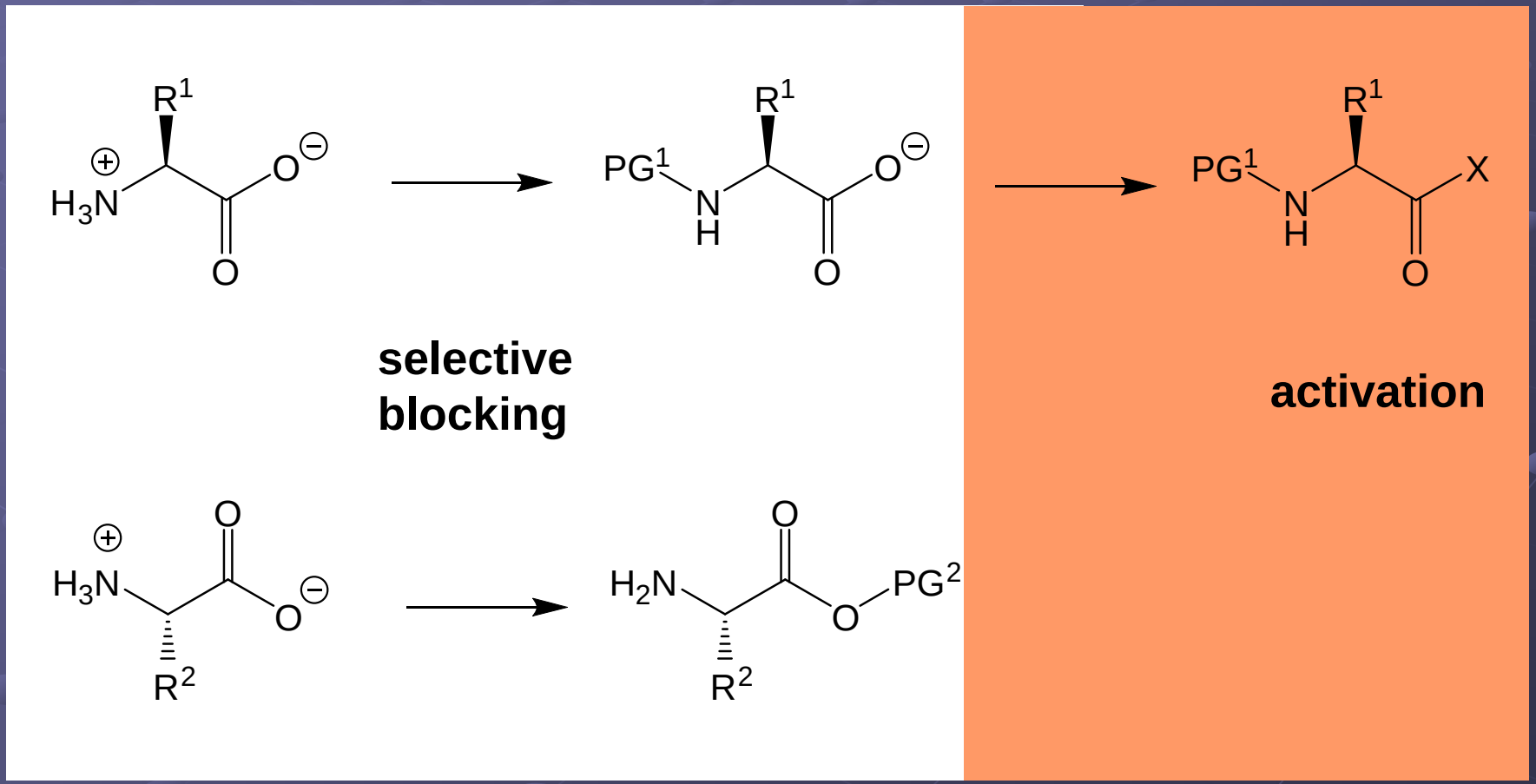
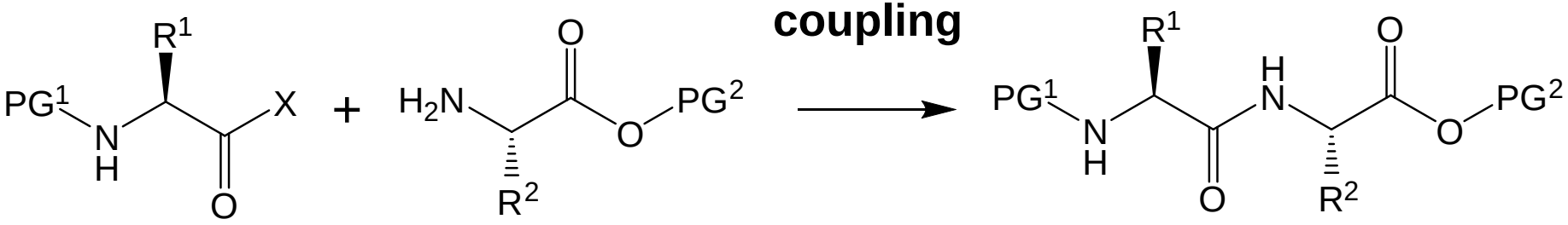
CHEMICAL SYNTHESIS OF BIOPOLYMERS

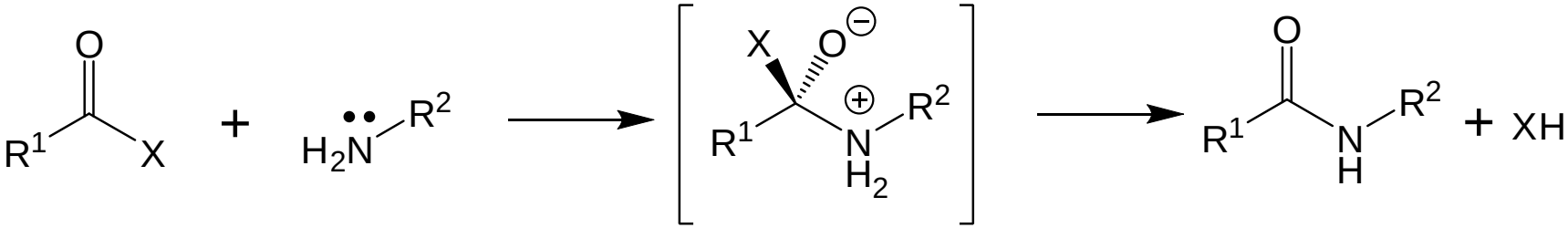
Activation and peptide bond formation



CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS



The peptide coupling reaction must be performed under **mild conditions**, and at room temperature in order to **avoid side-reaction and racemization**.

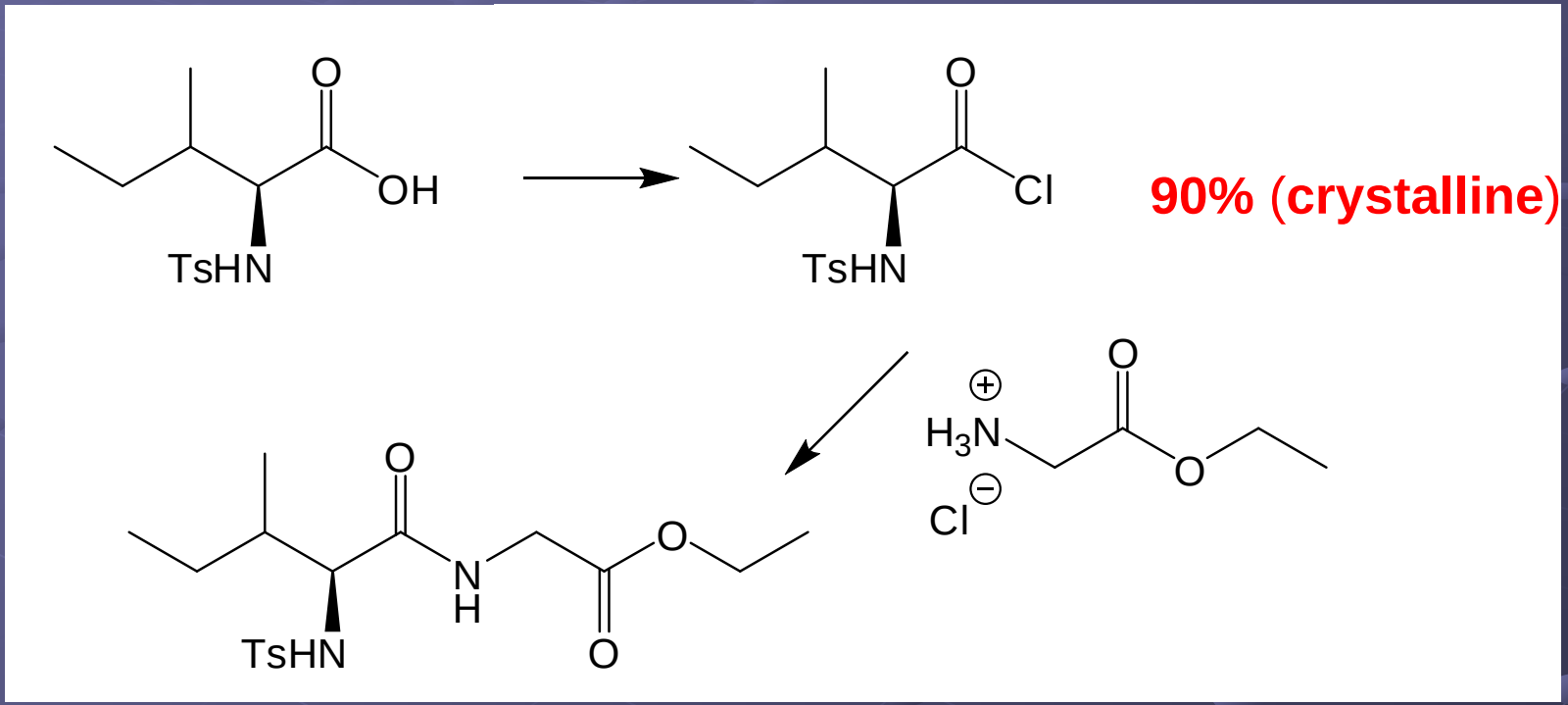
However, **carboxylic acids** react at room temperature **with amines** to give **ammonium salt** instead of carboxamide. Therefore, activation is necessary.

Activation is achieved with moieties which exert either an **negative inductive (-I) effect** or **negative mesomeric (-M) effect (or both)** to decrease the electron density at the C=O group.

The **leaving group capacity** (**nucleofugicity**) is another factor which influences the reaction rate.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The acid chloride procedure



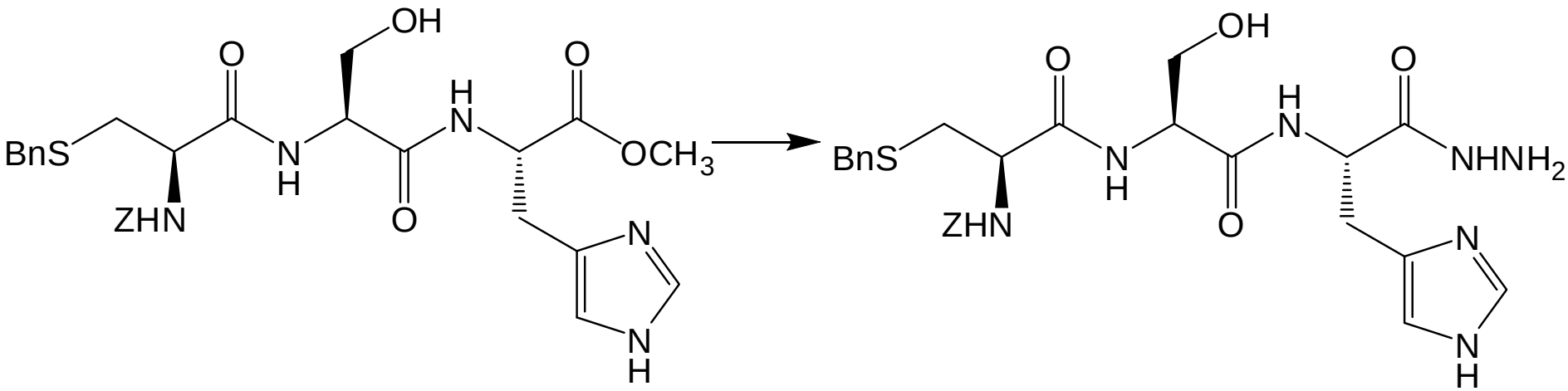
Procedure:

1) PCl_5,dry ether,20 °C, $O=PCl_3$, is removed by evaporation on an oil pump;
2) dry ether, Et_3N, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The azide procedure

Precursor – peptide hydrazides through hydrazinolysis of esters

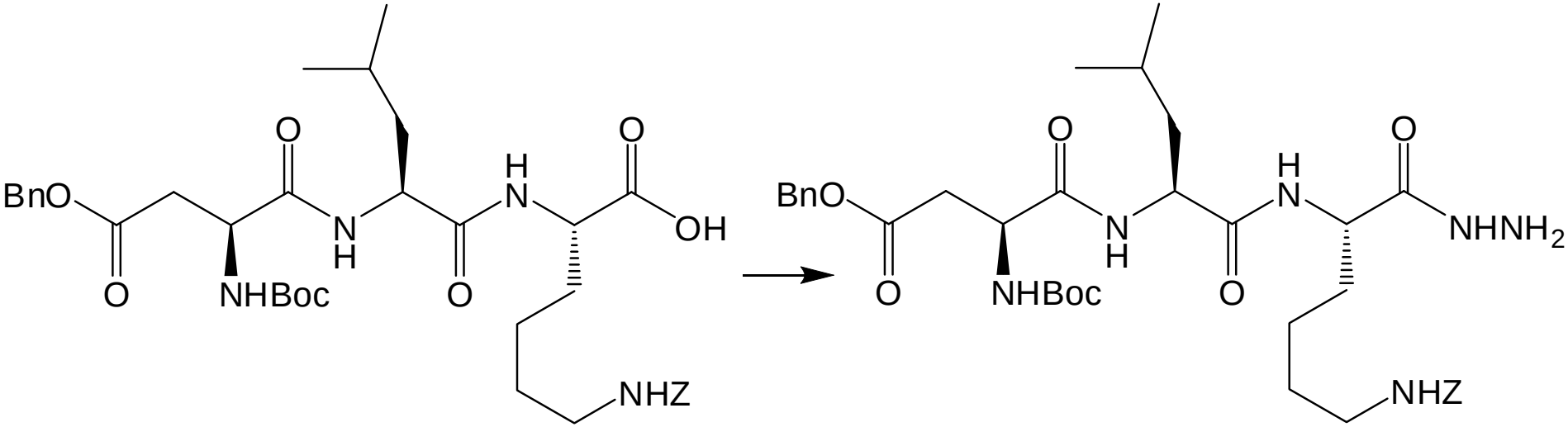


88% (crystalline)

Procedure:
Hydrazine hydrate,methanol, 20 °C, 3 d.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Peptide hydrazides from carboxylic acids

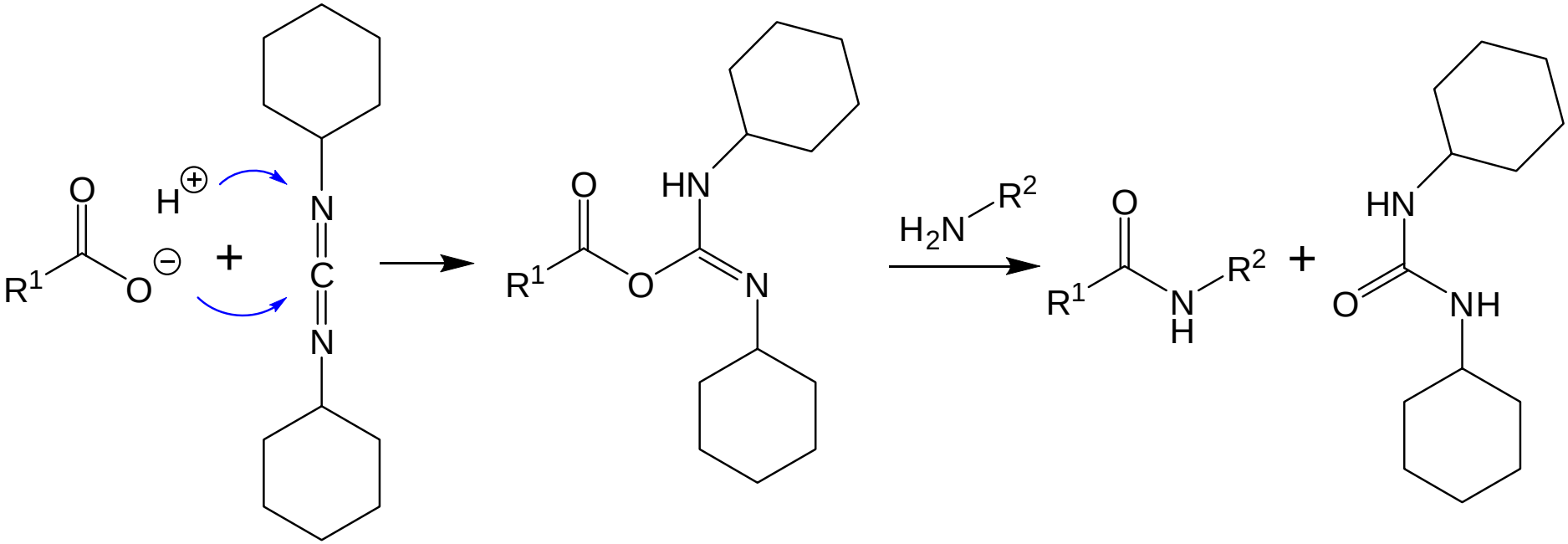


71% (crystalline)

Procedure:
Anhydr hydrazine,1-hydroxybenzotriazole,dicyclohexylcarbodiimide, DMF, 0 °C → 20 °C,12 h.

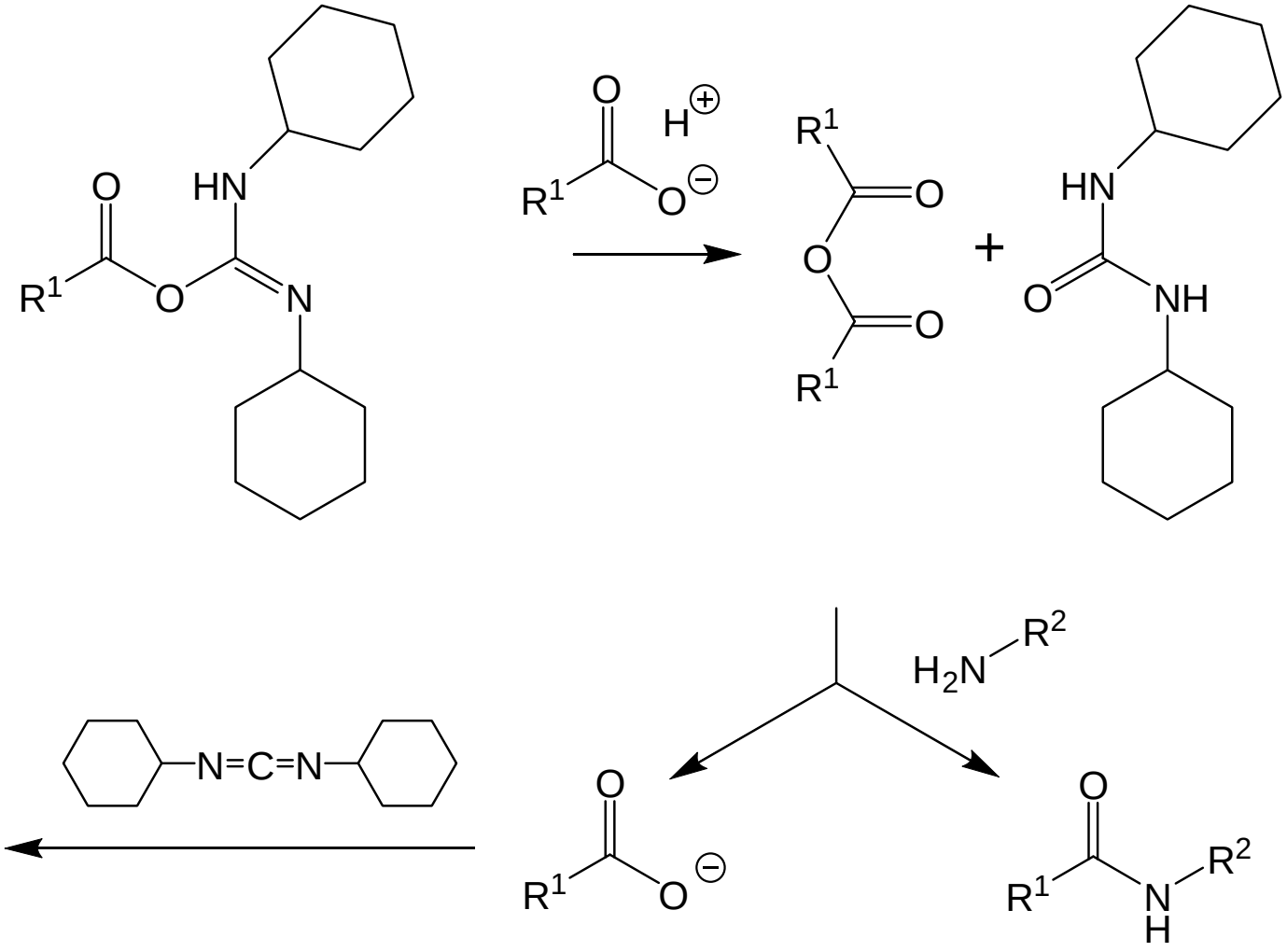
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Problems of the carbodiimide procedure



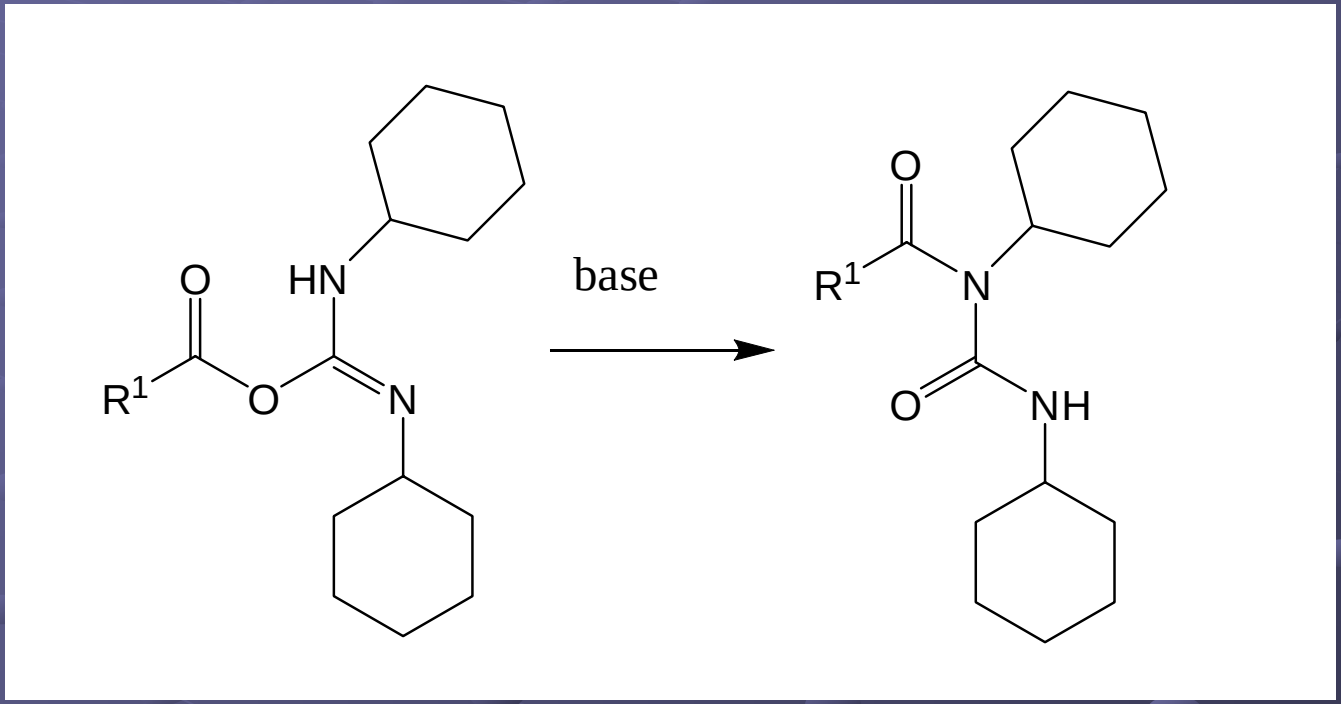
The **carboxylate** anion adds to the **protonated carbodiimide** with formation of a highly reactive ***O*-acylisourea**. This intermediate reacts with the **amino component** to produce in this case a hydrazide and the urea derivative.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Undesired side-reaction

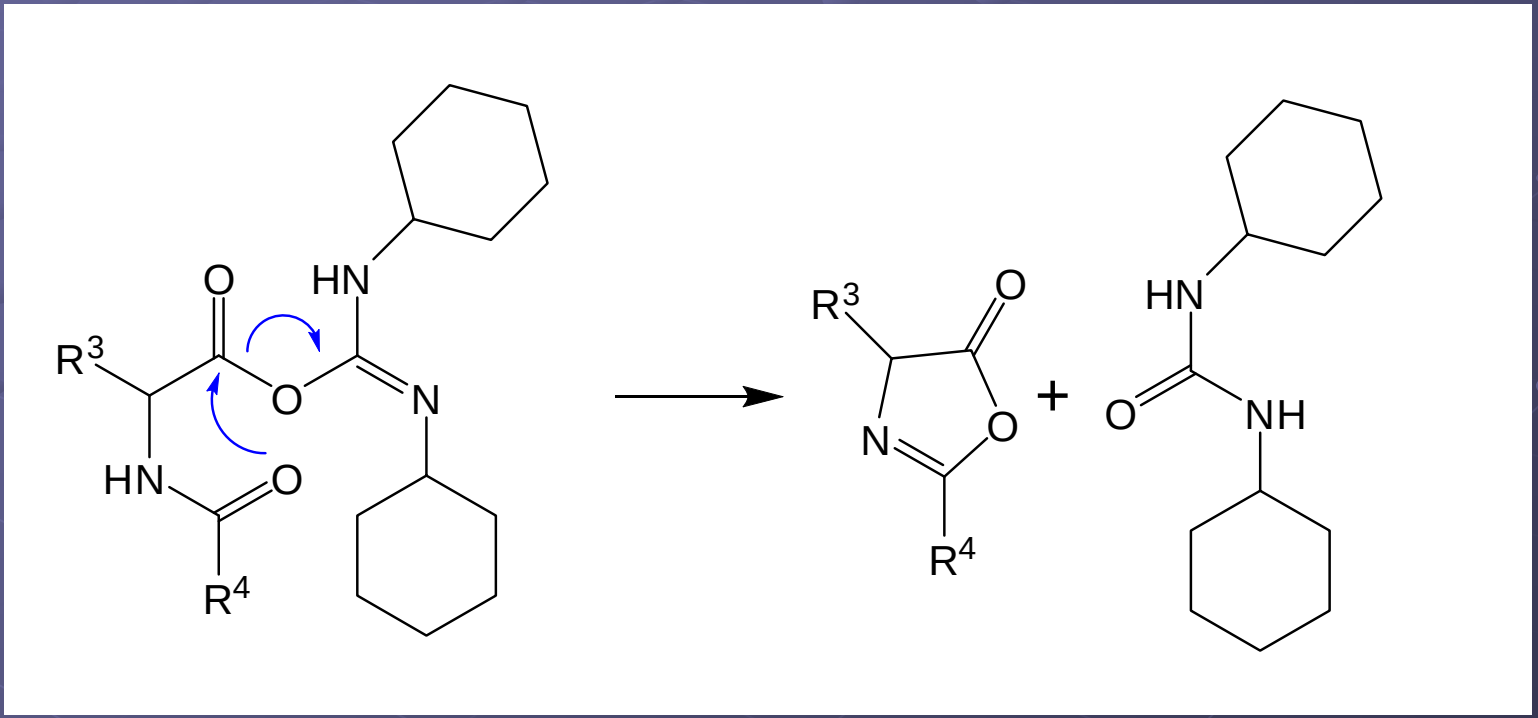


The ***O* → *N* acyl migration is catalyzed** even by the **basic amino component of the carbodiimide**. The ***N*-acylurea does not undergo further reactions!**

Polar solvents additionally **favour this reaction pathway**.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Undesired side-reaction in the case of N-acyl-protected amino acids and peptides

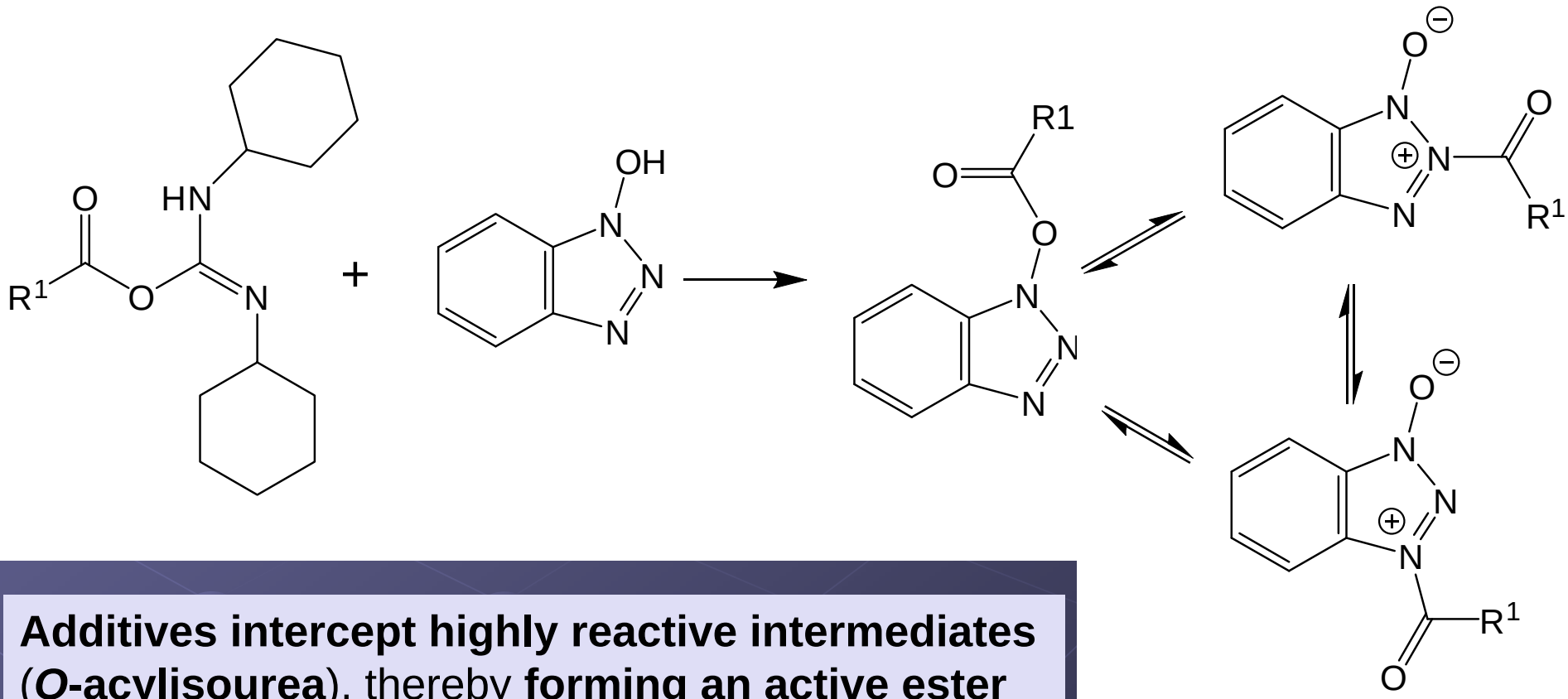


R^4-C=O can also be **another amino acid residue**.

5-Oxazolones are prone to racemization.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

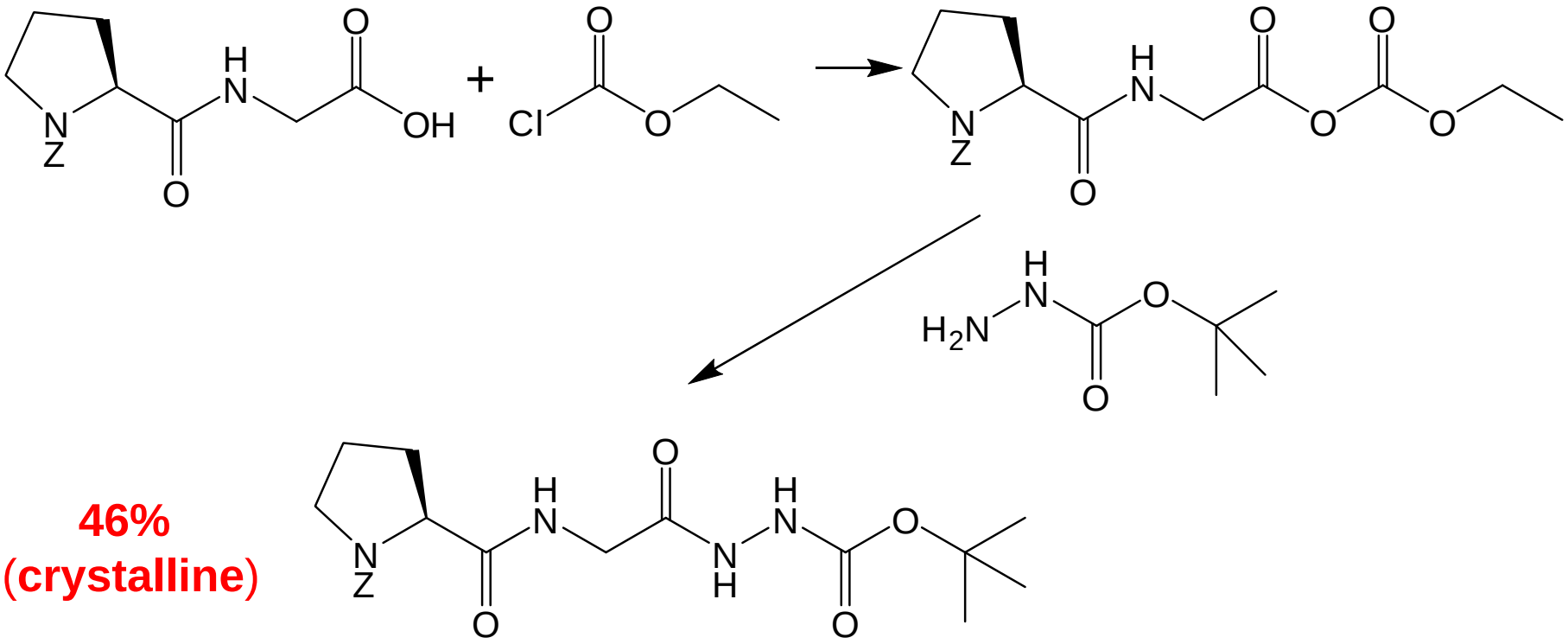
Application of appropriate additives to avoid side-reaction



Additives intercept highly reactive intermediates (***O*-acylisourea**), thereby **forming an active ester** which has **lower reactivity** but is still sufficiently potent for rapid amide bond formation.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Protected Hydrazides

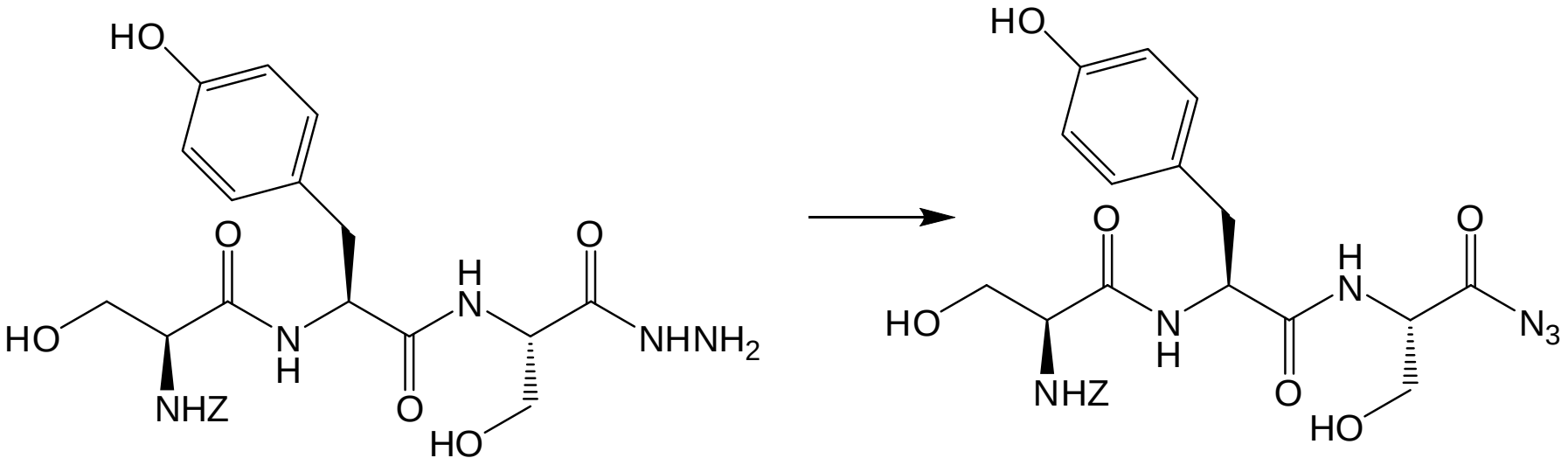


46%
(**crystalline**)

Procedure:
Et_3N, dry THF, successively ethyl chlorocarbonate, *tert*-butylcarbazate, -10 °C → 20 °C, 5 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

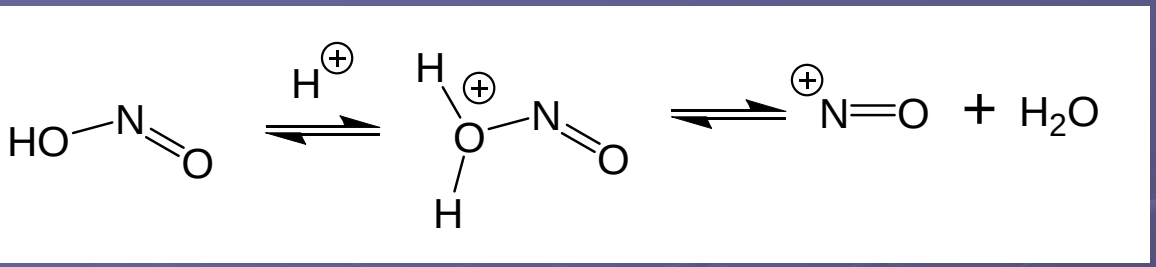
Generation of the azide with the aid of sodium nitrite and *coupling without isolation of the azide*

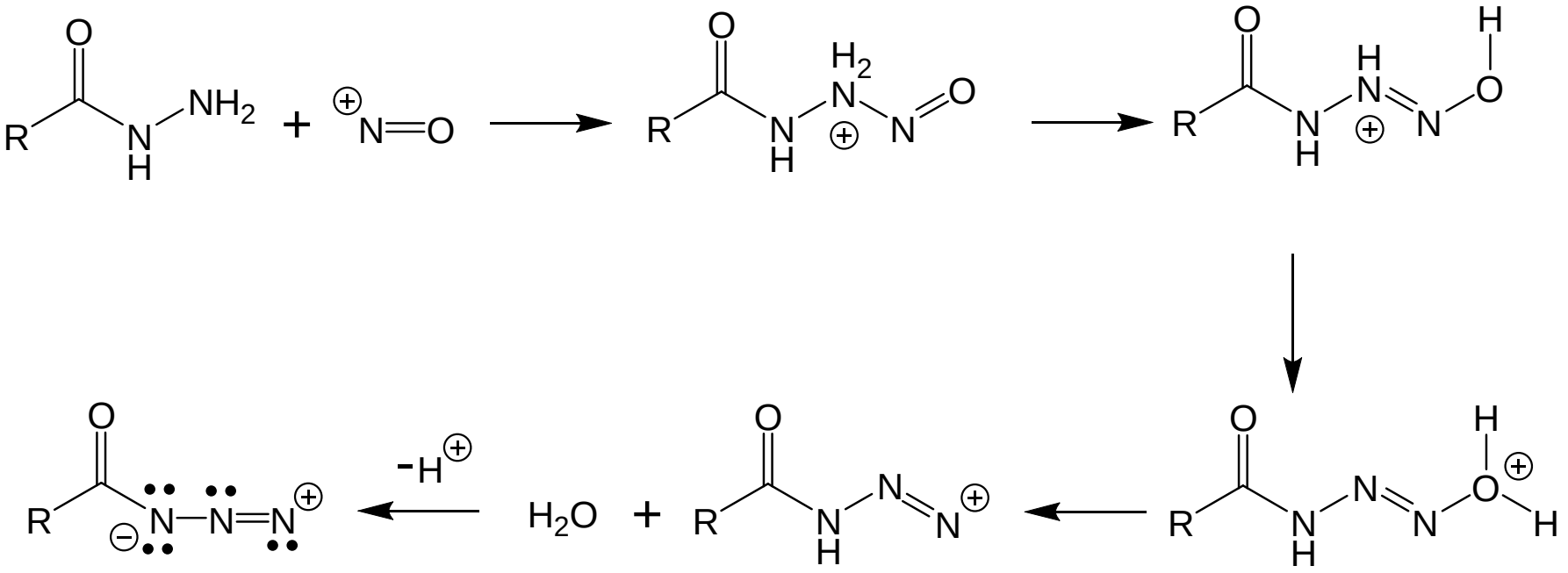


Procedure:

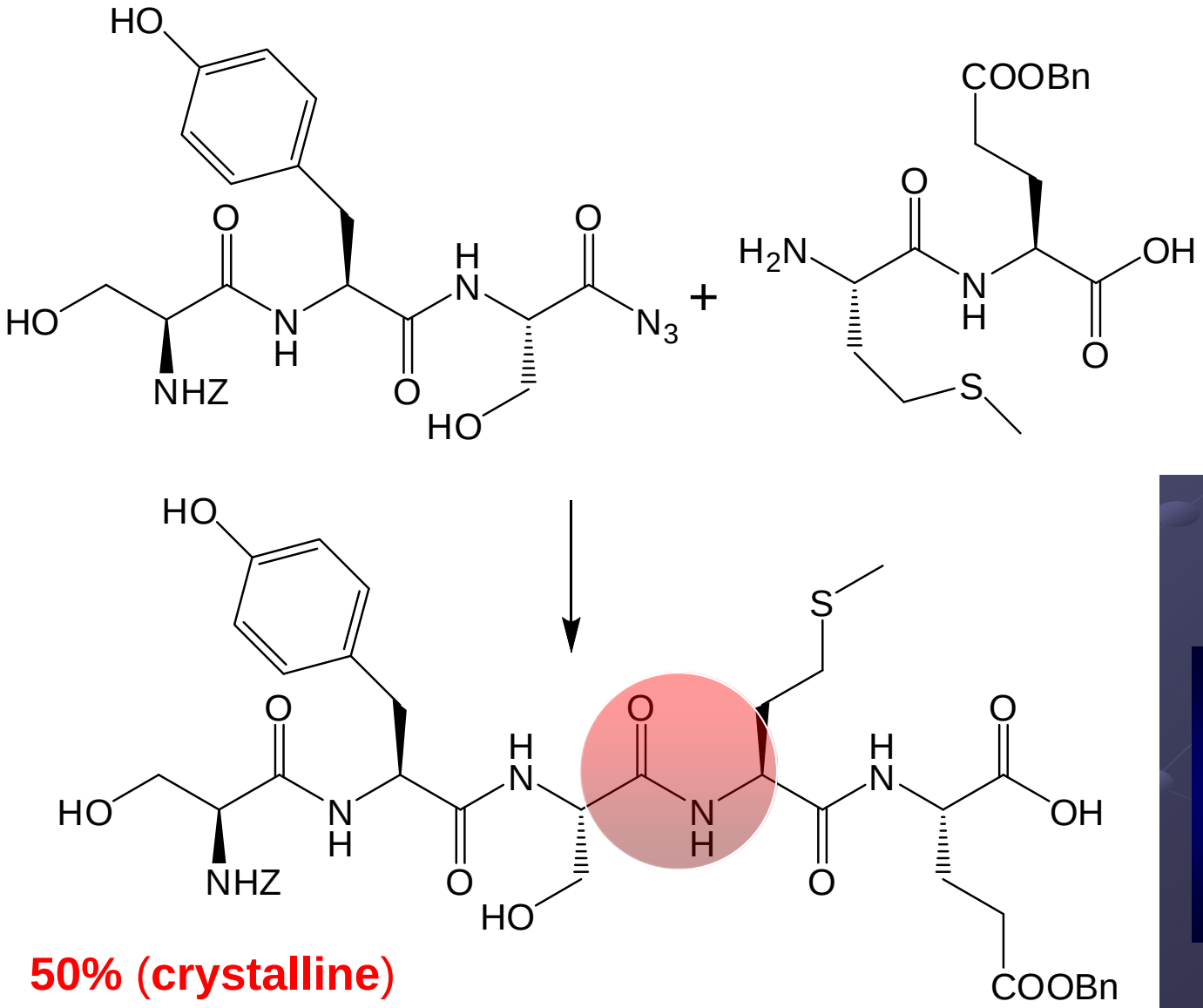
1) $NaNO_2$, 4 M HCl, DMF, -5 °C → 0 °C, 5 min;
2) Et_3N (neutralization of the excess HCl).

CHEMICAL SYNTHESIS OF BIOPOLYMERS





CHEMICAL SYNTHESIS OF BIOPOLYMERS



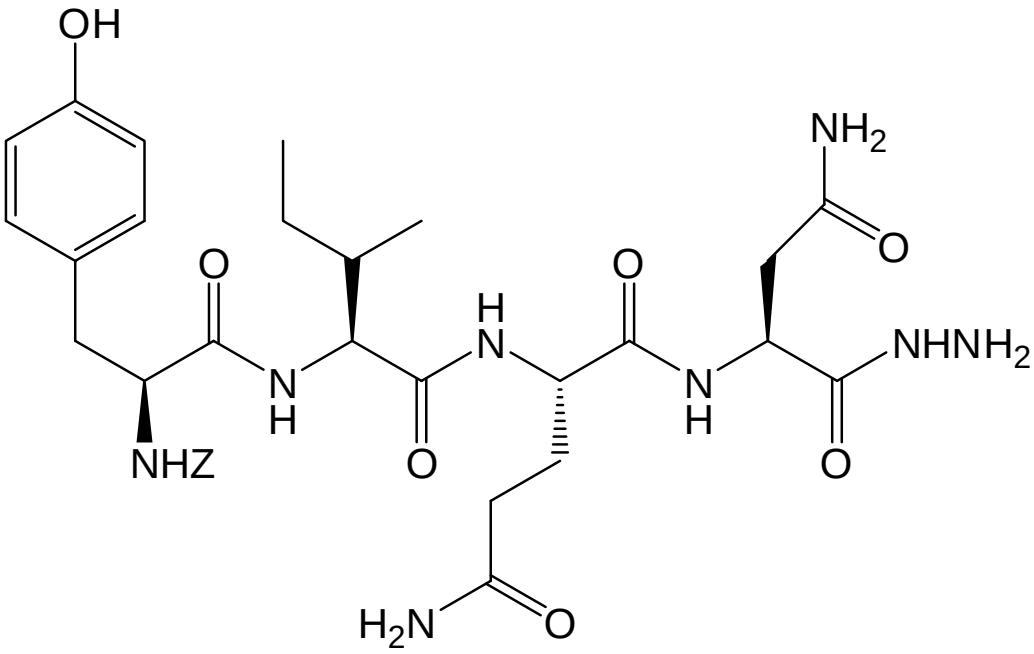
50% (crystalline)

Procedure:

1) DMF, 0 °C, 36 h;
2) O.5 M NH_4OH
3) ion-exchange resin

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Generation of the azide with the aid of butyl nitrite and *coupling of the azide in situ*



Z-Tyr-Ile-Gln-Asn-$NHNH_2$

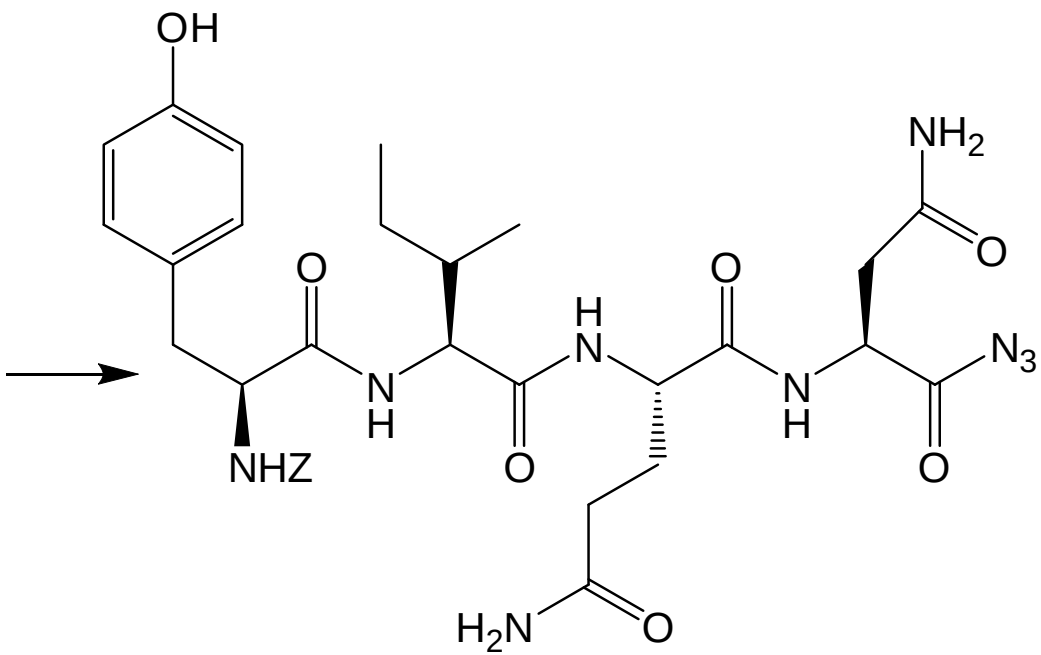
Procedure:

1) *n*-BuONO, 6.3 M HCl in THF, DMF, -30 °C, 4 min;

2) amino component, *N*-ethylpiperidine pH 8-9, 0 °C, 12 h.

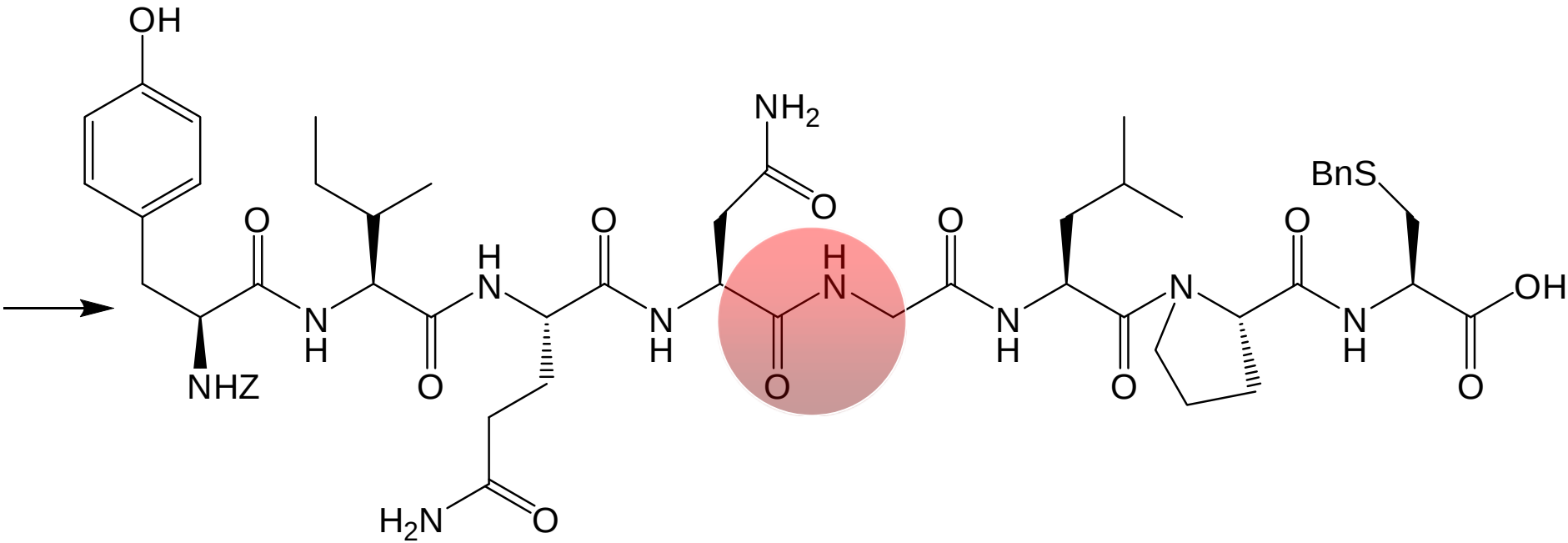
3) washing with 1 M HCl, 0,5 M $KHCO_3$, and water.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



H-Cys(Bn)-Pro-Leu-Gly-NH_2

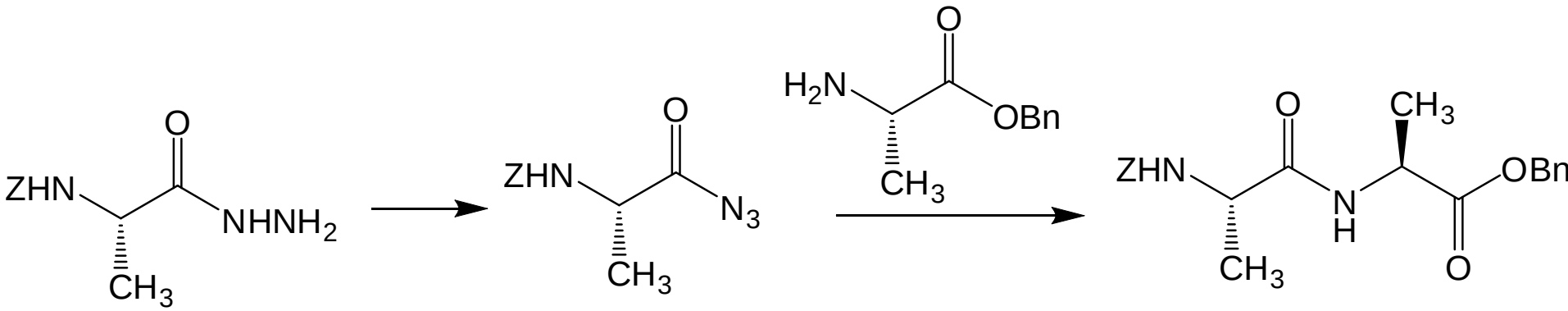
CHEMICAL SYNTHESIS OF BIOPOLYMERS



79% (crystalline)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Generation of the azide with the aid of sodium nitrite
followed by coupling with the isolated azide



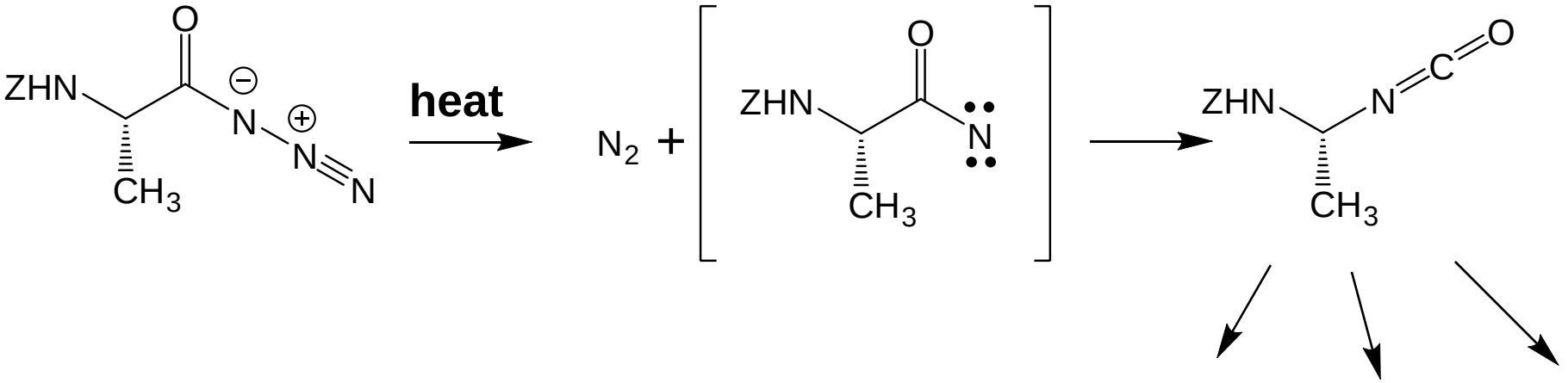
80% (crystalline)

Procedure:

1) $NaNO_2$, 5 M HCl in AcOH – H_2O, DMF, -5 °C, few min;
2) extraction with ether, and the etheral solution of the azide is then used;
3) amino compound in $CHCl_3$, 20 °C, 20 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

In generally, lower temperature (e.g. 0 °C) is preferable for coupling from the point of view of by-product formation through **CURTIUS rearrangement**, but **this type of side-reaction cannot be completely stopped**.



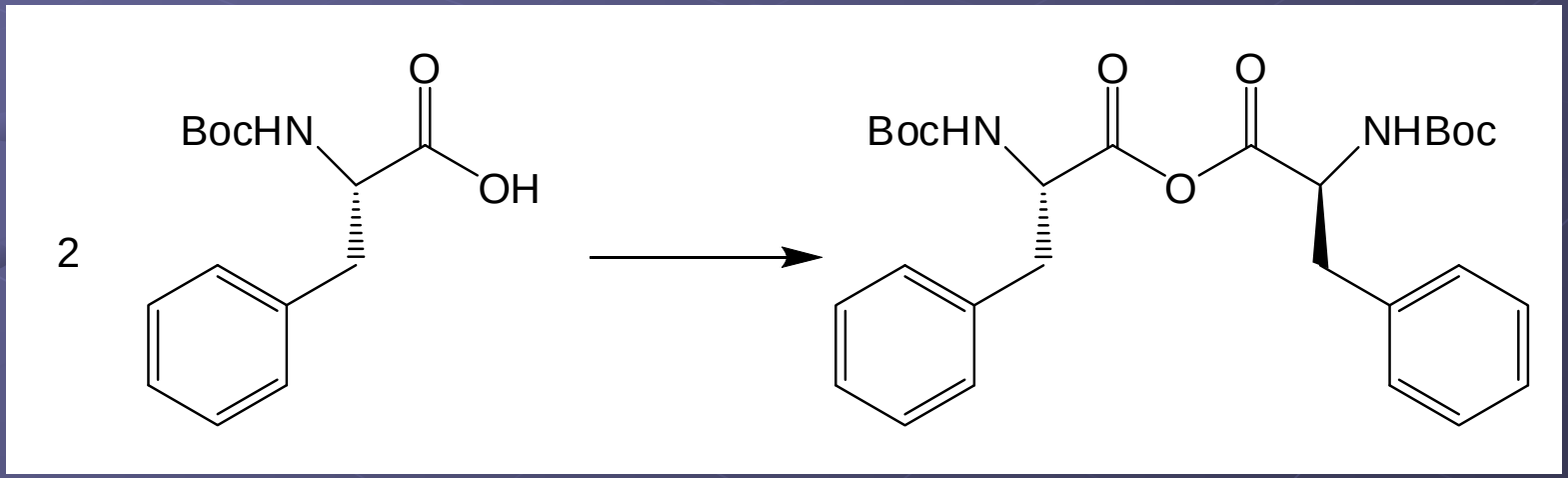
consecutive reactions

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The anhydride procedure

Symmetrical anhydrides

The general interest in **symmetrical anhydrides** for stepwise peptide chain elongation increased as Boc-protected amino acid derivatives became commercially available at reasonable prices.



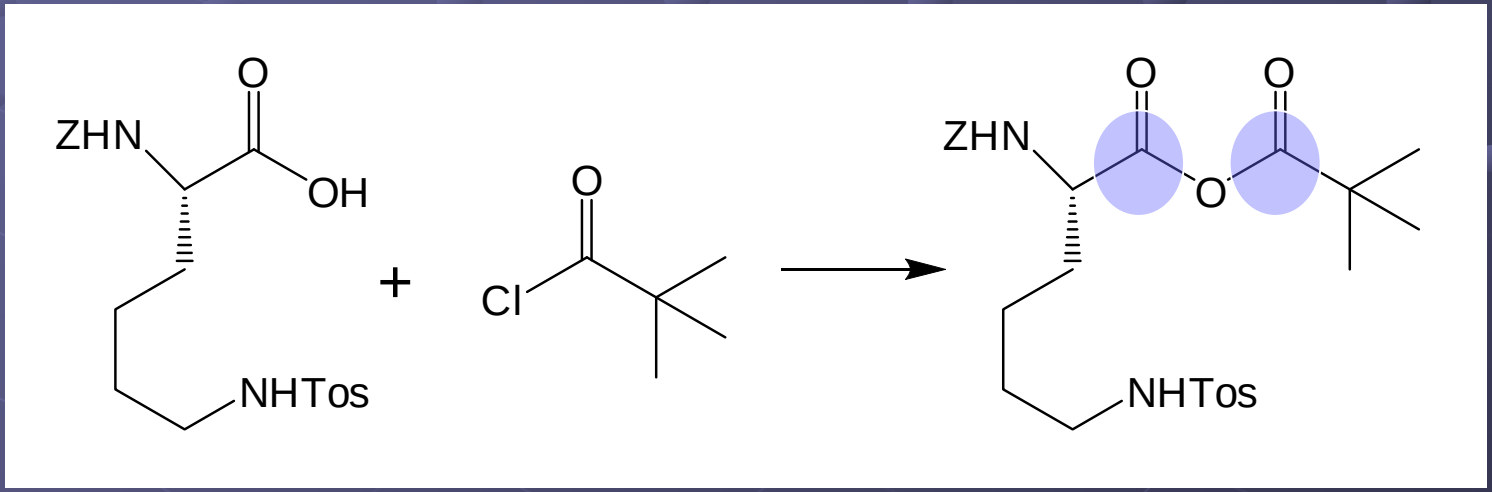
76%
(crystalline)

Procedure:
N-ethyl-*N'*-3-dimethylaminopropylcarbodiimide hydrochloride, CH_2Cl_2, 0 °C, 2 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Pivalic acid mixed anhydrides

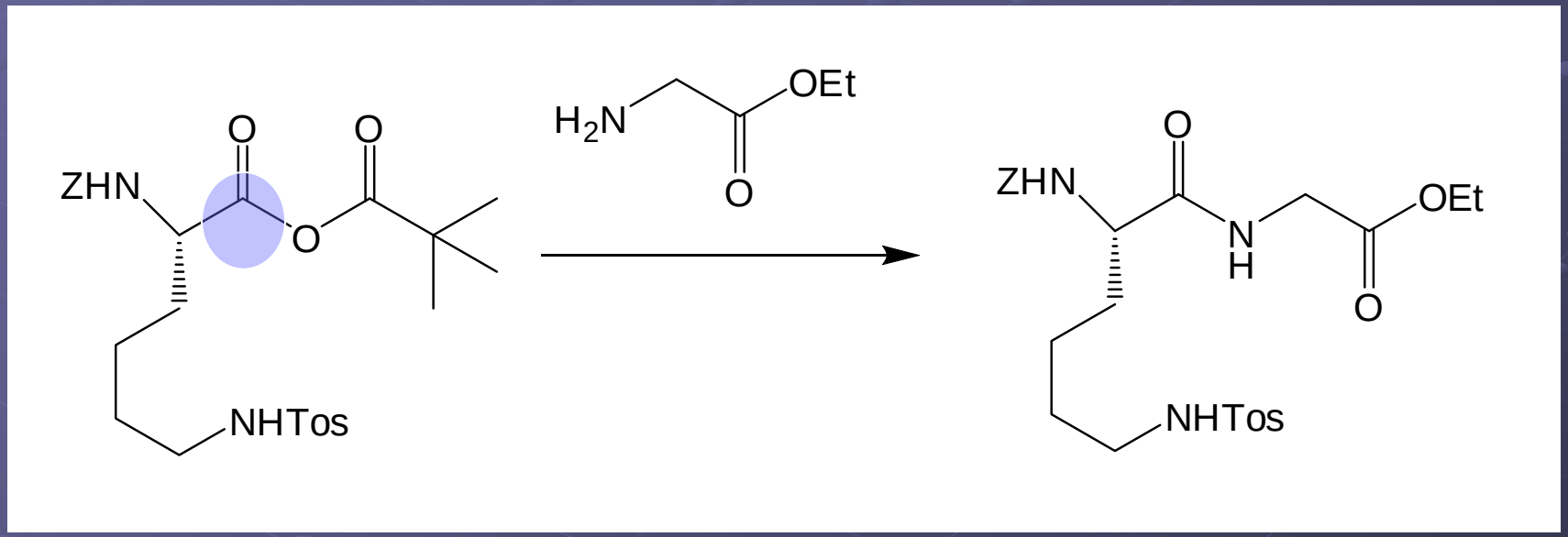
The regioselectivity of the aminolysis reaction of the mixed anhydride depends on the **electrophilicity**, **stability** and/or **steric situation** of the two competing carboxy groups.



in situ

Procedure:
Pyridine, $CHCl_3$, -3 °C, 15 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

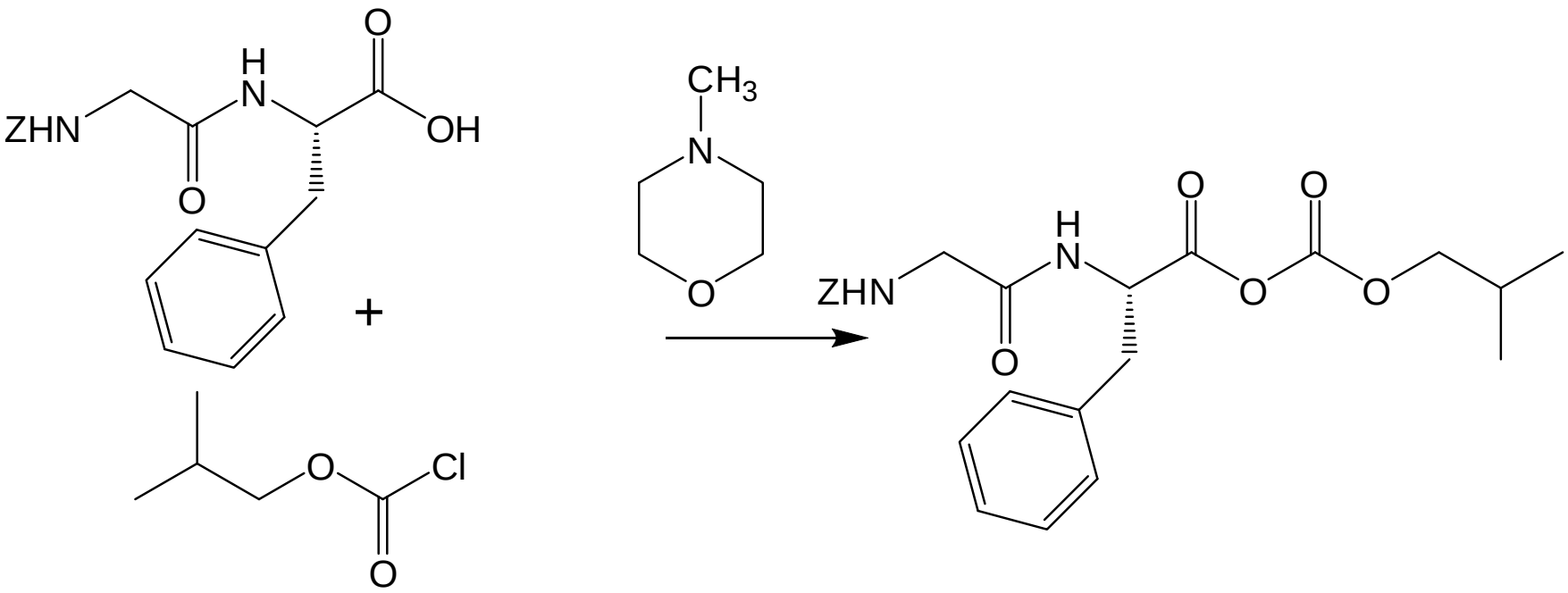


81% (crystalline)

Procedure:
Glycine ethylester, pyridine, $CHCl_3$, -10 °C → 20 °C, 1 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

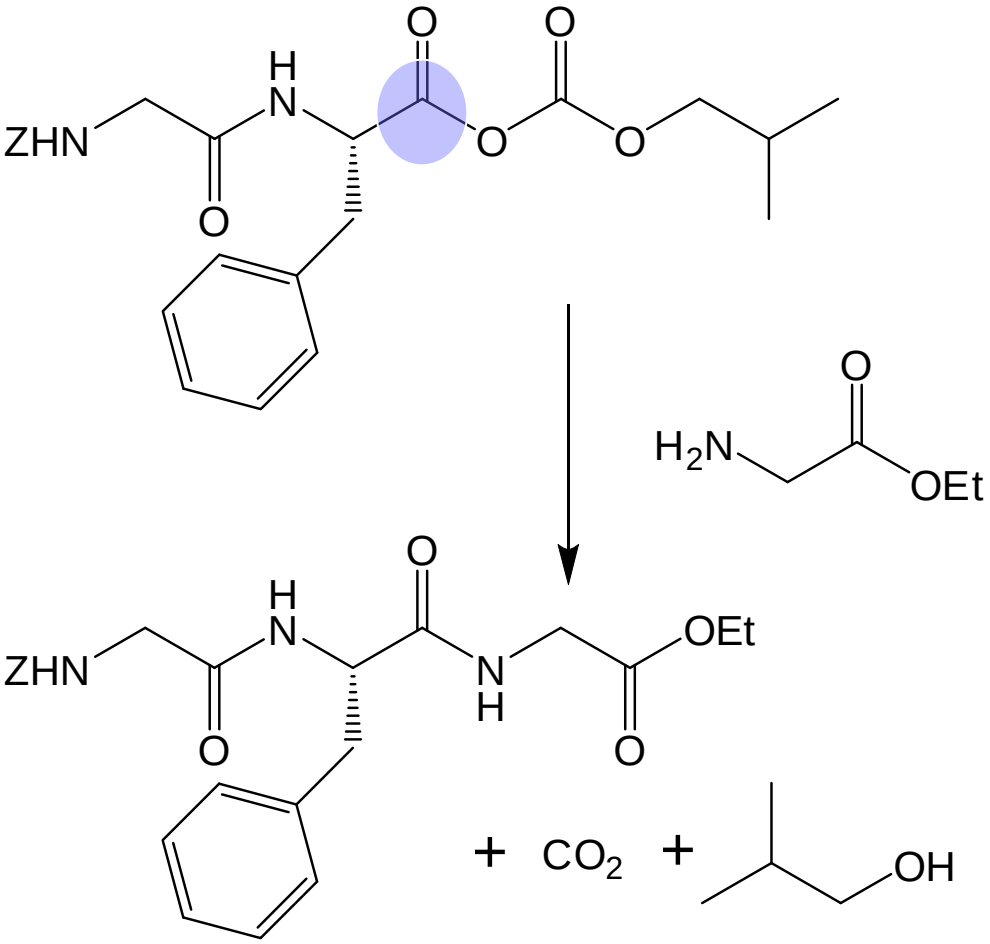
Isobutylcarbonic acid mixed anhydrides



in situ

Procedure:
Isobutyl chlorocarbonate, *N*-methylmorpholine, dry THF, -15 °C, 2 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



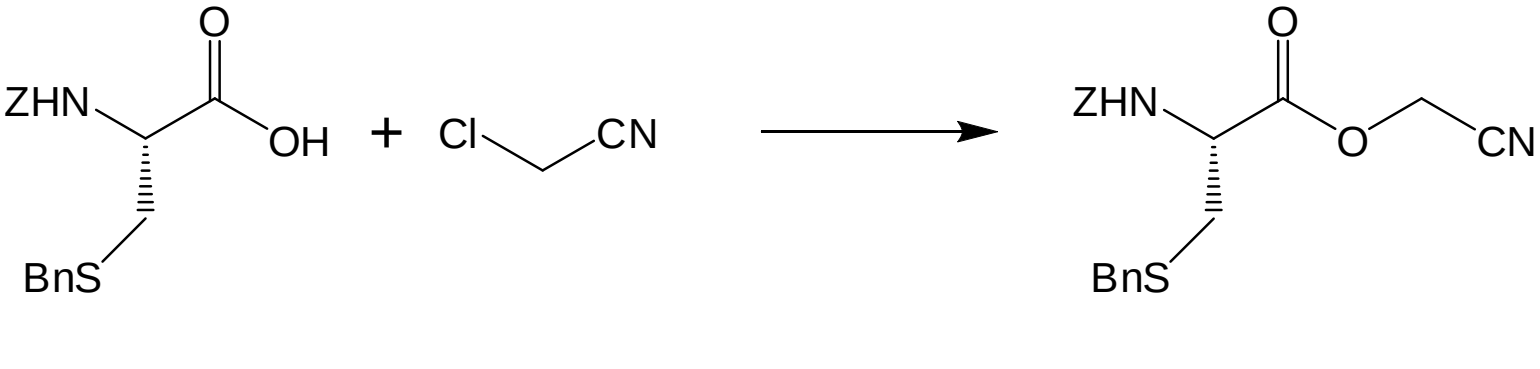
91% (crystalline)

Procedure:
Et_3N, dry DMF, -15 °C → 20 °C, 2 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

The active ester procedure

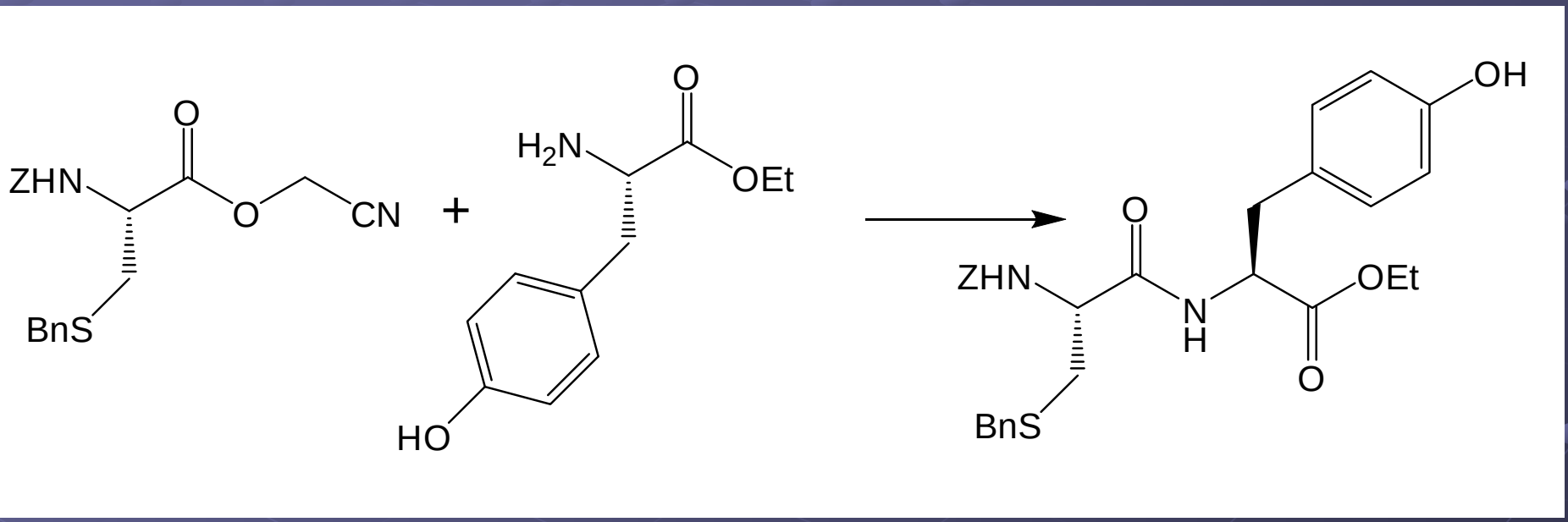
Cyanomethyl esters



81%
(crystalline)

Procedure:
Chloroacetonitrile (reagent and solvent), Et_3N, 0 °C → 20 °C, 15 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

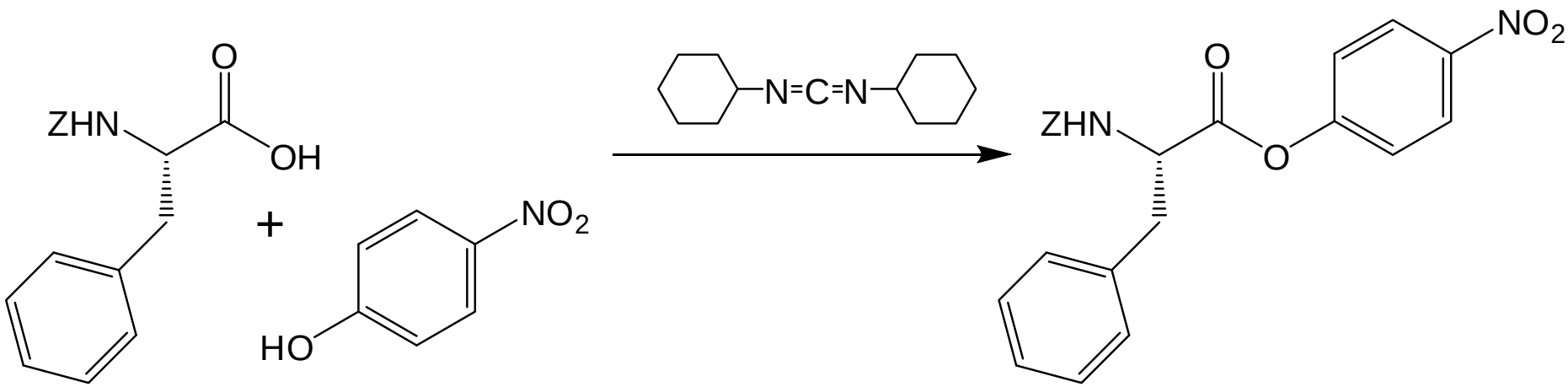


95%

Procedure:
dry THF, cat AcOH, 20 °C, 2 d.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

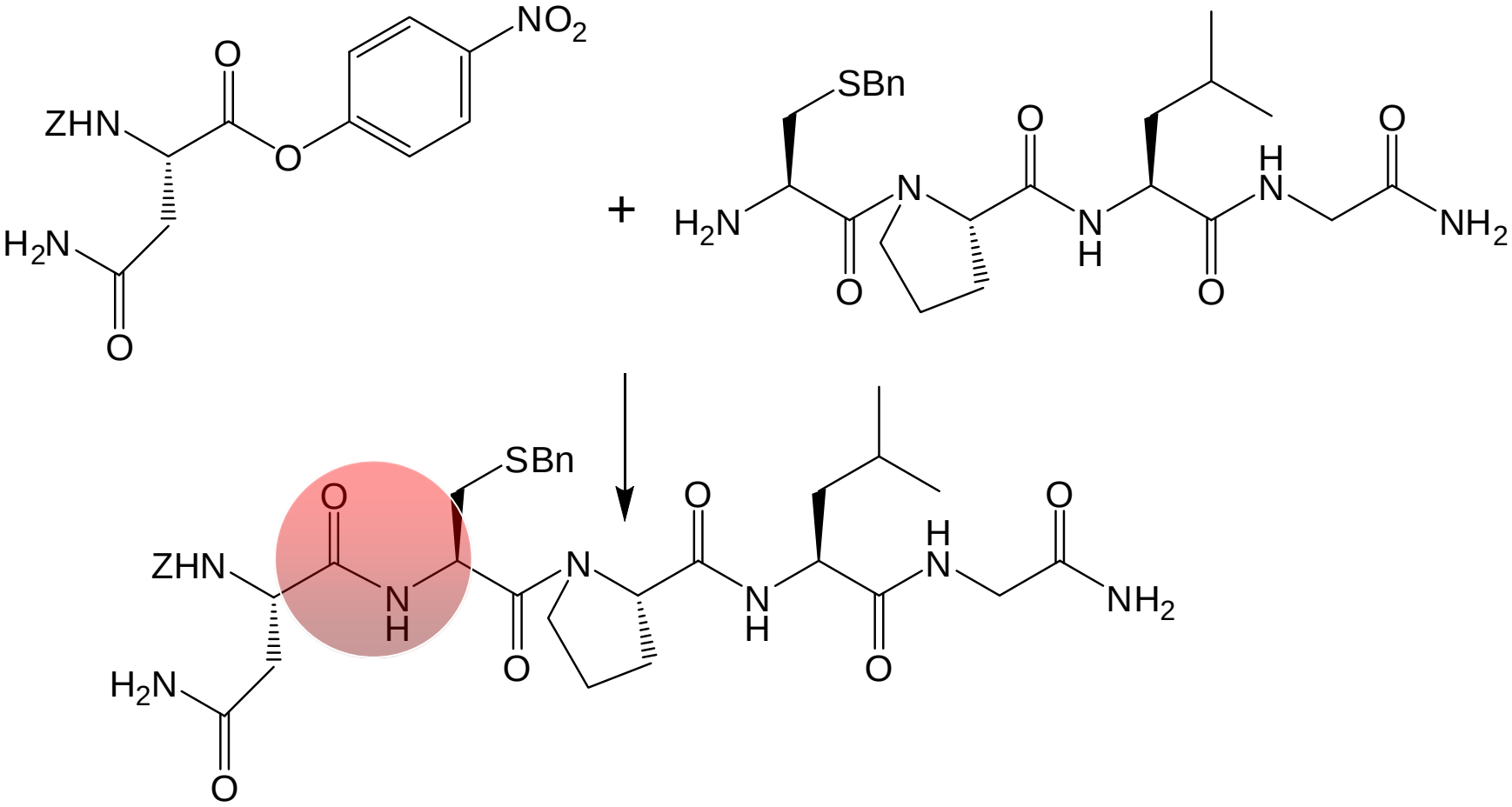
p-Nitrophenyl esters



75%
(crystalline)

Procedure:
p-Nitrophenol, ethyl acetate, DCC, 0 °C → 20 °C, 3 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

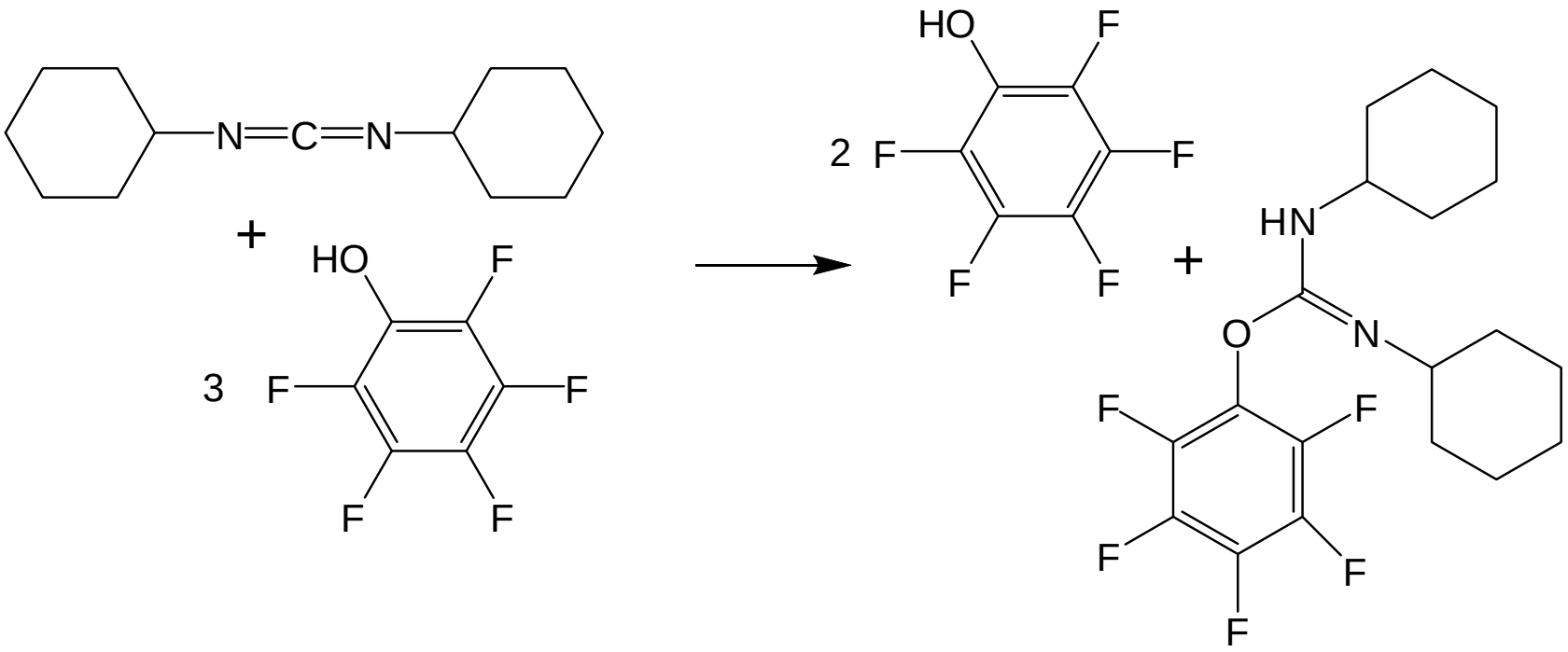


Procedure:
Ethyl acetate, 20 °C, 2 d.

98% (crystalline)

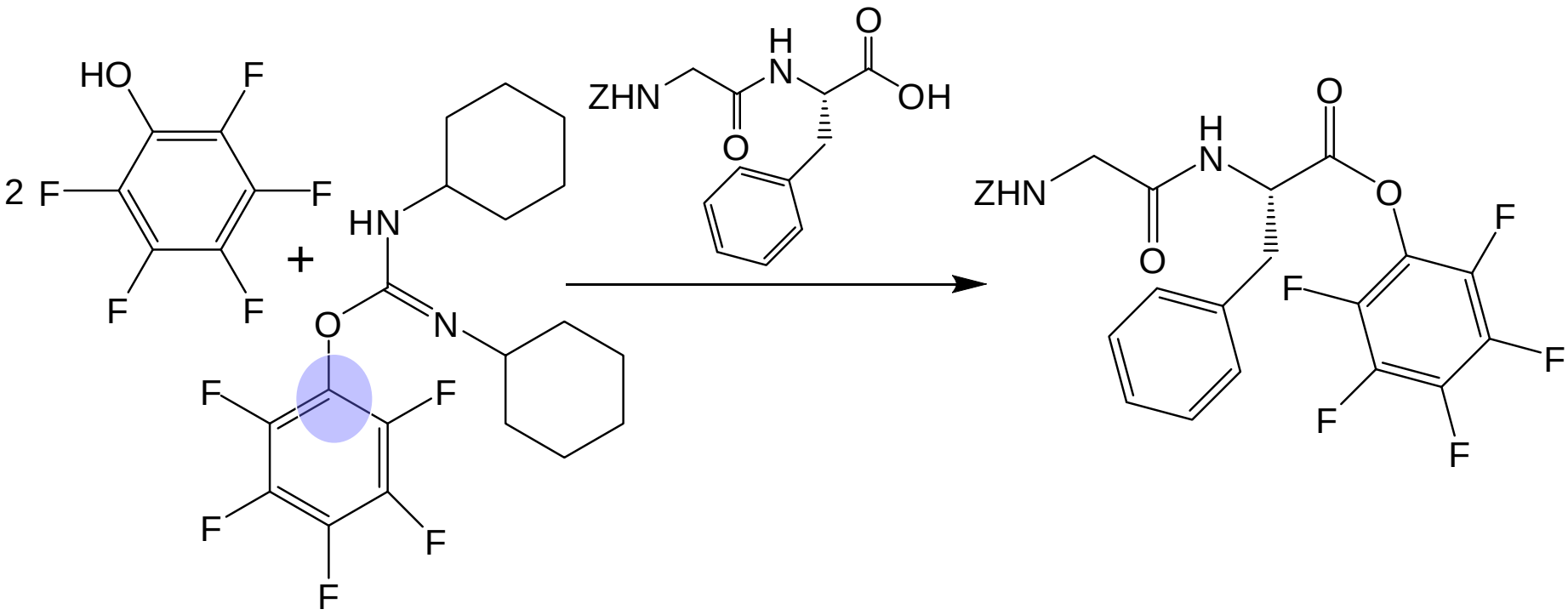
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Pentafluorophenyl esters



Procedure:
Pentafluorophenol, ethyl acetate, DCC, 0 °C, 5 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



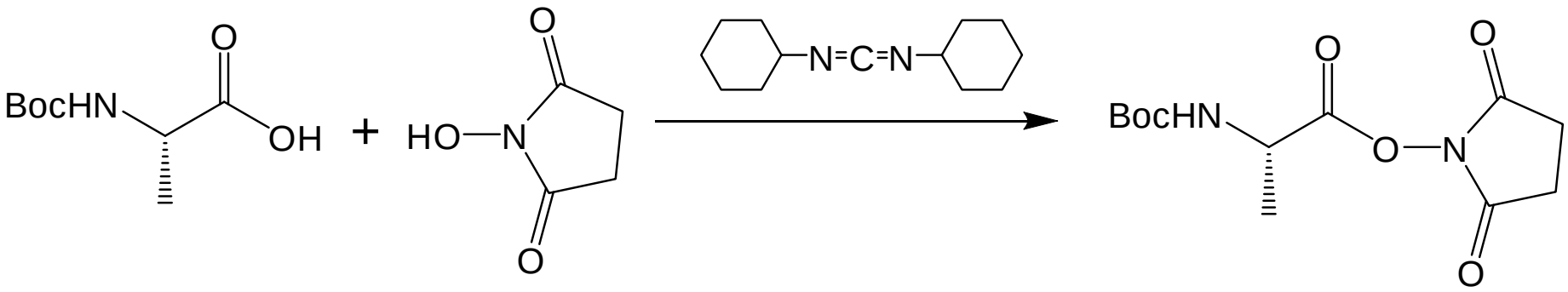
Procedure:
0 °C, 1.5 h.

92%
(crystalline)

CHEMICAL SYNTHESIS OF BIOPOLYMERS

New type of active esters

N-hydroxysuccinimide esters

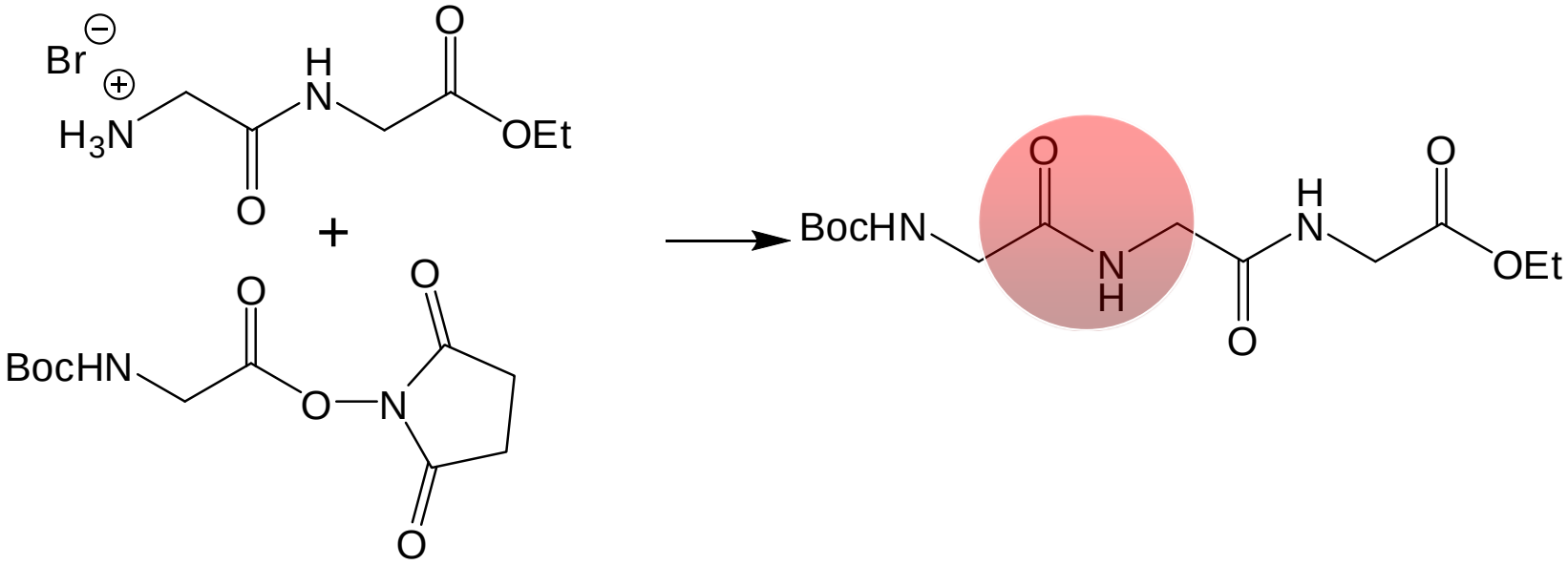


71% (crystalline)

Procedure:
DCC, dry 1,2-dimethoxyethane, 0 °C - 5 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling in organic media

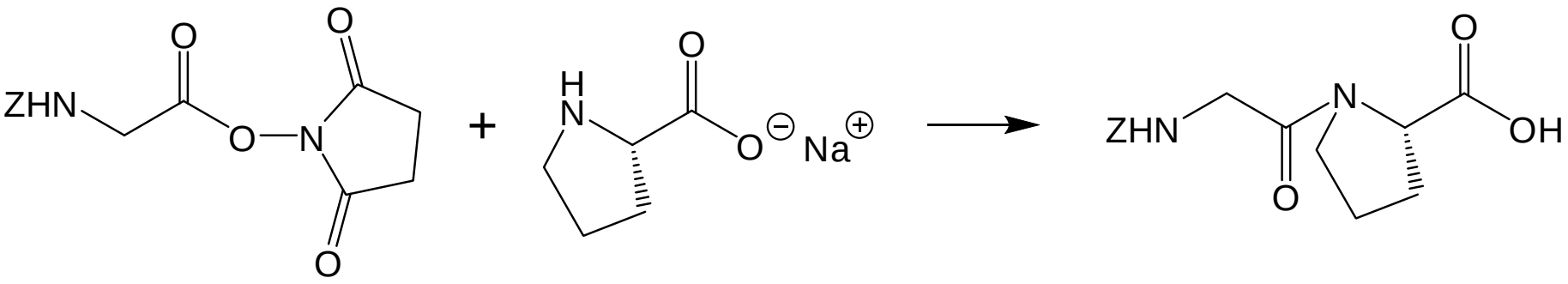


85% (crystalline)

Procedure:
Et_3N, dry 1,2-dimethoxyethane, 20 °C, 20 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling in organic-aqueous media



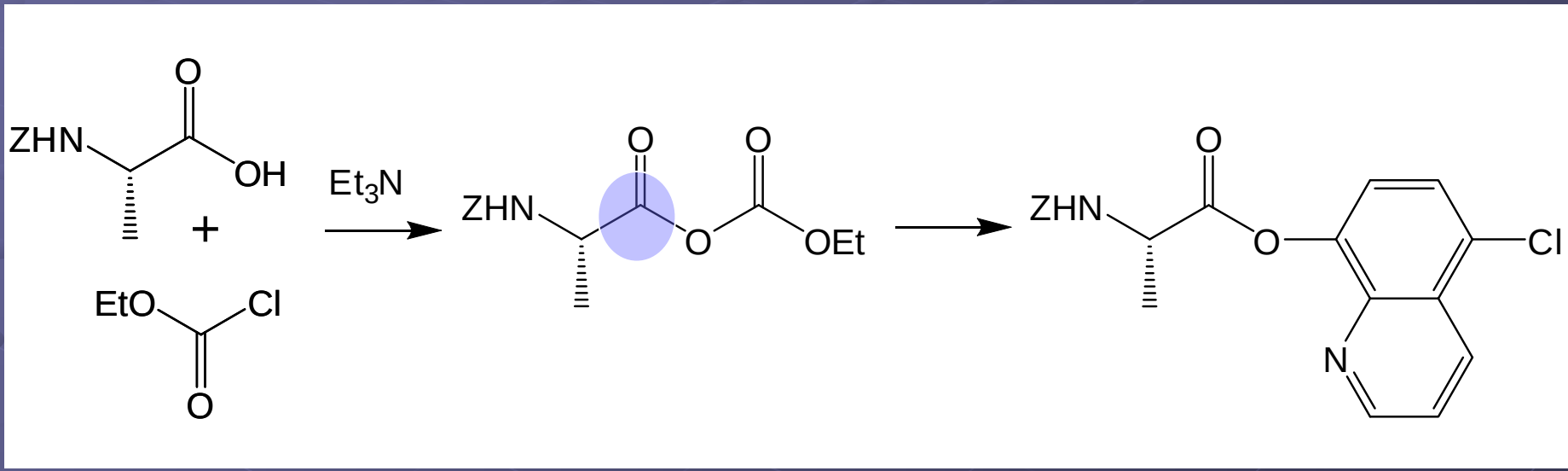
75% (crystalline)

Procedure:

1) $NaHCO_3$, H_2O – 1,2-dimethoxyethane (4:5 v/v) , 20 °C, 1 h.
2) 2 M HCl.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Esters of 5-chloro-8-hydroxy-quinoline



84% (crystalline)

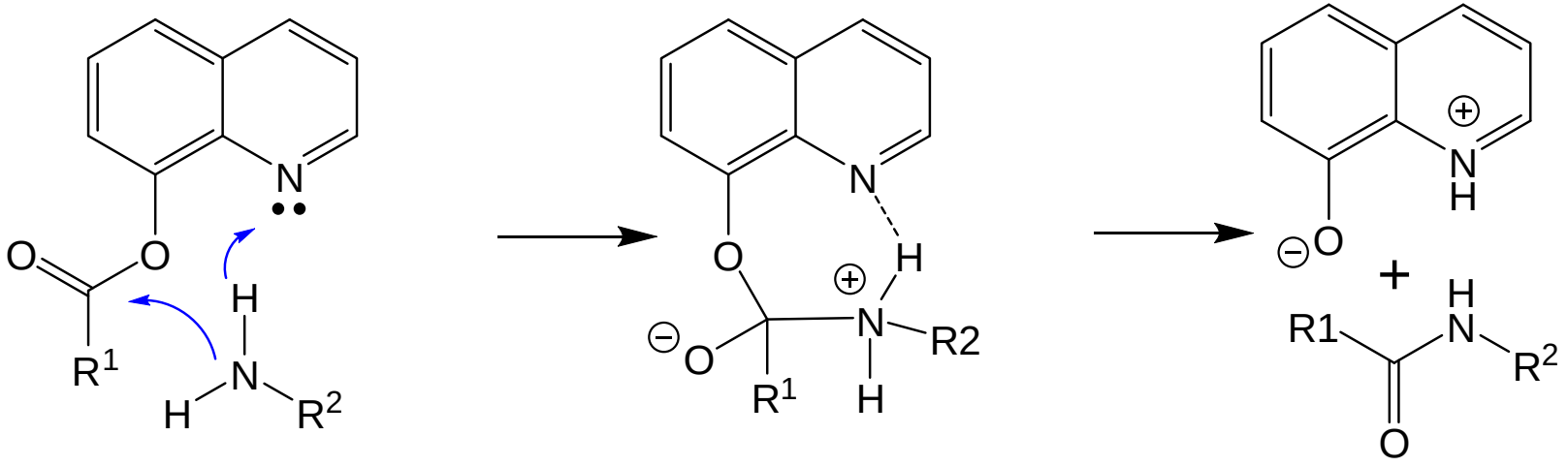
Procedure:

1) Et_3N, dry THF, ethyl chlorocarbonate,-15 °C, 15 min;
2) 5-chloro-8-hydroxy-quinoline, -15 °C → 20 °C, 2 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Esters of ***N*-hydroxysuccinimide** (HOSu), **8-hydroxy-quinoline** (HOQ) and **1-hydroxy-benzotriazole** (HOBt) present a new type of active esters.

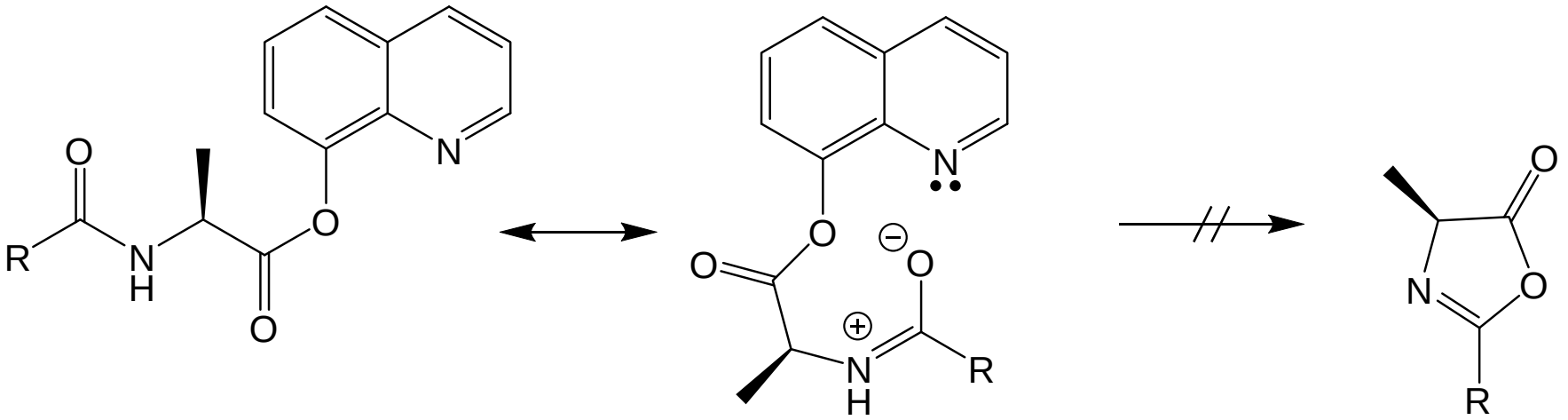
As an example, 8-quinolyl esters display significantly high aminolysis reactivity because nucleophilic attack of the amino group to the carboxy group of the ester is favoured by the **anchimeric assistance** of the quinoline nitrogen.



Intramolecular base catalysis

CHEMICAL SYNTHESIS OF BIOPOLYMERS

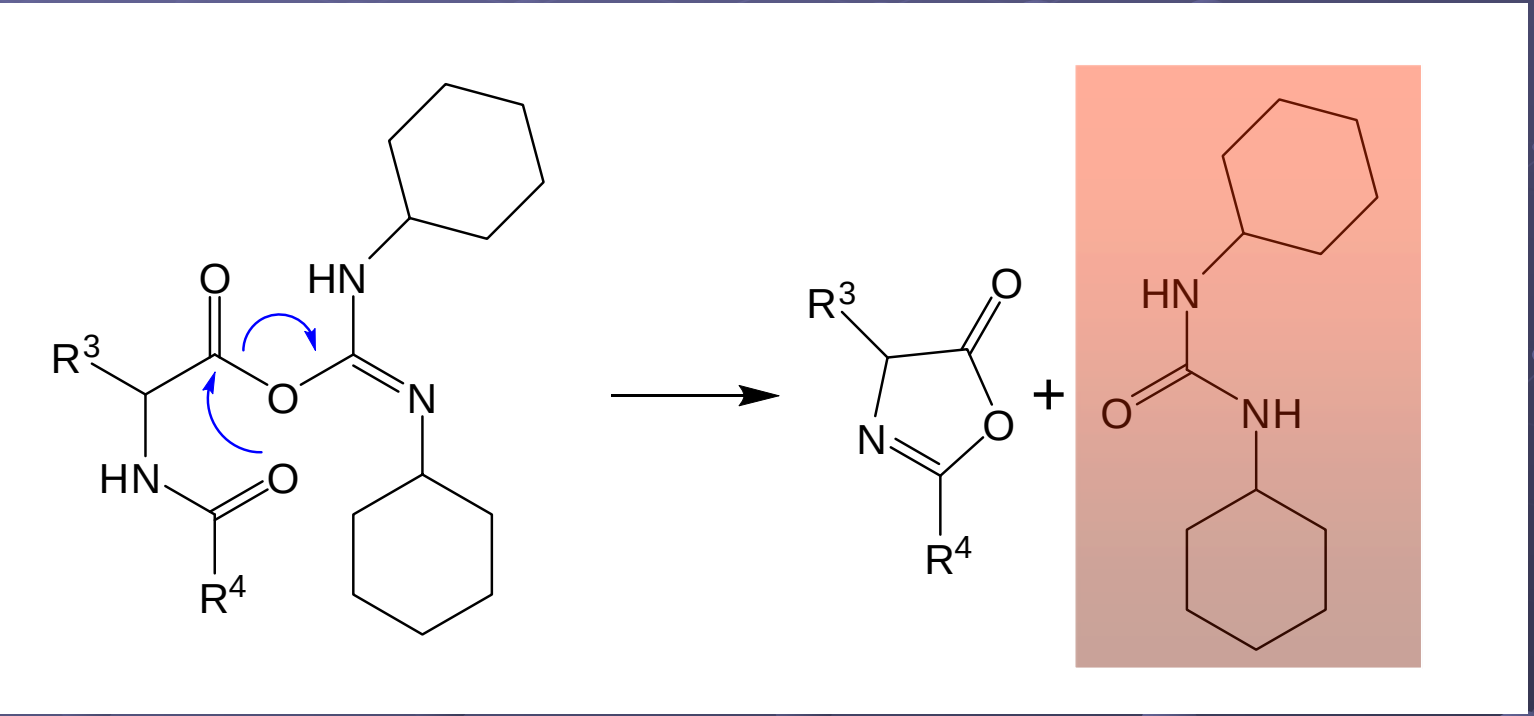
Furthermore, **racemization** of **8-quinolyl esters** is disfavoured, because the amide oxygen cannot intramolecularly attack the **weakly electophiic ester group** to give oxazolones which favours racemization.



Tautomeric forms and/or the formation of **hydrogen bonds** to quinoline nitrogen seem to be disfavoured.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Undesired side-reaction in the case of N-acyl-protected amino acids and peptides

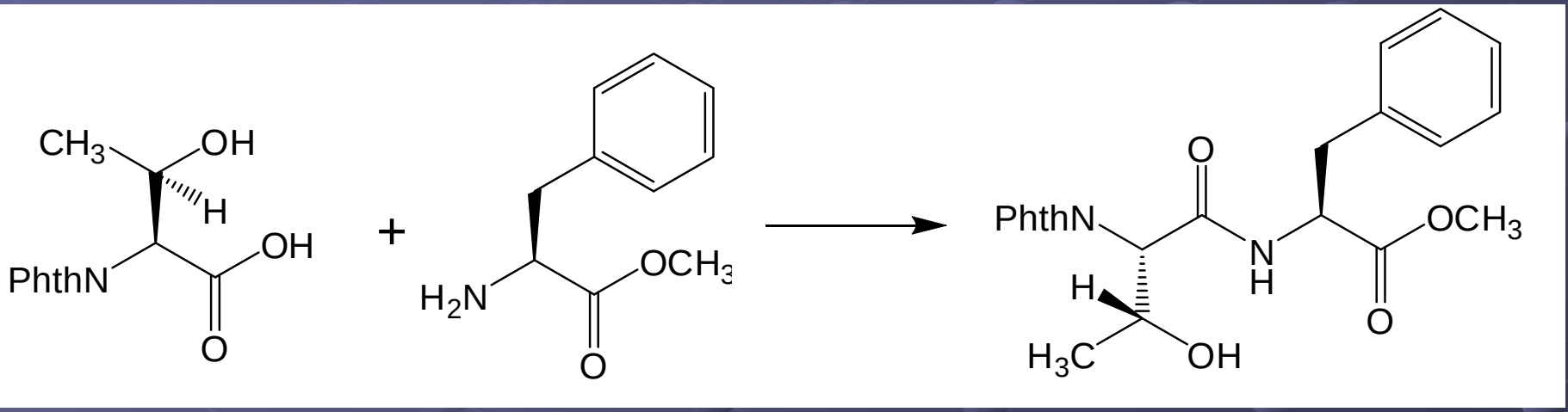


5-Oxazolones are prone to racemization.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Peptide bond formation with the aid of coupling reagents

Coupling with DCC



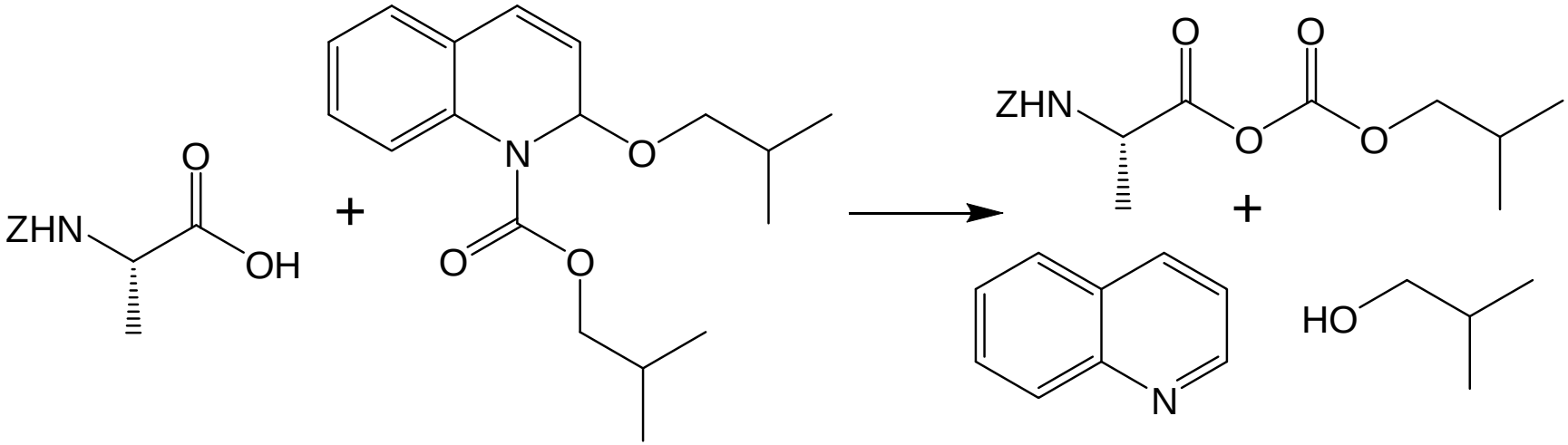
Procedure:
DCC, CH_2Cl_2, 20 °C, 5 h.

91% (crystalline)

The coupling with DCC can be carried out in the presence of **1-hydroxy-benzotriazole or *N*-hydroxysuccinimide** to avoid high reactive *N*-acyl-isourea derivatives.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

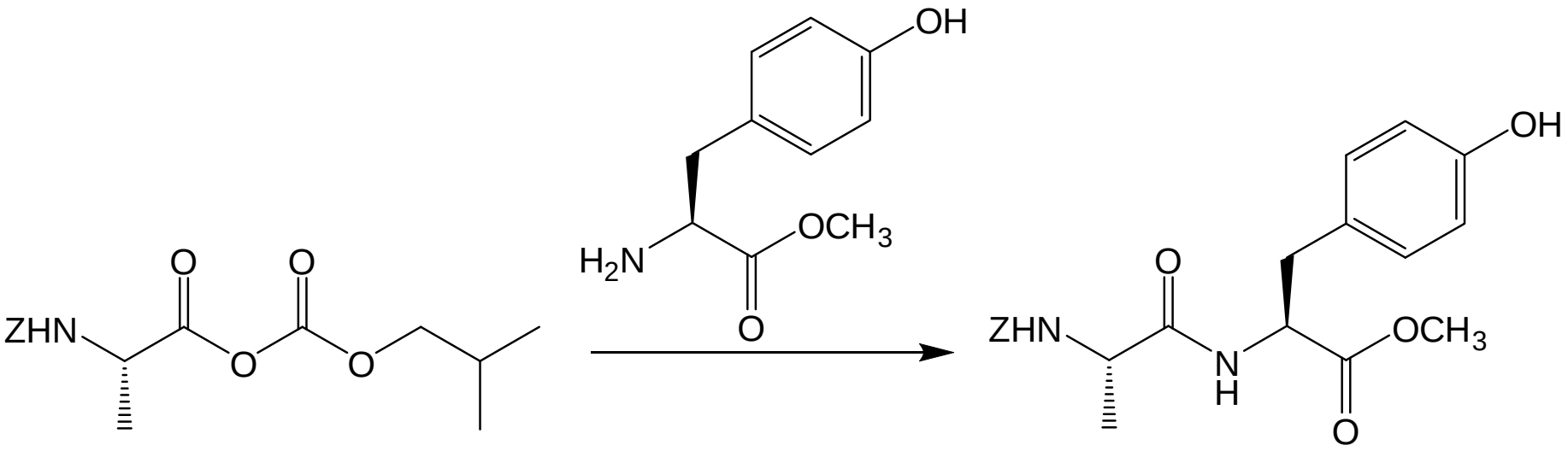
Coupling with 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)



in situ

Procedure:
DMF, 20 °C, 24 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

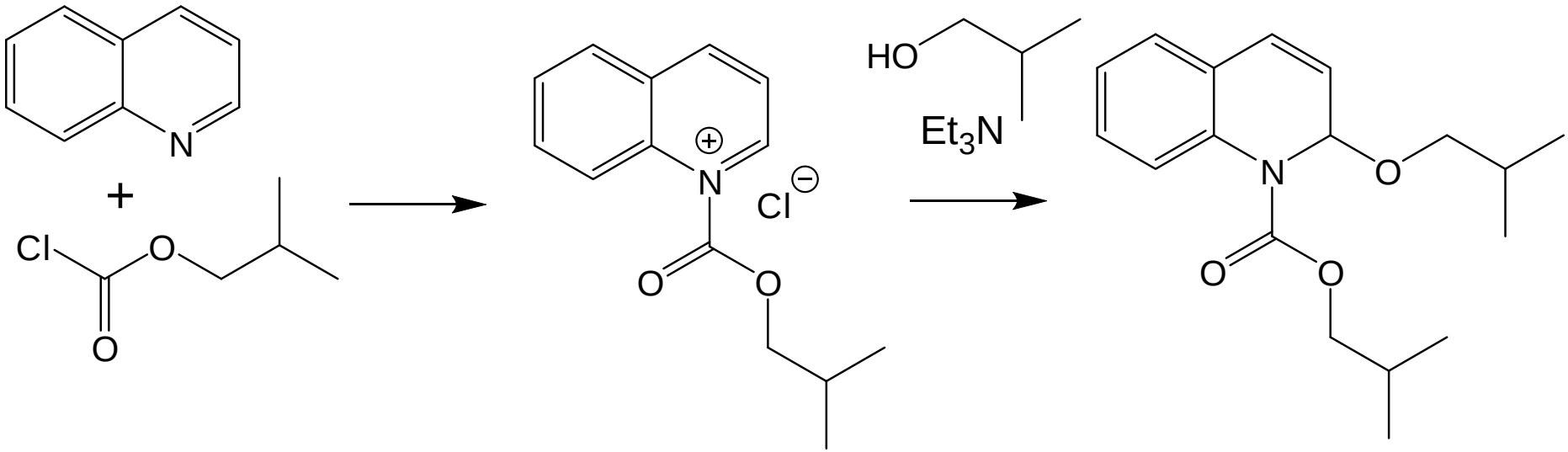


88% (crystalline)

Procedure:
DMF, 20 °C, 24 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Preparation of 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)



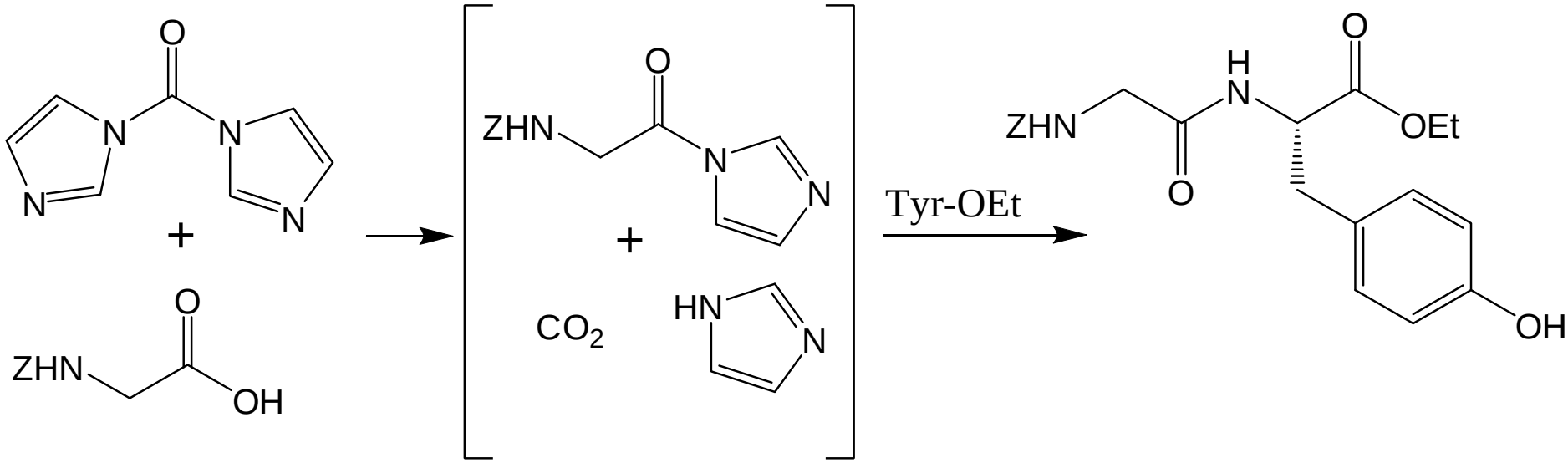
54% (oil)

Procedure:

1) Isobutyl chlorocarbonate, dry diethyl ether, -5 °C, 10 min;
2) Isobutyl alcohol, Et_3N, - 5 °C. 30 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling with carbodiimidazole



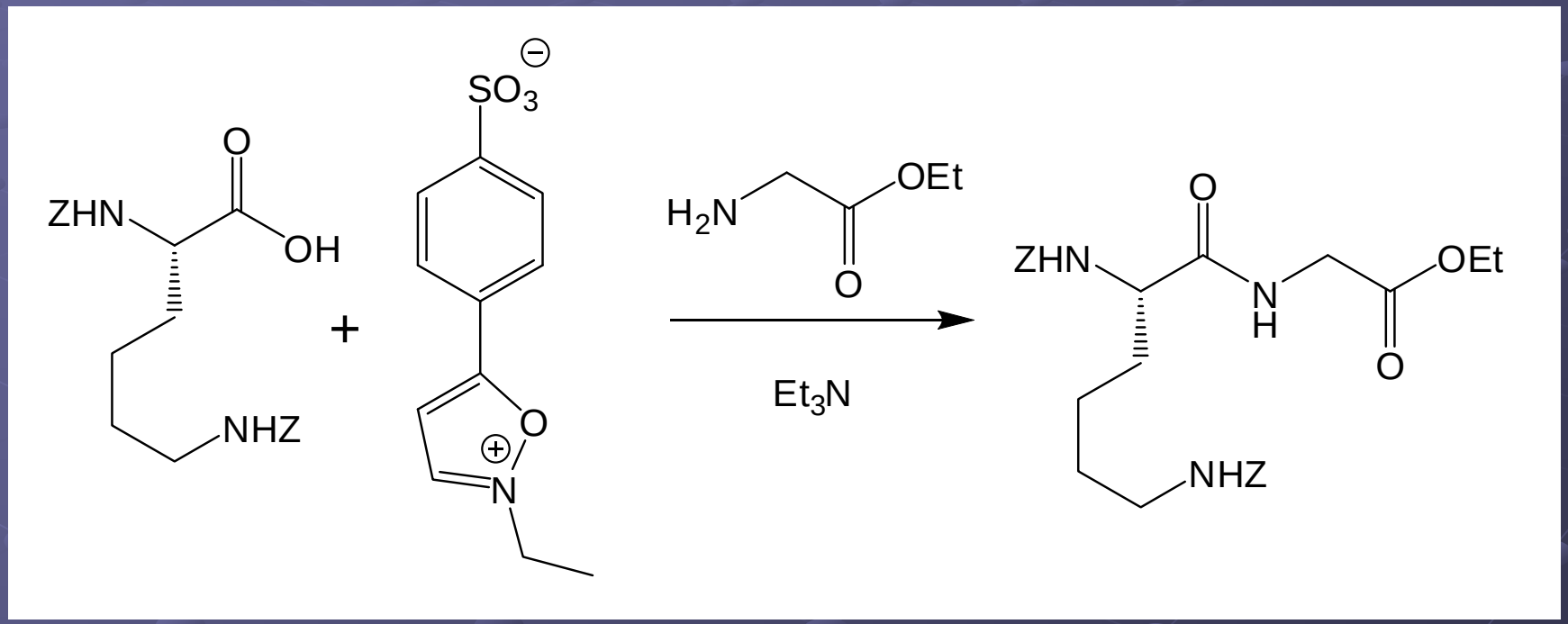
98% (crystalline)

Procedure:

1) Carbodiimidazole, dry THF, 20 °C, 30 min;
2) amino component, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling with *N*-ethyl-5-phenylisoxazolium-3'-sulfonate (Woodward's Reagent K)



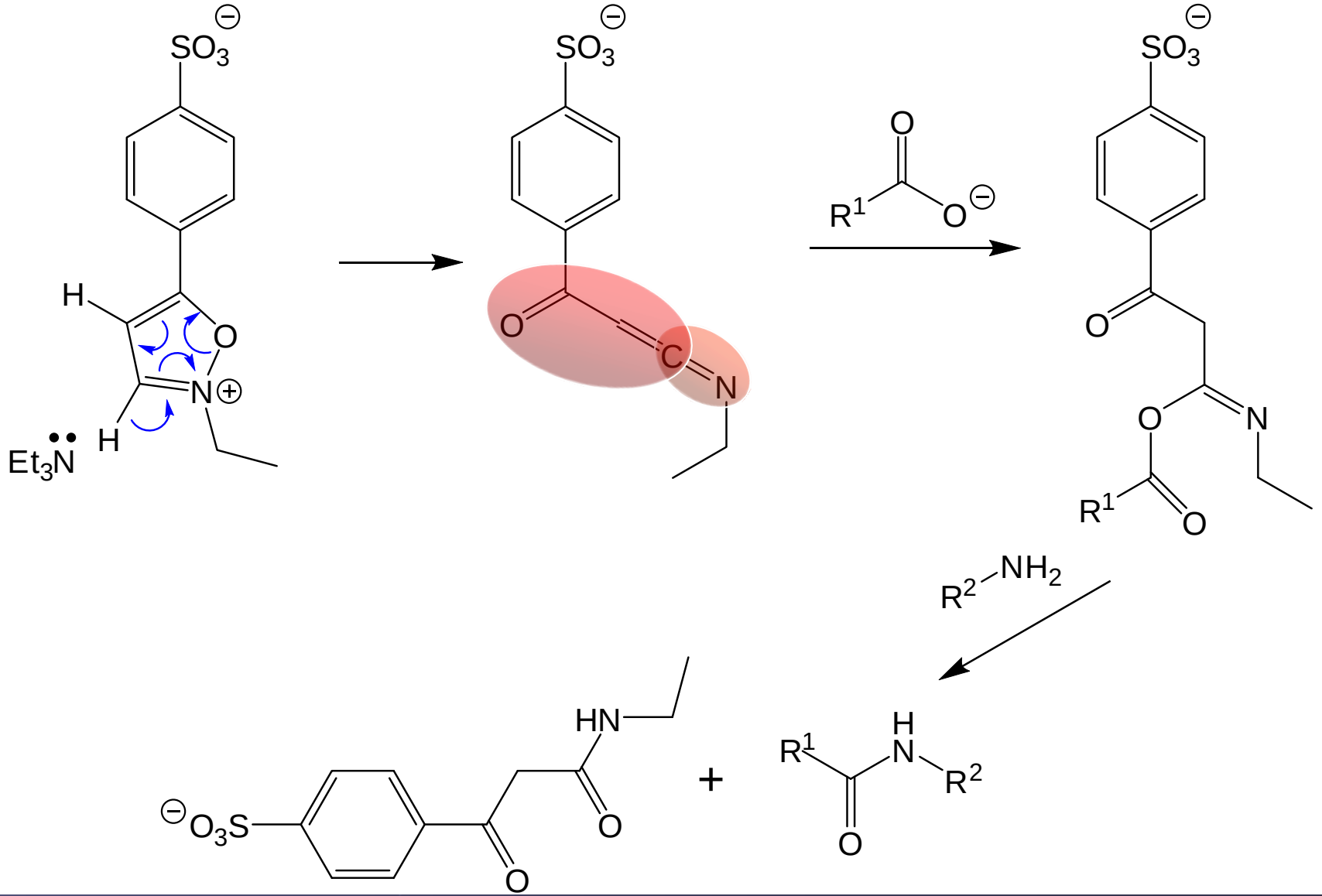
98% (crystalline)

Procedure:

1) *N*-ethyl-5-phenylisoxazolium-3'-sulfonate, acetonitrile, Et_3N, 0 °C, 1 h;

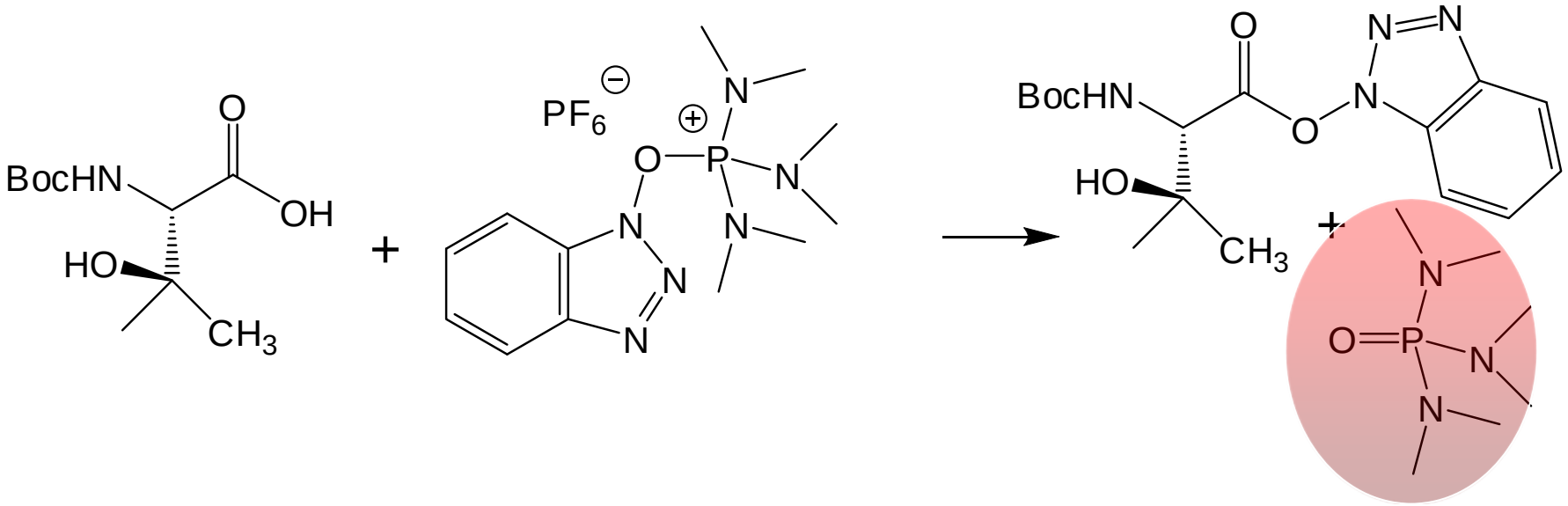
2) amino component, 20 °C, 12 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



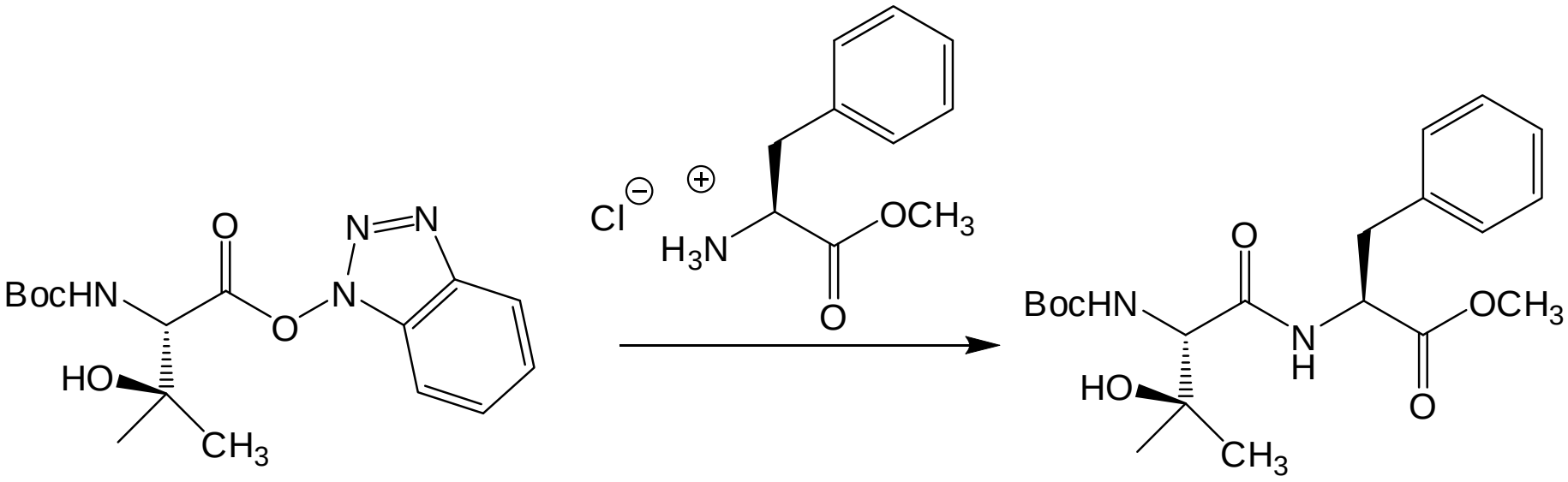
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling with 1-benzotriazolyl-tri-dimethylaminophosphonium hexa-fluorophosphate (BOP Reagent)



Procedure:
BOP-reagent, acetonitrile, Et_3N, 20 °C, 2 h.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

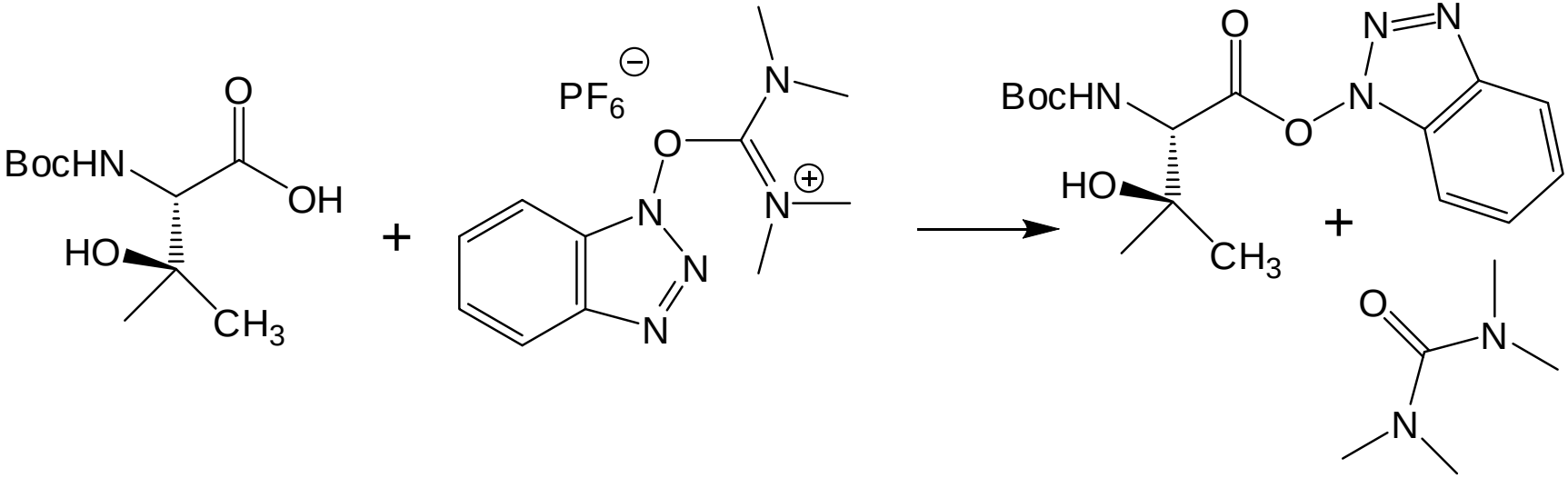


98% (crystalline)

Procedure:
BOP-reagent, acetonitrile, Et_3N, 20 °C, 2 h.

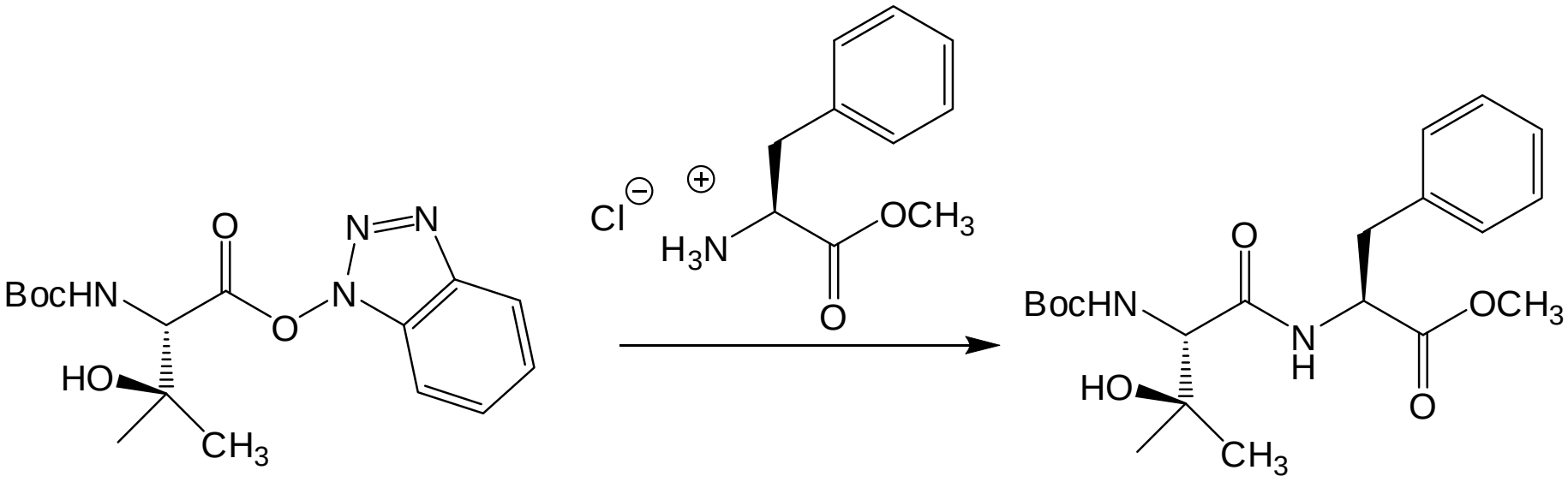
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling with *O*-benzotriazol-tetramethyluronium hexafluoro-phosphate (HBTU)



Procedure:
HBTU-reagent, acetonitrile, Et_3N, 20 °C, 15 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

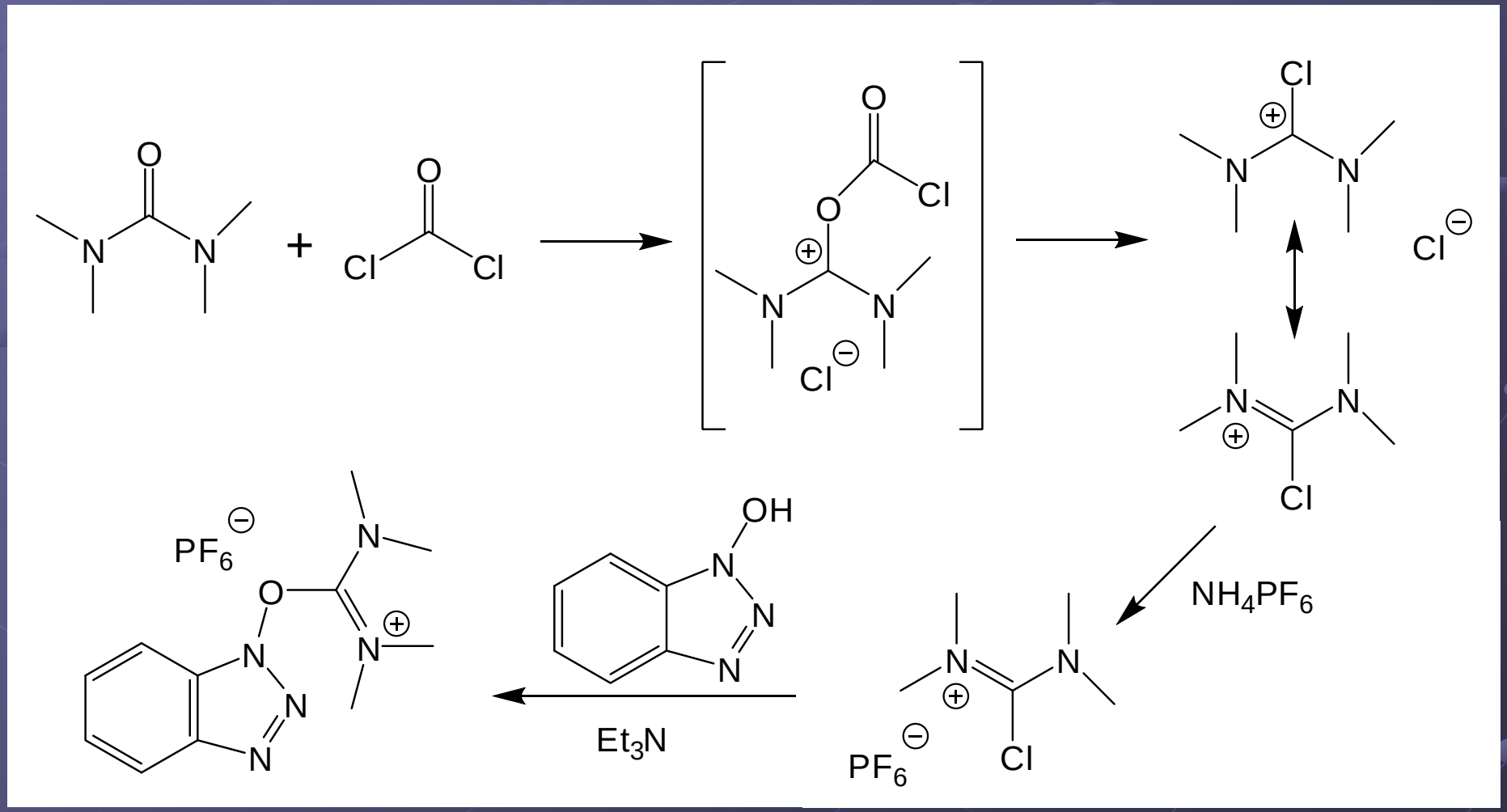


98% (crystalline)

Procedure:
HBTU-reagent, acetonitrile, Et_3N, 20 °C, 15 min.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Preparation of *O*-benzotriazol-tetramethyluronium hexafluoro-phosphate (HBTU)



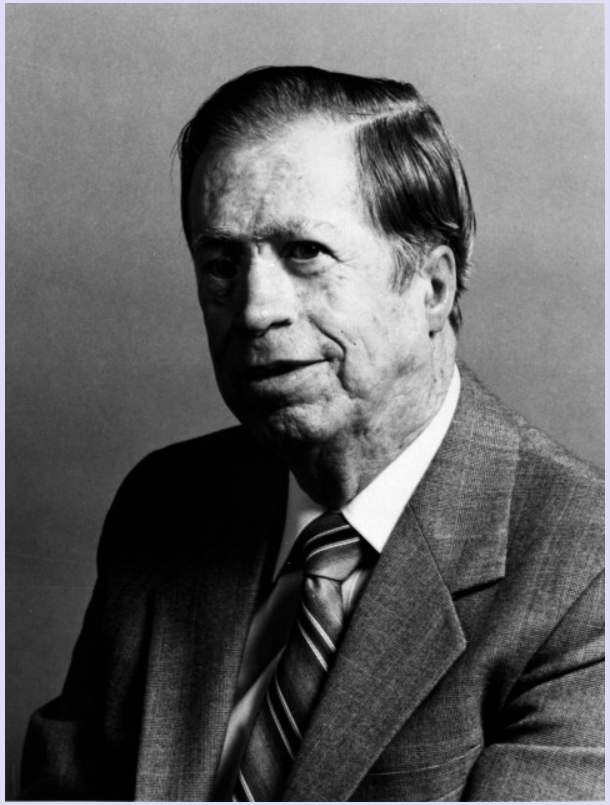
CHEMICAL SYNTHESIS OF BIOPOLYMERS

2.3 Solid-phase peptide synthesis (SPPS; Merrifield synthesis)

The ingenious **concept of peptide synthesis** on a solid support was developed by **Robert B. Merrifield in 1963**, and provided a **major breakthrough in peptide chemistry**.

The **amazingly simple concept** is that the first amino acid of the peptide to be synthesized is connected via its carboxyl group to an insoluble polymer that may be easily separated from either reagents or dissolved products by the use of filtration.

A **necessary prerequisite is that linkers are introduced into the polymer**.



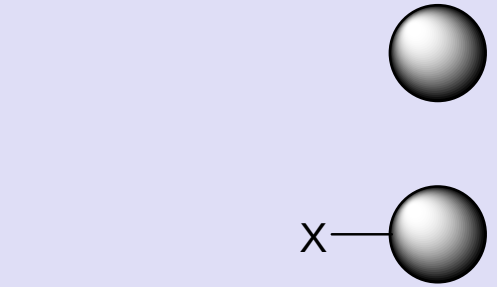
Robert Bruce Merrifield
Nobel Prize in Chemistry
1984

CHEMICAL SYNTHESIS OF BIOPOLYMERS

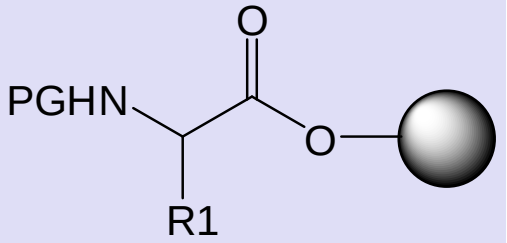
CHEMICAL SYNTHESIS OF BIOPOLYMERS

Polymer

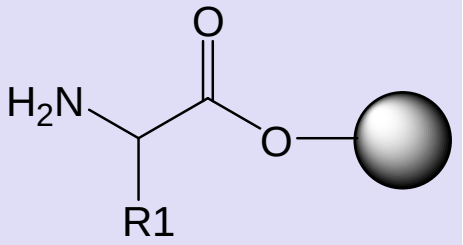
- **Step 1: introduction of suitable anchor group**



- **Step 2: attachment of the first *N*-protected amino acid by esterification**

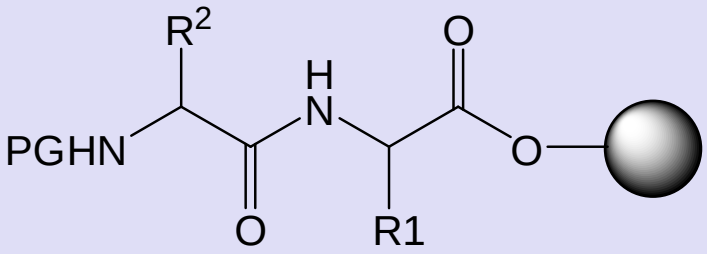


- **Step 3: selective cleavage of the *N*-protecting group**

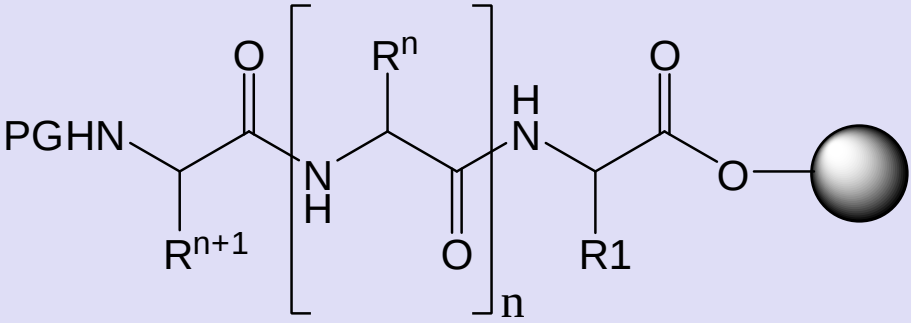


CHEMICAL SYNTHESIS OF BIOPOLYMERS

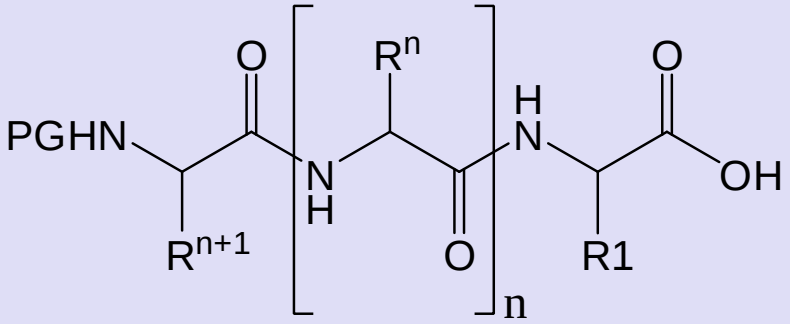
- **Step 4: addition of the next of the *N*-protected amino acid**



- **Step 5: n-fold repetition of step 3 to 4**



- **Step 6: cleavage of the *N*-protecting group and polymer removal**

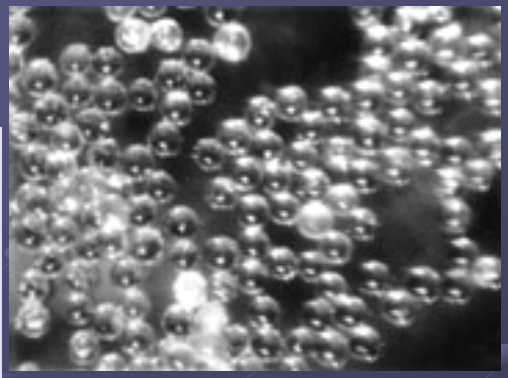


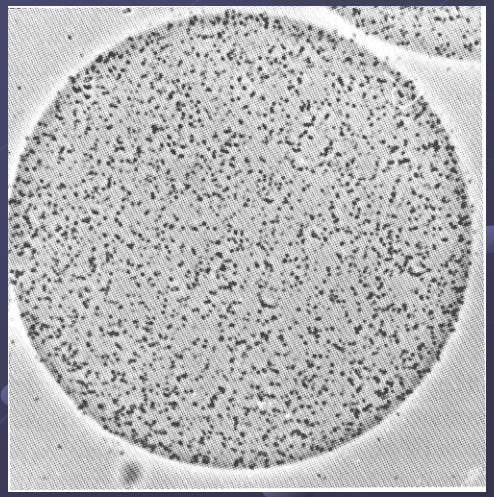
Free peptide

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Solid supports and linker systems

The classical **Merrifield resin** (copolymer of polystyrene with 1-2% divinyl benzene as cross-linker) complies well with the requirements of suitable polymeric support materials, but **is not optimal** with respect to **mechanical stability**, **loading capacity**, **diffusional problems**, and differences in **solvation** between the polymer and the peptide.





^{3}H-labeled peptides in a single bead, as revealed by auto-radiography

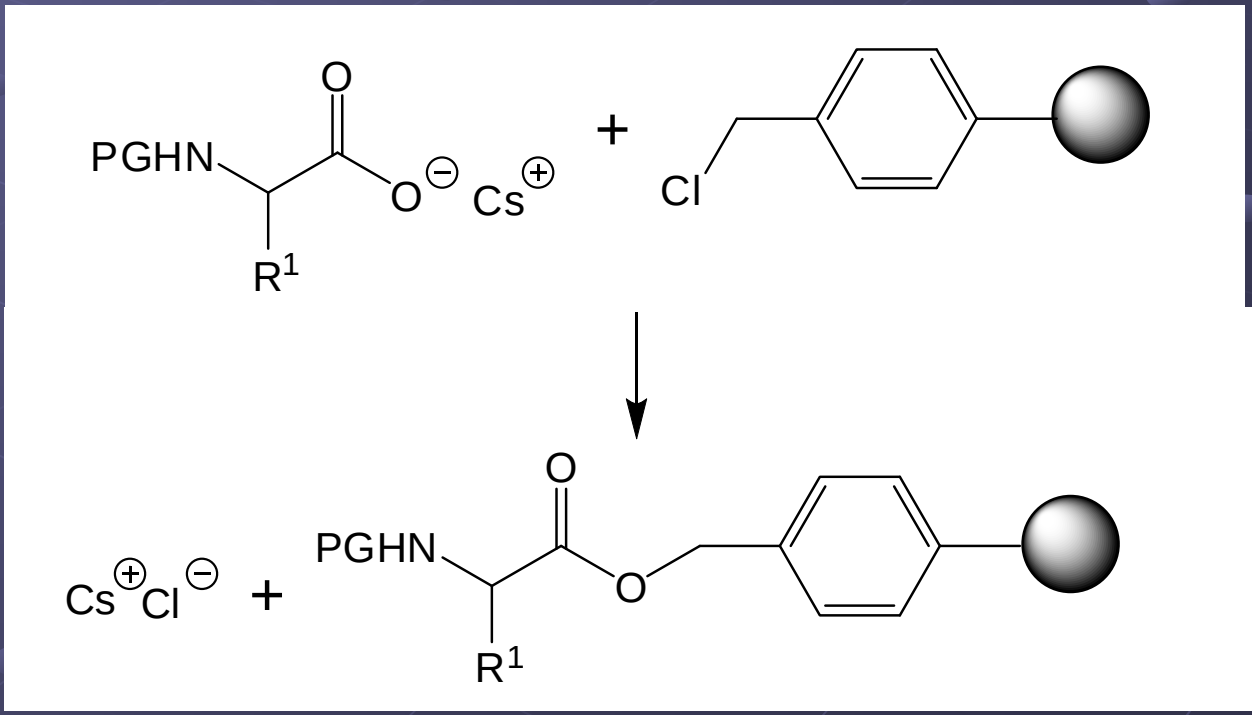
CHEMICAL SYNTHESIS OF BIOPOLYMERS

The **chloromethyl group** is the classical anchoring moiety present in the Merrifield resin.

Attachment of the first amino acid is performed as a nucleophilic substitution reaction of chloride by the amino acid carboxylate in ethanol, THF, or dioxane.

Often, **cesium salts** or **alkylammonium salts** are used.

Many different variants For chemical functionalization of polymers have been identified, and the number is steadily increasing.



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Protection Schemes

Boc/Bn-protecting groups scheme (Merrifield tactics)

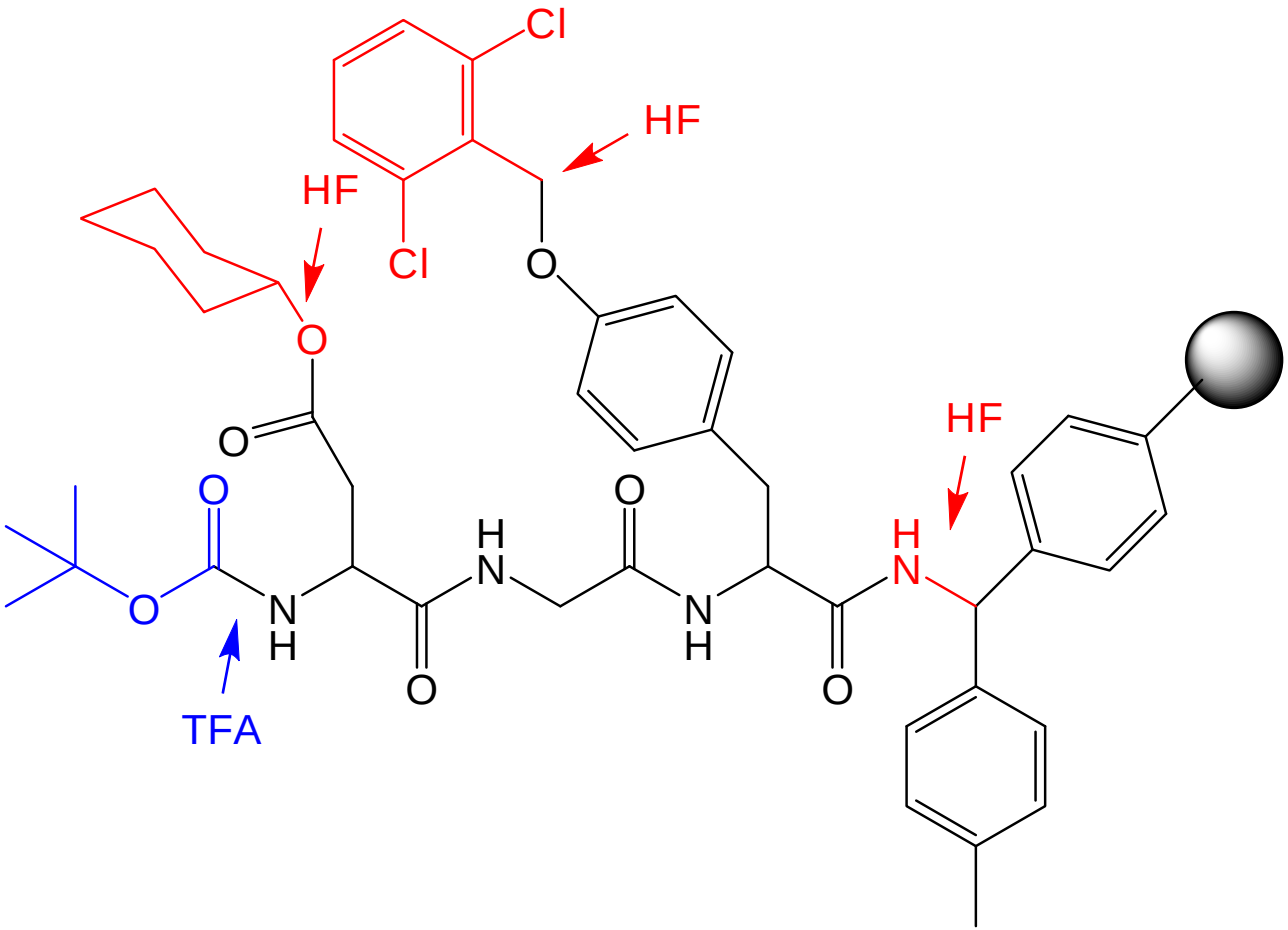
The **standard Merrifield system** is based on **Boc-protecting group tactics**, which rely on **selective acidolytic cleavage**.

The **Boc group** is usually cleaved with **trifluoroacetic acid** (TFA; 20 – 50 %).

The **semipermanent benzyl-type groups** must be stable under the conditions of repetitive Boc-group cleavage.

Because cleavage of the temporary and semipermanent protecting groups as well as of the linker proceed mechanistically in a very similar manner, **fine-tuning of the cleavage kinetics** is required.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



4-methylbenzhydrylamine resin

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Fmoc/*t*Bu-protecting groups scheme (Carpino tactics)

Louis Carpino

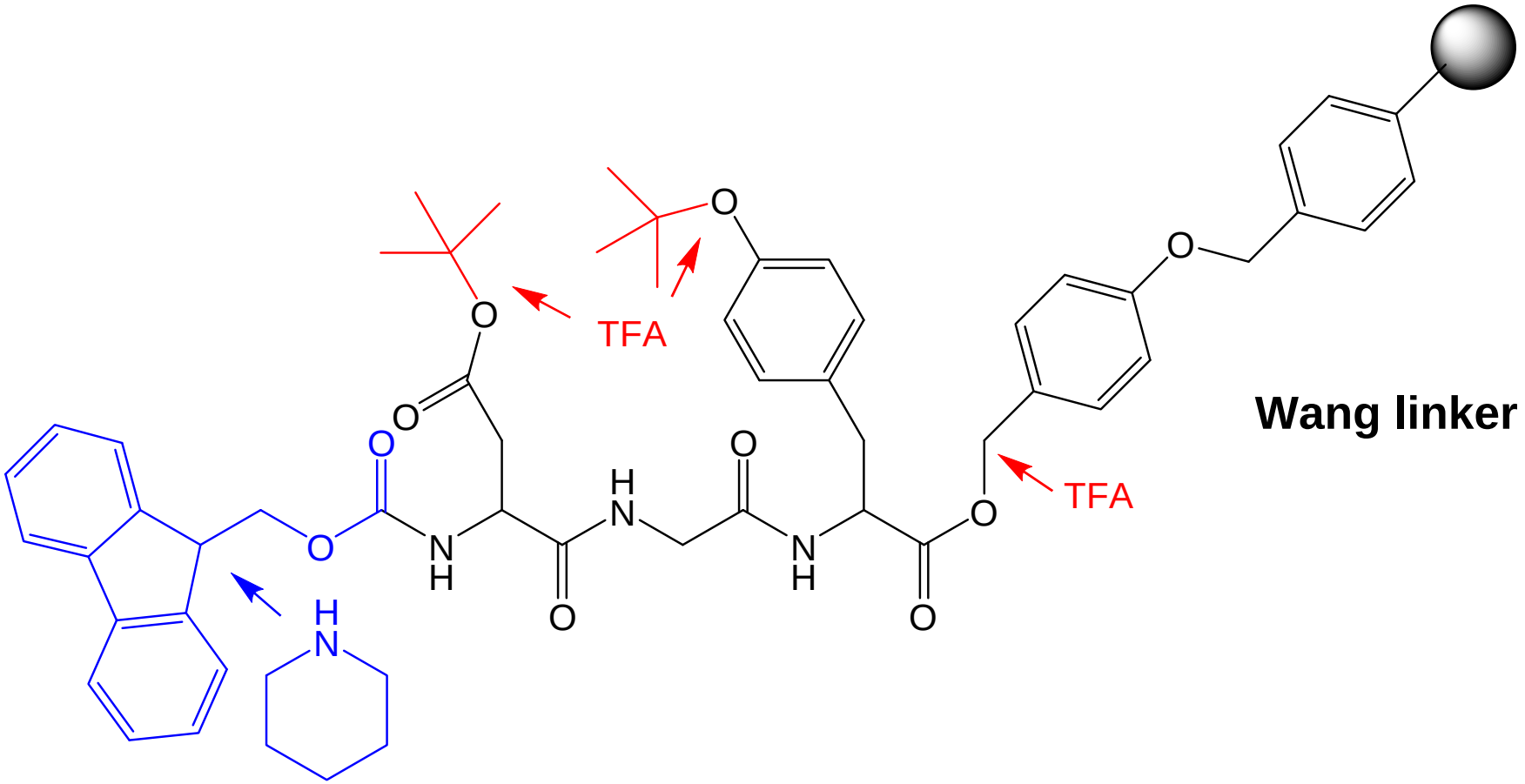
The **Fmoc-protecting group** tactics makes use of the **base lability** of the **fluorenyl-9-methyloxycarbonyl group** (Fmoc).

It is **widely applied alternative** to the Merrifield tactics with **two-dimensional orthogonality**.

The **semipermanent side chain-protecting groups** are mostly of the ***tert*-butyl type**, and can be cleaved under **relatively mild reaction** conditions with **TFA**.

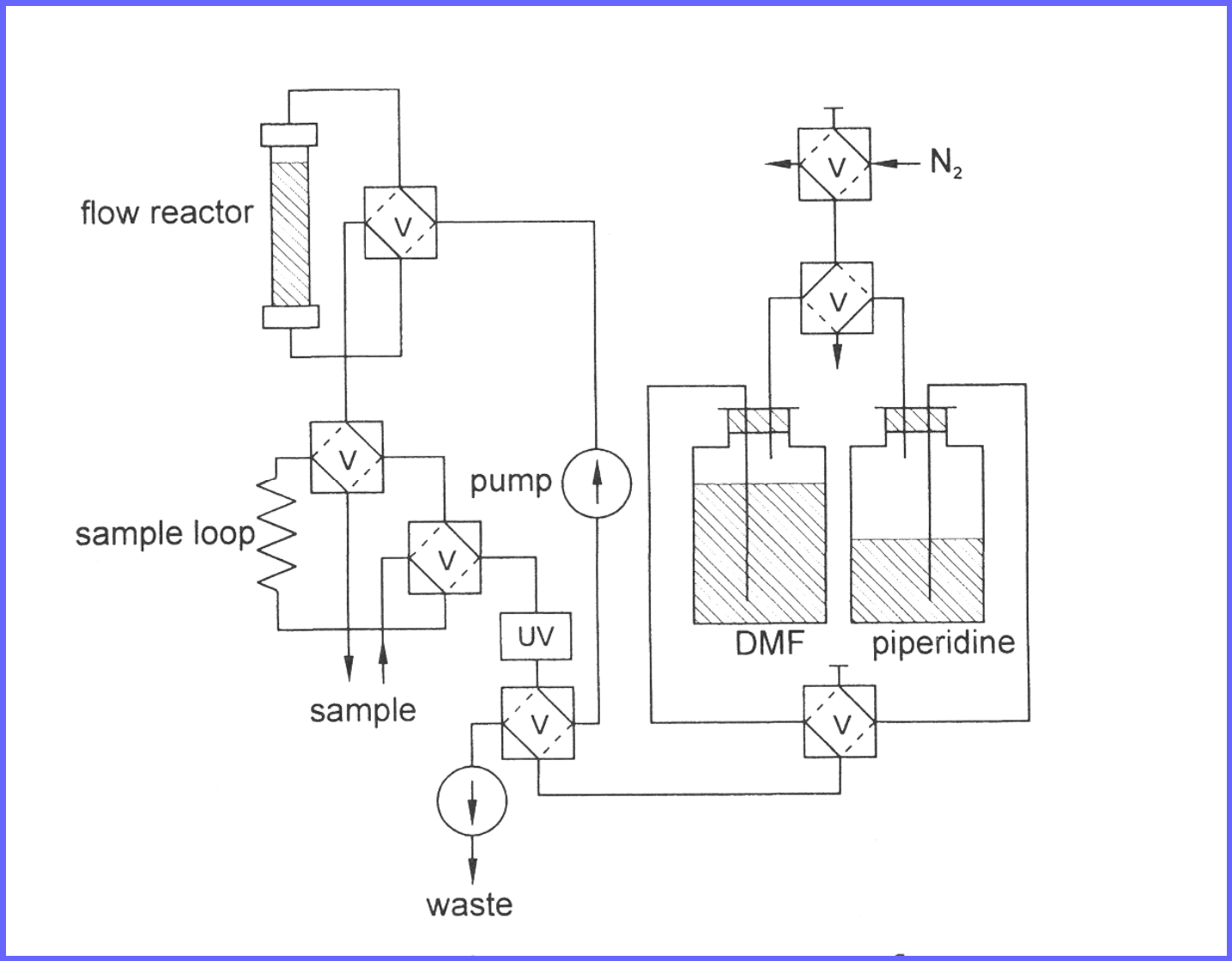
Linker moieties displaying **comparable acid lability** are mainly used.

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

CHEMICAL SYNTHESIS OF BIOPOLYMERS



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Coupling methods

The **carbodiimide/HOBt coupling method** is often used solid-phase peptide synthesis.

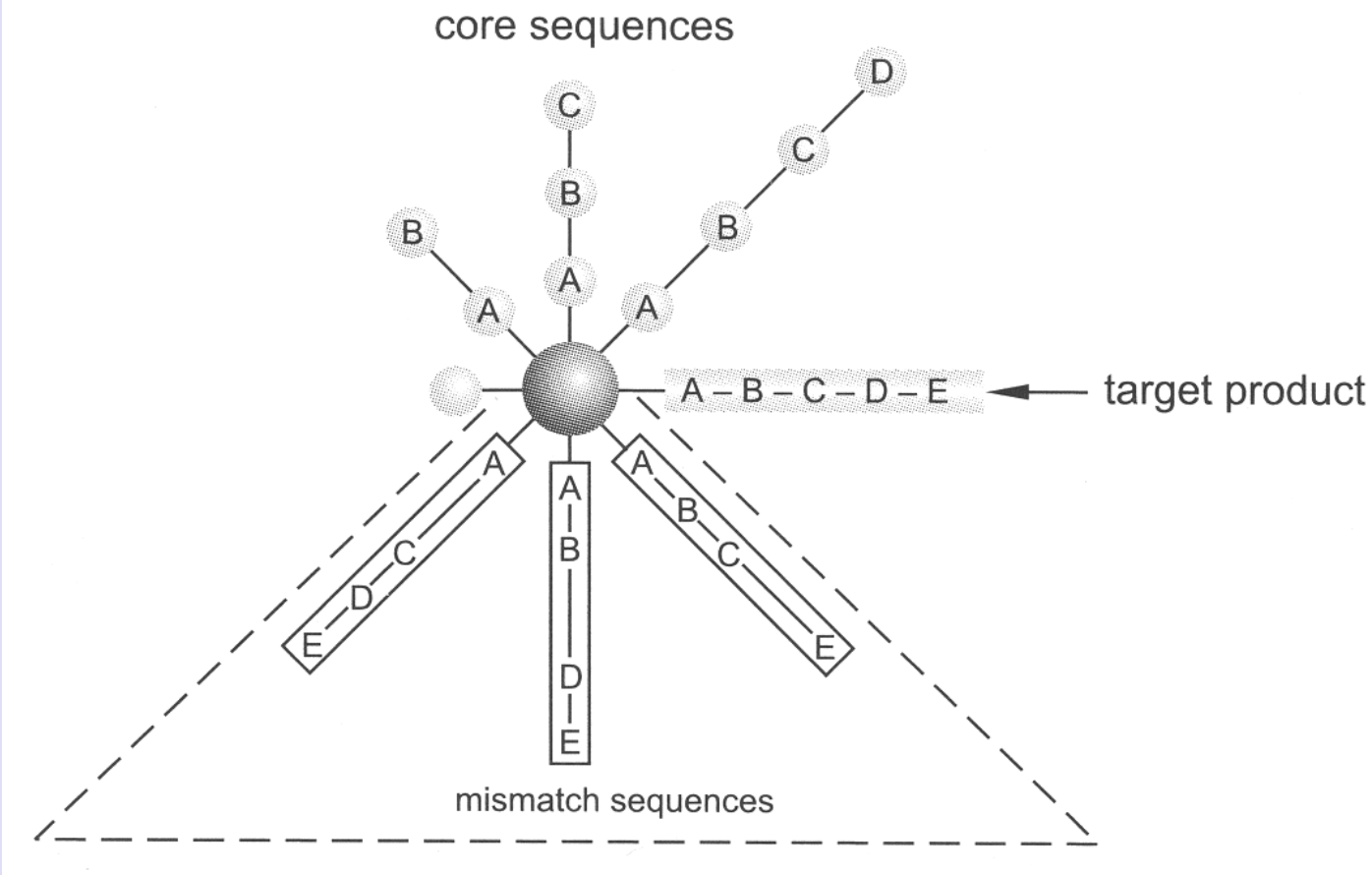
The reactions are usually performed in **dichloromethane**, sometimes with addition of **DMF**, and at **ambient temperature**.

Because complete conversion is the basic precondition for the formation of a homogeneous final product, **coupling reagents are applied in excess** (usually **three-fold**).

Beside the carbodiimide/HOBt method all the **other coupling procedures** (symmetrical anhydrides, active esters, reagents of the uronium or phosphonium type) are used/proved to be efficient coupling methods.

CHEMICAL SYNTHESIS OF BIOPOLYMERS

Undesired problems during elongation



CHEMICAL SYNTHESIS OF BIOPOLYMERS

Mismatch sequences arise when **acylation or deprotection are incomplete** and one or more amino acid components are skipped in the chain elongation.

Mismatches sequences may also be formed when **acylation of nucleophilic side-chain** occurs after partial deblocking.

The **separation of undesired side** products from the target peptide on completion of the synthesis **is very tedious**, and **often impossible on a preparative scale.**

Preventative measures to avoid mismatch sequences are **double coupling steps** (limited by side-reaction) and **capping**.

The **permanent and irreparable risk of side-reactions on all synthetic steps**, is, despite all advantages, one of the **peculiarities of SPPS**.