AMINO ACIDS CHEMISTRY

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Amino acids chemistry

LD-1

Chemistry and Biology. Chirality. α-world

Secondary structures of α-peptides

Chemistry: Protecting groups

Chemistry: Peptide Coupling. Solid-phase synthesis

LD-2

Unnatural world

β-amino acids and higher homologues of α-amino acids

Secondary structures of β and γ-peptides

Homologation of α-amino acids

LD-3

Unnatural world

Sulfur and Selenium containing unnatural amino acids

Proline based unnatural amino acids

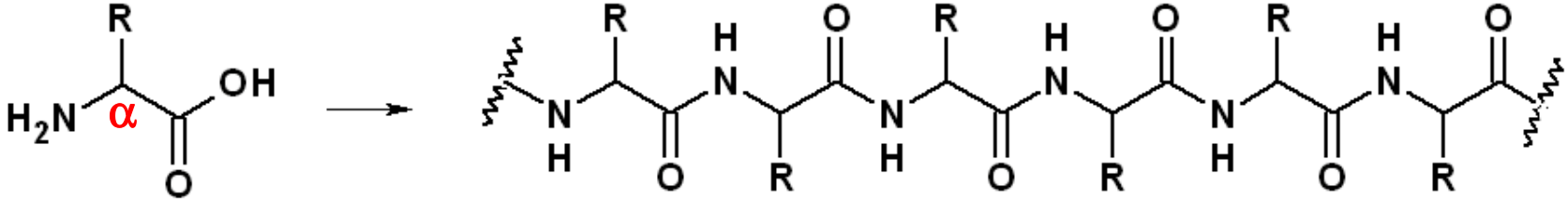
Cystathionine and Homocysteine families

LD-4

Peptidomimetics Chemistry

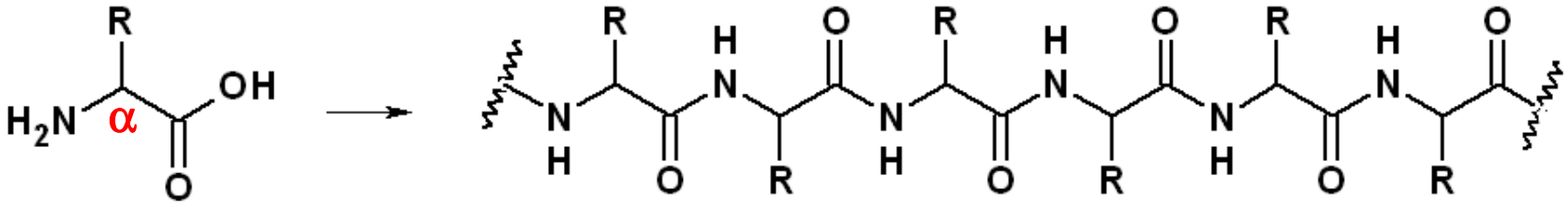
Protein Synthesis: Chemical Ligation

Chemistry and Biology



Chemistry and Biology

α-world

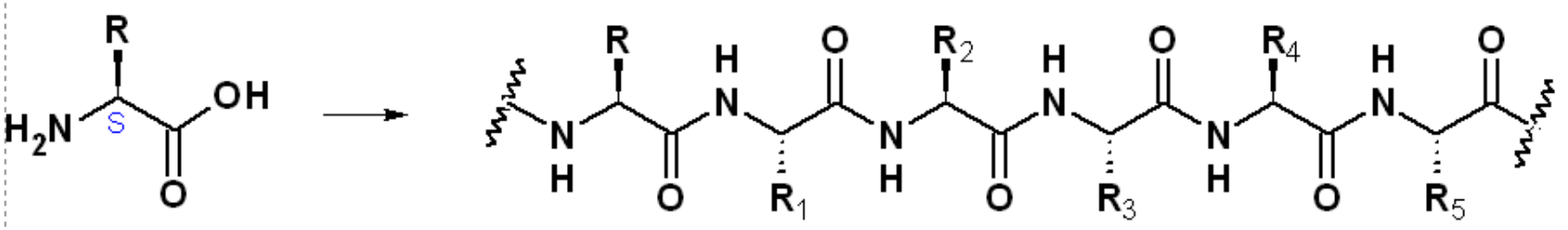


Metabolism

Structure and functions

Chemistry and Biology

α-world



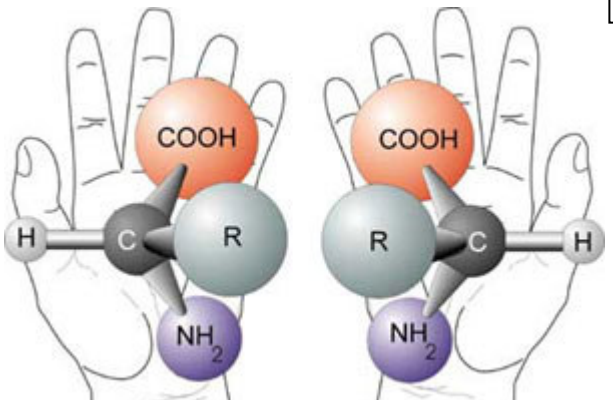
- 20 R have been found in protein
  (22 with selenocysteine and pyrrolysine)

- α-Carbon stereochemistry: (*S*) in protein
  Both configuration in many natural products

Chemistry and Biology

α-world

Life and chirality



nature chemistry

ARTICLES

PUBLISHED ONLINE: 1 NOVEMBER 2009 | DOI: 10.1038/NCHEM.416

Complete chiral symmetry breaking of an amino acid derivative directed by circularly polarized light

Wim L. Noorduin[1], Arno A. C. Bode[1], Maarten van der Meijden[2], Hugo Meekes[1], Albert F. van Etteger[1], Willem J. P. van Enckevort[1], Peter C. M. Christianen[1], Bernard Kaptein[3], Richard M. Kellogg[2], Theo Rasing[1] and Elias Vlieg[1]*

Chemistry and Biology

α-world

Life and chirality

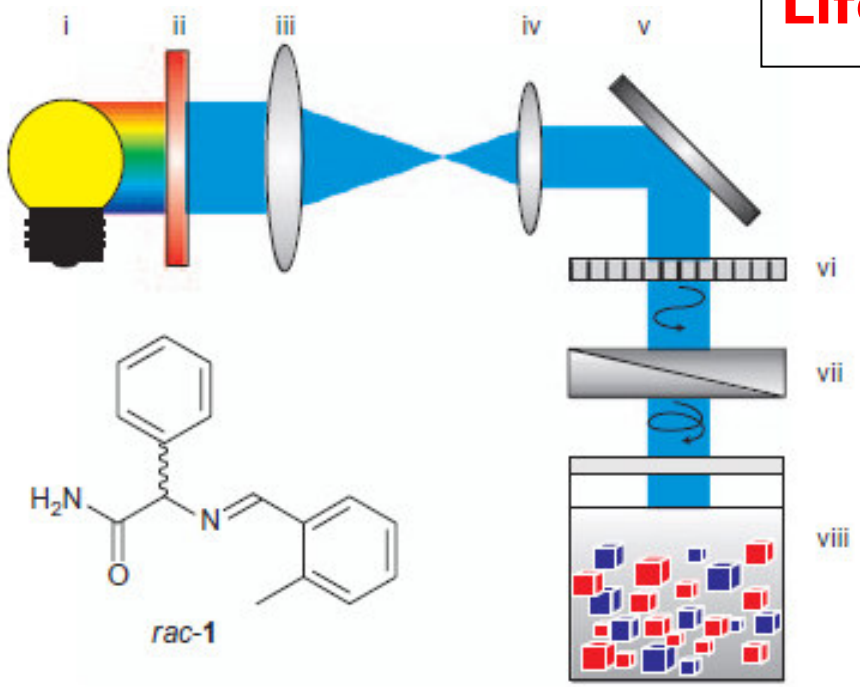


Figure 1 | Experimental set-up for CPL-driven deracemization. Light from a 200 W Hg (Xe) light source (i) is filtered through a 300–400 nm filter (ii), then focused using two quartz lenses (iii, iv). The focused light is then directed using an ultraviolet mirror (v) through a linear polarizer (vi), followed by a Babinet-Soleil compensator (vii) to generate either *r*- or *l*-CPL. The light is used to irradiate solutions and/or suspensions of compound **1** in a reaction vessel (viii) with a quartz window at the top. Inset shows the structural formula of **1**.

Chemistry and Biology

α-world

Life and chirality

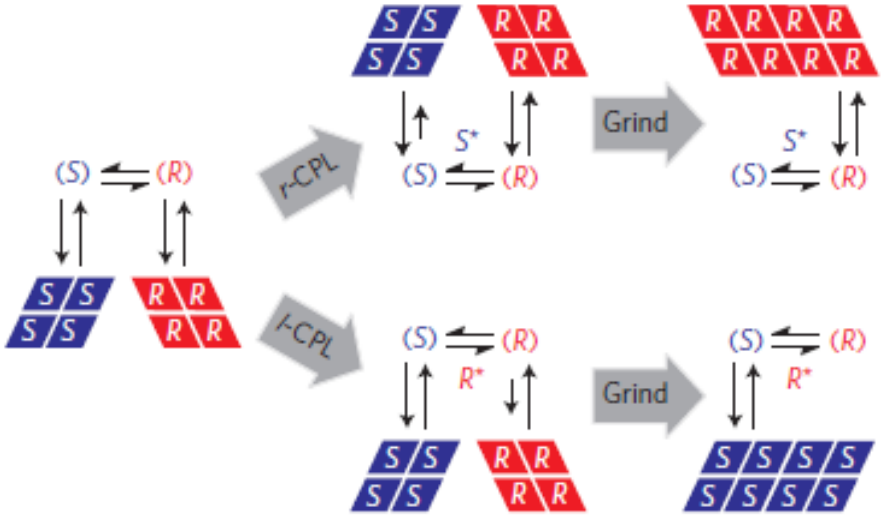


Figure 3 | Cascade of events during the CPL-directed deracemization of racemic 1. Initially, dissolved enantiomers of **1**, represented by (*R*) and (*S*), are in equilibrium with the corresponding crystal phase. During the *l*-CPL irradiation of a solution–solid mixture of racemic **1** an enantioselective growth inhibitor *R** is formed (lower route). This compound *R** enantioselectively hampers the growth of (*R*)-**1** crystals so that the solid phase enriches in (*S*)-**1**. This chiral imbalance is fully amplified on abrasive grinding, which results in the complete deracemization of racemic **1** to give a solid phase of (*S*)-**1**. A similar, but opposite, process occurs on *r*-CPL irradiation (upper route).

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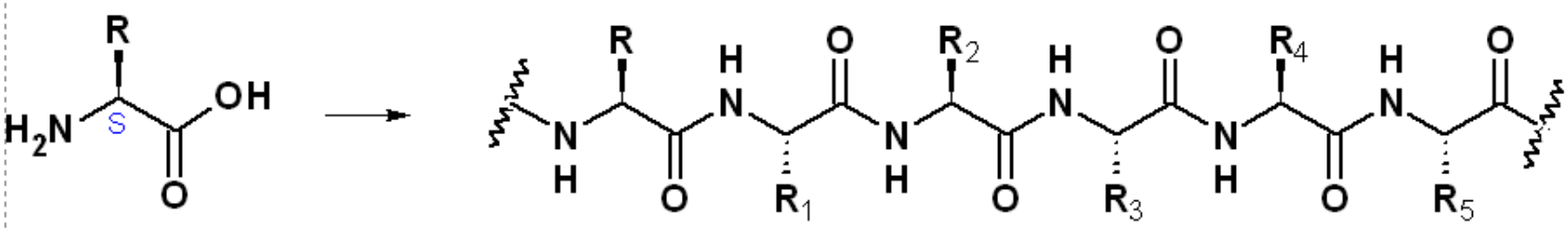
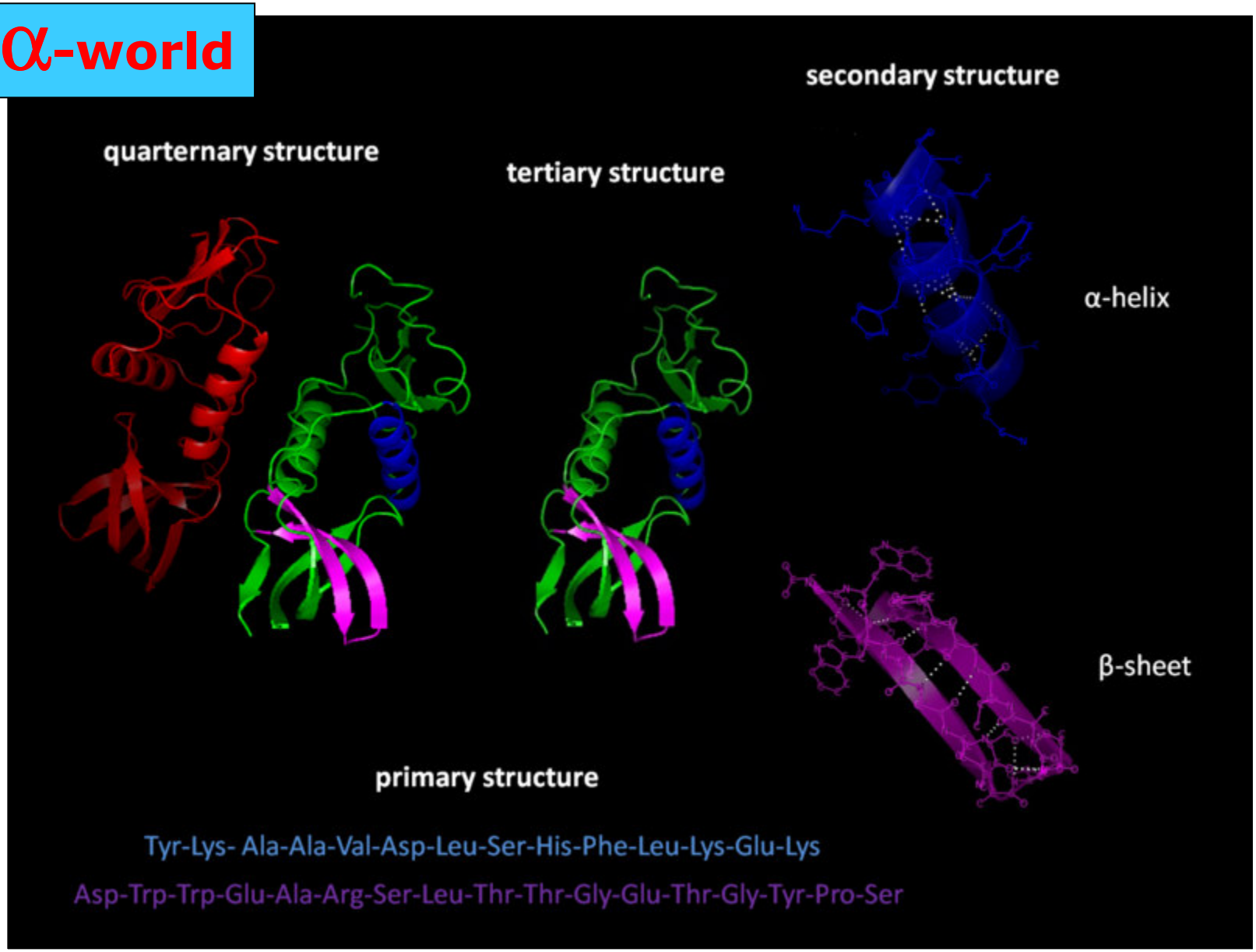
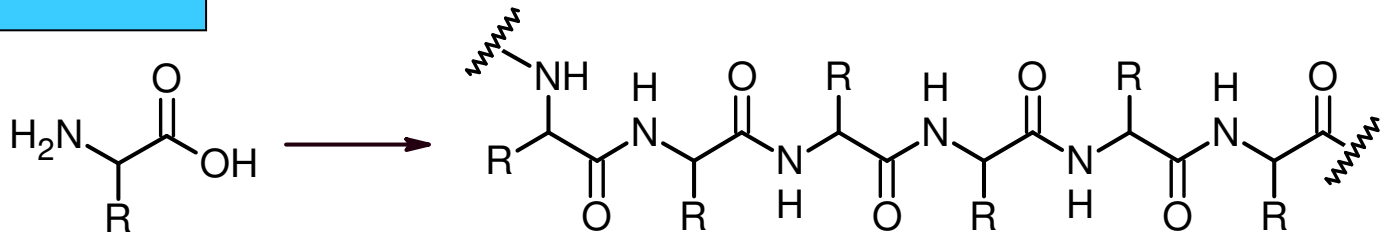


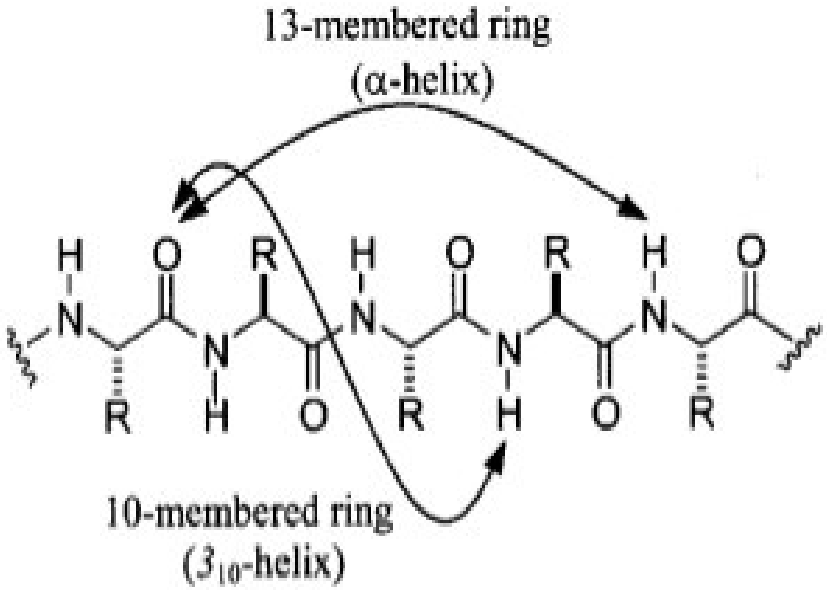
Chemistry and Biology



Chemistry and Biology

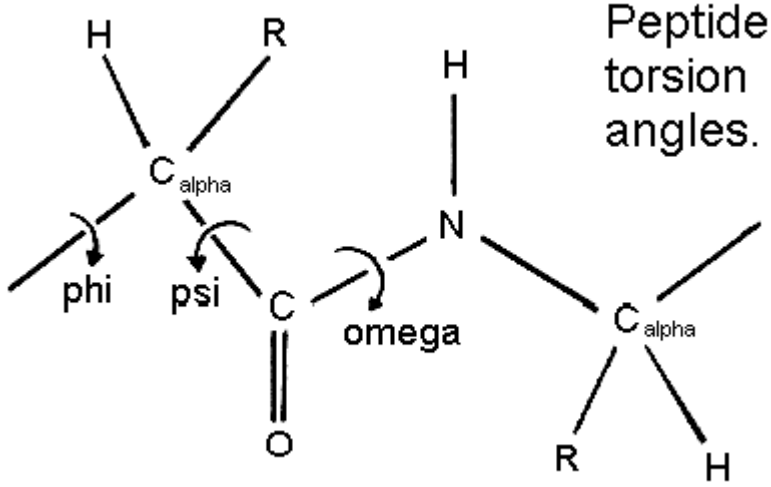
α-world

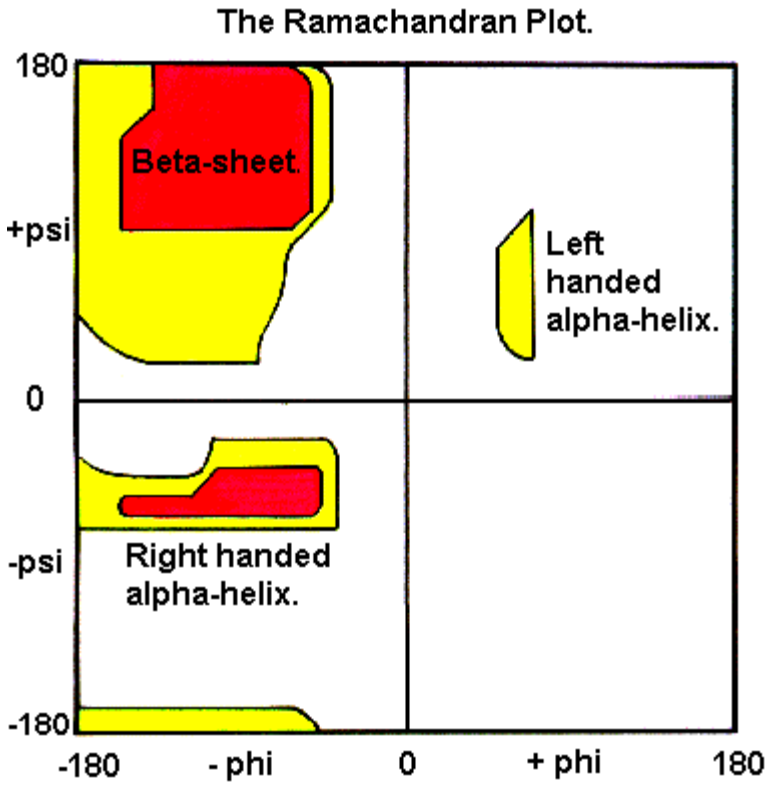




H-bond patterns associated with helical secondary structures in α-polypeptides

α-world


H
R
C alpha
phi
psi
C
O
omega
H
N
C alpha
R
H
Peptide torsion angles.


The Ramachandran Plot.
180
+psi
0
-psi
-180
-180
- phi
0
+ phi
180
Beta-sheet.
Left handed alpha-helix.
Right handed alpha-helix.

Chemistry and Biology

α-world

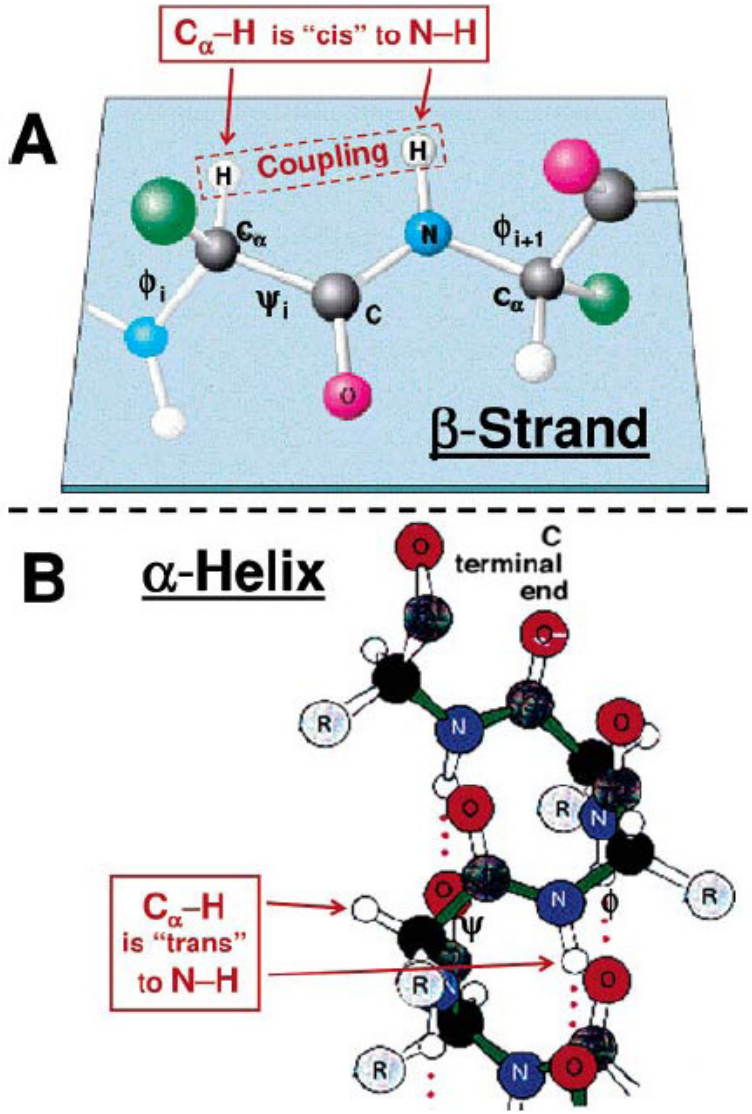
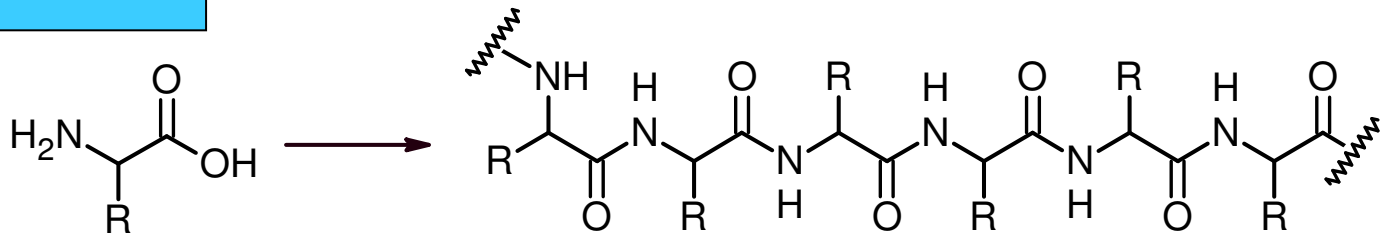


Figure 1. Model of a polypeptide chain (A) at β-strand-like and (B) at α-helix-like Ψ and Φ Ramachandran angles. The distance between C_α–H and N–H hydrogens depends on the Ψ Ramachandran angle.

Chemistry and Biology

α-world

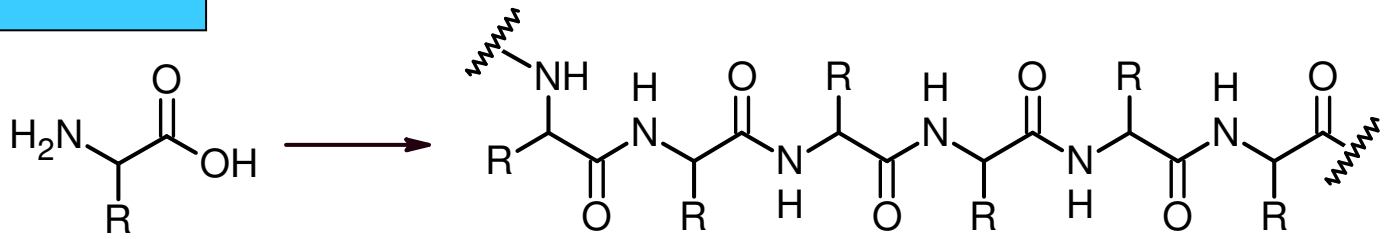


$$\#\text{protein} = (n)^{L}$$

n = # AA
L = protein length

Chemistry and Biology

α-world



$$\#\text{protein} = (n)^{L}$$

n = # AA
L = protein length

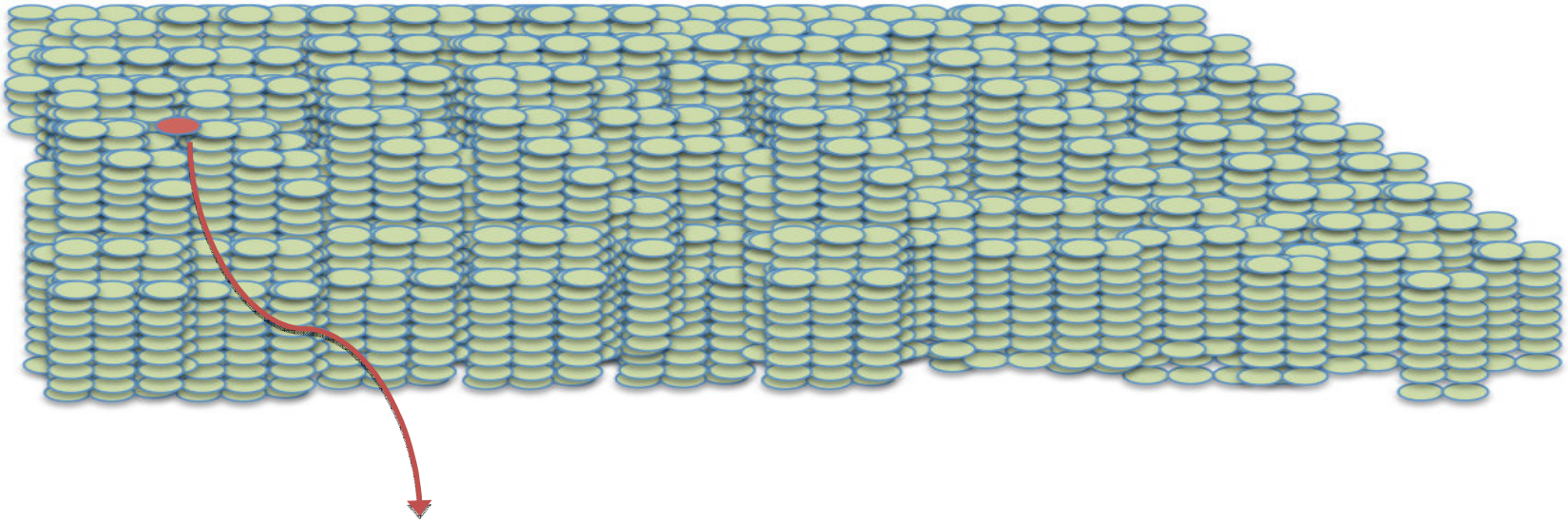
n = 20
L = 100 ➔ 20^{100} ➔ 10^{130} protein

Complex organism know < 10^{6} ?

Chemistry and Biology

α-world

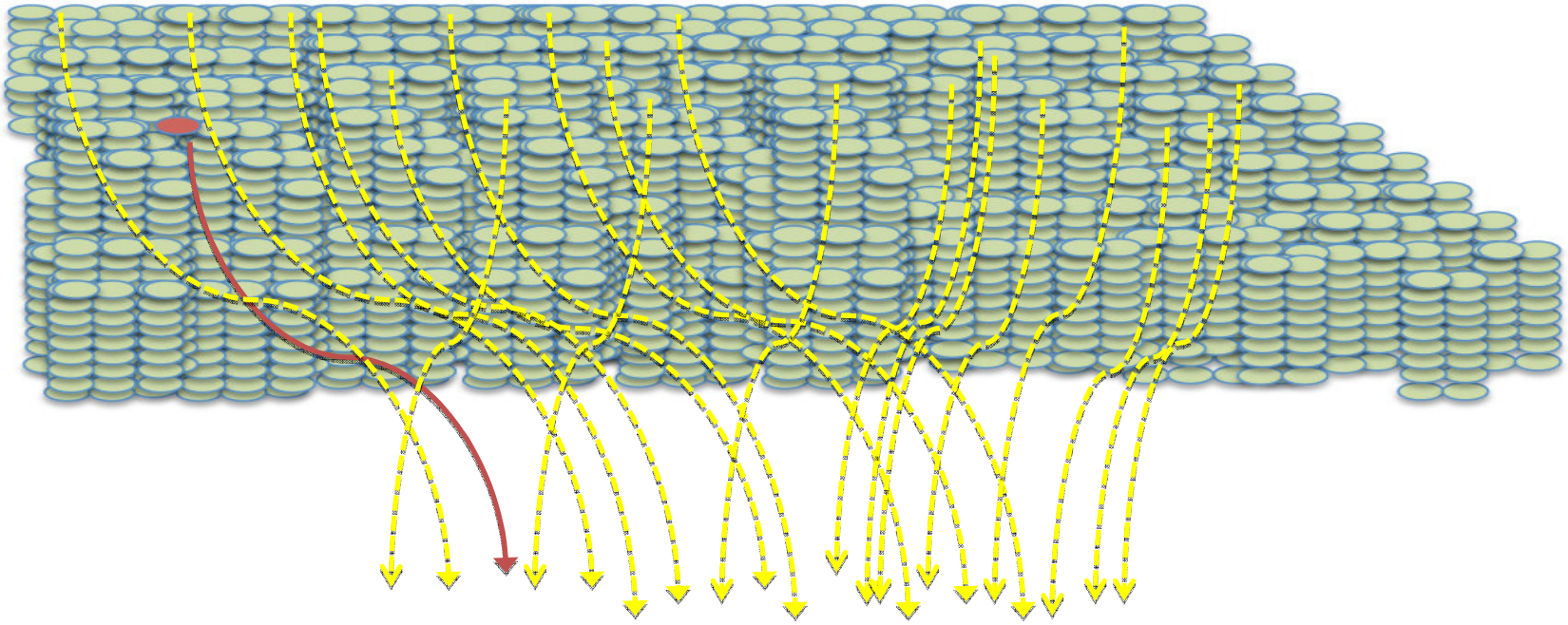
n = 20
L = 100 ➔ 10^{130} protein



Chemistry

α-world

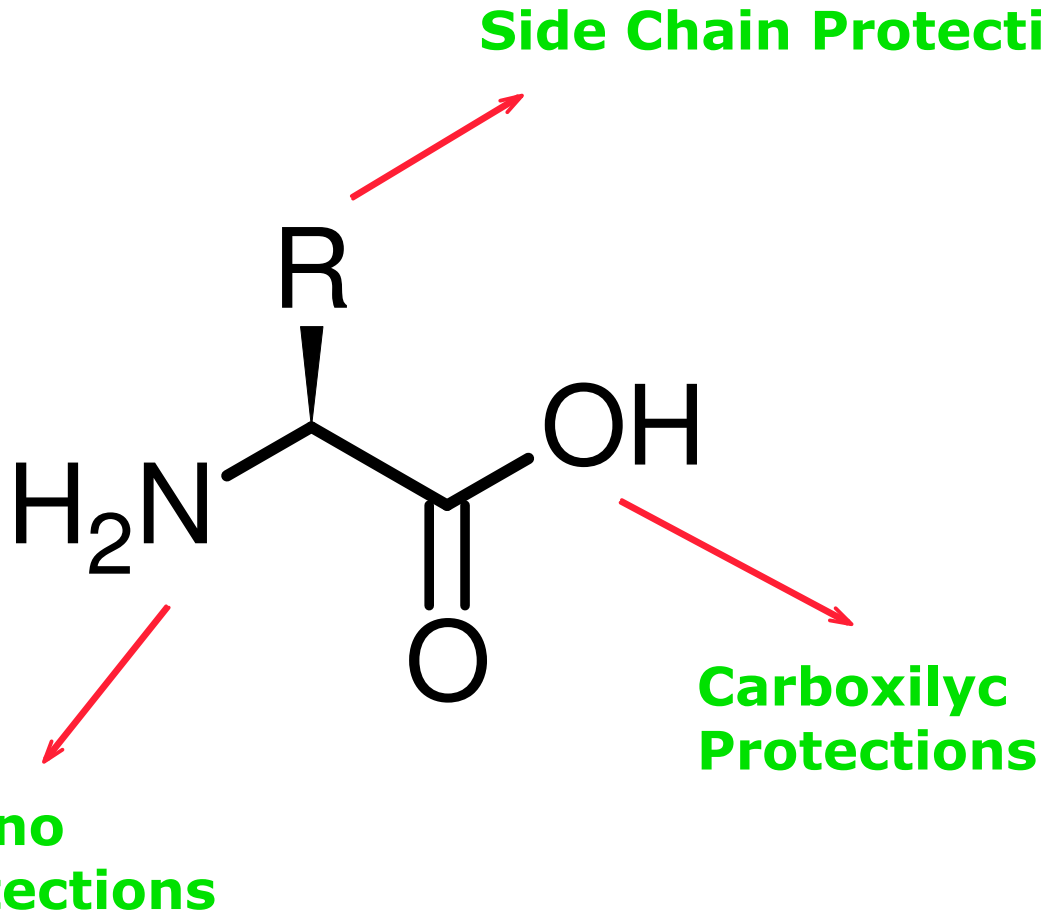
n = 20
L = 100 ➔ 10^{130} protein



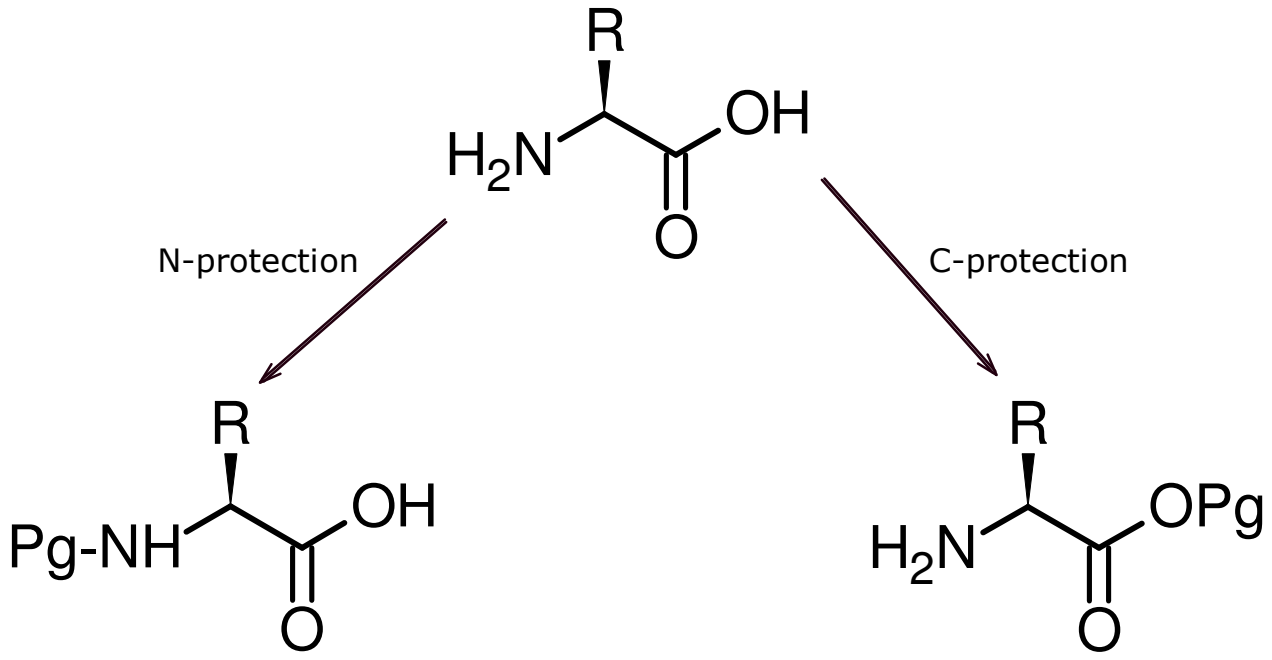
Accessible by chemical synthesis

- **SPPS Methods**
- **Chemical Ligation**

Protecting Groups



Protecting Groups

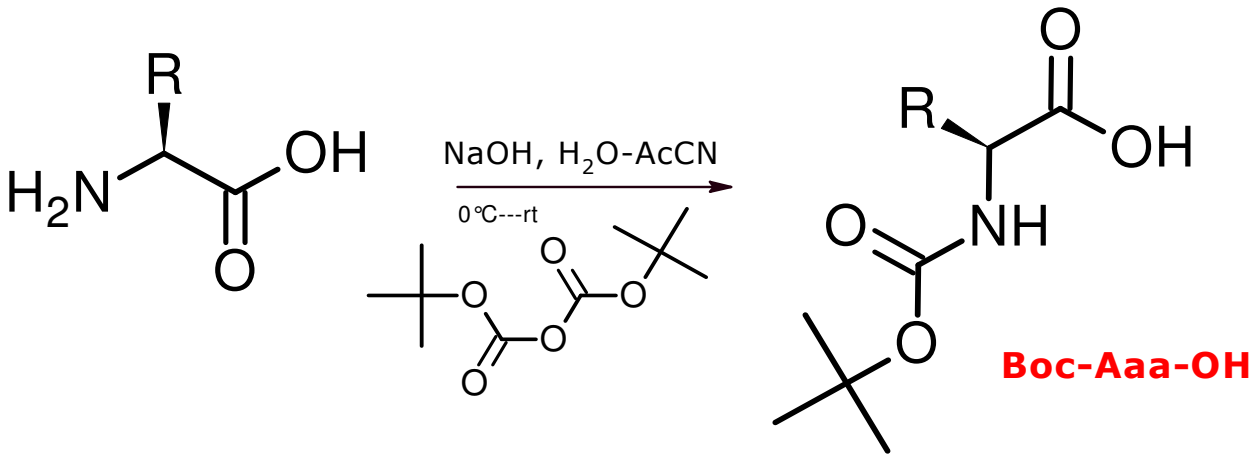


equivalent to free carboxylic acid

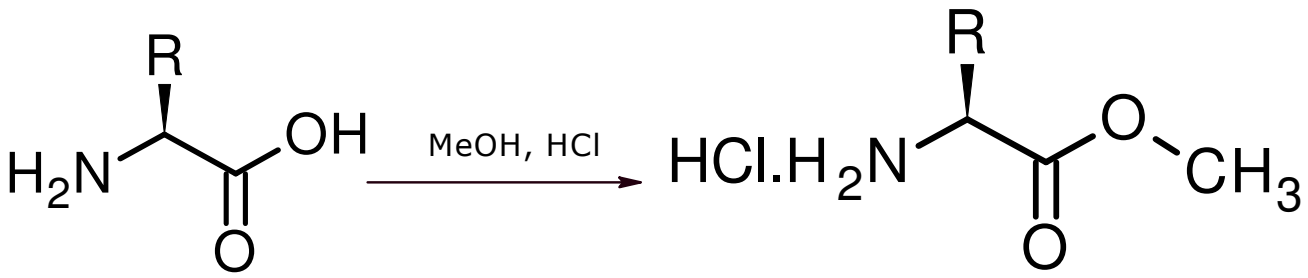
equivalent to free amine

Chemistry

Protecting Groups



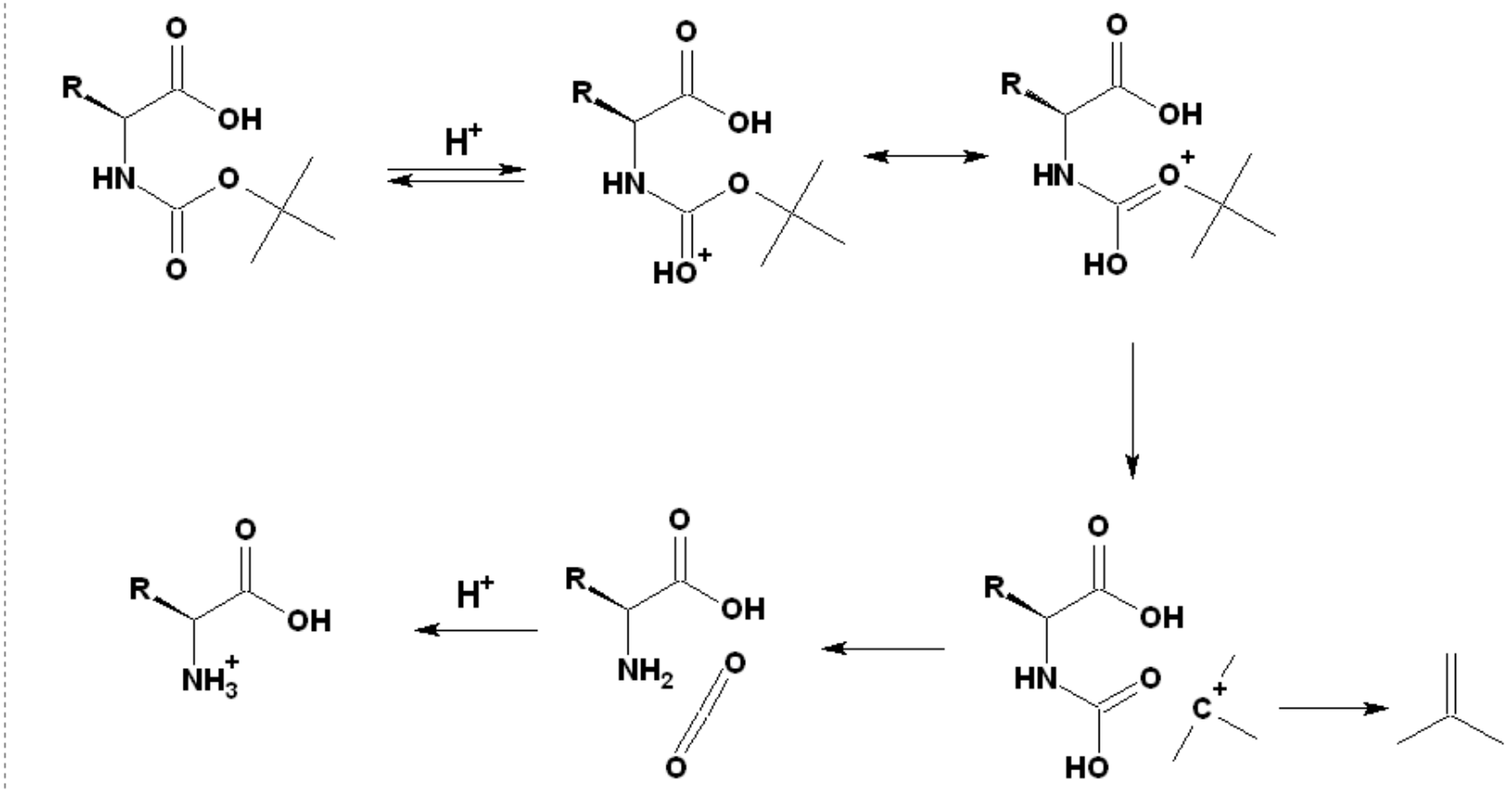
Boc-Aaa-OH



H-Aaa-OMe·HCl

Protecting Groups

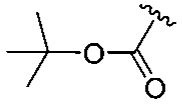
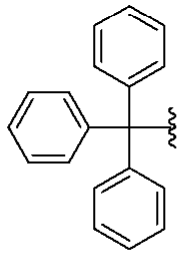
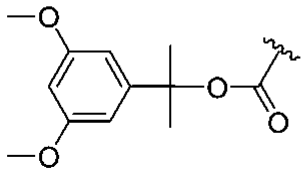
Boc deprotection



Chemistry

Protecting Groups

Table. Amino-Protecting Groups Removed by Acid

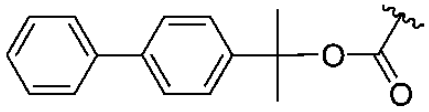
Name and Structure	Removal conditions	Stability to the removal of
tert-Butyloxycarbonyl (Boc) 	1) 25-50% TFA-DCM 2) 4 M HCl in dioxane 3) 2 M $MeSO_3H$ in dioxane 4) 1 M TMS-Cl, 1 M phenol-DCM	Fmoc, Z,[a] Trt, Alloc, *p*NZ
Trityl (Trt) 	1) 1% TFA-DCM 2) 0.1 M HOBt-TFE 3) 0.2% TFA, 1% H_2O-DCM 4) 3% TCA-DCM	Fmoc, Alloc
3,5-Dimethoxyphenylisoproxycarbonyl (Ddz) 	1-5% TFA-DCM	Fmoc, Alloc

Chem. Rev. 2009, 109, 2455–2504

Chemistry

Protecting Groups

Table. Amino-Protecting Groups Removed by Acid

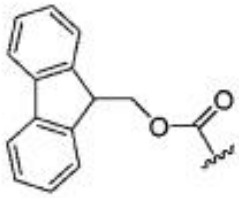
Name and Structure	Removal conditions	Stability to the removal of
2-(4-Biphenyl)isopropoxycarbonyl (Bpoc) 	0.2-0.5%-TFA	Fmoc, Alloc
2-Nitrophenylsulfenyl (Nps) 	1) Diluted solutions of HCl-$CHCl_3$-AcOH 2) 2-Mercaptopyridine-AcOH-MeOH, DMF or DCM 3) Ni Raney column in DMF	Fmoc

Chem. Rev. 2009, 109, 2455–2504

Chemistry

Protecting Groups

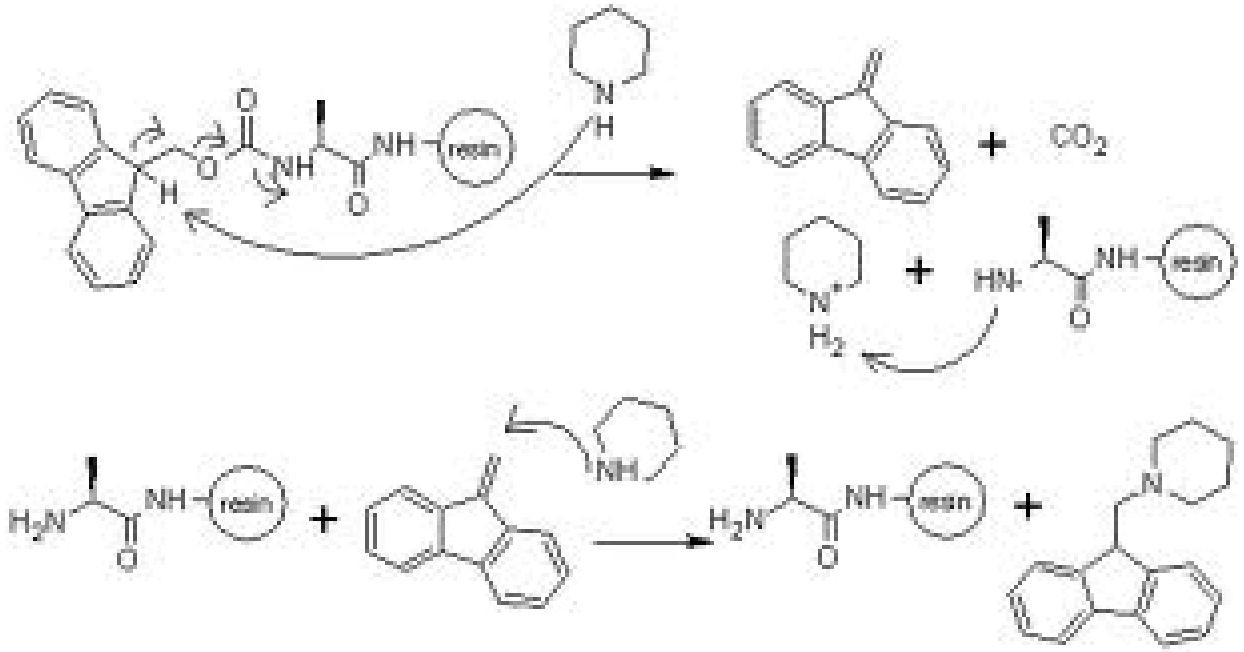
Table. Amino-Protecting Groups Removed by Base

Name and Structure	Removal conditions	Stability to the removal of
9-Fluorenylmethoxycarbonyl (Fmoc) 	Solid phase: 1) 20% piperidine-DMF 2) 1-5% DBU-DMF 3) morpholine-DMF (1:1) 4) 2% HOBt, 2% hexamethyleneimine, 25% *N*-methylpyrrolidine in DMSO-NMP (1:1) Solution: 1) NH_3 (10 h) 2) morpholine or piperidine in organic solvents (within minutes) 3) 10% DEA,DMA (2 h) 4) polymeric secondary amines (i.e. piperidine, piperazines) in organic solvents	Boc, Z,[a] Trt, Alloc, *p*NZ [a]

Chemistry

Protecting Groups

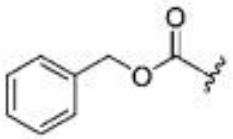
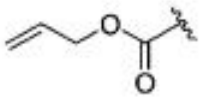
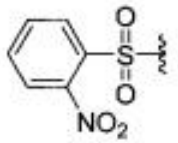
Fmoc deprotection



Chemistry

Protecting Groups

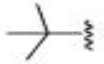
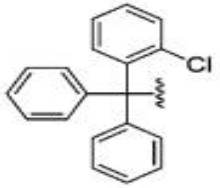
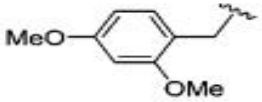
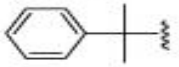
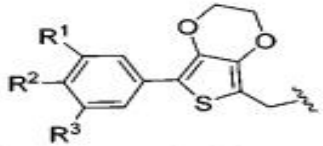
Table. Other Amino-Protecting Groups

Name and Structure	Removal conditions	Stability to the removal of
Benzyloxycarbonyl (Z) 	1) H_2 cat 2) Strong acids such as: HBr in AcOH, TFA at high temperatures, TFA-thioanisole or liquid HF 3) BBr_3	Boc, Fmoc, Trt, Alloc, *p*NZ [a]
Allyloxycarbonyl (Alloc) 	$Pd(PPh)_3$ cat., scavengers: $H_3N{\cdot}BH_3$, $Me_2NH{\cdot}BH_3$ or $PhSiH_3$ in organic solvents	Boc, Fmoc, Trt, *p*NZ [a]
o-Nitrobenzenesulfonyl (oNBS) 	1) 5% thiophenol-DMF, 2 eq. of K_2CO_3 (primary amines) 2) β-mercaptoethanol and DBU (secondary amines)	Boc, Fmoc, Trt

Chemistry

Protecting Groups

Table. Carboxylic Acid-Protecting Groups Removed by Acid

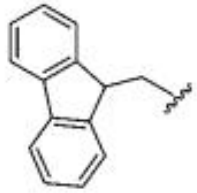
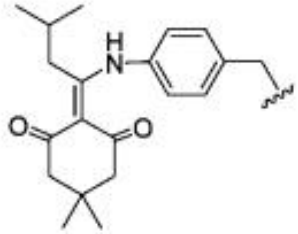
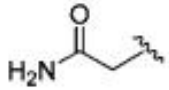
Name and Structure	Removal conditions	Stability to the removal of
tert-Butyl (^{t}Bu) 	90% TFA-DCM (solid phase and solution) or 4 M HCl in dioxane (solution)	Fmoc, Z,[a] Trt Alloc, *p*NZ,
2-Chlorotrityl (2-Cl-Trt) 	1% TFA-DCM	Fmoc, Alloc
2,4-Dimethoxybenzyl (Dmb) 	1% TFA-DCM	Fmoc, Alloc
2-Phenylisopropyl (2-Ph^{i}Pr) 	4% TFA-DCM	Fmoc, Alloc
5-Phenyl-3,4-ethylenedioxythenyl (Phenyl-EDOTn)  R^1=R^2=R^3= OMe; R^1=R^2= OMe, R^3= H; R^1=R^2= H, R^3= OMe or R^1=R^2=R^3= H.	0.01%-0.5% TFA-DCM and scavengers	Fmoc

lgl12LD1

Chemistry

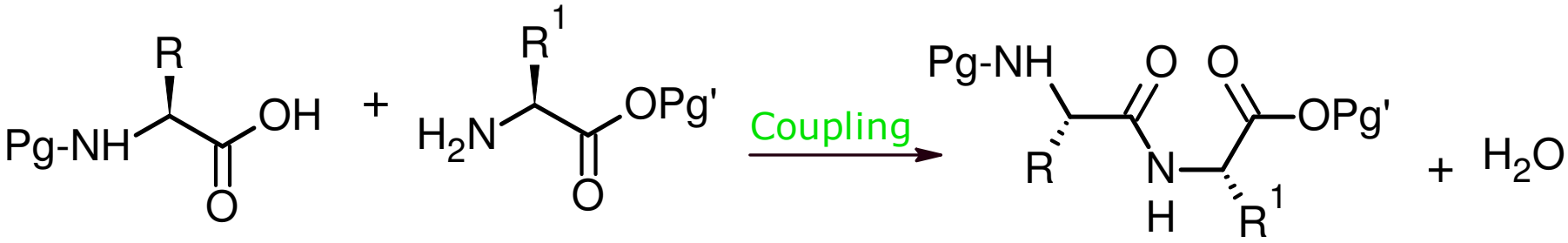
Protecting Groups

Table. Carboxylic Acid-Protecting Groups Removed by Base

Name and Structure	Removal conditions	Stability to the removal of
9-Fluorenylmethyl (Fm) 	15% DEA or 20% piperidine-DMF or DCM	Boc, Trt, Alloc
4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino)benzyl (Dmab) 	2% hydrazine-H_2O-DMF (1:1)	Boc, Fmoc, Trt,
Methyl (Me) and Ethyl (Et)	LiOH, NaOH or KOH	Boc, Z
Carbamoylmethyl (Cam) 	NaOH or Na_2CO_3-DMF-H_2O	Boc, Fmoc [a] Z^b

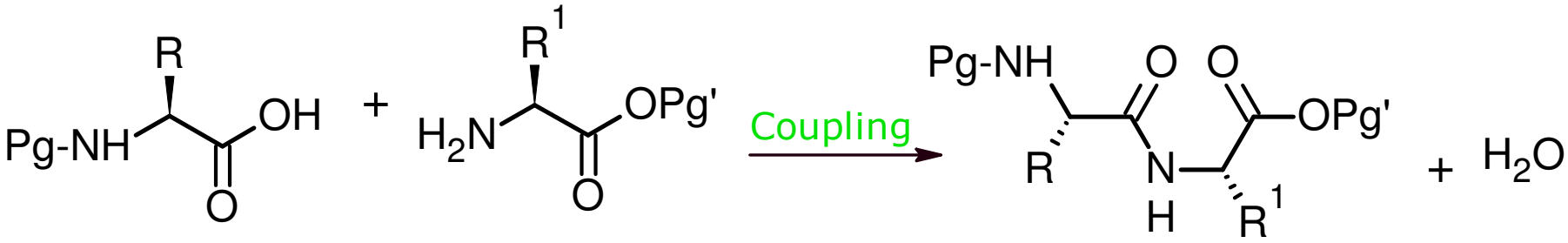
Peptide Coupling

Solution synthesis



Peptide Coupling

Solution synthesis



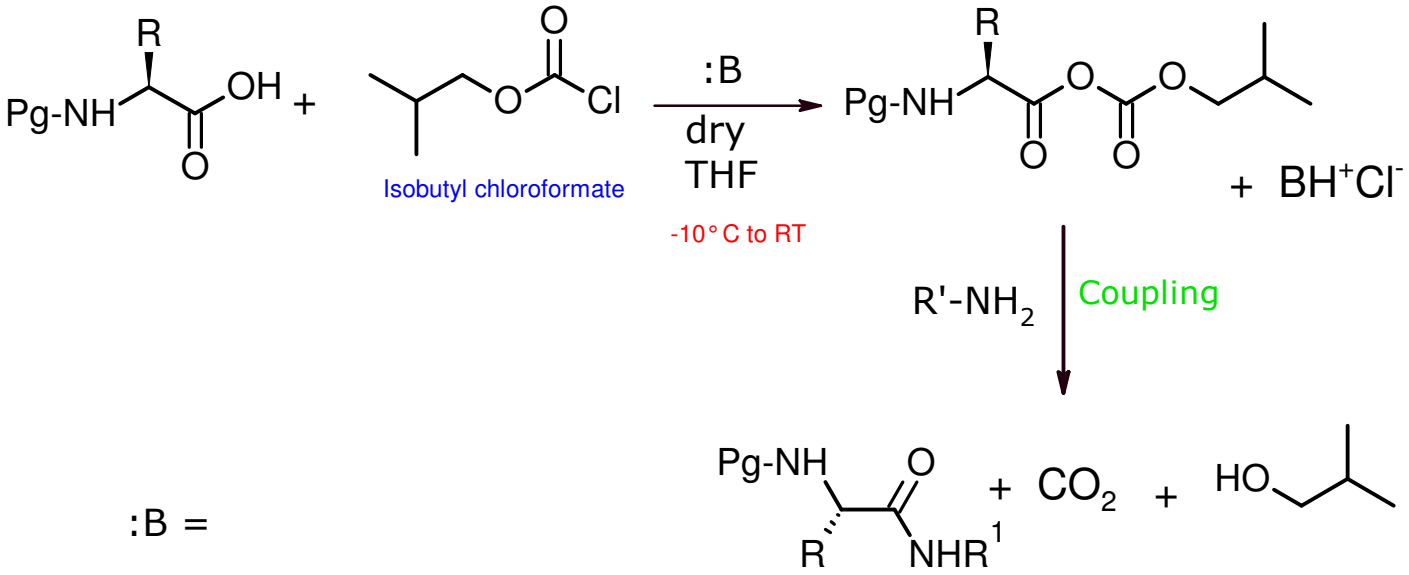
- **Mixed anhydrides**
- **Carbodiimmides**
- **Phosphonium salts**
- **Uronioum salts**

Chemistry

Peptide Coupling

Solution synthesis

- **Mixed anhydrides**
- Carbodiimmide
- Phosphonium salt
- Uronioum salt



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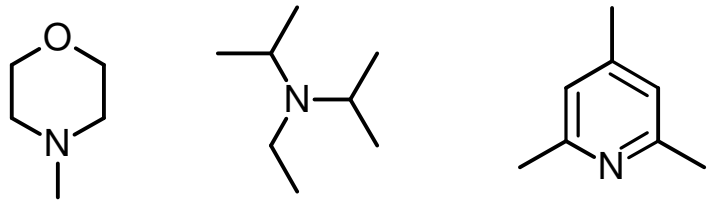


NMM DIPEA sym-collidine

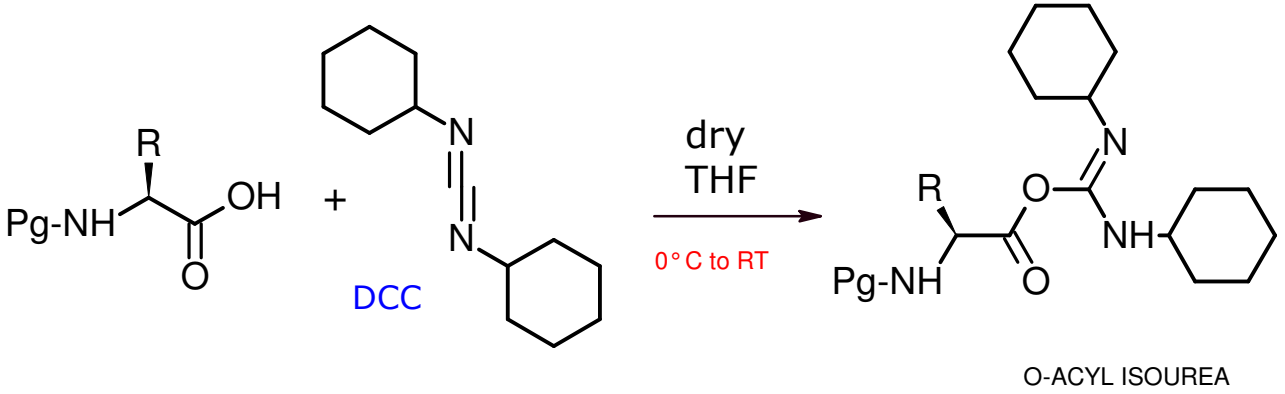
Fast process
High yields,
Easy work-up
Virtually racemization free
High regioselectivity
Substrate with free OH
are compatible

Chemistry

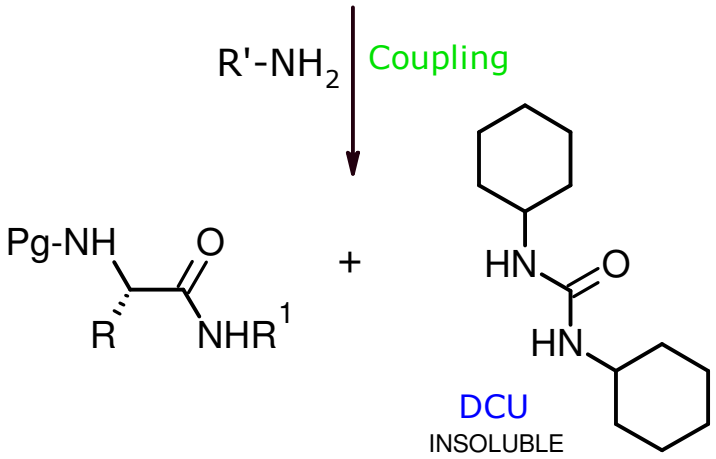
Peptide Coupling

Solution synthesis

- Mixed anhydrides
- **Carbodiimmides**
- Phosphonium salts
- Uronioum salts



High yields,
Easy work-up
High tendency to racemization
Low regioselectivity
Substrate with free OH
are not compatible



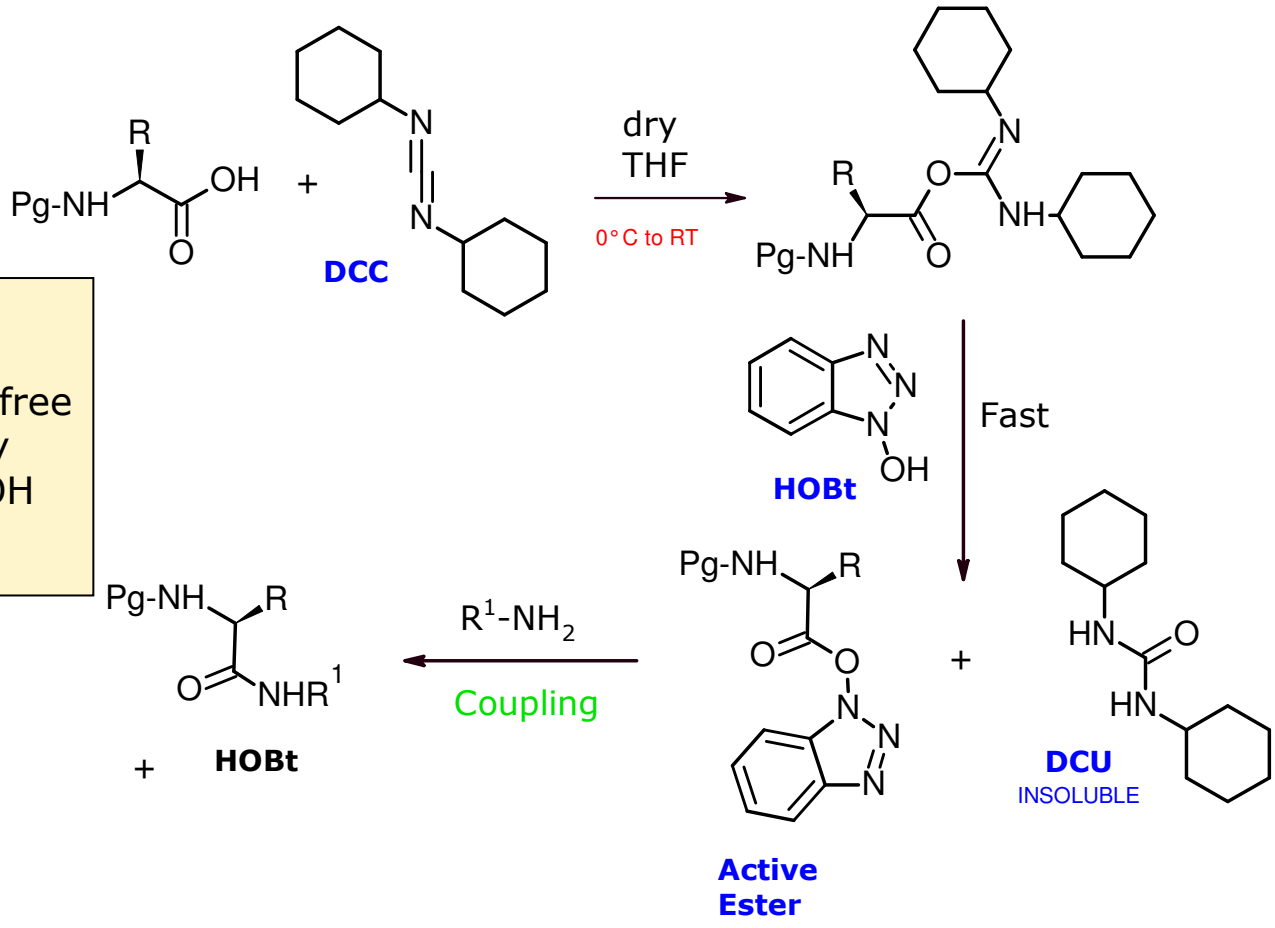
Chemistry

Peptide Coupling

Solution synthesis

Active esters ←

- Mixed anhydrides
- **Carbodiimmides**
- Phosphonium salts
- Uronioum salts



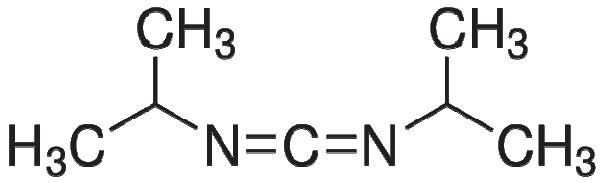
High yields,
Easy work-up
Virtually racemization free
Low regioselectivity
Substrate with free OH
are not compatible

Chemistry

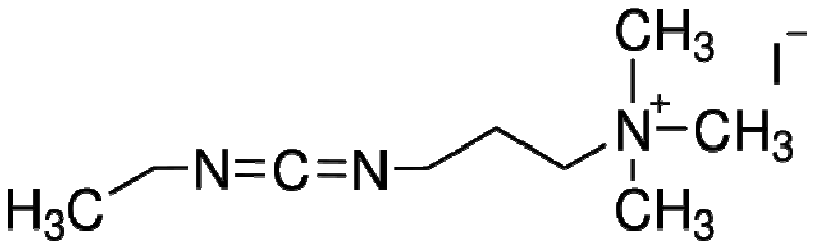
Peptide Coupling

Solution synthesis

- Mixed anhydrides
- **Carbodiimmides**
- Phosphonium salts
- Uronioum salts



***N,N´* -Diisopropylcarbodiimide**
Used specially in SPPS for attachment of first residue



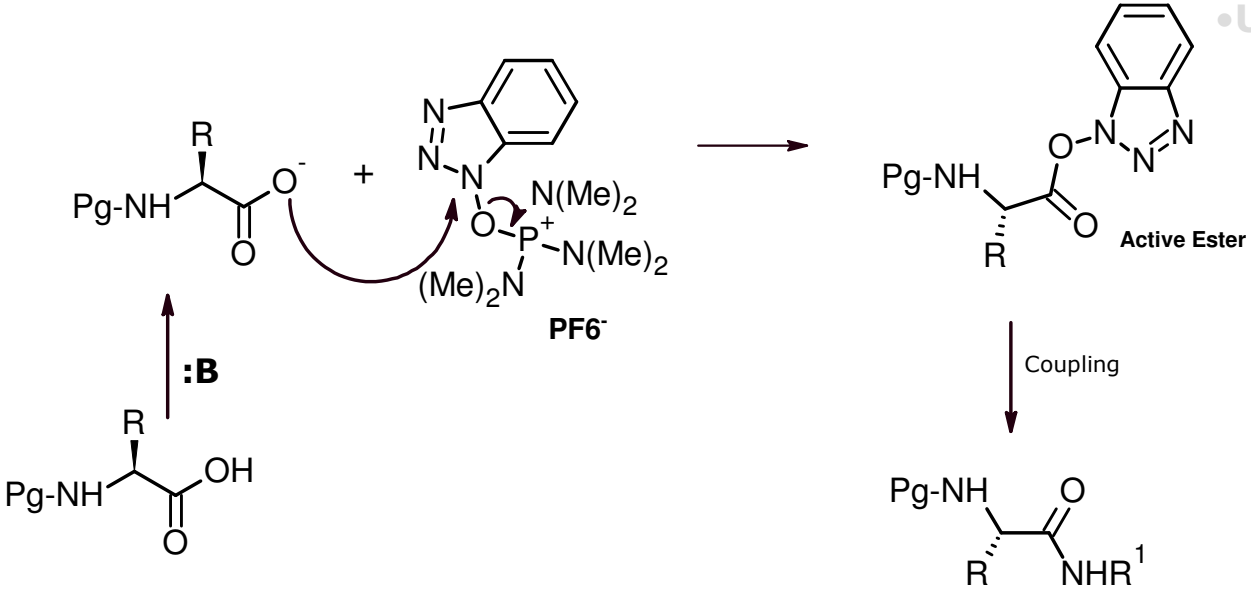
1-[3-(Dimethylamino)propyl]-
3-ethylcarbodiimide methiodide
Water-soluble carbodiimmide

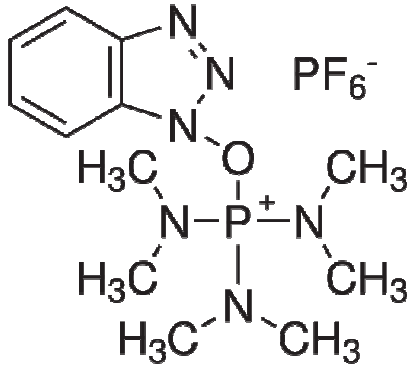
Chemistry

Peptide Coupling

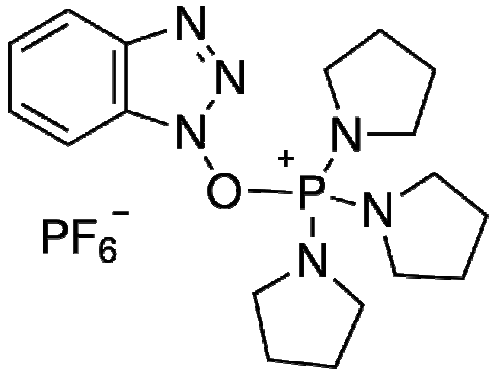
Solution ⍰ solid-phase synthesis

- Mixed anhydrides
- Carbodiimmides
- **Phosphonium salts**
- Uronioum salts

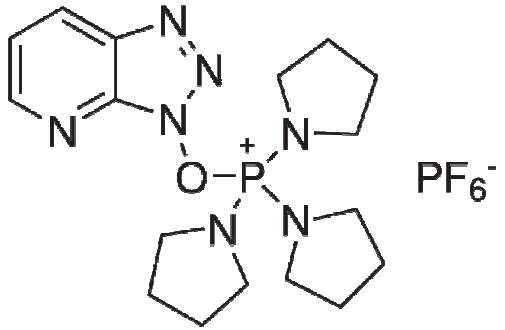




BOP Reagent, BOP, Castros reagent



PyBOP



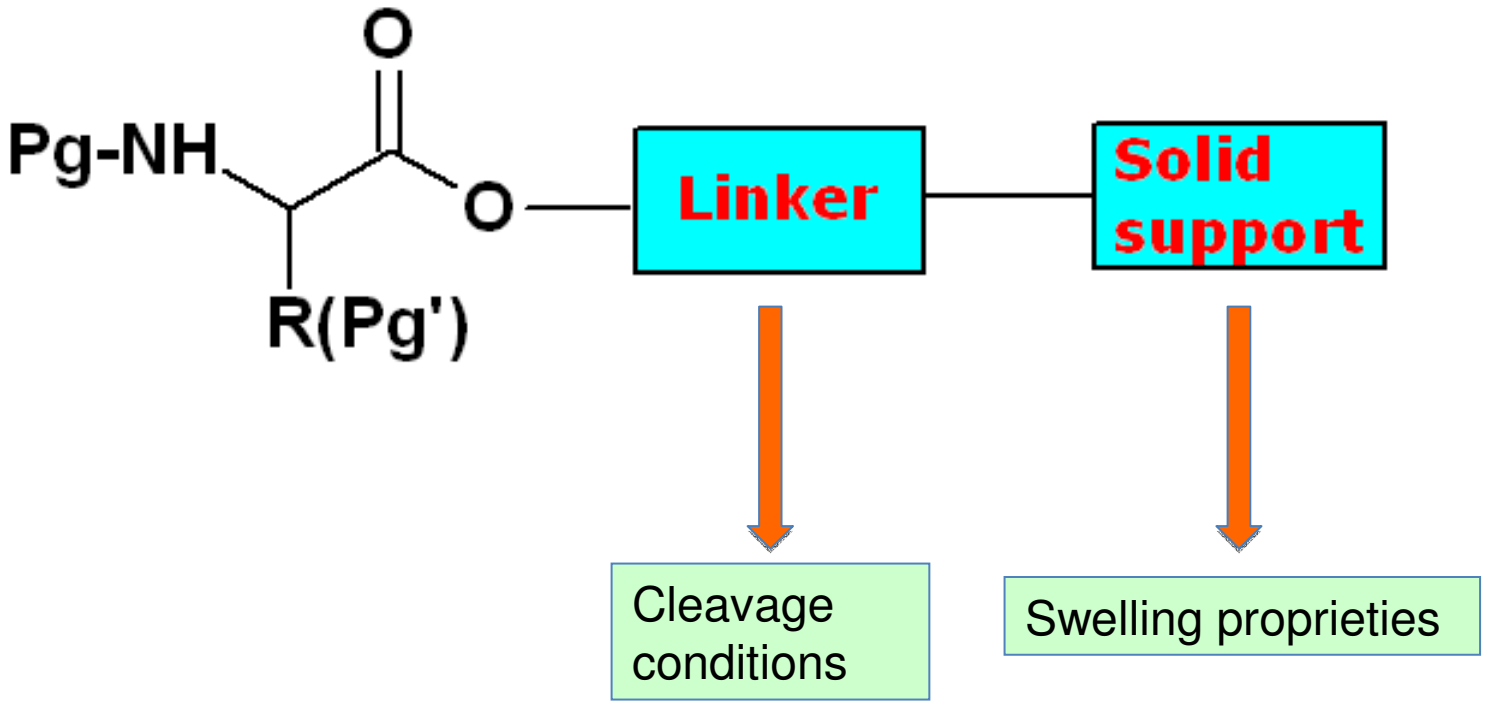
PyAOP

Chemistry

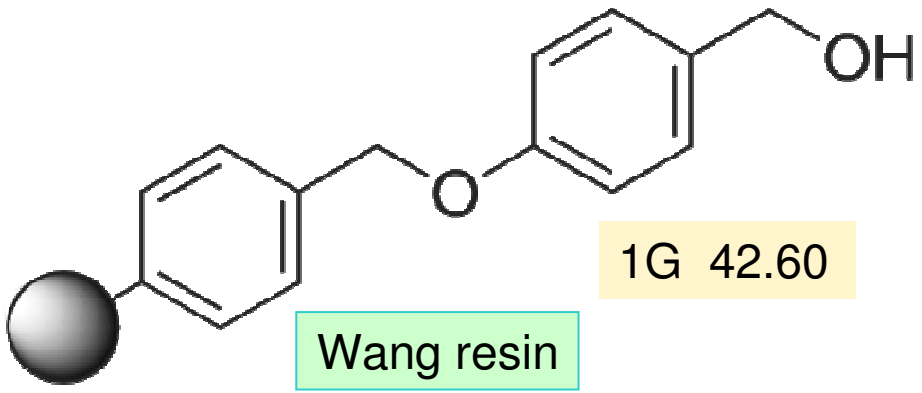
Peptide Coupling

solid-phase synthesis

- Mixed anhydrides
- Carbodiimmide
- **Phosphonium salts**
- Uronioum salt

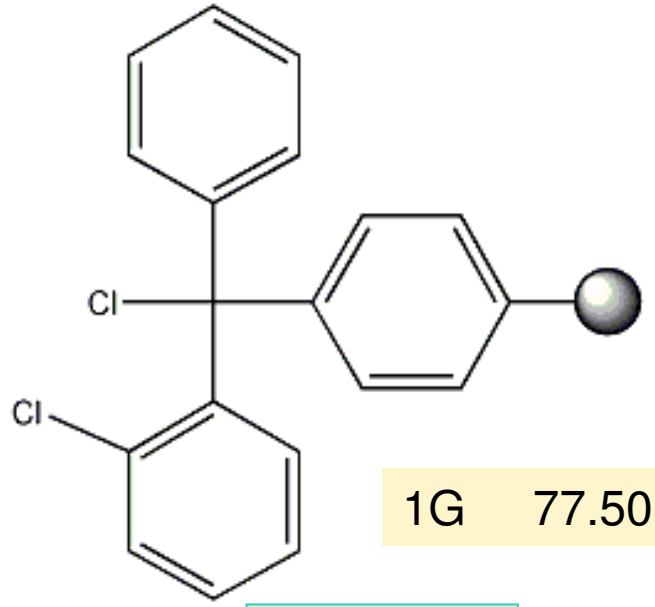


Chemistry



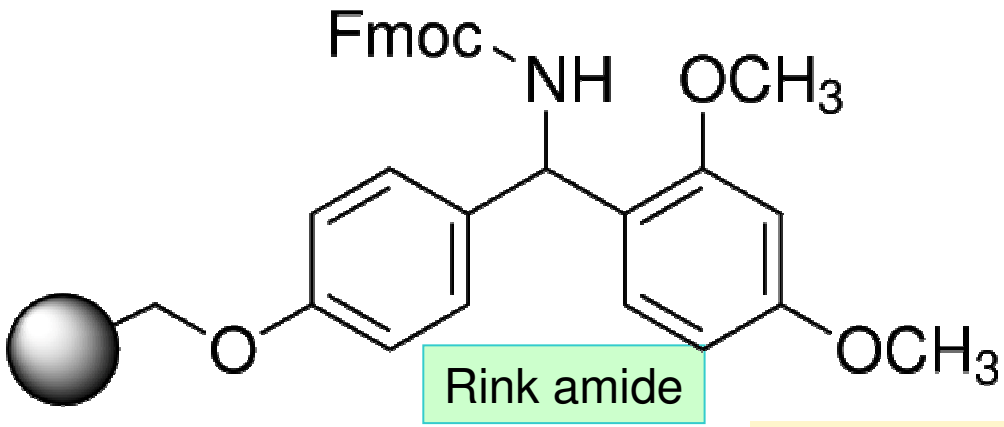
1G 42.60

Wang resin



1G 77.50

Trityl linker



Rink amide

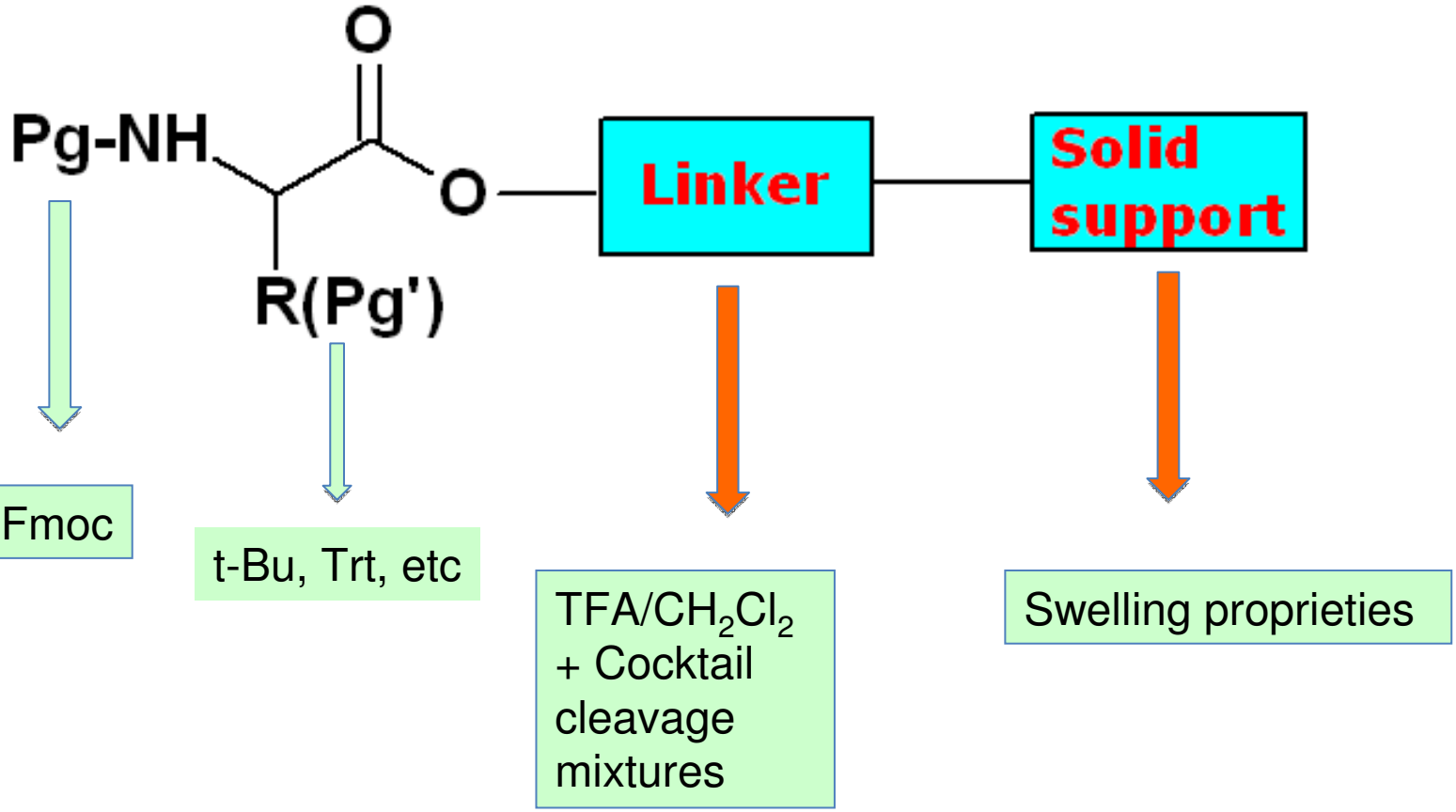
1G 93.00

Chemistry

Peptide Coupling

solid-phase synthesis

- Mixed anhydrides
- Carbodiimmides
- **Phosphonium salts**
- Uronioum salts

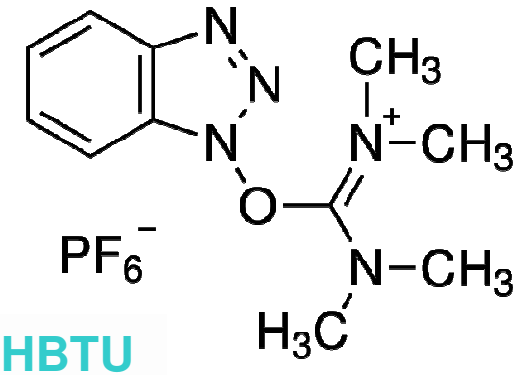


Chemistry

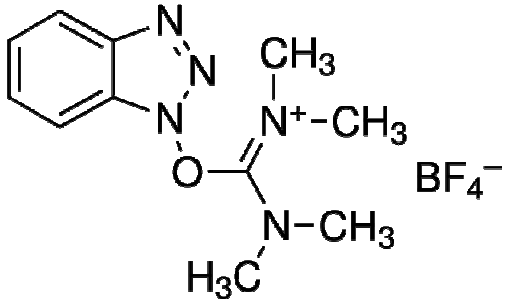
Peptide Coupling

solid-phase synthesis

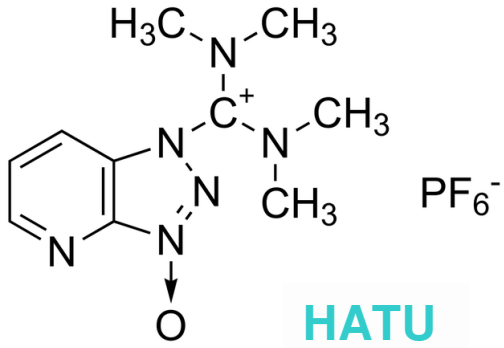
- Mixed anhydrides
- Carbodiimmides
- Phosphonium salts
- **Uronioum salts**



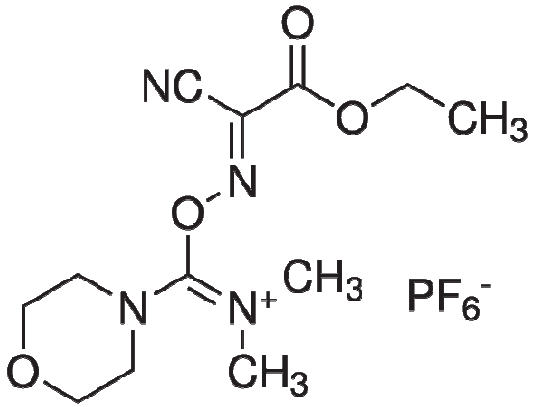
HBTU



TBTU



HATU



COMU

Advantages

- Equal or even superior performance to HATU
- Non-explosive (does not contain benzotriazole moiety)
- Suitable for solution phase & solid phase peptide synthesis
- Retention of configuration – low to non-existent racemization
- High solubility and stability in typical solvents
- Visual or colorimetric reaction monitoring possible
- Easy removal of water-soluble by-products

Chemistry

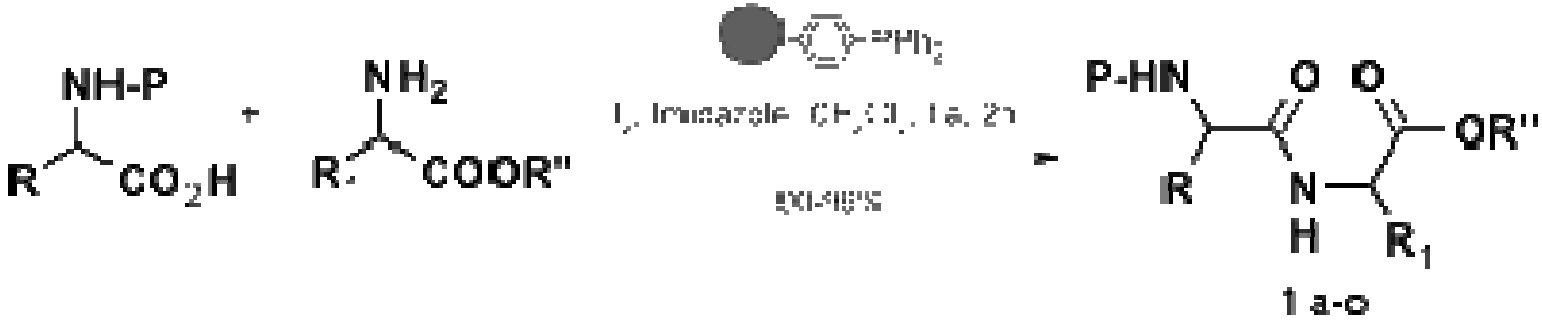
Peptide Coupling

- Mixed anhydrides
- Carbodiimmides
- **Phosphonium salts**
- Uronioum salts

Chemistry

Peptide Coupling

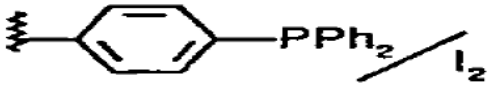
- Mixed anhydrides
- Carbodiimmides
- **Phosphonium salts**
- Uronioum salts



1 a-o

Chemistry

Peptide Coupling



P-Xxx-OH + H-Yyy-OR —(Imidazole / CH_2Cl_2, r.t., 2 h, 90-99%)→ P-Xxx-Yyy-OR

1 a-o

1	P	Xxx	Yyy	R
a	Fmoc	L-Ala	L-Leu	All
b	Cbz	L-Met	L-Ala	Me
c	Fmoc	L-Cys(Trt)	L-Cys(Trt)	Me
d	Boc	L-Leu	L-Phe	Bu^t
e	Fmoc	L-Phe	Gly	Bu^t
f	Fmoc	D,L-Phe	Gly	Bu^t
g	Fmoc	L-Val	L-Val	All
h	Fmoc	L-Phe	L-Leu	Bn
i	Boc	L-Trp	L-Leu	Me
l	Fmoc	L-Glu(Bu^t)	L-Phe	All
m	Cbz	L-Ala	L-Phe	All
n	Boc	L-Arg(Ts)	L-Phe	Me
o	Cbz	L-Thr(OH)	L-Val	Hept

Synthesis 1995, 141-143

Chemistry

Peptide Coupling

- Mixed anhydrides
- Carbodiimmide
- Phosphonium salt
- **Uronioum salts**

Solid-phase synthesis steps:

1. **Attachment of first residue** Fmoc-Aaa-OH, DIC, DMF
2. **Fmoc deprotection** 20% Piperidine in DMF
3. **Coupling** Fmoc-Aaa-OH, DIPEA,HBTU, HOBt, DMF
4. Iterative steps 2 and 3
5. **Cleavage** TFA in CH_2Cl_2, Thianisole, Triethylsilane, Precipitation in dry ether
6. **Analysis and purification** HPLC/MS, Preparative HPLC

Chemistry

Peptide Coupling

Primary Sequence of Recombinant α-gliadine

V[1]RVPVPQLQPQNPSQQQPQE[20]QVPLVQQQQF
LGQQQPFPPQ[40]QPYPQPQPFPSQQPQLQLQP[60]
FPQPQLPYSQPQPFRPQQPY[80]PQPQPQYSQPQ
QPISQQQQQ[100]QQQQQQQQQQQQQQQILQQI
[120]LQQQLIPCMDVVLQQHNIAH[140]GRSQVLQQ
STYQLLQELCCQ[160]HLWQIPEQSQCQAIHKVVH
A[180]IILHQQQKQQQQPSSQVSFQ[200]QPLQQYP
LGQGSFRPSQQNP[220]QAQGSVQPQQLPQFEEI
RNL[240]ALQTLPAMCNVYIPPYCTIT[260]PFGIFGT
N[268]

Chemistry

Peptide Coupling

Primary Sequence of Recombinant α-gliadine

VRVPVPQLQPQNPSQQQPQEQVPLVQQQQFLG
QQQPFPPQQPYPQPQPFPSQQPQLQLQPFPQPQ
LPYSQPQPFRPQQPYPQPQPQYSQPQQPISQQQ
QQQQQQQQQQQQQQQQQQILQQILQQQLIPCM
DVVLQQHNIAHGRSQVLQQSTYQLLQELCCQH
LWQIPEQSQCQAIHKVVHAIILHQQQKQQQQP
SSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGS
VQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCTIT
PFGIFGTN

Chemistry

Peptide Coupling

Primary Sequence of Recombinant α-gliadine

VRVPVPQLQPQNPSQQQPQEQVPLVQQQQFLG
QQQPFPPQQPYPQPQPFPSQQPQLQLQPFPQPQ
LPYSQPQPFRPQQPYPQPQPQYSQPQQPISQQQ
QQQQQQQQQQQQQQQQQILQQILQQQLIPCM
DVVLQQHNIAHGRSQVLQQSTYQLLQELCCQH
LWQIPEQSQCQAIHKVVHAIILHQQQKQQQQP
SSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGS
VQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCTIT
PFGIFGTN

Chemistry

Peptide Coupling

Primary Sequence of Recombinant α-gliadine

VRVPVPQLQPQNPSQQQPQEQVPLVQQQQFLG
QQQPFPPQQPYPQPQPFPSQQPQLQLQPFPQPQ
LPYSQPQPFRPQQPYPQPQPQYSQPQQPISQQQ
QQQQQQQQQQQQQQQQQQILQQILQQQLIPCM
DVVLQQHNIAHGRSQVLQQSTYQLLQELCCQH
LWQIPEQSQCQAIHKVVHAIILHQQQKQQQQP
SSQVSFQQPLQQYPLGQGSFRPSQQNPQAQGS
VQPQQLPQFEEIRNLALQTLPAMCNVYIPPYCTIT
PFGIFGTN

Chemistry

Peptide Coupling

Solid-Phase Peptide Synthesis
SPPS

26 PEPTIDES
L= 20

1 operator + 2
2 months
< 8000 €

Tabella 5: Dati analitici dei peptidi sintetizzati

	Peptide	Sequenza	Peso molecolare calcolato	Peso molecolare osservato
P1	1-20	VRVPVPQLQPQNPSQQQPQE	2296.192	2297.28
P2	10-29	PQNPSQQQPQEQVPLVQQQQ	2328.146	2329.18
P3	20-39	EQVPLVQQQQFLGQQQPFPP	2335.196	2336.23
P4	30-49	FLGQQQPFPPQQPYPQPQPF	2368.164	2369.22
P5	40-59	QQPYPQPQPFPSQQPYLQLQ	2411.191	2412.25
P6	50-69	PSQQPYLQLQPFPQPQLPYS	2355.190	2356.30
P7	60-79	PFPQPQLPYSQPQPFRPQQP	2376.201	2377.25
P8	70-89	QPQPFRPQQPYPQPQPQYSQ	2438.170	2439.26
P9	80-99	YPQPQPQYSQPQQPISQQQQ	2397.135	2398.18
P10	90-109	PQQPISQQQQQQQQQQQQQQ	2461.169	2462.18
P11	100-119	QQQQQQQQQQQQQQQQQILQQ	2549.233	2550.15
P12	111-129	QQQQQQILQQILQQQLIPCM	2436.260	2437.26
P13	120-139	ILQQQLIPCMDVVLQQHNIA	2303.210	2304.30
P14	130-149	DVVLQQHNIAHGRSQVLQQS	2256.172	2257.28
P15	141-160	GRSQVLQQSTYQLLQELCCQ	2324.125	2325.17
P16	150-169	TYQLLQELCCQHLWQIPEQS	2459.161	2460.16
P17	160-179	QHLWQIPEQSQCQAIHKVVH	2408.217	2409.20
P18	170-189	QCQAIHKVVHAIILHQQQKQ	2349.280	2350.31
P19	180-199	AIILHQQQKQQQQPSSQVSF	2322.208	2323.26
P20	190-209	QQQPSSQVSFQQPLQQYPLG	2287.123	2288.15
P21	200-219	QQPLQQYPLGQGSFRPSQQN	2300.130	2301.24
P22	210-229	QGSFRPSQQNPQAQGSVQPQ	2168.030	2169.25
P23	220-239	PQAQGSVQPQQLPQFEEIRN	2293.140	2294.34
P24	230-249	QLPQFEEIRNLALQTLPAMC	2314.181	2315.32
P25	240-259	LALQTLPAMCNVYIPPYCTI	2223.114	2224.15
P26	250-268	NVYIPPYCTITPFGIFGTN	2116.034	2117.06