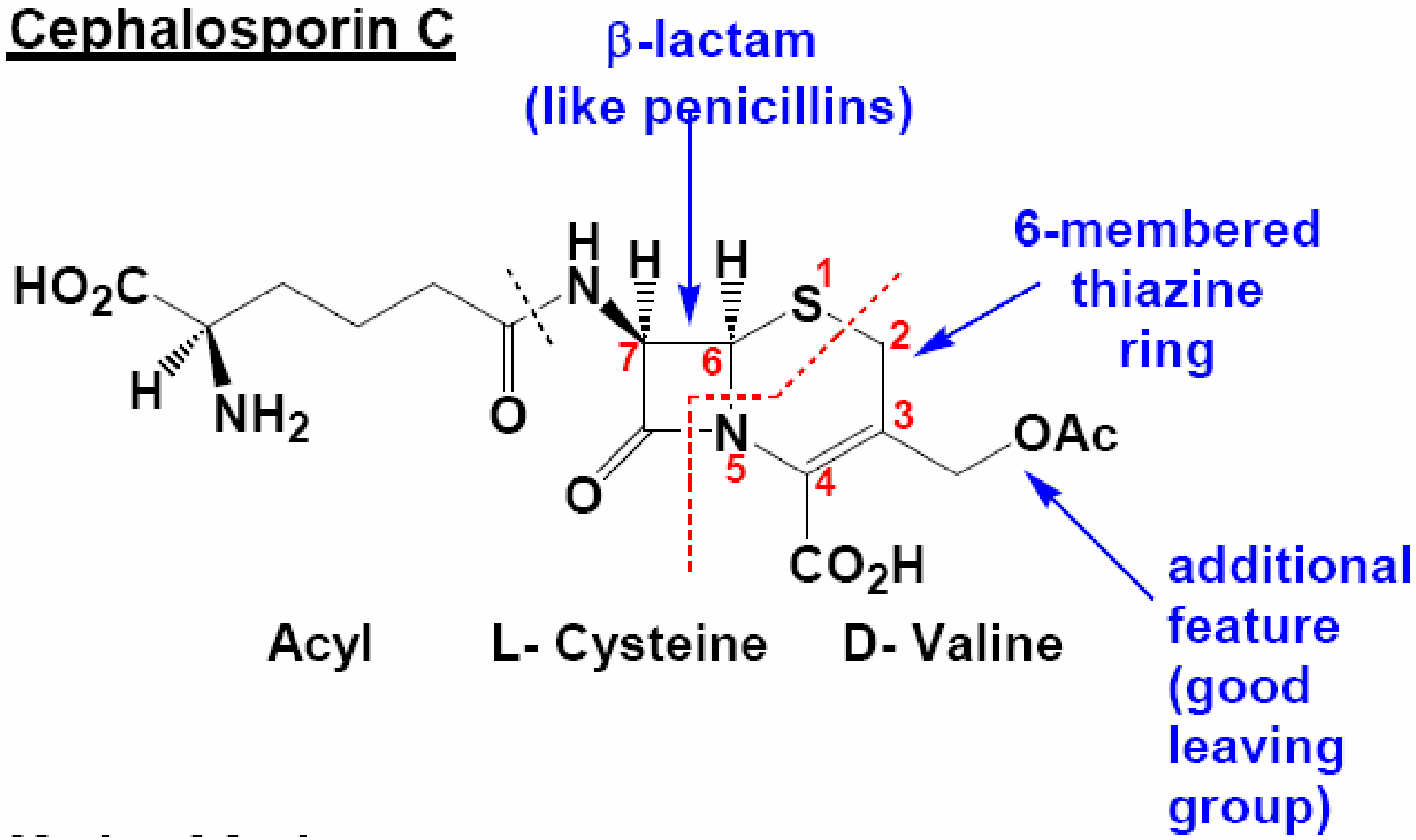
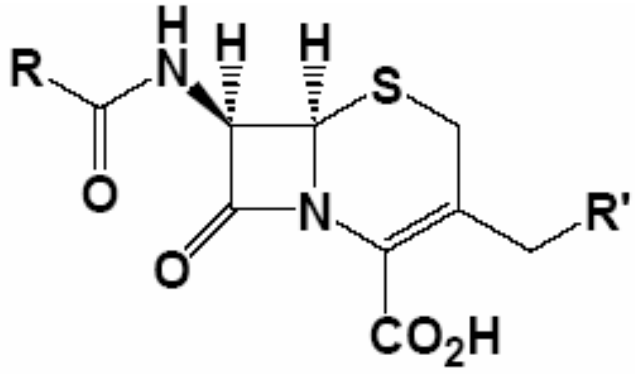
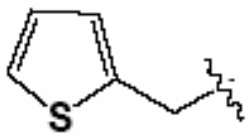
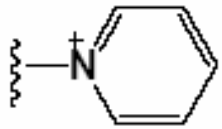
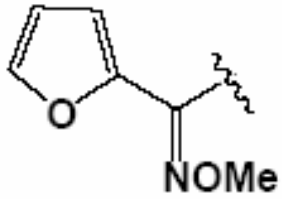
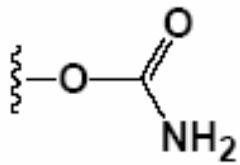
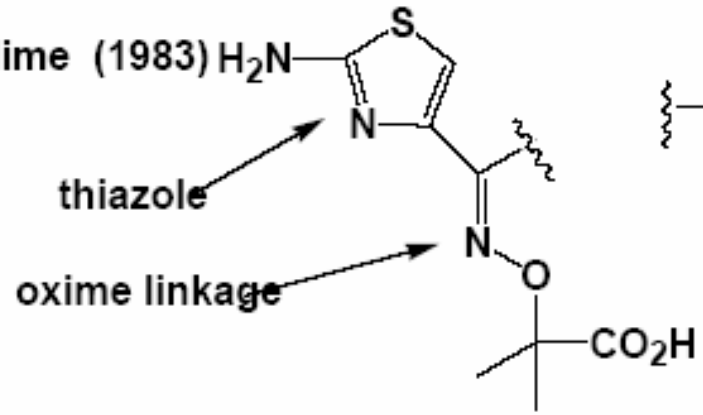
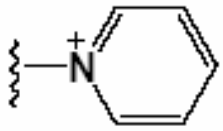
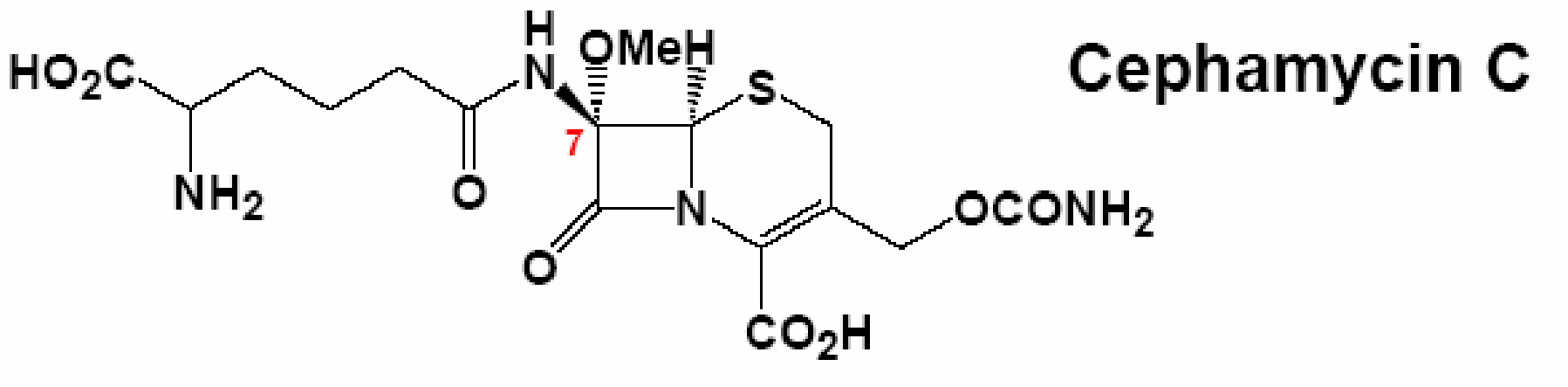
III. Sản xuất chất kháng sinh CEPHALOSPORIN

1. Giới thiệu chung



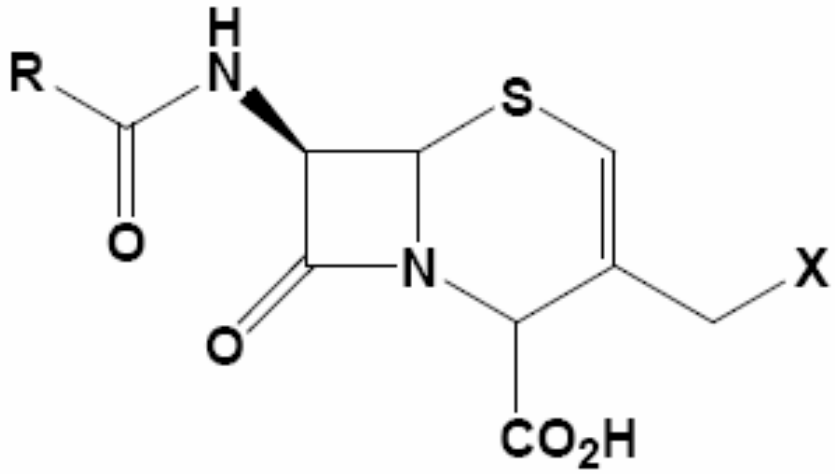


	R	R'	activity	β-lactamase resistance	*Pseudomonas* activity
cefaloidine (1966)			✓	✗	✗
cefuroxime (1977)			✓	✓	✗
ceftazidime (1983)			✓	✓	✓



Cephamycin C

- The presence of the conjugated double bond is also important



is not active

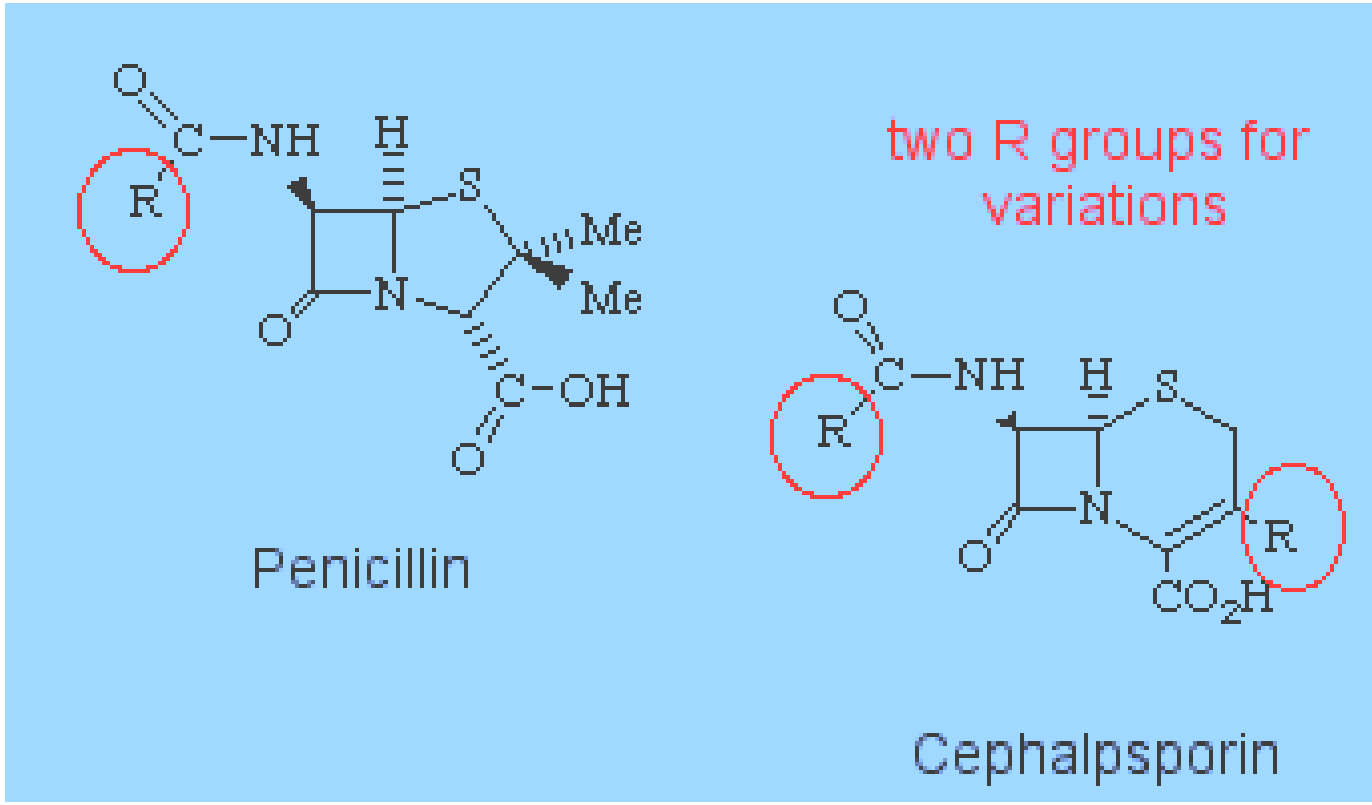
2. Phân loại Cephalosporin

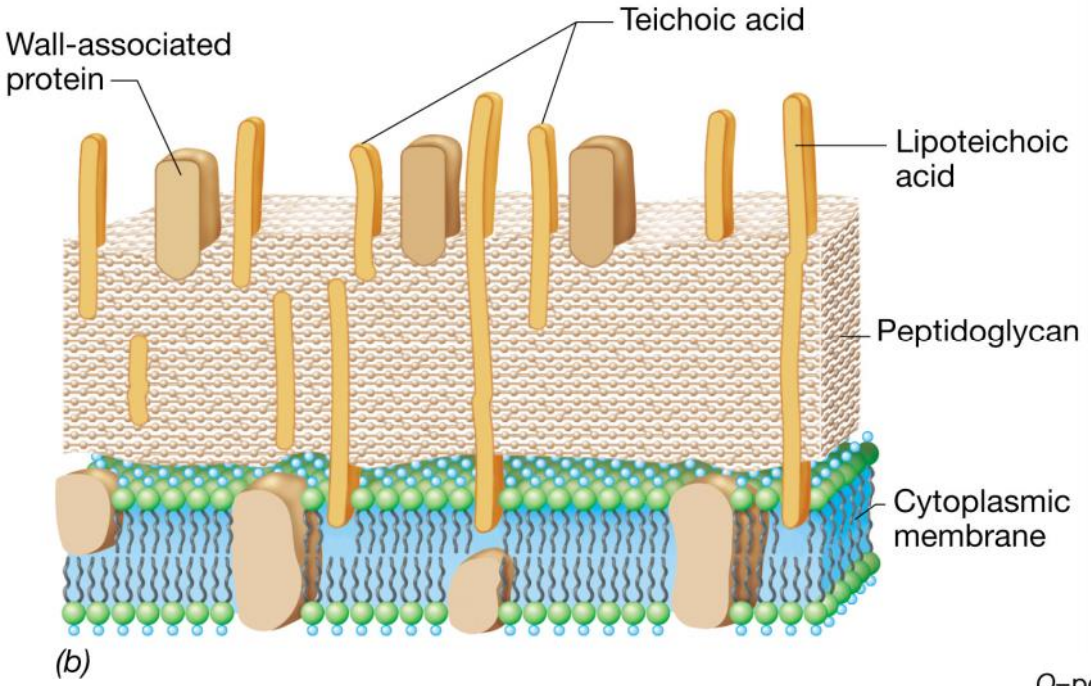
- Cephalosporin thế hệ 1
- Cephalosporin thế hệ 2
- Cephalosporin thế hệ 3
- “Cephalosporin thế hệ 4”

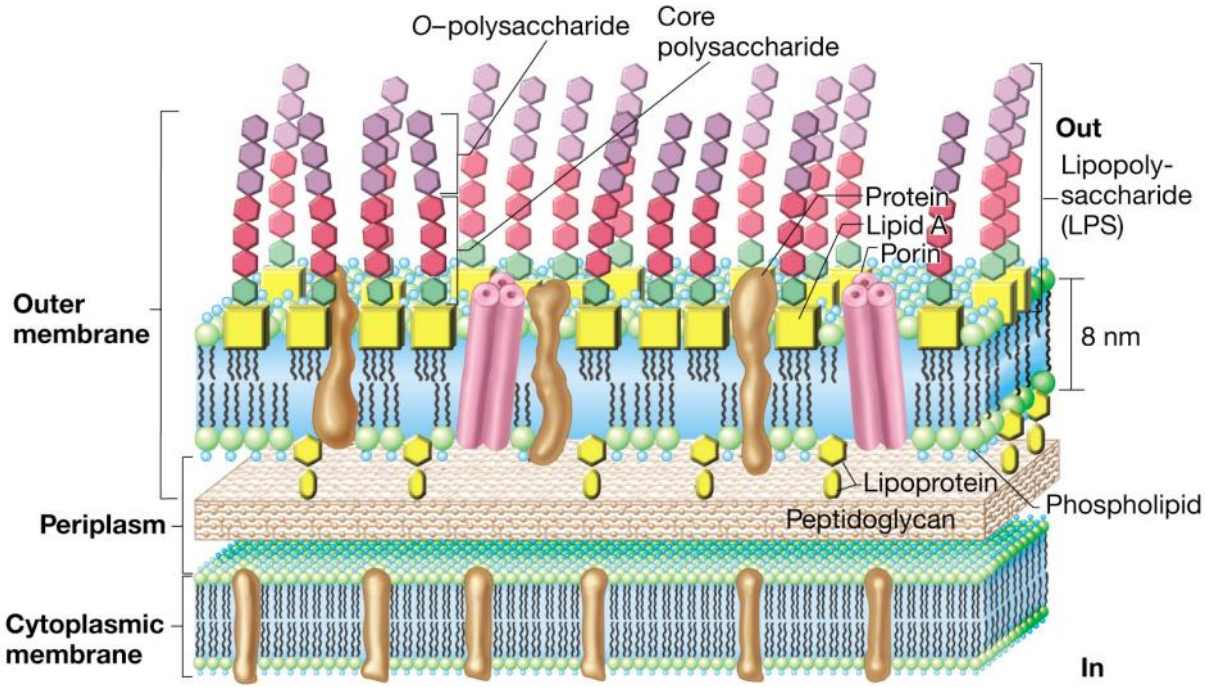
Classification	Spectrum
Ist generation Cefazolin (Ancef, kezol..) Cephalexin (Keflex etc) Cefadroxil (Duricef) Cephradine (Velosef)	**good against Gram (+); modest against Gram (-)** *Streptococci (except penn-resistant); Staphylococcus (except Methicillin-resistant strain)*
IInd generation Cefuroxime (Zinacef) Cefoxitin (Mefoxin) Cefprozil (Cefzil) Cefaclor (Ceclor) Cefuroxime acetil (Ceftin) Loracarbef (Lorabid) Cefotetan (Cefotan) Cefranide (Precef)	**Increased activity against Gram (-) but much less active than IIIrd generation** *Gram (-) e.g., Enterobacter sp, Klebsiella sp., haemophilus influenza; Not active against gram + as Ist generation*

IIIrd generation Cefotaxime (Claforan) Cefpodoxime proxetil (Vantin) Cefibuten (Cedax) Cefdinir (Omnicef) Cefditren pivoxil (Spectracef)	**Less active than Ist against Gram (+) but more active against** *Enterobactericeae including β-lactamase producing bacteria*
Ceftriaxone (Rochephin) Ceftizoxime (Cefizox) Cefoperazone (Cefobid) Ceftazidime (Fortaz)	*Active against Pseudomonas*
IV generation Cefepime (Maxipime)	*Extended spectrum of activity than IIIrd generation and have increased stability against hydrolysis by β-lactamase*

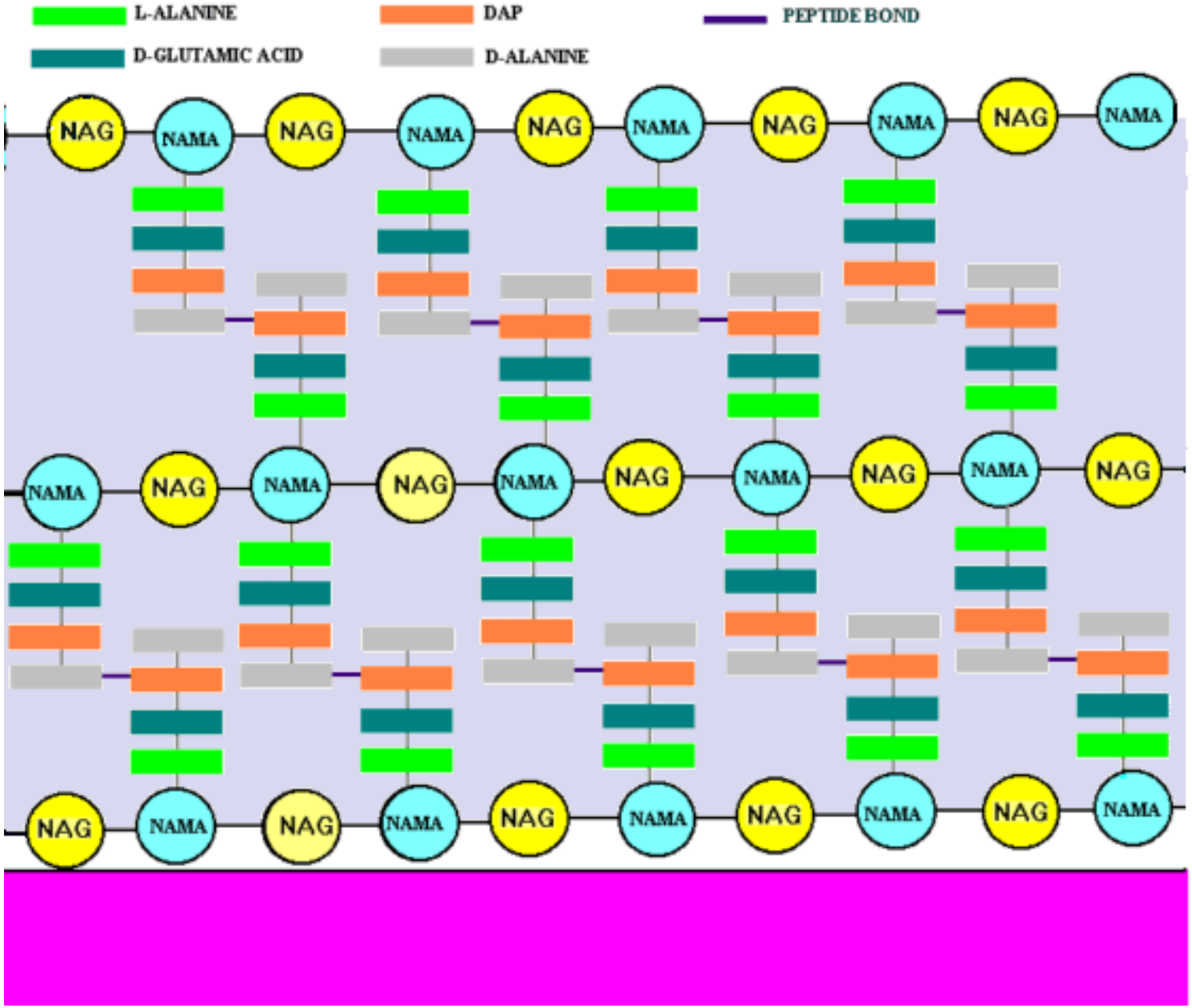
3. Cơ chế tác dụng

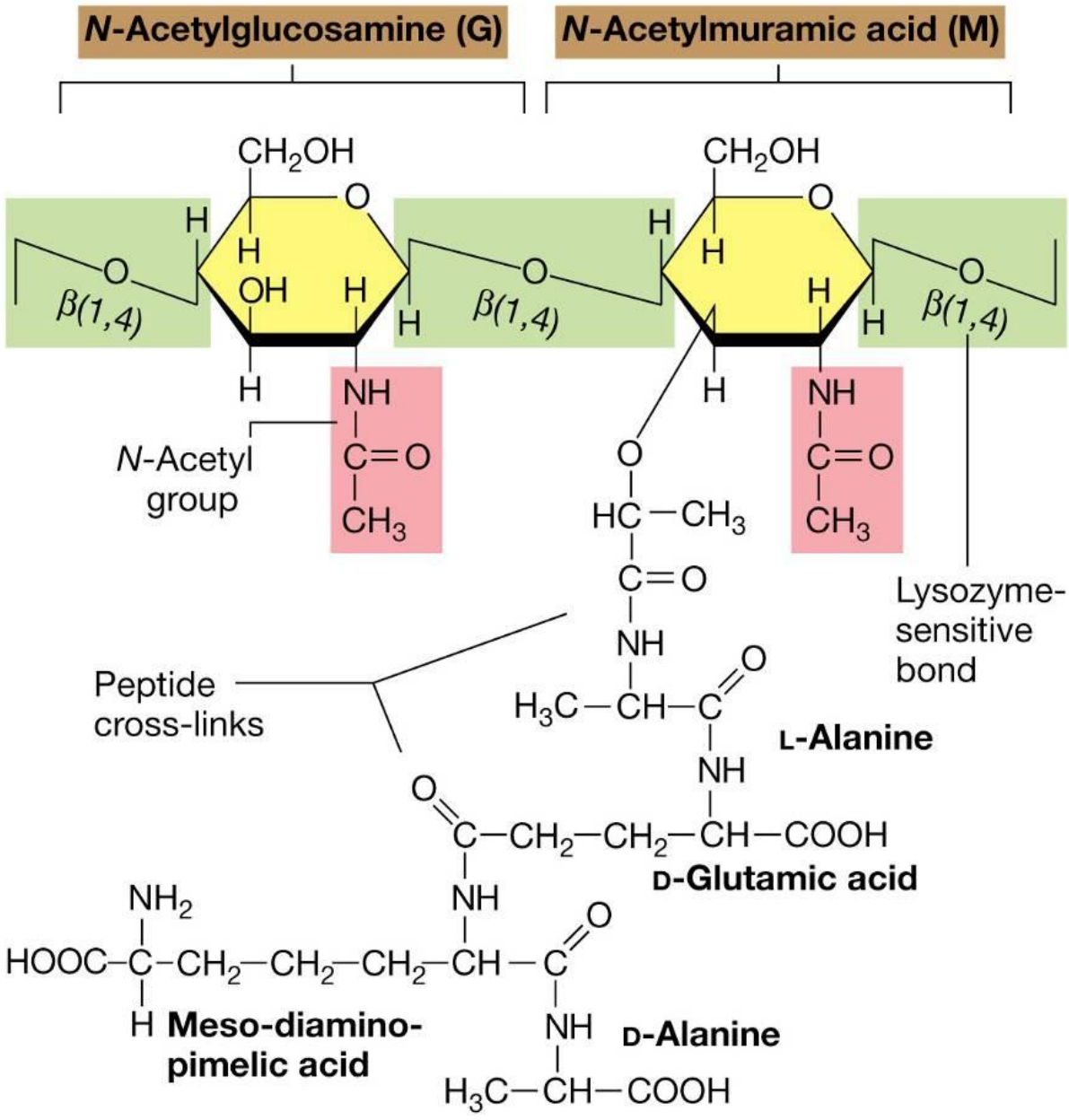




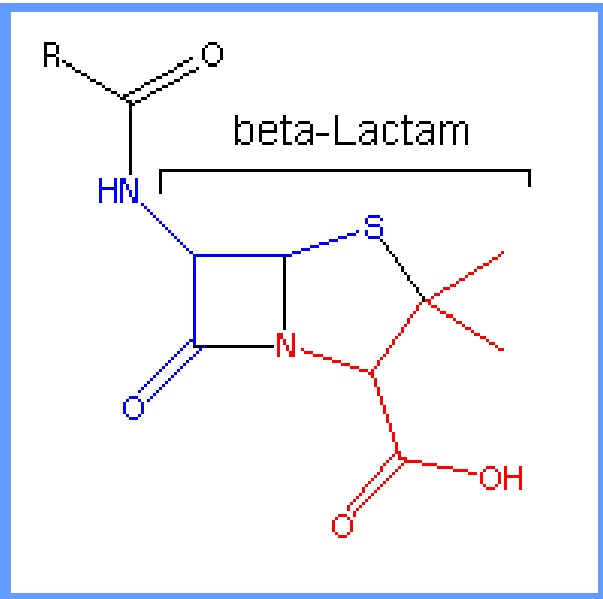


THE GRAM(+) CELL WALL



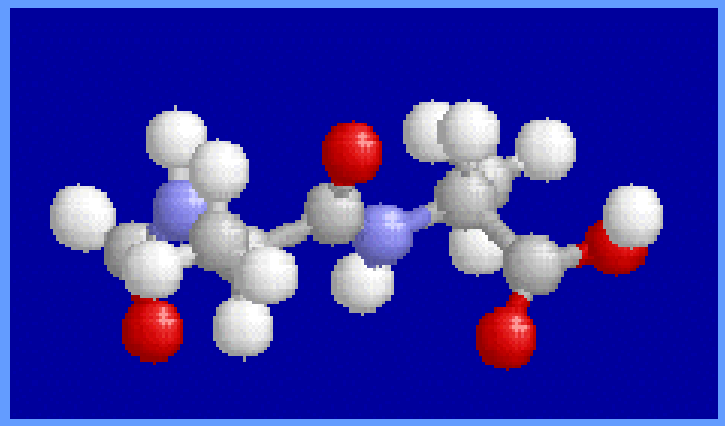


Similarity in structure of cell wall peptide and penicillin

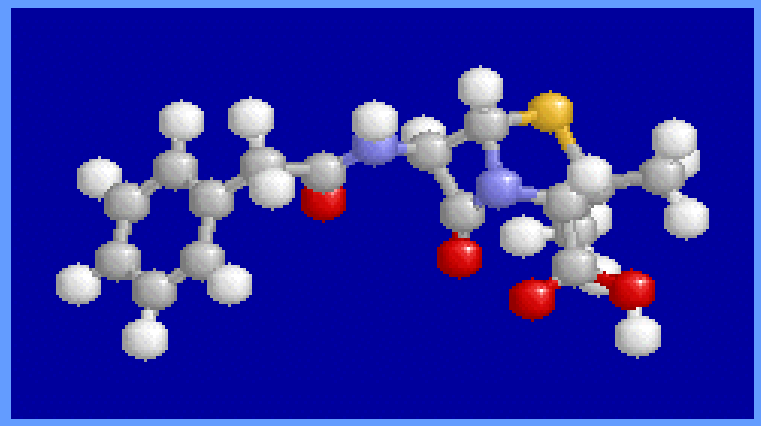


Trace red oxygen and blue nitrogen backbone

D-alanine-alanine



Penicillin

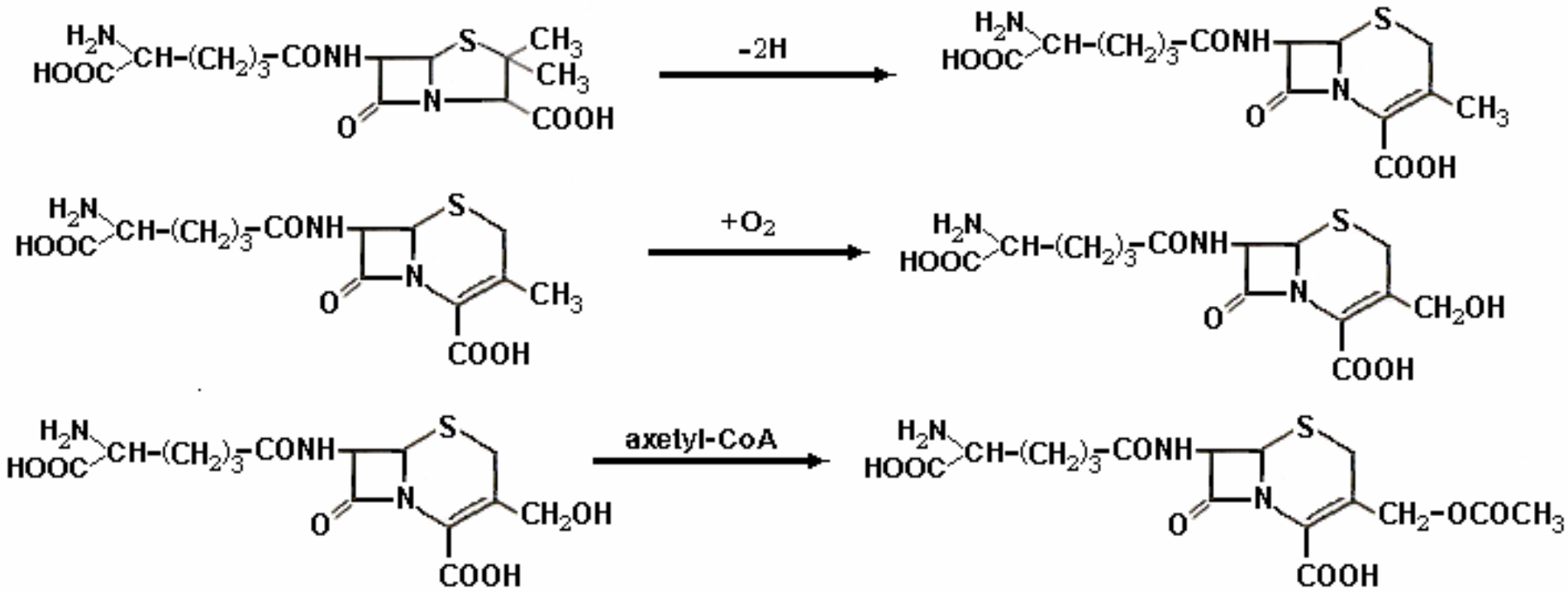


C. Ophardt, c. 2003

4. Sản xuất cephalosporin

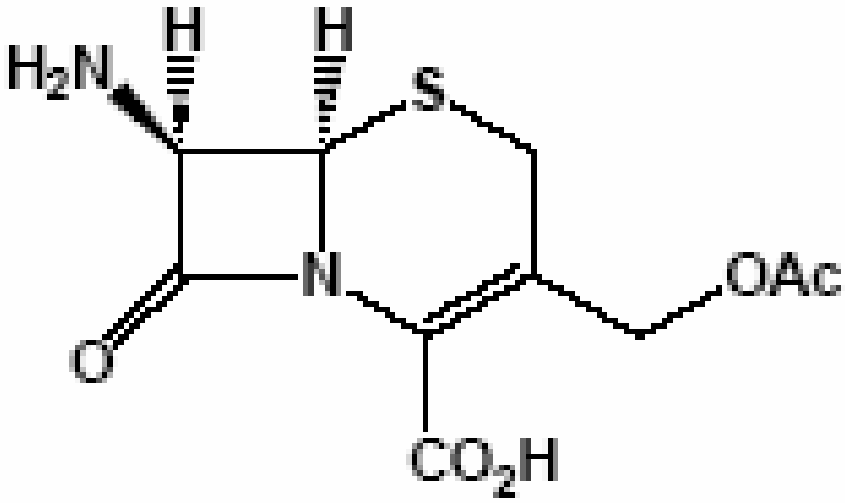
- Sinh tổng hợp
- Bán tổng hợp

Sinh tổng hợp từ *Cephalosporium acremonium*



Cephalosporin C được sx bởi *Paecilomyces persicinus* (Amato *et al*, 1976); *Steptomyces clavuligerus* (Aharonowitz and Demain, 1977); Acremonium chemostat (Karaffa *et al*, 1996)

Bán tổng hợp

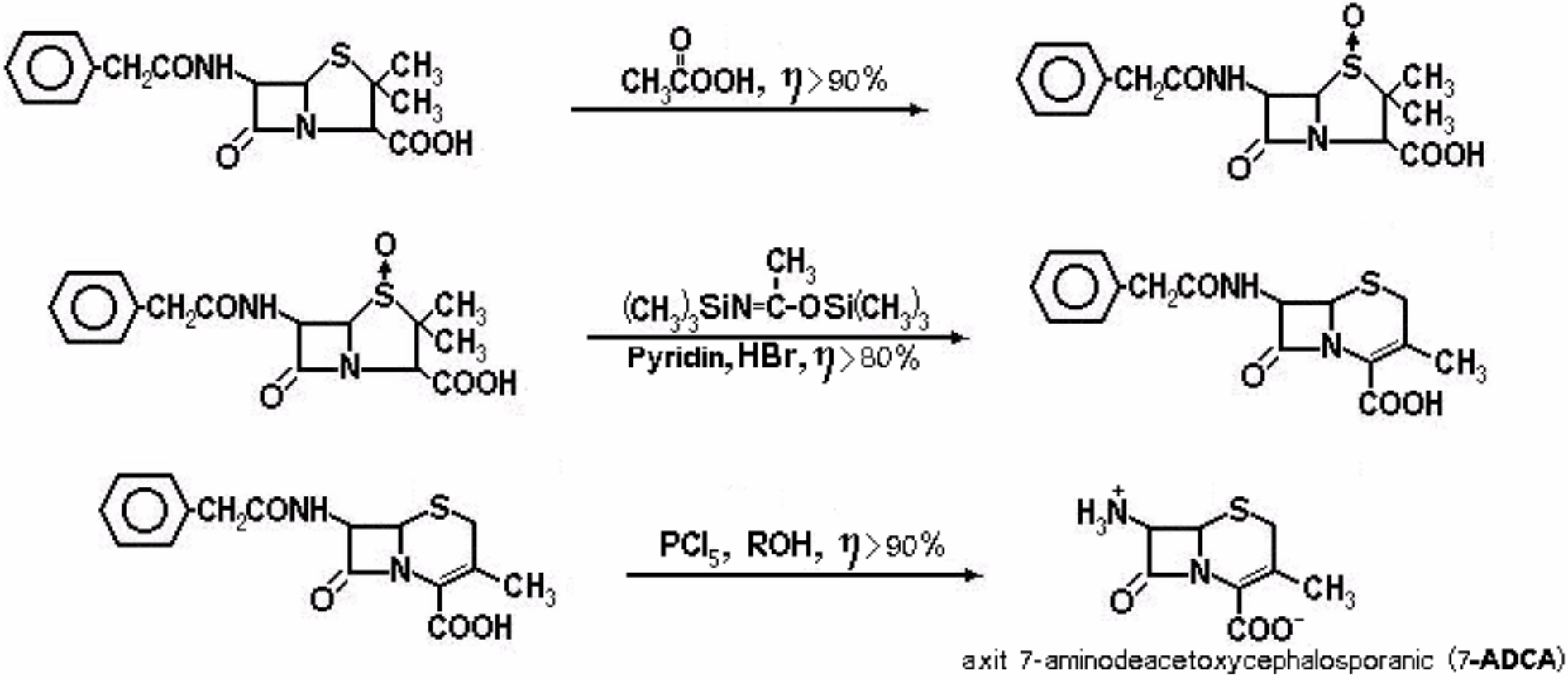


7-ACA

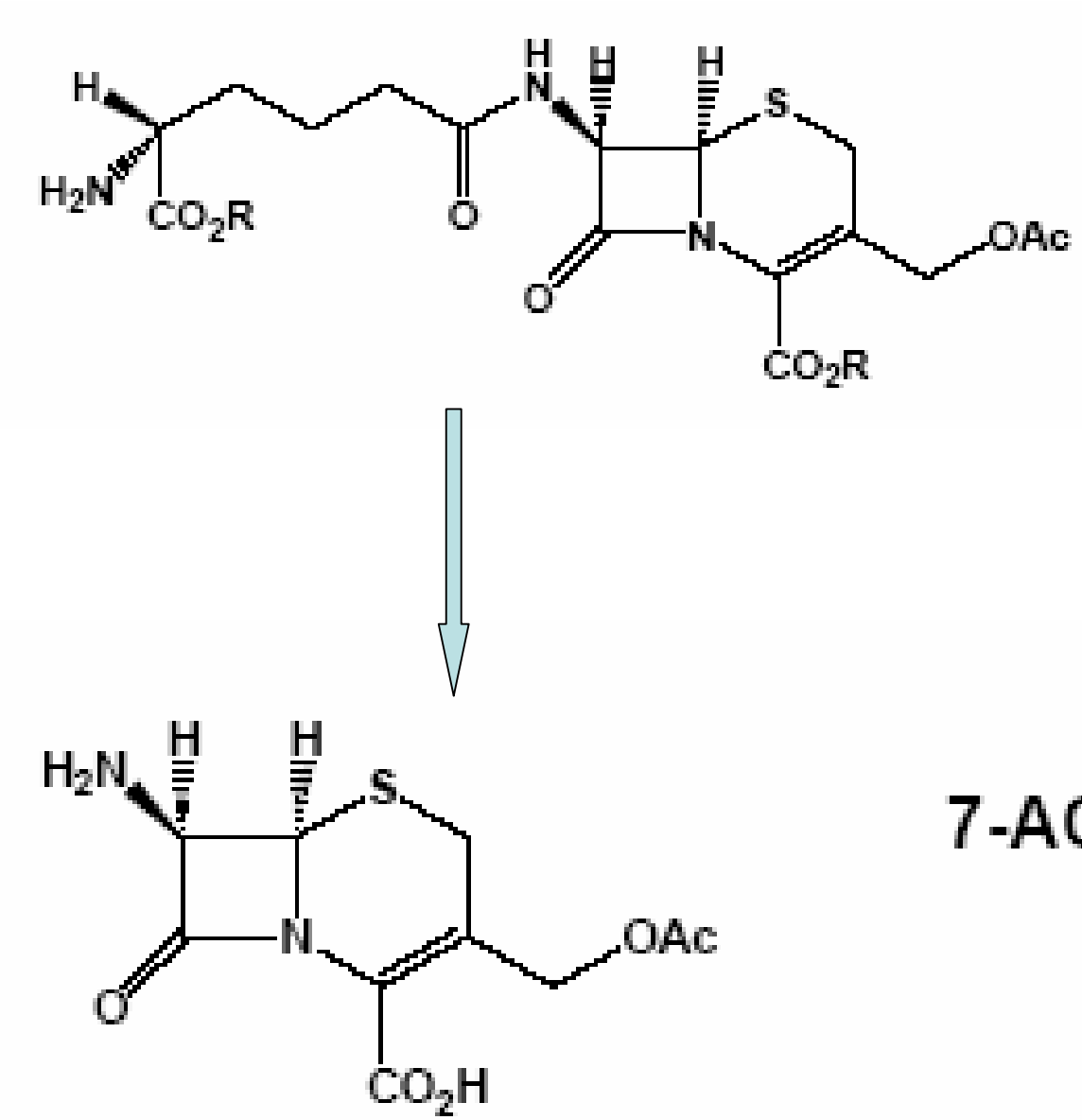
Bán tổng hợp

- **1. Từ penicilin**

- **2. Từ Cephalosporin C**

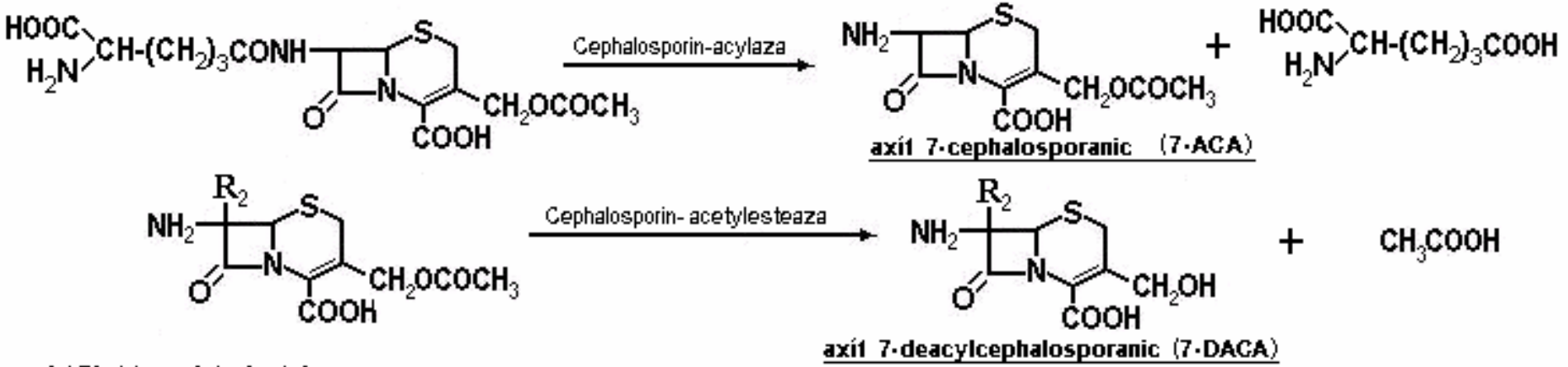


axit 7-aminodeacetoxycephalosporanic (7-**ADCA**)

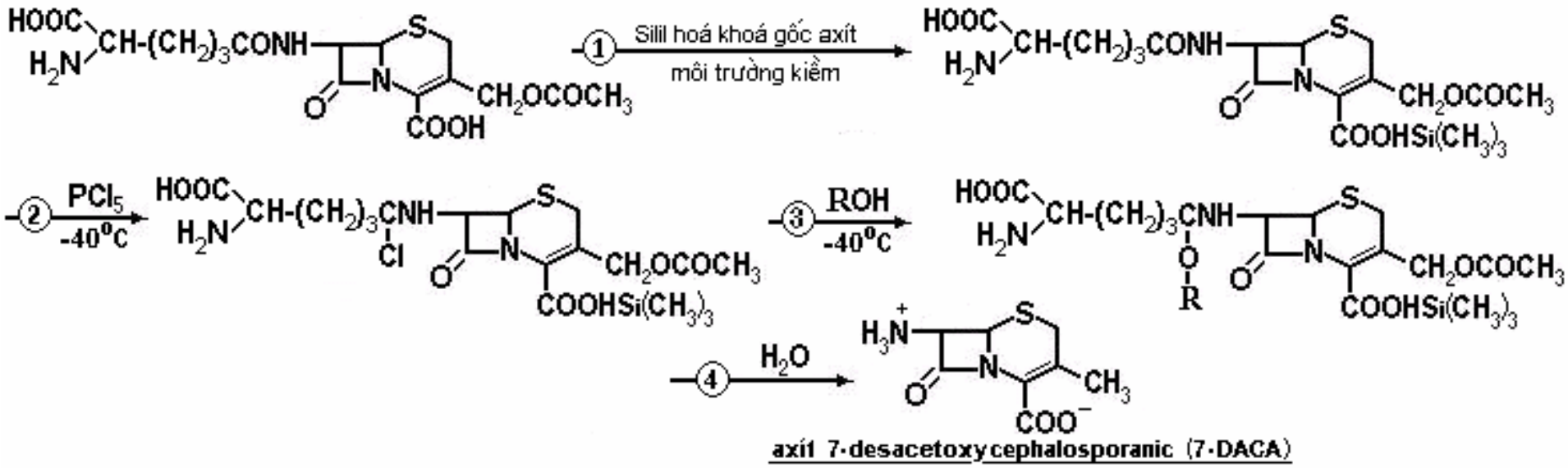


7-ACA

a/ Phương pháp enzym



b/ Phương pháp hoá học

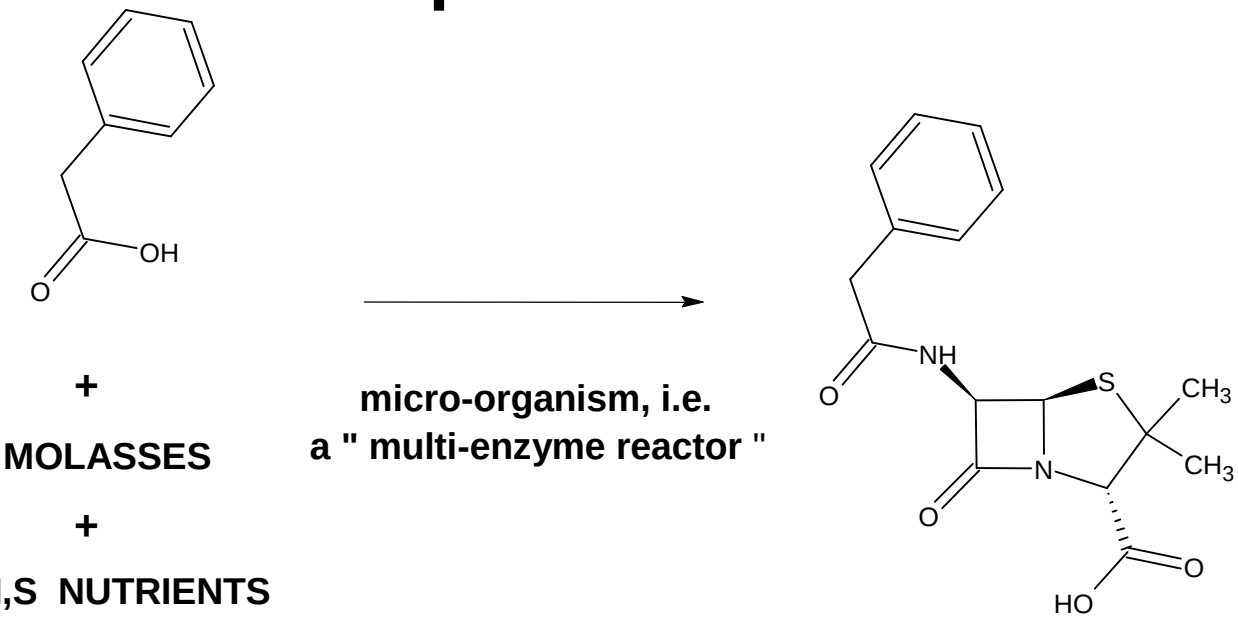


7-ADCA. The old process

- In the old process the penicillin nucleus is obtained via fermentation.
- Six chemical transformations were necessary to selectively convert peniccilin G into 7-amino-desacetoxy-cephalosporic acid.

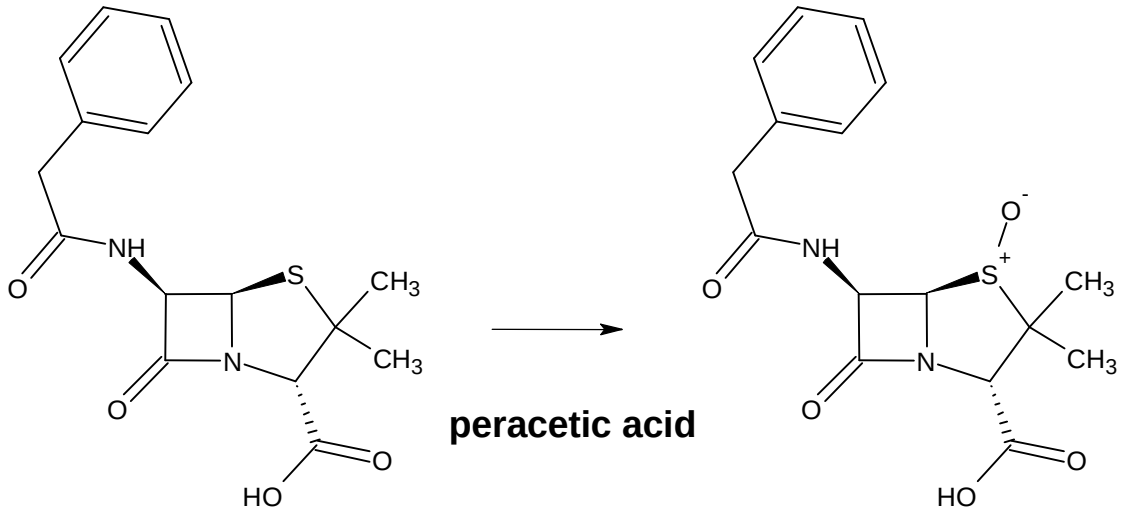
1st generation 7-ADCA proces: step 1

1970-1990



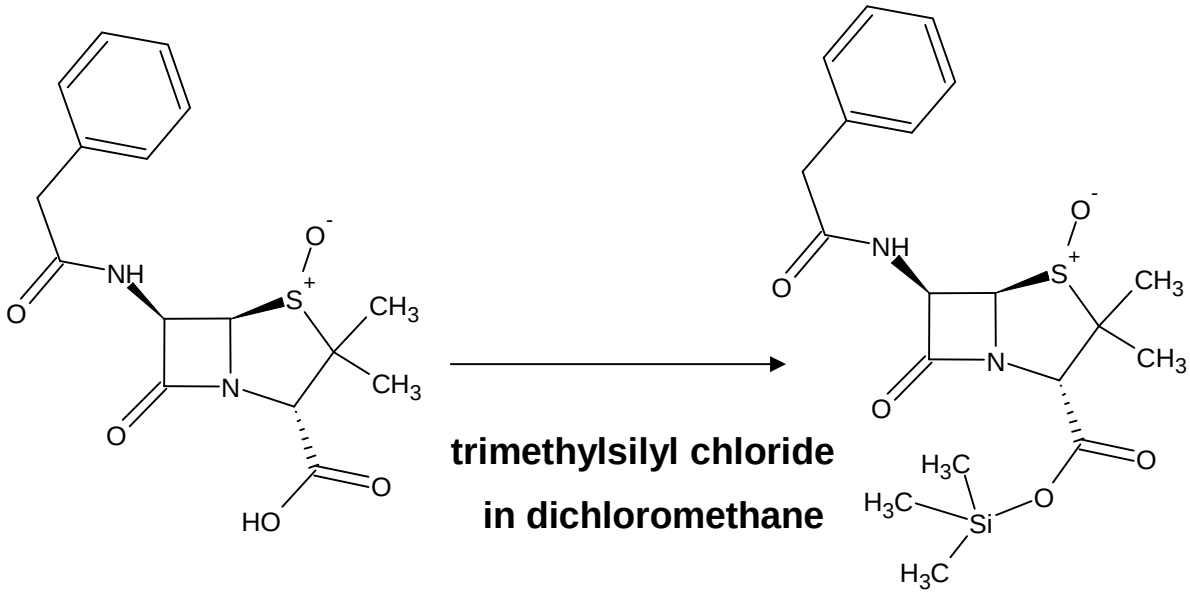
Penicillin G is produced by fermentation in the presence of phenylacetic acid (This side-chain makes isolation easier than the natural side-chain)

Step 2. Oxidation of sulfur



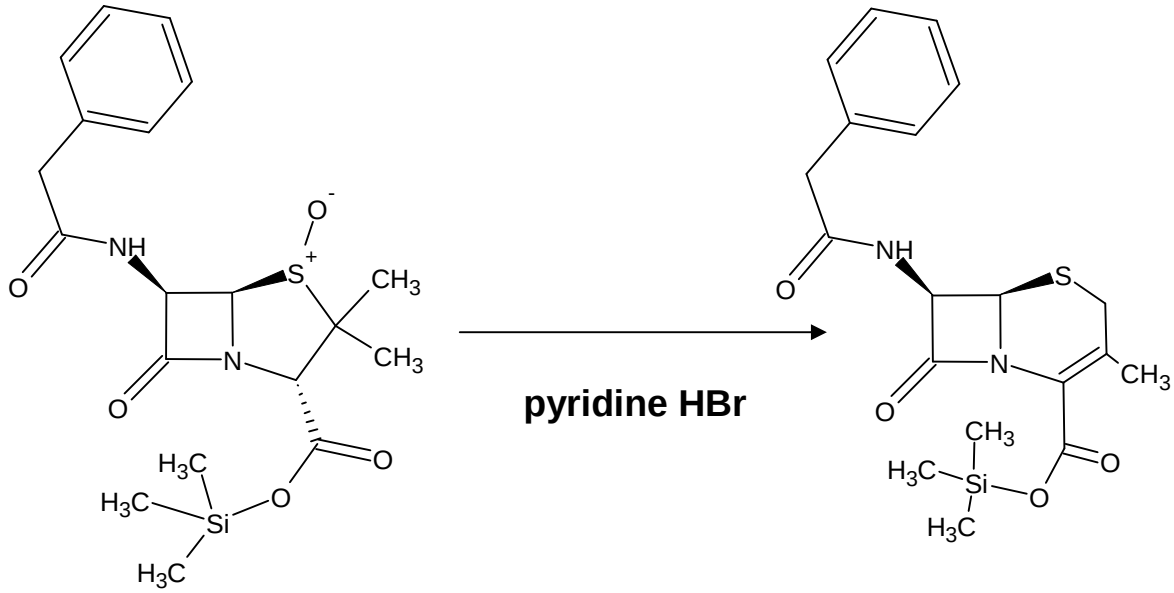
Oxidation of sulfur with peracetic acid to create the sulfoxide. This is necessary to create a leaving group.

Step 3. Acid protection



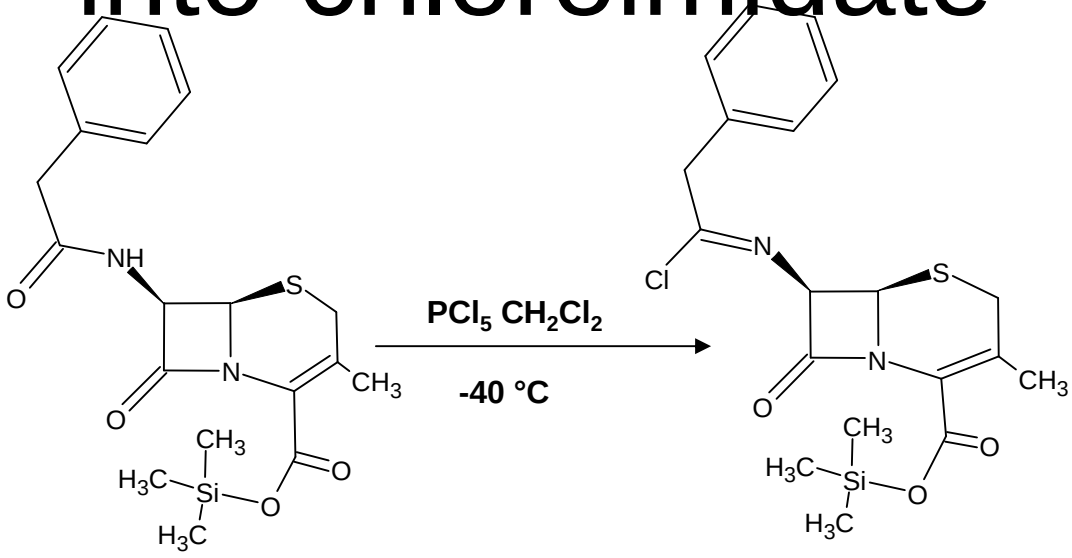
To prevent intereference of the acidic group in the next chemistry it is protected as a trimethylsilyl ester.
Treatment with water will restore the acid group.

Step 4. Ring expansion



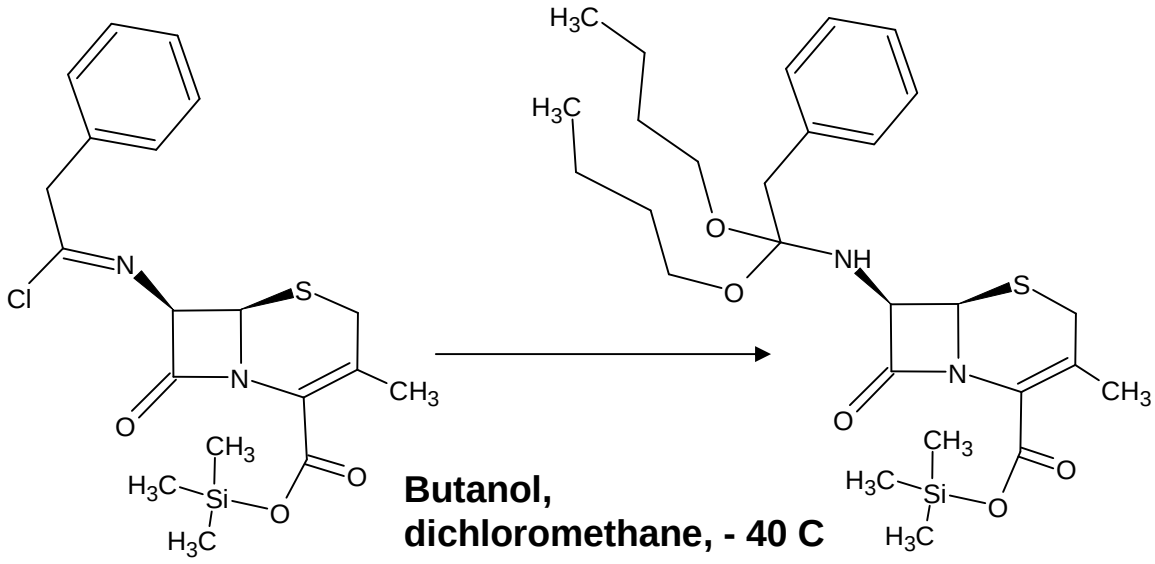
The rearrangment of the sulfoxide to the ring-expanded product is catalysed by a very mild acid: pyridinium.HBr salt.
The catalyst ends up in the waste stream.

Step 5. Conversion of amide into chloroimidate



To split off the amide group a selective reaction is needed that can discriminate between the amide group and the beta-lactam (a cyclic amide) group.

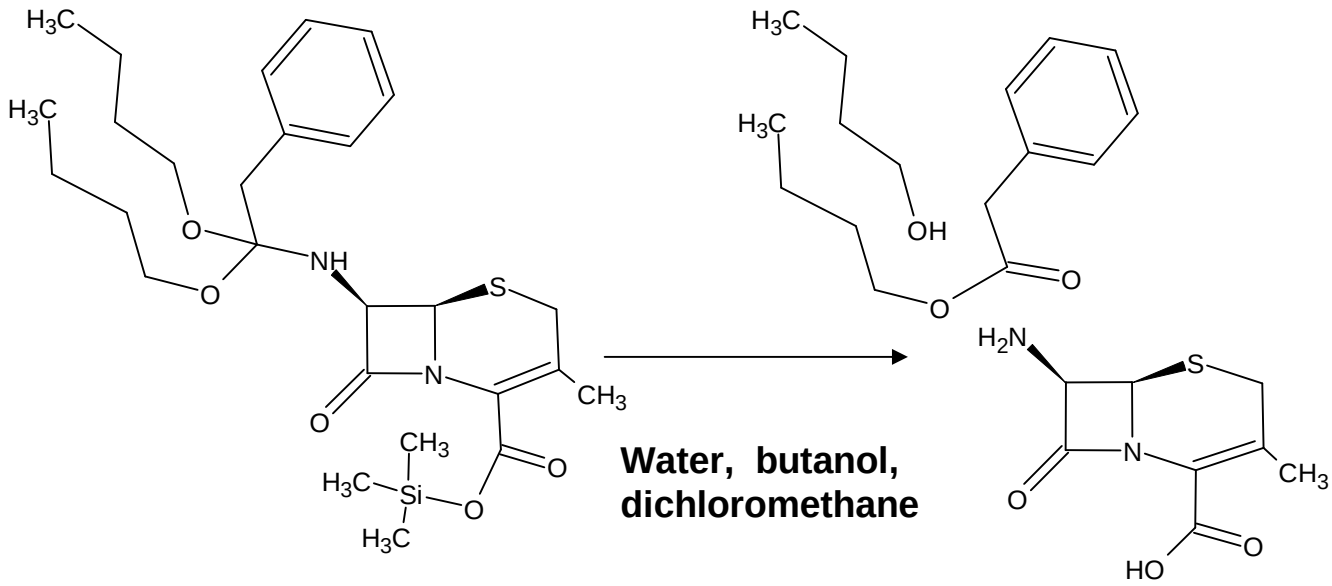
Stap 6. Reaction with BuOH



Butanol, dichloromethane, - 40 C

It is still not possible to hydrolyse the chloro-imidate selectively. It needs to be converted into a highly labile ortho ester derivative which can be hydrolysed at low temperature (-40 °C).

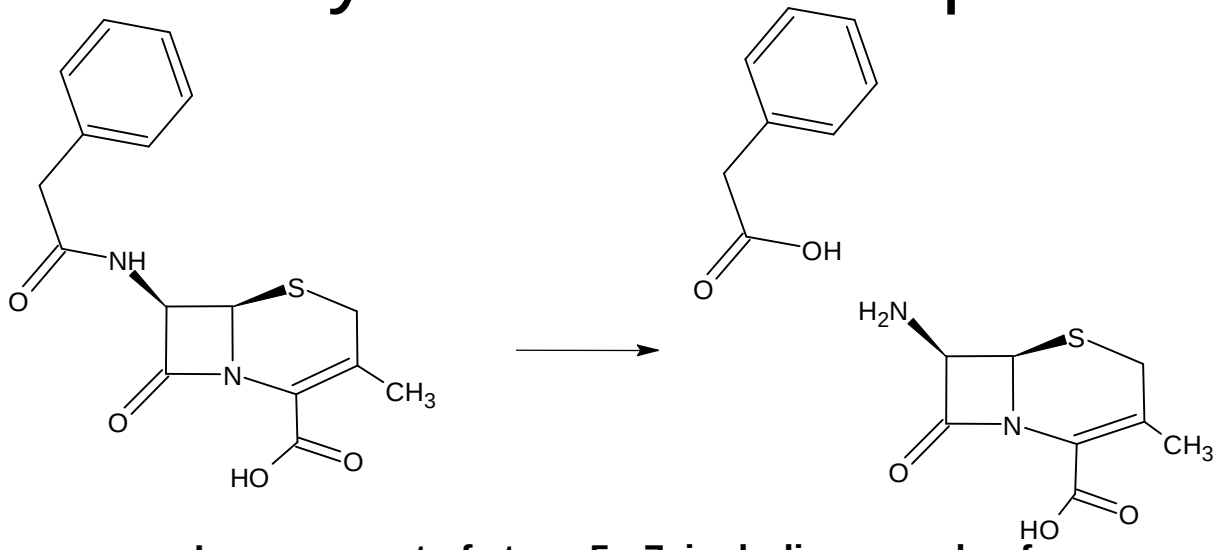
Step 7. Hydrolysis



- **Addition of water hydrolyses both the ortho-ester, as well as the trimethylsilyester.**
- **Phenylacetic acid, butanol and the trimethylsilyl rest go to waste.**

2nd Generation 7-ADCA process: Use of an enzyme saves 2 steps

1990-2000

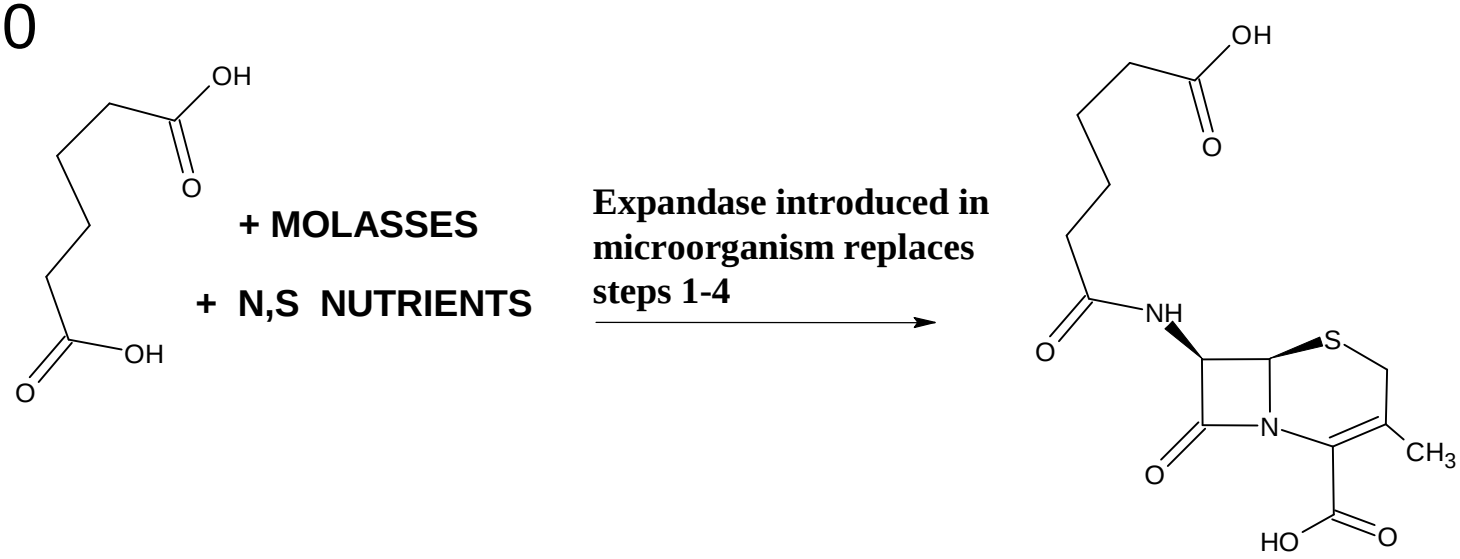


Improvement of steps 5 - 7, including recycle of phenylacetic acid for Pen-G fermentation:
in water; immobilized enzyme (acylase)

- **Use of a hydrolytic enzyme immediately generates 7-ADCA.**
- **An additional advantage is that the phenylacetic acid can now be recycled.**

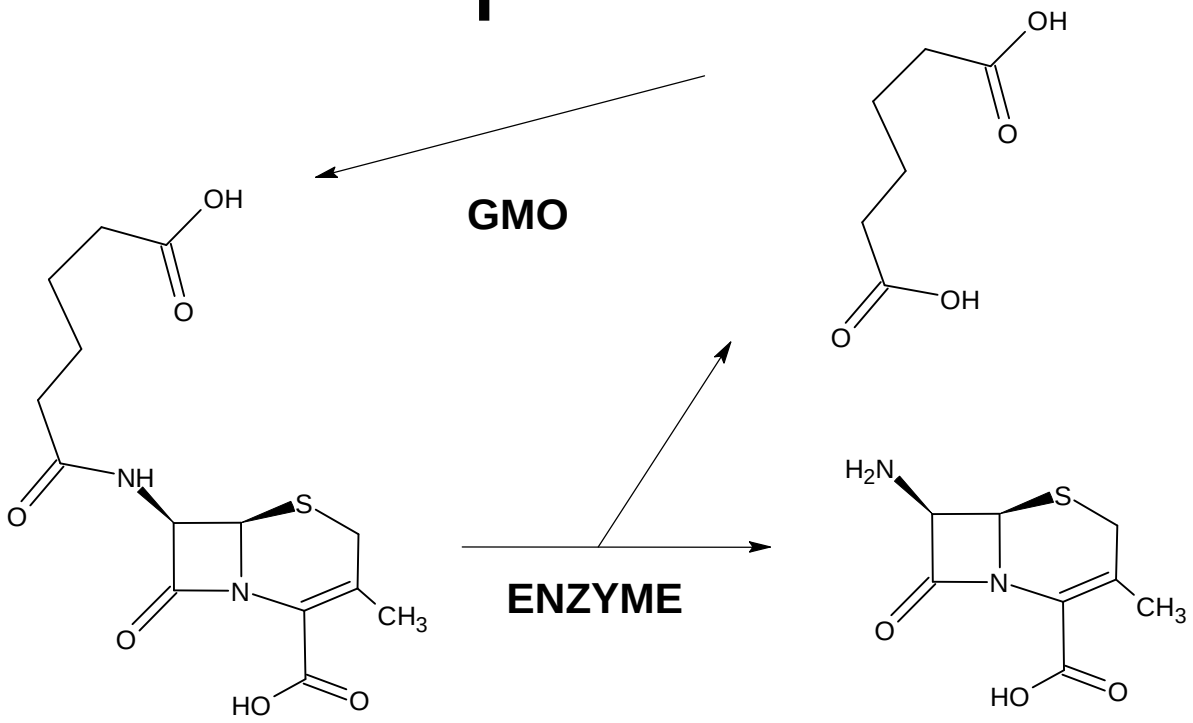
3e Generation 7-ADCA proces: Use of a GMO avoids 3 more steps

>2000



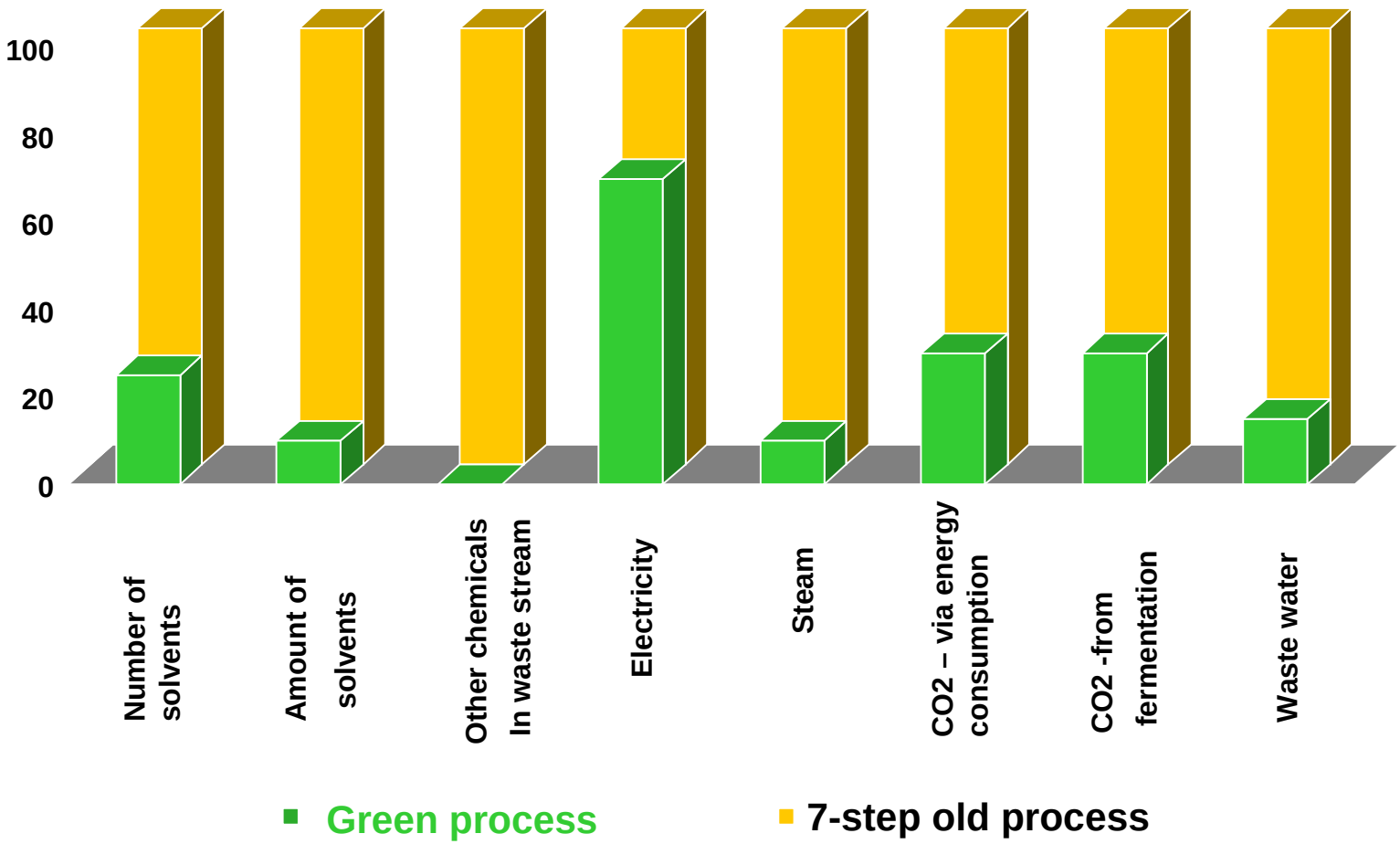
- **Fermentation using a genetically modified organism (GMO) leads to the immediate formation of N-adipyl 7-ADCA.**
- **The genome of the penicillin strain has been supplemented with the gene for an "expandase" enzyme. This enzyme fulfills the role of chemical steps 2 and 4.**

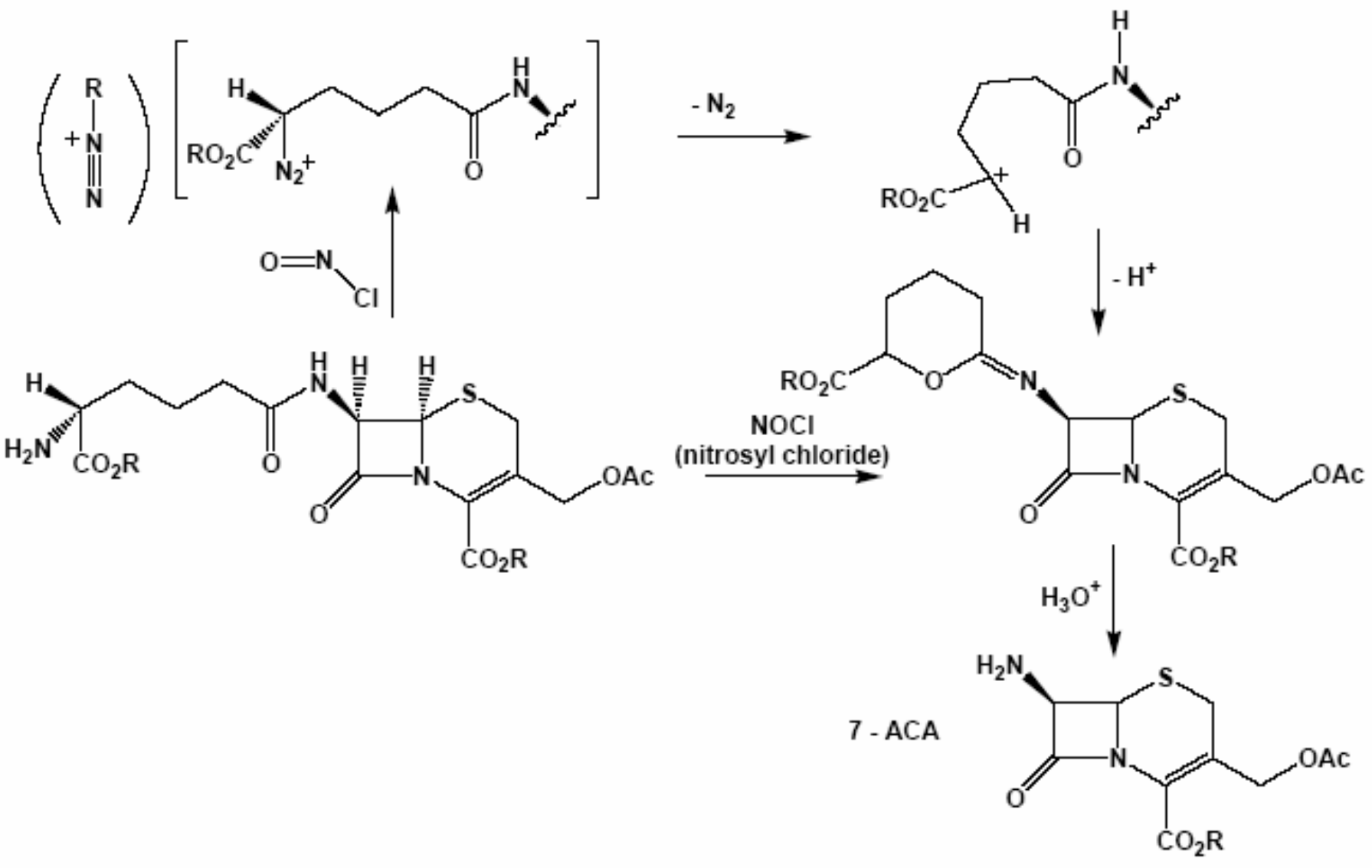
Overview > 2000 7-ADCA proces



- **Only 2 steps in water as solvent!**
- **Adipic acid can be recycled.**
- **No large chemical waste streams.**
- **New process is both more economic as well as environmentally friendly.**

Green 7-ADCA process





Ceftazidime