Peptide chemistry

Yssy Baker
ysobel.baker@chem.ox.ac.uk

Talk outline

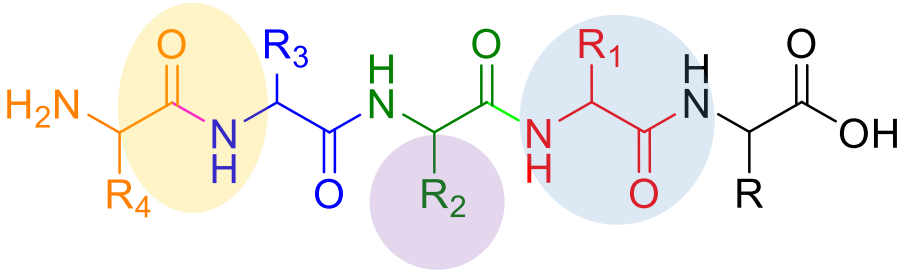
- **Introduction to peptide chemistry**
  - Peptides and proteins
  - Peptide synthesis
  - Protecting group strategies
- **Applications of peptide chemistry**
  - Synthesis and semisynthesis of modified proteins using chemical ligation
  - Cyclic peptides- analogues of marine snail venom for medicinal chemistry
  - Chemical probes- synthesis of modified ubiquitin for proteomic studies

Peptides and proteins

peptide bond

amino acid residue

N-terminus



C-terminus

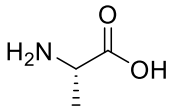
amino acid side chain

Peptide: short chain of two or more amino acids linked by amide bonds

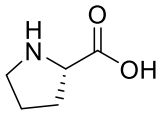
Protein: longer polymer of amino acids made of peptide subunits (polypeptides)

Exact number of amino acids for defining a peptide or protein is arbitrary

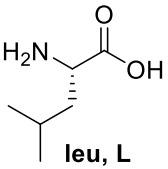
Amino acids



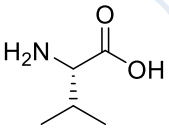
ala, A
alanine



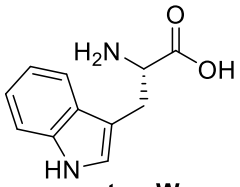
pro, P
proline



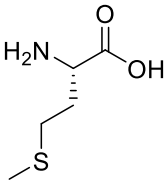
leu, L
leucine



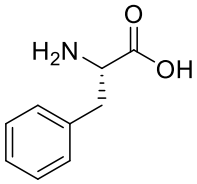
val, V
valine



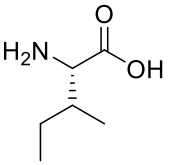
trp, W
tryptophan



met, M
methionine

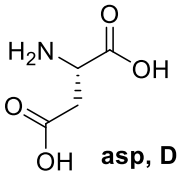


phe, F
penylalanine

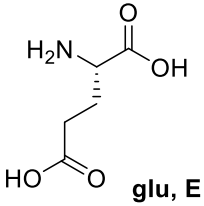


ile, I
isoleucine

hydrophobic

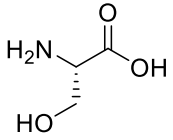


asp, D
aspartic acid

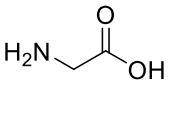


glu, E
glutamic acid

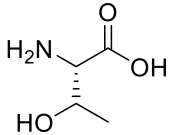
acidic



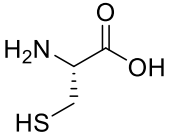
ser, S
serine



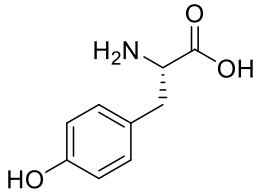
gly, G
glycine



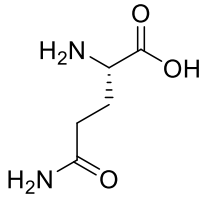
thr, T
threonine



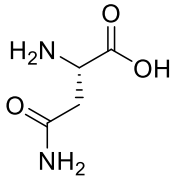
cys, C
cysteine



tyr, Y
tyrosine

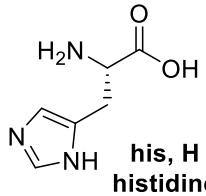


gln, Q
glutamine

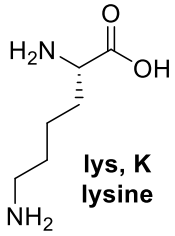


asn, N
asparagine

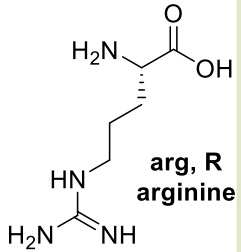
neutral/polar



his, H
histidine



lys, K
lysine



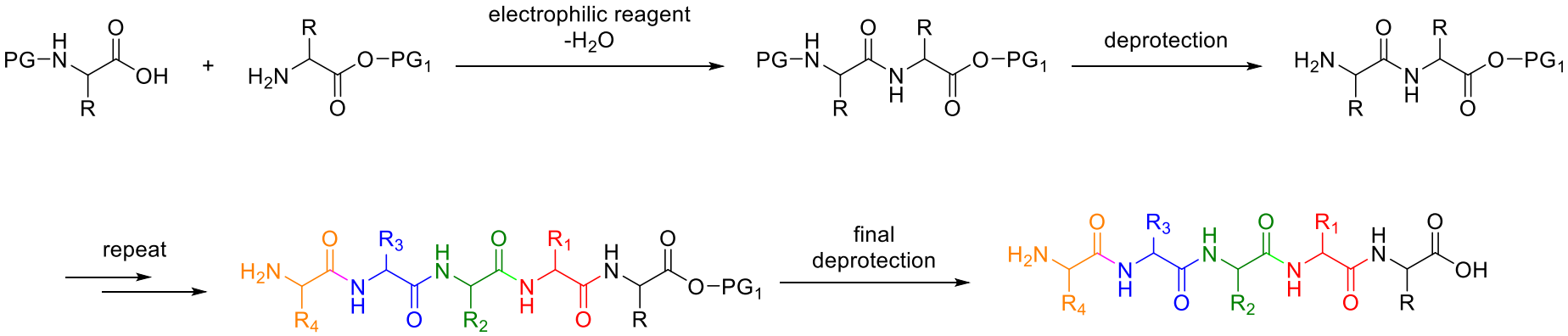
arg, R
arginine

basic

Importance of peptides and peptidomimetics

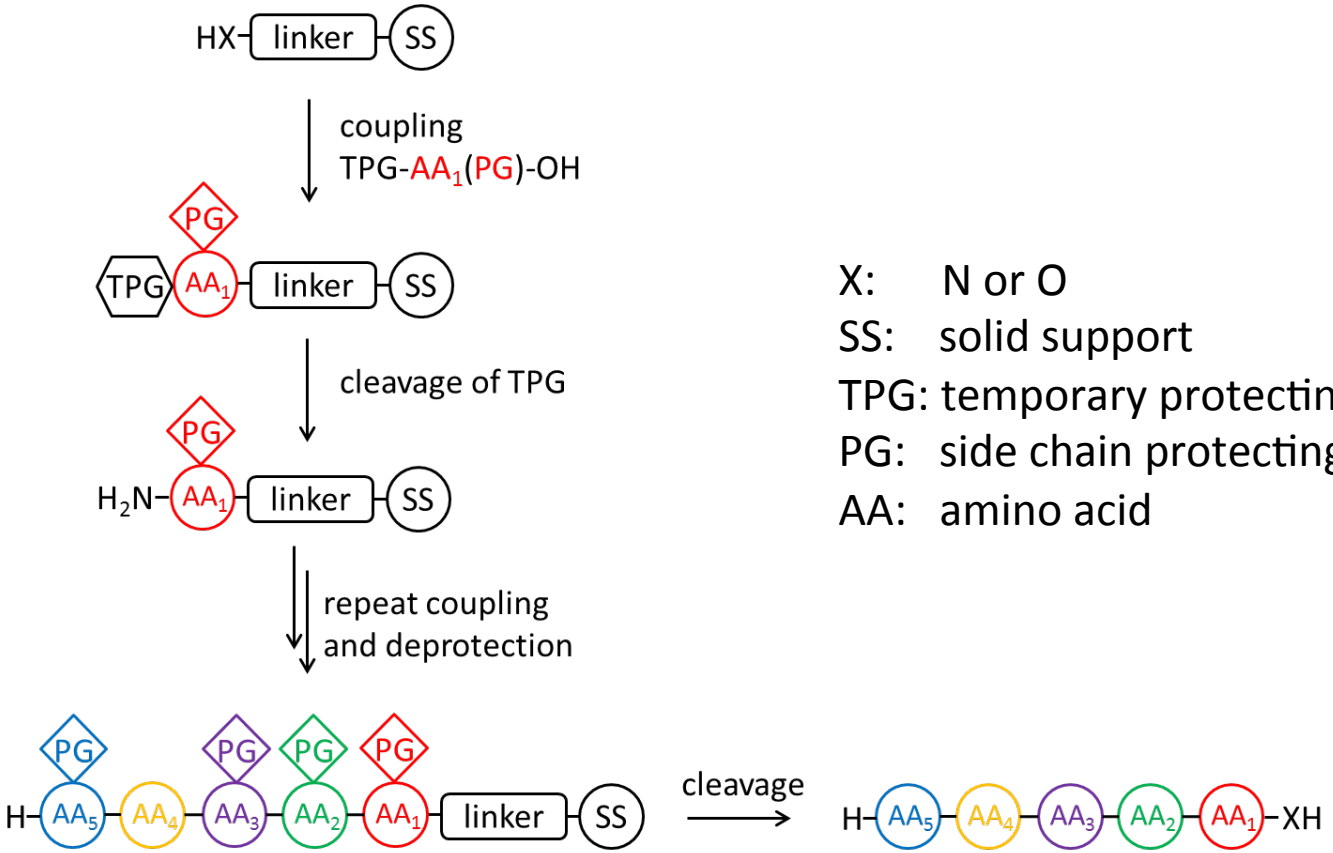
- Amide bonds are ubiquitous in nature
- Many natural products have a peptide framework
- Broad spectrum of biological activity
- Non-natural amino acid mimics paramount for drug discovery and scientific advancement
- Therapeutic agents- many peptide based therapeutics in development
- Chemical probes- studying biological systems

Synthesis solution phase



- Traditional approach
- Side chains often require protecting groups
- Limited by purification after each step, yields and solubility of intermediates

Solid Phase Peptide Synthesis (SPPS)

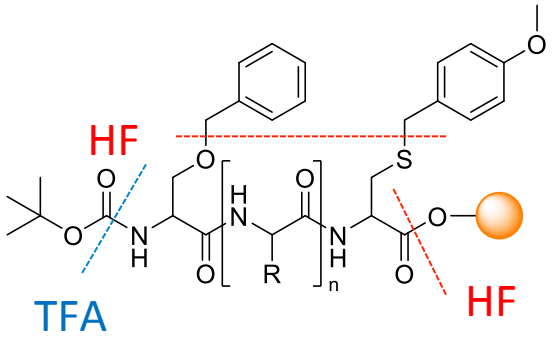


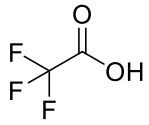
X: N or O
SS: solid support
TPG: temporary protecting group
PG: side chain protecting group
AA: amino acid

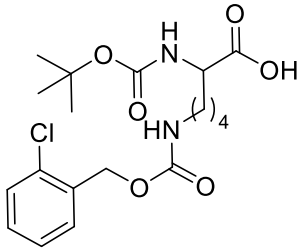
- long peptides could be synthesized without issues of solubility
- Fast: impurities and unreacted reagents removed by washing and filtering the resin
- High yielding as large excesses of reagents can be used

Merrifield awarded the Nobel Prize in 1984

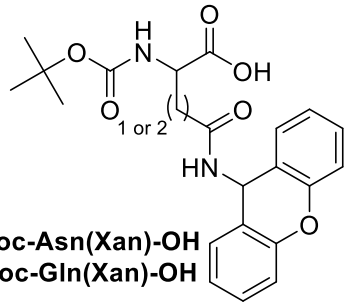
Boc SPPS: Protecting group strategy



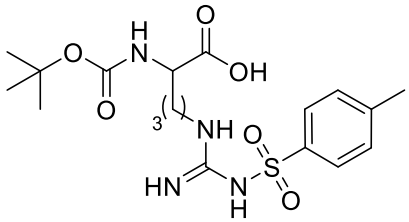




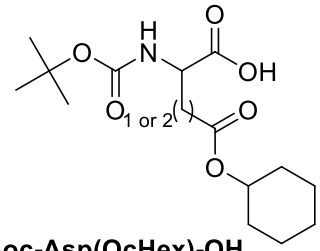
Boc-Lys(2-ClZ)-OH



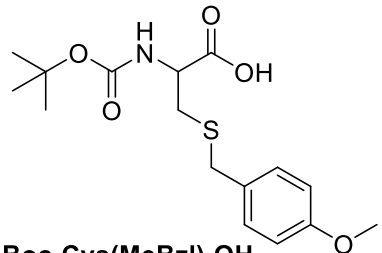
Boc-Asn(Xan)-OH
Boc-Gln(Xan)-OH



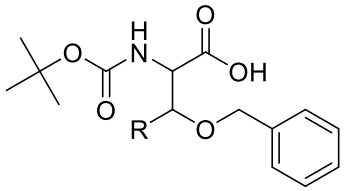
Boc-Arg(Tos)-OH



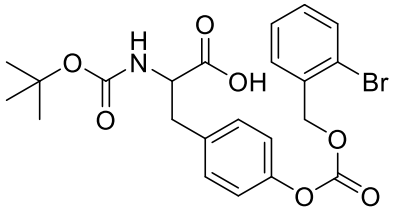
Boc-Asp(OcHex)-OH
Boc-Glu(OcHex)-OH



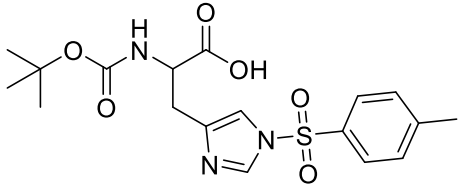
Boc-Cys(MeBzl)-OH



Boc-Ser(Bzl)-OH
Boc-Thr(Bzl)-OH

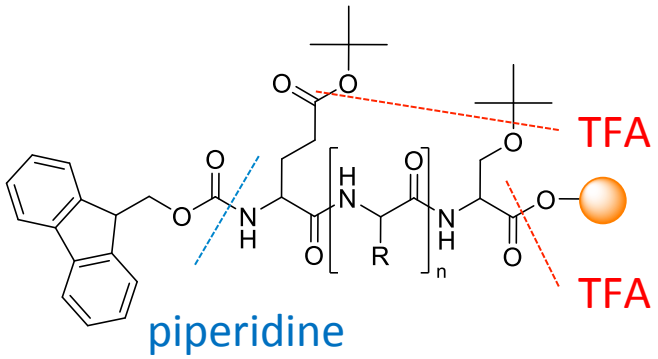


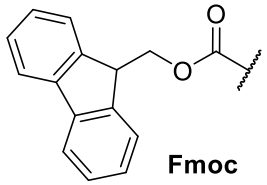
Boc-Tyr(2-BrZ)-OH



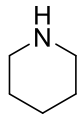
Boc-His(Tos)-OH

Fmoc SPPS: Protecting group strategy

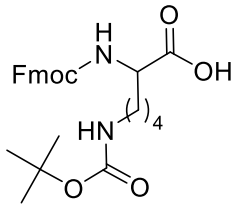




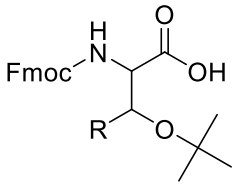
Fmoc



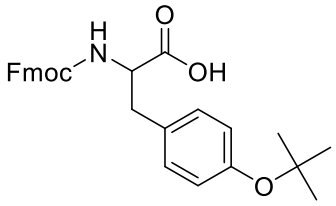
piperidine



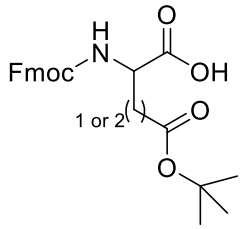
Fmoc-Lys(Boc)-OH



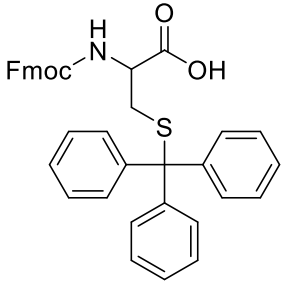
Fmoc-Ser(tBu)-OH
Fmoc-Thr(tBu)-OH



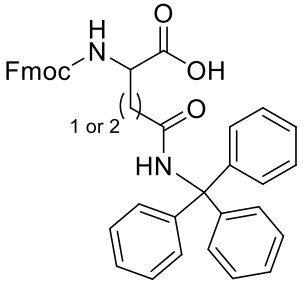
Fmoc-Tyr(tBu)-OH



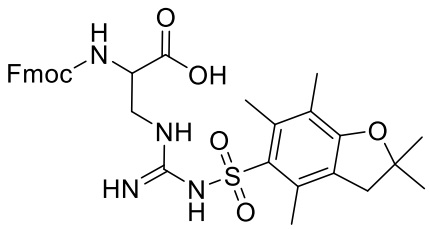
Fmoc-Asp(OtBu)-OH
Fmoc-Glu(OtBu)-OH



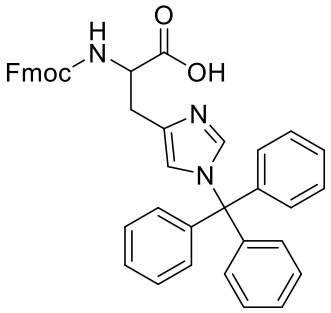
Fmoc-Cys(Trt)-OH



Fmoc-Asn(Trt)-OH
Fmoc-Gln(Trt)-OH



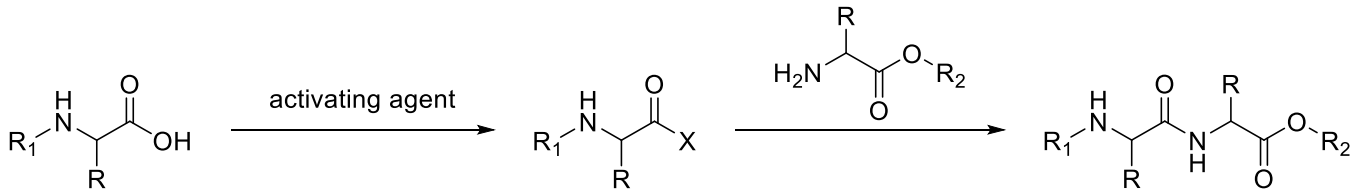
Fmoc-Arg(Pbf)-OH



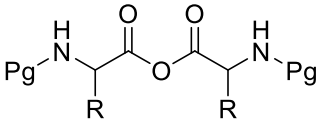
Fmoc-His(Trt)-OH

Peptide bond formation

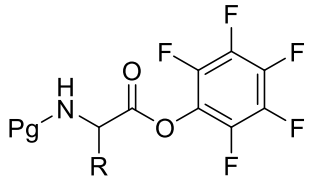
General strategy for peptide bond formation



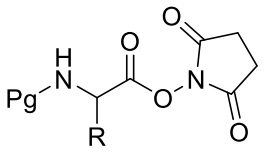
commonly used active esters



symmetric anhydride

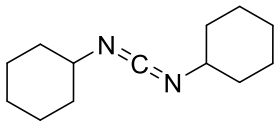


pentafluorophenyl active ester



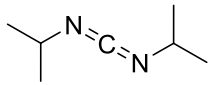
hydroxysuccinimido active ester

Peptide bond formation: Carbodiimides



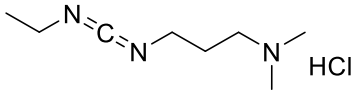
DCC

insoluble urea by-product
removed by filtration
not suitable for SPPS



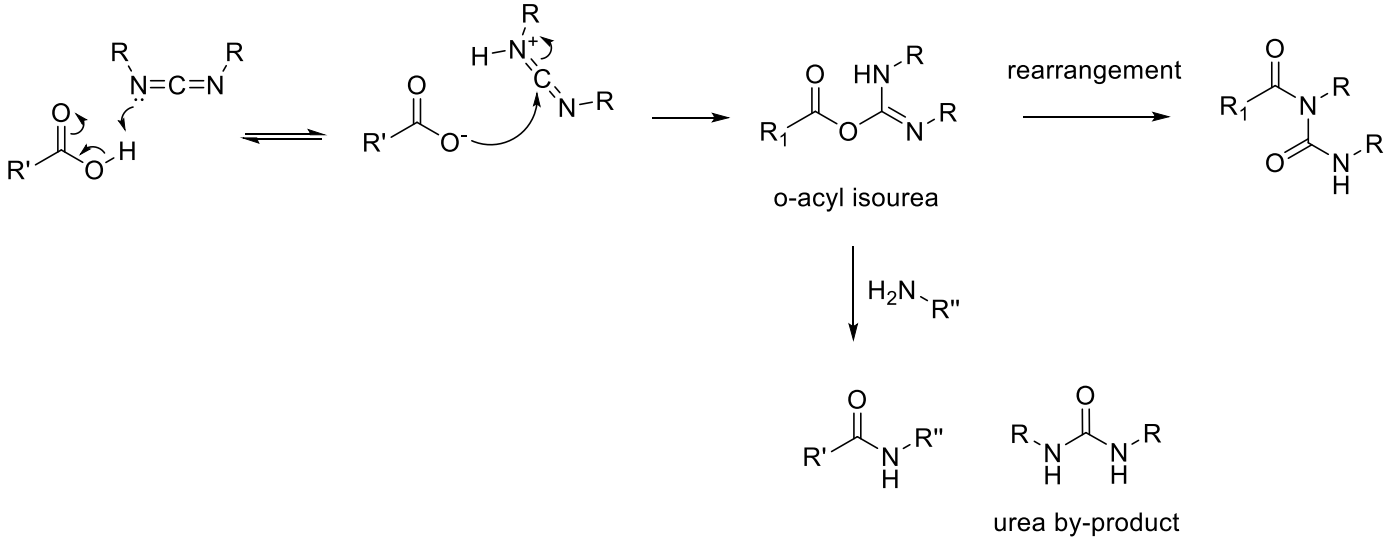
DIC

soluble by-product
can be removed by
washing resin in SPPS



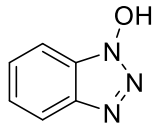
EDC

useful for solution phase
reactions
water soluble by-product
removed in work-up



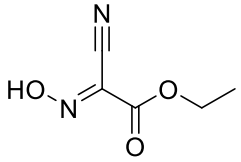
Peptide bond formation: Additives

Additives: accelerate coupling reaction and suppress by-product formation



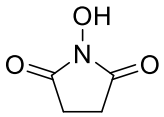
HOBt

very good but explosive



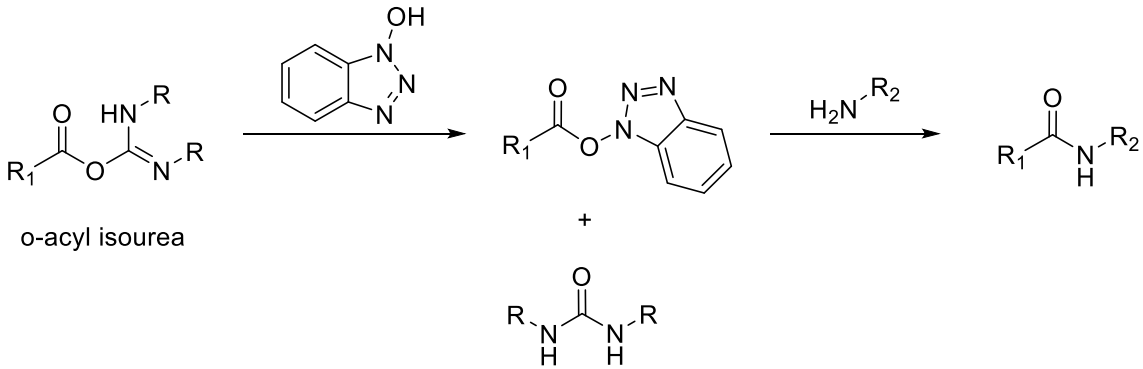
Oxyma Pure^{R}

non-explosive alternative



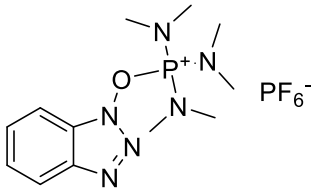
HOSu

water soluble

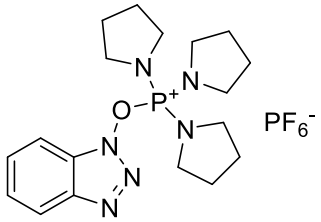


o-acyl isourea

Peptide bond formation: Coupling agents

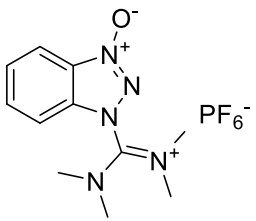


BOP

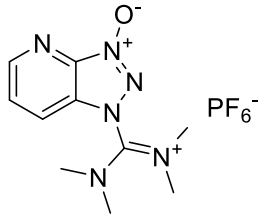


PyBOP

phosphonium reagents



HBTU



HATU

uronium-imonium reagents

Structures contain the additive

HBTU is the more cost effective alternative and is acceptable for most coupling applications

HATU is the most reactive uronium reagent, but expensive

More information: http://documents.bachem.com/coupling_reagents.pdf

Comparing Boc and Fmoc SPPS

Fmoc/tBu

- Orthogonal N^{α}/side chain protection
- Only requires TFA for final cleavage
- Deprotection and coupling can be monitored by UV chromophores
- Final cleavage possible in SPPS reaction vessel

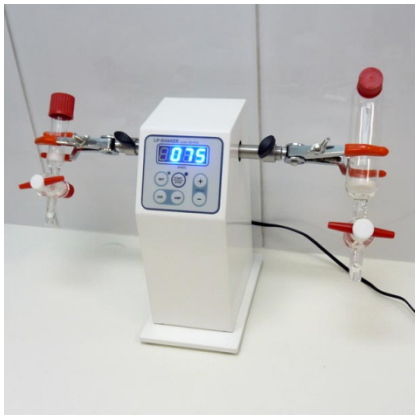
especially good for acid sensitive peptides & derivatives

Boc/Bzl

- N^{α}/side chain protection both acid labile
- Requires repetitive TFA cleavage and a final HF cleavage
- Monitored only by Ninhydrin test
- Requires specialist equipment for final cleavage

base labile peptides, “difficult sequences” repetitive TFA treatment impedes aggregation

Peptide synthesis setup



manual

multichannel

microwave

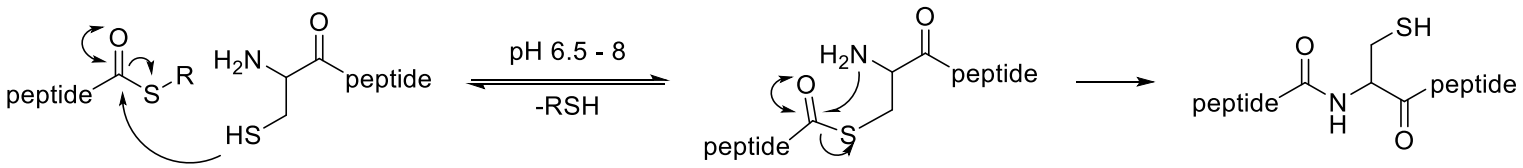
Current limitations of peptide synthesis

- SPPS is limited by yields
- Upper limit typically around 70 amino acids: if a 99% yield per step: final yield <50% for a 70 amino acid chain
- Synthetic difficulty also is sequence dependent; typically amyloid peptides and proteins are difficult to make.
- Cost- large excess of reagents and need for protecting groups make large scale synthesis expensive and not environmentally friendly

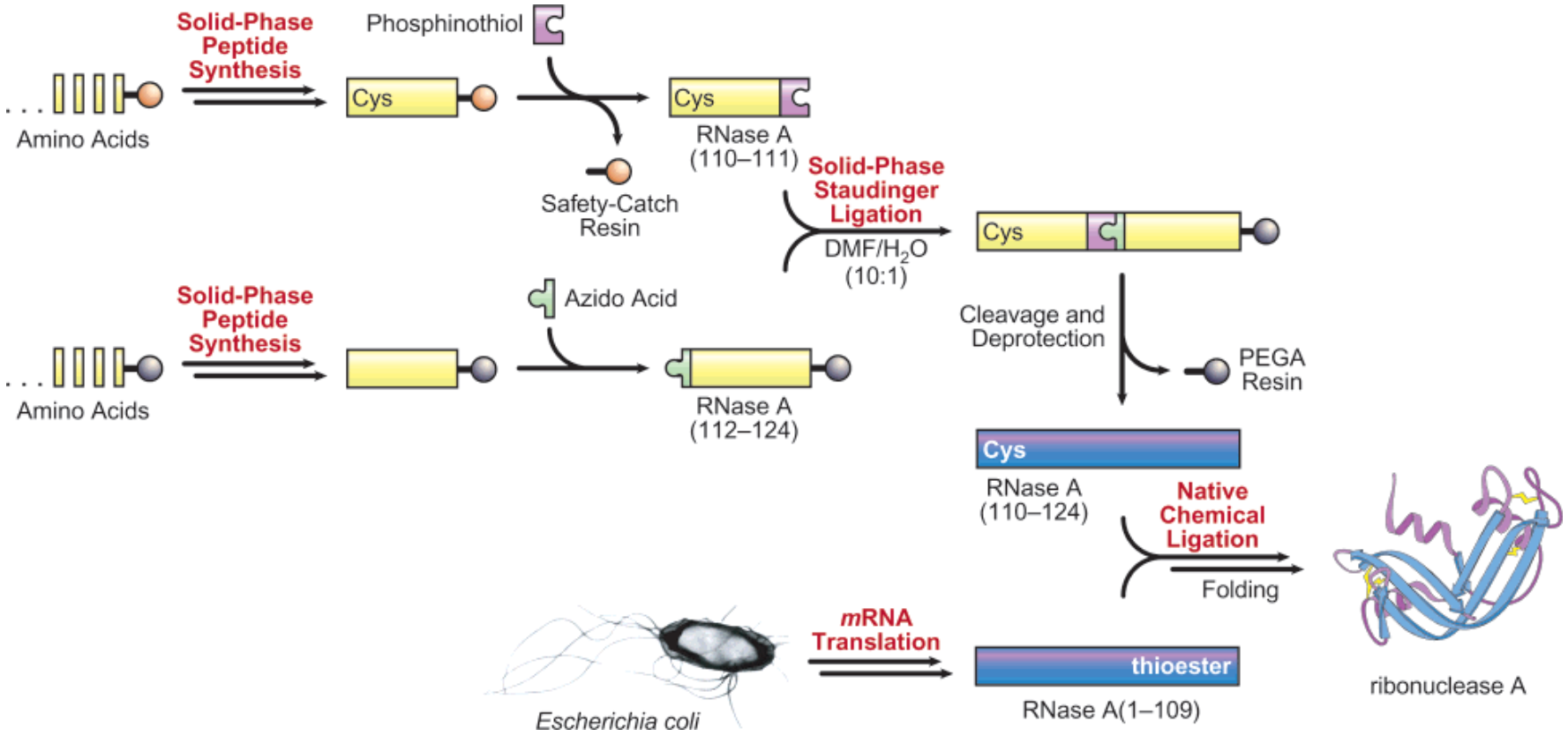
Chemical synthesis of longer peptides and proteins

Native chemical ligation (NCL)

- Native chemical ligation can also be used in the assembly of synthetic peptide fragments to give of long peptides and proteins
- Allows for controlled location of modifications



Example: Orthogonal ligation to give semisynthetic Rnase A



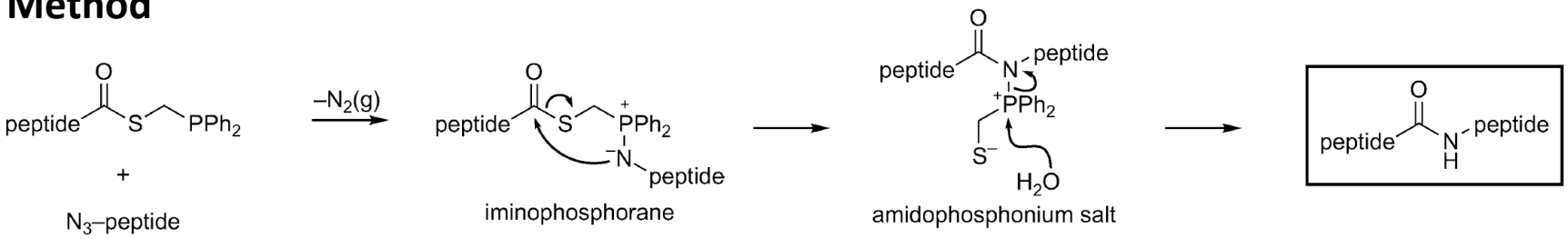
[$^{13}C'$,$^{13}C^{\alpha}$,^{15}N]proline was inserted at position 114

k_{cat}/K_M = 0.94 x 10^7 M^{-1} s^{-1} (recombinant DNA technology k_{cat}/K_M = 1.1 x 10^7 M^{-1} s^{-1}

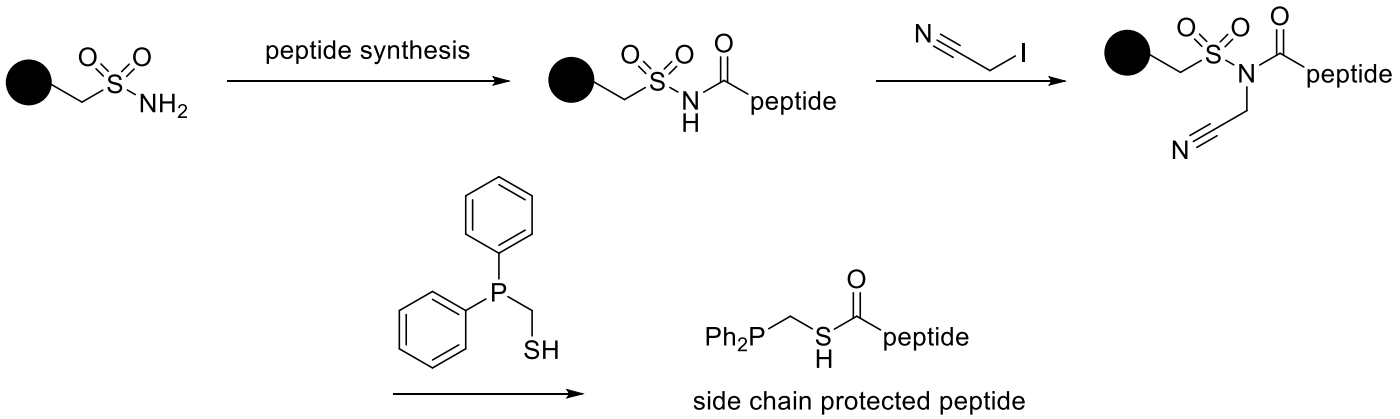
B. L. Nilsson, R. J. Hondal, M. B. Soellner, R.T. Raines *J. Am. Chem. Soc.* **2003** *125* (18), 5268-5269

Peptide ligation using the Staudinger ligation

Method



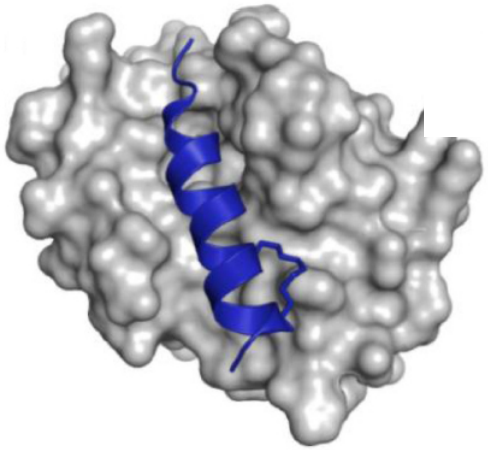
Synthesis of C-terminal phosphinothioester peptide "safety-catch" linker



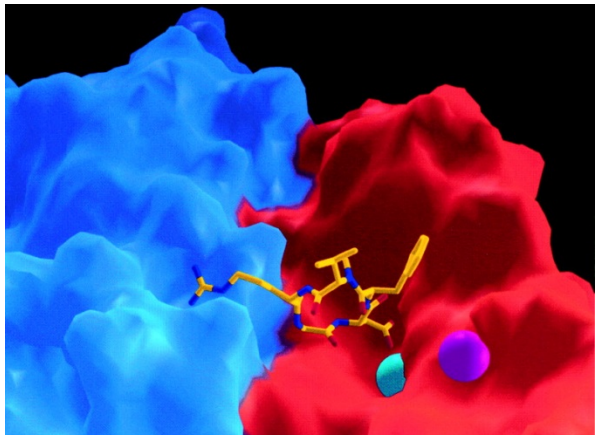
B. L. Nilsson, R. J. Hondal, M. B. Soellner, R.T. Raines *J. Am. Chem. Soc.* **2003** *125* (18), 5268-5269

Synthesis of cyclic peptides

- applications that range from drug discovery to nanomaterials
- biostability and resistance to proteolytic digestion in physiological environments
- exhibit high potency and low toxicity
- Many cyclic peptides are notoriously difficult to prepare

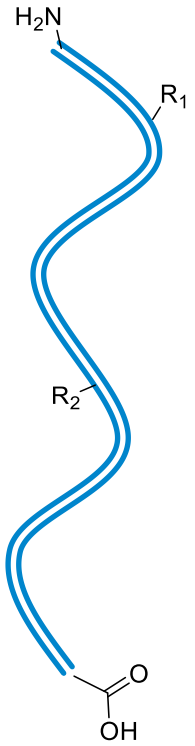


BH3 stapled peptide (blue) bound to MCL-1
N. S Robertson et. al. *Rep. Org Chem.*, **2015**, *5*, 65-74

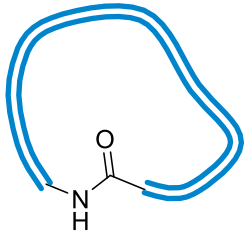


Cyclic RGD peptide binding integrin
J. P. Xiong et al. *Science* **2002**, *296,* 151-155

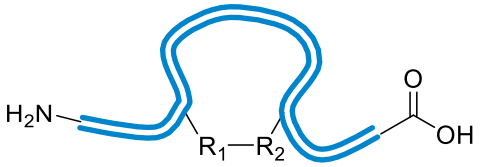
Types of cyclisation



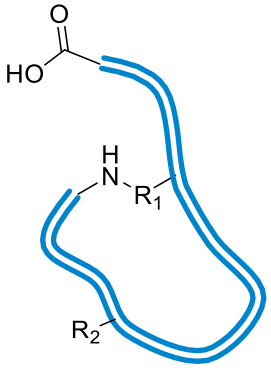
cyclisation →



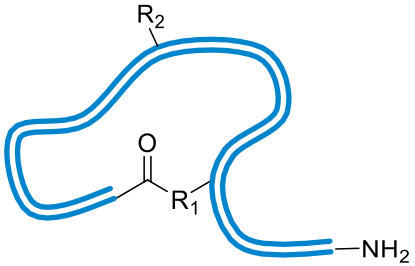
head to tail



side chain to side chain



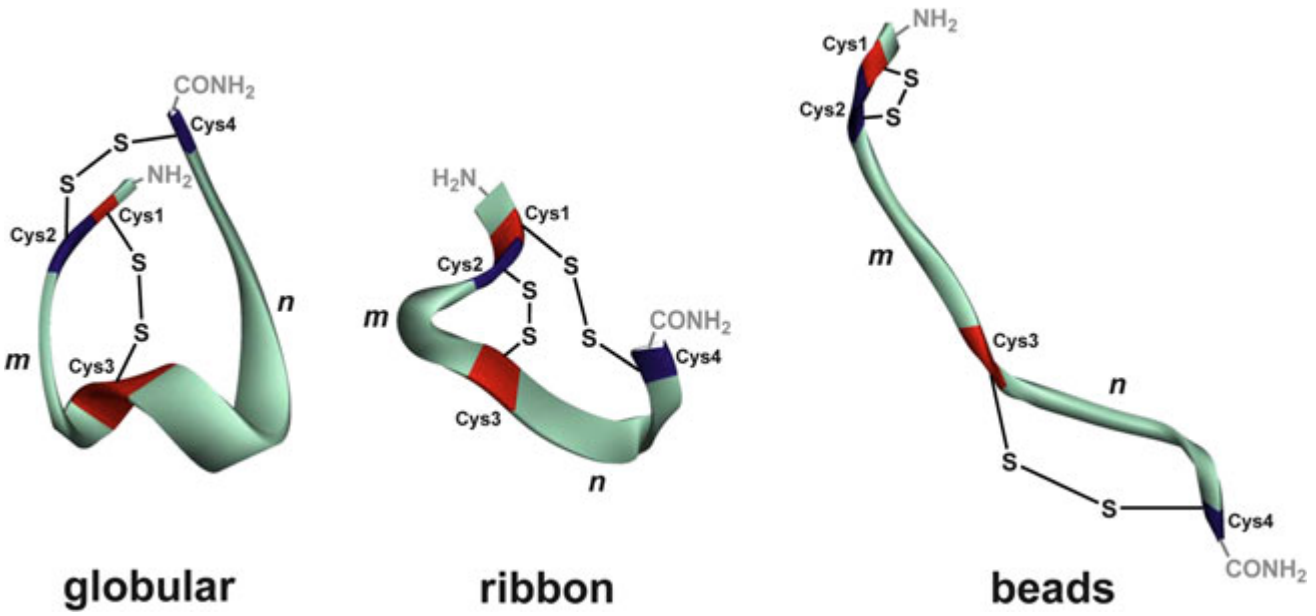
side chain to head



side chain to tail

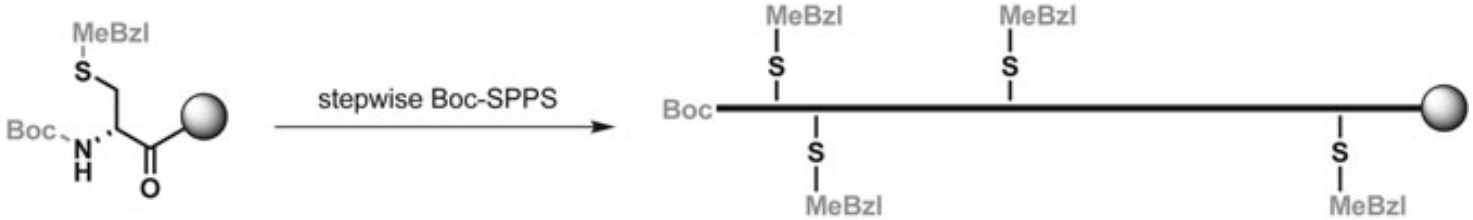
α-Conotoxins

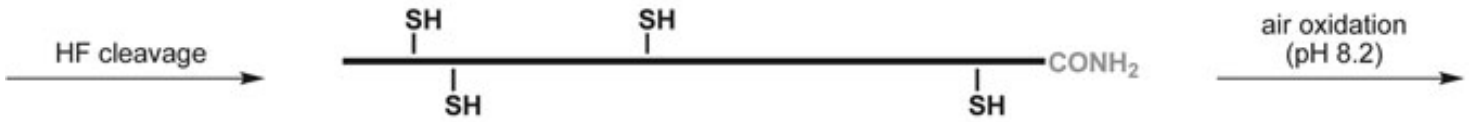
- Peptide neurotoxins isolated from the venom ducts of carnivorous marine cone snails
- 12–20 amino acids with a conserved cysteine framework
- Potent selective binders of certain nicotinic acetylcholine receptor subtypes
- Poor biochemical stability and resistance to proteolytic digestion

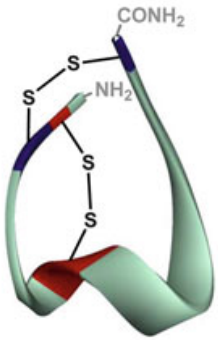


Book: Peptide Modifications to Increase Metabolic Stability and Activity [electronic resource]

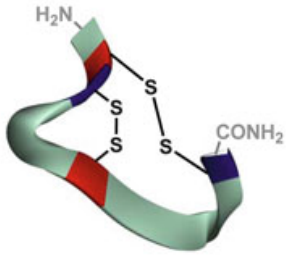
α-Conotoxin synthesis: non-directed cyclisation



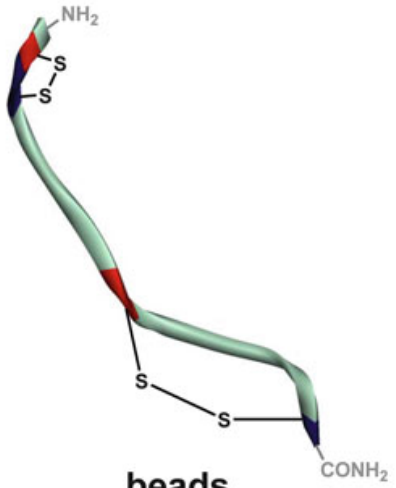




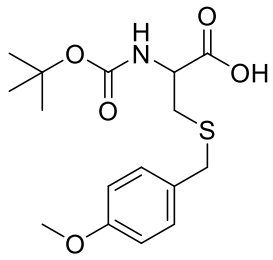
globular



ribbon

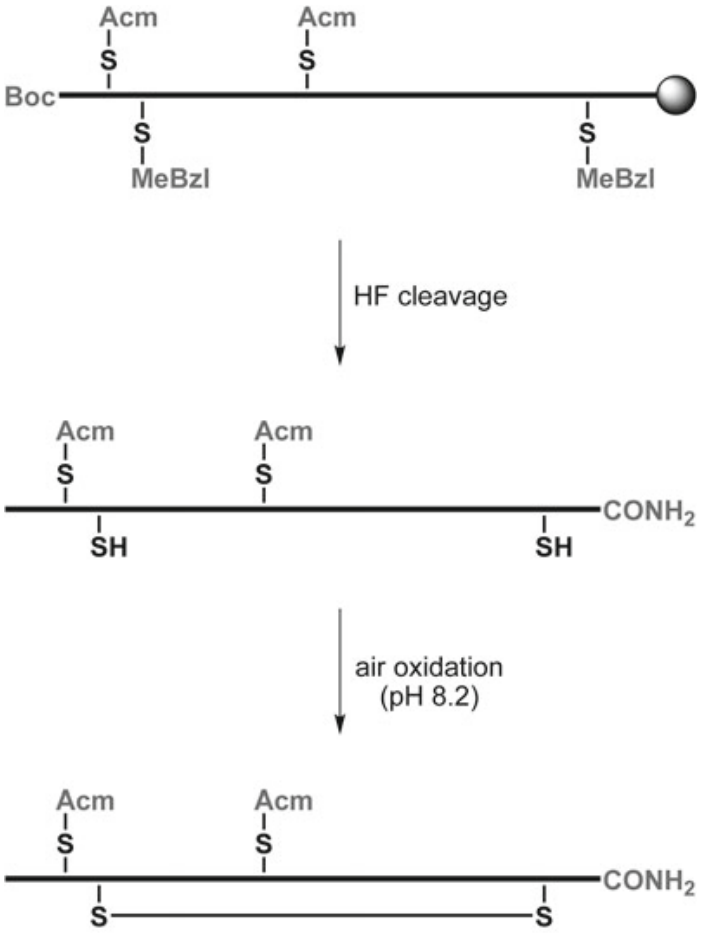


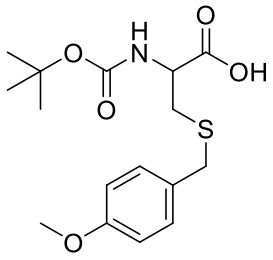
beads



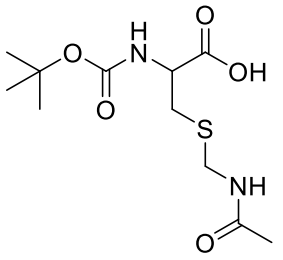
Boc-Cys(MeBzl)-OH

α-Conotoxin synthesis: Directed cyclisation

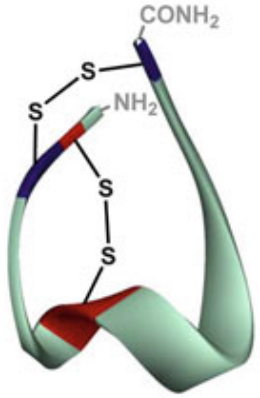




Boc-Cys(MeBzl)-OH

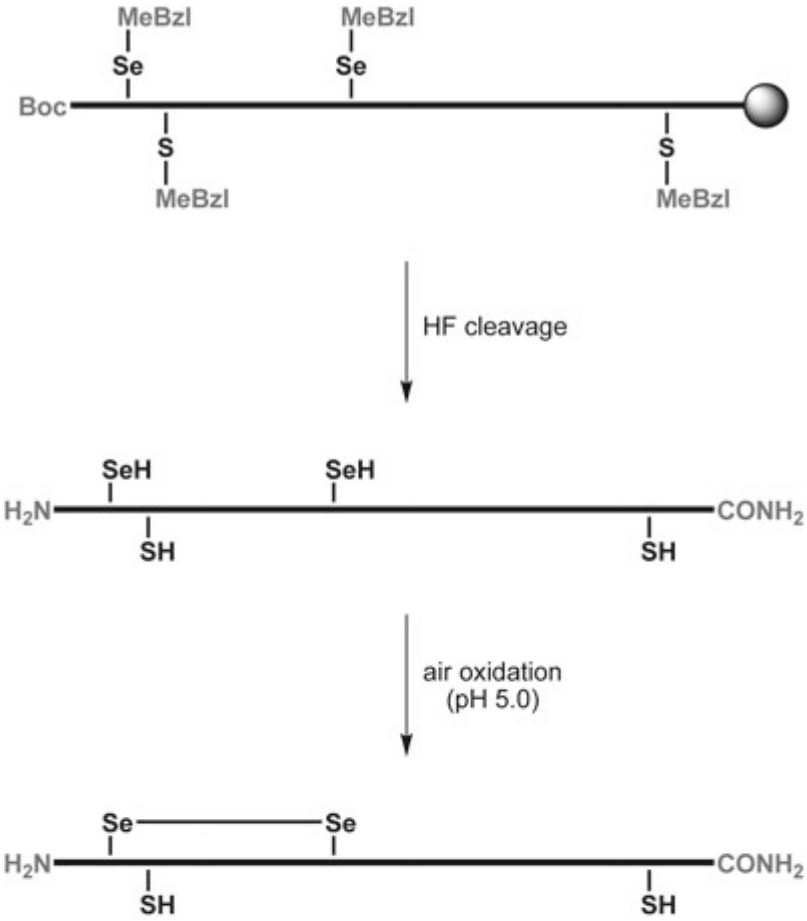


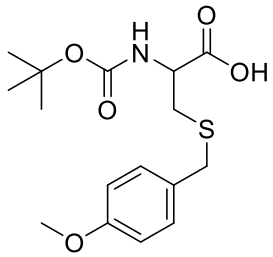
Boc-Cys(Acm)-OH



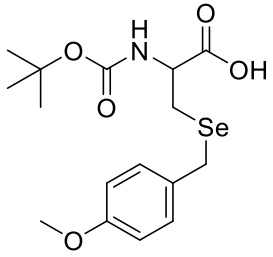
globular

α-Conotoxin synthesis: Selenocysteine directed folding



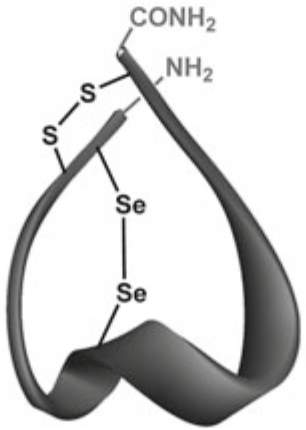


Boc-Cys(MeBzl)-OH

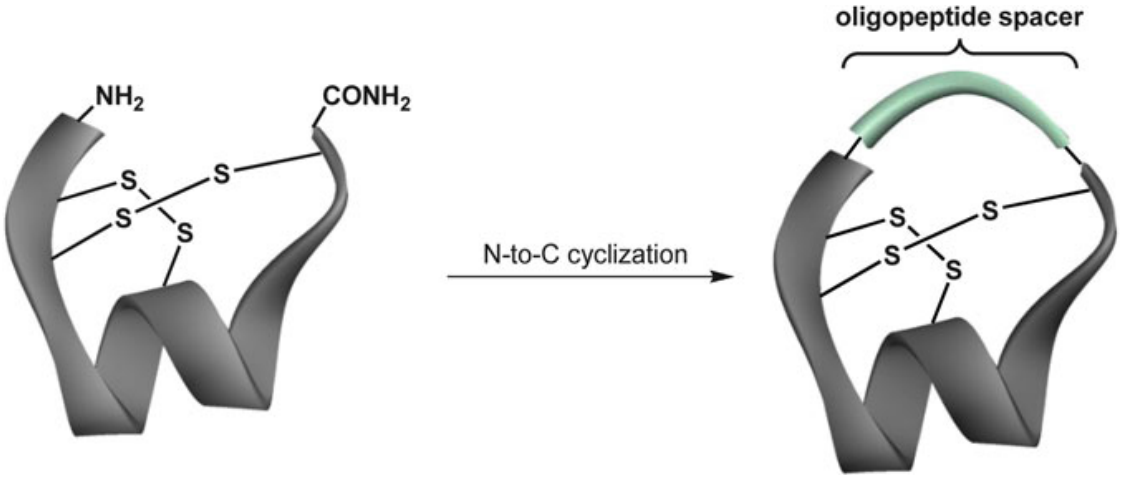


Boc-Sec(MeBzl)-OH

air oxidation
(pH 8.2)



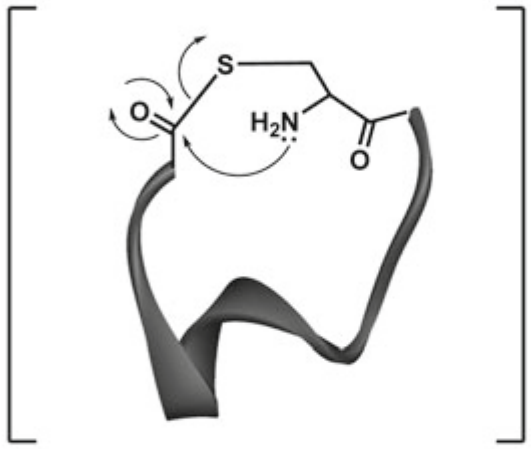
α-Conotoxin synthesis: Head to tail cyclisation



- improved stability
- retained pharmacological activity of the native conotoxin
- loss of flexibility, while preserving key structural characteristics

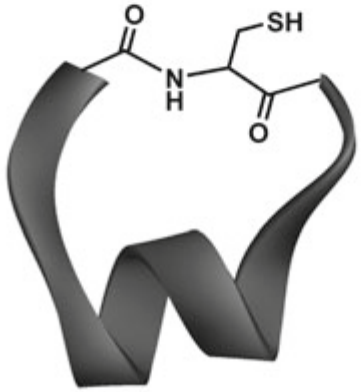
α-Conotoxin synthesis: Head to tail cyclisation

Intramolecular thioester exchange



Spontaneous rearrangement

native chemical ligation



Native peptide amide bond + free cysteine thiol

Chemical synthesis of ubiquitin, ubiquitin-based probes, and diubiquitin

Ubiquitin is a small protein that is found in almost all cellular tissues in humans and other eukaryotic organisms

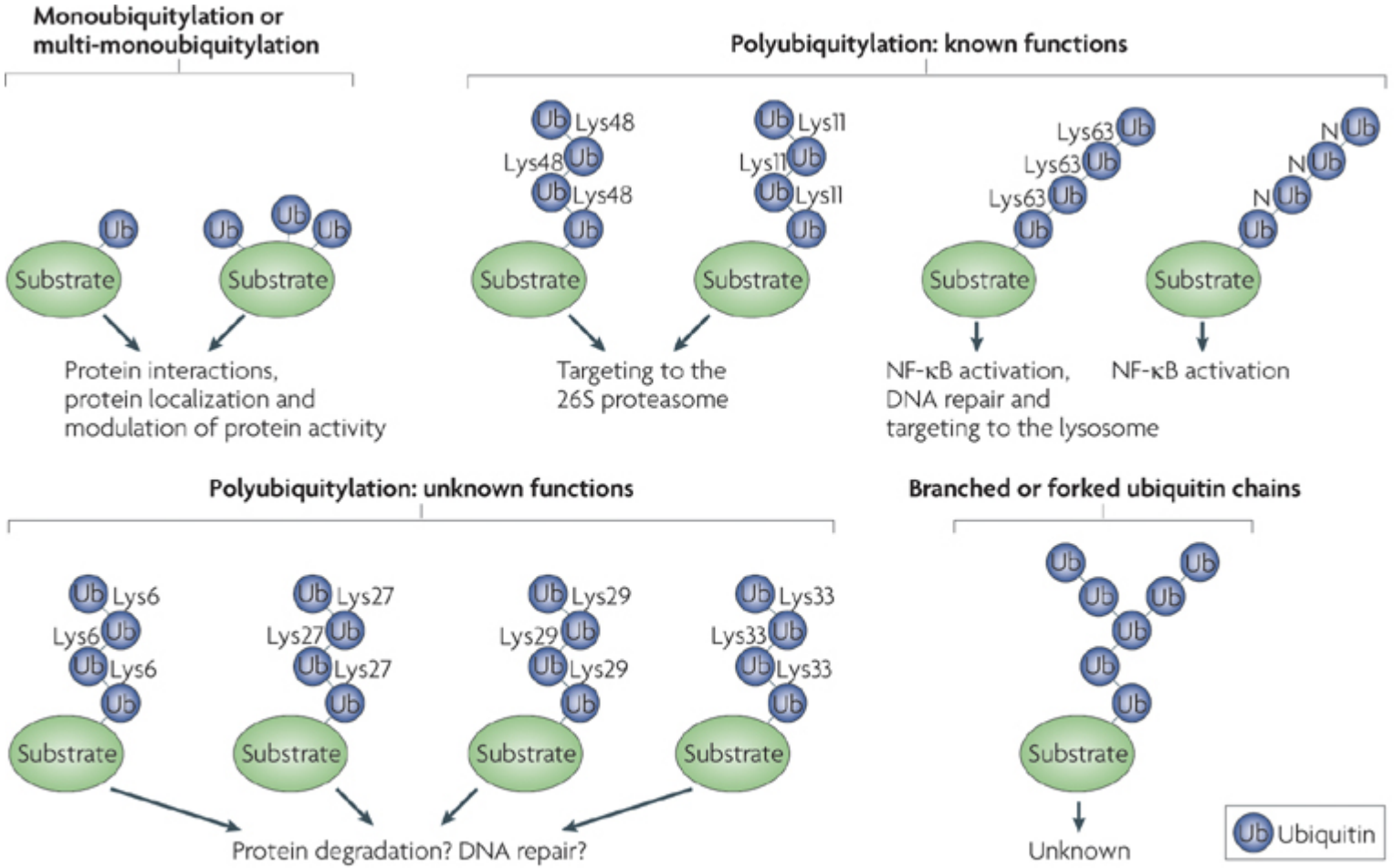
Post-translational modification of proteins with ubiquitin controls many functions including:

- protein breakdown by the proteasome
- cellular localization of proteins
- transcriptional activity
- DNA repair

Sequence of ubiquitin

MQIFV**K**TLTG**K**TITLEVEPSDTIENV**K**A**K**IQD**K**EGIPPDQQRLIFAG**K**QLEDGRTLSDYNIQ**K**ESTLHLVLRLRGG

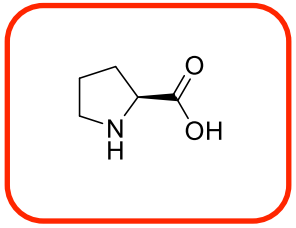
Post-translational modification of proteins with ubiquitin



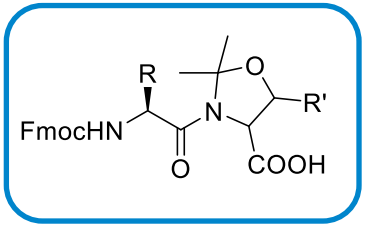
Nature Reviews Molecular Cell Biology **2009**, *10*, 755-764

Chemical synthesis of ubiquitin

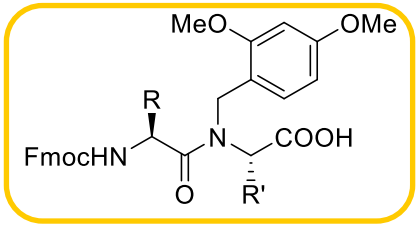
- 76 amino acid protein
- Chemical synthesis required to generate a library of modified protein
- Attempts at Fmoc SPPS failed to give the defined product



proline disrupts β-sheets and α-helices



pseudoproline dipeptide building block



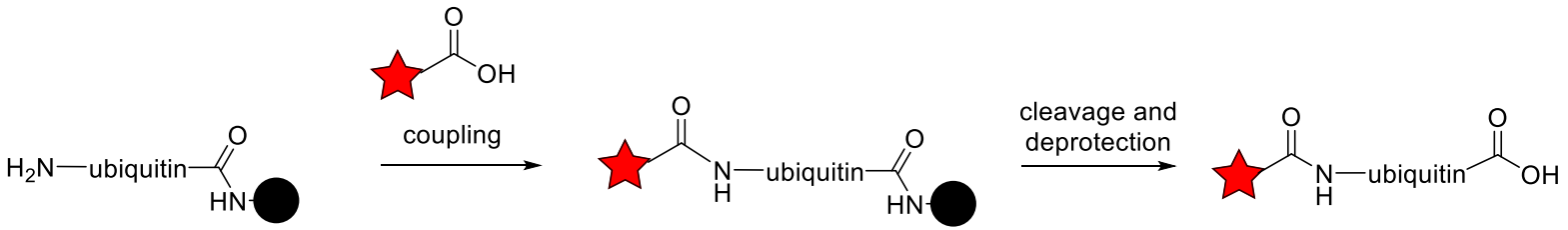
dimethoxybenzyl dipeptide building block

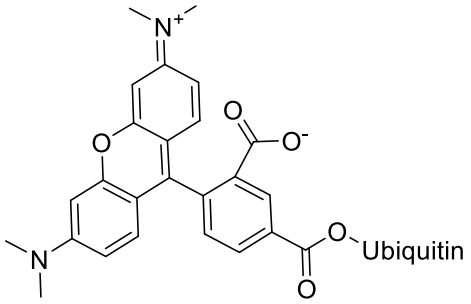
MQIFV**K**T**LT**G**K**T**IT**LEVEPSDTIENV**K**A**K**IQD**K**EGI**PP**DQQRLIF**AG**K**K**QLE**DG**RT**LS**DYNIQ**K**E**ST**LHLVLRLRGG

F. el Oualid et. al. *Angew. Chem. Int. Ed.*, **2010**, *49* (52), 10149–10153

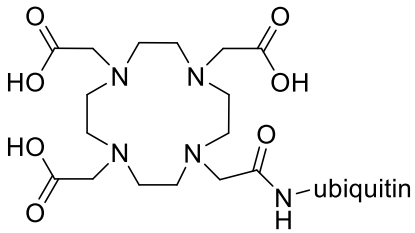
Chemical synthesis of modified ubiquitin

- N- terminus modification can be achieved before cleavage





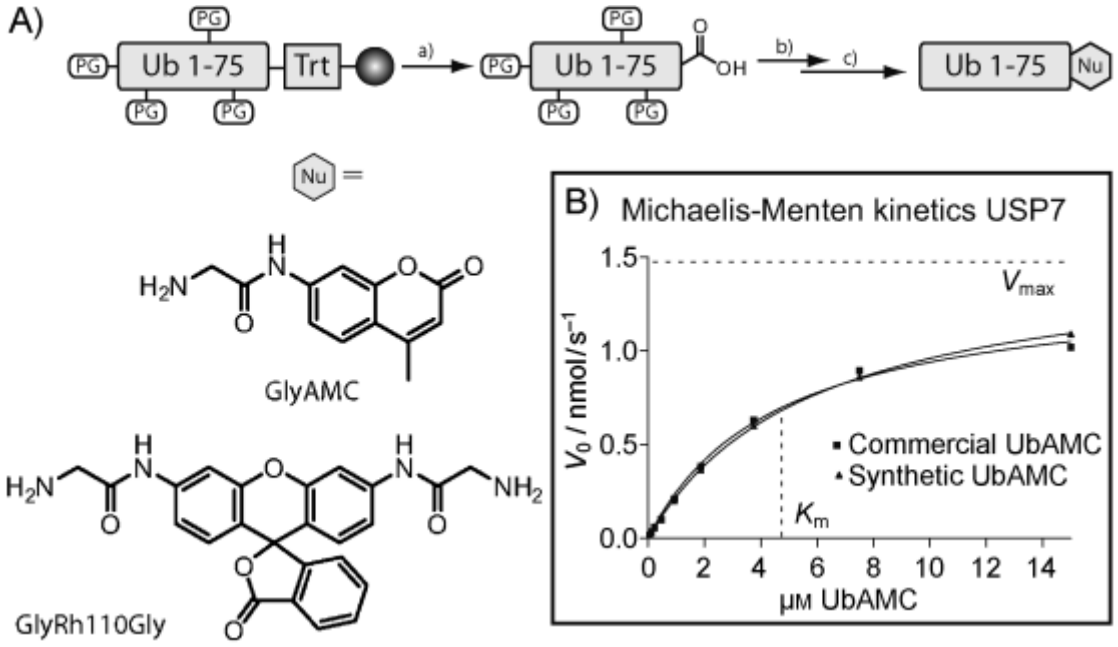
5 TAMRA-Ub
5-carboxytetramethylrhodamine-ubiquitin

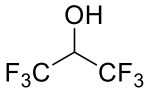


5 DOTA-Ub
1,4,7,10-tetraazacyclododecane-
1,4,7,10-tetraacetic acid-ubiquitin

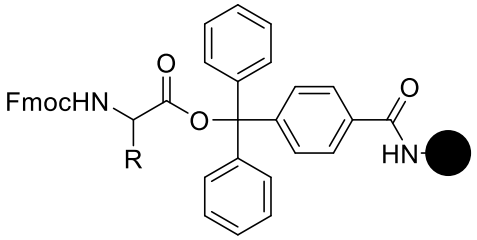
F. el Oualid et. al. *Angew. Chem. Int. Ed.,* **2010***, 49* (52), 10149–10153.

Chemical synthesis of modified ubiquitin





HFIP
Hexafluoro-2-propanol



Trt
acid sensitive trityl resin

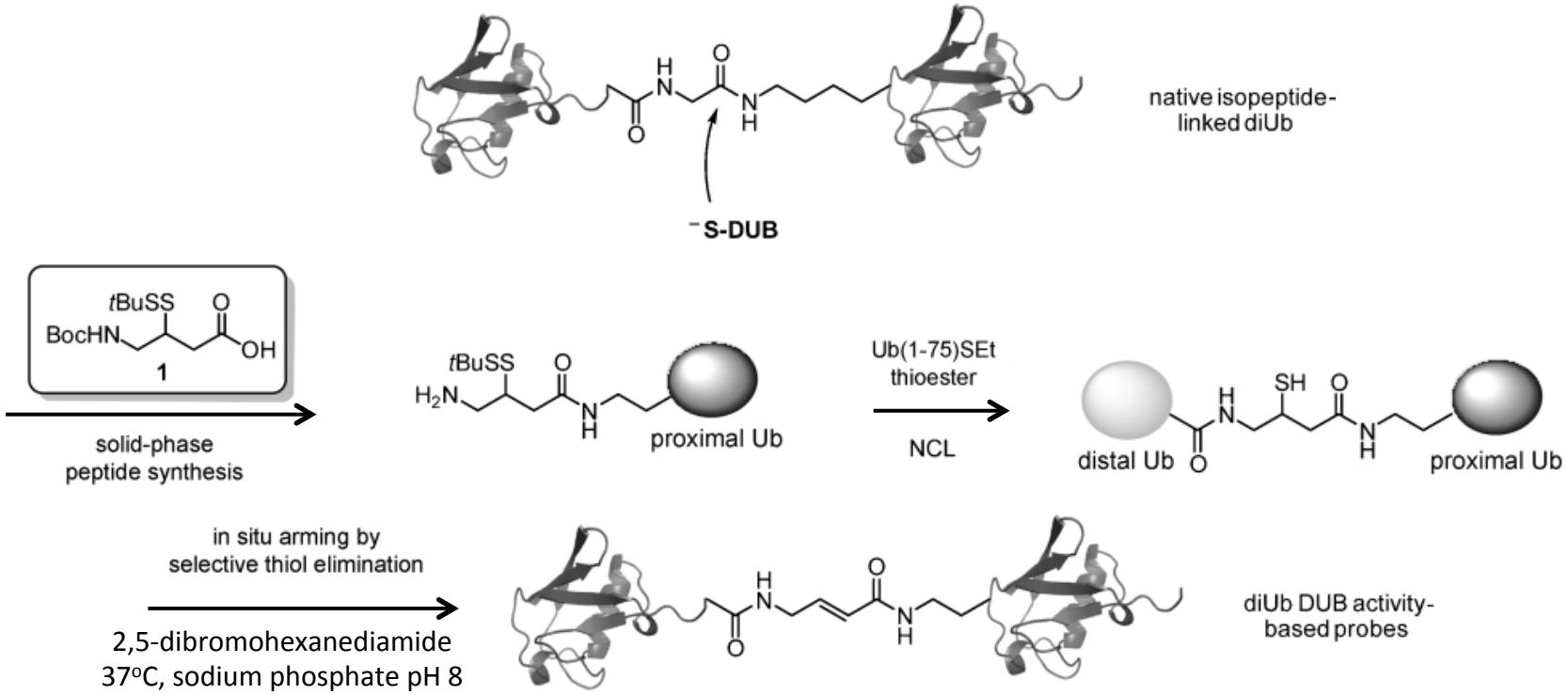
a) HFIP/CH_2Cl_2, 30 min, RT; b) PyBOP, DIPEA, Nu, CH_2Cl_2, 16 h, RT; c) TFA/iPr$_3$SiH/H_2O, 3 h, RT.

F. el Oualid et. al. *Angew. Chem. Int. Ed.,* **2010***, 49* (52), 10149–10153.

Chemical synthesis of a modified diubiquitin probe to measure deubiquitylating enzyme activity

Deubiquitylating enzymes (DUBs) are proteases that remove ubiquitin from a substrate

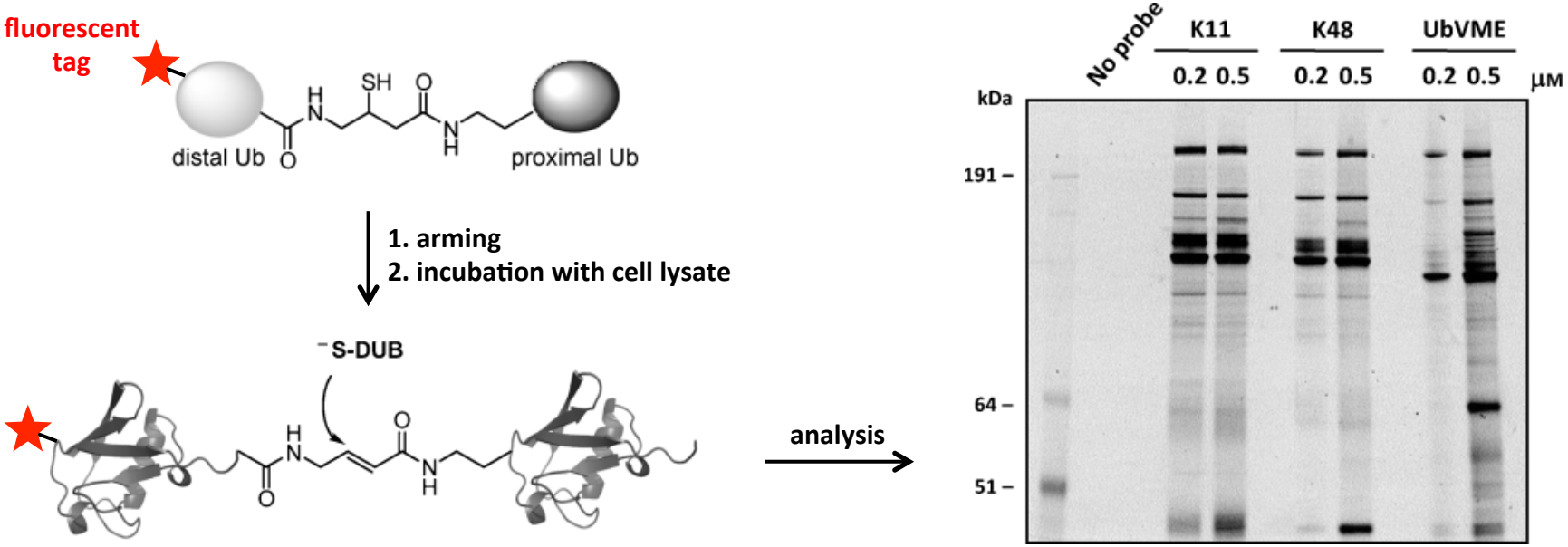
DUBs have a catalytic Cys residue in the active site



M. P. C. Mulder, T. El Oualid, J. ter Beek, H. Ovaa, *ChemBioChem*, **2014**, *15*, 946–949
J. M. Chalker, L. Lercher, N. R. Rose, C. J. Schofield, B. G. Davis, *Angew. Chem. Int. Ed*. **2012**, *51*, 1835–1839

Application of modified diubiquitin probe

- The diubiquitin probe was fluorescently labelled during Fmoc-SPPS
- After arming, the resulting fluorescent probe was incubated with cell lysates and analysed by SDS-PAGE
- The fluorescent intensity of the bands can then be used to measure deubiquitylating enzyme activity



M. P. C. Mulder, T. El Oualid, J. ter Beek, H. Ovaa, *ChemBioChem*, **2014**, *15*, 946–949

Emil Fischer's 1902 Nobel Prize lecture

"Of the chemical aids in the living organism the ferments—mostly referred to nowadays as enzymes– are so pre-eminent that they may justifiably be claimed to be involved in most of the chemical transformations in the living cell. The examination of the synthetic glucosides has shown that the action of the enzymes depends to a large extent on the geometrical structure of the molecule to be attacked, that the two must match like lock and key. Consequently, with their aid, the organism is capable of performing highly specific chemical transformations which can never be accomplished with the customary agents. To equal Nature here, the same means have to be applied, and I therefore foresee the day when physiological chemistry will not only make extensive use of the natural enzymes as agents, but when it will also prepare synthetic ferments for its purposes."

Fischer's vision is becoming reality