CHEMICAL SYNTHESIS OF BIOPOLYMERS

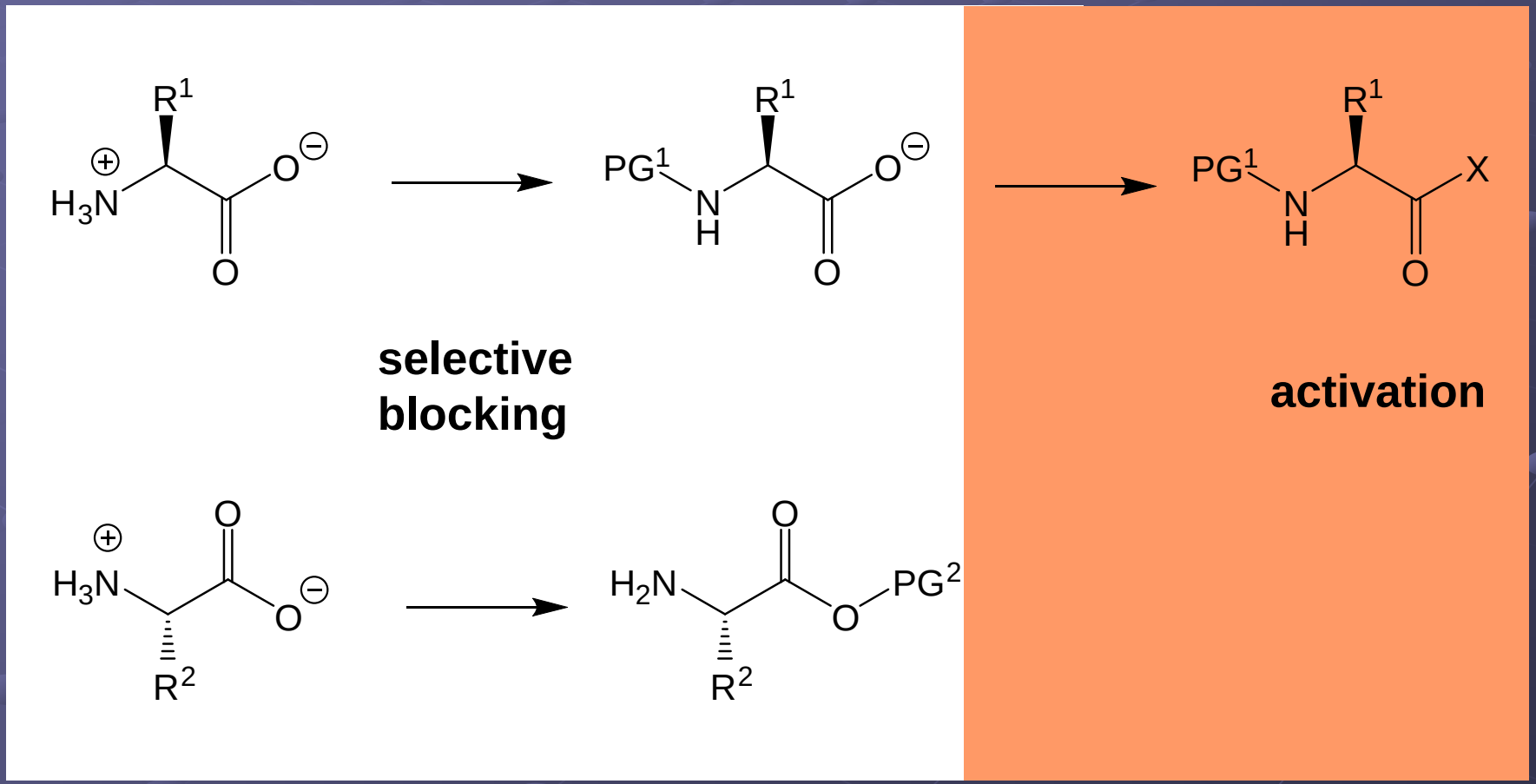
2.2 Peptide Synthesis

Main targets of peptides synthesis:

- Confirmation of suggested primary structures
- Design of bioactive drugs
- Preparation of pharmacologically active peptides and proteins
- Synthesis of model peptides

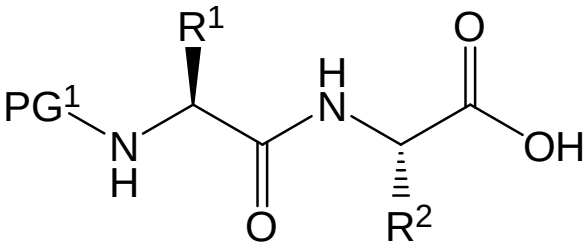
CHEMICAL SYNTHESIS OF BIOPOLYMERS

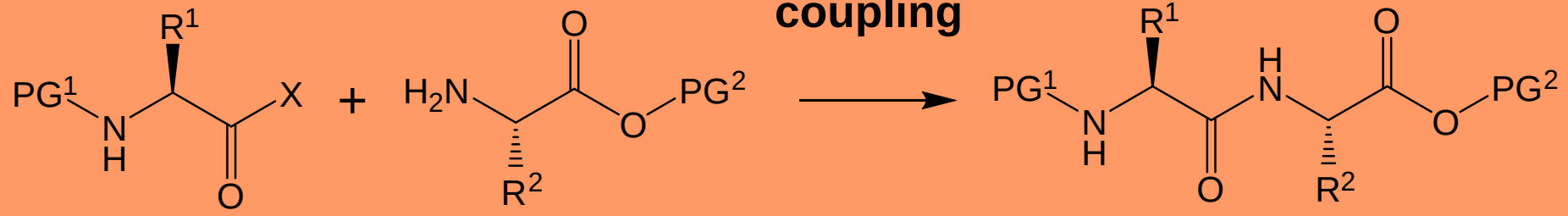
Basic principles of peptide bond formation



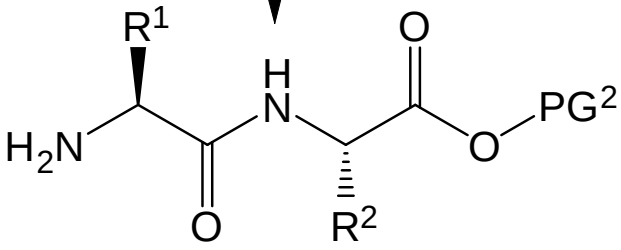
CHEMICAL SYNTHESIS OF BIOPOLYMERS

selective deblocking
new carboxy component





selective deblocking
new amino component



CHEMICAL SYNTHESIS OF BIOPOLYMERS

The peptide synthesis is a three-step procedure:

1. Step – preparation of partially protected amino acids, **temporary** saved at the amino or carboxyl function. The proteinogenic amino acids **Ser, Thr, Tyr, Asp, Glu, Lys, Arg, His, Sec,** and **Cys** have functionalities in the side chain which need to be selectively protected by **semipermanent protecting groups**.

2. Step – **activation** of the *N*-protected amino acid at the carboxyl function followed by the **formation of the peptide bond**. The coupling reaction can be performed either as a one-pot reaction or in to separate, consecutive steps.

3. Step – **selective deprotection** in order to continue the peptide synthesis. Finally, **total deprotection** when the peptide chain has been fully assembled.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Activation of the carboxyl component and subsequent bond formation should occur at a **high reaction rate** and **without racemization or formation of by-products**.

High yields are required upon application of **equimolare amounts** of both components.

The **number of appropriate methods** applicable to practical synthesis is **rather small** compared to the high number of principle coupling methods for peptide bonds.

The **peptide synthesis** can be distinguished in **stepwise synthesis** and **fragment condensation**. The **solid-phase peptide synthesis** is in its original version a variant of the stepwise synthesis. Nowadays, fragment condensations may also be performed on polymeric support.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Protecting group manipulations

N^{α}-amino protection

Several hundred different amino-protecting groups have been developed during the past decades – which confirms the non-existence of an universal, ideal amino-protecting group.

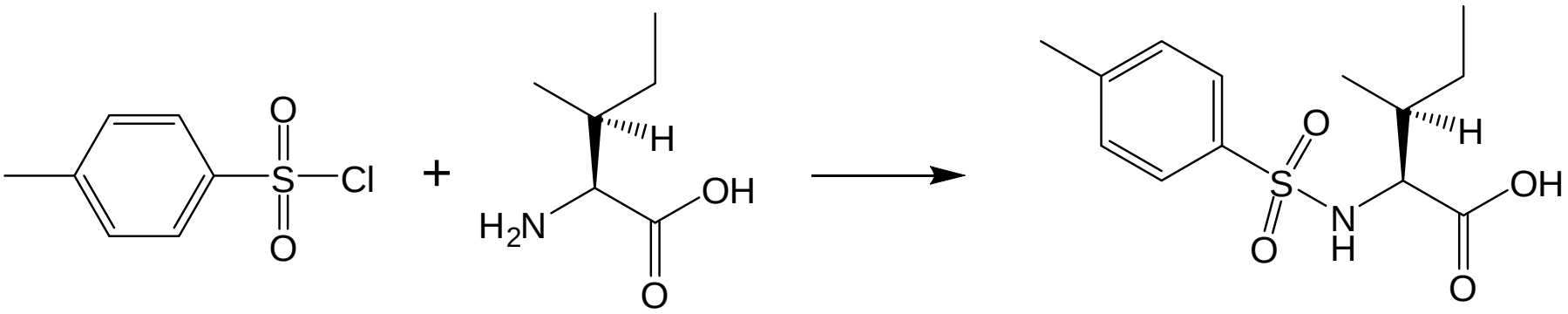
Protection of the amino group can be achieved easily by **acylation** or **alkylation**. The **disadvantage of the simple acyl residue** is that the carboxyamide group of the protecting group is chemically very similar to the peptide bond, which renders **selective cleavage very difficult or even impossible**.

The procedures of protecting group manipulations were taken from the book ***The Practice of Peptide Synthesis***, M. Bodanzky and A. Bodanzky, sec. revised ed., Springer Verlag, 1994 or from original literature.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Acyl-type protecting groups

p-Toluenesulfonyl group



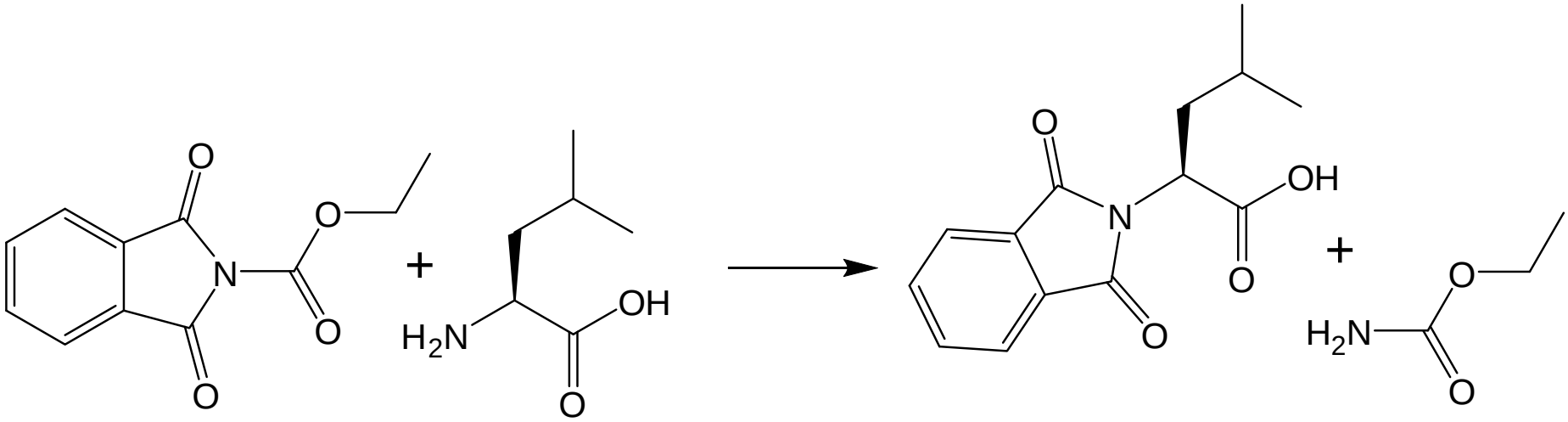
88% (crystalline)

Procedure:

1) 1 M NaOH, *p*-toluenesulfonyl chloride (several small portions), H_2O, pH 9, +20 °C (cooling is necessary), 3 h;
2) 5 M HCl

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Phthaloyl group (Phth)



93% (crystalline)

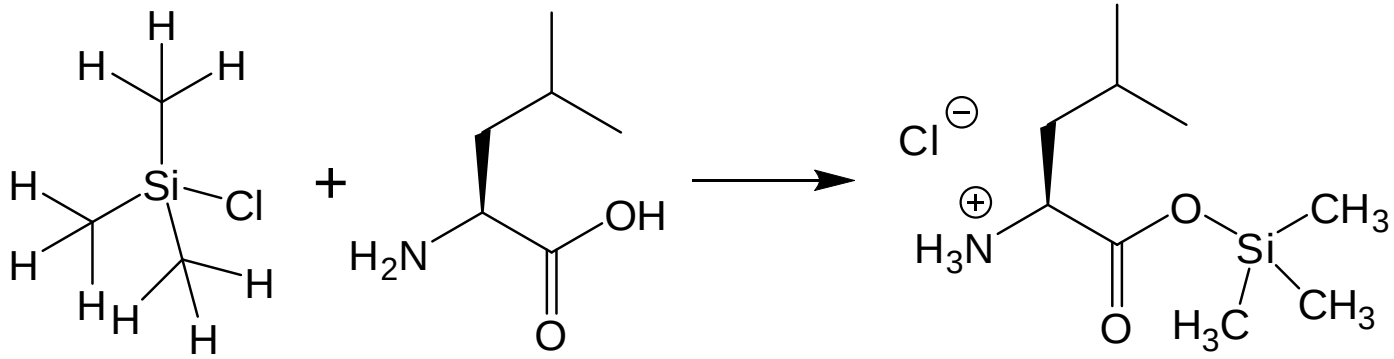
Procedure:

1) Na_2CO_3, *N*-ethyloxycarbonylphthalimide, H_2O, +20 °C, 15 min;
2) 6 M HCl

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Alkyl-type protecting groups

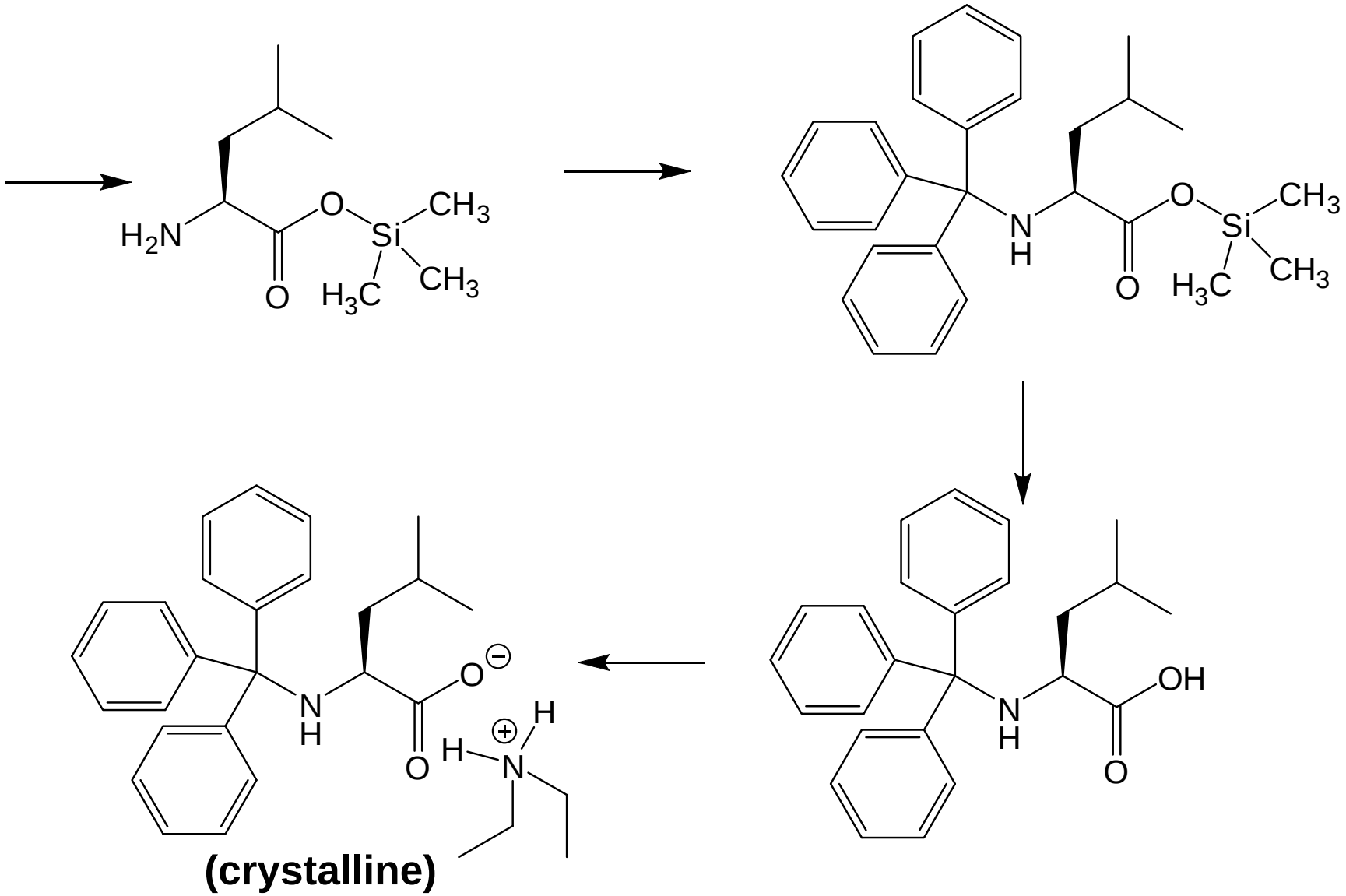
Triphenylmethyl group (Tr)



Procedure:

1) $(H_3C)_3SiCl$, chloroform – acetonitrile (38 : 1), reflux, 2 h;
2) Et_3N, triphenylmethyl chloride, 25 °C, 1 h;
3) 5% aq citric acid
4) Et_2NH (for storage)

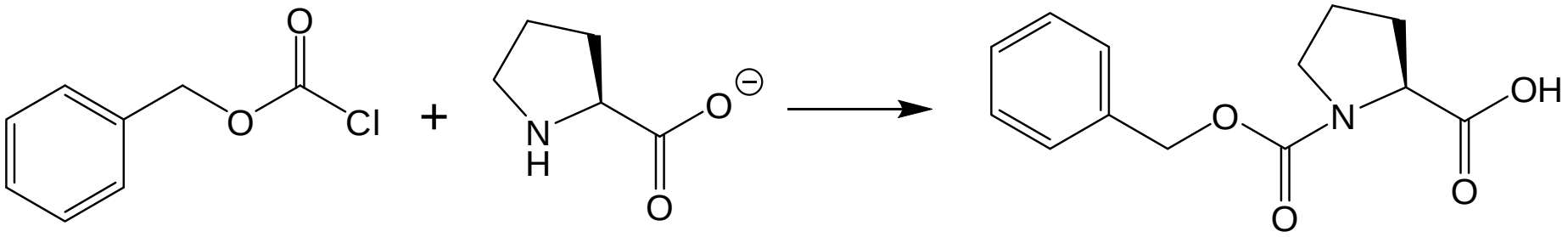
CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Alkoxycarbonyl-type (urethane-type group)

Benzyloxycarbonyl group
(Cbz or Z in honour of its inventor, Leonidas Zervas)

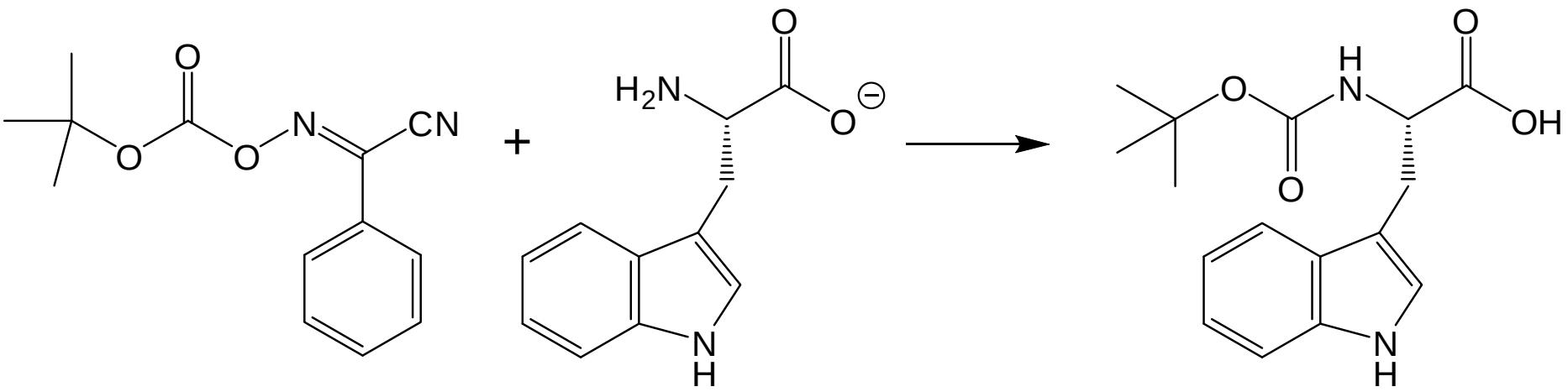


92% (crystalline)

Procedure:
1) 2 M NaOH, benzyloxycarbonyl chloride, several portions, +5 → +25 °C, 2 h;
2) 5 M HCl

CHEMICAL SYNTHESIS OF BIOPOLYMERS

tert-Butoxycarbonyl group (Boc)

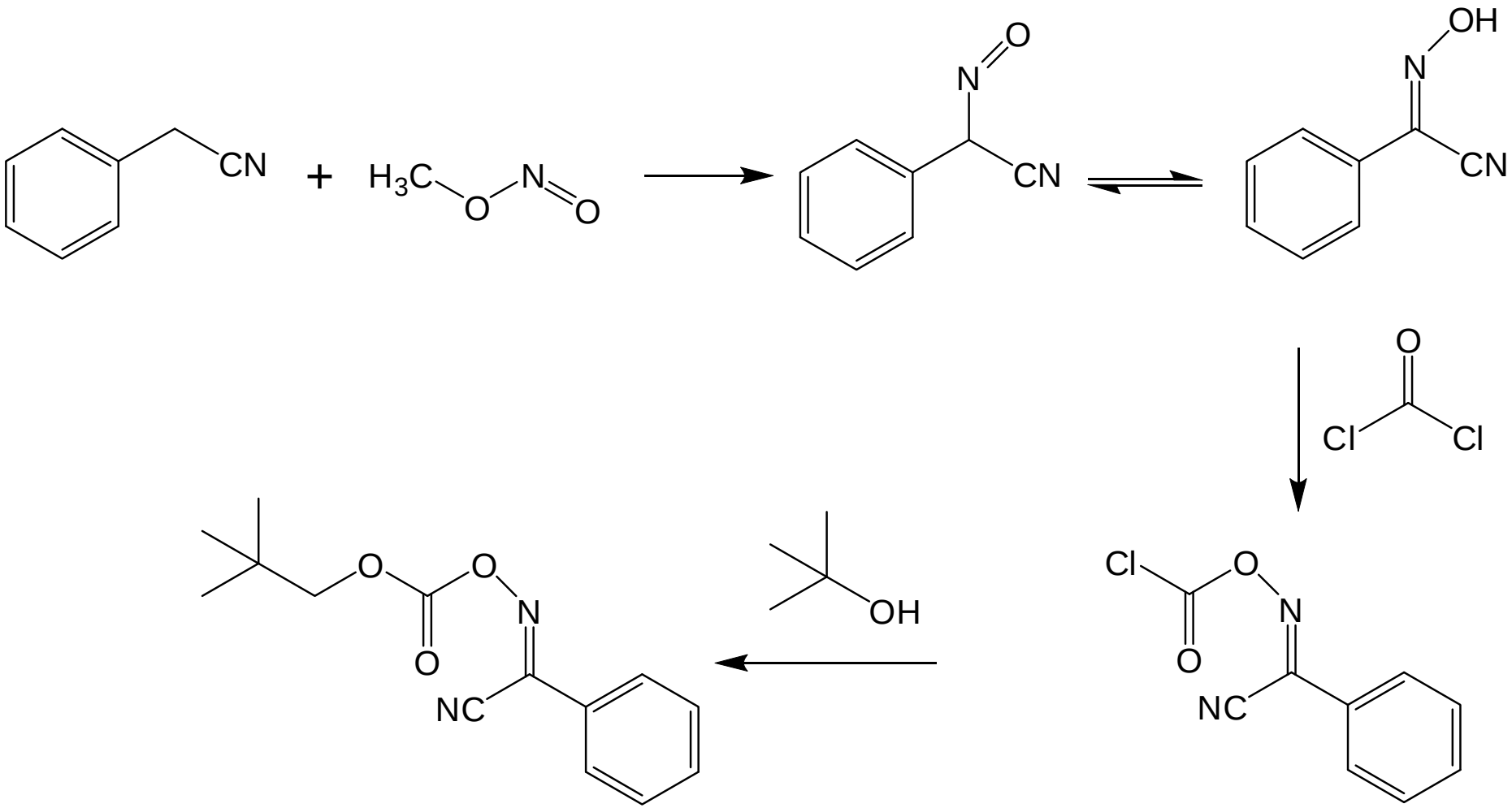


98% (crystalline)

Procedure:

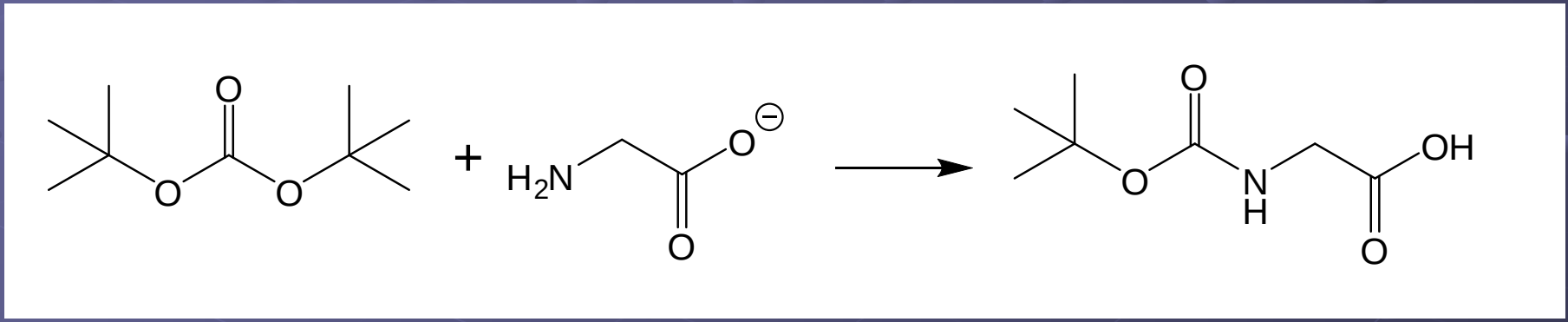
1) Et_3N, 2-*tert*-butyloxycarbonyloximino-2-phenylacetonitrile, water – dioxane (1:1), 25 °C, 3 h;
2) 5% citric acid

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

***tert*-Butoxycarbonyl group (Boc)**



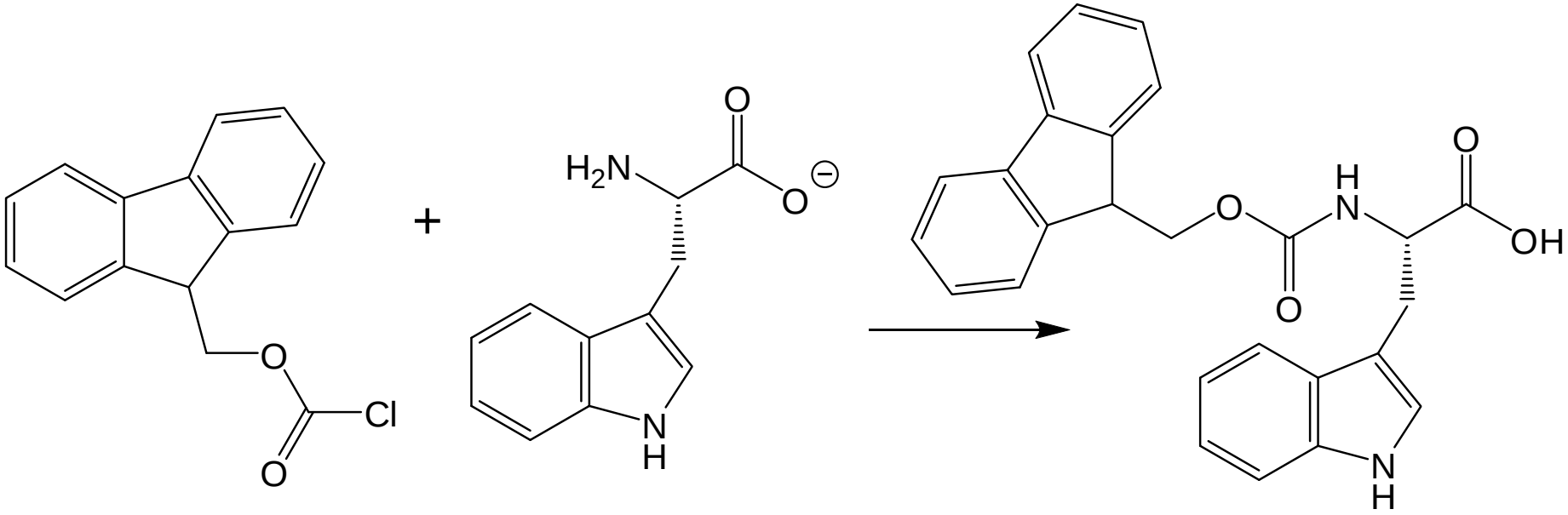
85% (crystalline)

Procedure:

1) 1 M NaOH, Di-*tert*-butyl dicarbonate, water – dioxane (1:2), 0 °C → 25 °C, 30 min;
2) $KHSO_4$

CHEMICAL SYNTHESIS OF BIOPOLYMERS

9-Fluorenylmethoxycarbonyl group (Fmoc)



91% (crystalline)

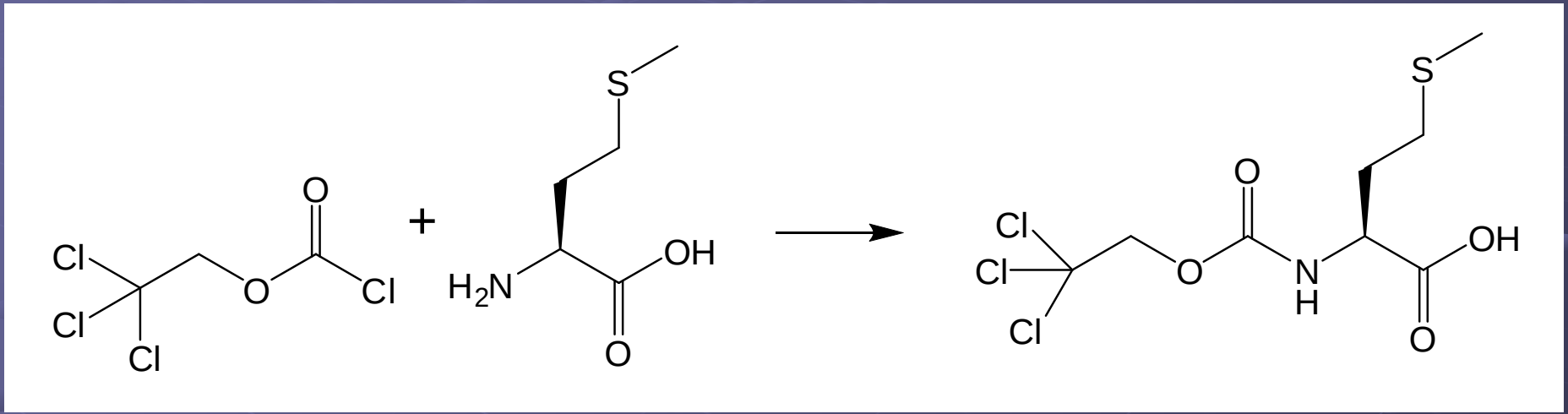
Procedure:

1) Na_2CO_3, 9-fluorenylmethyl chlorocarbonate, water – dioxan (2:1), 0 °C ⟶ 25 °C, 12 h;

2) conc HCl

CHEMICAL SYNTHESIS OF BIOPOLYMERS

2,2,2-Trichloroethoxycarbonyl group (Troc)



76% (crystalline)

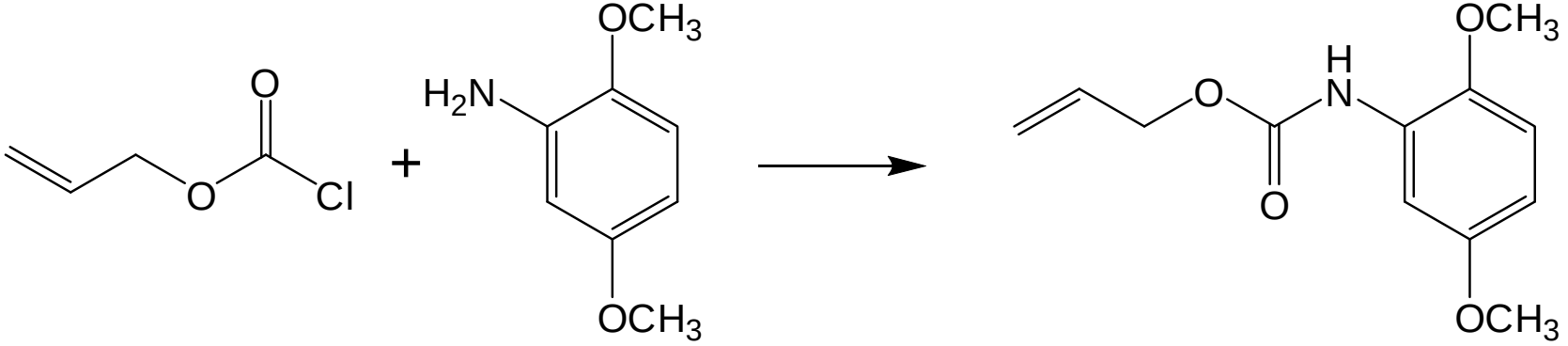
Procedure:

1) 1 M NaOH, 2,2,2-trichloroethyl chlorocarbonate, water, Ar, 0 °C → 25 °C, 20 h;
2) conc HCl

Lit.: Carson, J. F. *Synthesis* **1981**, 268-270.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Allyloxycarbonyl group (Aloc)



95%

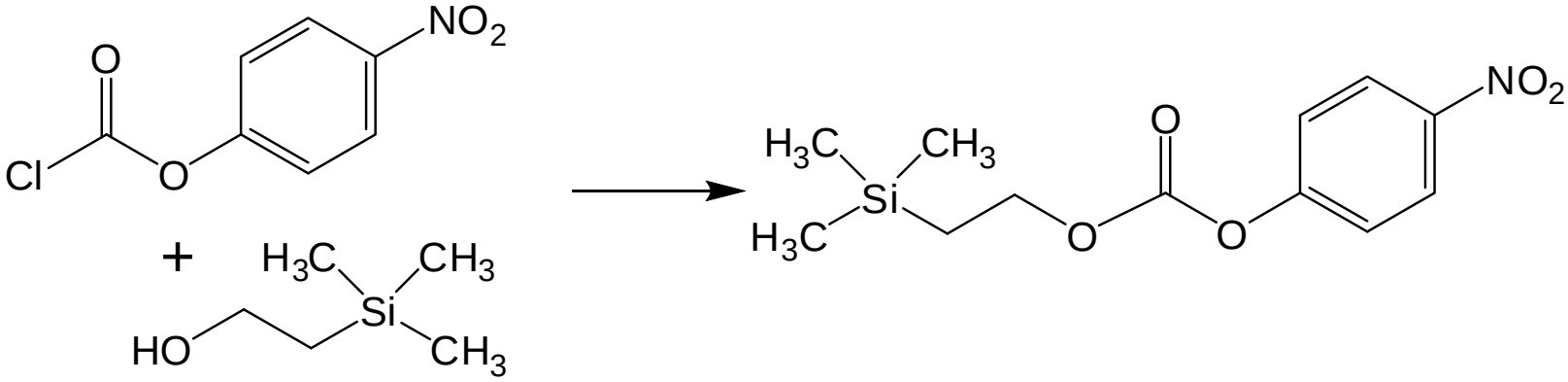
Procedure:

1) Et_3N, allyl chloroformate, THF – water (5 :1), 0 °C ⟶ 21 °C, 11 h;
2) 1 M HCl

Lit.: Wipf, P.; Kim, Y.; Jahn, H. *Synthesis* **1995**, 1549-1561.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

2-(Trimethylsilyl)ethoxycarbonyl group (Teoc)



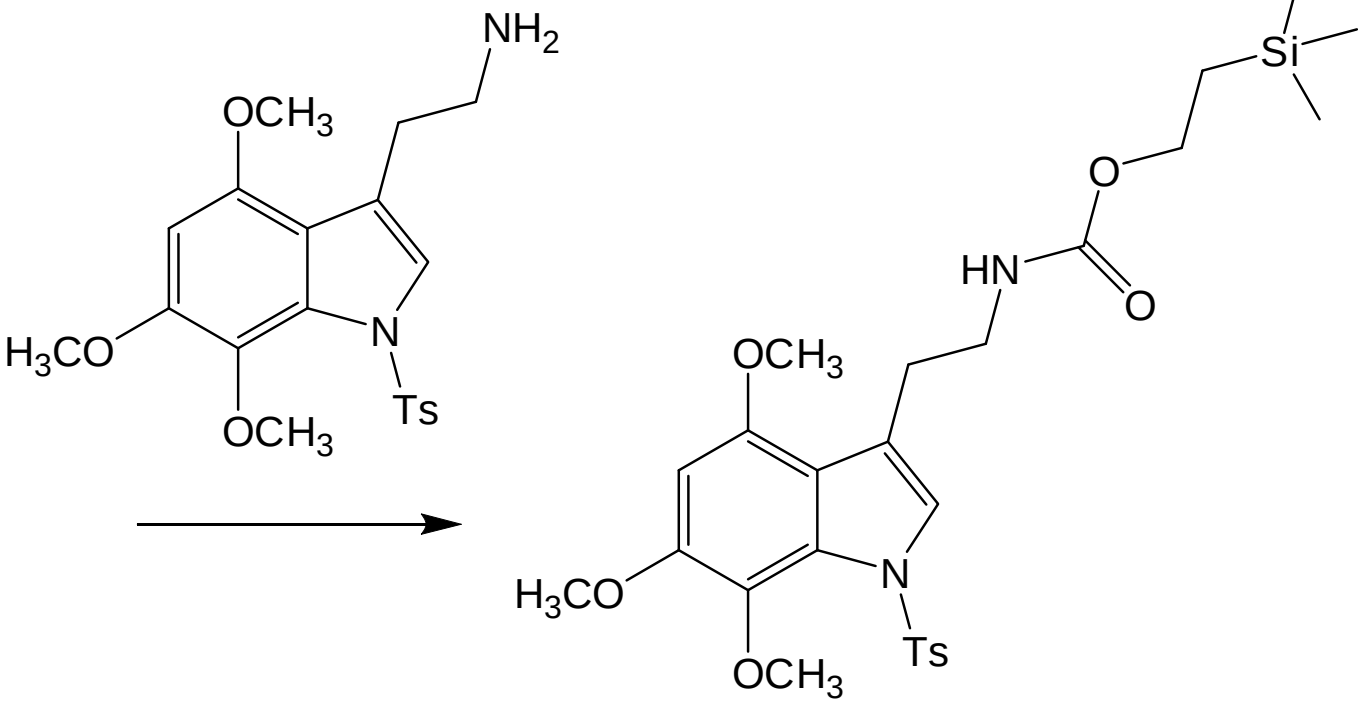
Procedure:

1) 2-(Trimethylsilyl)ethanol, 4-nitrophenyl chloroformate, CH_2Cl_2, pyridine,
2) 2-(trimethylsilyl)ethyl) 4-nitrophenyl carbonate, acetonitrile, N_2, reflux, 8 h

Lit.: Sadanandan, E., V.; Pillai, S. K.; Lakshmikantham, M. V.; Billimoria, A. D.; Culpepper, J. S. Cava, M. P. *J. Org. Chem.* **1995**, *60*, 1800-1805.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

2-(Trimethylsilyl)ethoxycarbonyl group (Teoc)



85% (oil)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

C^{α}-carboxy protection

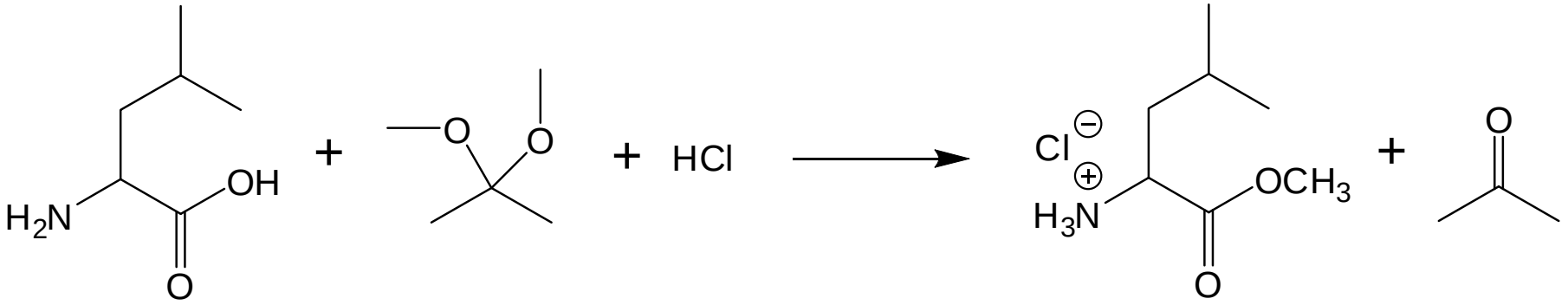
There are **few exceptions** from the usual synthetic strategy where a **peptide chain is assembled from the C-terminus to the N-terminus**.

C^{α}-carboxy-protecting groups in solution-phase synthesis and also **as linker moieties in solid-phase synthesis** must be **orthogonal** to the temporary N^{α}-protecting group.

In many cases, **esters** of the **methyl, ethyl, benzyl, *tert*-butyl, or aryl type** are appropriate for the reversible blocking of the carboxy group.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Methyl ester

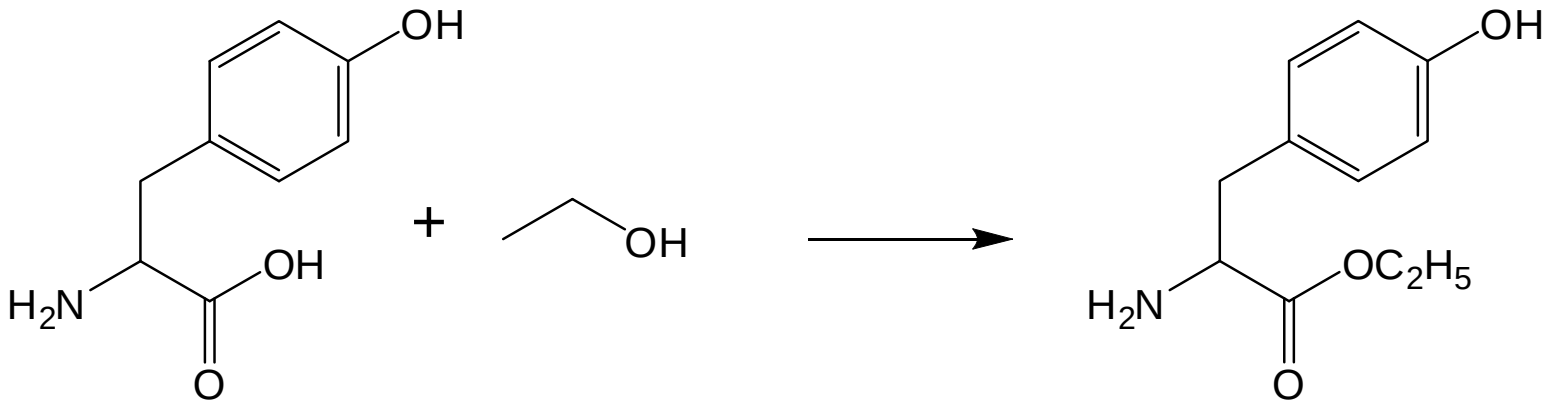


90% (crystalline)

Procedure:
2,2-Dimethoxypropane, conc HCl, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Ethyl ester

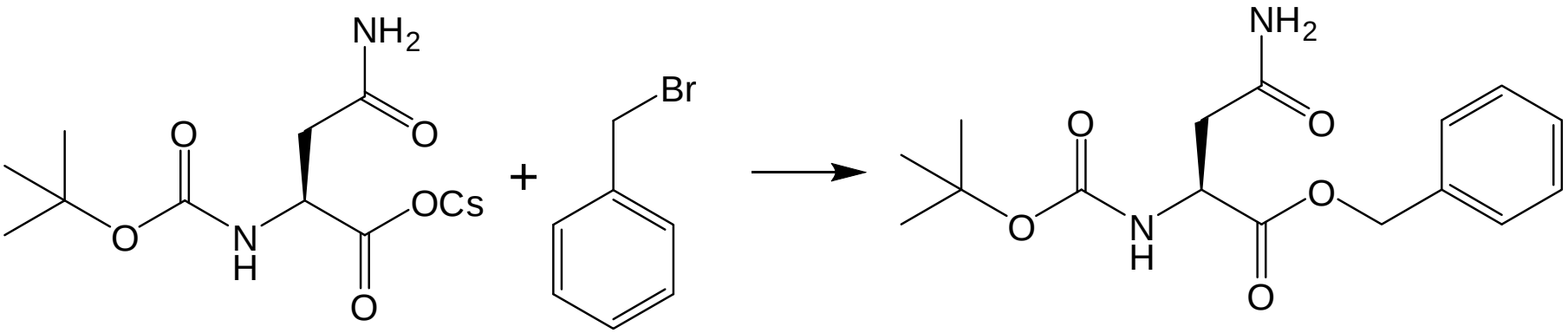


81% (crystalline)

Procedure:
1) abs Ethanol, dry HCl gas, 20 °C → reflux, 7h.;
2) 5 M NaOH

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Benzyl ester



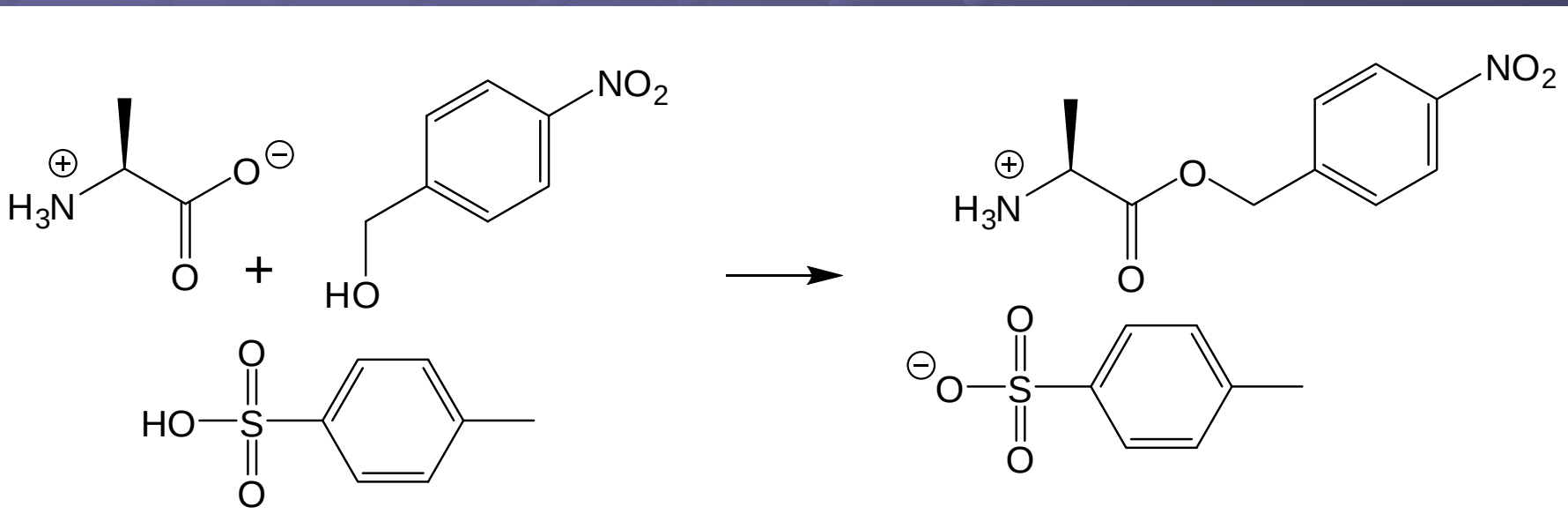
90% (crystalline)

Procedure:

1) $CsCO_3$, H_2O – methanol,
2) abs DMF, benzyl bromide, 20 °C, 6 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

4-Nitrobenzyl ester

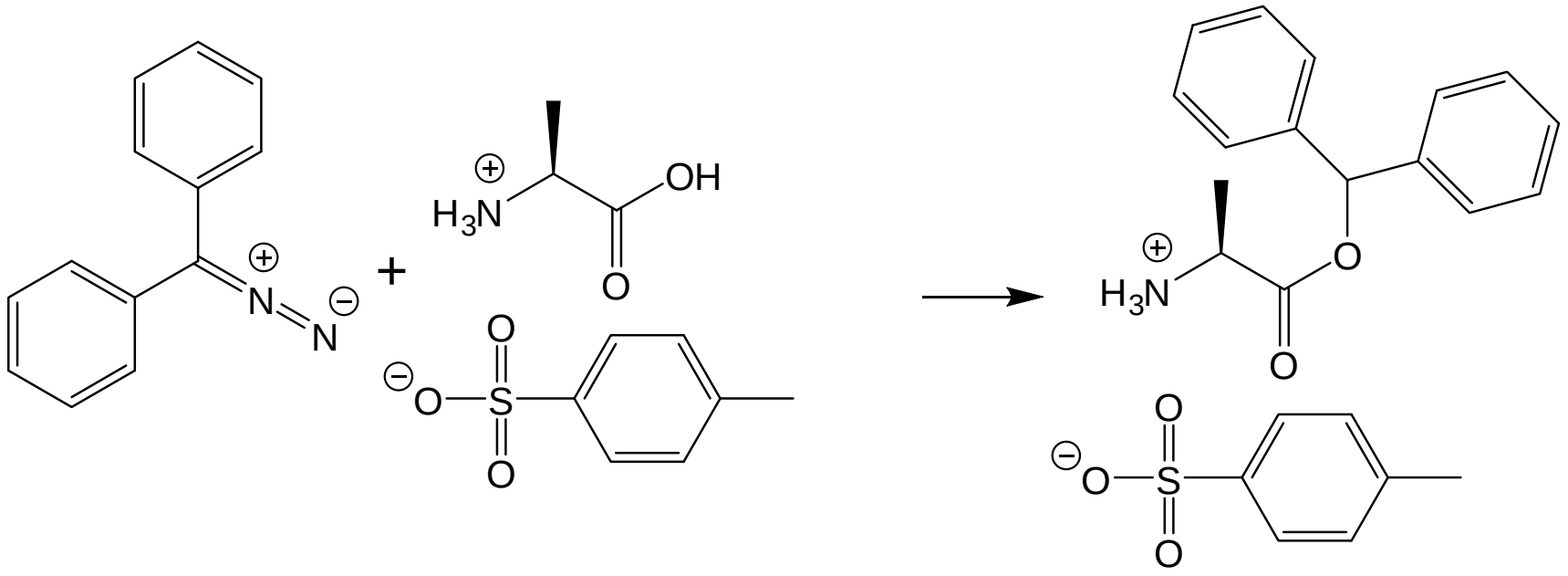


92% (crystalline)

Procedure:
4-Nitrobenzyl alcohol, *p*-toluenesulfonic acid monohydrate, abs chloroform, reflux (Soxhlet or Dean-Stark, molecular sieves), 6 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Benzhydryl ester (diphenylmethyl ester)



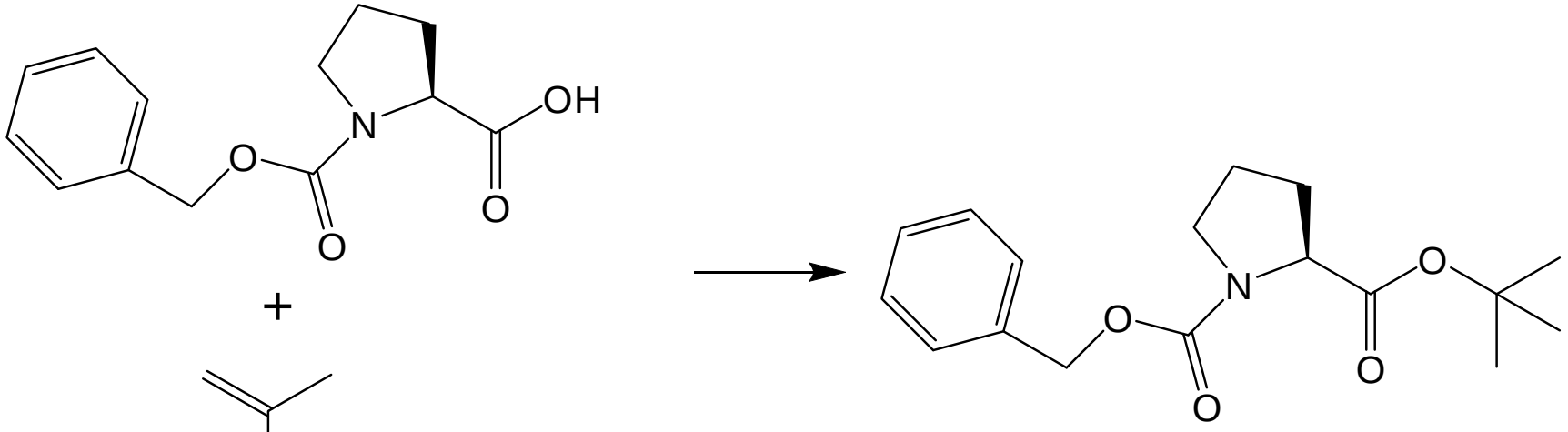
80% (crystalline)

Procedure:

1) *p*-toluenesulfonic acid monohydrate,
2) diazodiphenylmethane, abs DMF, 50 °C, 15 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

***tert*-Butyl ester**

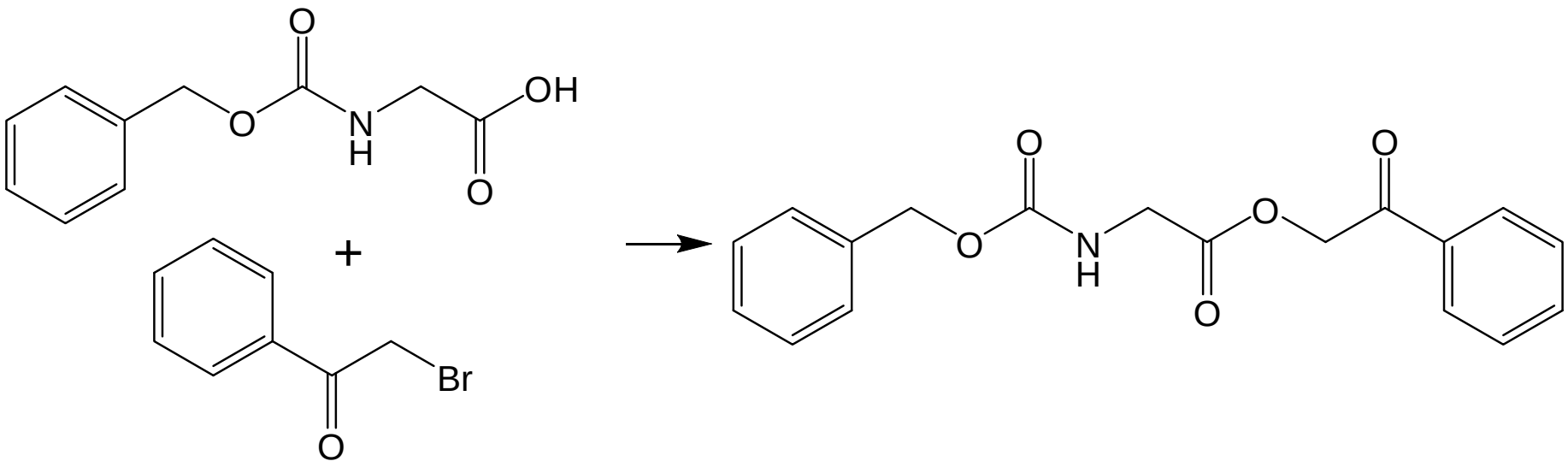


94% (crystalline)

Procedure:
CH_2Cl_2, cat H_2SO_4, saturated with isobutylene, 20 °C, 3 days.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Phenacyl ester

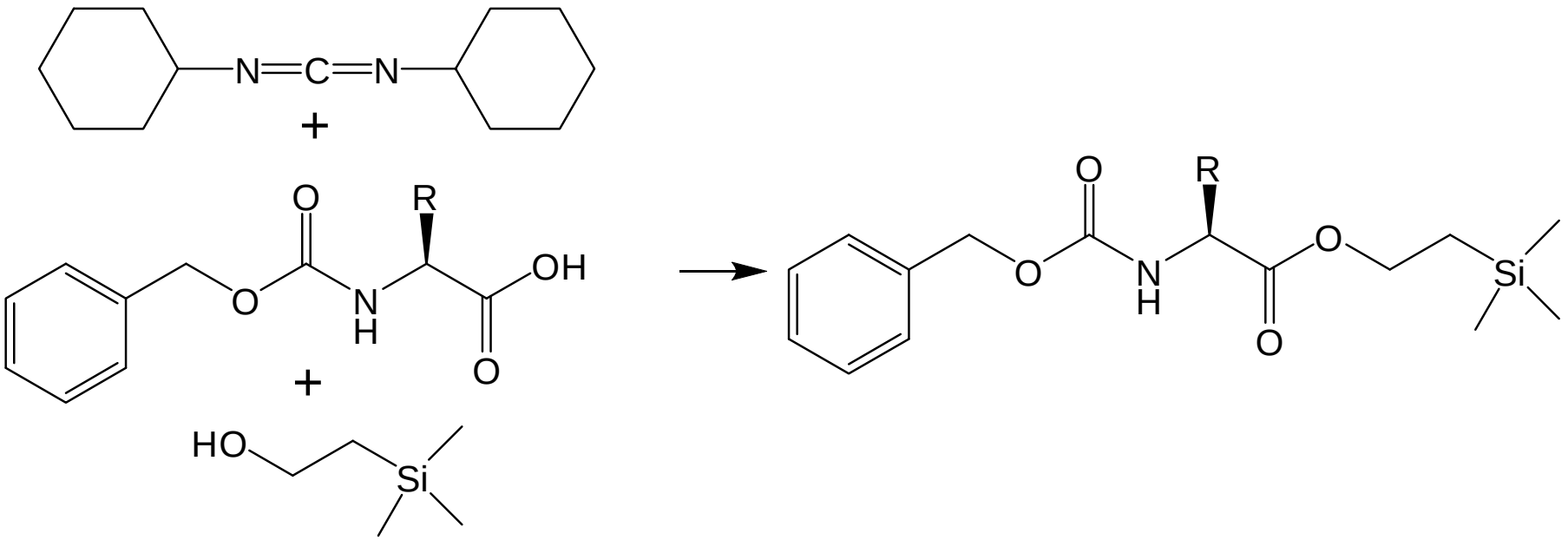


83% (crystalline)

Procedure:
Et_3N, ethyl acetate, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

2-Trimethylsilylethyl ester

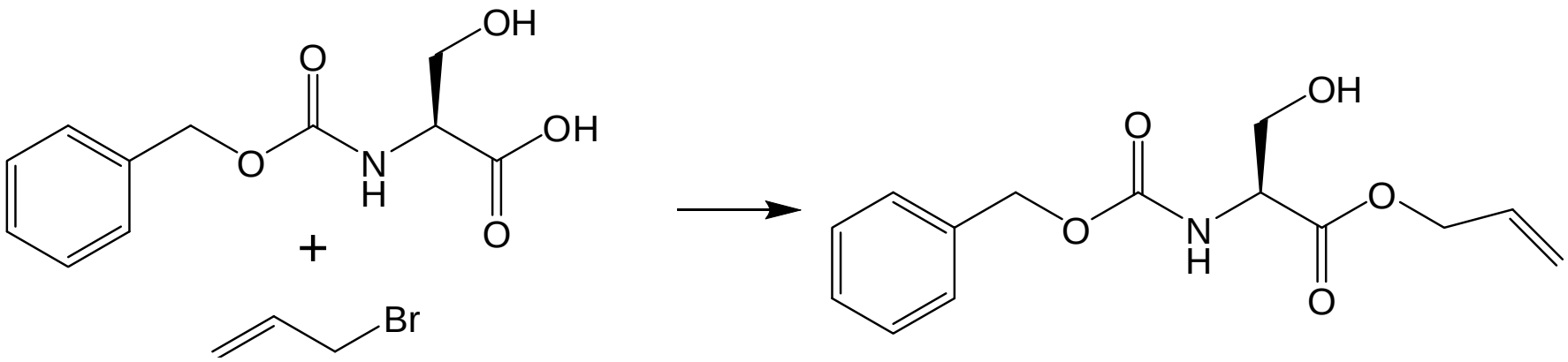


70 - 90%
(most of the esters remained as oils)

Procedure:
2-Trimethylsilylethanol, dicyclohexylcarbodiimide, acetonitrile, 0 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Allyl ester

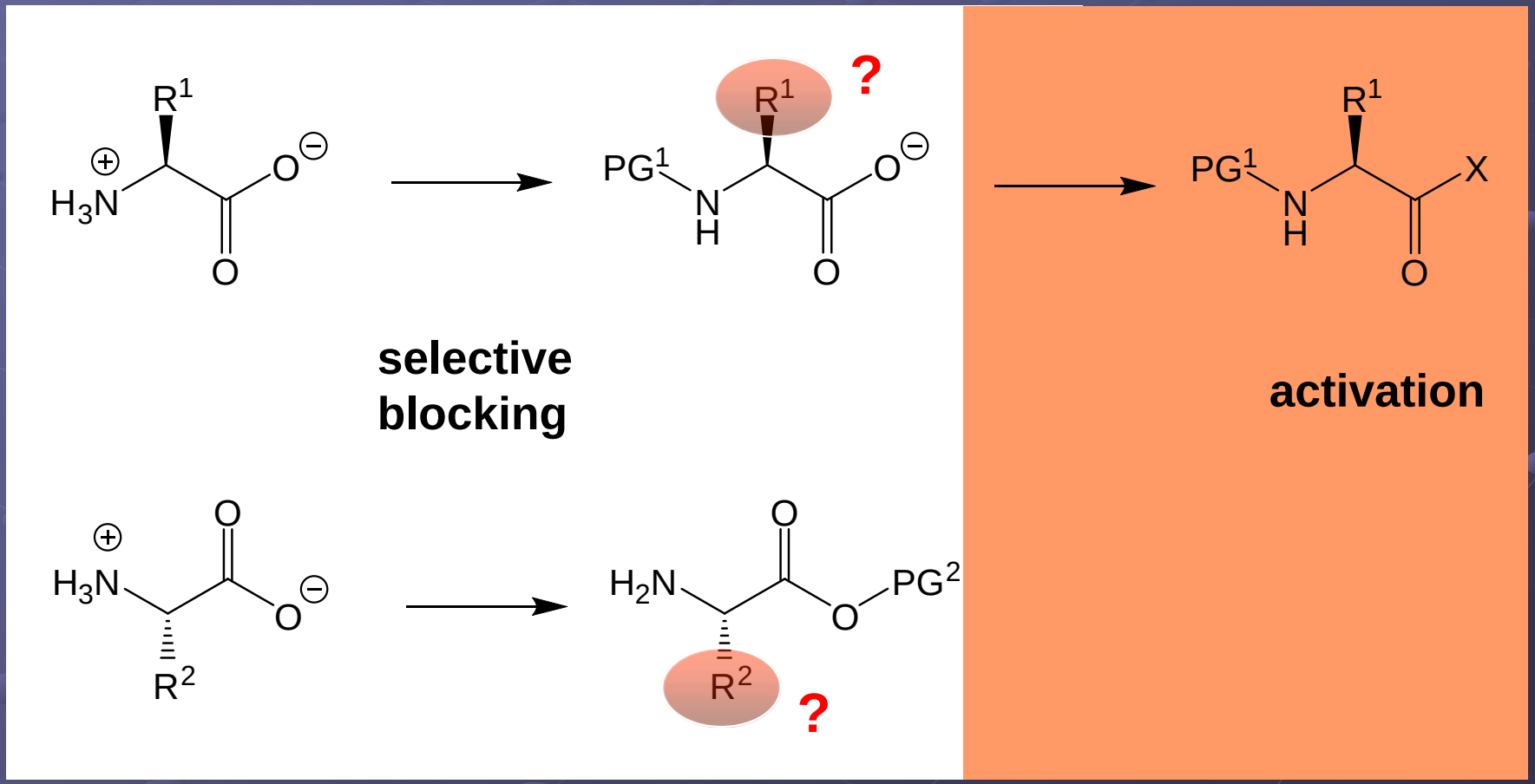


83% (colourless oil)

Procedure:
5% aq $NaHCO_3$, H_2O, CH_2Cl_2, allyl bromide, tri-*n*-octylmethylammonium chloride, vigorously stirring, 20 °C, 72 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

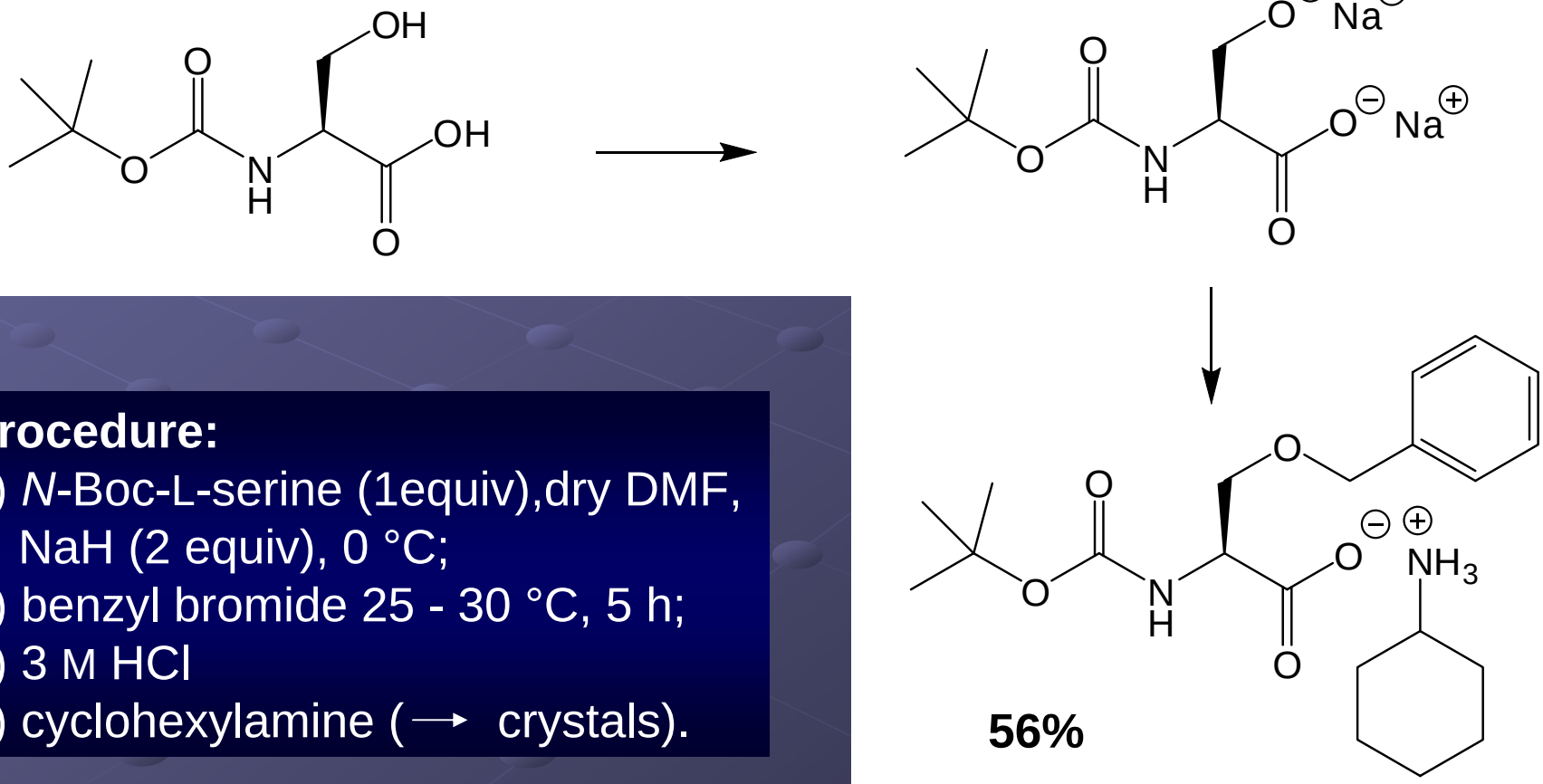
Basic principles of peptide bond formation



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Protection of the side chain functions

L-Serine ethers

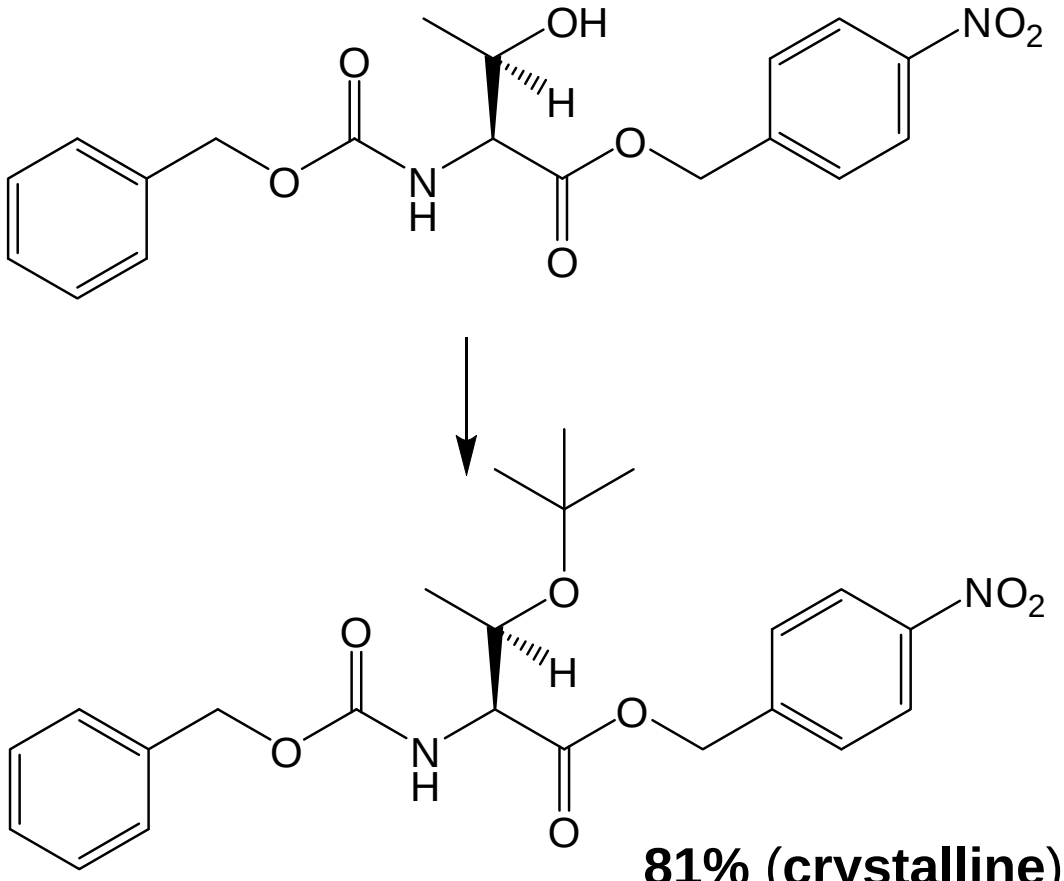


Procedure:

1) *N*-Boc-L-serine (1equiv),dry DMF, NaH (2 equiv), 0 °C;
2) benzyl bromide 25 - 30 °C, 5 h;
3) 3 M HCl
4) cyclohexylamine (⟶ crystals).

CHEMICAL SYNTHESIS OF BIOPOLYMERS

L-Threonine ethers

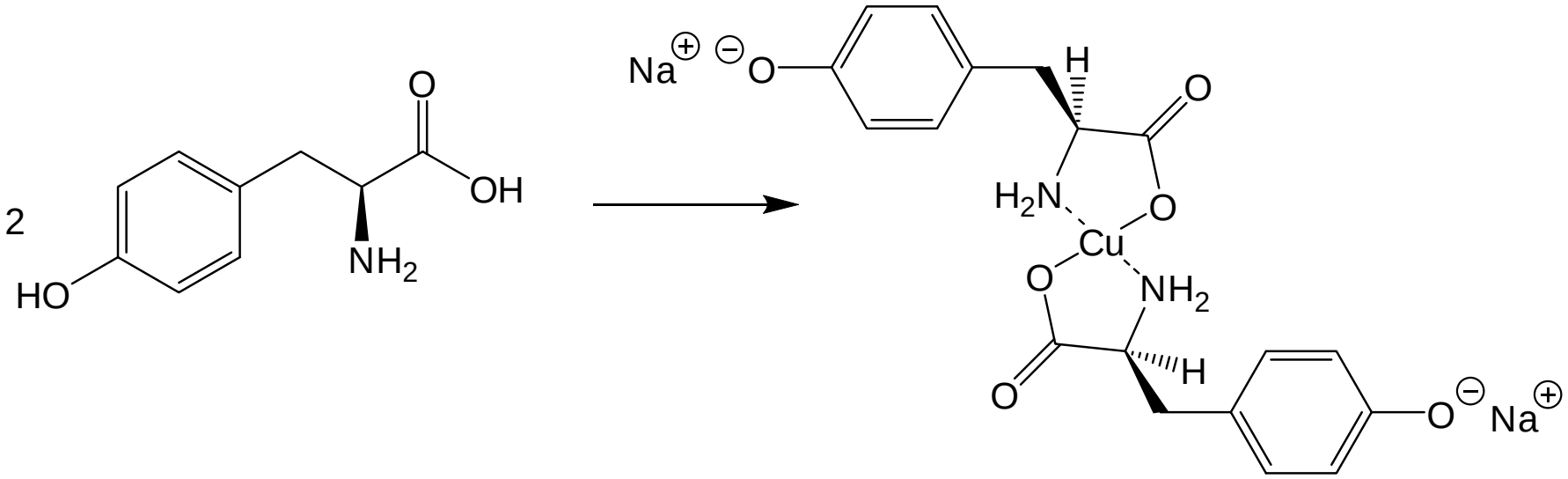


81% (crystalline)

Procedure:
Isobutene, cat H_2SO_4,
CH_2Cl_2, 0 °C → 20 °C, 4 d.

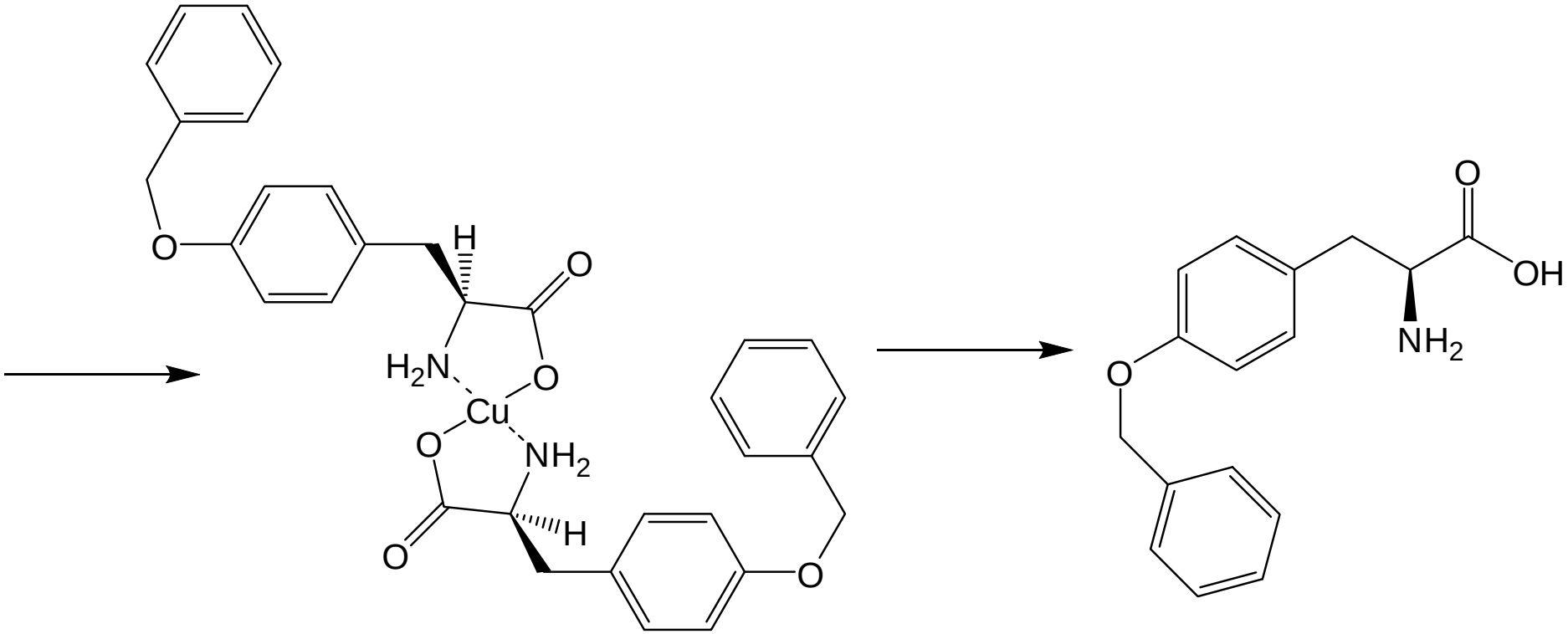
CHEMICAL SYNTHESIS OF BIOPOLYMERS

L-Tyrosine ethers



Procedure:
$CuSO_4$, 2 M NaOH, H_2O, 60 °C;

CHEMICAL SYNTHESIS OF BIOPOLYMERS



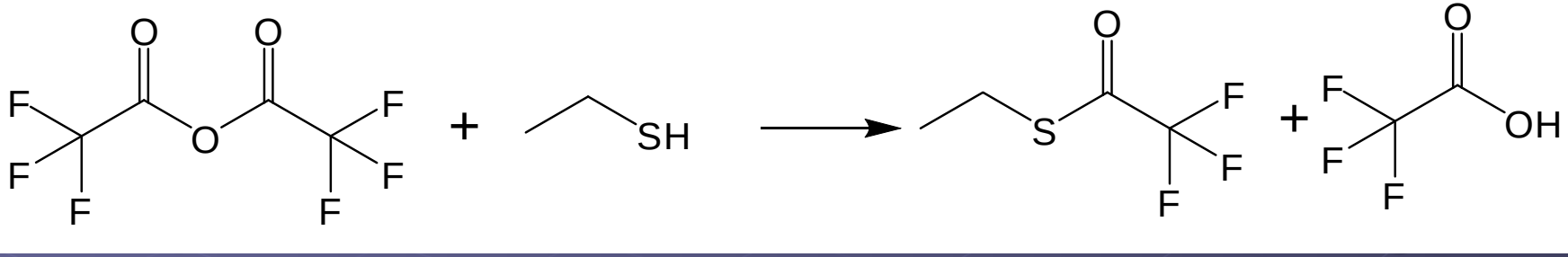
Procedure:

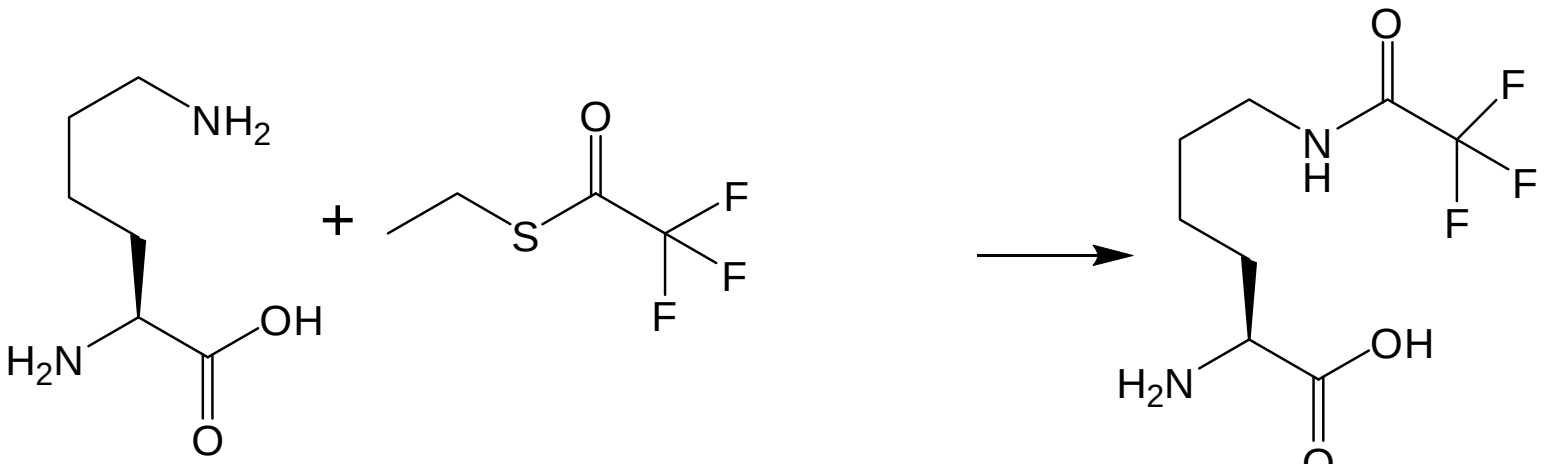
1) 2 M NaOH, benzyl bromide, 30 °C, 2 h;
2) 1 M HCl
3) 1.5 M NH_4OH

64% (crystalline)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

*N*ε-Trifluoroacetyl-L-lysine





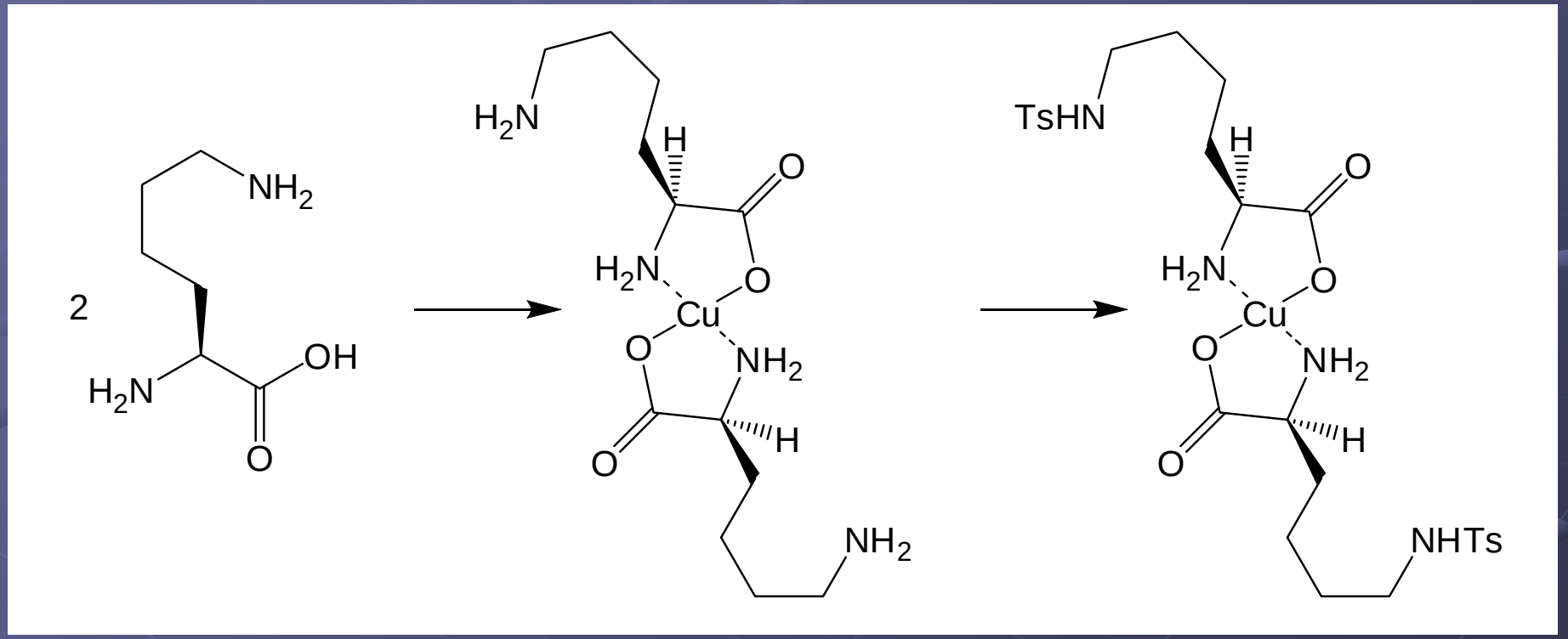
Procedure:

1) $(CF_3CO)_2O$, EtSH, 0 °C → 100 °C, 5 h;
2) ethyl thioltrifluoroacetate, 1 M NaOH, 20 °C, 6 h.

52% (crystalline)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

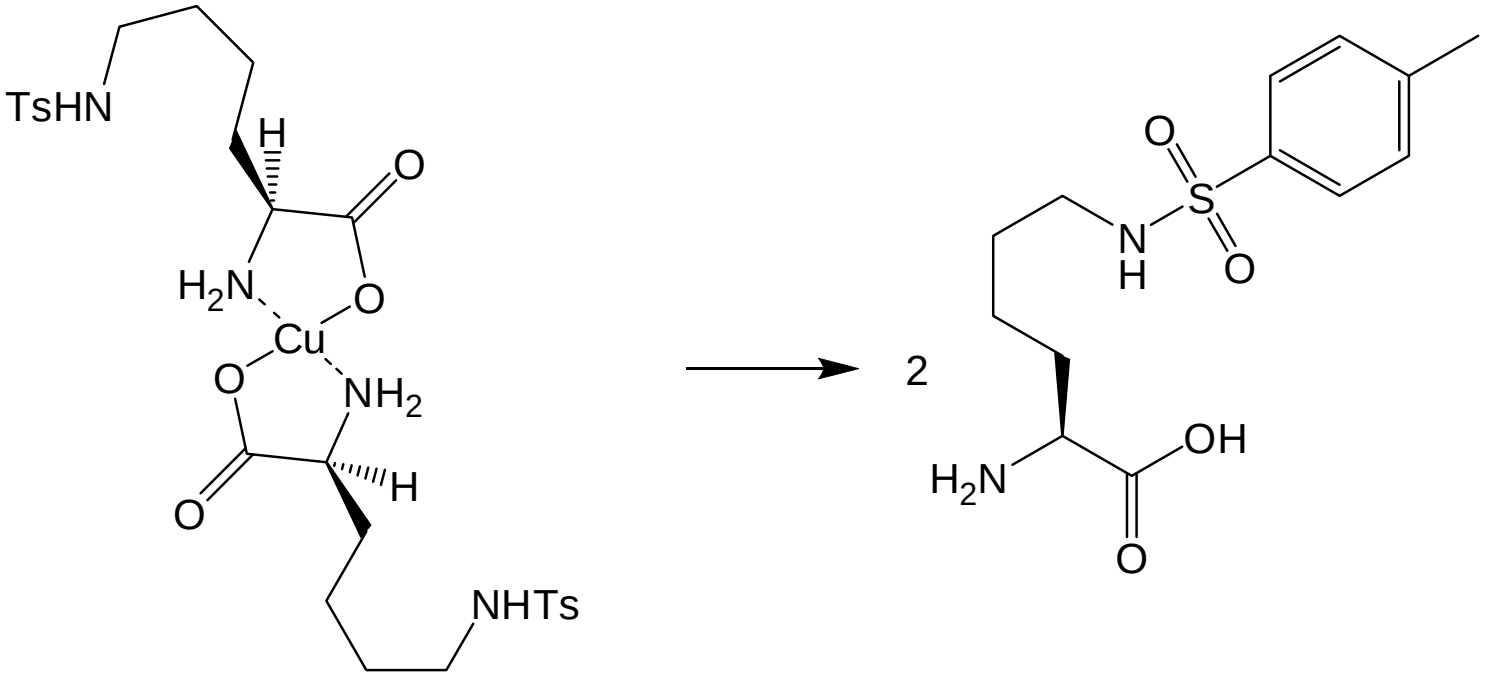
N^{ε}-*p*-Toluenesulfonyl-L-lysine



Procedure:

1) ($CuCO_3$/$Cu(OH)_2$, H_2O,reflux, 2 h;
2) $NaHCO_3$, acetone, *p*-toluenesulfonyl chloride, 20 °C, 12 h;

CHEMICAL SYNTHESIS OF BIOPOLYMERS

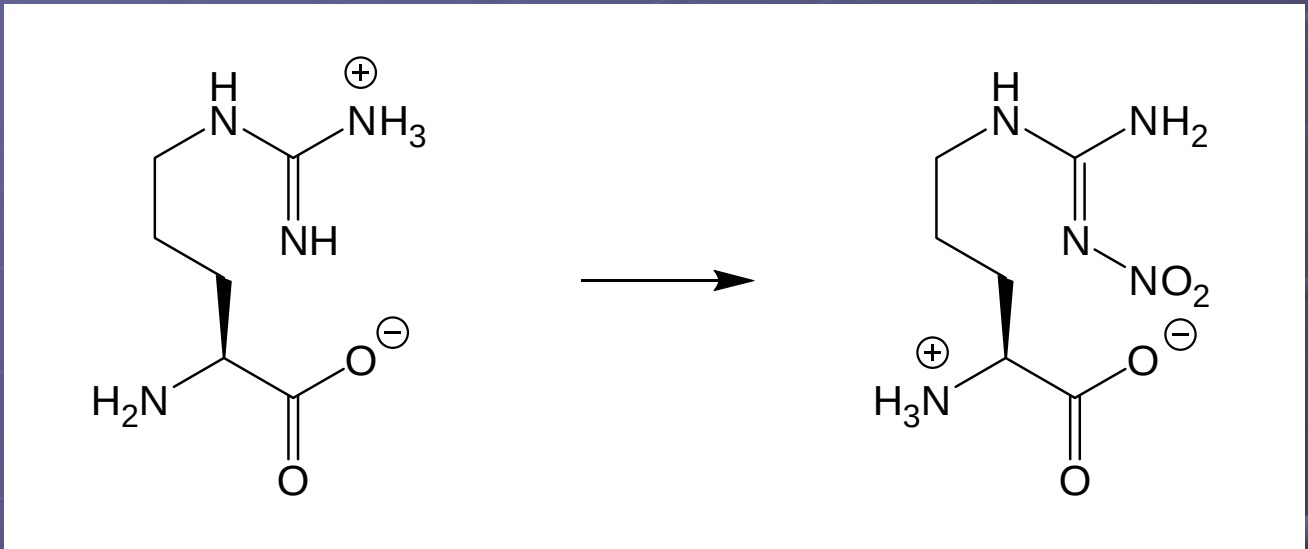


55% (crystalline)

Procedure:
3) H_2S, H_2O, reflux,

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Nitro-L-arginine

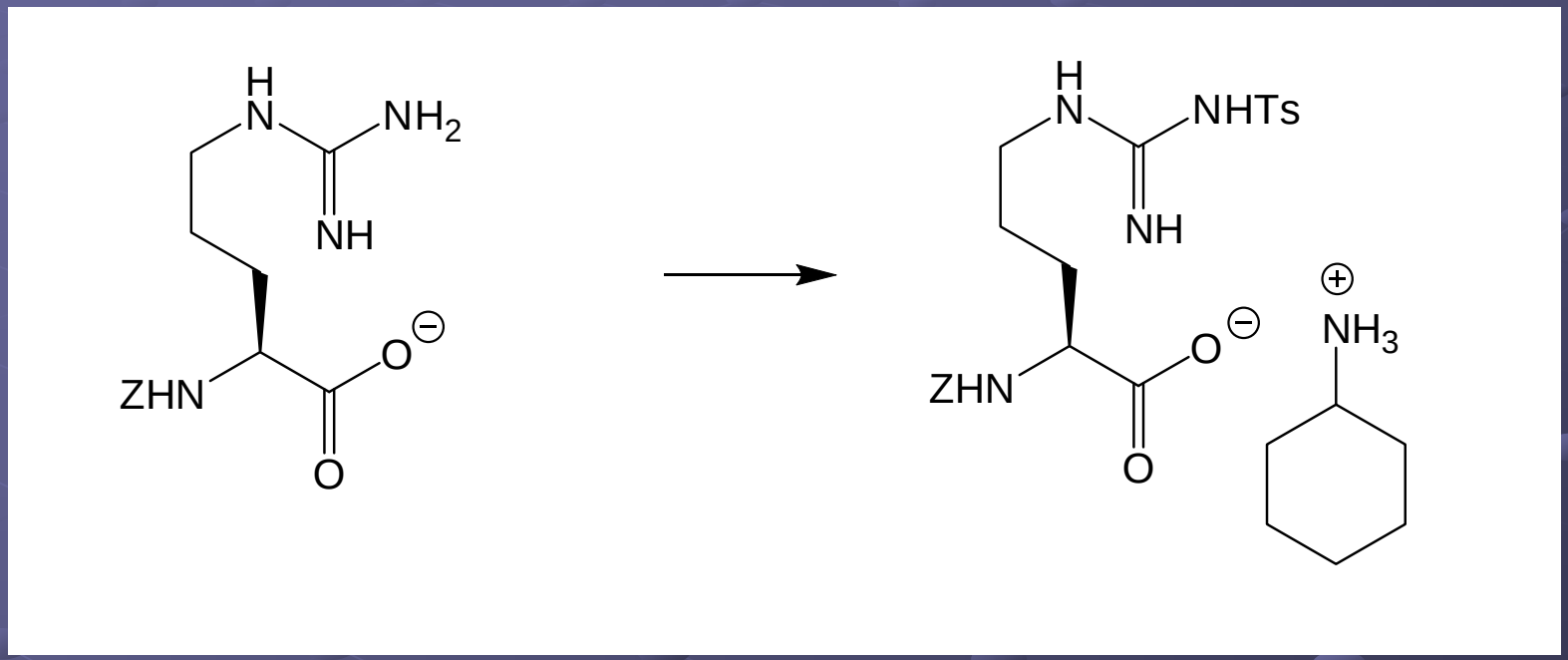


82% (crystalline)

Procedure:
Fuming sulfuric acid (30% SO_3),fuming nitric acid, 0 °C, 2 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

N^{α}-Benzyloxycarbonyl- N^{G}-*p*-toluenesulfonyl-L-arginine



48% (crystalline)

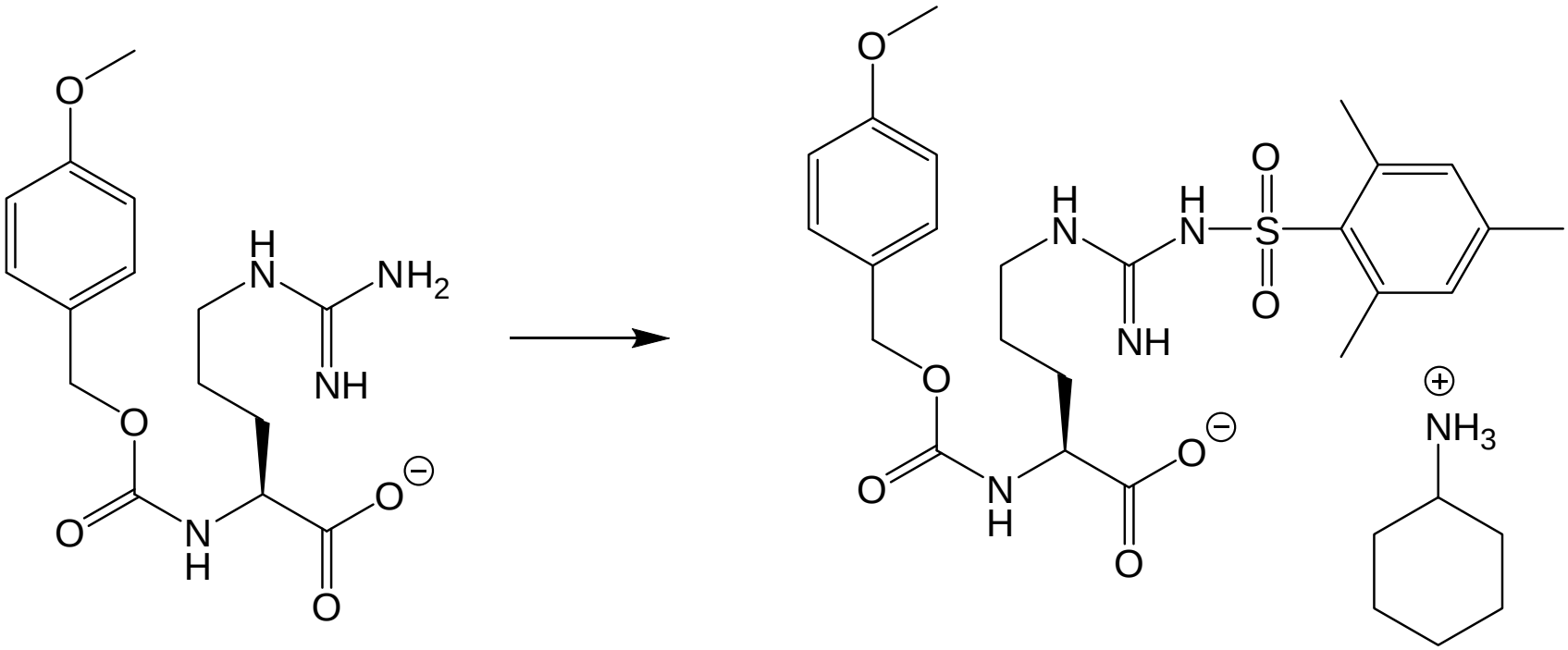
Procedure:

1) *p*-toluene sulfonyl chloride, acetone-H_2O, 4 M NaOH, 0 °C, 4 h;

2) cyclohexylamine

CHEMICAL SYNTHESIS OF BIOPOLYMERS

N^{α}-p-Methoxy-benzyloxycarbonyl- N^{G}-mesitylenesulfonyl-L-arginine



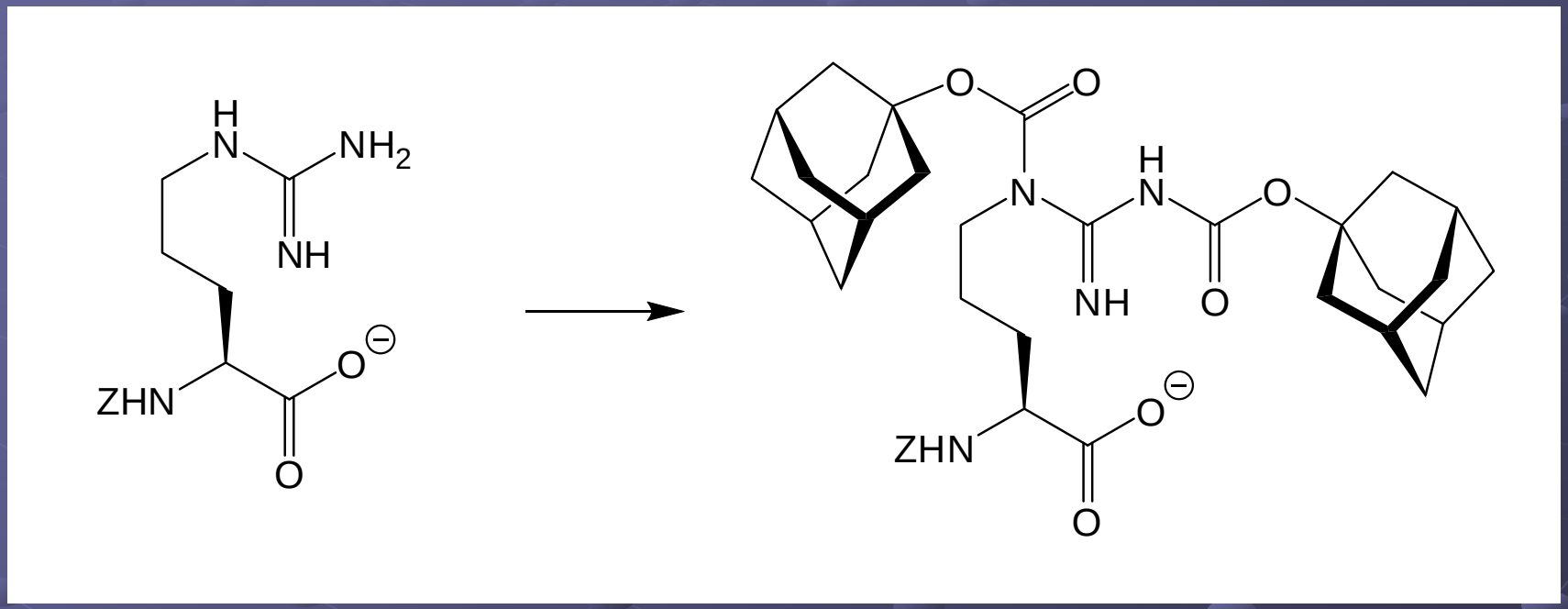
61% (crystalline)

Procedure:

1) Mesitylene-2-sulfonyl chloride, acetone-H_2O, 4 M NaOH, 0 °C, 4 h;
2) cyclohexylamine

CHEMICAL SYNTHESIS OF BIOPOLYMERS

N^{α}-Benzyloxycarbonyl-N^{δ},N^{ω}-bis-(1-adamantyl-oxycarbonyl)-L-arginine



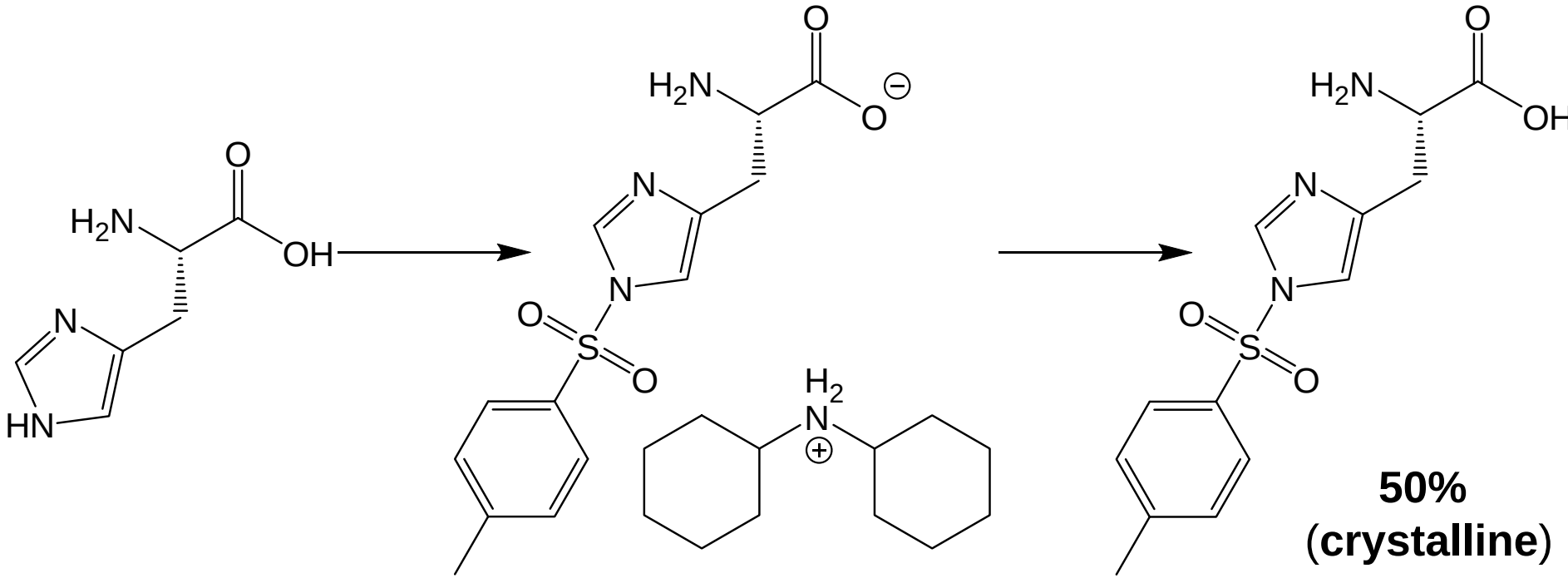
90% (crystalline)

Procedure:

1) 1-adamantyl chlorocarbonate, dioxane - 2 M NaOH, 7 °C, 4 h;
2) citric acid

CHEMICAL SYNTHESIS OF BIOPOLYMERS

N^{α}-Benzyloxycarbonyl-N^{im}-*p*-toluenesulfonyl-L-histidine

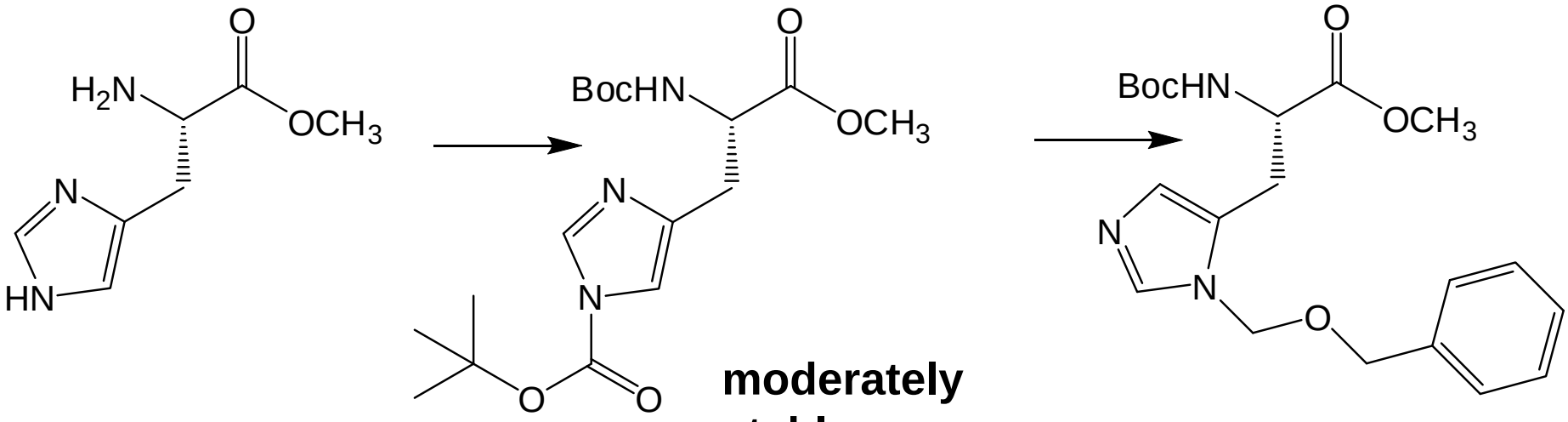


Procedure:

1) *p*-toluenesulfonyl chloride, $NaCO_3$, H_2O, 10 °C → 20 °C, 5 h;
2) 0.5 M H_2SO_4, 20 °C, 6 h;
3) dicyclohexylamine;
4) HBr-AcOH.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

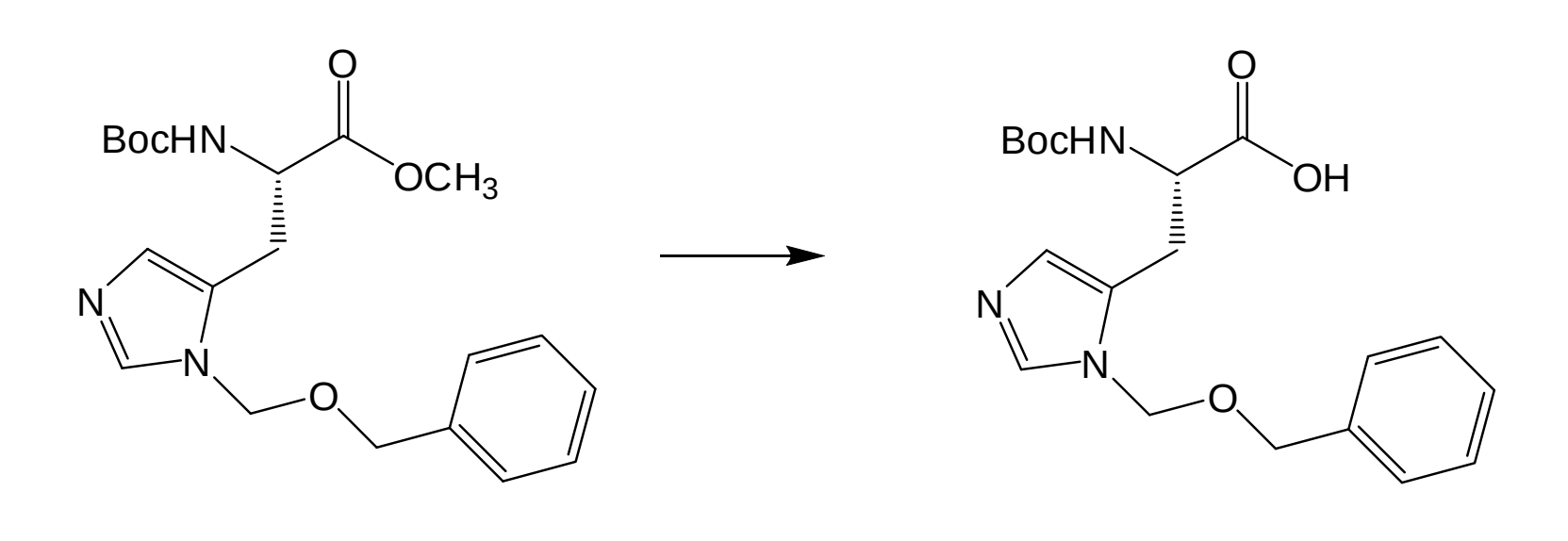
N^{ε}-*tert*-Butyloxycarbonyl-N^{π}-benzyloxymethyl-L-histidine



Procedure:

1) $[(CH_3)_3O\text{-}CO]_2O$, Et_3N, methanol,20 °C, 12 h;
2) benzyl chloromethyl ether, CH_2Cl_2, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

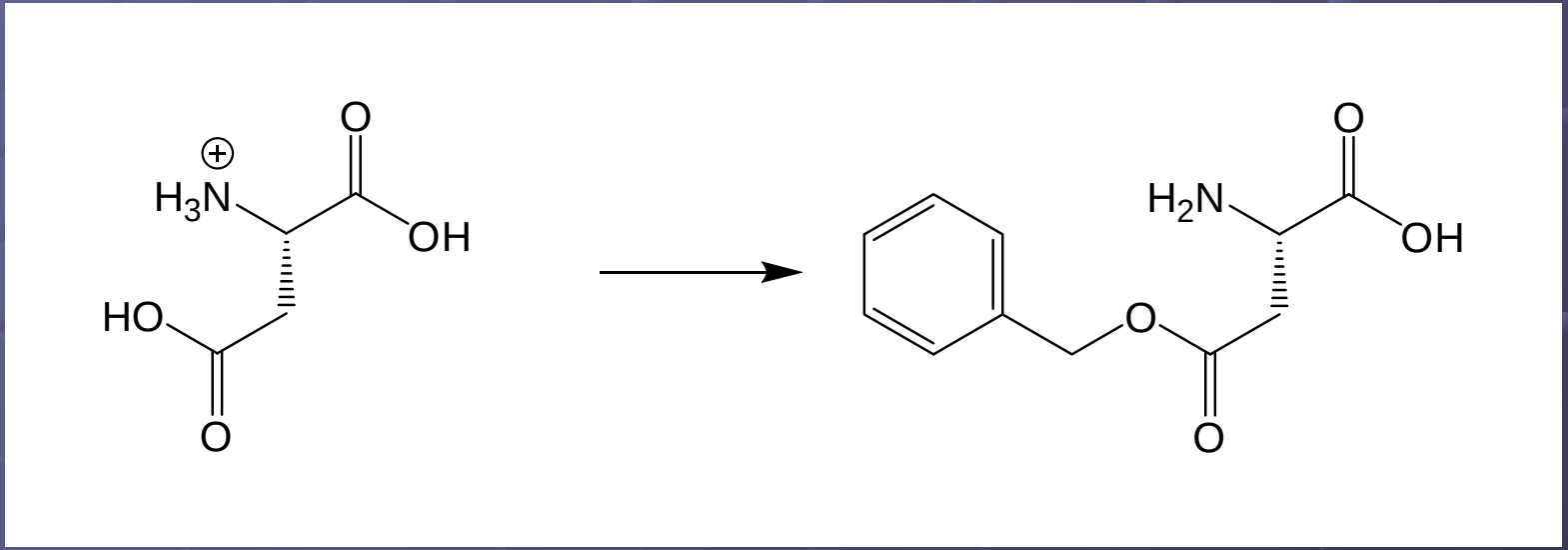


54% (crystalline)

Procedure:
3) 1 M NaOH, methanol, 15 min;
4) 1 M HCl.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

β-Benzyl L-aspartate



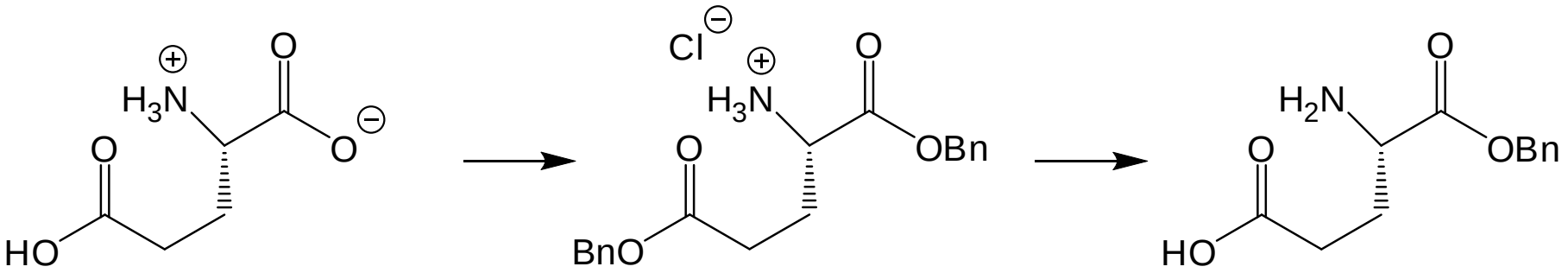
40% (crystalline)

Procedure:

1) Benzyl alcohol, cat H_2SO_4, 20 °C, 24 h;
2) pyridine.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

L-Glutamic acid α-benzyl ester (starting material for protecting group manipulation on next slide)



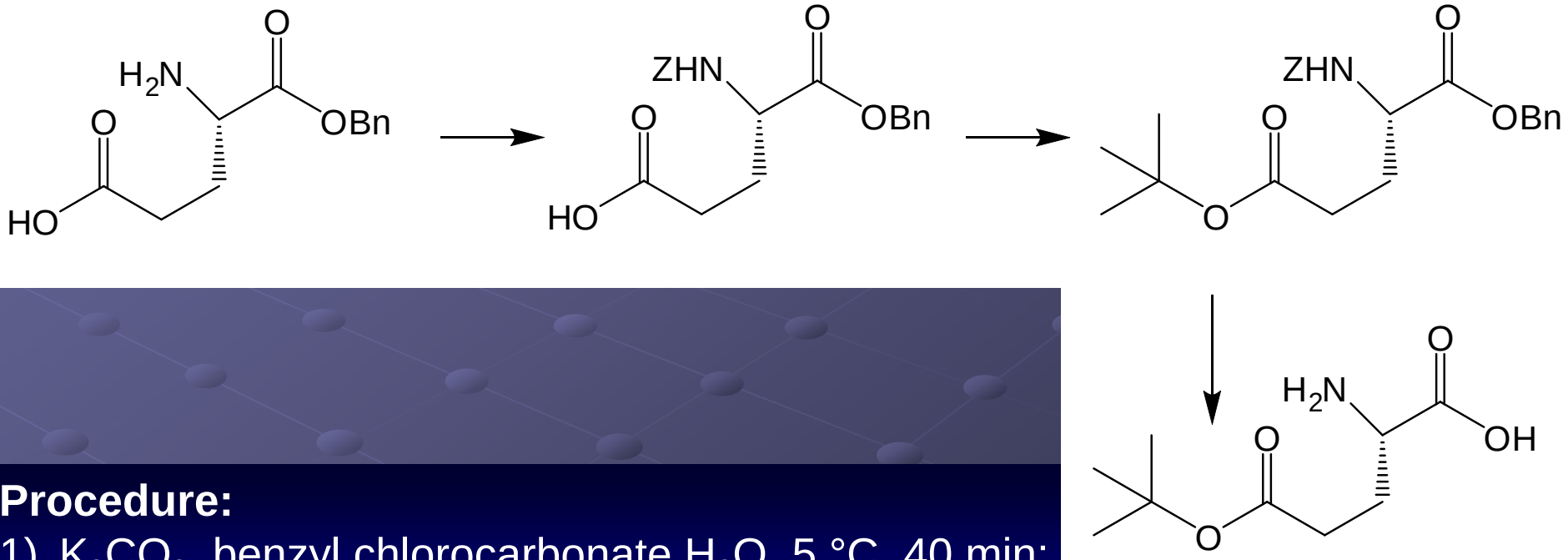
60% (crystalline) **67% (crystalline)**

Procedure:

1) dry HCl gas, benzyl alcohol, removal of H_2O by repeated distillation with benzene in vacuo, 55 °C → 85 °C, 4 h;
2) HI, acetic acid, 50 °C, 6 h;
3) tri-*n*-butylamine.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

L-Glutamic acid γ-*tert*-butyl ester



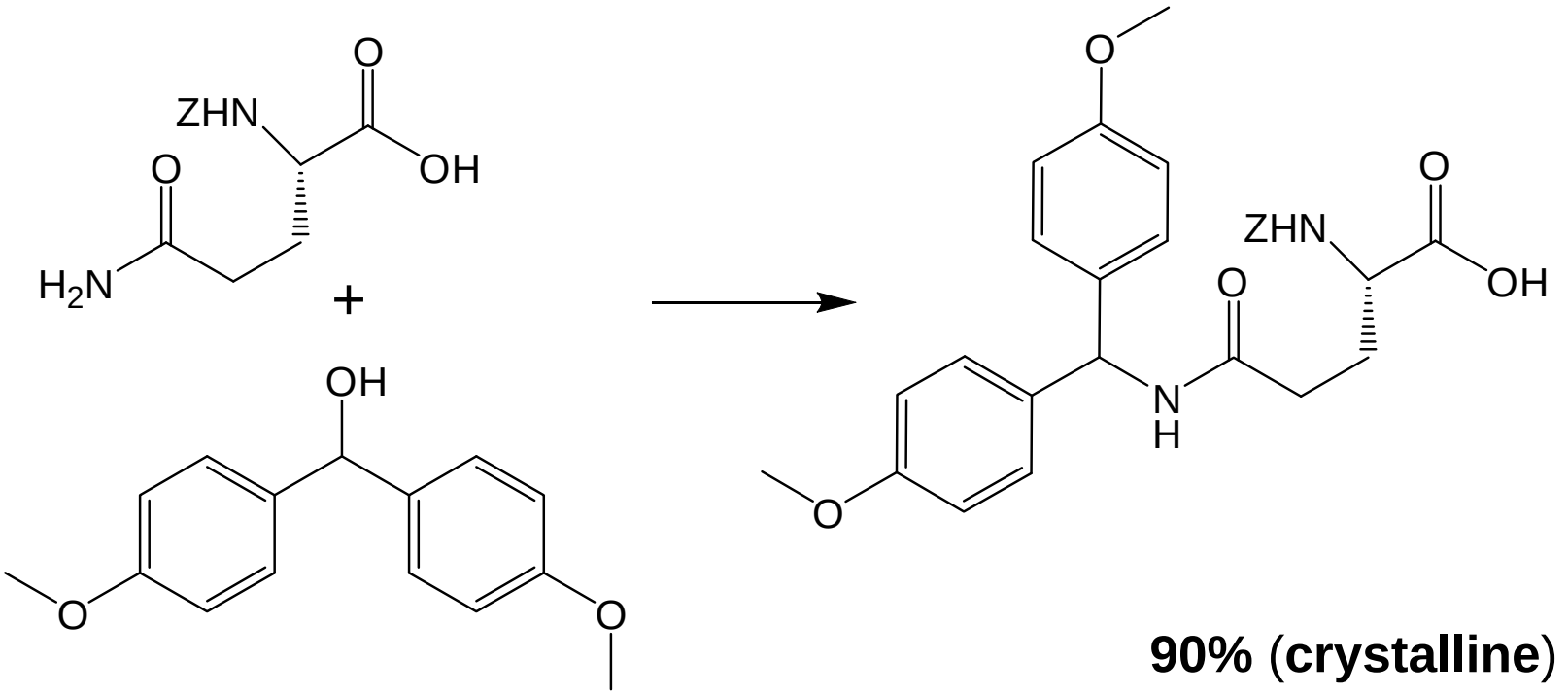
Procedure:

1) K_3CO_3, benzyl chlorocarbonate,H_2O, 5 °C, 40 min;
2) 6 M HCl;
3) isobutylene, cat H_2SO_4; -15 °C → 20 °C, 20 h;
4) 10% Pd/C, H_2, methanol, 20 °C, 2h.

45% (crystalline)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Benzyloxycarbonyl-4,4’-dimethoxybenzhydryl-L-glutamine

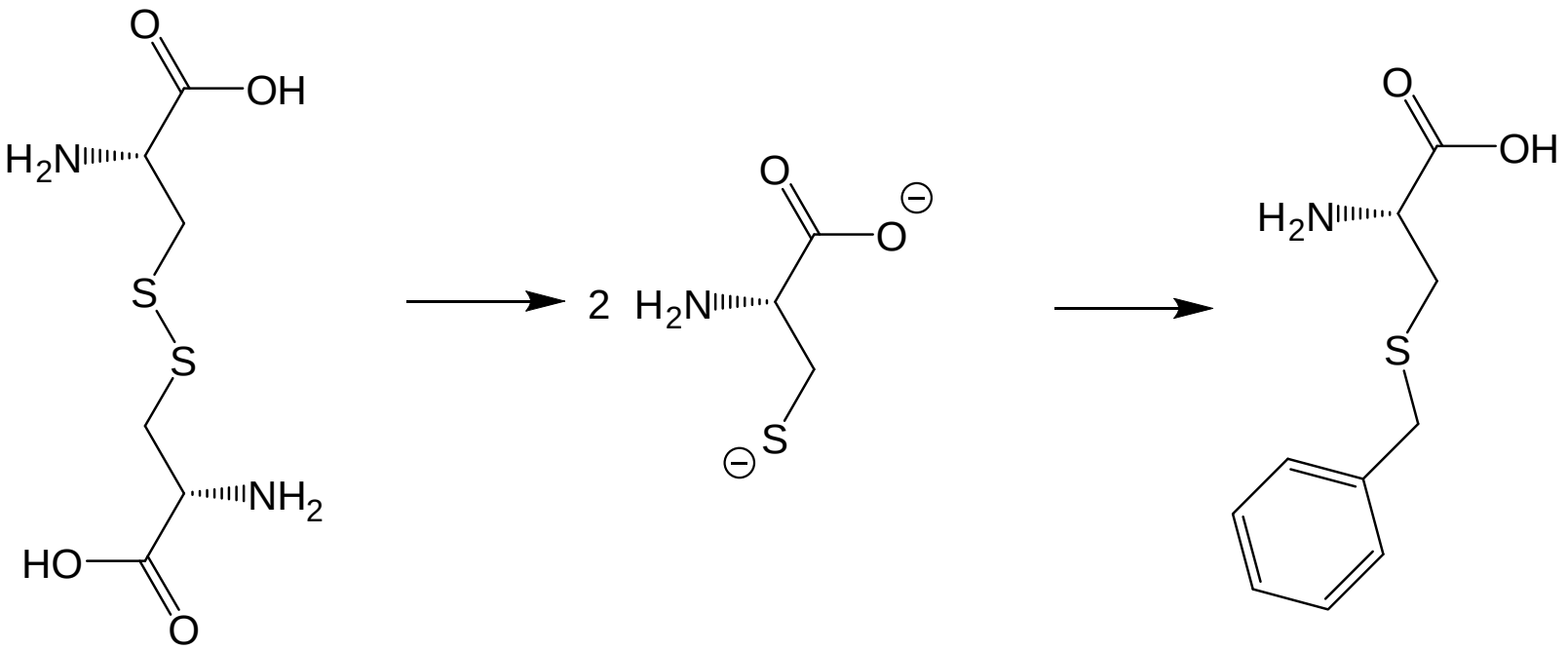


Procedure:

1) 4,4’-Dimethoxybenzhydrol, acetic acid, cat H_2SO_4, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

S-Benzyl-L-cysteine



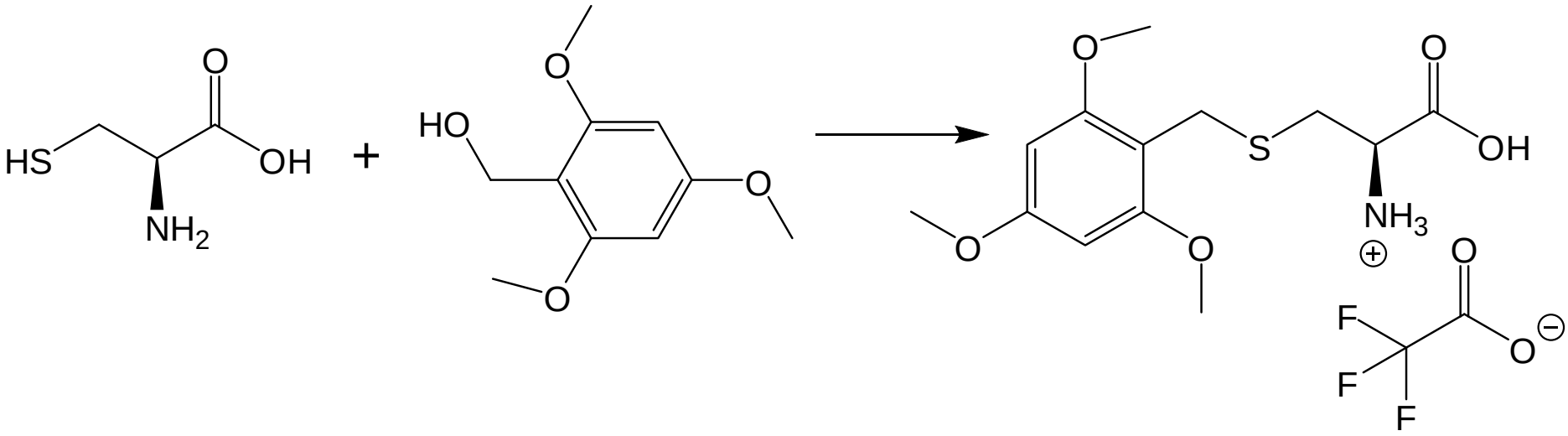
Procedure:

1) anhydr liq NH_3, Na, -40 °C, 30 min;
2) benzyl chloride; -40 °C → 50 °C, 12 h;
3) 6 M HCl.

85% (crystalline)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

S-2,4,6-Trimethoxybenzyl-L-cysteine (Tmob protecting group)



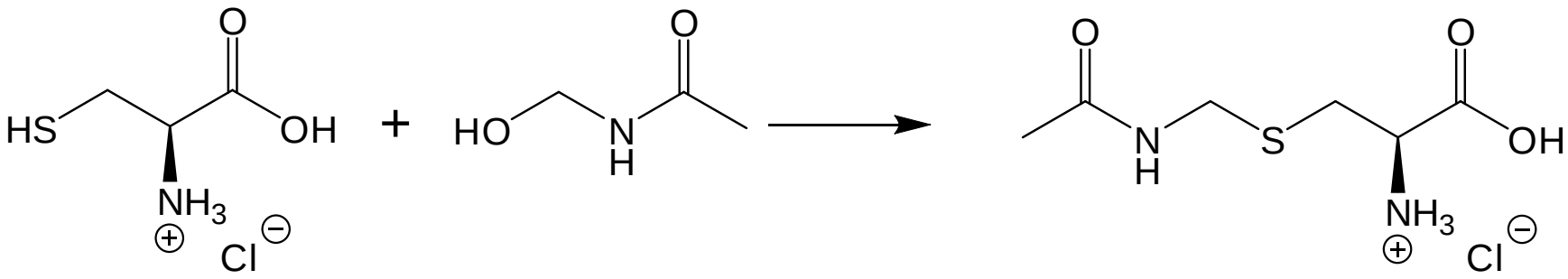
84% (crystalline)

Procedure:
CF_3COOH, 2,4,6-trimethoxybenzyl alcohol, CH_2Cl_2, 5 °C, 10 min.

Lit.: Munson, M. C.; Garcia-Echeverria, C.; Albericio, F.; Barany, G. *J. Org. Chem.* **1992**, *57*, 3013-3018.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

S-Acetamido-methyl-L-cysteine (Acm protecting group)



71% (crystalline)

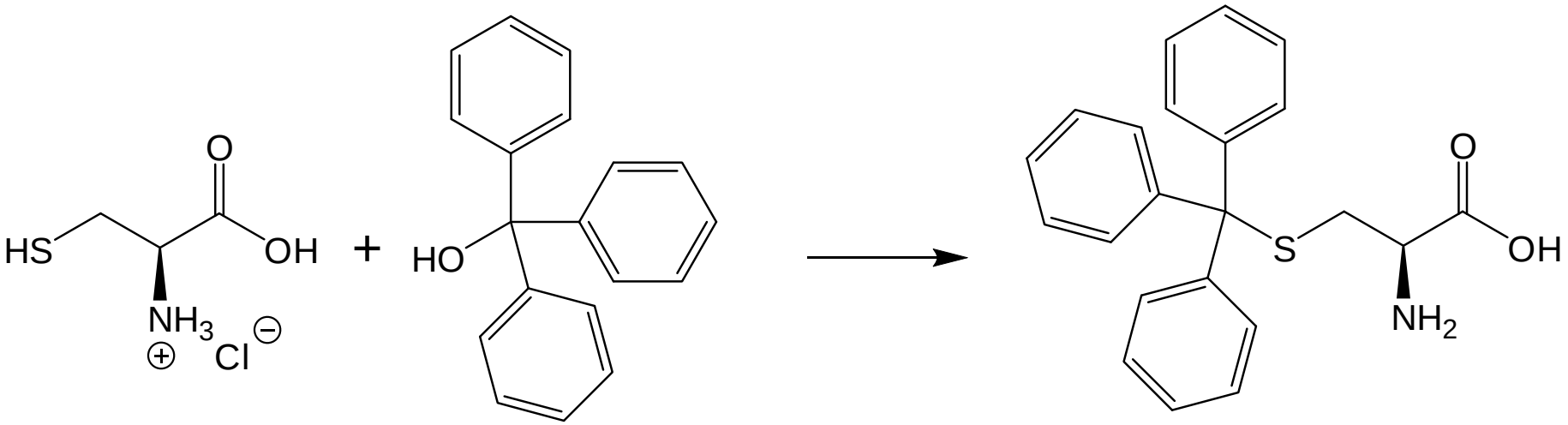
Procedure:

1) *N*-Hydroxymethyl-acetamide, CF_3COOH, 20 °C, 30 min;

2) 1 M HCl.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

S-Triphenylmethyl-L-cysteine



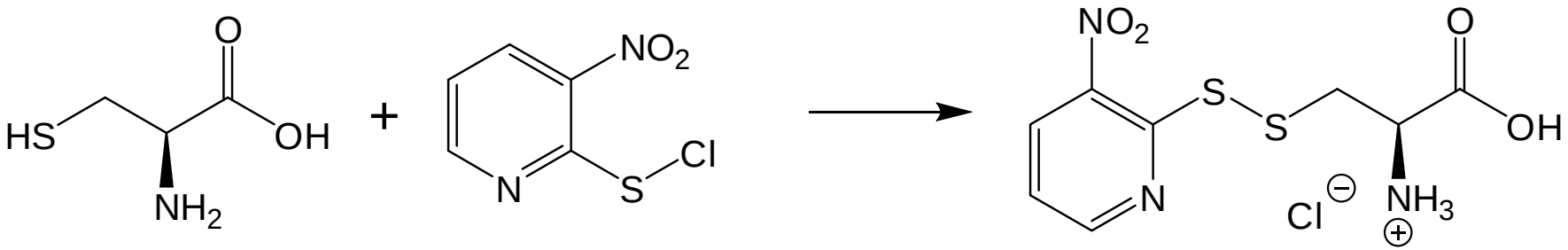
85% (crystalline)

Procedure:

1) Triphenylmethanol, acetic acid, BF_3etherate,60 °C → 80 °C, 30 min;
2) Ethanol – water, NaOAC.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

***S*-(3-Nitro-2-pyridinesulfenyl)-L-cysteine**

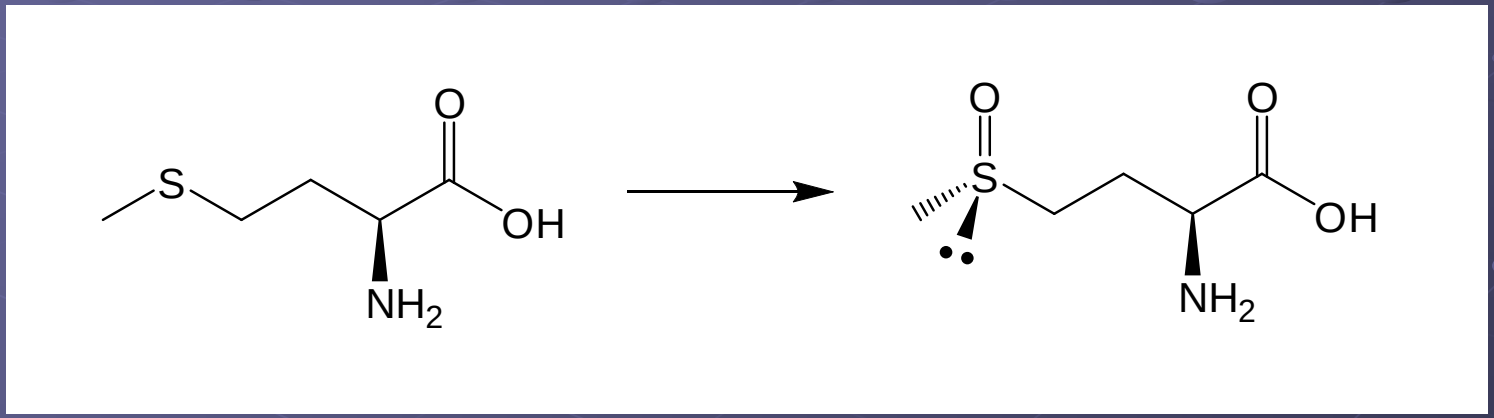


70% (crystalline)

Procedure:
90% HCOOH, 3-nitro-2-pyridinesulfenyl chloride, 20 °C, 1 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

L-Methionine sulfoxide



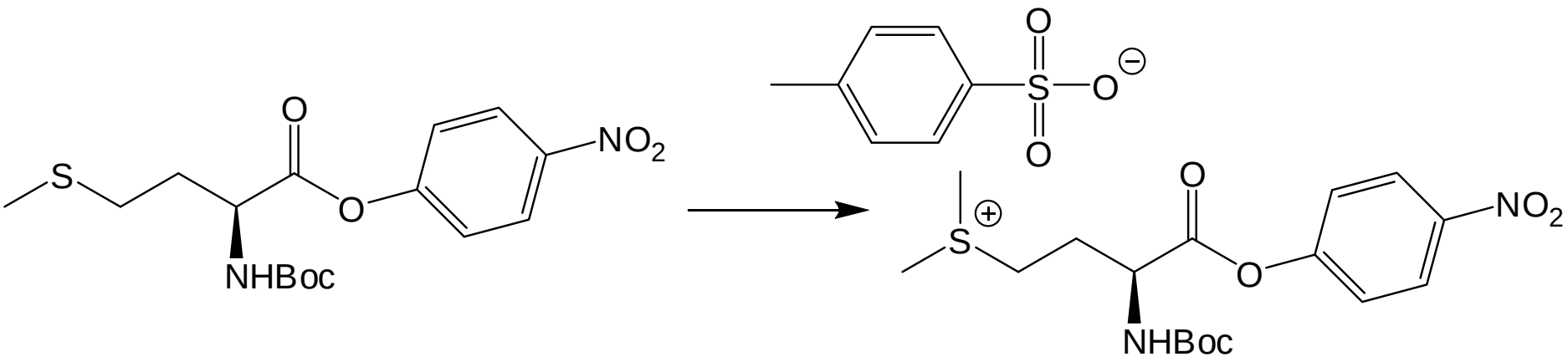
97% (crystalline)

Procedure:

1) 30% H_2O_2, H_2O, 20 °C, 1.5 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

***N-tert*-Butyloxycarbonyl-*S*-methyl-L-methionine *p*-nitrophenyl ester *p*-toluenesulfonate**



85% (crystalline)

Procedure:
Methyl *p*-toluenesulfonate,ethyl acetate, 20 °C, 4 d.