27.14
The Strategy of Peptide Synthesis

General Considerations

Making peptide bonds between amino acids is not difficult.

The challenge is connecting amino acids in the correct sequence.

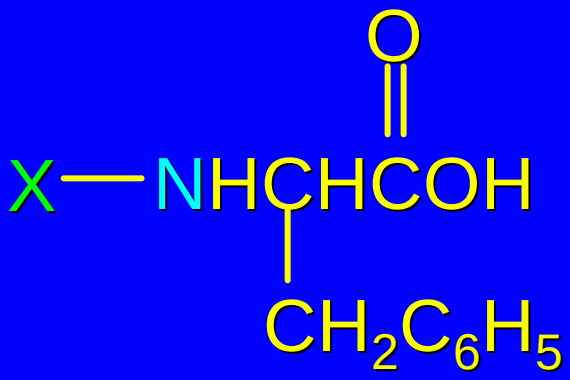
Random peptide bond formation in a mixture of phenylalanine and glycine, for example, will give four dipeptides.

Phe—Phe Gly—Gly Phe—Gly Gly—Phe

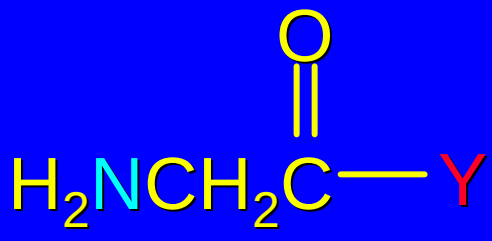
General Strategy

1. Limit the number of possibilities by "protecting" the nitrogen of one amino acid and the carboxyl group of the other.

N-Protected
phenylalanine

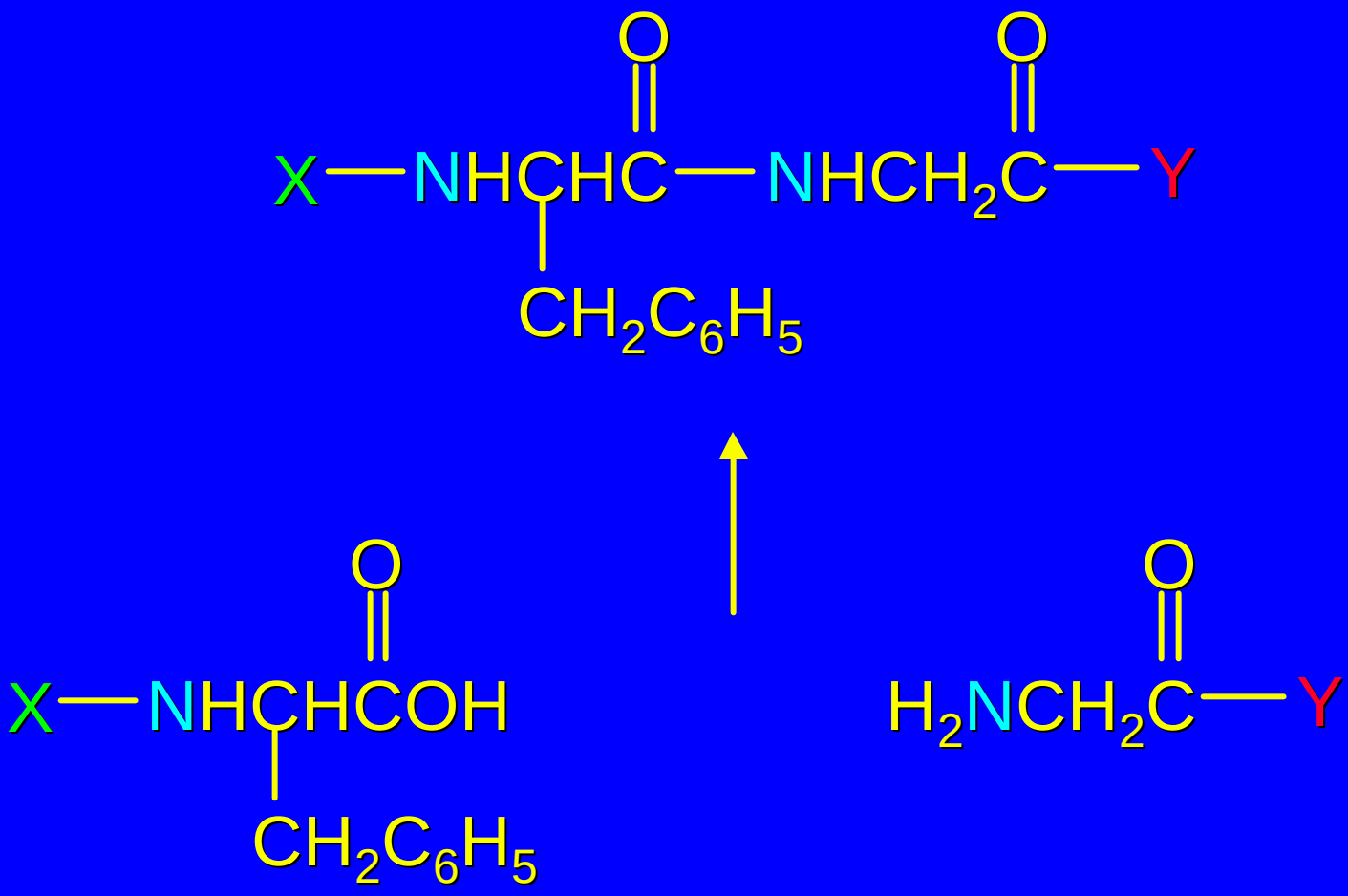


C-Protected
glycine



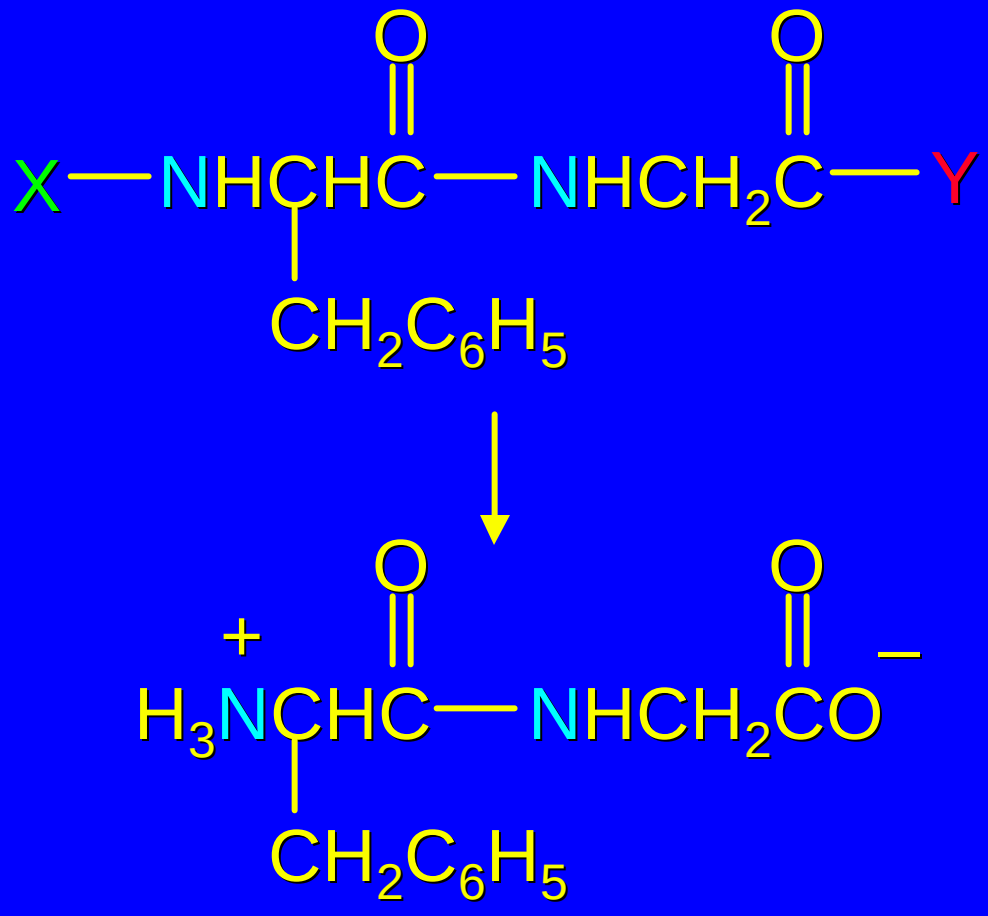
General Strategy

2. Couple the two protected amino acids.



General Strategy

3. Deprotect the amino group at the N-terminus and the carboxyl group at the C-terminus.



Phe-Gly

27.15
Amino Group Protection

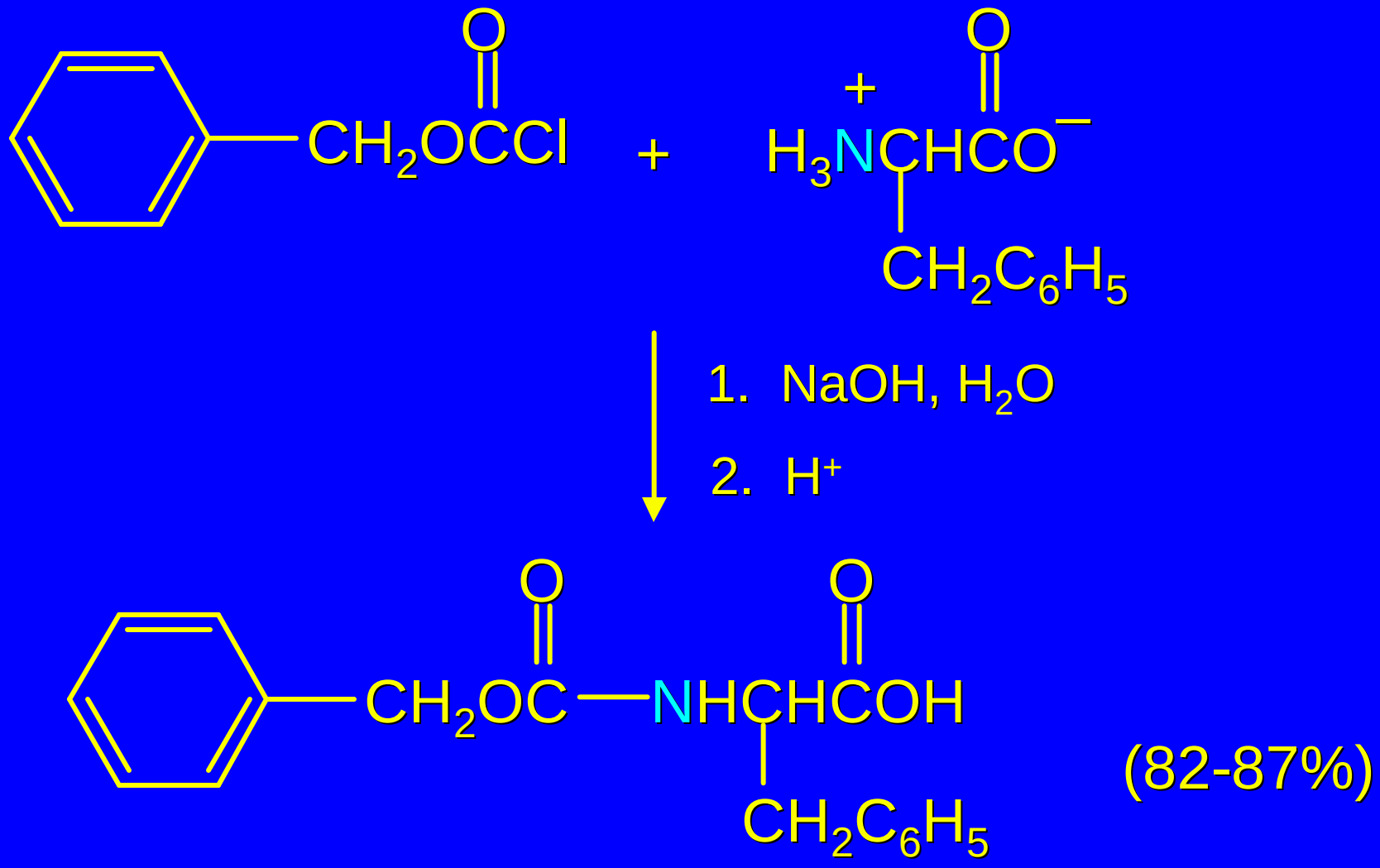
Protect Amino Groups as Amides

Amino groups are normally protected by converting them to amides.

Benzyloxycarbonyl ($C_6H_5CH_2O$—) is a common protecting group. It is abbreviated as *Z*.

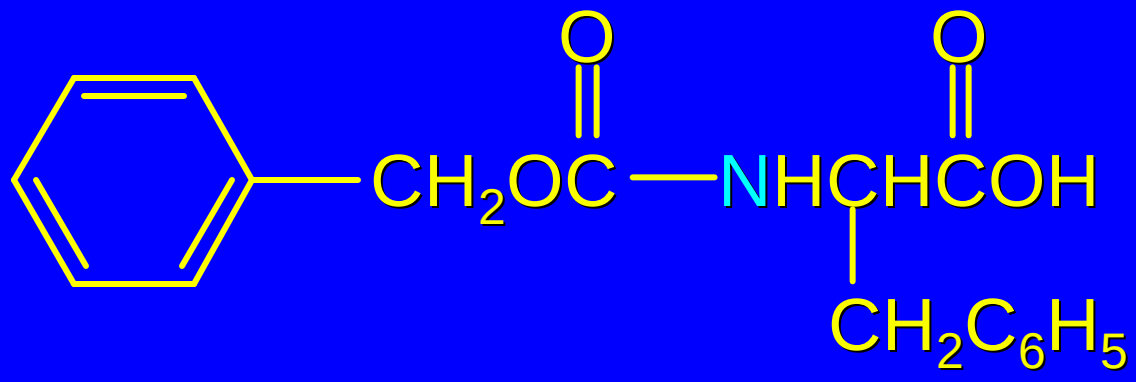
Z-protection is carried out by treating an amino acid with benzyloxycarbonyl chloride.

Protect Amino Groups as Amides

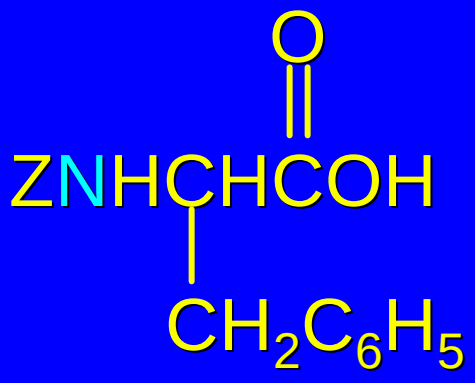


(82-87%)

Protect Amino Groups as Amides



is abbreviated as:



or Z-Phe

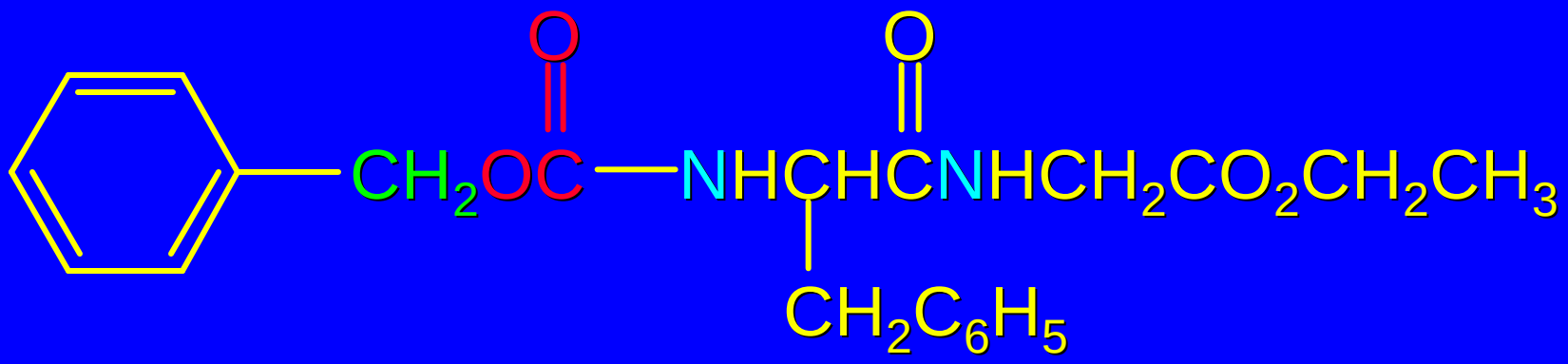
Removing Z-Protection

An advantage of the benzyloxycarbonyl protecting group is that it is easily removed by:

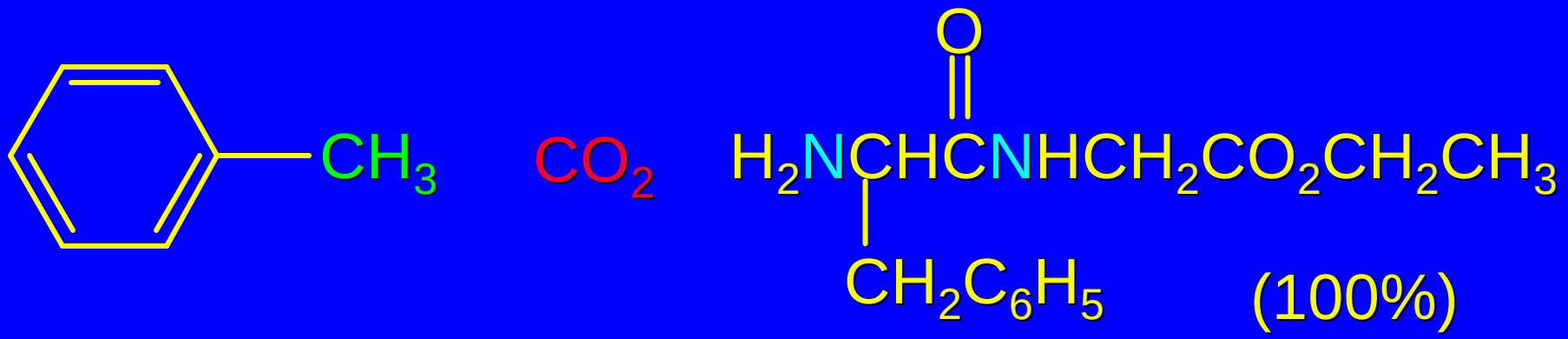
a) hydrogenolysis

b) cleavage with HBr in acetic acid

Hydrogenolysis of Z-Protecting Group

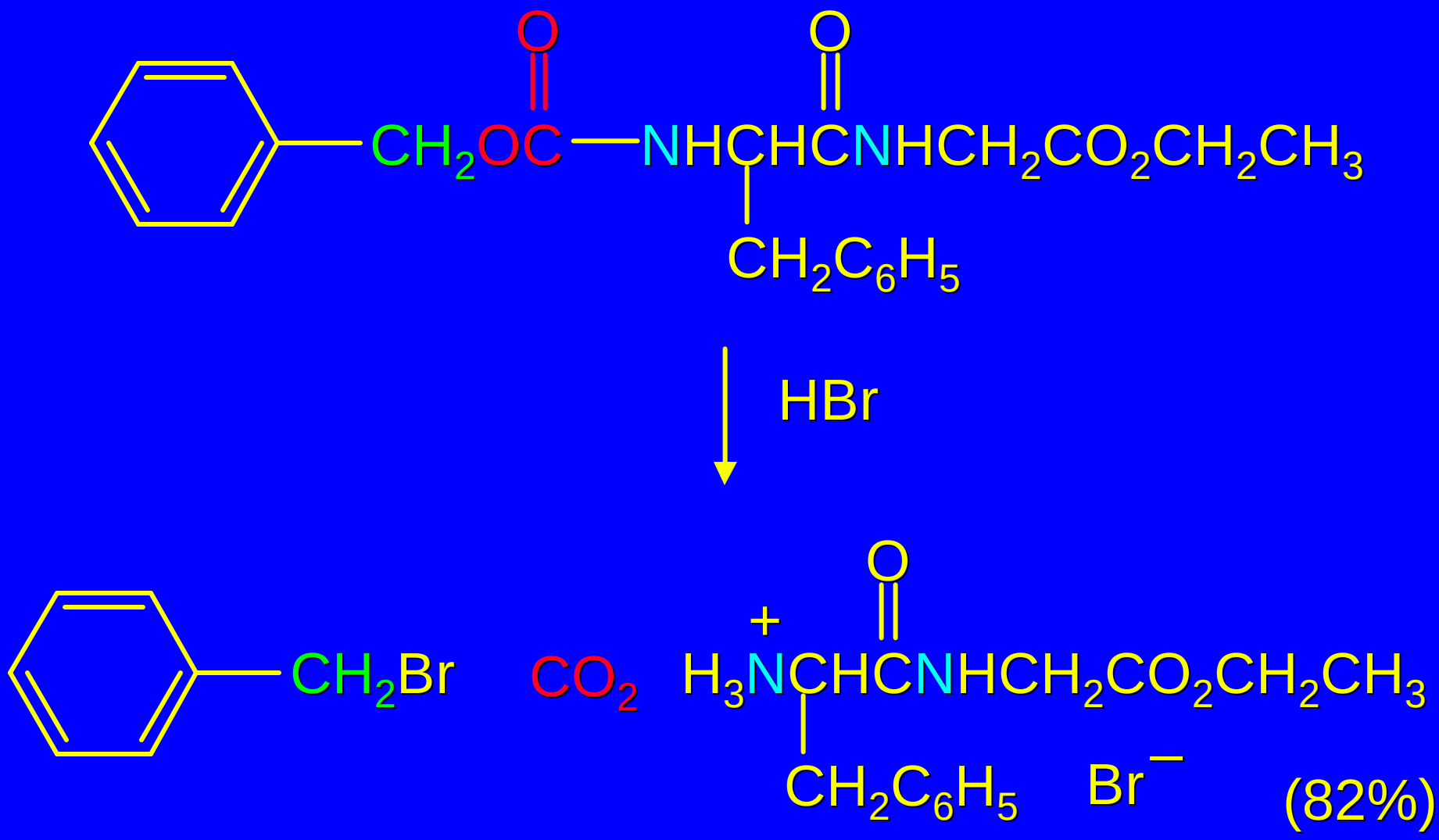


H_2, Pd

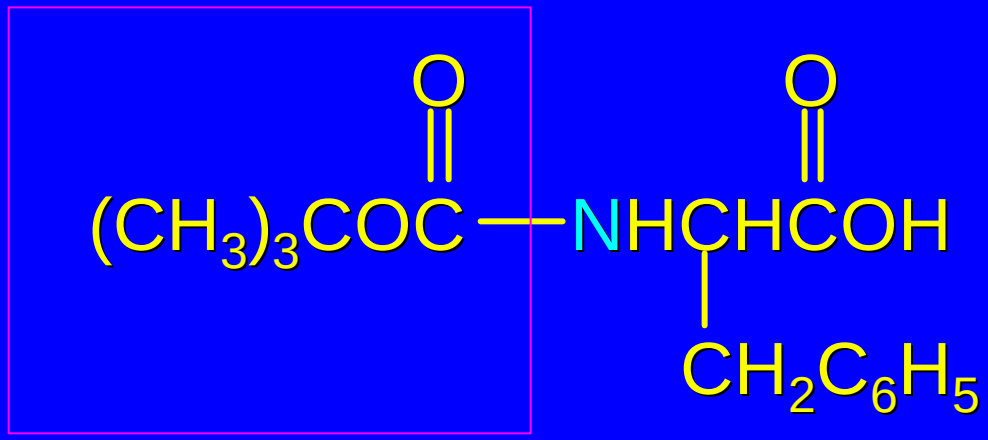


(100%)

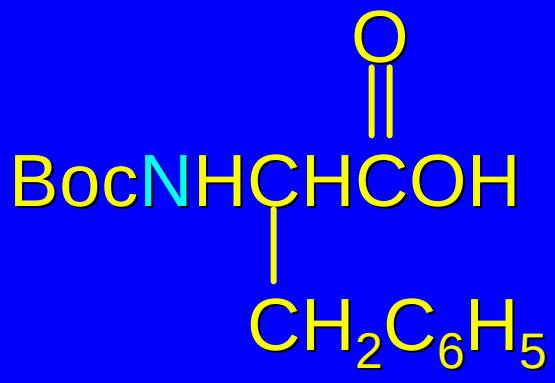
HBr Cleavage of Z-Protecting Group



The tert-*Butoxycarbonyl Protecting Group*

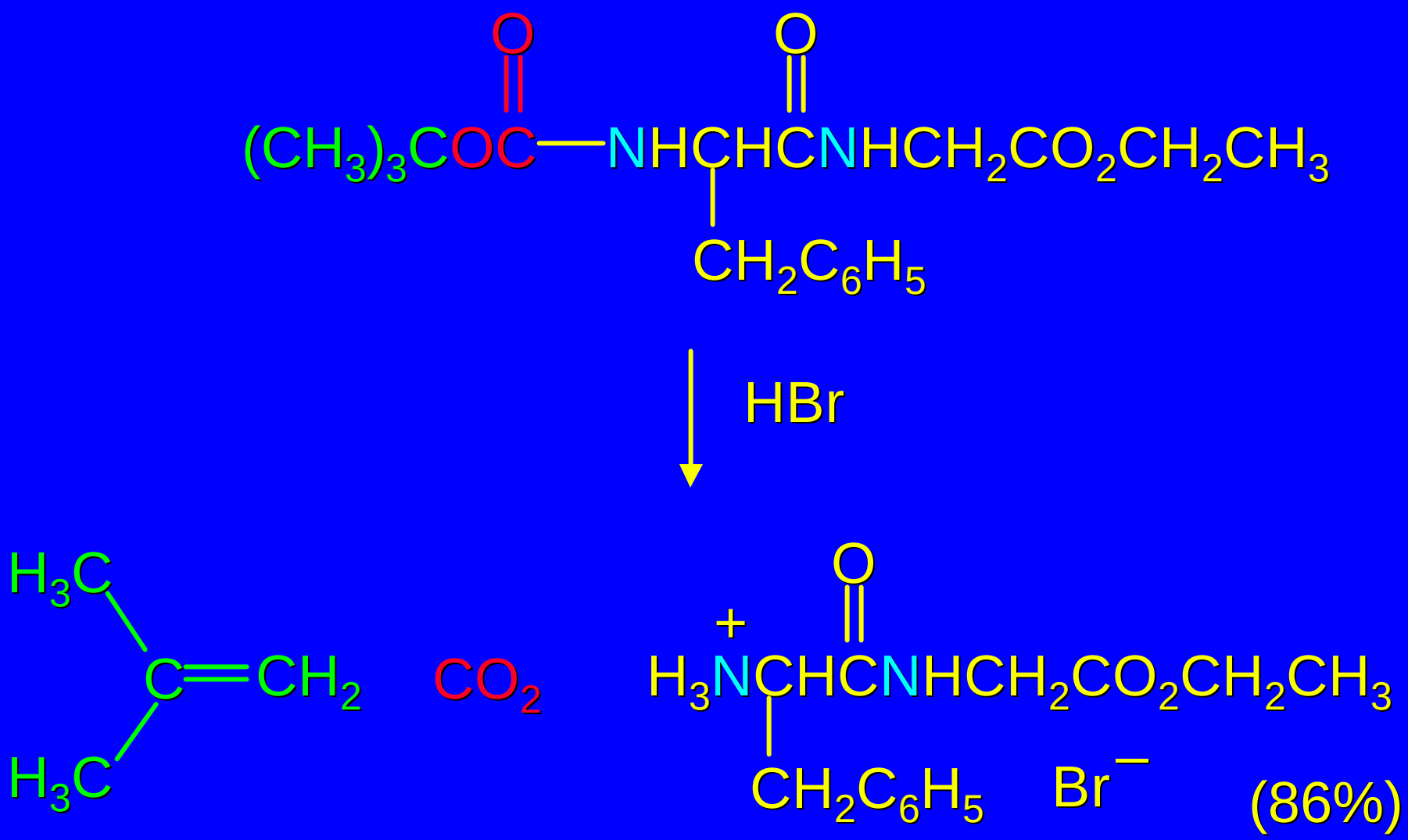


is abbreviated as:



or Boc-Phe

HBr Cleavage of Boc-Protecting Group



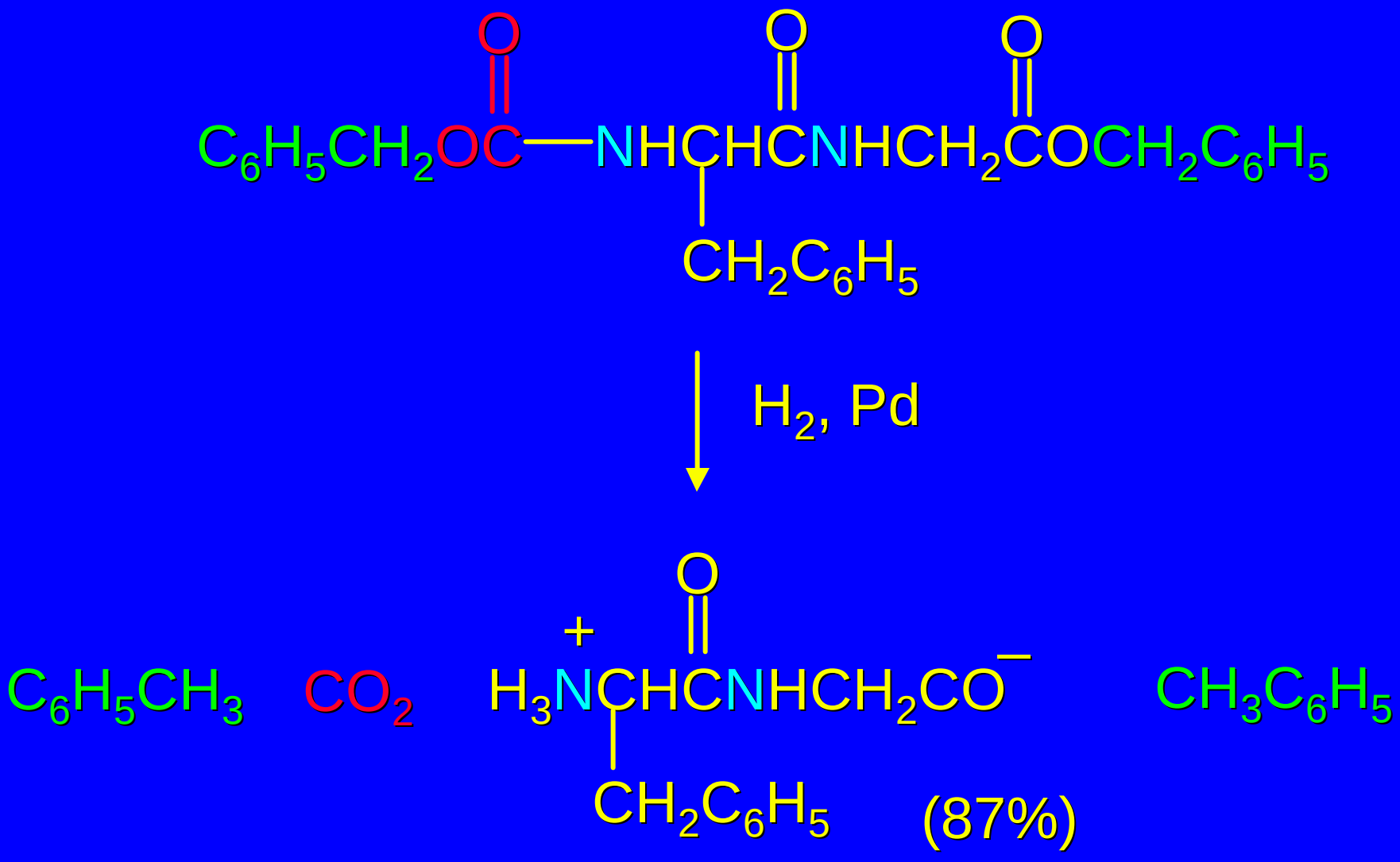
27.16
Carboxyl Group Protection

Protect Carboxyl Groups as Esters

Carboxyl groups are normally protected as esters.

- Deprotection of methyl and ethyl esters is by hydrolysis in base.
- Benzyl esters can be cleaved by hydrogenolysis.

Hydrogenolysis of Benzyl Esters



27.17
Peptide Bond Formation

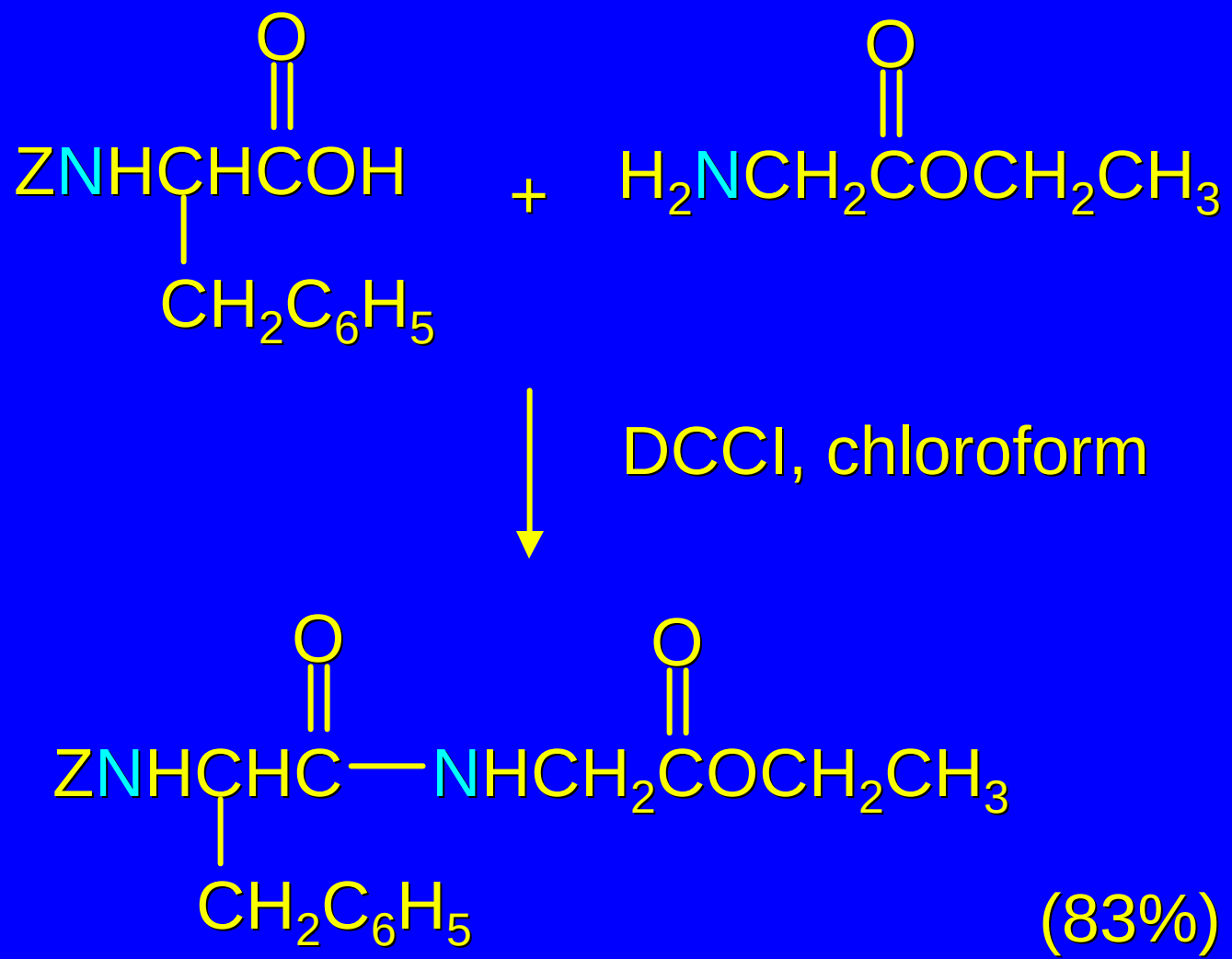
Forming Peptide Bonds

The two major methods are:

1. coupling of suitably protected amino acids using *N*,*N'*-dicyclohexylcarbodiimide (DCCI)

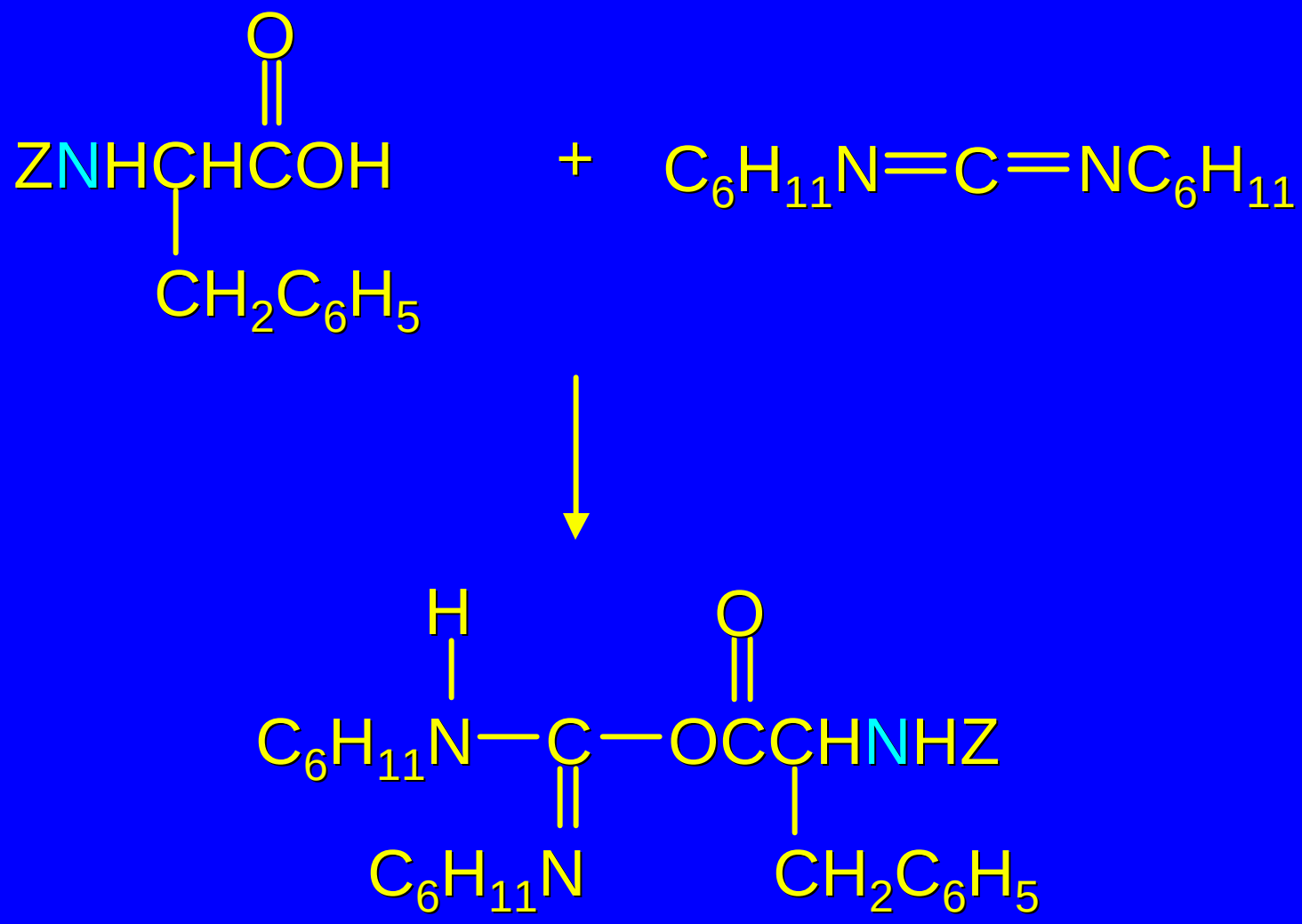
2. via an *active ester* of the N-terminal amino acid.

DCCI-Promoted Coupling



(83%)

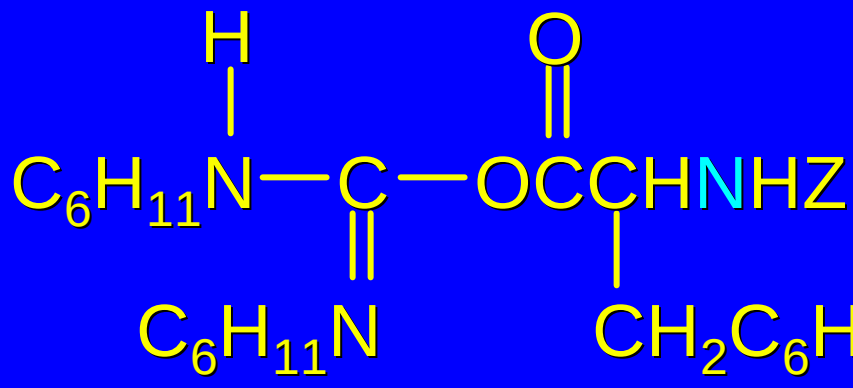
Mechanism of DCCI-Promoted Coupling



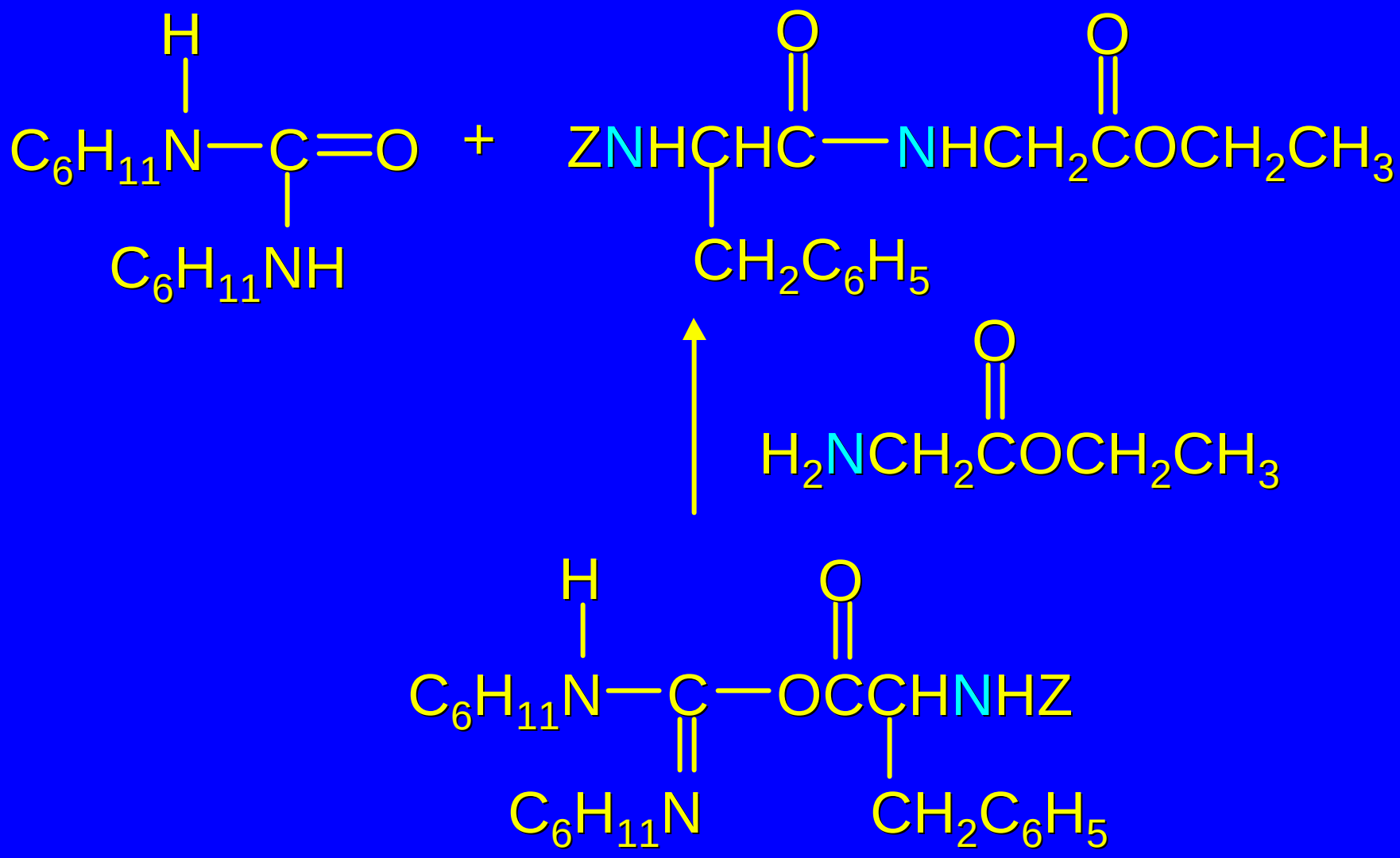
Mechanism of DCCI-Promoted Coupling

The species formed by addition of the Z-protected amino acid to DCCI is similar in structure to an acid anhydride and acts as an acylating agent.

Attack by the amine function of the carboxyl-protected amino acid on the carbonyl group leads to nucleophilic acyl substitution.



Mechanism of DCCI-Promoted Coupling



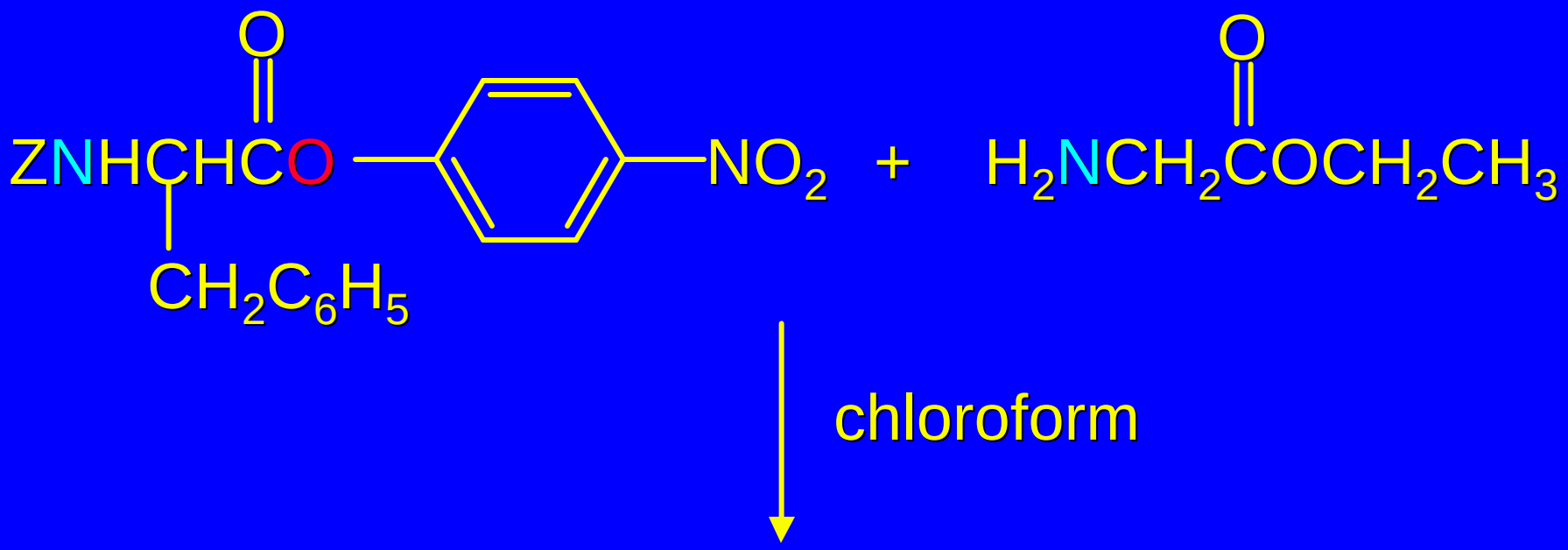
The Active Ester Method

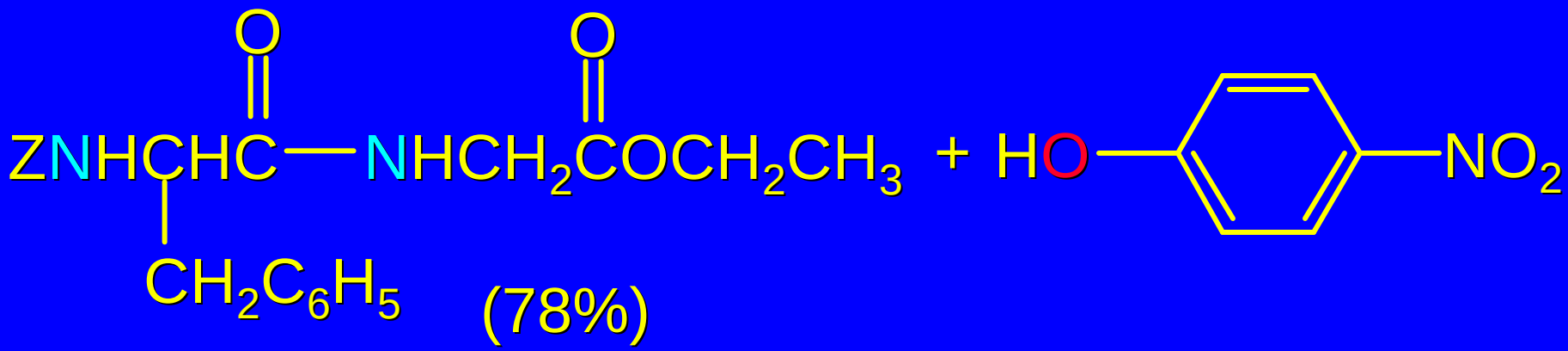
A *p*-nitrophenyl ester is an example of an "active ester."

p-Nitrophenyl is a better leaving group than methyl or ethyl, and *p*-nitrophenyl esters are more reactive in nucleophilic acyl substitution.

The Active Ester Method

The Active Ester Method





(78%)

27.18
Solid-Phase Peptide Synthesis: The Merrifield Method

Solid-Phase Peptide Synthesis

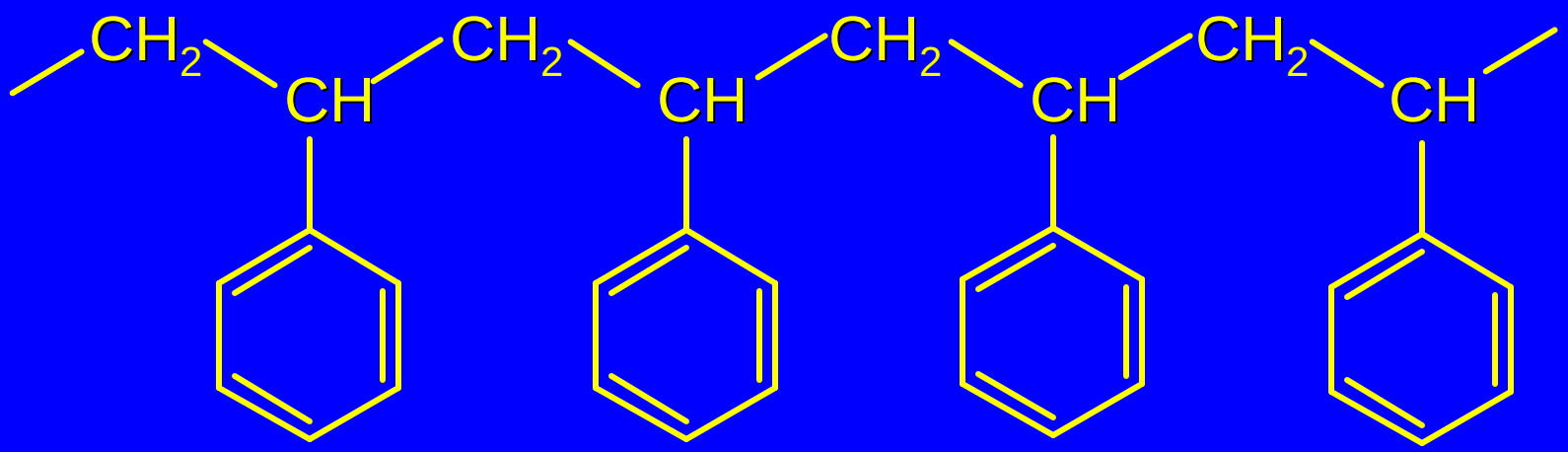
In solid-phase synthesis, the starting material is bonded to an inert solid support.

Reactants are added in solution.

Reaction occurs at the interface between the solid and the solution. Because the starting material is bonded to the solid, any product from the starting material remains bonded as well.

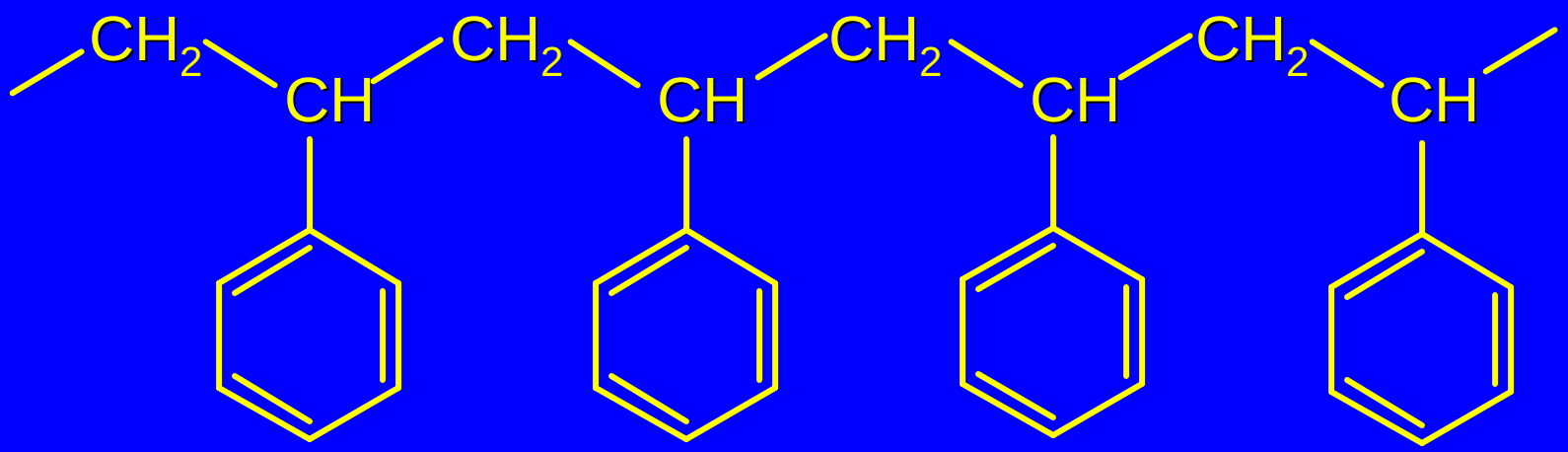
Purification involves simply washing the byproducts from the solid support.

The Solid Support



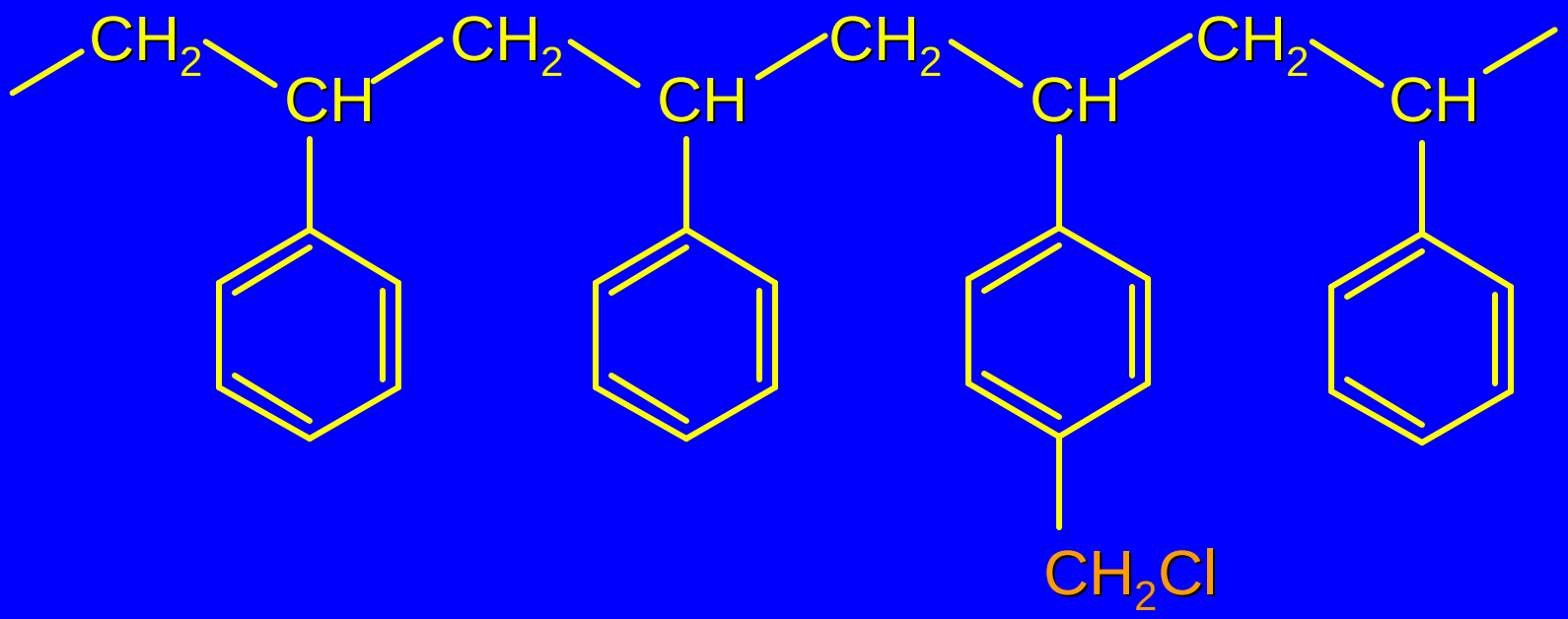
The solid support is a copolymer of styrene and divinylbenzene. It is represented above as if it were polystyrene. Cross-linking with divinylbenzene simply provides a more rigid polymer.

The Solid Support



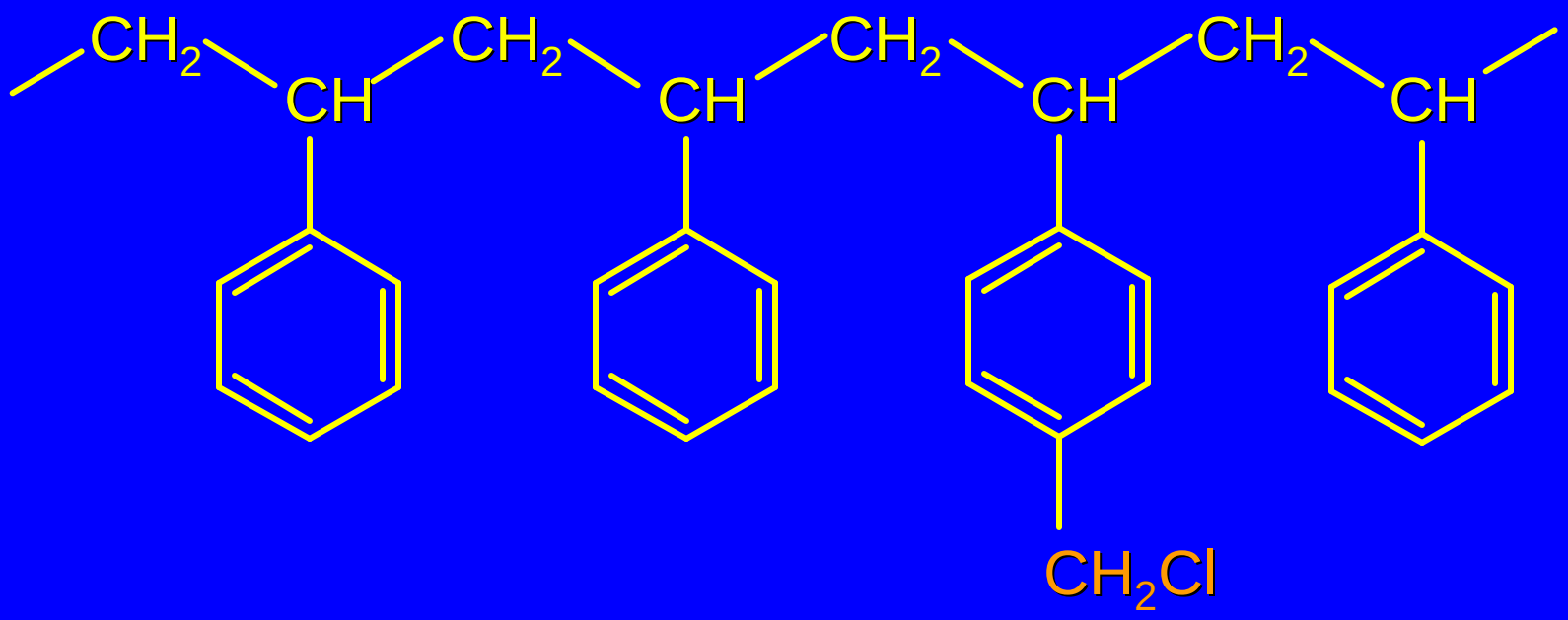
Treating the polymeric support with chloromethyl methyl ether ($ClCH_2OCH_3$) and $SnCl_4$ places $ClCH_2$ side chains on some of the benzene rings.

The Solid Support



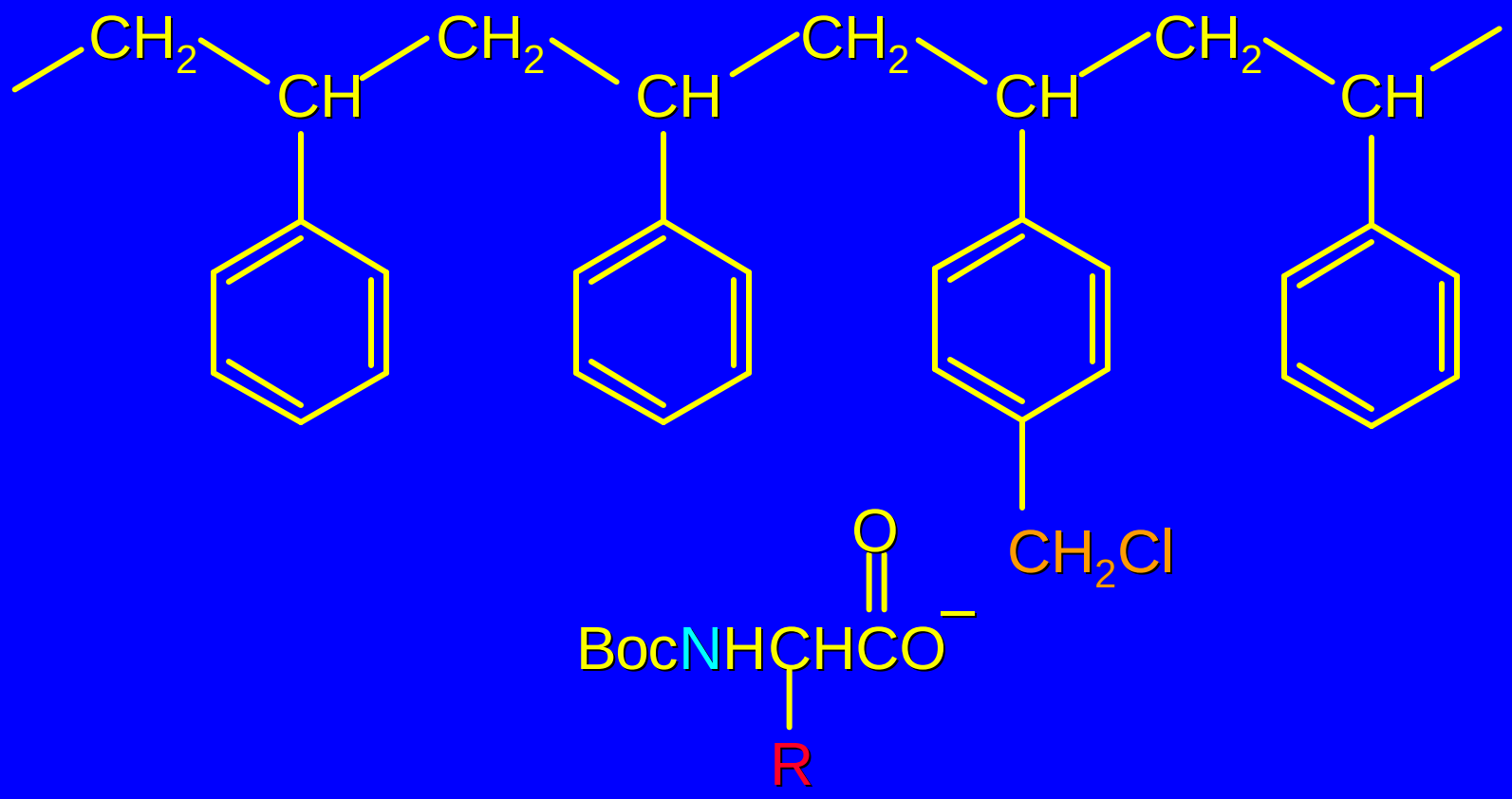
The side chain chloromethyl group is a benzylic halide, reactive toward nucleophilic substitution (S_N2).

The Solid Support

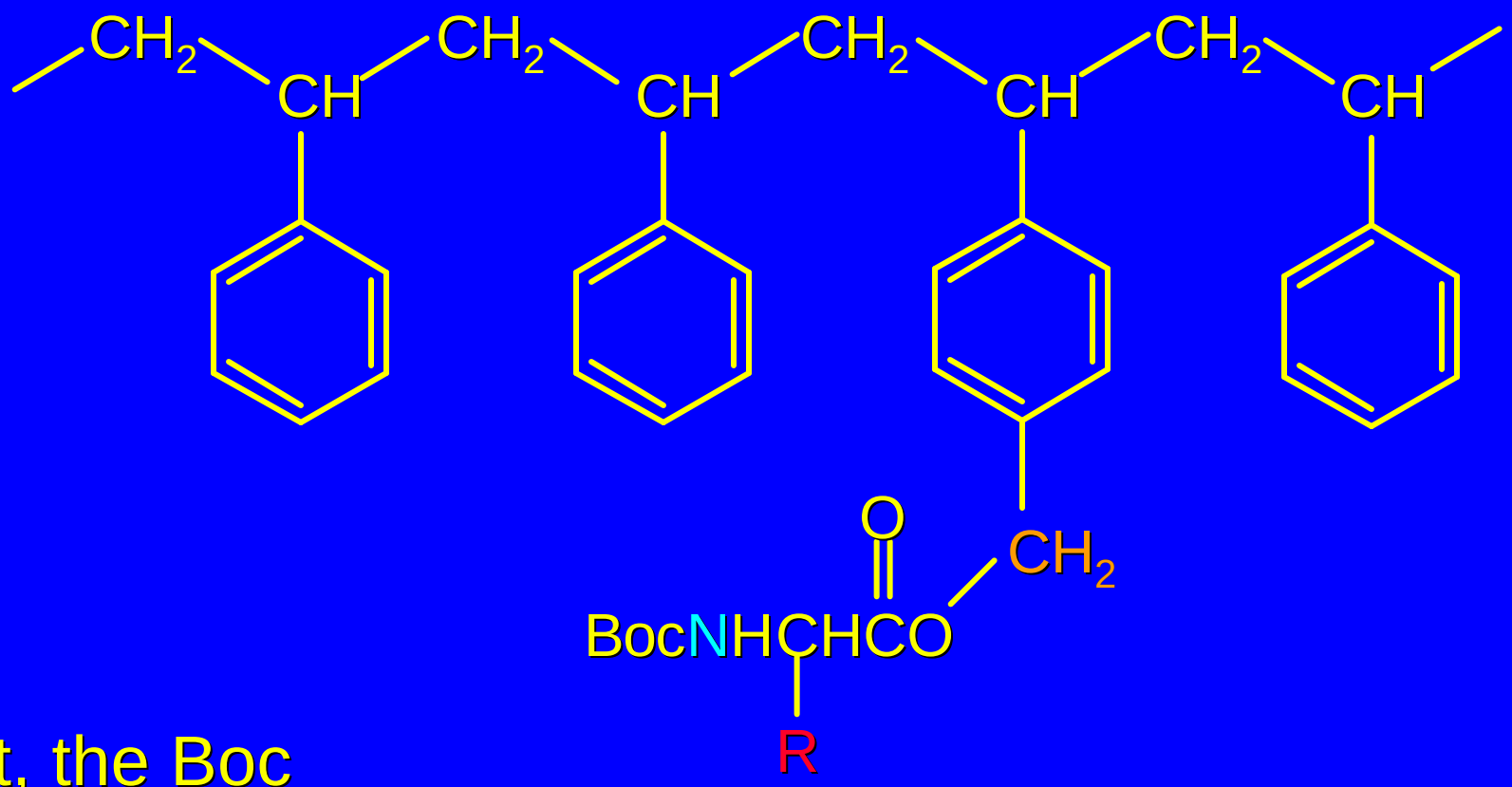


The chloromethylated resin is treated with the Boc-protected C-terminal amino acid. Nucleophilic substitution occurs, and the Boc-protected amino acid is bound to the resin as an ester.

The Merrifield Procedure

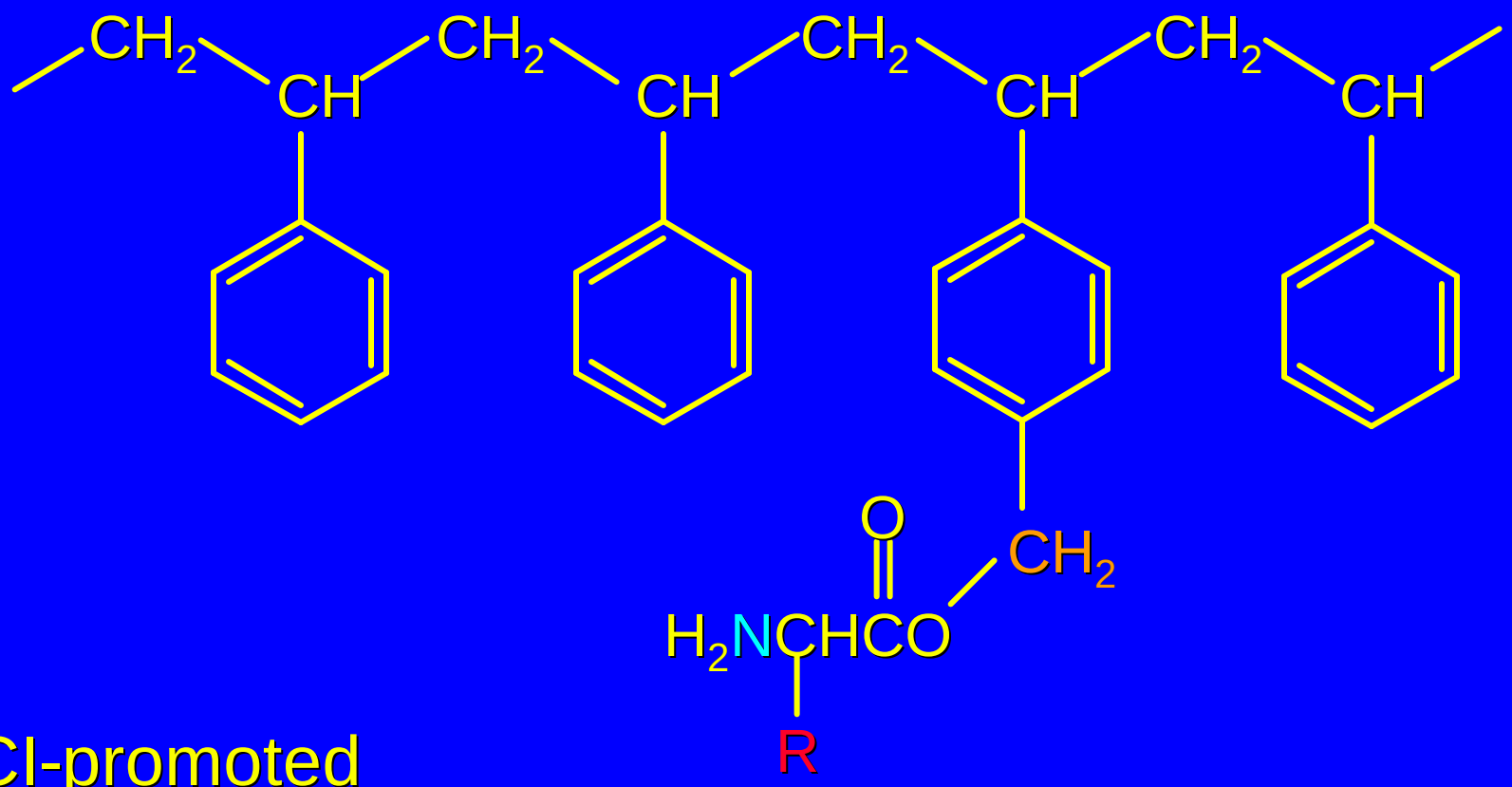


The Merrifield Procedure



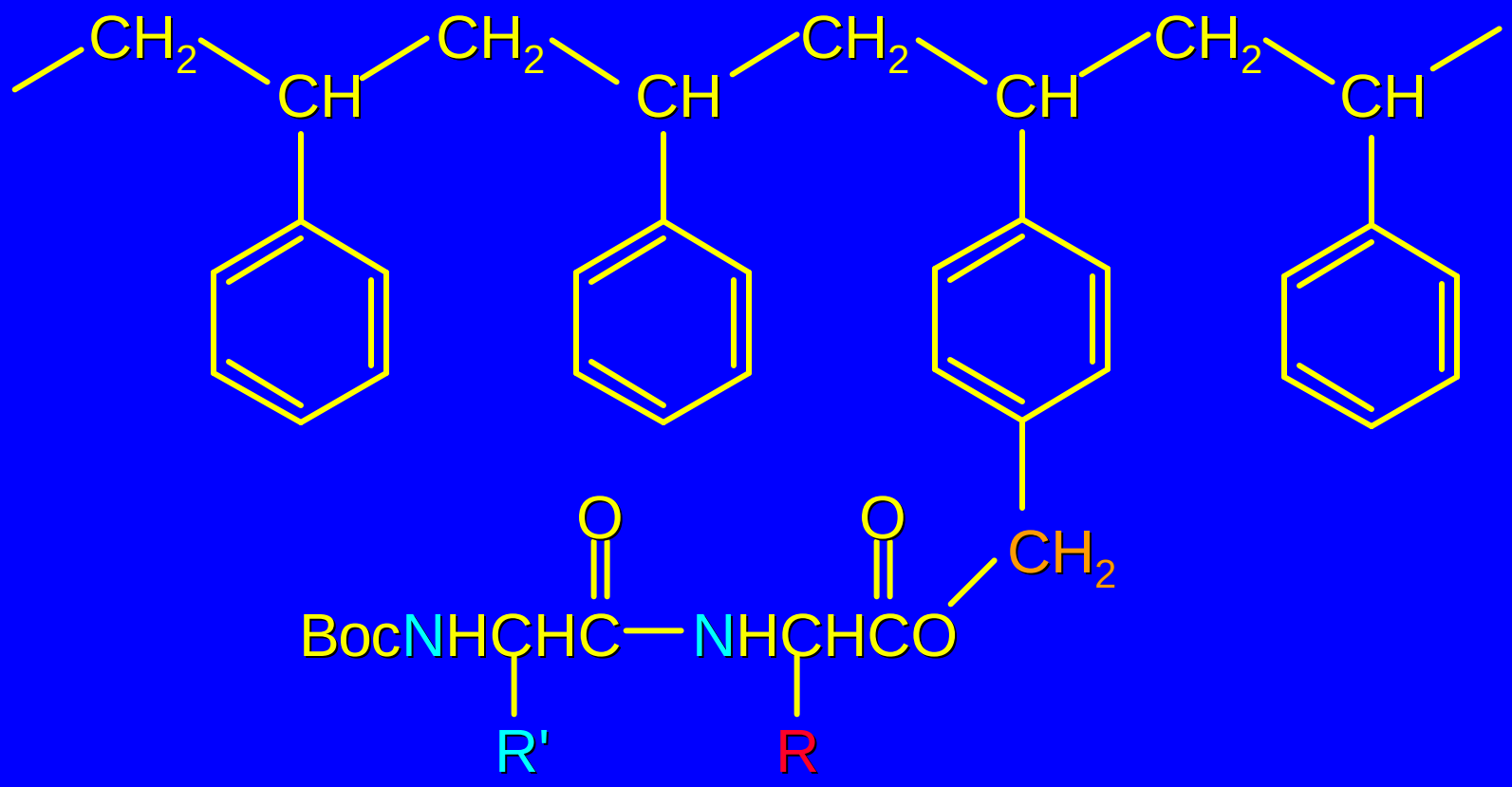
Next, the Boc protecting group is removed with HCl.

The Merrifield Procedure



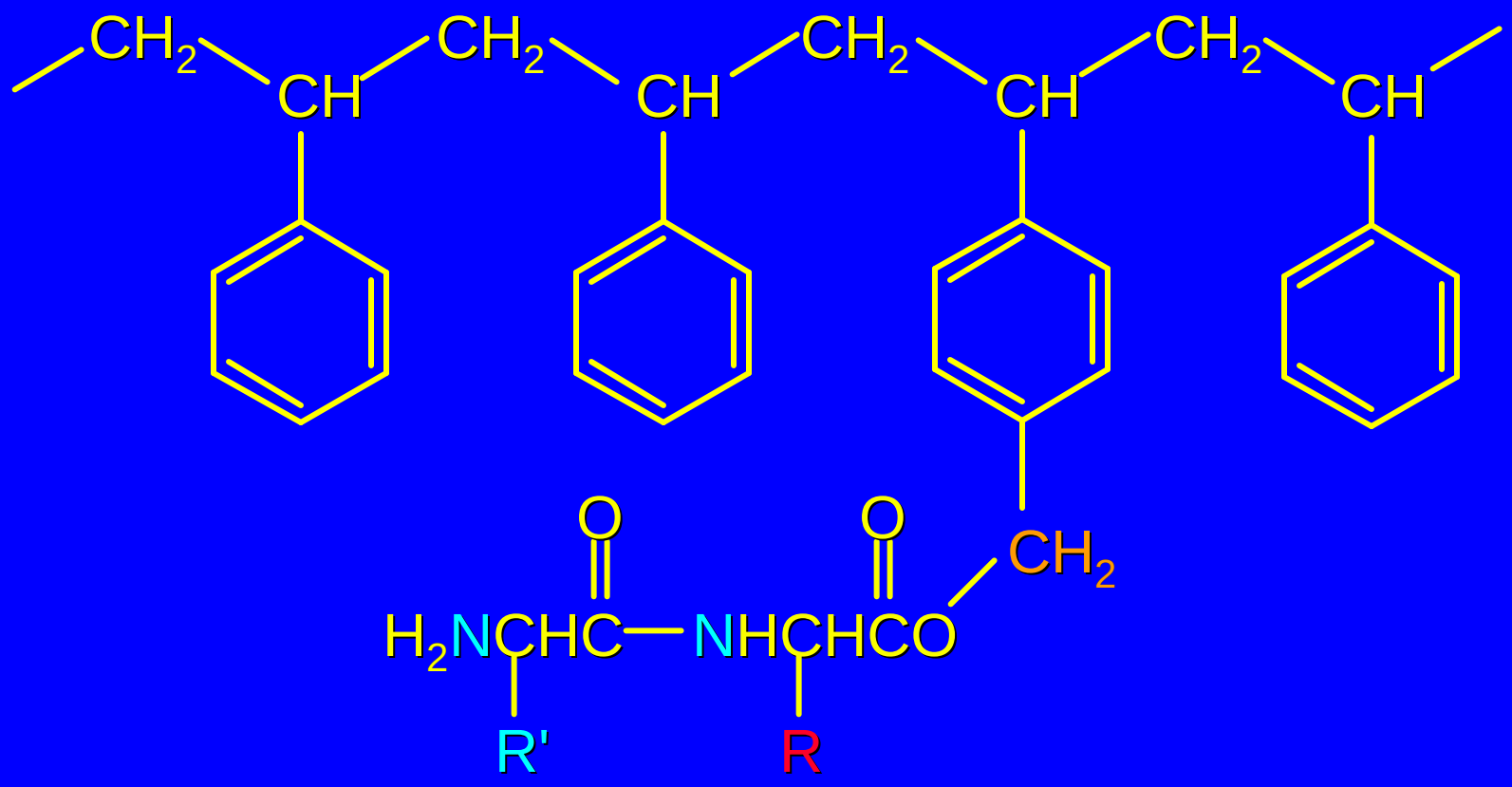
DCCI-promoted coupling adds the second amino acid

The Merrifield Procedure



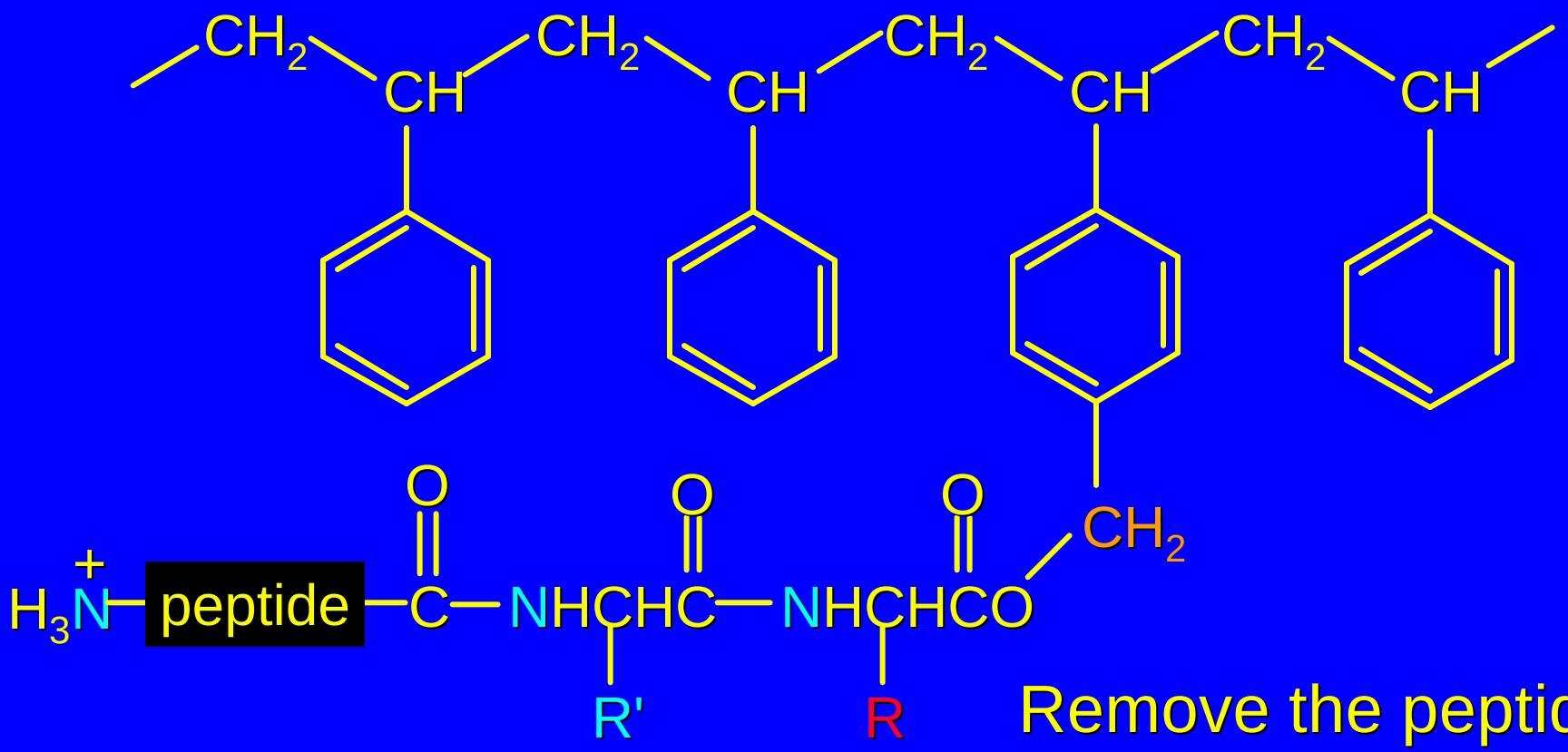
Remove the Boc protecting group.

The Merrifield Procedure



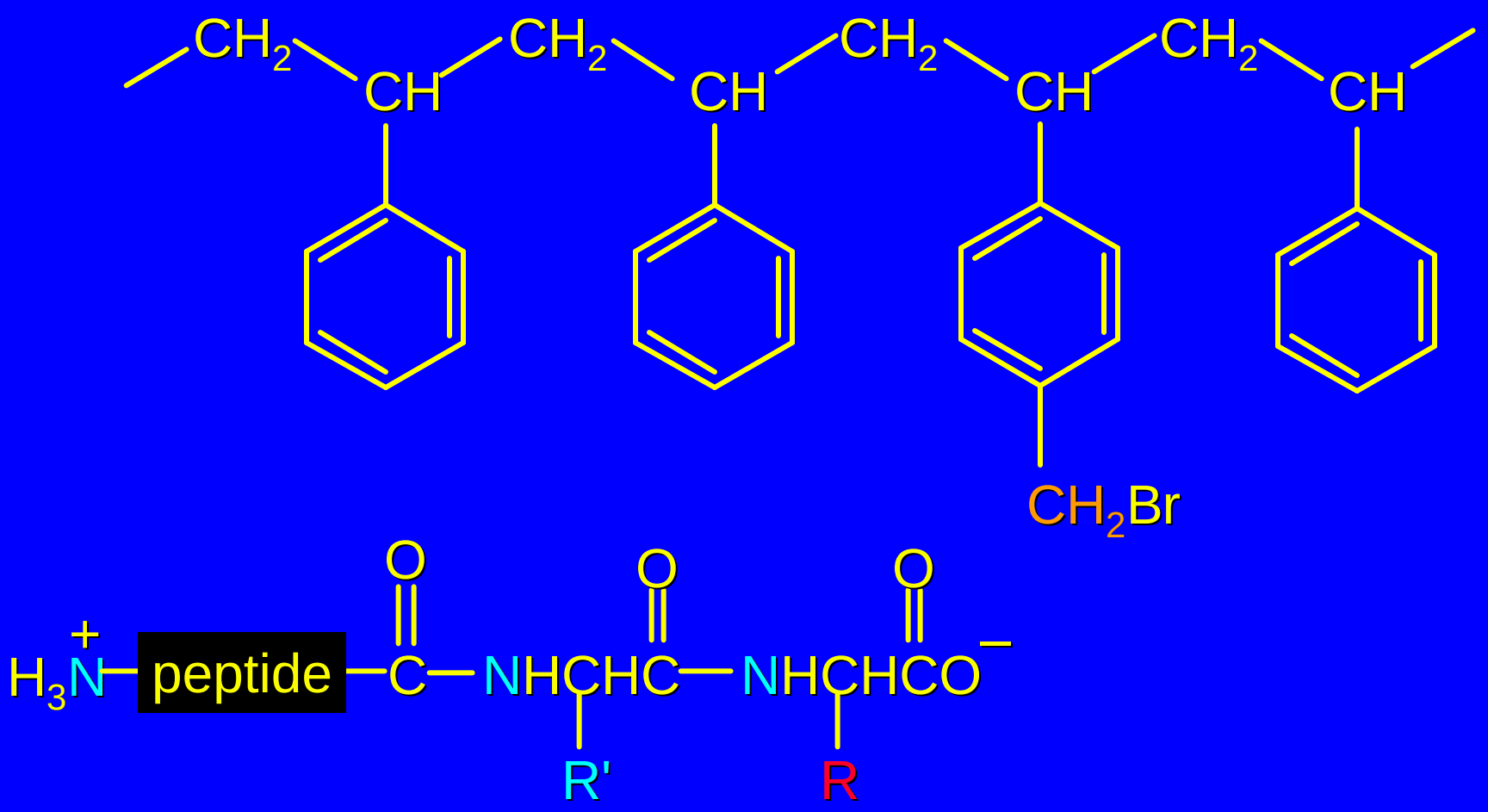
Add the next amino acid and repeat.

The Merrifield Procedure



Remove the peptide from the resin with HBr in CF_3CO_2H

The Merrifield Procedure



The Merrifield Method

Merrifield also automated his solid-phase method.

Synthesized a nonapeptide (bradykinin) in 1962 in 8 days in 68% yield.

Synthesized ribonuclease (124 amino acids) in 1969.

369 reactions; 11,391 steps

Nobel Prize in chemistry: 1984