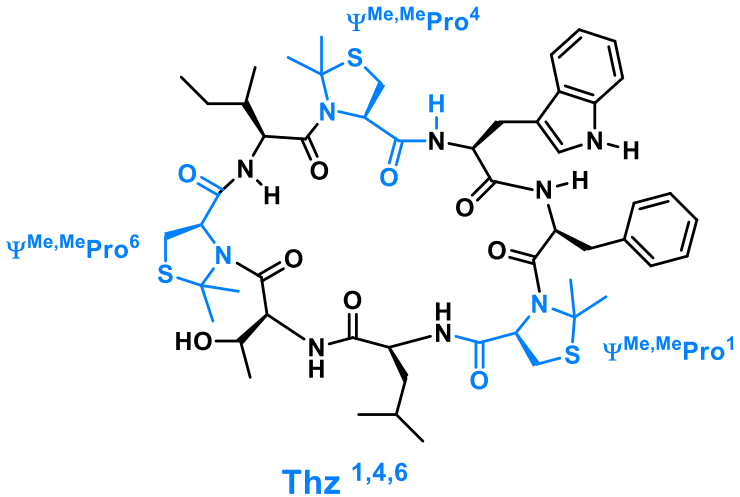


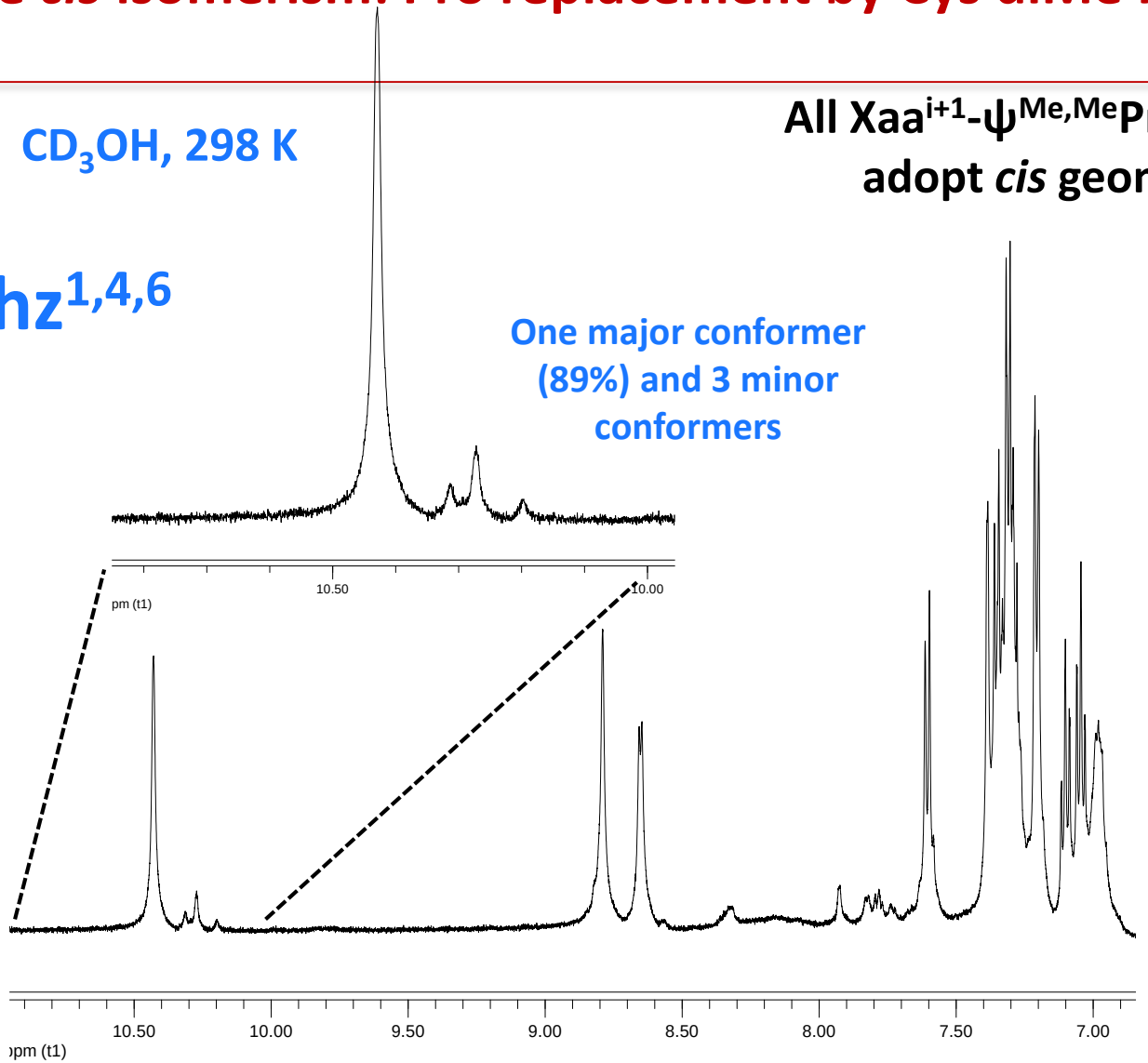
Thz 1,4,6

CD_3OH, 298 K

Thz^{1,4,6}

All Xaa^{i+1}-ψ^{Me,Me}Pro^{i} bonds adopt *cis* geometry

One major conformer (89%) and 3 minor conformers



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Acid-Labile Cys Protecting Groups: Scavengers

[Contribution from the Laboratory of Organic Chemistry, University of Athens, Greece]

On Cysteine and Cystine Peptides. I. New S-Protecting Groups for Cysteine[1,2]

By Leonidas Zervas and Iphigenia Photaki

Received May 31, 1962

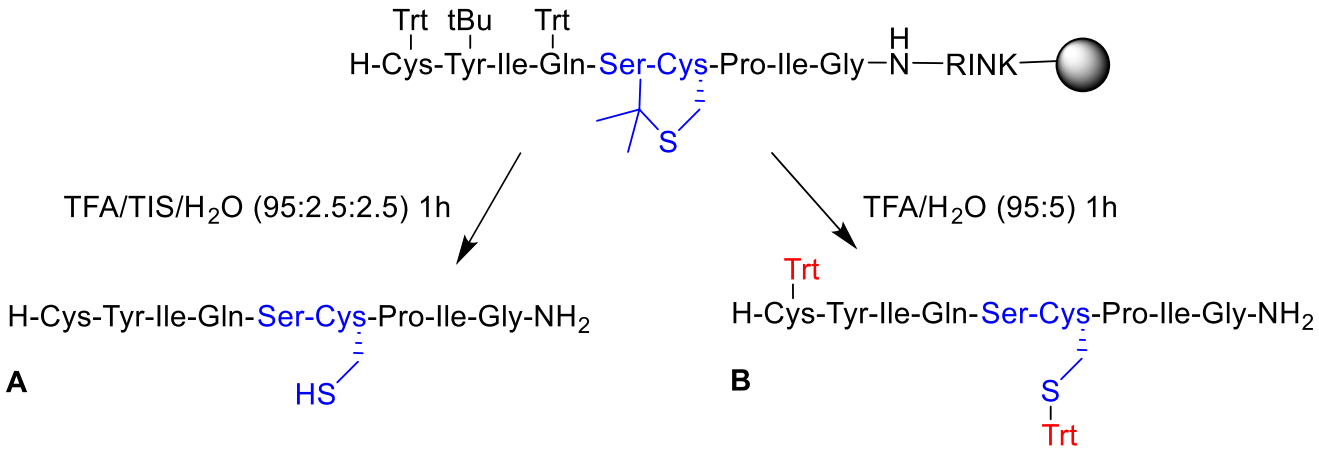
The problem of the synthesis of unsymmetrical cystine peptides with two or more cystine –S–S– bridges is discussed. For a solution of this problem, the following requirements must be fulfilled: (a) cysteines bearing different S-protecting groups selectively removable must be available and (b) procedures must be worked out for preventing the rearrangement of cystine chains during synthesis until their final incorporation in a multimembered ring system. Concerning the first of the above requirements, S-diphenylmethyl-L-cysteine (I) and S-trityl-L-cysteine (II) are proposed as the most suitable S-protected cysteines for the incorporation of cysteine residues in a peptide chain. The S-trityl group can be easily split off with heavy metal salts at room temperature, whereas the removal of the S-diphenylmethyl group is also easily effected by the action of trifluoroacetic acid. The SH– groups thus liberated can be oxidized to the corresponding –S–S– derivatives. Several peptides of cysteine and cystine have been synthesized in this way.

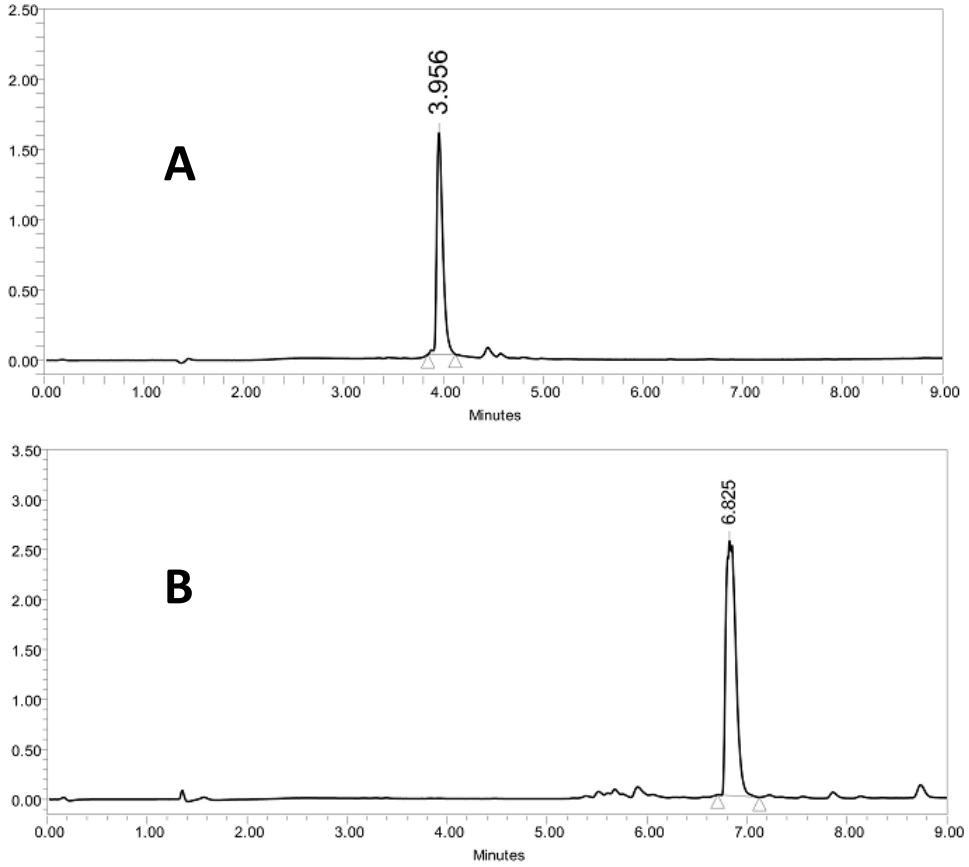
The cleavage of the S-DPM– group requires a higher concentration of hydrogen bromide (2 *N* HBr), higher temperature (about 55°) and longer reaction time (90 minutes). Even under these conditions the cleavage is seldom more than 50%. If the temperature is kept at 20° and the reaction time is reduced to about 20 minutes, the removal of the S-DPM group from S-DPM-N-carbobenzoxycysteine (IVb) amounts to an extent of only 8–10%, and it is easy to isolate S-DPM-cysteine (I) in high yield (85%).

Boiling trifluoroacetic acid very rapidly converts S-DPM-cysteine (I) and S-Tr-cysteine (II) quantitatively to cysteine, but in contrast to hydrogen bromide it splits off the S-protecting group from II a little more slowly than from I (I in 15 minutes and II in 30 minutes); under the same conditions N-carbobenzoxy-S-DPM-cysteine (IVb) is transformed to cysteine only to an extent of 75% and this cannot be substantially increased even if the

Acid-Labile Cys Protecting Groups: Scavengers

- Model peptide to study deprotection conditions

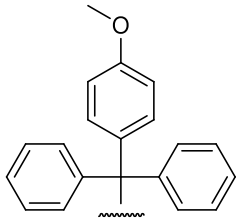
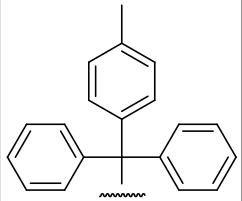
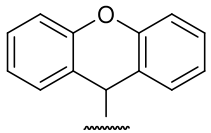
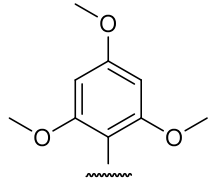
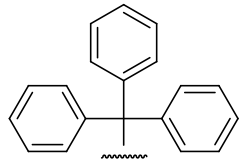
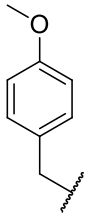




Conclusion: Don't use scavengers if Trt should be kept

Acid-Labile Protecting Groups for Cys

TFA-labile protecting groups for Fmoc/*t*Bu strategy:

					
Mmt	Mtt	Xan	Tmob	Trt	Mob
4-methoxytrityl	4-methyltrityl	xanthenyl	2,4,6-trimethoxybenzyl	trityl	4-methoxybenzyl
0.5-1% TFA scavengers	1% TFA scavengers	1% TFA scavengers	5% TFA scavengers	5-25% TFA scavengers	95% TFA Scavengers > 40 ° C HF

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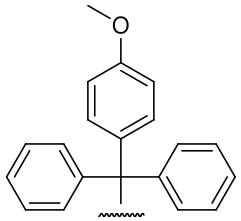
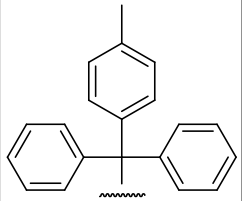
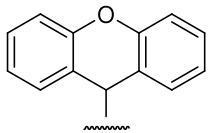
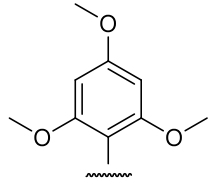
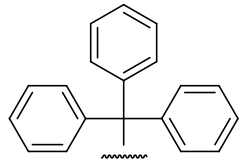
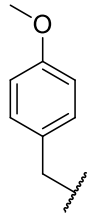
Acid-Labile Protecting Groups for Cys

TFA-labile protecting groups for Fmoc/*t*Bu strategy:

					
Mmt	Mtt	Xan	Tmob	Trt	Mob
4-methoxytrityl	4-methyltrityl	xanthenyl	2,4,6-trimethoxybenzyl	trityl	4-methoxybenzyl
0.5-1% TFA scavengers	1% TFA scavengers	1% TFA scavengers	5% TFA scavengers	5-25% TFA scavengers	95% TFA Scavengers > 40 ° C HF

Protecting Group Gap …

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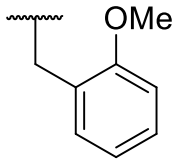
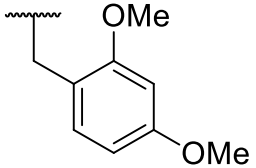
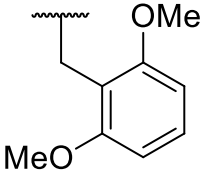
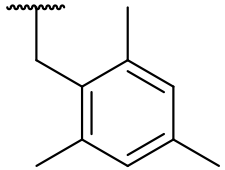
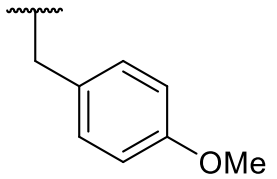
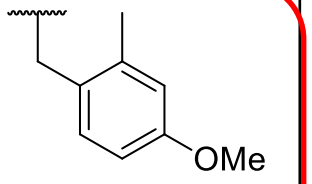
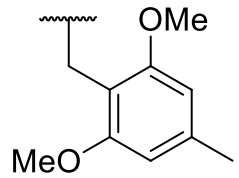
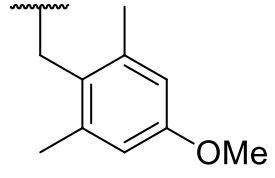
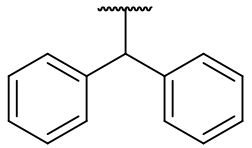
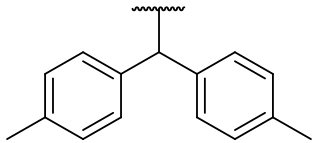
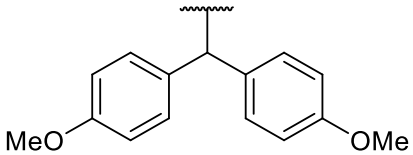
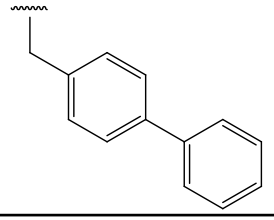
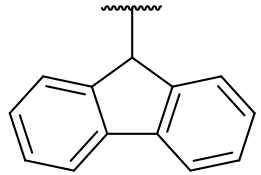
Acid-Labile Protecting Groups for Cys

				
2MeOBn	2,4diMeOBn	2,6diMeOBn	TMeb	Mob
NO TFA-labile	20% TFA 2.5%TIS + 2.5%H_2O for 30 min at RT	60% TFA 2.5%TIS + 2.5%H_2O for 1 h at RT	NO TFA-labile	95% TFA 2.5%TIS + 2.5%H_2O > 2 h at 40 ˚C

TFA-lability tests
[Fmoc-Ala-Cys(**PG**)-Leu-NH_2]:

		
4MeO-2MeBn	2,6diMeO-4MeBn	2,6diMe-4MeOBn
60% TFA 2.5%TIS + 2.5%H_2O for 1 h at RT	20% TFA 2.5%TIS + 2.5%H_2O for 30 min at RT	20% TFA 2.5%TIS + 2.5%H_2O for 30 min at RT

Acid-Labile Protecting Groups for Cys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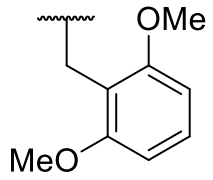
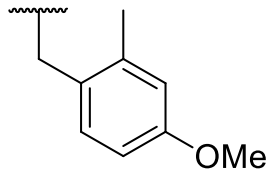
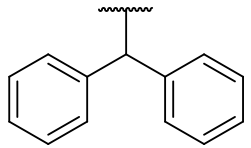
TFA-lability tests
[Fmoc-Ala-Cys(**PG**)-Leu-NH_2]:



2,6diMeOBn



4MeO-2MeBn



Dpm

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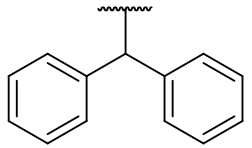
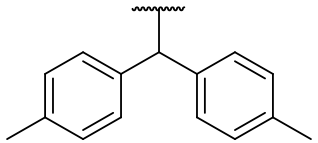
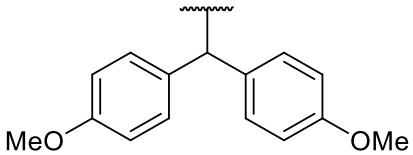
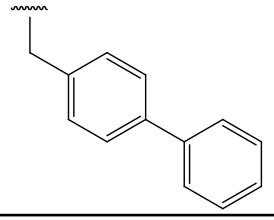
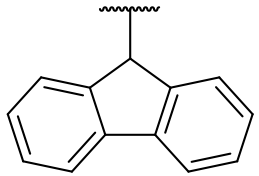
Acid-Labile Protecting Groups for Cys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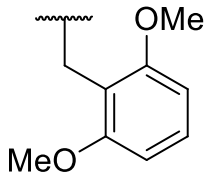

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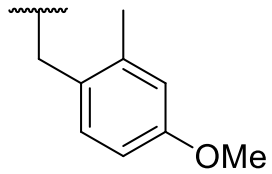

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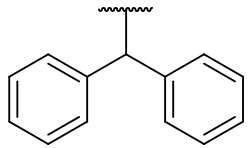

Dpm

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Acid-Labile Cys Protecting Groups: Scavengers

[CONTRIBUTION FROM THE LABORATORY OF ORGANIC CHEMISTRY, UNIVERSITY OF ATHENS, GREECE]

On Cysteine and Cystine Peptides. I. New S-Protecting Groups for Cysteine[1,2]

BY LEONIDAS ZERVAS AND IPHIGENIA PHOTAKI

RECEIVED MAY 31, 1962

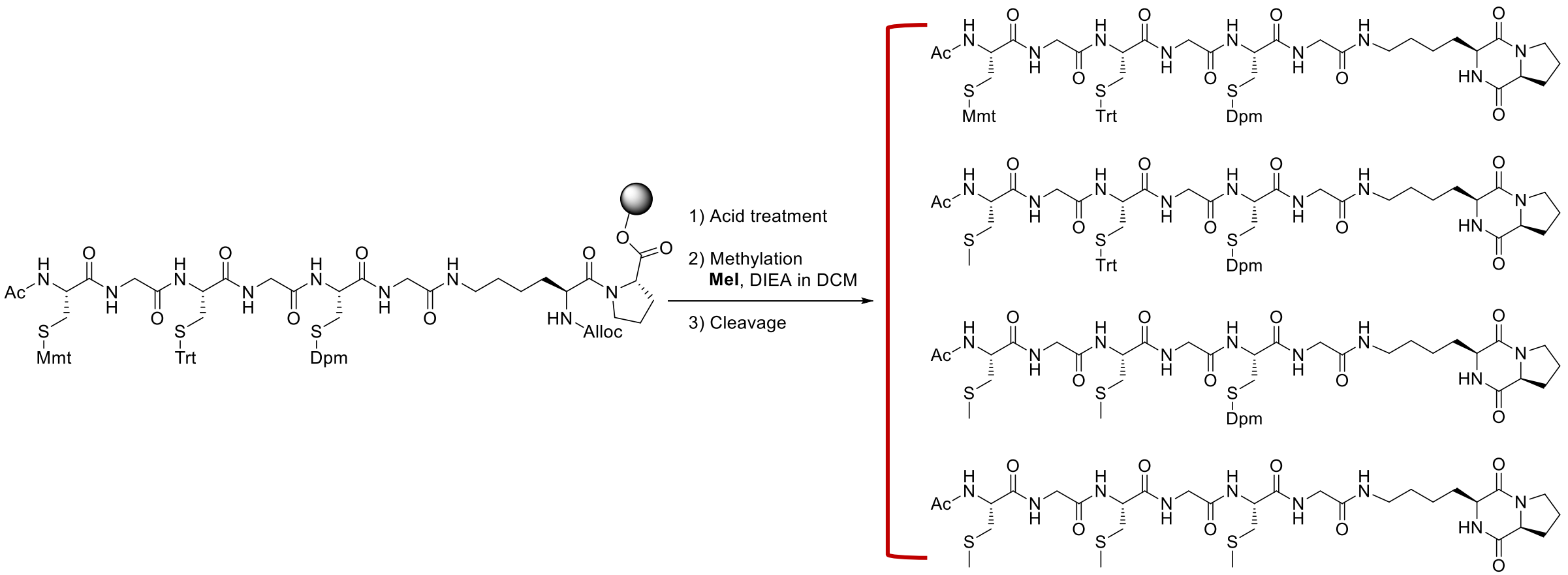
The problem of the synthesis of unsymmetrical cystine peptides with two or more cystine –S–S– bridges is discussed. For a solution of this problem, the following requirements must be fulfilled: (a) cysteines bearing different S-protecting groups selectively removable must be available and (b) procedures must be worked out for preventing the rearrangement of cystine chains during synthesis until their final incorporation in a multimembered ring system. Concerning the first of the above requirements, S-diphenylmethyl-L-cysteine (I) and S-trityl-L-cysteine (II) are proposed as the most suitable S-protected cysteines for the incorporation of cysteine residues in a peptide chain. The S-trityl group can be easily split off with heavy metal salts at room temperature, whereas the removal of the S-diphenylmethyl group is also easily effected by the action of trifluoroacetic acid. The SH– groups thus liberated can be oxidized to the corresponding –S–S– derivatives. Several peptides of cysteine and cystine have been synthesized in this way.

Leonidas Zervas
1902-1980

The cleavage of the S-DPM– group requires a higher concentration of hydrogen bromide (2 *N* HBr), higher temperature (about 55°) and longer reaction time (90 minutes). Even under these conditions the cleavage is seldom more than 50%. If the temperature is kept at 20° and the reaction time is reduced to about 20 minutes, the removal of the S-DPM group from S-DPM-N-carbobenzoxycysteine (IVb) amounts to an extent of only 8–10%, and it is easy to isolate S-DPM-cysteine (I) in high yield (85%).

Boiling trifluoroacetic acid very rapidly converts S-DPM-cysteine (I) and S-Tr-cysteine (II) quantitatively to cysteine, but in contrast to hydrogen bromide it splits off the S-protecting group from II a little more slowly than from I (I in 15 minutes and II in 30 minutes); under the same conditions N-carbobenzoxy-S-DPM-cysteine (IVb) is transformed to cysteine only to an extent of 75% and this cannot be substantially increased even if the

Compatibility of Mmt, Trt, and Dmp



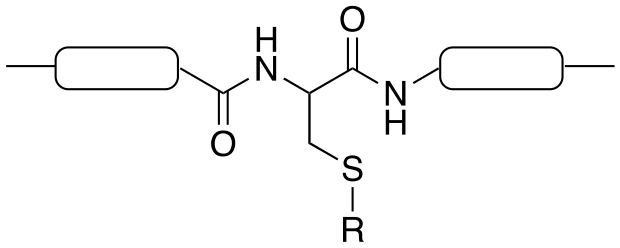
M. Góngora-Benítez, L. Mendive-Tapia, I. Ramos-Tomillero, A.C. Breman, J. Tulla-Puche, F. Albericio. Org. Lett., 14, 5472 (2012)
I. Ramos-Tomillero, L. Mendive-Tapia, M. Góngora-Benítez, E. Nicolas, J. Tulla-Puche, F. Albericio. Molecules 18, 5155 (2013)

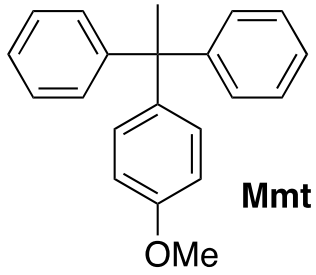
Compatibility of Mmt, Trt, and Dmp

Acid Treatment	mono-Met Peptide	di-Met Peptide	tri-Met Peptide	Fully Protected Peptid
10% TFA 2.5% TIS en DCM (2 x 10 min)	-	++++	-	-
90% TFA 2.5% TIS en DCM (1 x 1 h)	-	-	++++	-
60% TFA 2.5% TIS en DCM (1 x 1 h)	-	+	++	-

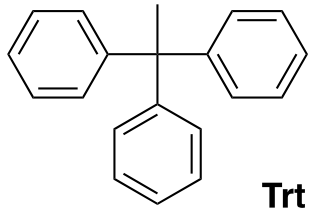
M. Góngora-Benítez, L. Mendive-Tapia, I. Ramos-Tomillero, A.C. Breman, J. Tulla-Puche, F. Albericio. Org. Lett., 14, 5472 (2012)

Dpm for Cys, labile with 90% TFA

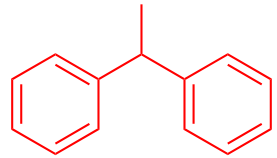




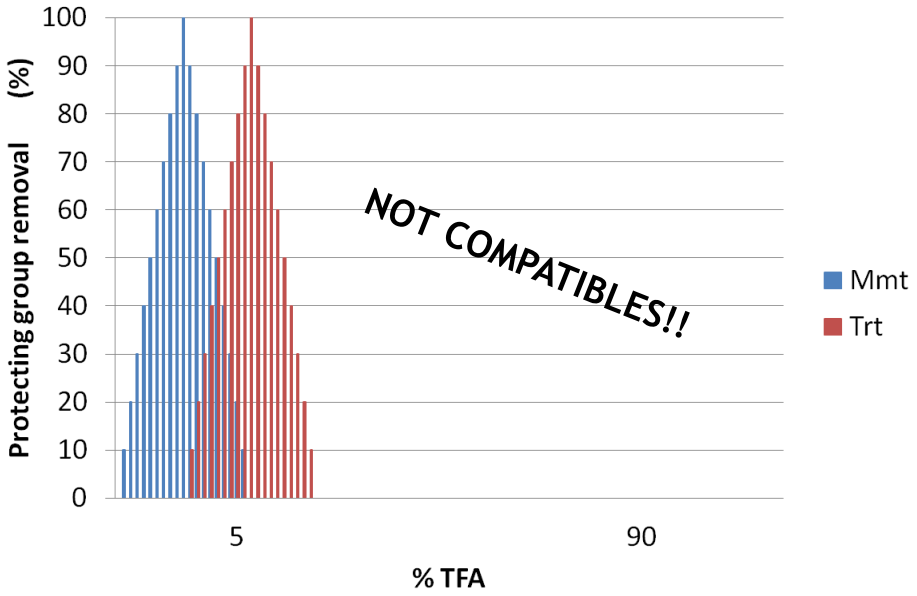
Mmt

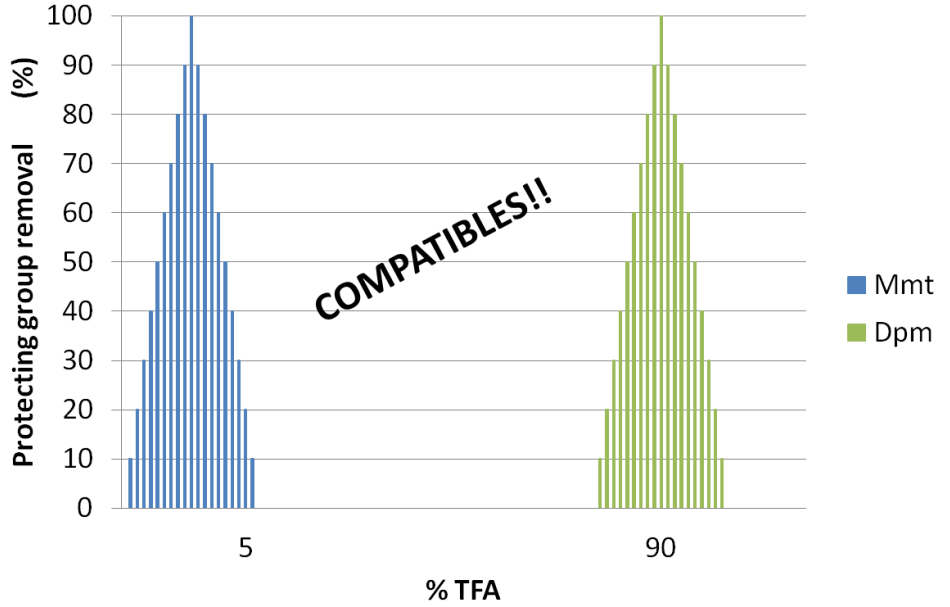


Trt



Dpm

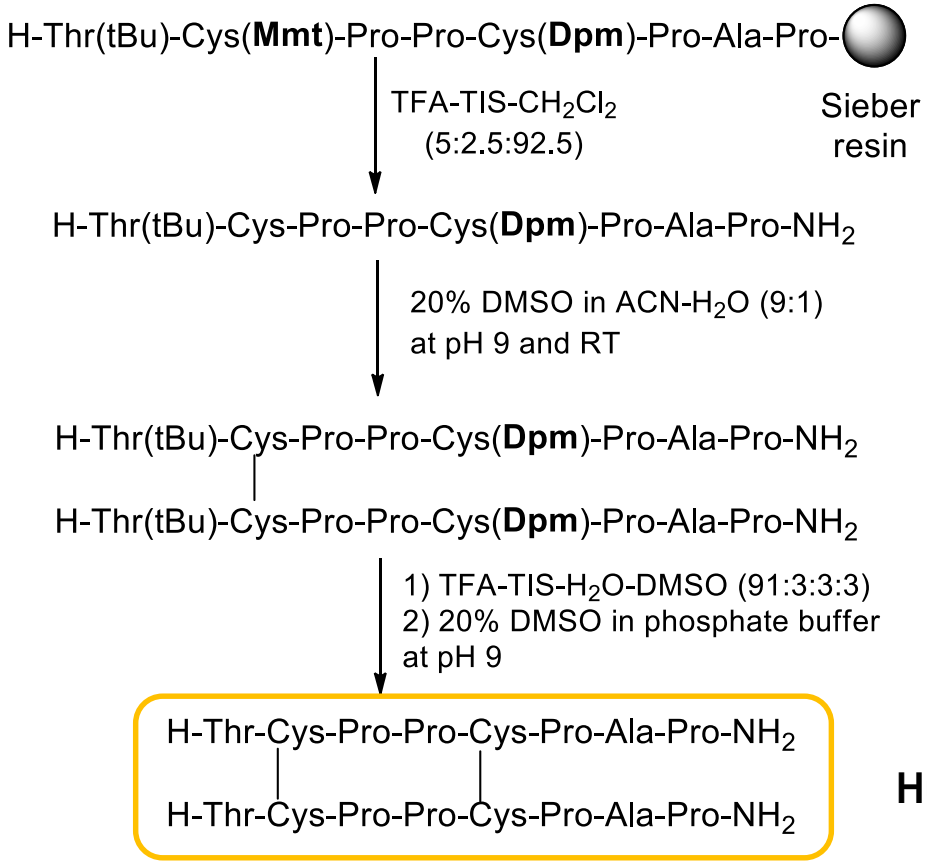


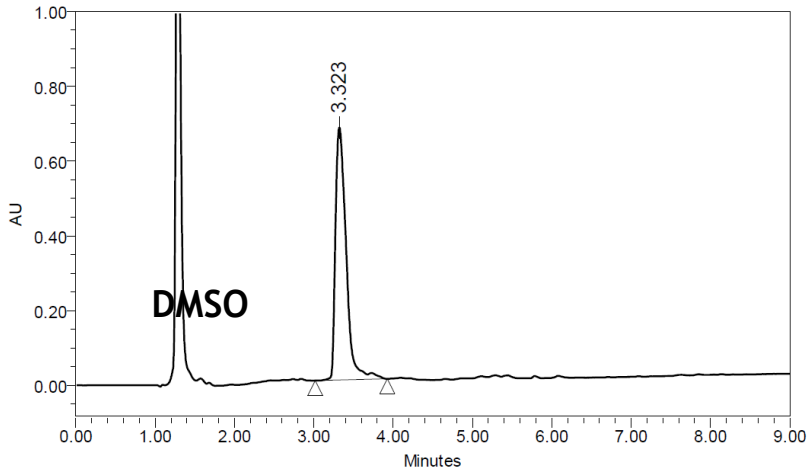


Iris Biotech

M. Góngora-Benítez, L. Mendive-Tapia, I. Ramos-Tomillero, AC. Breman, J. Tulla-Puche, F. Albericio. Org. Lett., 14, 5472 (2012)

Dpm for Cys, labile with 90% TFA



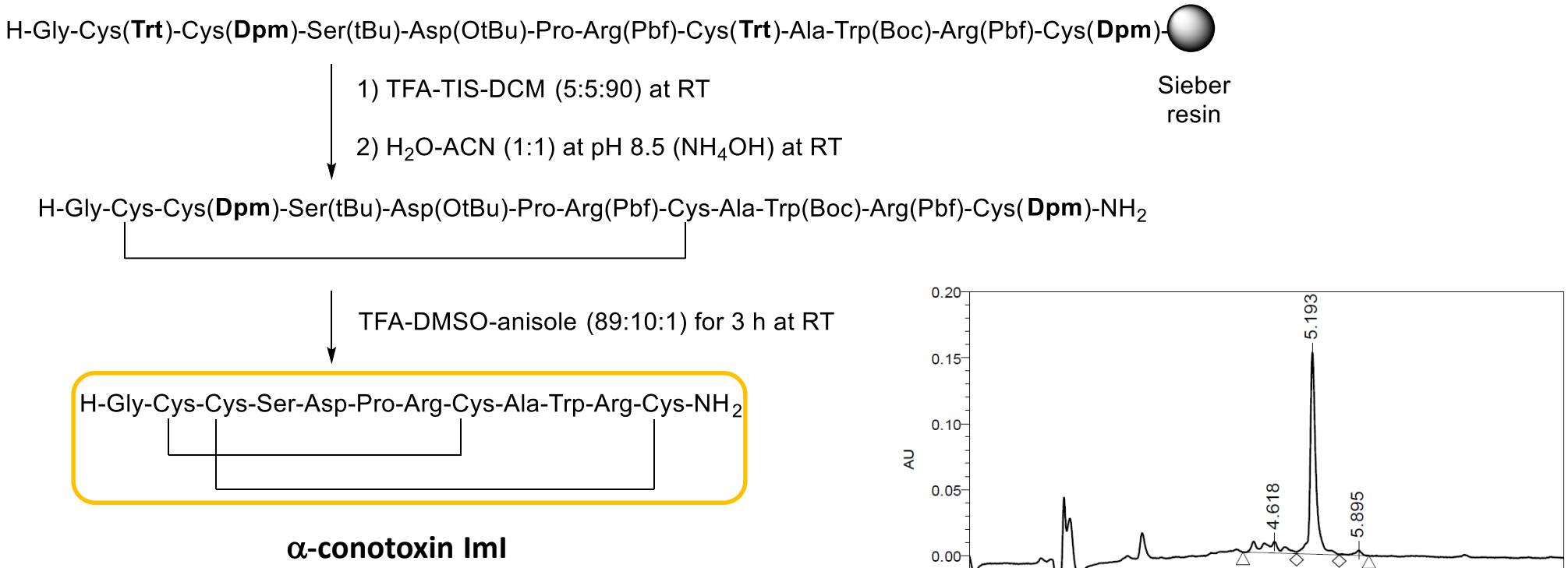


Human IgG1 hinge fragment

Iris Biotech

M. Góngora-Benítez, L. Mendive-Tapia, I. Ramos-Tomillero, AC. Breman, J. Tulla-Puche, F. Albericio. *Org. Lett.*, **14**, 5472 (2012).

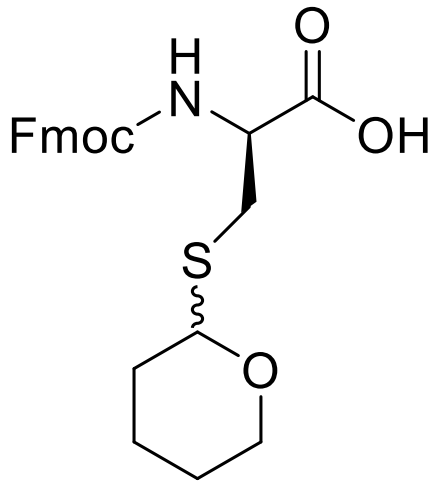
Dpm for Cys, labile with 90% TFA



α-conotoxin ImI

M. Góngora-Benítez, L. Mendive-Tapia, I. Ramos-Tomillero, AC. Breman, J. Tulla-Puche, F. Albericio. *Org. Lett.*, **14**, 5472 (2012)

THP better than Trt Cys Protecting Group?



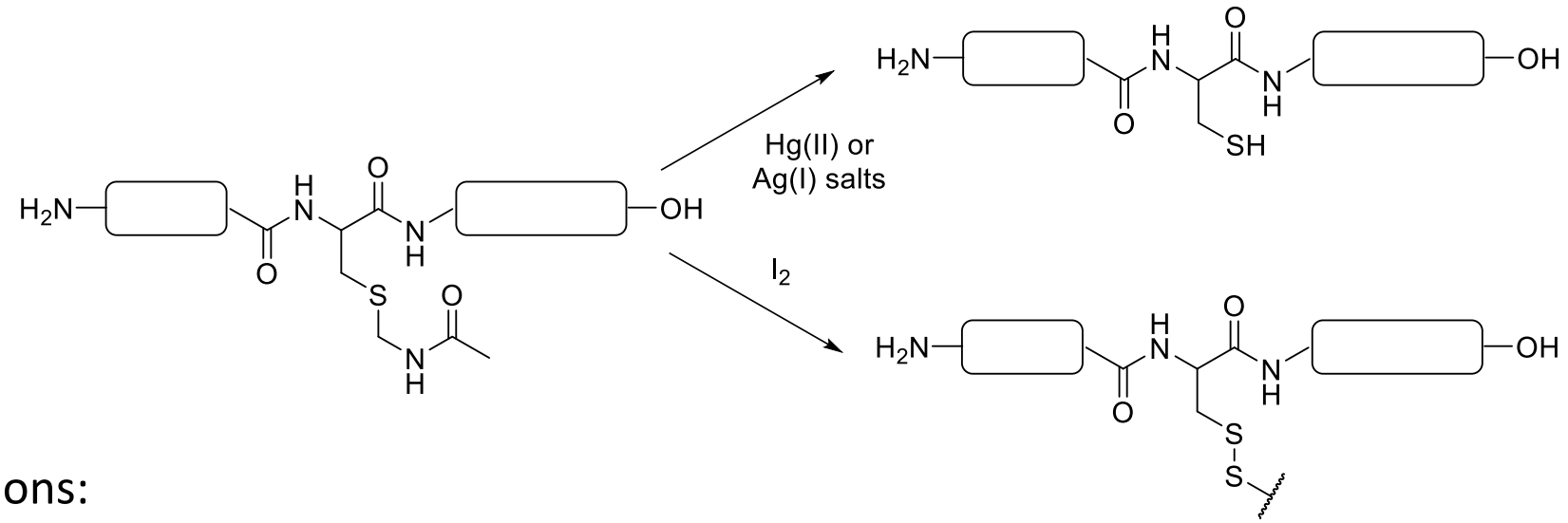
Fmoc-Cys(THP)-OH

Similar acid lability as Trt

I. Ramos-Tomillero, H. Rodriguez, F. Albericio. Org. Lett., 17, 1680- (2015).

Acm Replacement

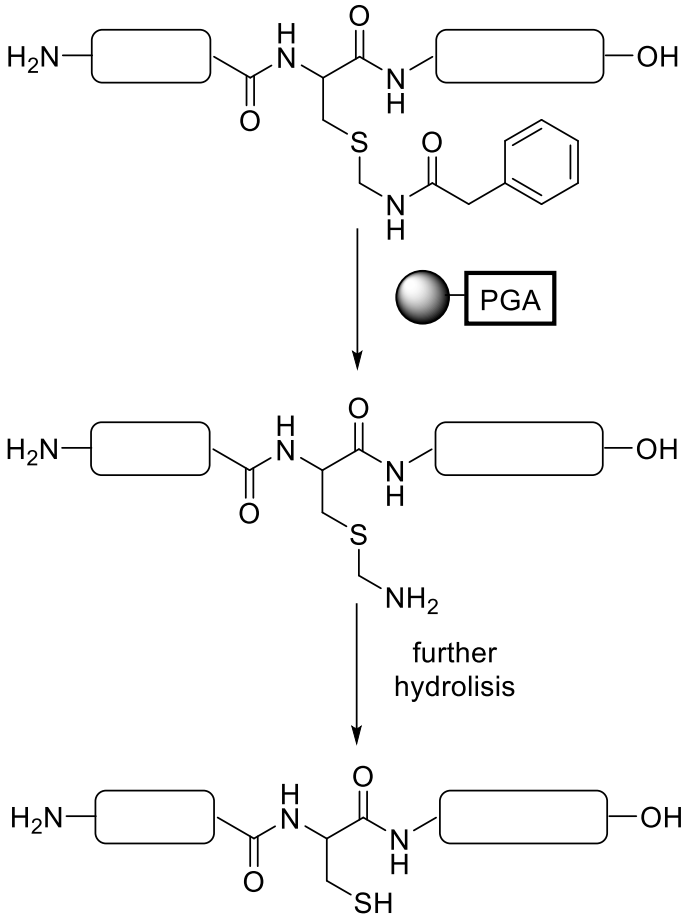
S-Acm protectin group for the synthesis of Cys-containing peptides



Side reactions:

- Hg(II) and Ag(I) are not acepted by the pharmaceutical industry
- S → N and S → O transfer of Acm group onto side chains
- Modification of sensitive residues such as Trp, Tyr or Met
- Tryptophan-2-yl sulfide as a side product
- Overoxidation

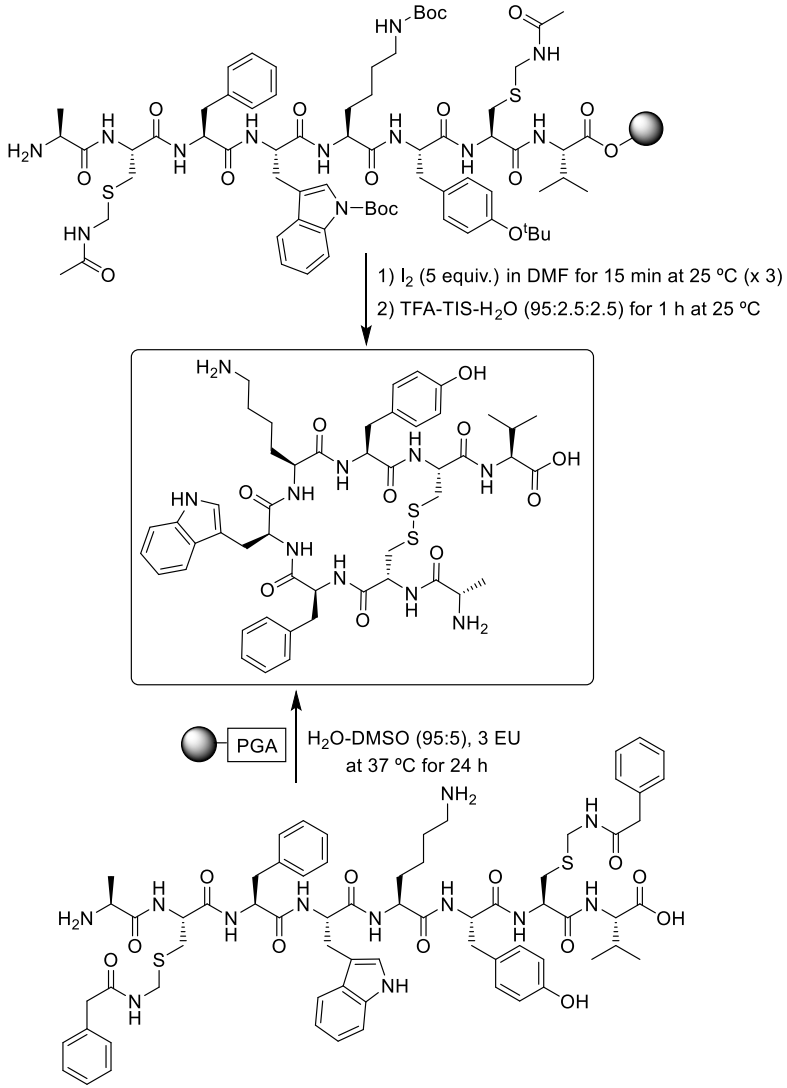
Enzyme-Labile Cys Protecting Group: S-Phacm

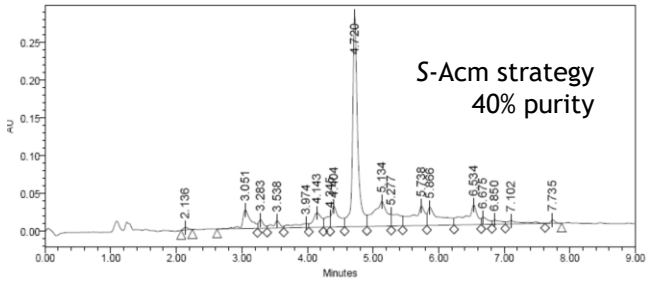


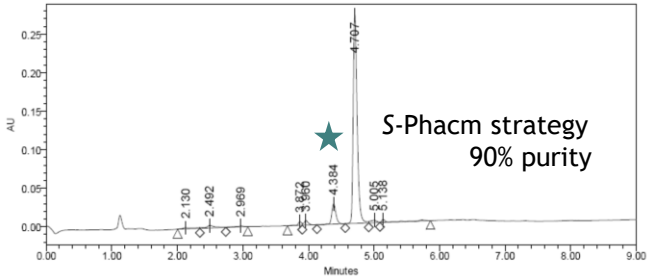
- Phenylacetamidomethyl is orthogonal to both Boc and Fmoc strategies.
- *S*-Phacm can be removed in similar conditions than Acm and, in addition, by the action of the PGA enzyme.
- Phacm can be mildly deblocked, while the resulting thioaminal is hydrolyzed to free Cys residue.

M. Royo, J. Alsina, E. Giralt, U. Slomcyznska, F. Albericio, *J. Chem. Soc. Perkin Trans. 1* 1995, 1095-1102

Enzyme-Labile Cys Protecting Group: S-Phacm







(phenylacetic acid: ★)

M. Góngora-Benítez, A. Basso, T. Bruckdorfer, M. Royo, J. Tulla-Puche, F. Albericio. Chem. Eur J., 18, 16166 (2012)

Enzyme-Labile Cys Protecting Group: S-Phacm

	reaction media	pH	totally deprot.	fully oxidized
1	H_2O	7.0	OK	-
2	H_2O/DMSO (95/5)/(80/20)	7.0	OK	OK
3	H_2O/MeCN (95/5)	7.0	OK	-
4	H_2O/MeOH (95/5)/(80/20)	7.0	OK	-
5	H_2O/2-propanol (95/5)	7.0	OK	-
6	H_2O/DMF (95/5)/(90/10)	7.0	OK	-
7	H_2O/ethylene glycol (95/5)/(80/20)	7.0	OK	-
8	H_2O/glycerol (95/5)/(80/20)	7.0	OK	-
9	H_2O/Et_2O(95/5)	7.0	OK	-
10	0.1 mM phosphate	7.8	OK	-
11	0.1 mM phosphate/DMSO (95/5)	7.8	OK	OK
12	0.1 mM phosphate	5.3	OK	-
13	0.1 mM phosphate/DMSO (95/5)	5.3	OK	OK

All experiments were performed at $8x10^{-5}$ M of URP peptide concentration and 25 mg (3 EU) Immobilised PGA, (hydrolytic activity U/g_{wet} 130) at 37 °C for 24 h.

Co-solvent and pH influence on the biocatalytic reaction

The enzymatic activity and stability remained almost intact in the presence of an variety of organic co-solvents.

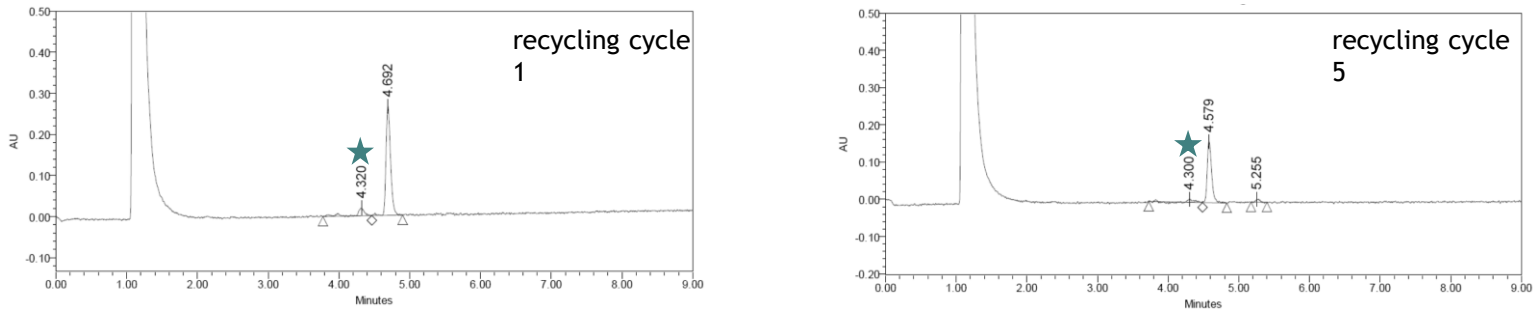
A wide range of pH is tolerated by the immobilized-PGA enzyme.

The use of DMSO as a co-solvent promotes oxidation of thiols to disulfide.

M. Góngora-Benítez, A. Basso, T. Bruckdorfer, M. Royo, J. Tulla-Puche, F. Albericio. Chem. Eur J., 18, 16166 (2012)

Enzyme-Labile Cys Protecting Group: S-Phacm

Reuse of the immobilized PGA enzyme

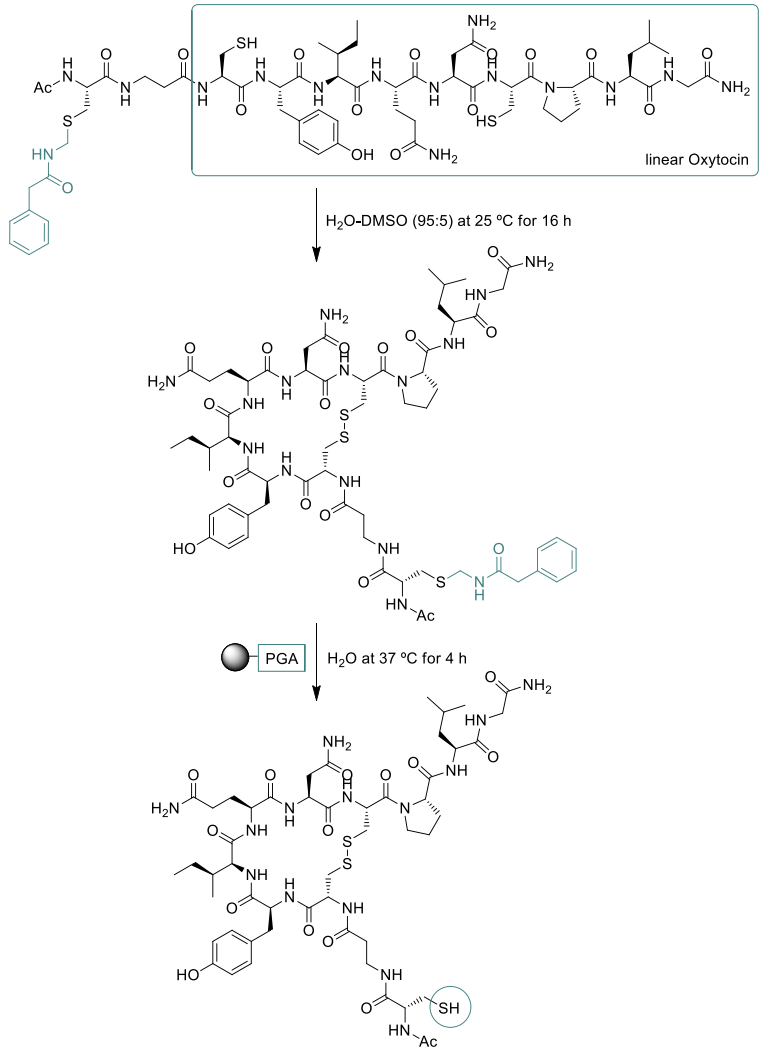


Chromatographic profiles of the oxidized URP peptide: recycling cycles 1 and 5.
(phenylacetic acid:★)

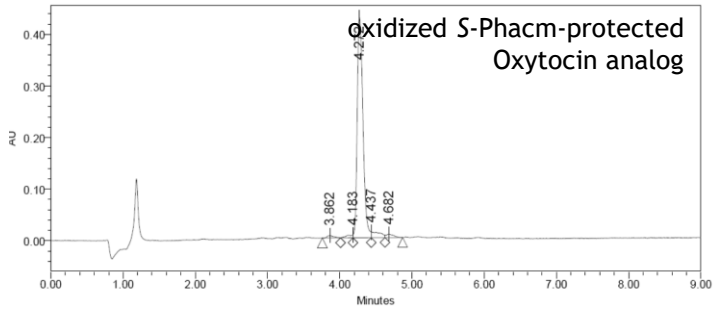
The recycling potential of immobilized PGA was evaluated by reusing the immobilized biocatalyst for the synthesis of URP for up to five cycles. At the end of each reaction cycle, immobilized enzyme was filtered from the reaction media and then washed with a phosphate buffer. After five recycling cycles, 100% enzyme activity was fully recovered.

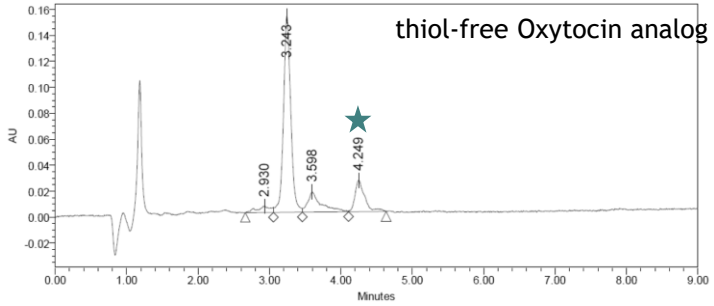
M. Góngora-Benítez, A. Basso, T. Bruckdorfer, M. Royo, J. Tulla-Puche, F. Albericio. Chem. Eur J., 18, 16166 (2012)

Enzyme-Labile Cys Protecting Group: S-Phacm



Compatibility of *S*-Phacm removal with S-S



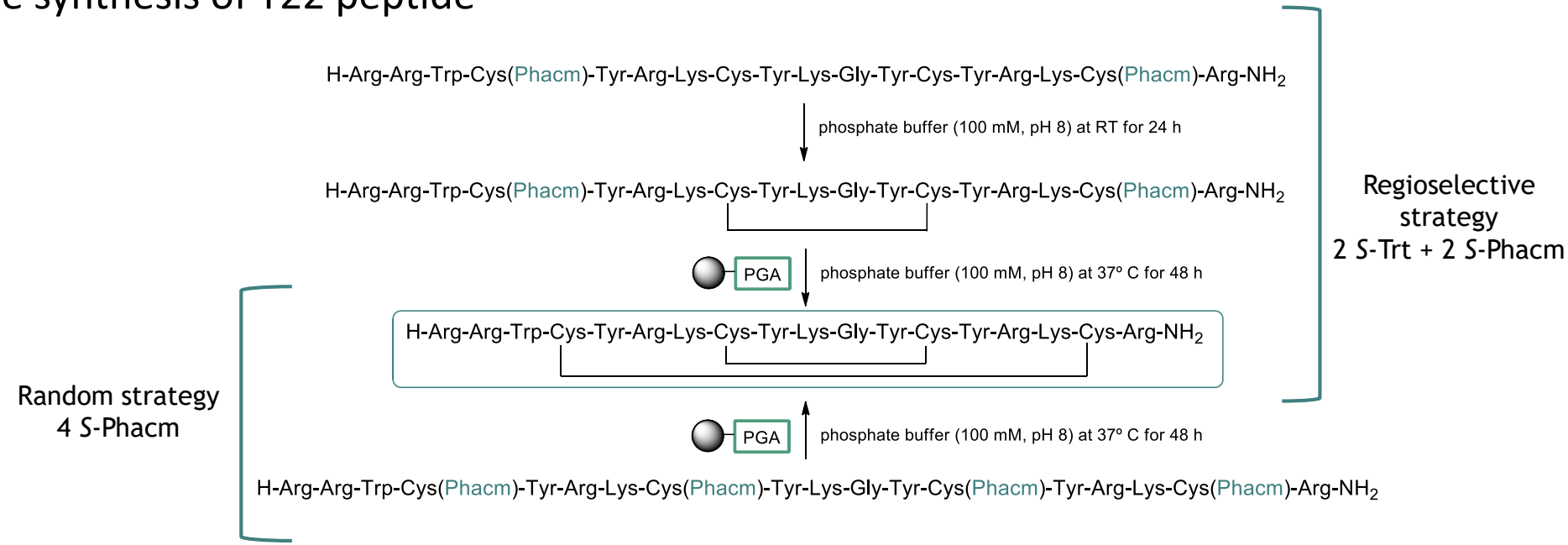


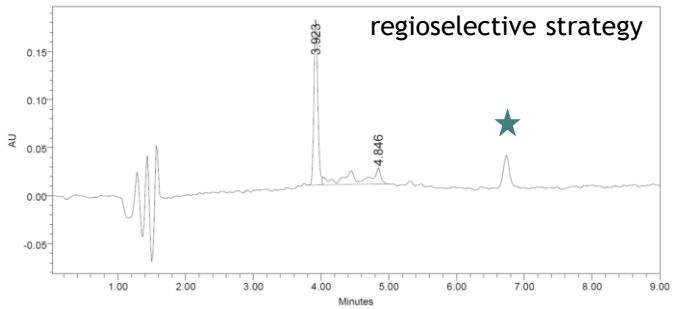
(phenylacetic acid: ★)

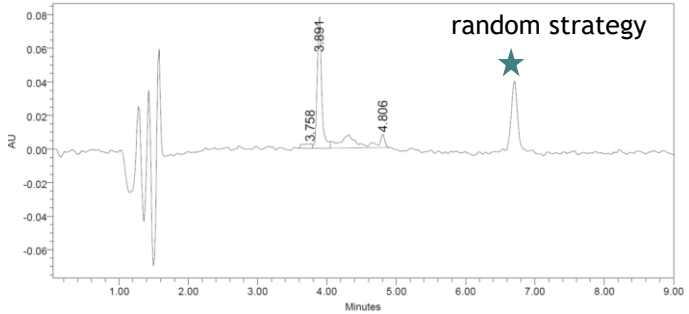
M. Góngora-Benítez, A. Basso, T. Bruckdorfer, M. Royo, J. Tulla-Puche, F. Albericio. Chem. Eur J., 18, 16166 (2012)

Enzyme-Labile Cys Protecting Group: S-Phacm

S-Phacm for the synthesis of T22 peptide



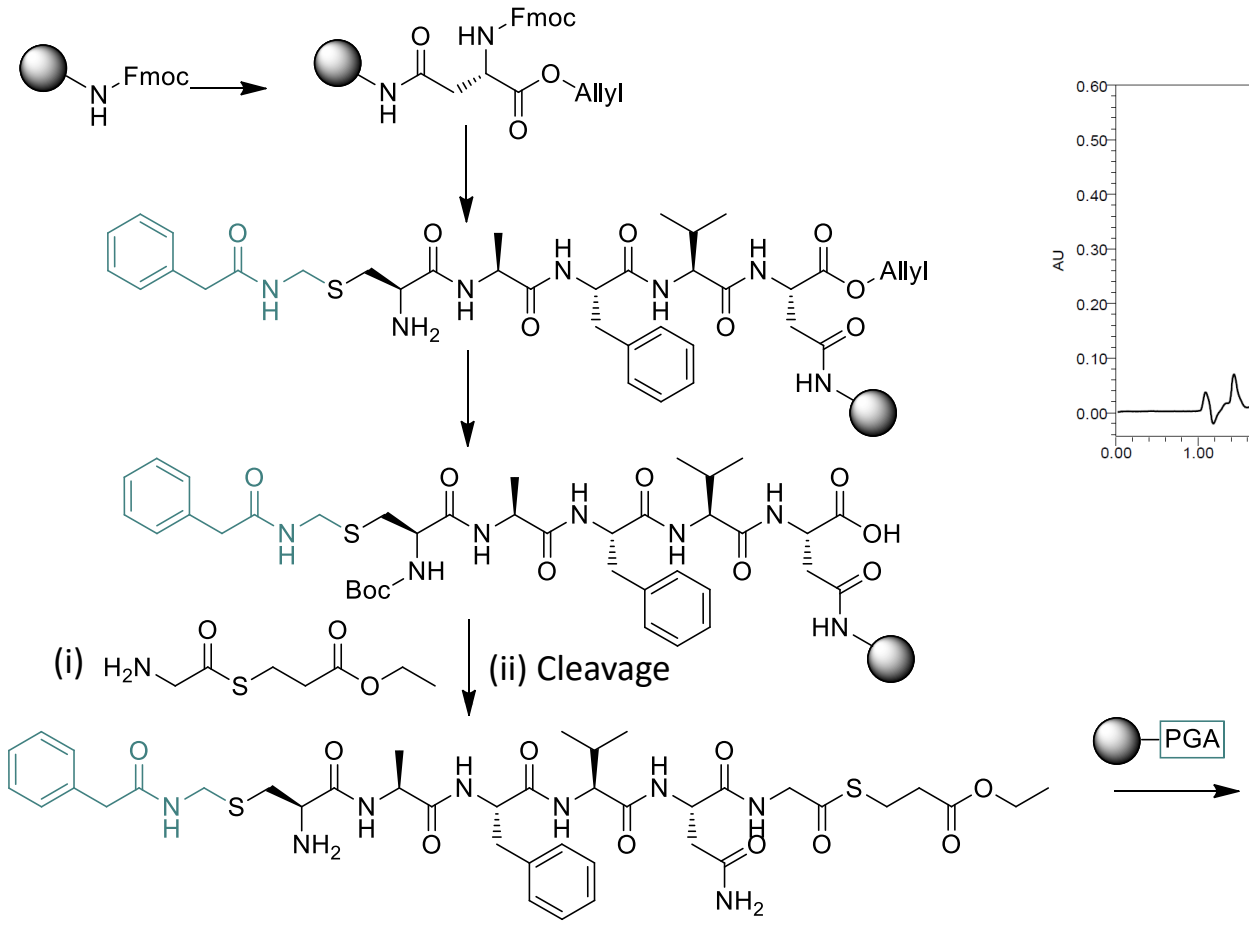


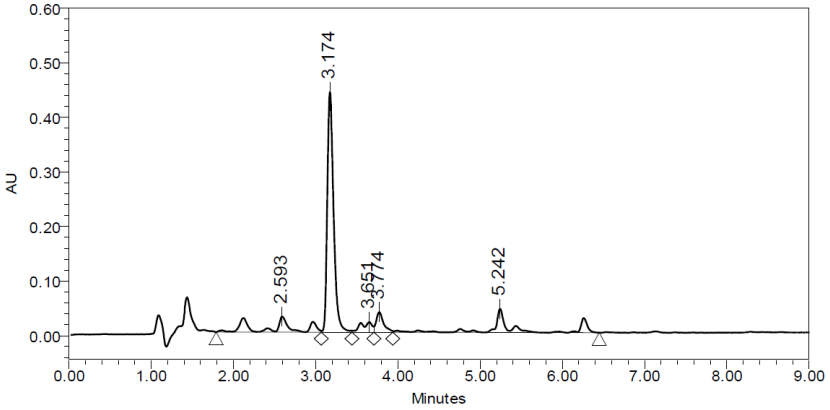


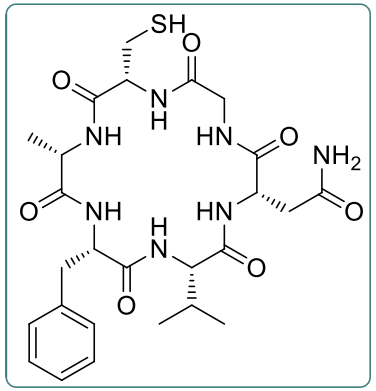
Chromatographic profile of the final bicyclic T22 peptide (phenylacetic acid: ★)

Enzyme-Labile Cys Protecting Group: S-Phacm

S-Phacm for Native Chemical Ligation

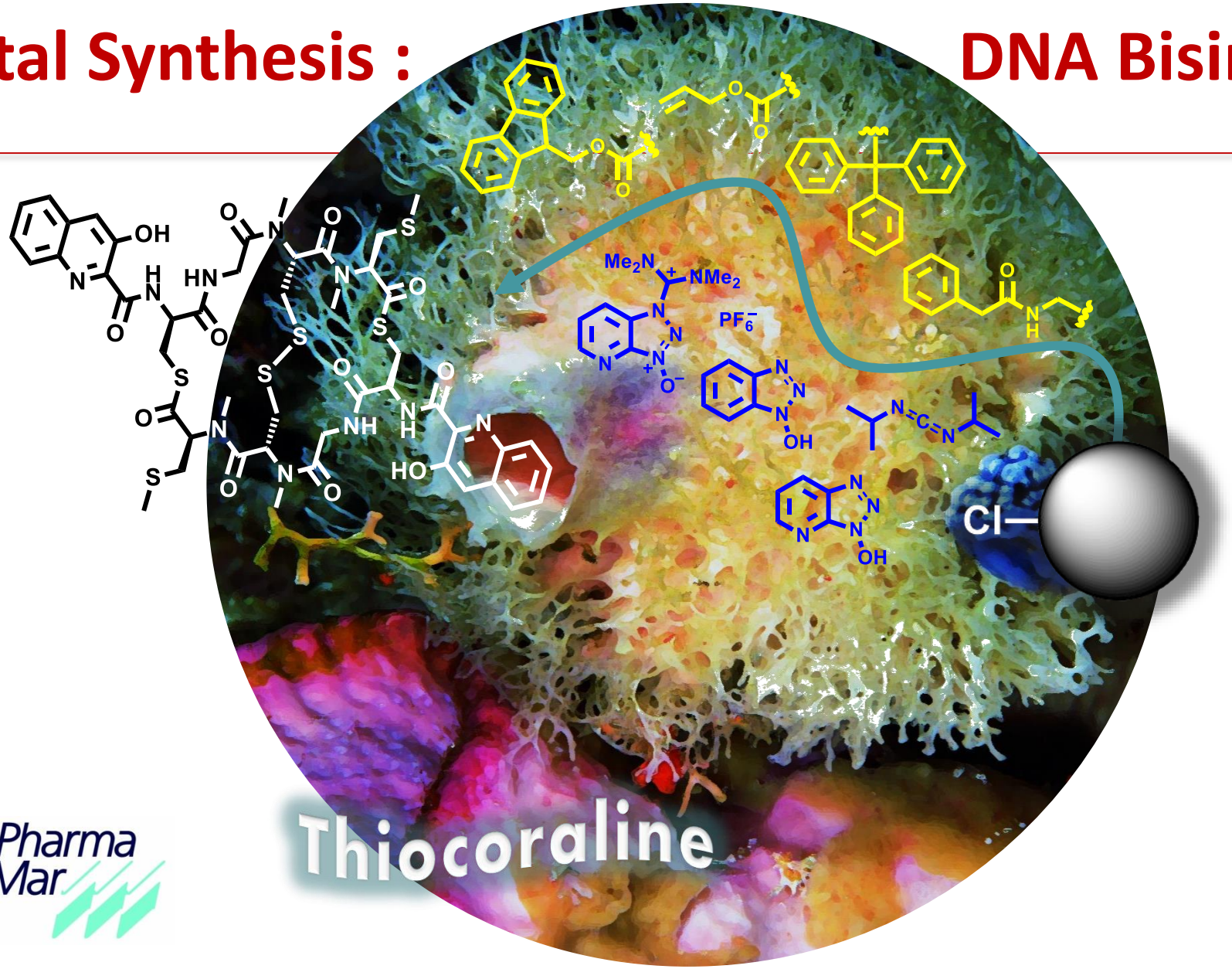






M. Góngora-Benítez, A. Basso, T. Bruckdorfer, M. Royo, J. Tulla-Puche, F. Albericio. Chem. Eur J., 18, 16166 (2012)

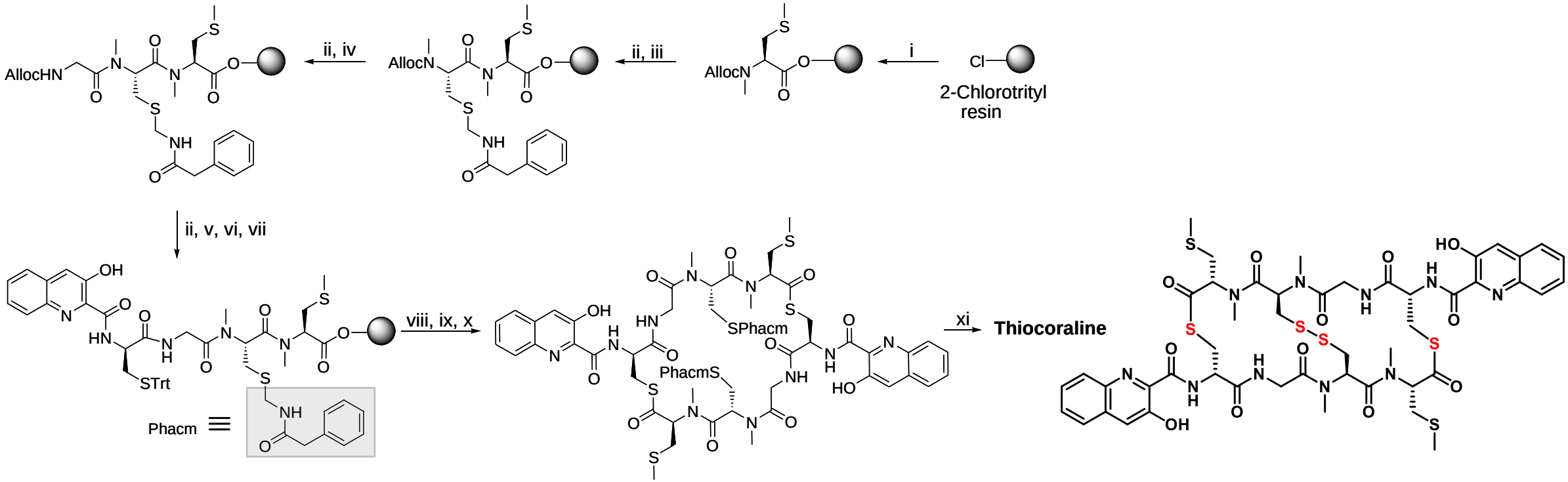
Total Synthesis : DNA Bisintercalators



Pharma Mar

J. Tulla-Puche, M. Góngora-Benítez, N. Bayó-Puxan, A. Francesch, C. Cuevas, F. Albericio. *Angew. Chemie,* **52,** 5726-5730 (2013)

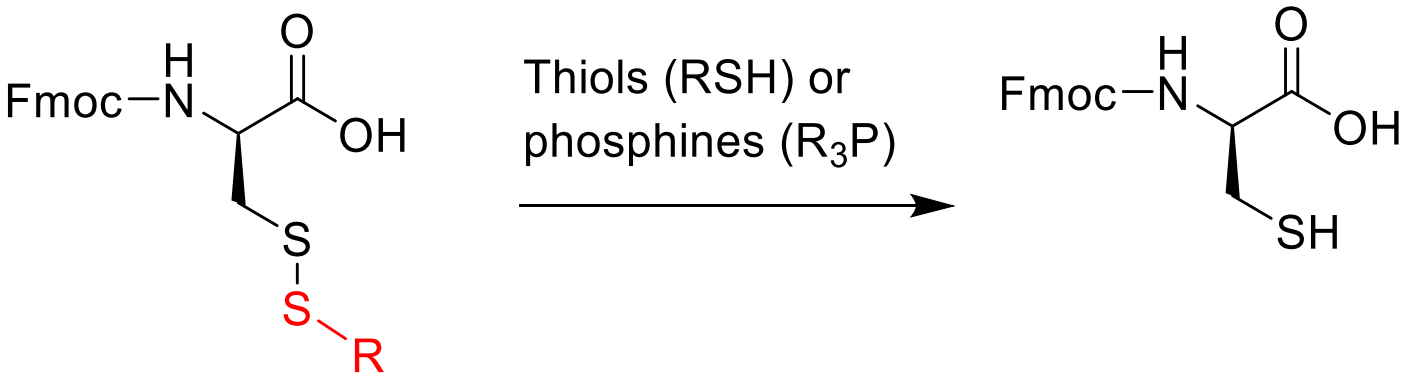
S-Phacm for the Synthesis of Thiocoraline



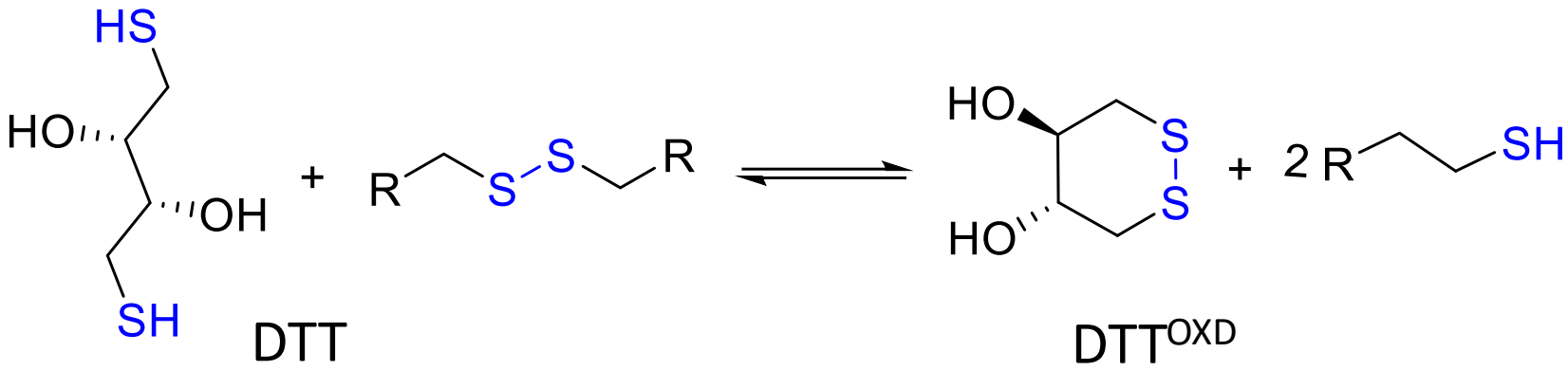
(i) Alloc-*N*Me-Cys(Me)-OH, DIEA, CH_2Cl_2; MeOH; (ii) $Pd(PPh_3)_4$, $PhSiH_3$, CH_2Cl_2; (iii) Alloc-*N*Me-Cys(Phacm)-OH, HATU, HOAt, DIEA, DMF; (iv) Alloc-Gly-OH, HATU, HOAt, DIEA, DMF; (v) Fmoc-D-Cys(Trt)-OH, HATU, HOAt, DIEA, DMF; (vi) piperidine-DMF (1:4); (vii) 3-hydroxyquinaldic acid, DIPCDI, HOBt, CH_2Cl_2; (viii) TFA-Me_2S-TIS-CH_2Cl_2 (2:0.5:0.5:97); (ix) TFA-TIS-CH_2Cl_2 (10:2.5:87.5); (x) DIPCDI, HOAt, DMF-CH_2Cl_2; (xi) immobilized PGA, H_2O-DMSO (9:1), pH 6.7.

J. Tulla-Puche, M. Góngora-Benítez, N. Bayó-Puxan, A. Francesch, C. Cuevas, F. Albericio. *Angew. Chemie,* **52,** 5726-5730 (2013)

Cys Protecting Groups Labile by Reducing Agents



Reducing Agents, DABDT as Substitution for DTT



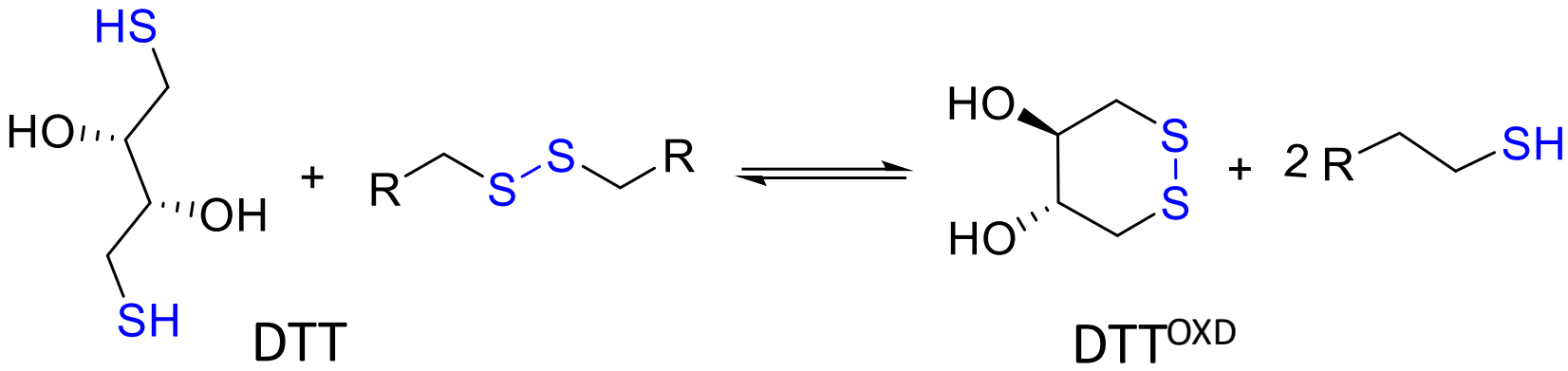
DTT DTT^{OXD}

- Complete reduction of disulfides
- Absence of the mixed disulfide intermediates
- Solid form
- High efficiency

- Odorous
- Expensive
- Very prone to air oxidation
- Non-functional at pH below 7

The stability of the formed cyclic six-membered oxidized product is the driving force to shift the equilibrium of the reaction

Reducing Agents, DABDT as Substitution for DTT



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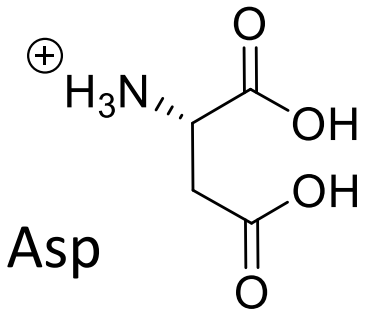
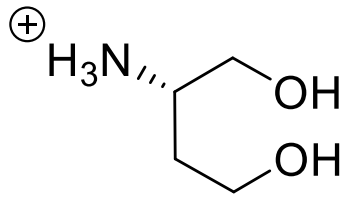


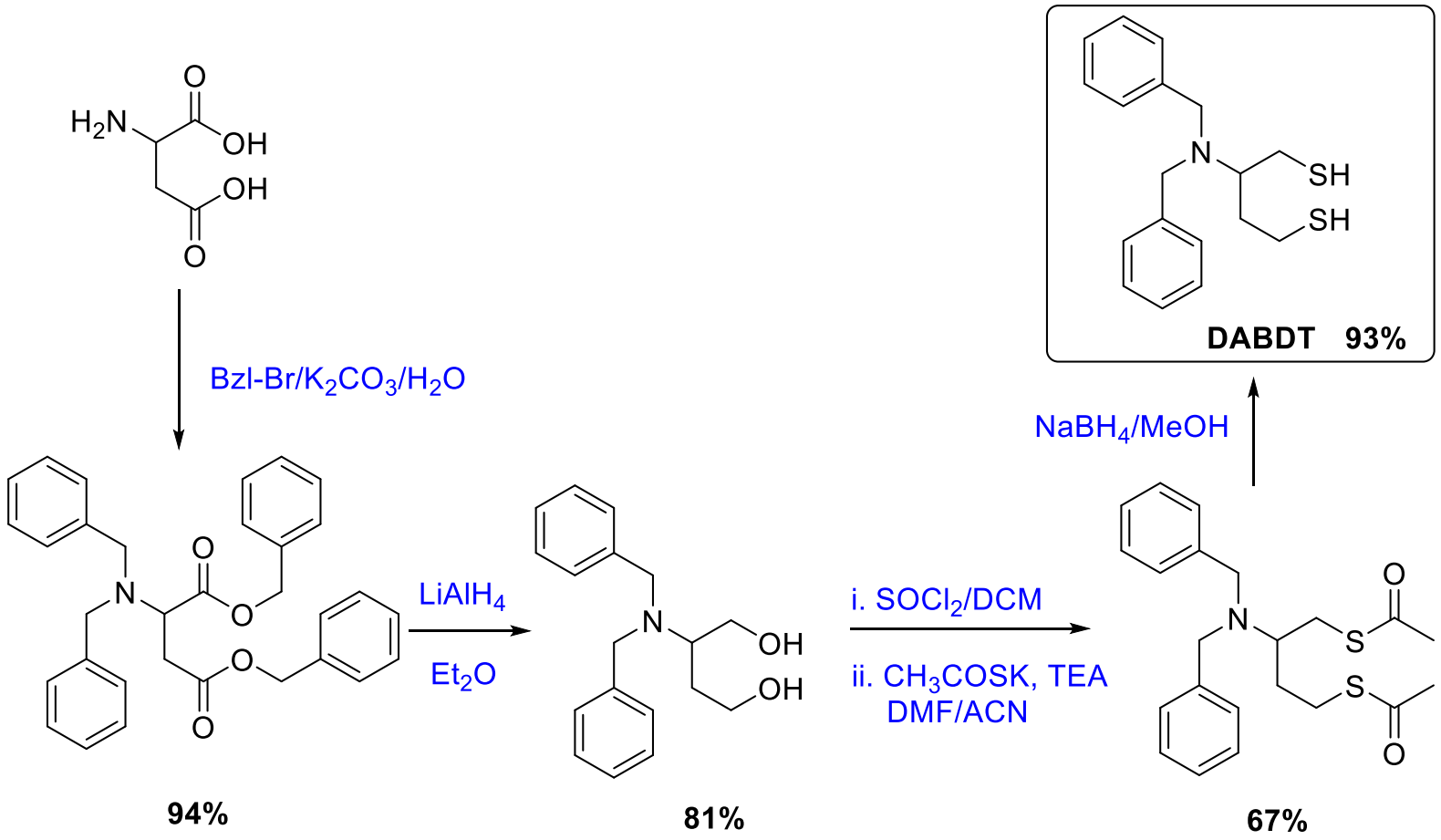
Asp



DTBA

JC. Lukesh III, MJ. Palte, RT Raines. J. Am. Chem. Soc., 134, 4057 (2012)

Reducing Agents, DABDT as Substitution for DTT



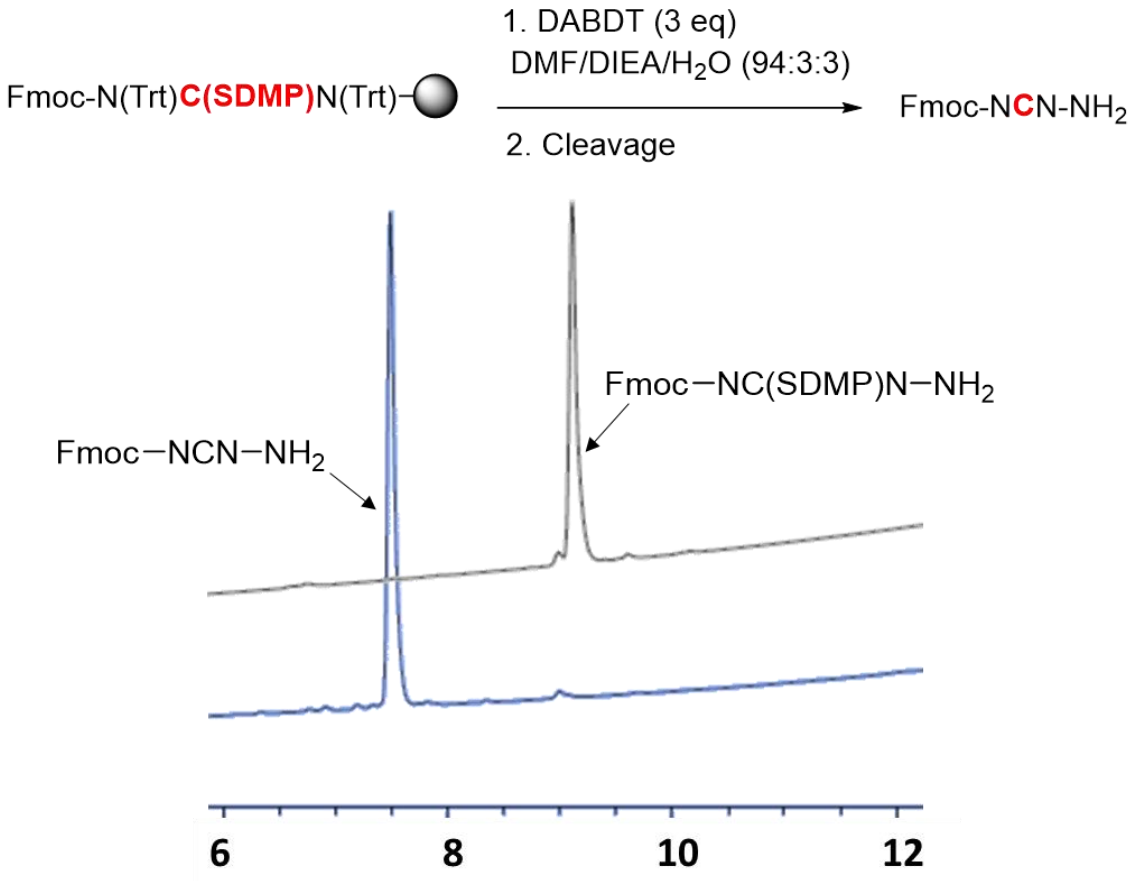
- Non-malodorous
- High efficiency
- Not readily oxidized by air
- Soluble in a wide range of solvents:

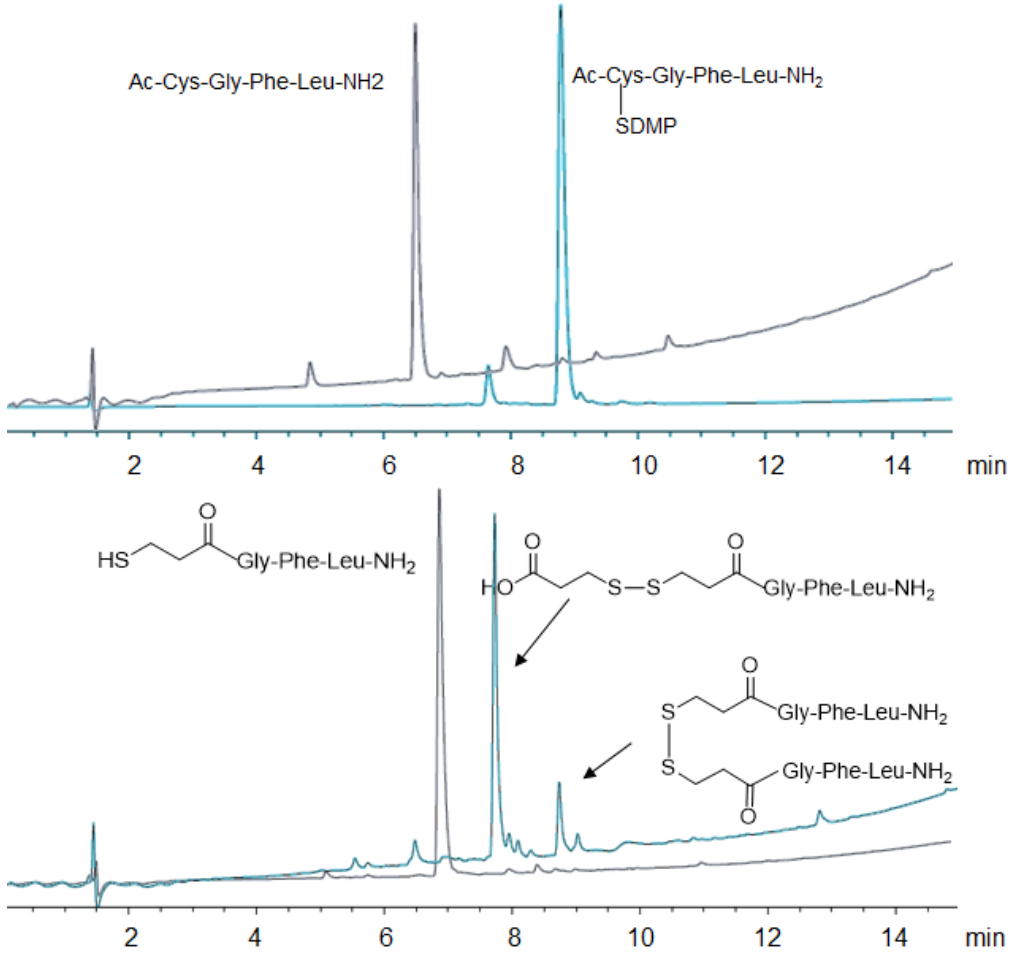
ACN,DMF, DCM, toluene, dioxane, THF, MeOH

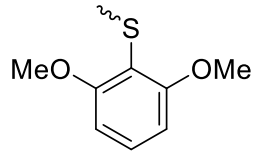
SN. Mthembu, A. Sharma, F. Albericio, BG. de la Torre, Org. Lett., 21, 10111 (2019)

Reducing Agents, DABDT as Substitution for DTT

On solid-phase reduction using DABDT in DIEA-H_2O-ACN (3:3:94), 2x5 min



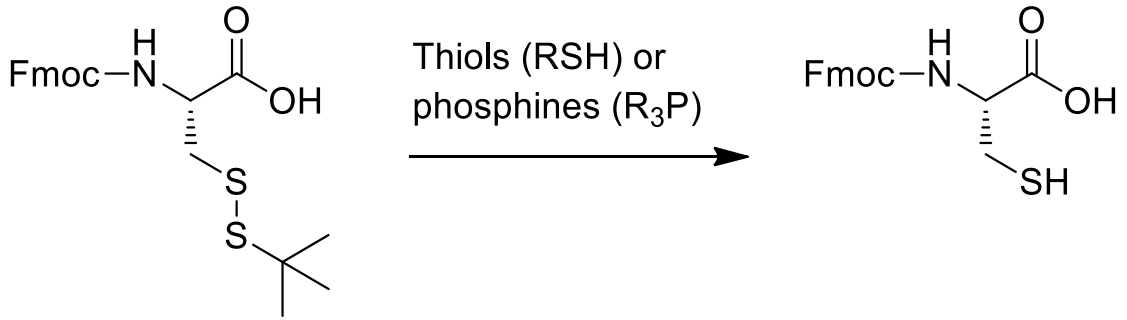




DMP

SN. Mthembu, A. Sharma, F. Albericio, BG. de la Torre, Org. Lett., 21, 10111 (2019)

S-tBu, a Cys Protecting Group Labile by Reducing Agents

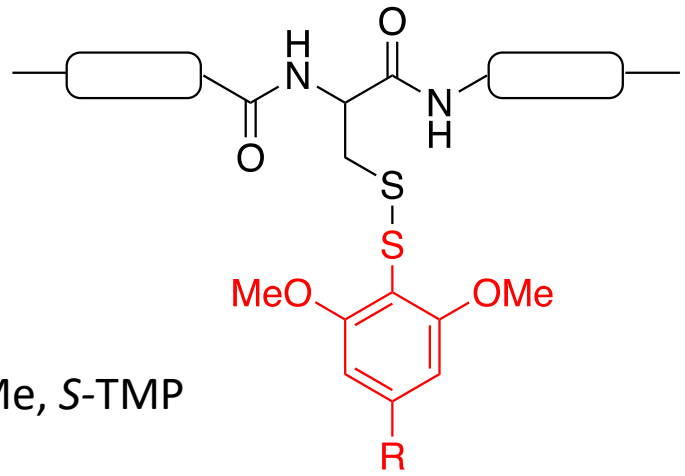


- S-*tert*-butylmercapto (S-^{t}Bu) is a commercially available thiol labile Cys protetcing group (PG)
- Compatible with Fmoc/^{t}Bu chemistry
- "Labile" to reducing agents but long removal times (6-24h) and very often it is stable
- Its lability is sequence dependent
- Novel, increasingly thiol labile Cys PGs would allow utilization of thiol labile PGs in routine peptide synthesis

TB. Postma, F, Giraud, F. Albericio. *Org. Lett.,* 14, 5468 (2012)

A. Chakraborty, A. Sharma, F. Albericio, BG. de la Torre. Org. Lett., 22, 9644 (2020)

S-DMP & S-TMP for Cys, labile by Reducing Agents



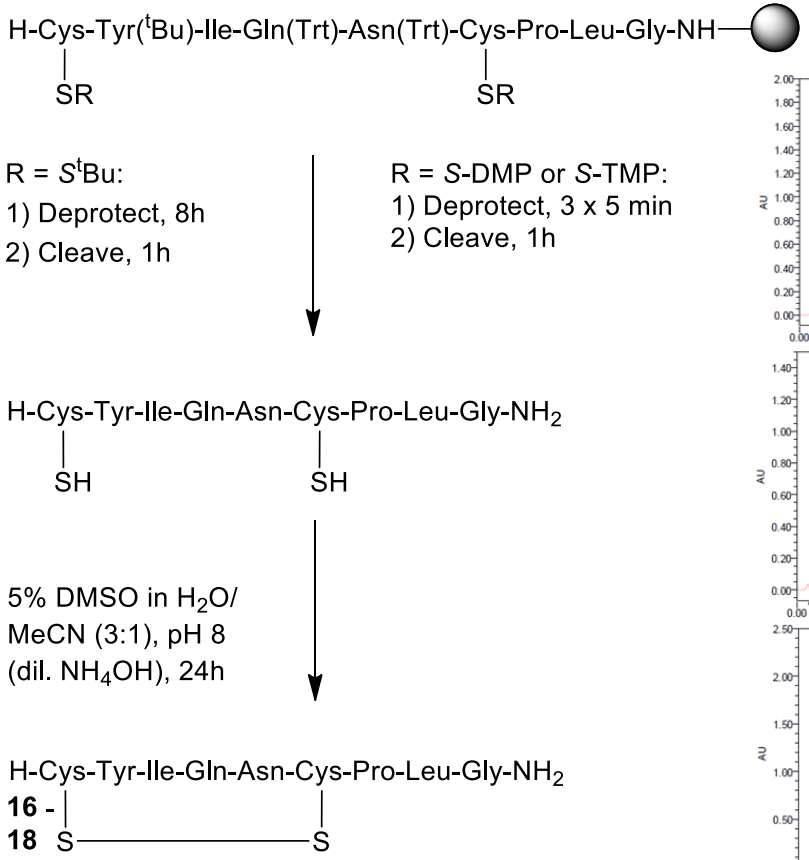
R = H, *S*-DMP; R + OMe, *S*-TMP

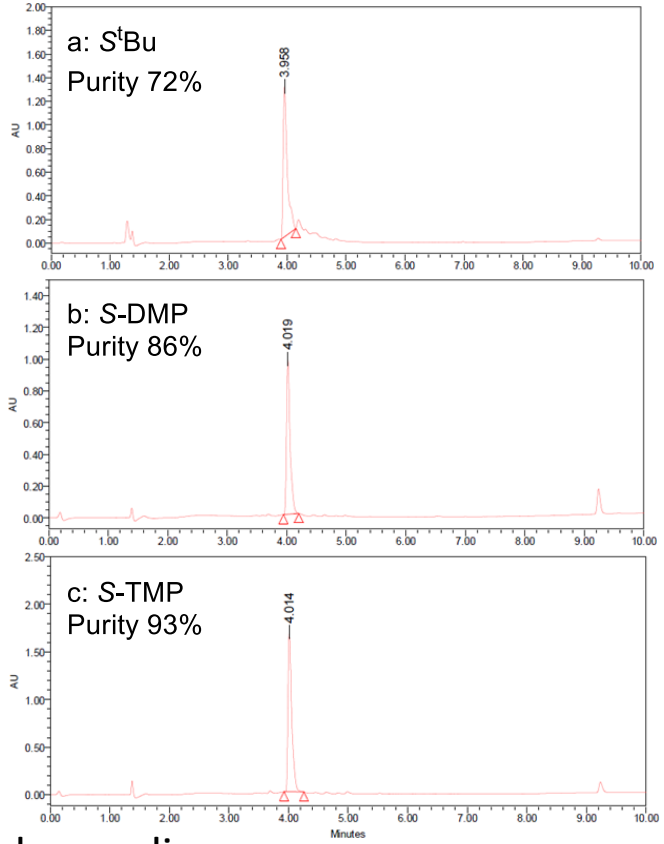
- The protecting group was found stable to base for 4h (20% piperidine in DMF)
- S-DMP was quantitatively removed by 20% β-mercaptoethanol/ 0.1 M NMM in DMF (3X5 min) *vs* 3h for S-^{t}Bu or by 5% DTT/ 0.1 M NMM in DMF (3x5 min)

TMM. Postma, F, Giraud, F. Albericio. *Org. Lett.,* 14, 5468 (2012)

S-DMP & S-TMP for Cys, labile by Reducing Agents

Oxytocin





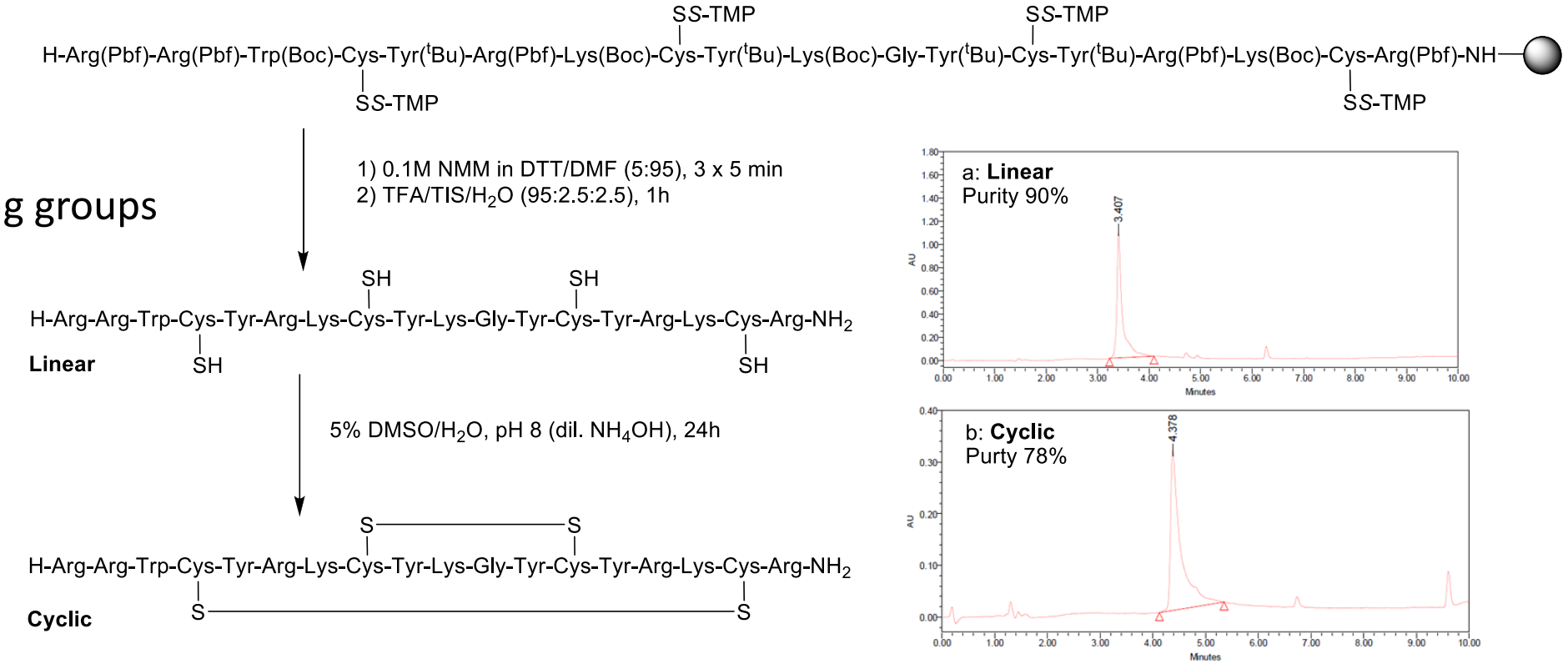
- S-DMP Synthesis is tedious and demanding
- Lack of full stability in front of 20% piperidine in DMF

T. Postma, M. Giraud, F. Albericio, *Org. Lett.*, 14, 5468-5471 (2012)

S-DMP & S-TMP for Cys, labile by Reducing Agents

T22

4 *S*-TMP protecting groups



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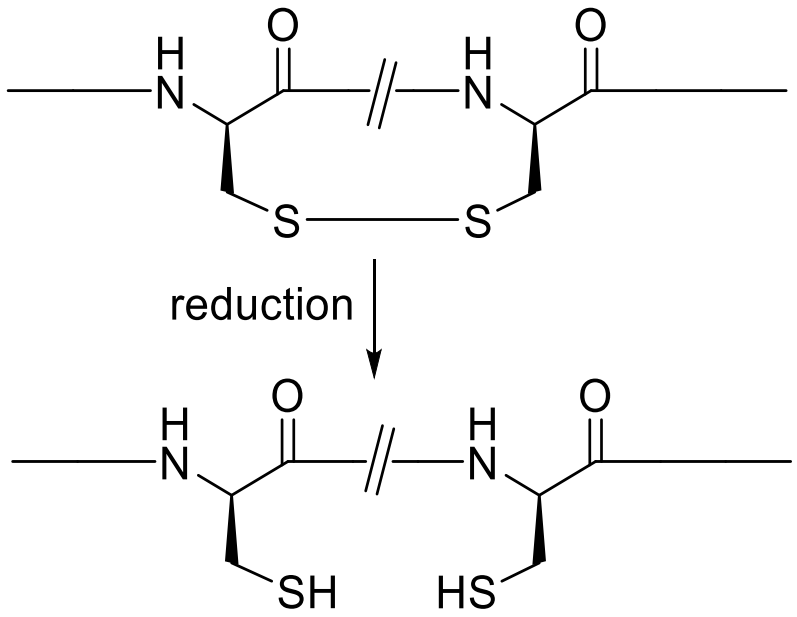
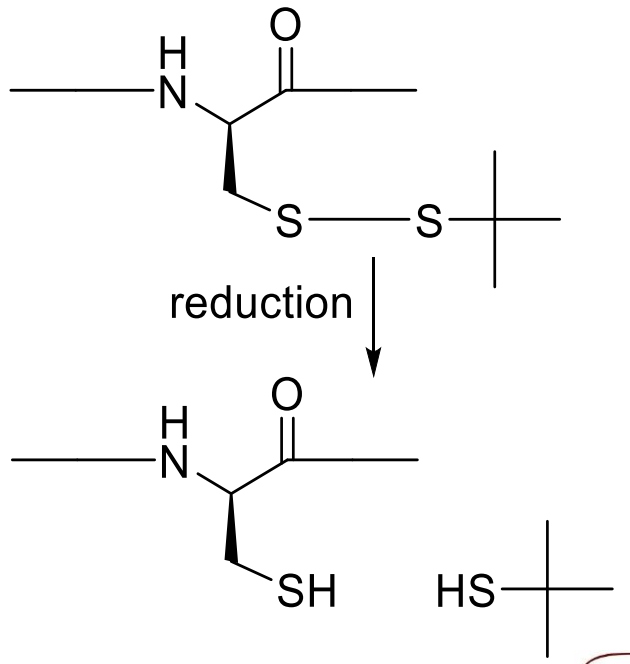
Rationale on a New PG for Cys, labile by Reducing Agents





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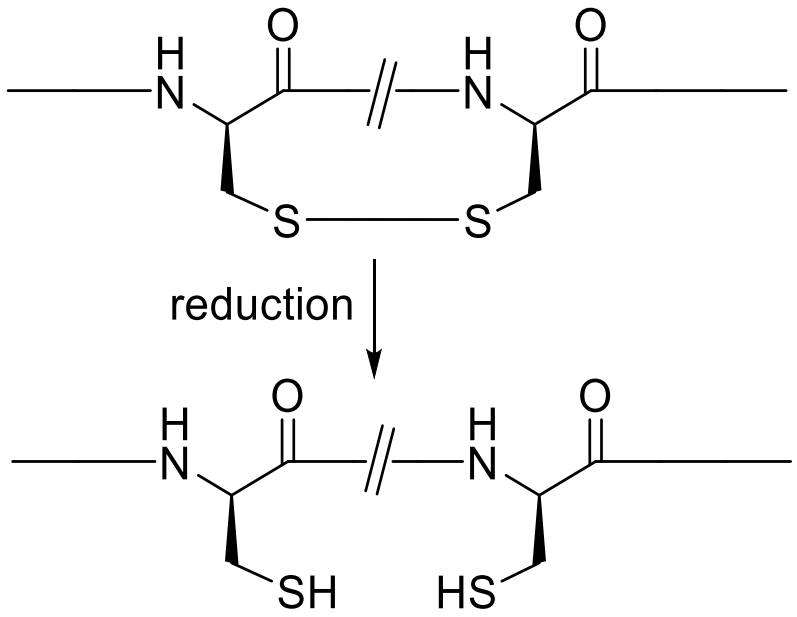

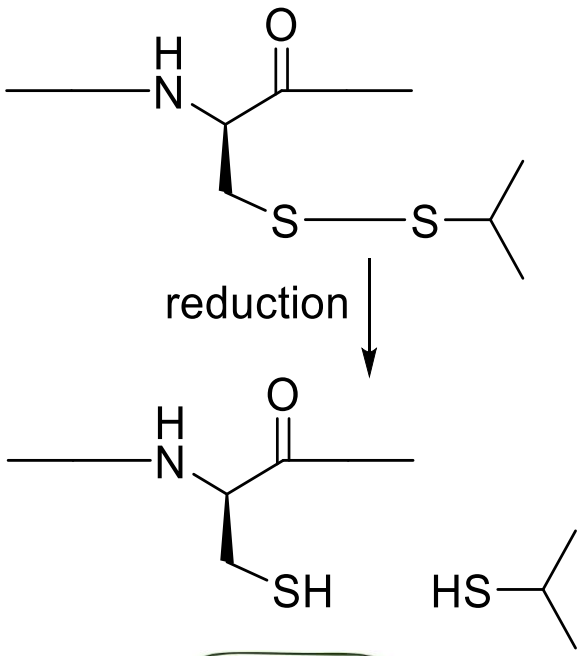

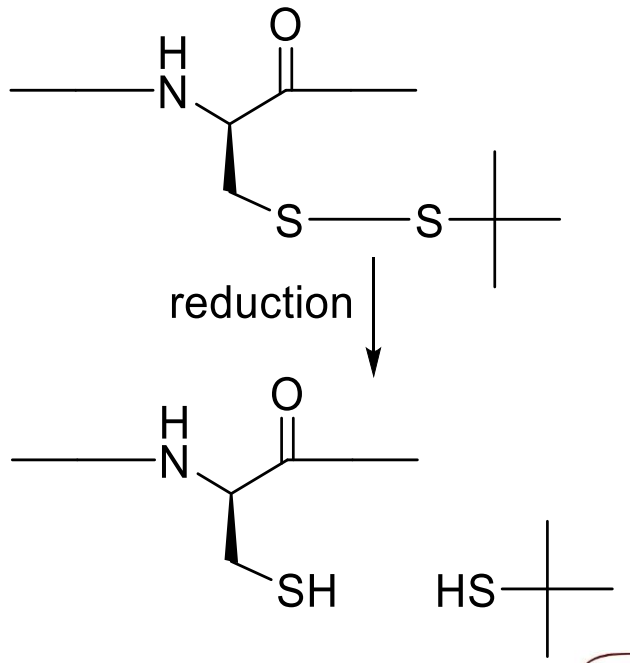

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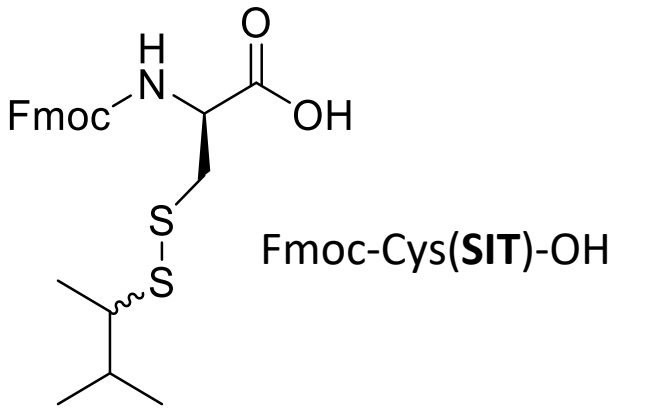
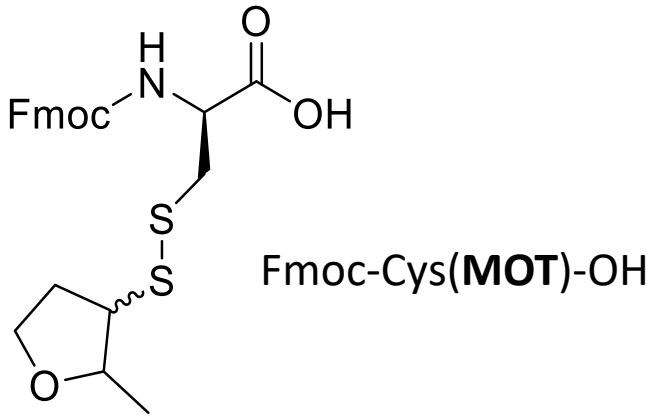
SIT & MOT New PGs for Cys, labile by Reducing Agents



Fmoc-Cys(**SIT**)-OH



Fmoc-Cys(**MOT**)-OH

Iris Biotech

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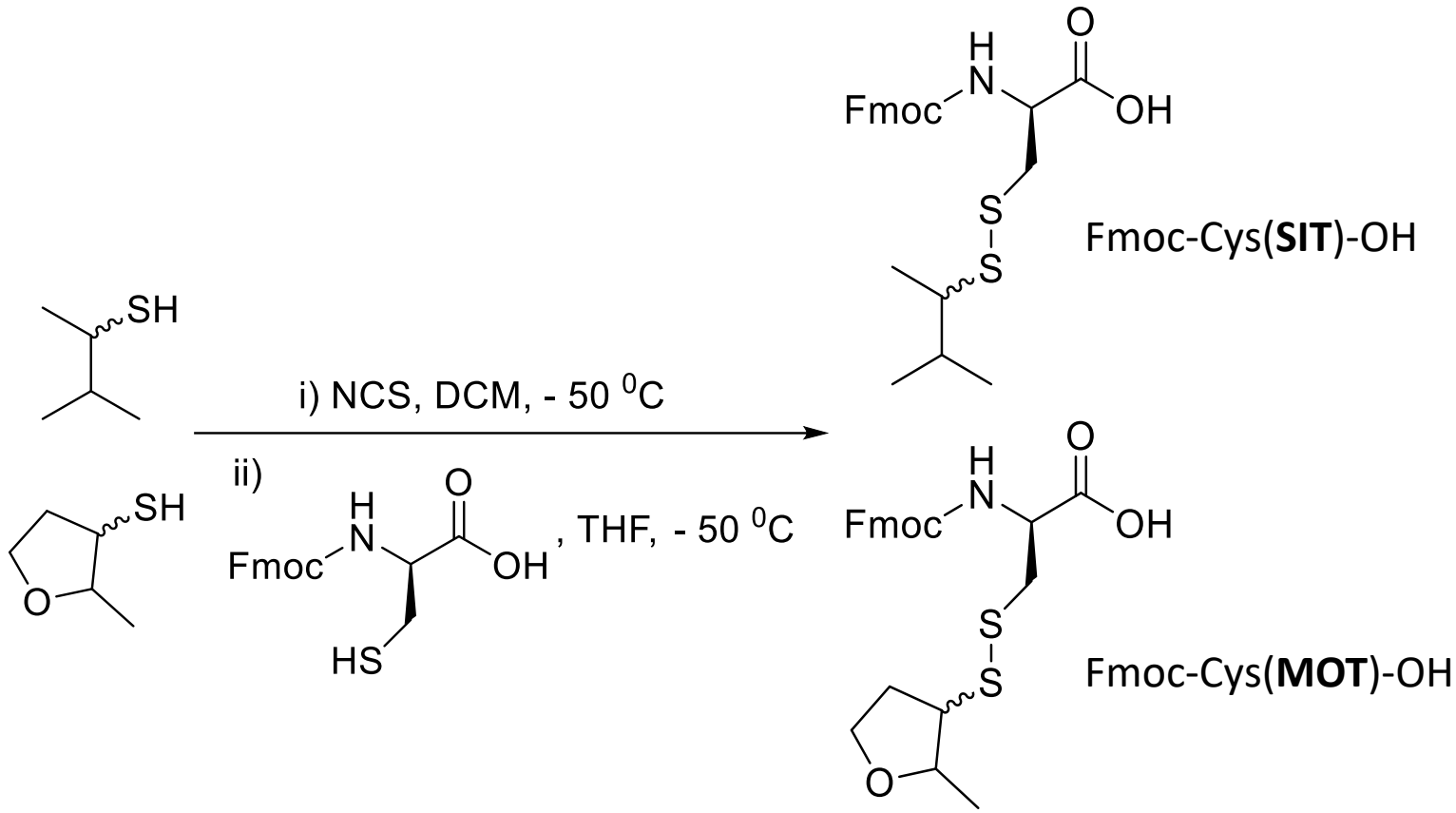

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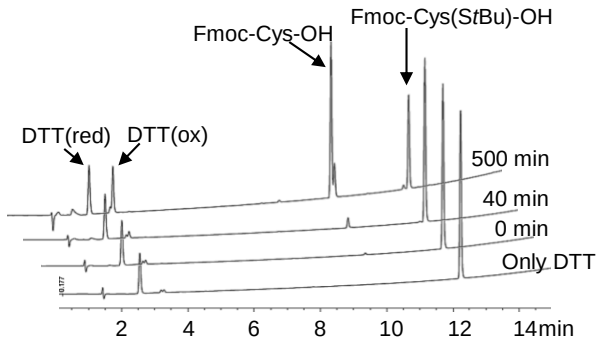
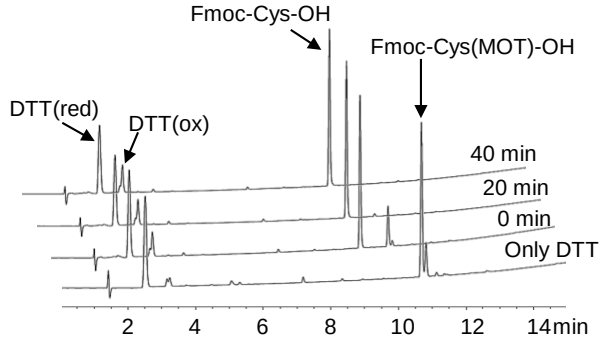
SIT & MOT New PGs for Cys, labile by Reducing Agents

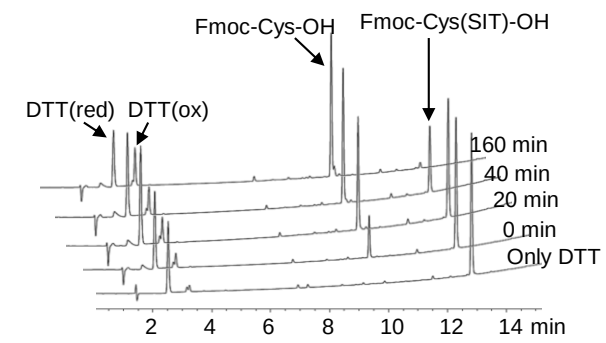
Boc-Cys(**PG**)-Gly-Phe-Leu-RinkAmide- 

Fmoc-Cys(S*t*Bu)-OH | Fmoc-Cys(MOT)-OH | Fmoc-Cys(SIT)-OH

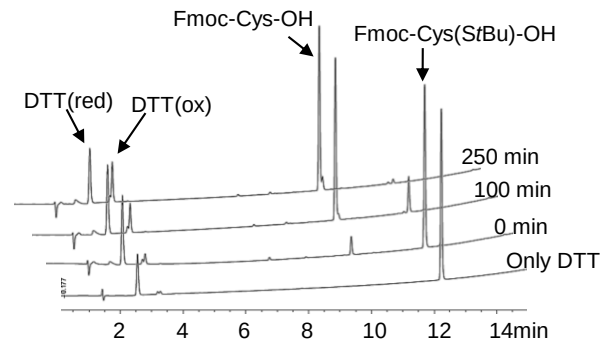
Reduction in presence of DTT + DIEA

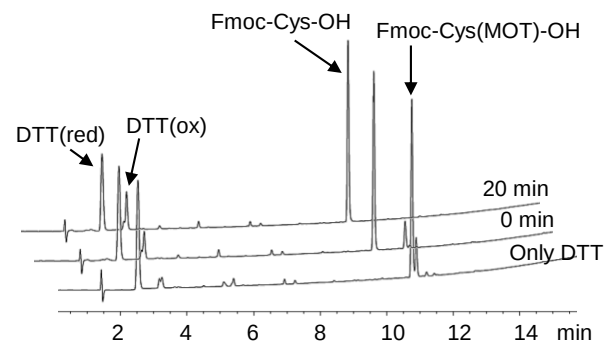


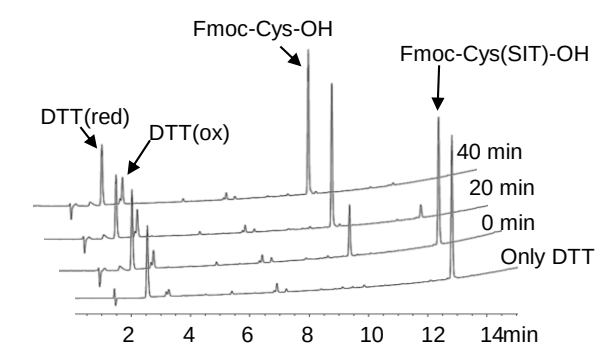




Reduction in presence of DTT + DIEA + H_2O







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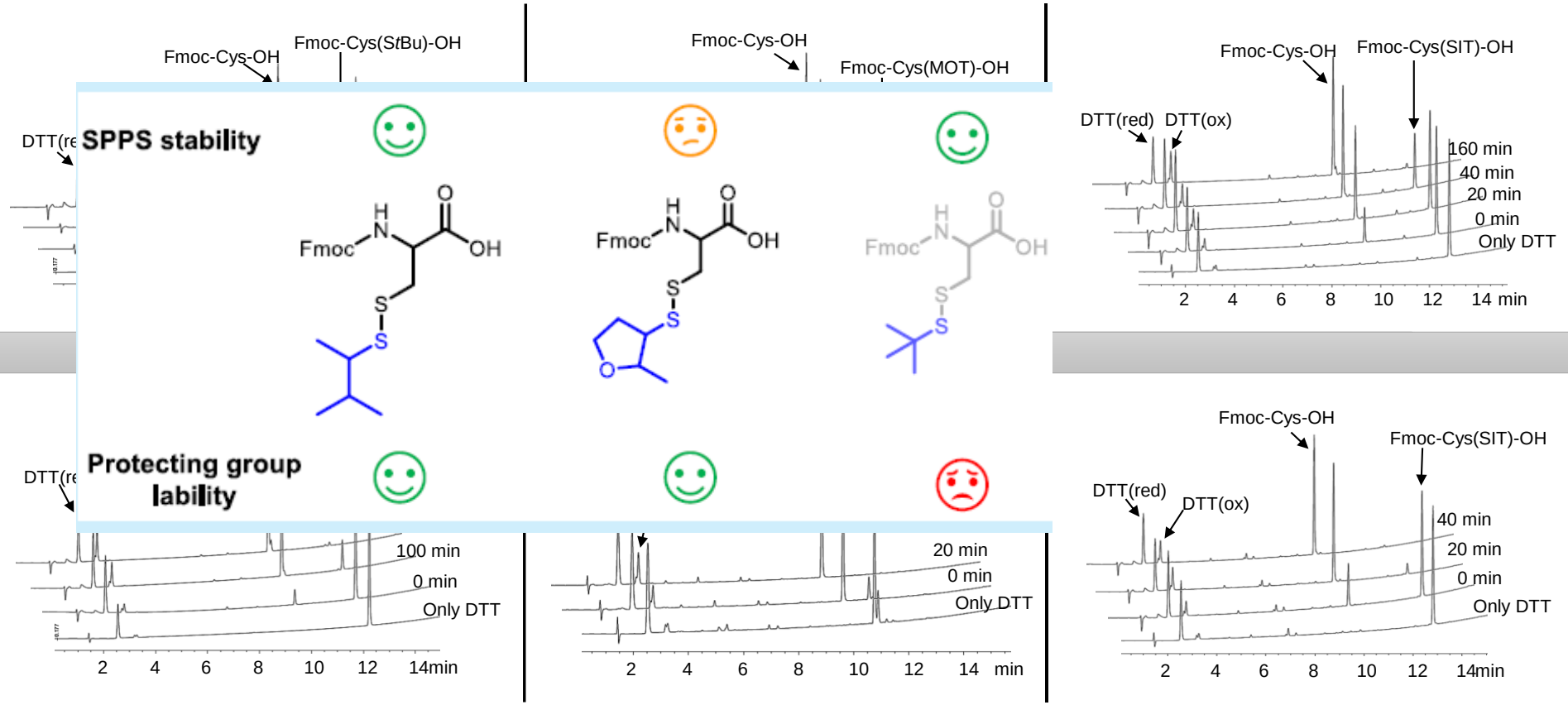
SIT & MOT New PGs for Cys, labile by Reducing Agents

Boc-Cys(**PG**)-Gly-Phe-Leu-RinkAmide-

Fmoc-Cys(S*t*Bu)-OH | Fmoc-Cys(MOT)-OH | Fmoc-Cys(SIT)-OH

Reduction in presence of DTT + DIEA



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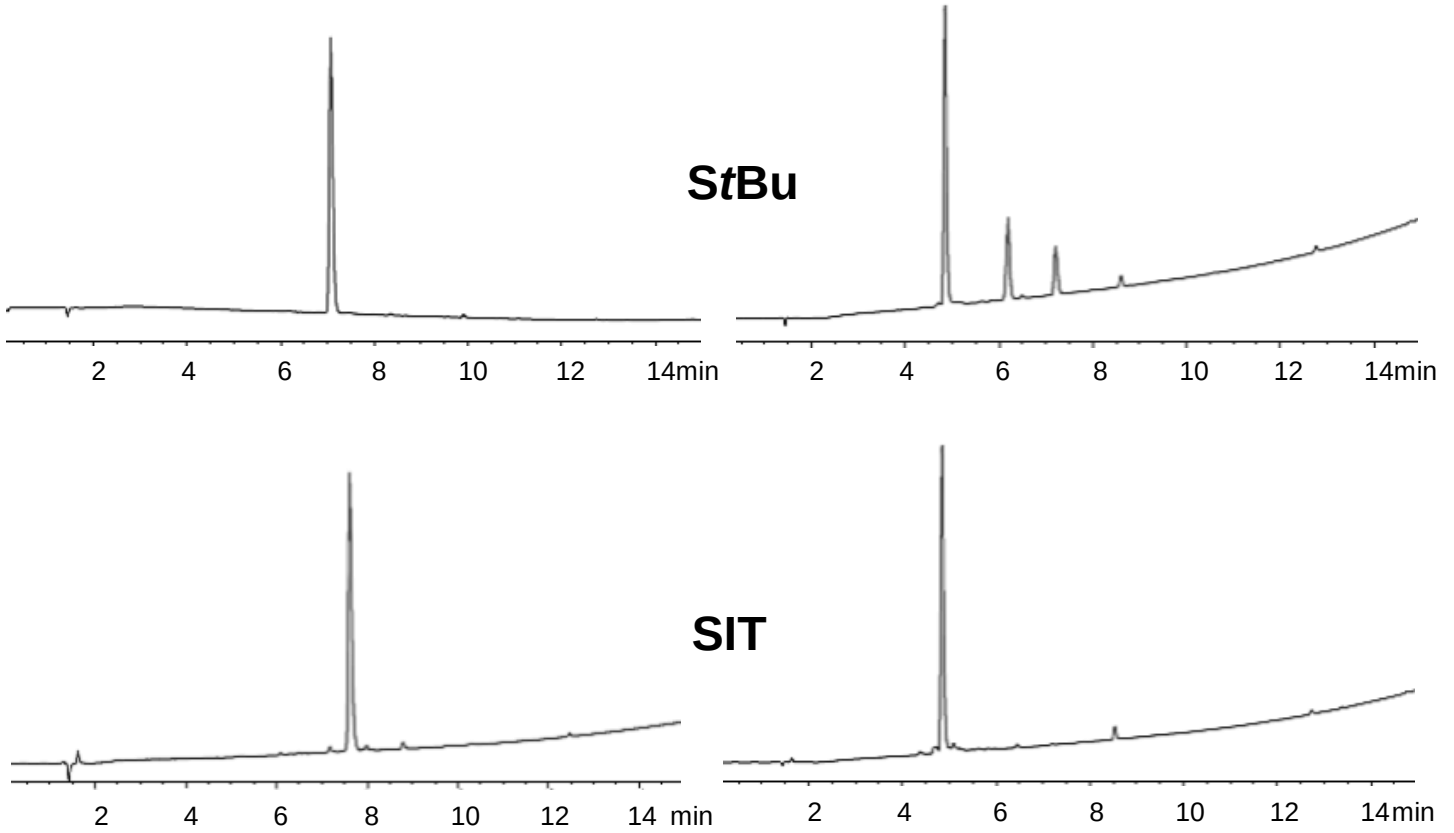
SIT New PG for Cys, labile by Reducing Agents

Vasopressin

H-Cys(**PG**)-Tyr(tBu)-Phe-Gln(Trt)-Asn(Trt)-Cys(**PG**)-Pro-Arg(Pbf)-Gly RinkAmide-

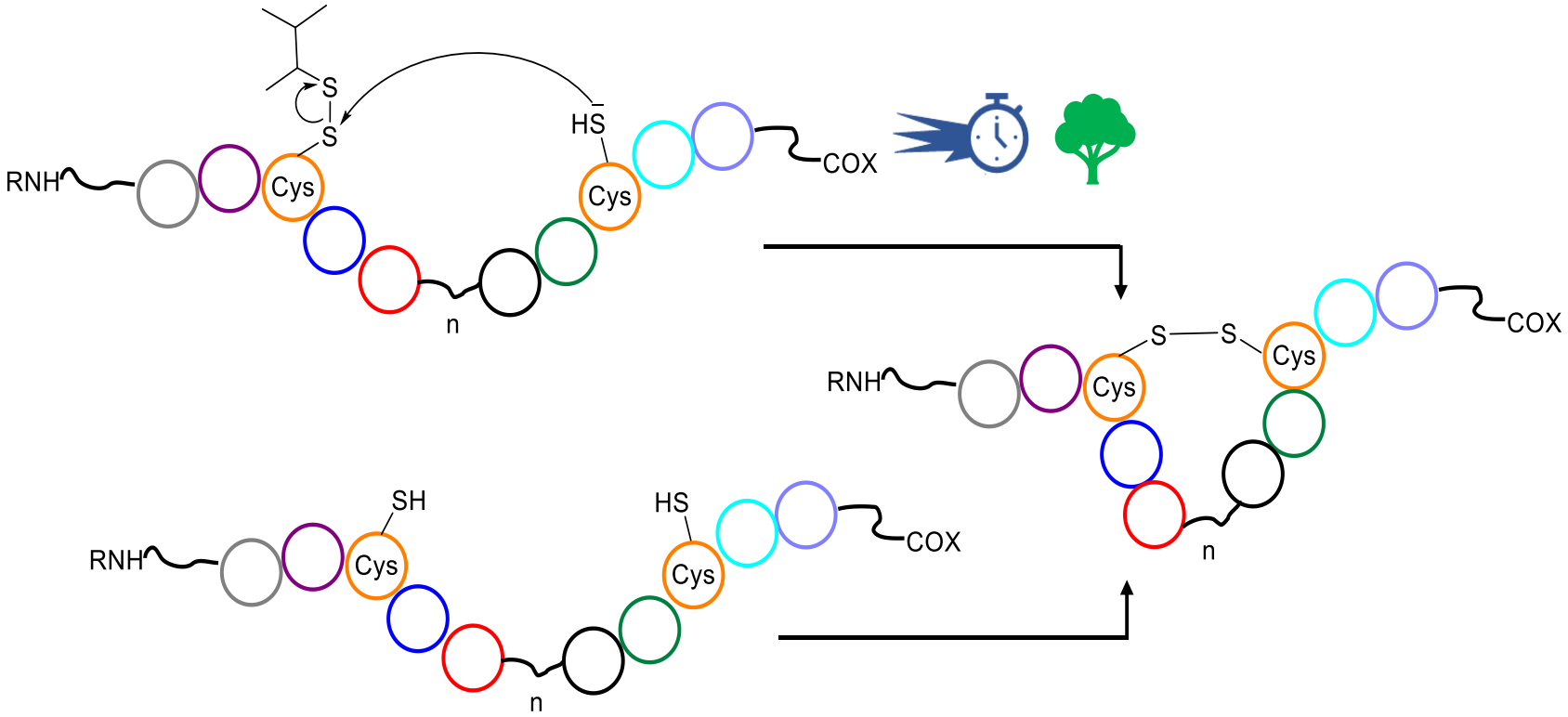
Removal Conditions:

3 x DTT (10 eq), 5 min
DMF-DIEA-H_2O (92:4:4)



A. Chakraborty, A. Sharma, F. Albericio, BG. de la Torre. Org. Lett., 22, 9644 (2020)

Chemoselective Disulfide Formation by Thiol-Disulfide Interchange in SIT-Protected Cysteinyl Peptides



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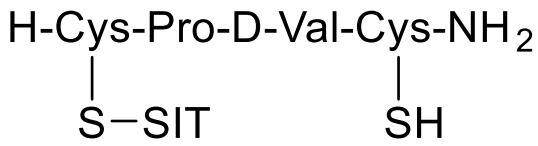

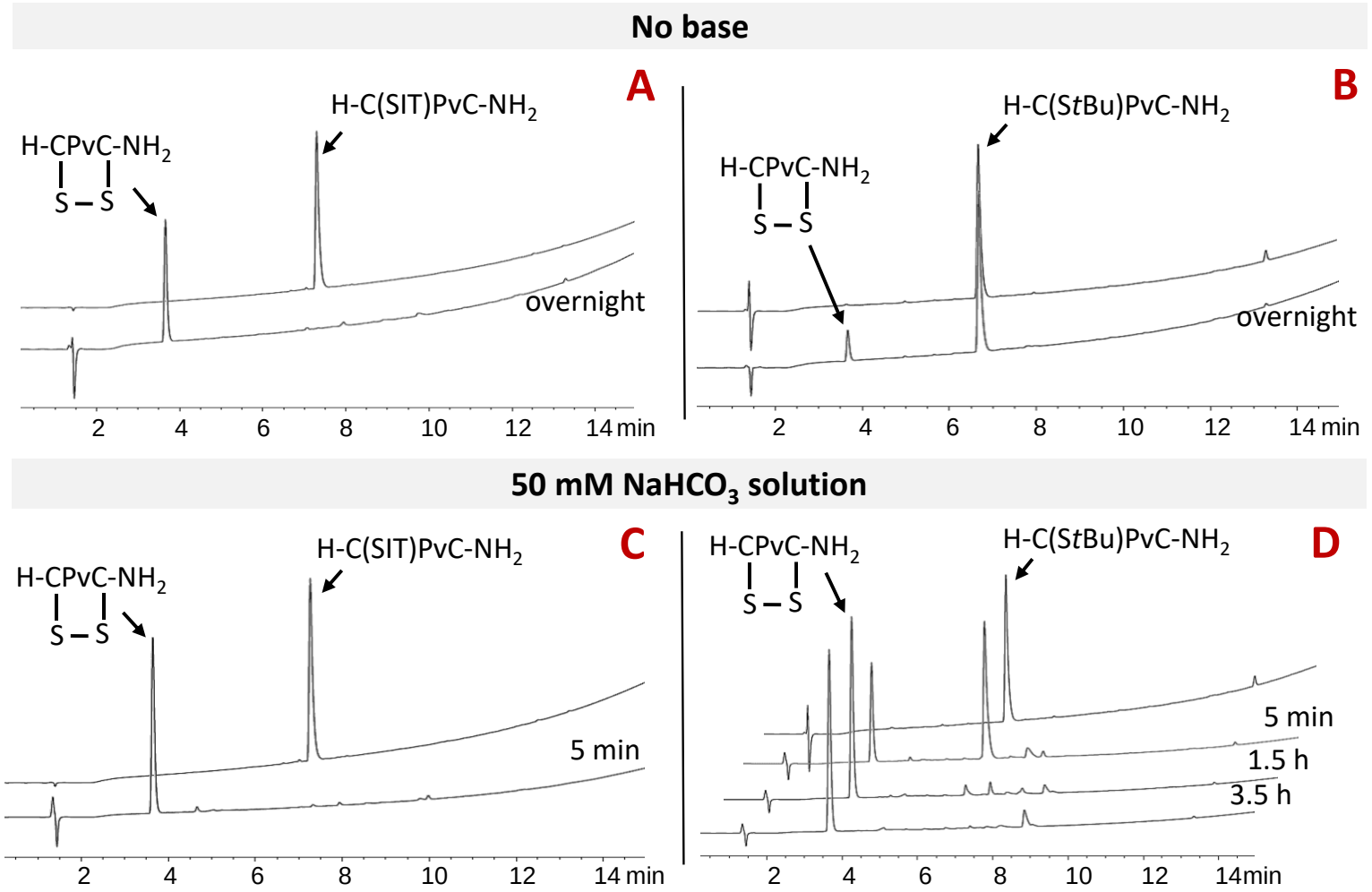

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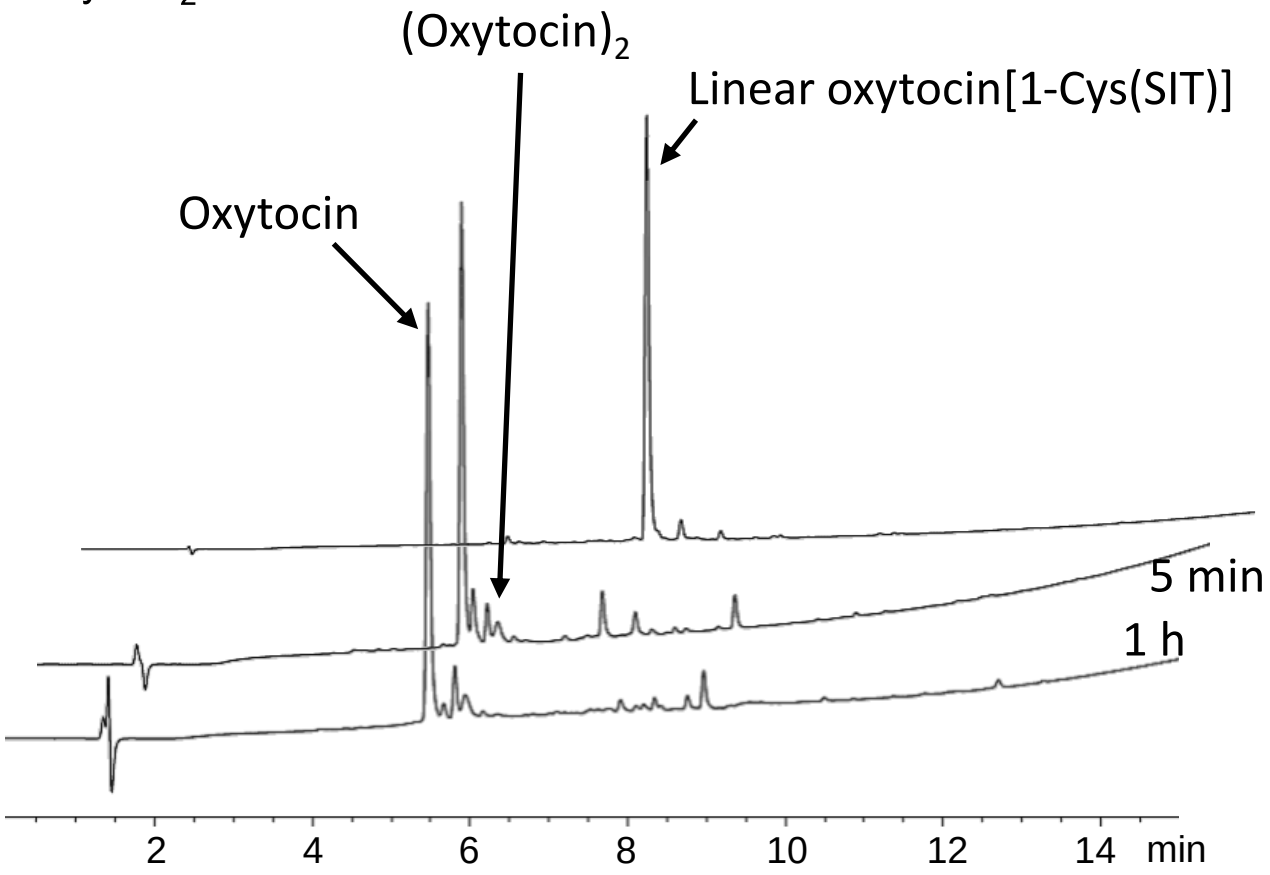
H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2

S–SIT SH

50 mM $NaHCO_3$



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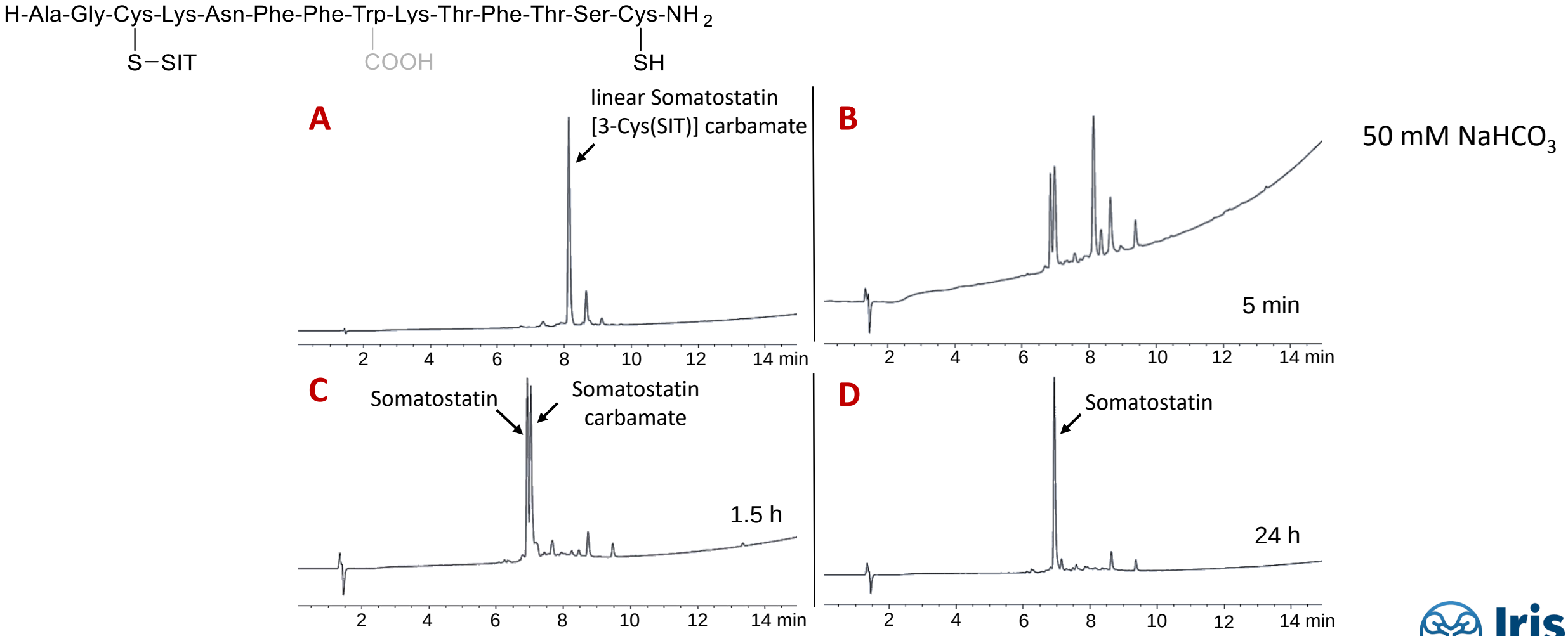

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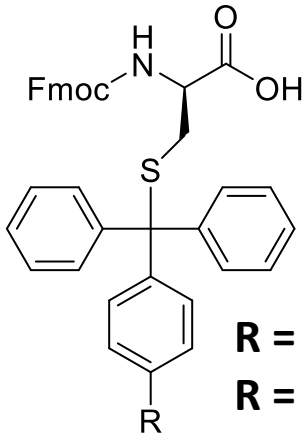
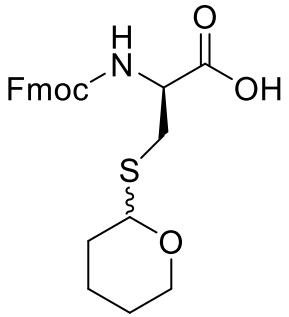
Cys Protecting Groups, Winners

Acid Labile

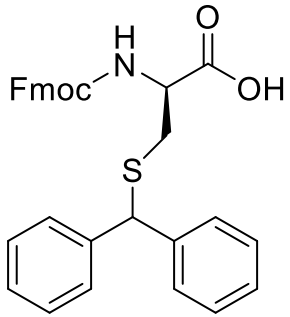


R = H, Trt

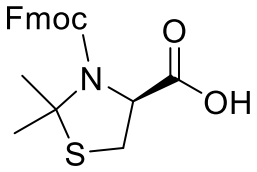
R = OMe, Mmt

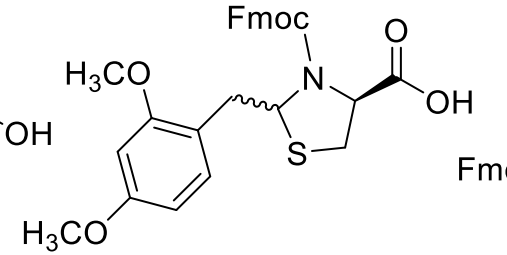


THP

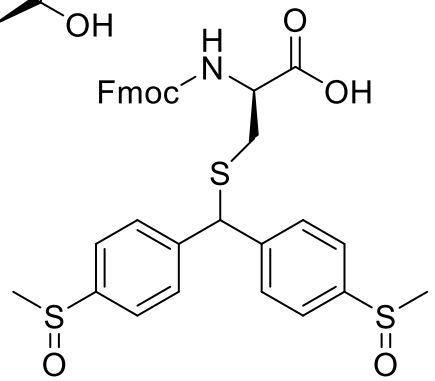


Dpm





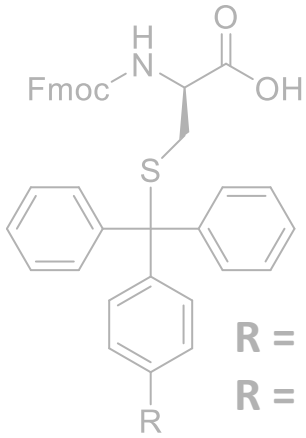
Ψ Pro



Msbh

Cys Protecting Groups, Winners

Acid Labile



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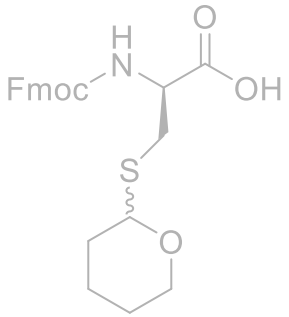

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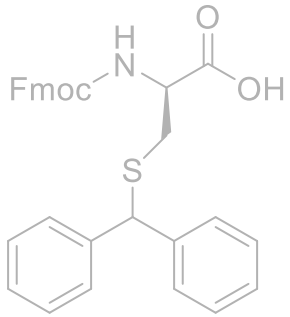

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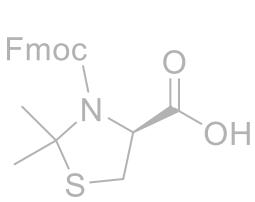

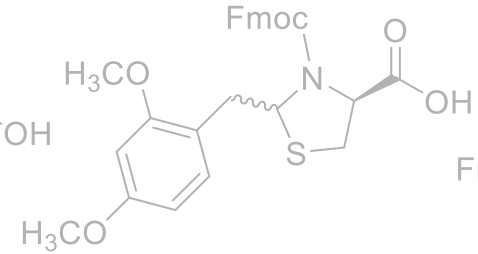

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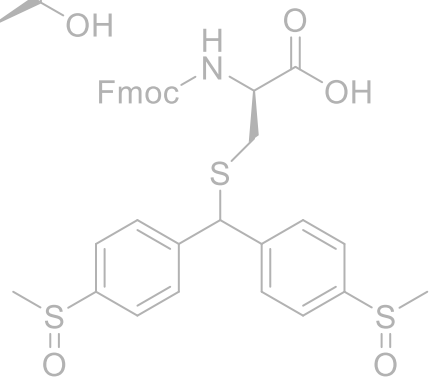
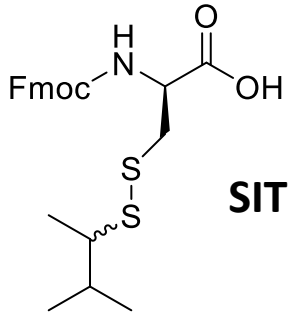


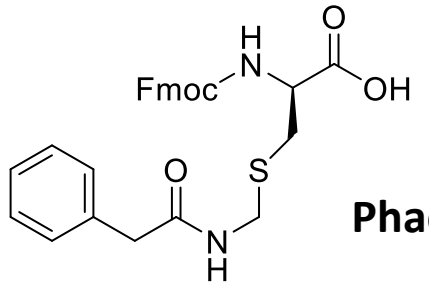
Msbh

Reducing Agent Labile



SIT

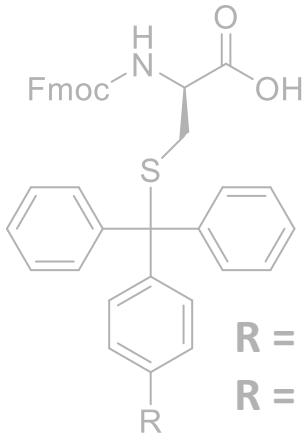
Enzymatically Labile



Phacm

Cys Protecting Groups, Winners

Acid Labile



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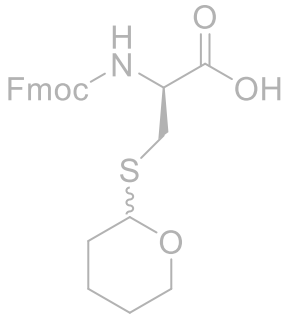

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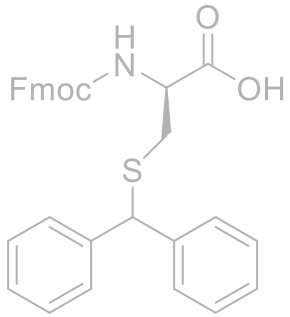

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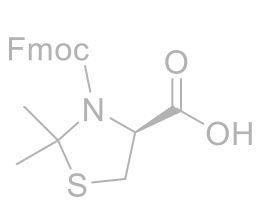
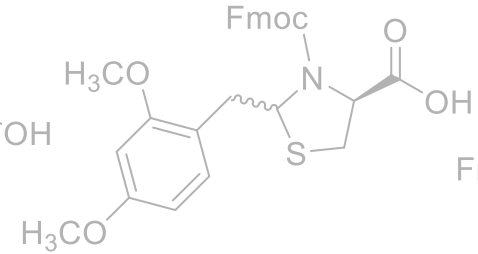
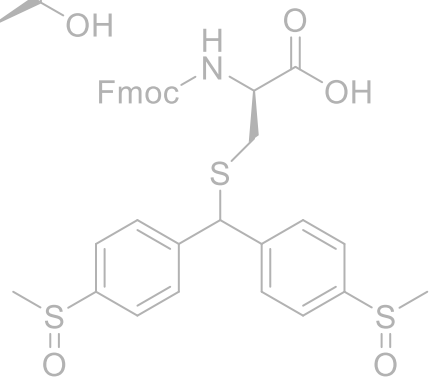


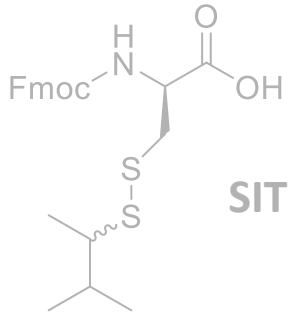


Ψ Pro



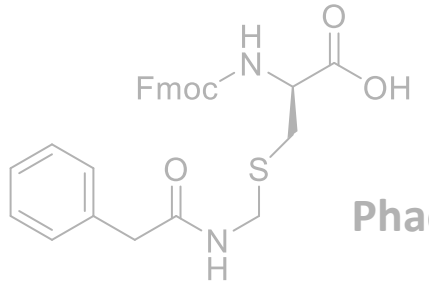
Msbh

Reducing Agent Labile



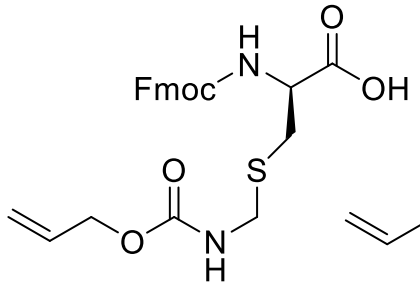
SIT

Enzymatically Labile

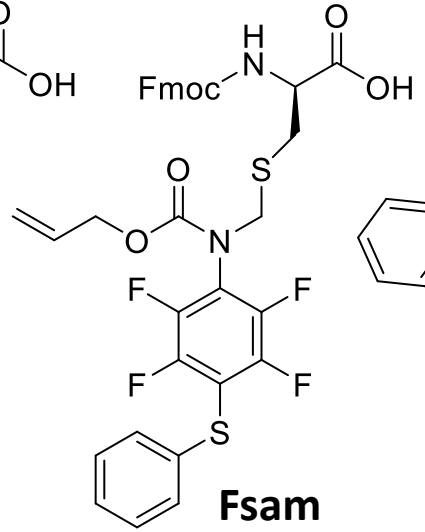


Phacm

Pd Labile

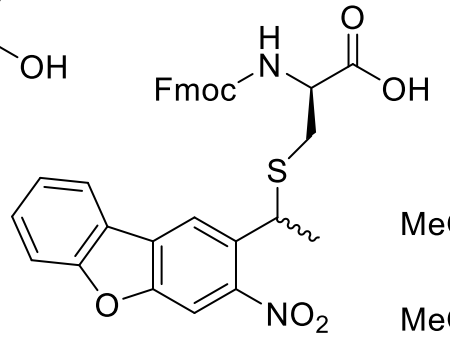


Allocam

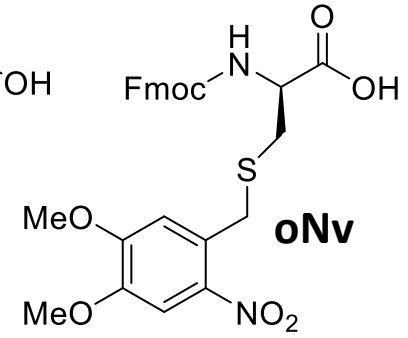


Fsam

Photolabile



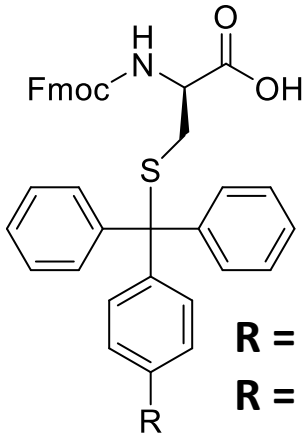
NDBF



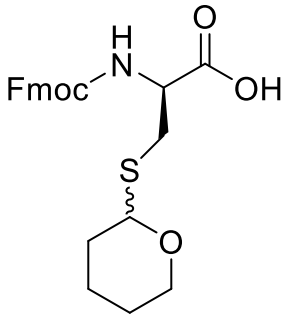
oNv

Cys Protecting Groups, Winners

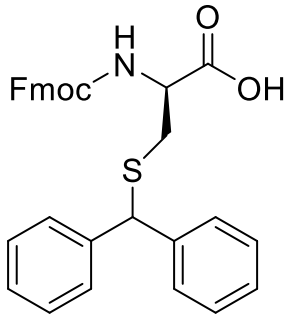
Acid Labile



R = H, Trt
R = OMe, Mmt

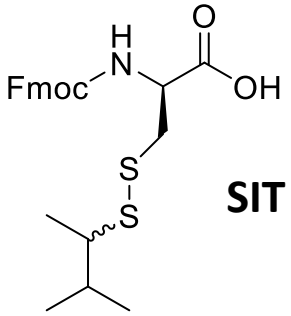


THP



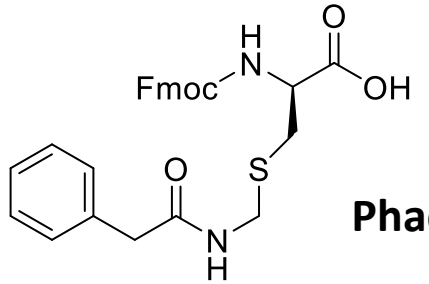
Dpm

Reducing Agent Labile

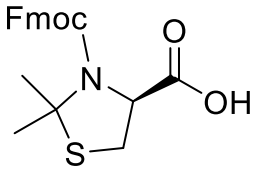


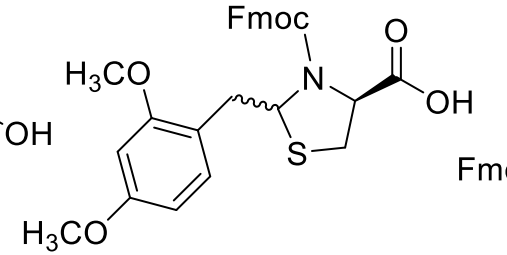
SIT

Enzymatically Labile

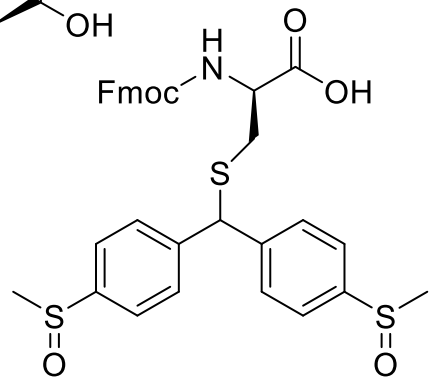


Phacm



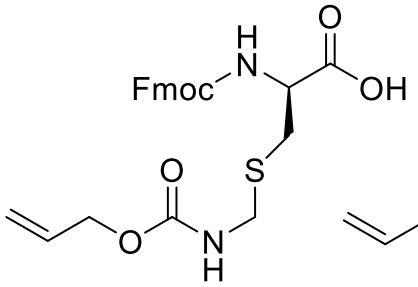


Ψ Pro

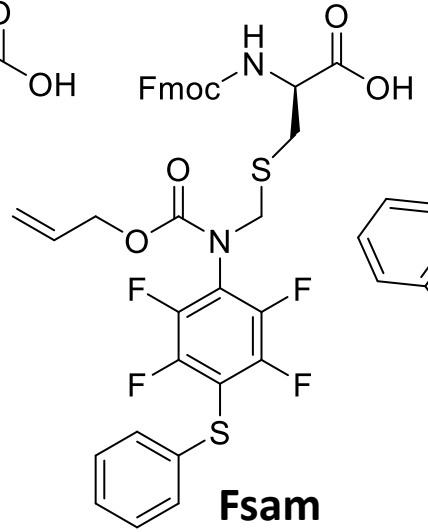


Msbh

Pd Labile

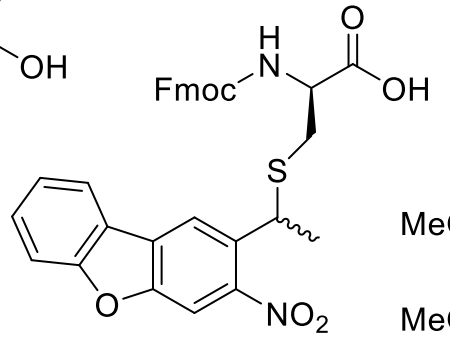


Allocam



Fsam

Photolabile



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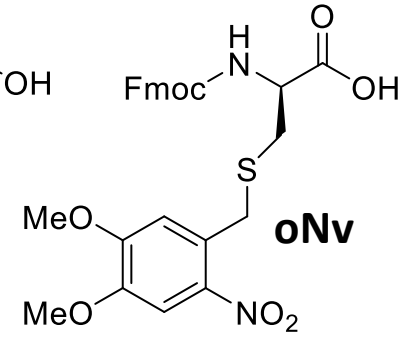
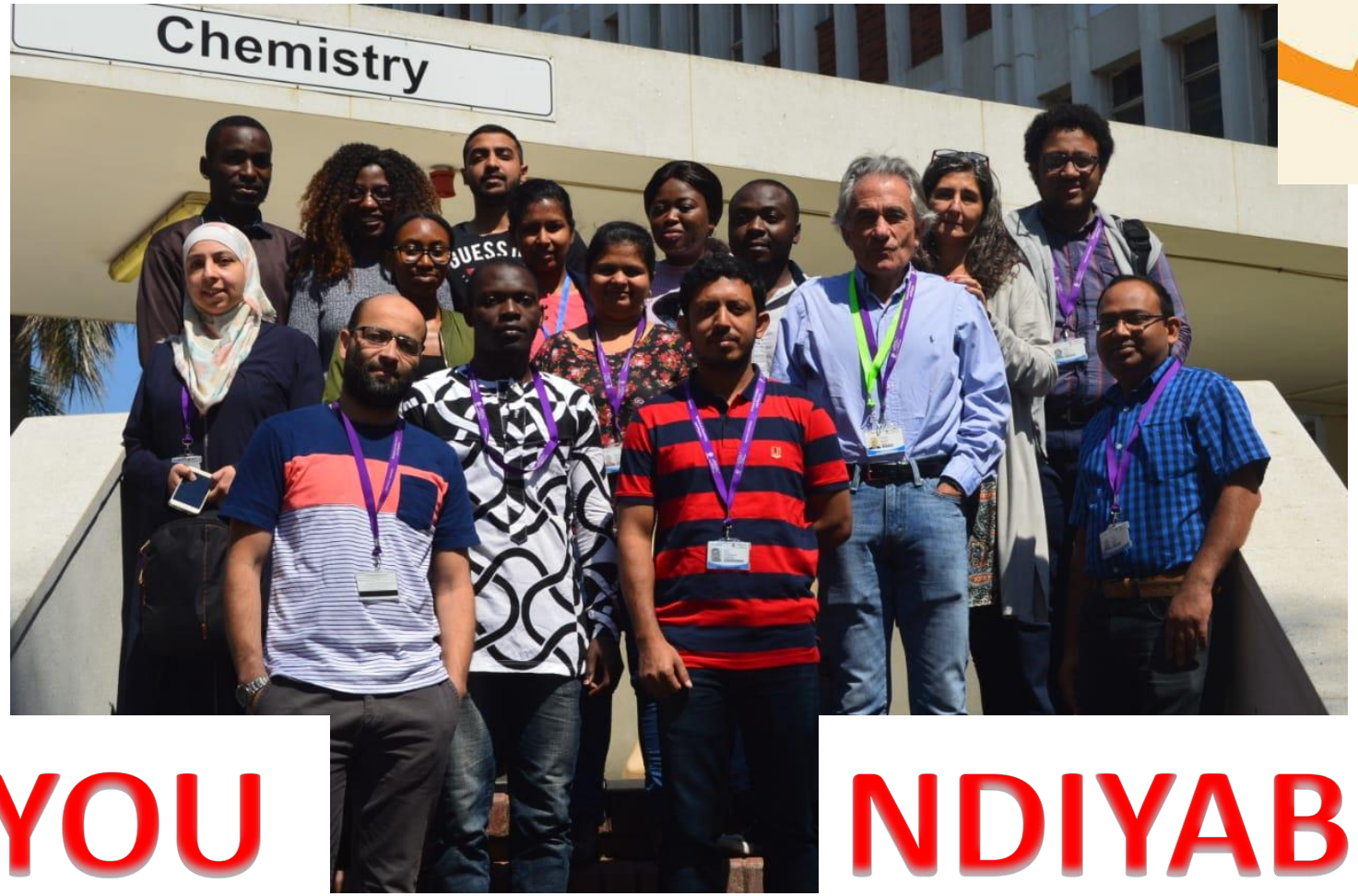


oNv

Iris Biotech

ACKNOWLEDGEMENTS



THANK YOU NDIYABULELA

ACKNOWLEDGEMENTS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