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SOME ASPECTS IN THE INDUSTRIAL SYNTHESIS OF β – LACTAM ANTIBIOTICS

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ABSTRACT

The recent discoveries of numerous significant new β – lactam antibiotics produced by fermentation, synthesis or semisynthesis have a fascinating and increasing role in antimicrobial chemotherapy. Cephalosporins and penicillins constitute about 63% of the total market in world-wide sales of antibiotics and will further predominate. The development in the field of β – lactam antibiotics is characterized by the fact that, despite the discovery and the structure elucidation of thousands of new antibiotics, only relatively few basic structures are to be found among the clinically important antibiotics. The variety of these β – lactam antibiotics includes penicillins, four generations of cephalosporins, especially aminothiazole cephalosporins, cephamycins and a number of nontraditional β - lactam antibiotics like 1-oxacephalosporins, penems, carbapenems, nocardicins, monobactams and irreversible inhibitors of β – lactamases.
Research and development work on the semisynthesis of β – lactam antibiotics is one of the most essential directions of KRKA's development. Industrial by important and known β – lactams were selected and synthesis and independent patents were elaborated. Own processes for synthesis of ampicillin, amoxycillin, talampicillin, flucloxacillin, piperacillin, cephalexin, cephamandole and cefotaxime were made in industrial scale. In this approach to the independent synthesis of acylation of 6-APA, 7-ACA and 7-ADCA by use of reactive intermediates of S-thiocarboxylic acid, 4-phenylthiazolidine-2,5-dione, or mixed thioanhydrides, were introduced and developed mostly in semiindustrial and industrial scale.
In KRKA's fundamental research program, a variety of new β – lactam antibiotics have been prepared, more specifically the "thiazolidine type" semisynthetic penicillins and cephalosporins, thiazolidine acylaminopenicillins and substituted phenylcephalosporins. The new β – lactam antibiotics were tested for their antibacterial, antifungal and antiviral activities.

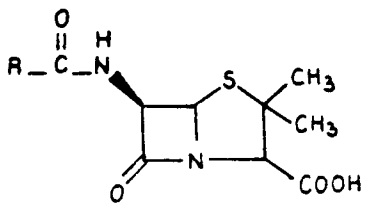
INTRODUCTION

The novelty of the β – lactam structure, the ensemble of diverse reactions and rearrangement of β – lactam antibiotics, the challenge posed by resistance in several susceptible, and the consequent need for improving the therapeutic value of available β – lactam antibiotics, have together been responsible for the continuing interest in this class of compounds.

During the last decades a variety of new β – lactams from natural sources has rekindled a great explosion in research work on organic synthesis and technology in order to find new (semi)synthetic β – lactam antibiotics with improved therapeutic values in medicinal chemotherapy of microbial infections (1-10). The basic structures of β – lactam antibiotics are shown in **Fig.1**. Penicillins and cephalosporins with penam (**1**) or cephem (**2**) structures are regarded as "classical" β – lactams. Carbapenems (**4**), penems (**8**), 1-oxacephalosporins (**3**), irreversible inhibitors of β – lactamases of clavam type (**5**), monocyclic β – lactam antibiotics with monolactam (**6**), nocardicin (**7**) structures, lactavicin (**9**), homocephems (**10**) are the most important "non-classical" β – lactams. The new β – lactams in the future will be possibly prepared by the semisynthesis from lactivicin, homocephems and pyrazolidinones.

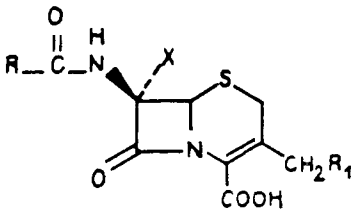
The medicinal use of classical penicillins (narrow-spectrum, broad-spectrum penicillins, carboxy penicillins, ureido penicillins), oral cephalosporins and all four generations of parenteral cephalosporins has very important growth and represents more than 65% of the total market in world-wide sales of antibiotics (estimated value 12 bill. US dollars).

The major international pharmaceutical companies have a patent monopoly, with extremely high profits in the whole food of industrial production of semisyn-



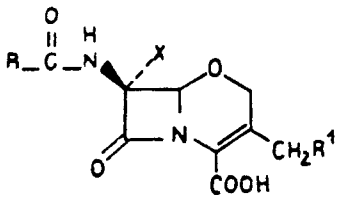
PENAM

1



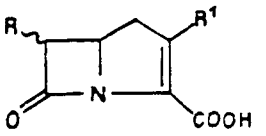
CEPHEM

2



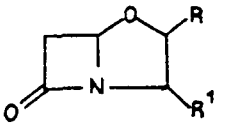
OXACEPHEM

3



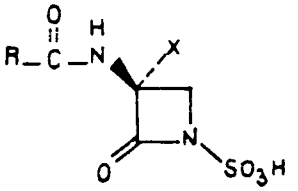
CARBAPENEM

4



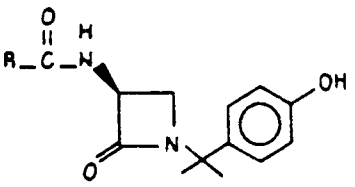
CLAVAM

5



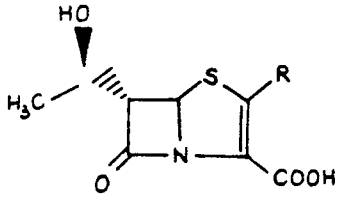
MONOLACTAM

6



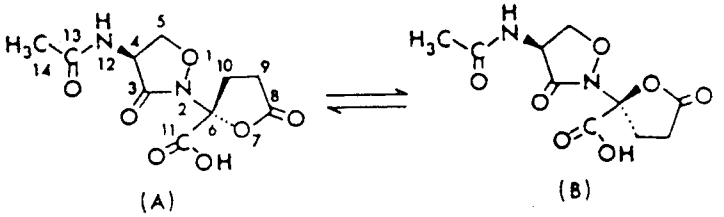
NOCARDICIN

7



PENEM

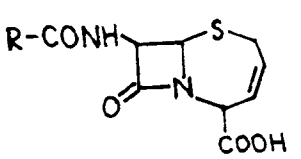
8



(A) (B)

LACTIVICIN

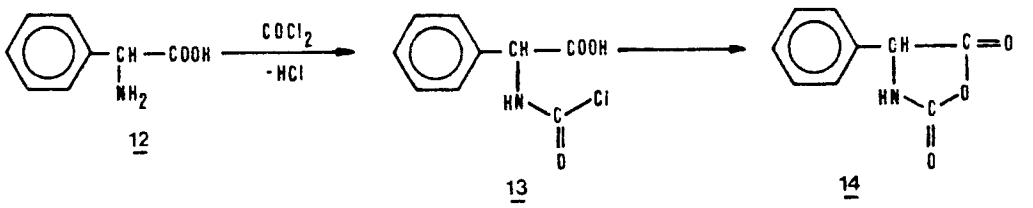
9



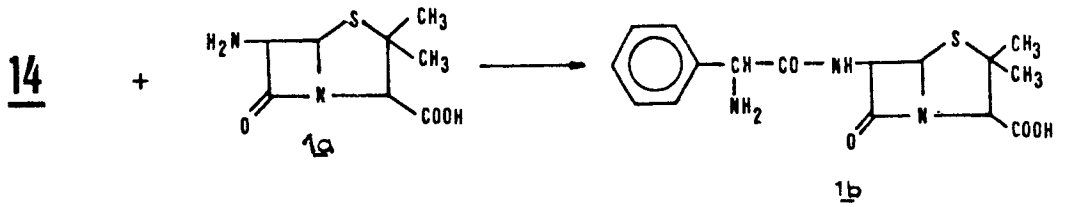
HOMOCEPHEMS

10

Fig 1. The basic structure of β – lactam antibiotics



Scheme 1



Scheme 2

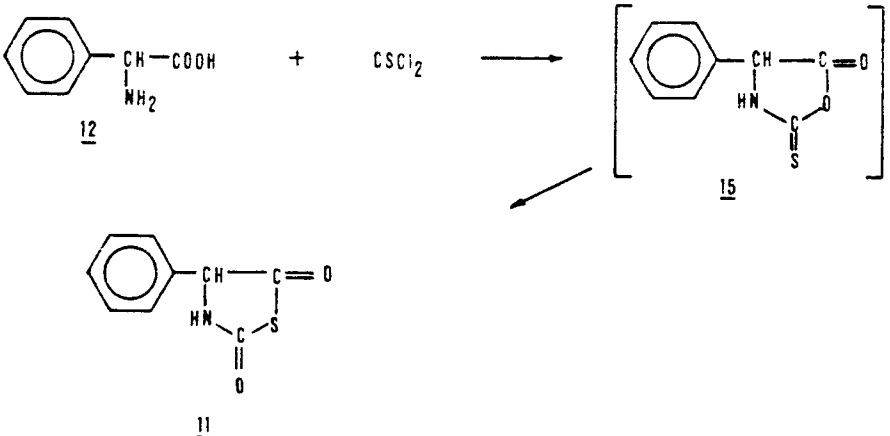
thetic penicillins and cephalosporins. For such reasons, it is the duty of Yugoslav chemists to develop industrial production of naturally occuring and semisynthetic penicillins and cephalosporins on the basis of their own and independent processes protected with patents and patent applications. Yugoslav pharmaceutical industry (Galenika, Pliva and Krka) has introduced the important part of the production of β – lactam antibiotics into industrial scale.

In KRKA special attention was given to research and development in industrially usable chemical and enzymatic transformations of natural penicillins and cephalosporins and further synthesis of semisynthetic β – lactams. The new and independent synthesis of ampicillin, amoxycillin, izoxazolyl penicillins, carbenicillin, piperacillin and cefotaxime were developed on the base of the new reaction pathways of the acylation of 6-aminopenicillinic acid (6-APA), 7-aminodesacetoxycephalosporanic acid (7-ADCA), 7-aminocephalosporanic acid (7-ACA) and ampicillin.

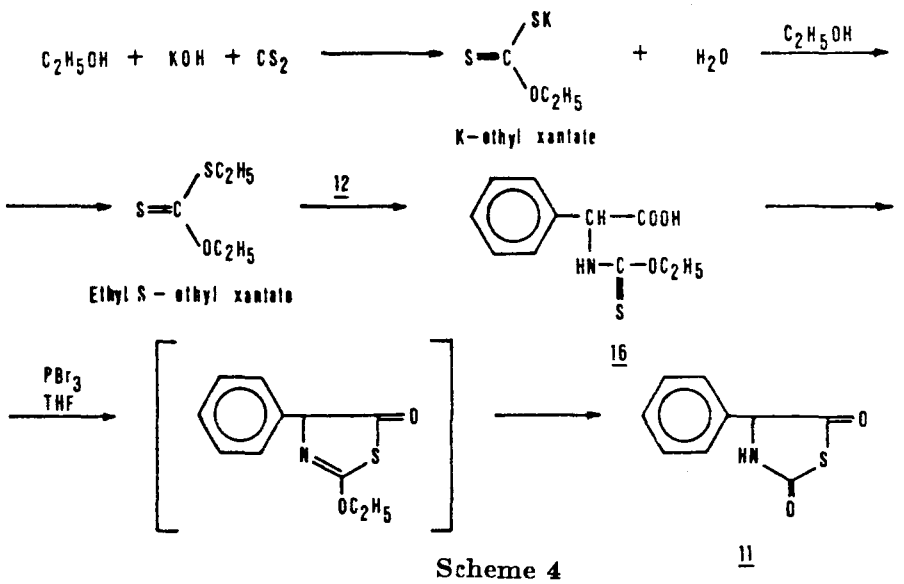
Finally, brief information of KRKA's research work on the synthesis of new β – lactams is given.

INDEPENDENT SYNTHESIS OF β – LACTAM ANTIBIOTICS

Industrial by important and known β – lactams were selected and independent synthesis in laboratory, pilot and industrial scale were developed in KRKA's R/D laboratories and its pilot and chemical plant.



Scheme 3



Scheme 4

The use of 4-Phenylthiazolidine-2,5-Dione in the Synthesis of β – Lactam Antibiotics

In our approach to the independent synthesis of ampicillin, talampicillin and cephalexin, new synthetic methods of acylation of 6-aminopenicillinic acid (**1a**) and 7-aminodesacetoxycephalosporanic acid (**2b**) were studied and special attention was given to the use of 4-phenylthiazolidine-2,5-dione (**11**) or N-thiocarboxyanhydride (NTA) of D-(-)-phenylglycine (**11-14**).

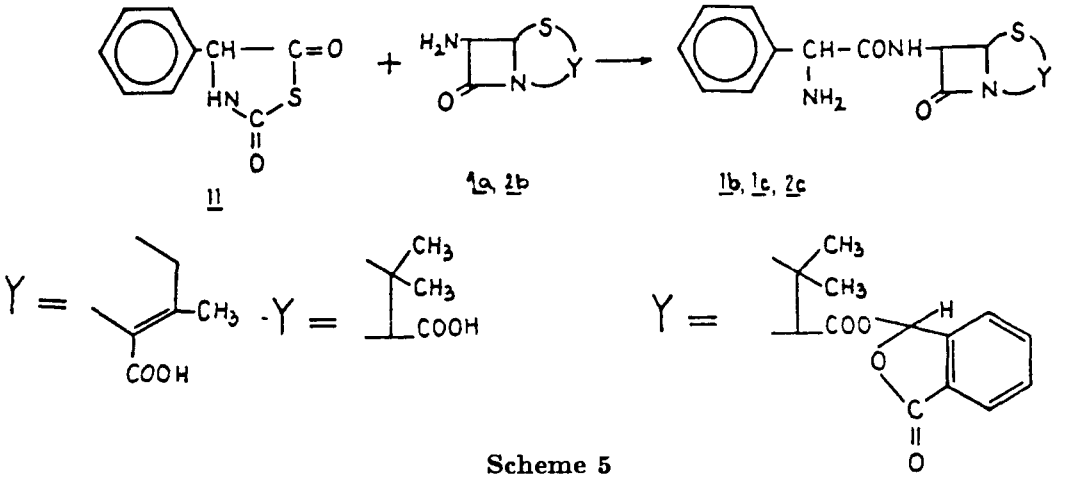
The use of α – amino acid N-carboxyanhydrides (NCA) was studied and introduced in peptide synthesis (15) and adapted in the synthesis of ampicillin (16). The basic intermediate 3-phenyl-1,3-oxazolidine-2,5-dione (14) was prepared by heterocyclization of D-(-)-phenylglycine (**12**) with phosgene, via corresponding carbamyl chloride derivative (**13**).

3-phenyl-1,3-oxazolidine-2,5-dione (**14**) reacted with 6-APA (**1a**) to form ampicillin (**1b**) but only in a modest yield.

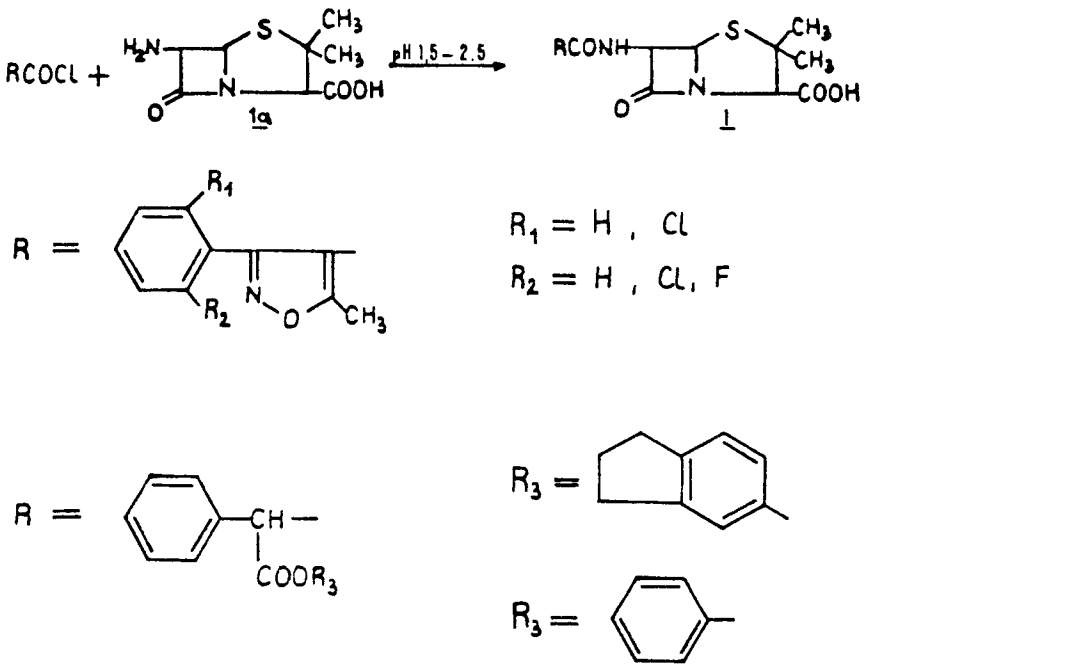
The use of α – amino acid N-carboxyanhydrides in the synthesis of peptides is usually complicated and limited, because decarboxylation occurs below pH 11, whereas at higher pH troublesome side reaction and formation of hydantoic acid takes place.

The recent use of α – amino acid N-thiocarboxyanhydride (**NTA'S**), the thio analogs of NCA'S is more successful in the peptide synthesis owing to increased stability of NTA'S at lower pH values (17,18).

The use of N-thiocarboxyanhydride of D-(-)-phenylglycine (**11**) in the synthesis of cephalexin (and ampicillin) has not been reported to the best of our knowledge. In order to find the independent synthesis of ampicillin, talampicillin and cephalexin, the syn-



Scheme 5



Scheme 6

thesis and use of 4-phenylthiazolidine-2,5-dione was studied. In this investigation, 4-phenylthiazolidine-2,5-dione (**11**) or NTA of D-(-)-phenylglycine has been prepared with the heterocyclization of D-(-)-phenylglycine (**12**) with thiophosgene in anhydrous dioxane (11,12,13,14). This reaction proceeded probably via intermediary formed phenyloxazolidine-2-thio-5-one (**15**).

The structure of 4-phenylthiazolidine-2,5-dione (11) was confirmed by NMR, IR and mass spectra and by indirect synthesis of the same compound.

A variant (indirect) synthesis of 4-phenylthiazolidine-2,5-dione was performed from correspoding thiouretane (Scheme 4).

4-Phenylthiazolidine-2,5-dione (**11**) was synthesized by heterocyclization of N-(ethoxythiocarbonyl) glycine (**16**) with phosphorus tribromide or trichloride (19,20). Thiourethane compound (**16**) was prepared by the reaction of D-(-)-phenylglycine (**12**) with ethyl-S-ethylxantate (21,22).

The condensation reactions of 4-phenylthiazolidine-2,5-dione with 6-APA (**1a**), phtalidyl ester or with 7-ADCA (**2b**) were performed in aqueous solutions or suspensions, depending on the pH values and reaction medium.

Finally ampicillin (**1b**), or talampicillin (**1c**) or cephalexin (**2c**), were isolated in good yields (12,13,23).

The Synthesis of β – lactam Antibiotics at Low pH Values

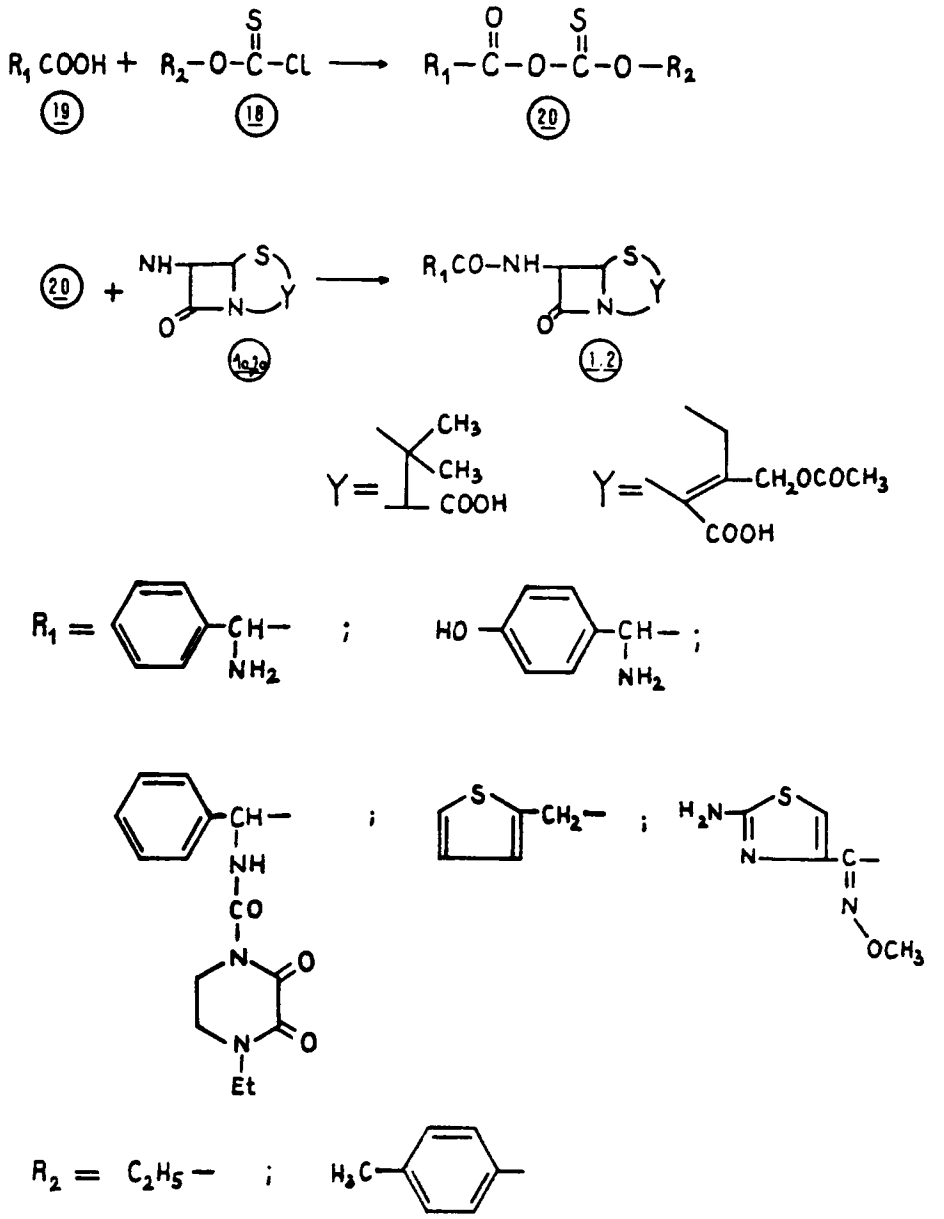
In our research work on the independent synthesis of isoxazolil penicillins (oxacillin, flucloxacillin, cloxacillin and dicloxacillin) the new synthetic approach was achieved by use of condensation reactions of corresponding methylisoxazolyl acid chlorides (17) with 6-APA at pH values from 1,8 to 2,5 in acetone solutions (24).

Our independent synthesis of carfecillin and carindacillin was done in a similar way (25).

The Use of Mixed Thioanhydride Method in the Synthesis of β – Lactam Antibiotics

Various chlorothioformates (**18**) were used in the independent synthesis of mixed thioanhydrides (**20**) which were the key intermediates for the further synthesis of important β – lactam antibiotics like ampicillin, amoxycillin, piperacillin and cefotaxime.

Chlorothioformates (**18**) react with different nucleophiles such as alcohols, thiols and primary amines giving thiocarbonates or thiocarbamates. Reaction and activation of acids with chlorothioformates have not yet been described in the literature. In our novel synthesis, after condensation reaction of selected chlorothioformates (**18**) with appropriate acids (**19**), few mixed thioanhydrides (**20**) were isolated



Scheme 7

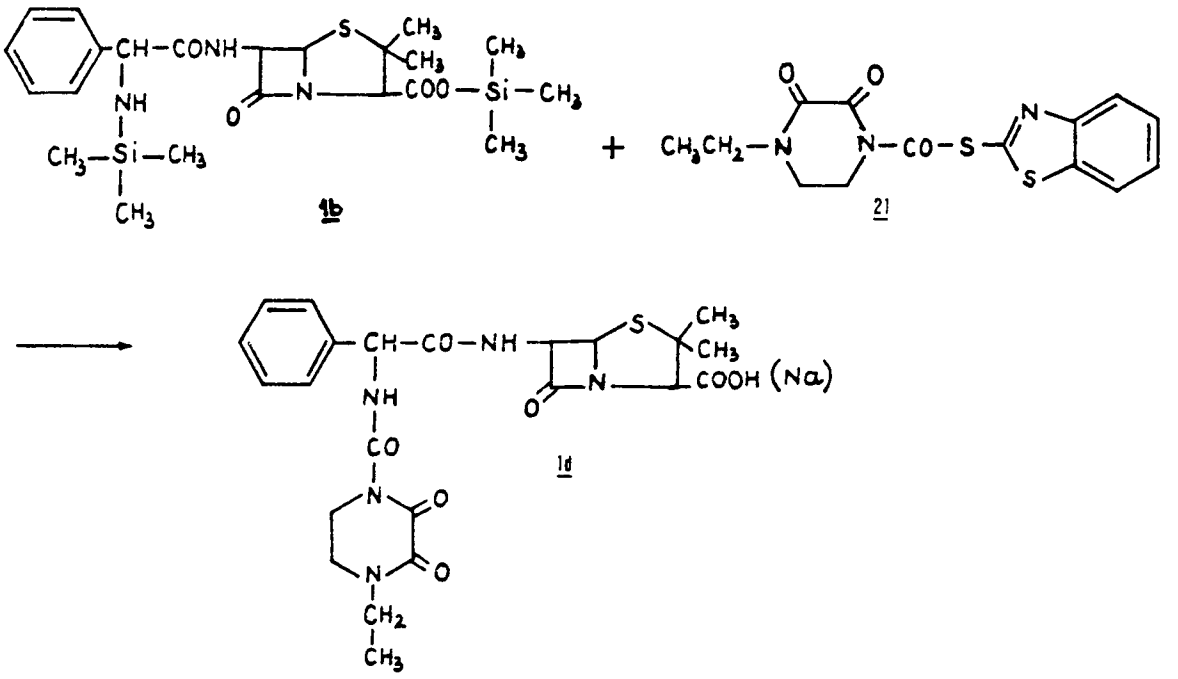
and reacted in further reaction step with 6-APA or 7-ACA to give final β – lactam semisynthetic antibiotics in moderate yields (**26-30**).

The synthesis and final semisynthetic β – lactams are represented in **Scheme 7**.

The Use of S-Thiocarboxylic Esters in the Synthesis of Piperacillin

In this approach to the independent industrial and economic synthesis of known ureido semisynthetic penicillins, especially piperacillin, the new and very stable reactive S-thiocarboxylic esters of 4-ethyl-2,3-dioxo-1-piperazine carboxylic acid (**21**) were for the first time used in the effective reaction of condensation with ampicillin (**1b**).

It is well known that 4-ethyl-2,3-dioxo-1-piperazine -4-carbonyl chloride, which is usually needed for the synthesis of piperacillin, is very unstable and must be



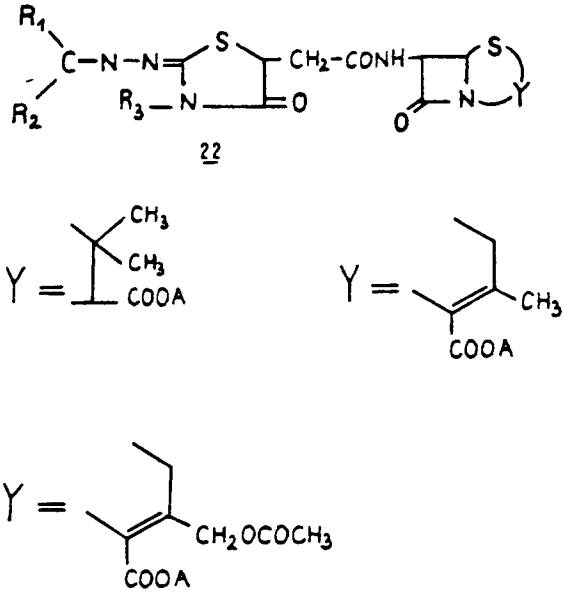
Scheme 8

used immediately after preparation in further synthesis of piperacillin (**1d**).

The main synthetic route is presented in **Scheme 8**.

The whole technology for the synthesis of piperacillin was developed in laboratory and pilot scale and effectively transferred to normal industrial scale production.

In the presented intensive and extensive R/D work S-2-benzothiazolyl-4-ethyl-2,3-dioxopiperazine



Scheme 9

(**23**) was selected as the best and the most stable intermediate for synthesis of piperacillin of very good quality and with over 90% yield of the whole synthesis.

THE SYNTHESIS OF NEW β – LACTAM ANTIBIOTICS

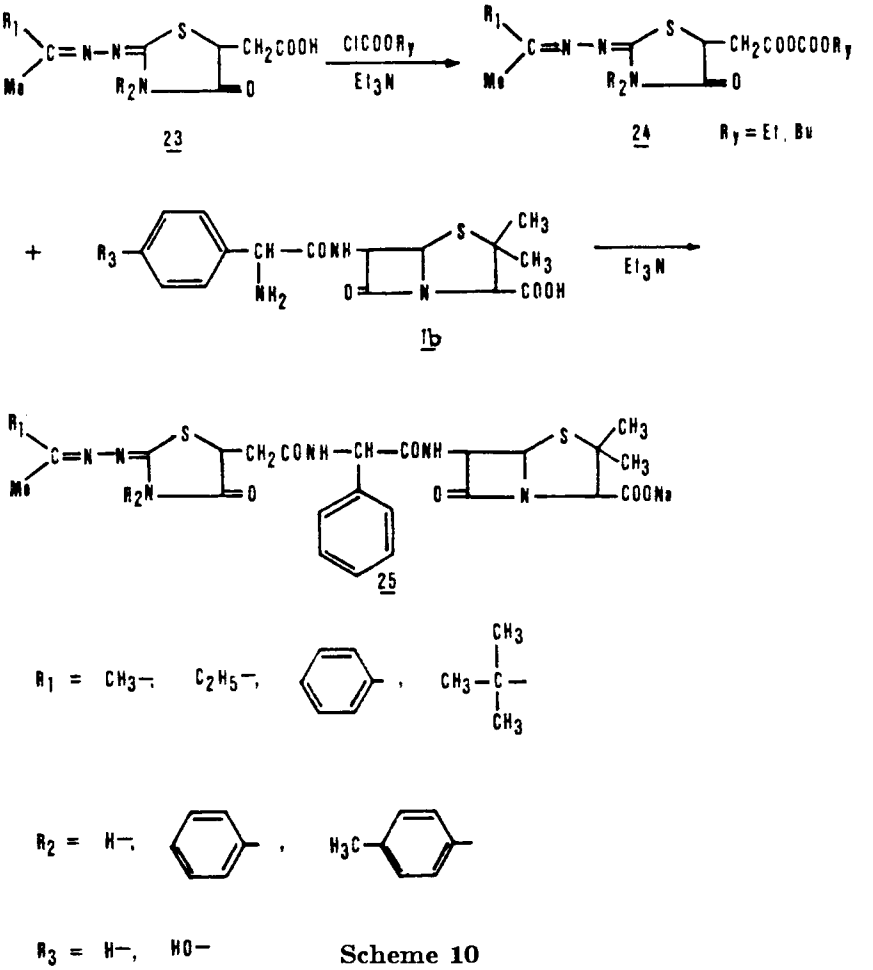
In KRKA's basic research work several new β – lactam antibiotics were prepared and tested for their antibacterial, antifungal and antiviral activities. The characteristic groups of new β – lactam antibiotics are as follows:

– "thiazolidine type" semisynthetic penicillins and cephalosporins,
– "thiazolidine" acylaminopenicillins,
– substituted phenylcephalosporins.

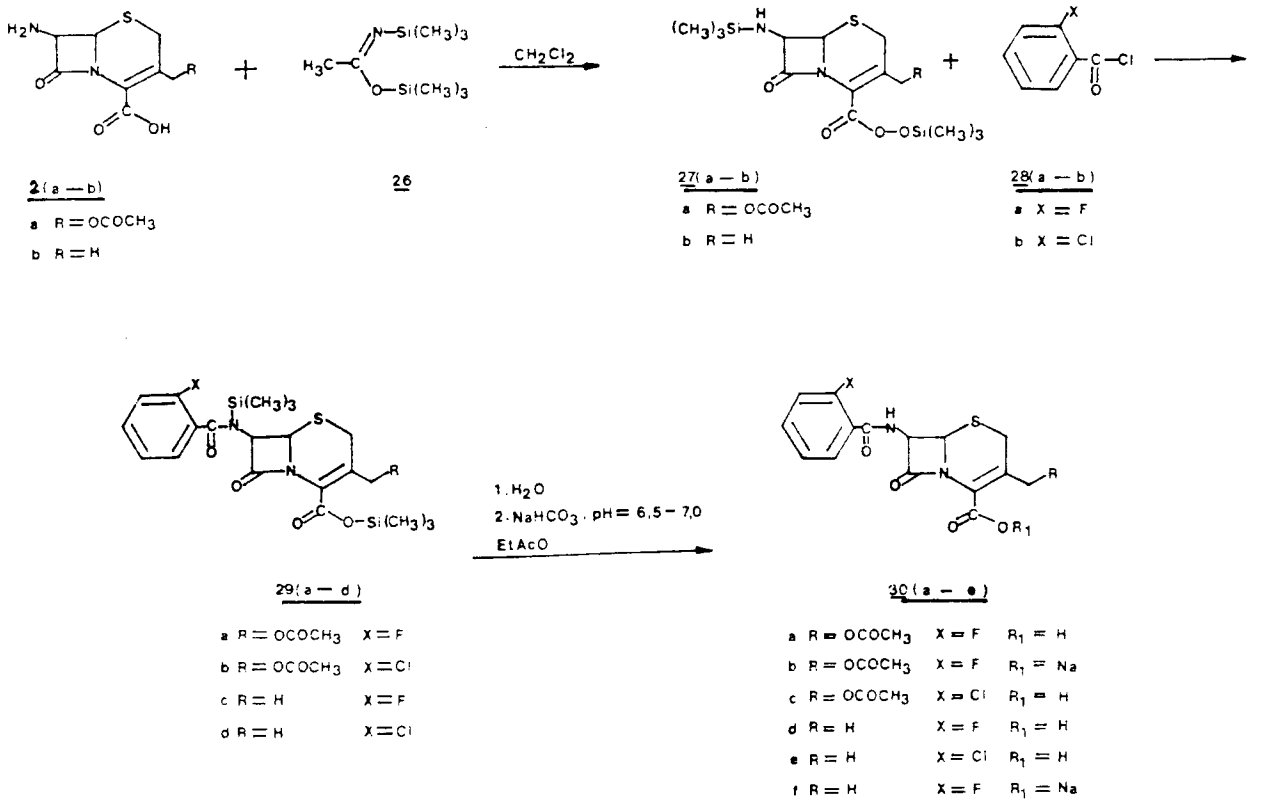
Synthesis of "Thiazolidine Type" Semisynthetic Penicillins and Cephalosporins (22)

In our previous research we have already reported that new substituted 2-alkyl(or aryl)idene hydrazino-4-oxothiazolidine-5-acetic acids are rather strong antiviral agents (31,32). In order to find new semisynthetic penicillins or cephalosporins with good antibacterial and antiviral activities, numerous semisynthetic penicillins (33,34) and cephalosporins (35-37) were prepared by the condensation reactions of the chlorides or mixed anhydrides of substituted 2-alkyl(or aryl)idene hydrazino-4-oxothiazolidine-5-acetic acid with 6-APA, 7-ACA, 7-ADCA (**Scheme 9**).

From the preliminary screening it can be seen that the new compounds exhibit a rather broad and strong bacteriostatic activity against Gram-negative and Gram-positive bacteria, as well as against Gram-positive cocci, with minimal inhibitory concentrations ranging from 0.19 μg ml^{-1}. Antiviral activity has been tested on a Herpes simplex strain, activated on humah embryo cell cultures and antiviral activity on the virus influenzae A_2, Belgrade strain, activated on choriallantoic membranes. It was observed that practically all the penicillins tested show inhibitory activities against both viruses.



Scheme 10



Scheme 11

Synthesis of "Thiazolidine Type" Acylaminopenicillins

The new semisynthetic acylaminopenicilline derivatives of 2-alkylidene or 2-arylideneazin-4-one-5-thiazolidine acetic acid were synthesized by acylation of α – amino group of ampicillin and amoxicillin (38, 39). The standart methods for acylation of α – amino group were used; acid chloride, mixed anhydride and carbodiimide method. The use of mixed anhydride method was the most successful synthetic method in the synthesis of new semisynthetic "thiazolidine-type" acylaminopenicillins (25). The synthesis of new antibiotics is presented in **Scheme 10.**

Synthesis and Structure-Activity Relationships of Substituted (2-F, 2-Cl) Phenylcephalosporins and Phenyldesacetoxycephalosporins (30)

In the research on the synthesis of new semisynthetic cephalosporins some phenylcephalosporins were prepared from 7-ADCA (**2b**) and 7-ACA (**2a**), which were transformed to corresponding silyl derivatives (**27**) and condensed with halogen substituted benzoylchlorides (**28**). The synthesis of new cephalosporins (**30**) is presented in **Scheme 11.**

The chemical structure of the synthesized compounds was established by IR, ^{1}H NMR, mass spectra and X-ray structure analysis. The new cephalosporins were tested for their antimicrobial activities.

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