

AUSTRALIA

Patents Act 1990

REQUEST FOR A STANDARD PATENT

AND NOTICE OF ENTITLEMENT

654384

The Applicant identified below requests the grant of a patent to the nominated person identified below for an invention described in the accompanying standard complete patent specification.

[70,71]Applicant and Nominated Person:

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[54]Invention Title:

PREPARATION OF A CEPHALOSPORIN ANTIBIOTIC USING THE SYN-ISOMER OF A THIAZOLYL INTERMEDIATE

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[31,33,32]

Details of basic application(s):-
757,108 UNITED STATES OF AMERICA US 10 September 1991

Applicant states the following:

1. The nominated person is the assignee of the actual inventor(s)
2. The nominated person is
~~the applicant~~
- the assignee of the applicant
~~authorised to make this application by the applicant~~
of the basic application.
3. The basic application(s) was/were the first made in a convention country in respect of the invention.

The nominated person is not an opponent or eligible person described in Section 33-36 of the Act.

9 September 1992

Bristol-Myers Squibb Company
By PHILLIPS ORMONDE & FITZPATRICK
Patent Attorneys
By

Our Ref : 302160

David B Fitzpatrick

5999q



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- (56) Prior Art Documents
AU 22844/92 C07J 501/46
- (57) Claim

1. A process for the preparation of an antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ^2 isomer which ~~comprises~~ ^{includes} reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in an aqueous-organic solvent at a pH of about 5.0 to 7.5.

2. A process of Claim 1 further ~~comprising~~ ^{including} the preparation of said syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer by reacting the anhydrous acid hydrochloride salt of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid with a mixture containing at least one molar equivalent of oxalyl chloride and at least one molar equivalent to a slight excess of dimethylformamide to an amount of said oxalyl chloride in an inert organic solvent at a temperature of less than -10°C .

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COMPLETE SPECIFICATION
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Complete Specification Lodged:
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Related Art:

Name of Applicant:

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Invention Title:

PREPARATION OF A CEPHALOSPORIN ANTIBIOTIC USING THE SYN-ISOMER OF A
THIAZOLYL INTERMEDIATE

Our Ref : 302160
POF Code: 129416/1490

The following statement is a full description of this invention, including
the best method of performing it known to applicant(s):

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BACKGROUND OF THE INVENTION

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1. Field of the Invention

The present invention provides a chemical acylation process and, in particular, an aqueous acylation process for the preparation of the antibiotic, cefepime dihydrochloride hydrate which is also known as 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate. The present invention also provides a stable, crystalline salt of the syn-isomer of a thiazolyl intermediate and a process for the preparation thereof which may be used in the preparation of useful broad spectrum antibacterial agents.

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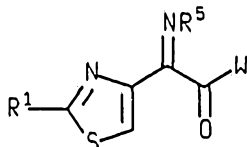
2. Background Art

A large number of cephalosporin antibiotics are known which contain a 2-(2-aminothiazol-4-yl)-(Z)-2-methoxyimino acetic acid side chain which is coupled to the 7-amino group of a cephalosporanic acid by well-known acylation procedures. In most instances, it is necessary to protect the amino moiety and activate the carboxylic acid of the side chain as part of the acylation procedure. Consequently, the art discloses a large number of amino-protecting groups for the 2-amino group of the thiazole ring and a large

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number of activating groups for the carboxylic acid. The search for newer protecting groups and activating groups to produce the desired antibiotic is still the subject of numerous publications in view of the costs
5 and toxicities associated with certain activating groups. Therefore, there is still a need in the preparation of useful broad spectrum antibiotics for a simple, stable, crystalline, economical, and non-toxic side chain having the desired geometric (Z)-isomer
10 which can be readily coupled to the 7-amino group of the cephalosporin nucleus. The following is representative of some of the thiazole side chain art.

In U.S. Patent No. 4,203,899 to Ochiai, et al.,
15 issued May 20, 1980, there is disclosed compounds of the Formula



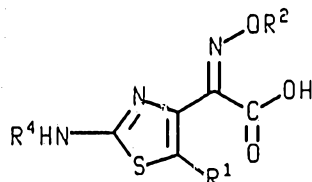
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wherein R^1 represents amino, protected amino, hydroxyl, or protected hydroxyl; R^5 represents
25 hydroxyl or protected hydroxyl; and W represents hydroxyl, C_{1-4} alkoxy, halogen, or OM wherein M is an alkali metal.

U.K. Patent Application GB-2,144,424, published
30 March 6, 1985, discloses the preparation of a series of pyridinium cephalosporin derivatives by various

methods, including the use of the compound of Formula

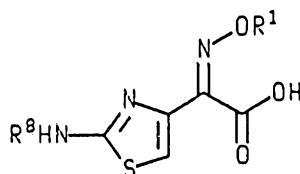
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or a salt thereof wherein R^1 denotes a hydrogen or
 10 halogen atom; R^2 denotes a hydrogen atom or a
 C_{1-6} alkyl radical, and R^4 represents hydrogen or an
 amino protective group, or with an activated
 derivative of this compound.

15 European Patent Application EP-160,546, published
 November 6, 1985, also discloses the preparation of a
 series of cephalosporin compounds by various methods,
 including the use of the substituted oximinothiazolyl
 acetic acid compounds of Formula

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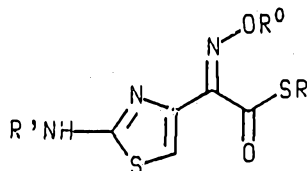


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or a reactive derivative thereof, wherein R^8
 represents a hydrogen atom or a protective group for
 an amino group. Suitable examples of such reactive
 30 derivatives which are disclosed are mixed acid
 anhydrides, acid anhydrides, acid halides, active
 esters, active amides, and acid azides.

In U.S. Patent No. 4,385,181 to Farge, et al., issued May 24, 1983, there is disclosed thiolesters of the Formula

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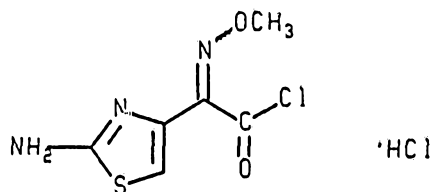


10 wherein R' represents hydrogen or a protecting radical, R⁰ represents hydrogen, alkyl, vinyl, cyanomethyl, or a protecting radical, and R represents alkyl, L-2-amino-2-carboxyethyl, phenyl, or a large number of various heterocyclic radicals listed in
 15 columns 4 to 8, as well as their syn- and anti-isomers and mixtures thereof.

In addition to the above-cited references, there exists a large number of references which disclose
 20 various protecting groups for the 2-amino substituent and an even larger number of activating groups/leaving groups of the carboxylic acid moiety which may be used in the acylation of a 7-amino cephalosporin compound.

25 However, the most relevant art is Czechoslovak Patent No. 238,950, published March 16, 1987, [Chemical Abstracts, Vol. 110, p. 544 (1989)] which discloses the compound of the instant invention having the Formula

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wherein the compound is alleged to have the syn configuration. The only evidence presented for the product in the patent is a chlorine content of 99% to 100.5% of the theoretical value.

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In connection with work on the development of new synthetic methods for the production of antibiotics, the present inventors, as well as others in the art, have felt the need for simple, convenient, economical, crystalline, stable, and non-toxic starting materials for use in the manufacture of antibiotics. Initial attempts to prepare and use the acid chloride of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid without the use of protecting groups were unsuccessful. However, the present inventors have now found that the compound of the instant invention can be prepared under specifically defined reaction conditions. This finding was further confirmed by the present inventors when they could not reproduce the teachings of the above-cited Czechoslovak patent. The desired syn-isomer of the acid chloride hydrochloride, which is necessary to produce the desired antibiotic, could not be repeated. Furthermore, additional experiments confirmed that the teachings of the art did not produce the hydrochloride salt of the desired syn-isomer of the acid chloride substantially free of the anti-isomer and having a proton nuclear magnetic resonance spectrum (^1H NMR) as described herein.

30 The broad spectrum antibiotic cefepime is disclosed by Aburaki, et al, in U.S. Patent No. 4,406,899, issued September 27, 1983 and its preparation is described by two Reaction Schemes in which the reactants and products required the use of blocking and deblocking groups. In the reaction

scheme actually exemplified, the product required a chromatographic purification step to separate the mixture of Δ^2 and Δ^3 isomers and the cefepime product thereby produced is in the zwitterion form. However, 5 the zwitterion form of cefepime is unstable at room and elevated temperatures.

Murray A. Kaplan et al, in U.S. Patent No. 4,910,301, issued March 20, 1990, discloses 10 temperature stable crystalline salts of cefepime in dry powder form having excellent room and superior elevated temperature stability compared to the zwitterion form of cefepime described by Aburaki et al, U.S. Patent No. 4,406,899.

15 In U.S. Patent No. 4,868,294, issued September 19, 1989, Brundidge et al, describe the preparation of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate salts substantially 20 free of the Δ^2 isomer and their use in an aqueous acylation procedure to prepare the antibiotic cefepime as the sulfate salt.

In U.S. Patent No. 4,754,031, issued June 28, 25 1988, Angerbauer et al, describe a process for the preparation of several cephalosporin antibiotics including cefepime in the zwitterion form. Although this process does not use protecting groups, it does use an anhydride for activation in an aqueous 30 acylation reaction which requires chromatographic purification steps to give the zwitterion form of cefepime.

In U.S. Patent No. 4,943,631, issued July 24, 1990, Brian E. Looker describes an improved process for the preparation of the antibiotic cefepime as a hydriodide salt. The process controls the formation
5 of the undesirable Δ^2 isomer by employing a cephalosporin sulfoxide intermediate. However, the process described in the patent remains costly and inefficient since it introduces two additional steps to the prior art process and continues to use
10 protecting groups which require blocking and deblocking procedures. Furthermore, the process requires the use of column chromatography as a purification method which is impractical on a manufacturing scale.

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The preparation of the crystalline sulfate salt and zwitterion of cefepime which are described in the art use essentially the same aqueous acylation process and use various blocking and deblocking groups and
20 active esters. In all instances, the preferred crystalline cefepime dihydrochloride hydrate form must be prepared via the purified zwitterion form of cefepime. Thus, there is a need to develop a simple, direct and cost effective acylation procedure which
25 will avoid reaction steps to add and remove protecting groups, stereochemical controlling steps and chromatography procedures and, more importantly, an acylation procedure which will produce the desired antibiotic cefepime dihydrochloride hydrate which is
30 substantially free of the anti-isomer and the Δ^2 isomer.

SUMMARY OF THE INVENTION

The present invention provides a chemical acylation process and, in particular, an aqueous
5 acylation process for the preparation of the antibiotic, cefepime dihydrochloride hydrate substantially free of the anti-isomer and the Δ^2 isomer. The present invention also provides a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-
10 yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer which is used in the acylation process for the preparation of the broad spectrum antibiotic, cefepime.

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DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the proton nuclear magnetic resonance spectrum of syn 2-(2-aminothiazol-4-yl)-2-
20 methoxyimino acetyl chloride hydrochloride of Example 10 in acetic acid-d₄ (100 MHz).

FIG. 2 shows the proton nuclear magnetic resonance spectrum of the product of Example 12 in
25 acetic acid-d₄ (100 MHz).

FIG. 3 shows the proton nuclear magnetic resonance spectrum of the product of Example 13 in
acetic acid-d₄ (100 MHz).

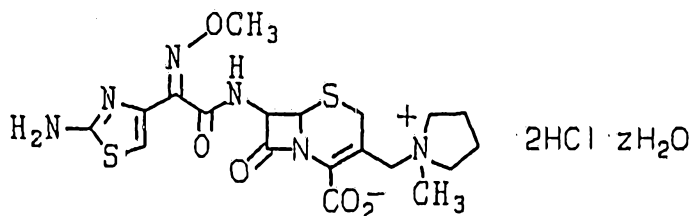
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FIG. 4 shows the proton nuclear magnetic resonance spectrum of the product of Example 14 in acetic acid-d₄ (100 MHz).

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides an aqueous acylation process for the N-acylation of 7-amino-3-
 5 [(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-
 carboxylate with the syn-isomer of 2-(2-aminothiazol-
 4-yl)-2-methoxyimino acetyl chloride hydrochloride
 which is substantially free of the anti-isomer to
 produce a temperature stable crystalline cefepime
 10 dihydrochloride hydrate substantially free of the
anti-isomer and the Δ^2 isomer and is represented by
 Formula V wherein z is 1 or 2.

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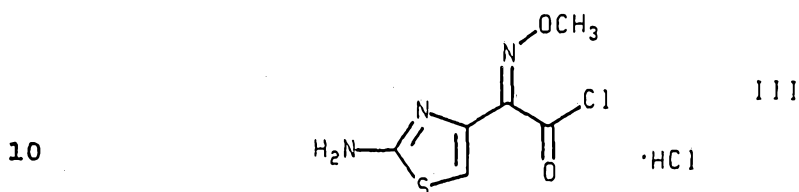
The advantages of the present aqueous acylation process become apparent and may be appreciated by
 25 those skilled in the art when all the advantages are
 combined and considered as a whole. The elimination
 of formal amino and carboxyl protecting groups and the
 corresponding elimination of the additional chemical
 steps required for blocking and deblocking offer a
 30 distinct advantage in overall process efficiency and
 cost of materials over the previously known art
 processes. The present process additionally provides
 and maintains control of the stereochemical
 configuration of methoxyimino isomer and the Δ^3 double
 35 bond of the cephalosporin nucleus without the need to

separate undesirable cephalosporin by-products by chromatography and without the need to use stereochemical controlling sulfoxide intermediates such as described in U.S. Patent No. 4,043,631.

- 5 Another advantage of the present invention is the preparation and use of the unprotected crystalline hydrochloride salt of syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride of Formula III which avoids unusual and sometimes complex organic
- 10 leaving groups described in the art. The use of the simple chloride ion as a leaving group avoids the use of potential toxic leaving groups such as 2-mercaptobenzothiazole. A further advantage of the preferred embodiment of the present aqueous acylation
- 15 process is to provide the desired temperature stable crystalline cefepime dihydrochloride hydrate directly from the reaction mixture of the acylation process without the need to prepare and isolate the sulfate salt or zwitterion of cefepime. A major advantage of
- 20 the preferred embodiment of the present aqueous acylation process is to provide the desired antibiotic without the need to use any silylating agents or any soluble silylated derivatives. The present process is advantageously carried out without the use of
- 25 protecting groups, stereochemical controlling groups, solubilizing silylated groups or chromatography to provide the water soluble crystalline cefepime dihydrochloride hydrate substantially free of the anti-isomer and the Δ^2 isomer in high yield directly
- 30 from the aqueous-organic reaction mixture.

The present invention also provides a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride which is substantially free of the anti-isomer and is

5 represented by Formula III.



As a result of being substantially free of the

15 anti-isomer, compound III is convertible to broad spectrum cephalosporins which themselves are substantially free of the anti-isomer, without the need for chromatographic separation of the syn- and anti-isomers. As a result of the enhanced stability,

20 compound III may be isolated and stored and, when desired, compound III may be converted to end products in a different solvent which is advantageous for the production of the desired antibiotic substantially free of the Δ^2 isomer. An additional advantage of the

25 intermediate of Formula III is that it does not require blocking (protection) of the amino group prior to acylation or deblocking (deprotection) of the amino group after acylation, thus, offering process efficiency. A further advantage of the acid chloride

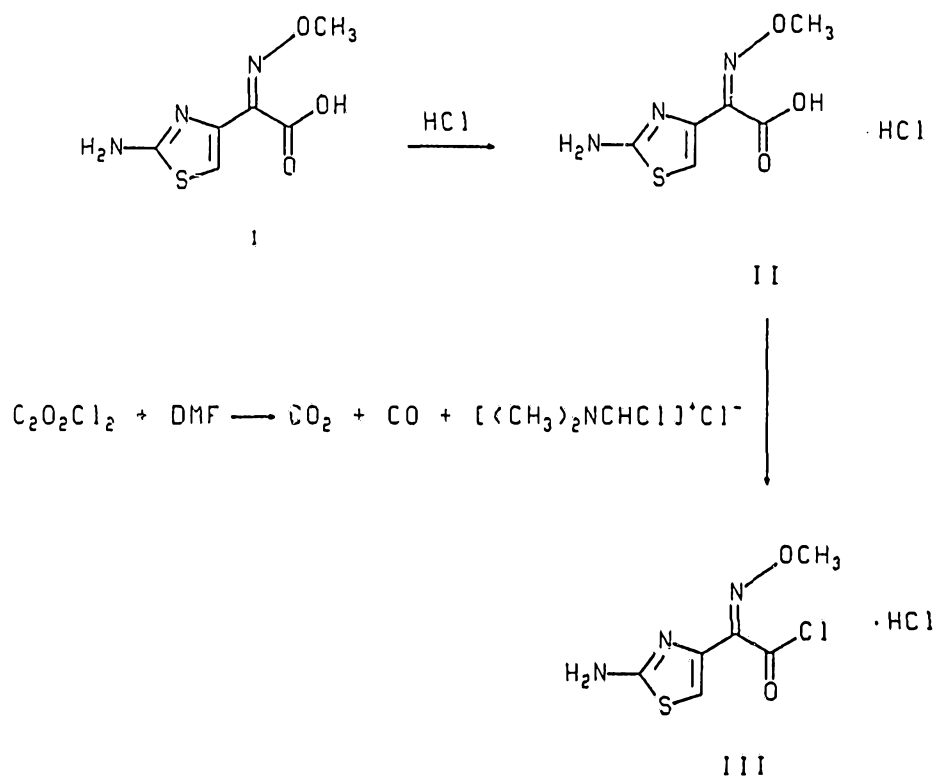
30 of Formula III is its use in the acylation process to produce broad spectrum cephalosporins. In contrast to other methods such as described in Aburaki, et al., U.S. Patent No. 4,406,899, the intermediate of Formula III has a chloride ion as a simple and non-toxic

35 leaving group with no precaution necessary to remove

it from the desired antibiotic as is the instance with most other leaving groups known in the art. Also, some of the intermediates known in the art which contain other leaving groups are difficult to prepare while other intermediates which contain leaving groups such as 2-mercaptobenzothiazole have been found to be toxic [Chem. Abstracts, 1989, Vol. 111 (3), 19243p].

Reaction Scheme 1

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The syn-isomer of the acid chloride hydrochloride of Formula III may be prepared from the syn-isomer of the acid of Formula I as outlined in Reaction Scheme 1. The acid of Formula I is first converted to
5 the corresponding hydrochloride salt of Formula II by methods known in the art and then, if desired, isolated as an anhydrous, crystalline compound of Formula II. The formation of the hydrochloride salt is advantageously carried out with at least one molar
10 equivalent of gaseous hydrogen chloride in an inert organic solvent such as toluene, acetonitrile, dichloromethane, acetone, benzene, xylene, cyclohexane, hexanes, dioxane or diethyl ether at a temperature of about -10°C to about 50°C. Preferably,
15 the reaction is carried out in toluene, dichloromethane or acetonitrile and the hydrochloride of Formula II thereby produced may be isolated or used in situ. When the reaction is conducted in acetonitrile, the resulting hydrochloride of Formula
20 II tends to retain loosely bound solvent. Consequently, it is advantageous to use the acid hydrochloride of Formula II from acetonitrile in a reasonable period of time in the next step to avoid solvate displacement by atmospheric moisture. Most
25 preferably, the reaction is carried out in toluene or dichloromethane at about 0°C to room temperature.

The acid salt of Formula II is then advantageously treated with a chlorinating agent and,
30 most preferably, with oxalyl chloride in combination with dimethylformamide to produce the stable, crystalline syn-isomer of compound III. As demonstrated herein, the use of other known chlorinating agents can result in isomerization to
35 give the undesirable anti-isomer or mixtures of

syn- and anti-isomers. Additionally, chlorinating agents, such as phosphorous pentachloride, may result in the chlorination of the 5-position on the thiazole ring which would then result in an undesirable
5 impurity in the antibiotic. The present inventors have discovered that, in addition to the preparation of the acid hydrochloride of Formula II, the appropriate selection of chlorinating agent and reaction conditions, such as solvent and temperature,
10 are critical in the process for the preparation of the syn-isomer of compound III which is substantially free of the anti-isomer.

Chlorinating methods, which are generally used
15 for activating acids, are well-known in the art. Phosphorous pentachloride, which is the most widely used chlorinating agent, is not suitable for the chlorination of compound II since it also results in the isomerization of the methoxyimino group to produce
20 the undesirable anti-isomer of compound III. This is clearly demonstrated in Examples 12, 13, 14, and 16 as described herein. Another known chlorinating method is the use of oxalyl chloride in combination with dimethylformamide. However, the present inventors
25 have discovered that the oxalyl chloride method, wherein dimethylformamide is used as a catalyst, does not produce substantial amounts of the desired syn-isomer of compound III. This is also clearly demonstrated in Example 15 as described herein. After
30 extensive studies, the present inventors have discovered that the use of dimethylformamide in an amount less than an equimolar amount relative to the amount of oxalyl chloride is deleterious to the production of the desired syn-isomer of the acid
35 chloride hydrochloride of Formula III. Most

preferably, the molar amount of dimethylformamide should exceed the molar amount of oxalyl chloride. The inventors have also found that the use of excess molar amounts of dimethylformamide is also deleterious to both the reaction and to the stability of the desired product. Thus, the inventors have discovered a method to control the instability of the reaction materials to either excess chloride ion generated by oxalyl chloride or excess dimethylformamide which are critical for production of the stable, crystalline syn-isomer of compound III which is substantially free of the anti-isomer. In the instance, when the conversion of the compound of Formula II to the compound of Formula III is not complete, there will remain a small amount of the syn-isomer of the acid of Formula IX in the isolated product of compound III. The presence of some unreacted compound II in the product of compound III and small amounts of the anti-isomer of compound III do not affect the subsequent acylation reaction for the successful production of the desired antibiotic which is substantially free of the anti-isomer of said antibiotic.

The present inventors have also found that the temperature and reaction solvent of the chlorinating reaction are also critical. Preferably, the reaction is conducted in an inert organic solvent, such as dichloromethane, chloroform or acetonitrile, at a temperature of less than -10°C . Most preferably, the reaction is advantageously carried out in dichloromethane at about -15°C to about -40°C .

The use of the syn-isomer of the acid chloride hydrochloride of Formula III to prepare useful broad-spectrum antibiotics by way of a general acylation

reaction is illustrated in Reaction Scheme 2. More specifically, Reaction Scheme 2 illustrates the use of the acid chloride of Formula III to prepare the broad spectrum antibiotic cefepime which is substantially free of the anti-isomer and the Δ^2 isomer. Furthermore, the acid chloride hydrochloride of Formula III may be used to prepare cephalosporin antibiotics having the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl attached to the 7-amino group of the cephalosporin nucleus such as cefodizime, cefmenoxime, cefotaxime, cefpirome, cefpodoxime, cefquinome, cefteteram, ceftiofur, cefetamet and cefuzonam.

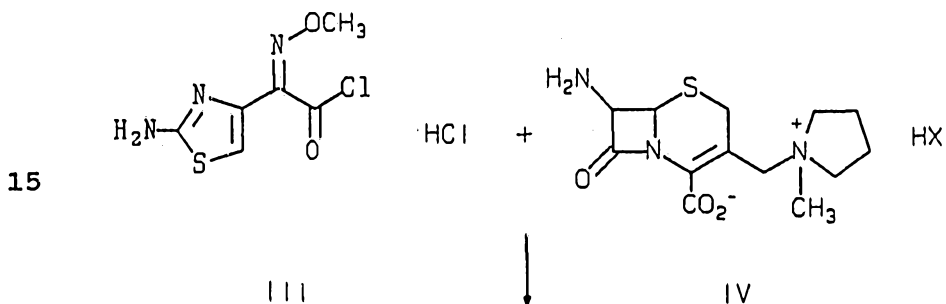
Furthermore, to confirm that the acid chloride hydrochloride product of the prior art is in the anti-isomer and not in the desired syn-isomer, the present inventors substituted the product of the prior art such as that produced in Example 14 for the syn-isomer of the compound of Formula III in the acylation reaction illustrated in Reaction Scheme 2. The resulting cephalosporin product which was produced as described in Examples 17 and 18 was compared to the cefepime antibiotic produced by the present invention. As can be seen by the comparison in Example 19, the anti-cefepime produced by teachings of the prior art is not the same as the useful broad-spectrum syn-cefepime produced by use of the present invention.

As defined herein and in the claims, the term "substantially free" means that the compound contains less than about 5% of the undesirable isomer. Preferably, the compound contains less than about 1% of the undesirable isomer.

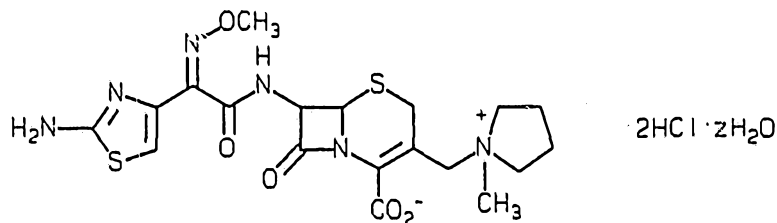
According to the process of the present invention, the broad-spectrum antibiotic cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ^2 isomer is prepared by the N-acylation of a compound of Formula IV with the syn-isomer of the acid chloride hydrochloride of Formula III as illustrated in Reaction Scheme 2.

Reaction Scheme 2

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The 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate salt which is substantially free of the Δ^2 isomer and represented by Formula IV wherein HX is HCl, HI or H₂SO₄ may be prepared by the general procedures described by S.P. Brundidge, et al. in U.S. Patent No. 4,868,294.

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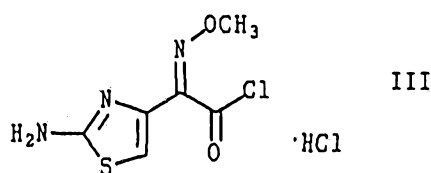
Suitable solvents which may be used in the aqueous acylation process are aqueous-organic solvents such as water with water-miscible organic solvents such as methanol, ethanol, isopropanol, butanol, acetone, tetrahydrofuran, acetonitrile, dioxane, dimethylacetamide, dimethylformamide or the like. The pH of the process is controlled by titrating with a suitable inorganic or organic base such as sodium hydroxide, sodium carbonate, sodium bicarbonate, potassium hydroxide, ammonium hydroxide, primary amine, secondary amine, tertiary amine or the like to neutralize the hydrochloric acid which is thereby produced. Preferably, the organic bases which may be used in the process are, for example, diethylamine, triethylamine, diisopropylethylamine, N-methylmorpholine, 2,6-lutidine, N,N-dimethylaniline, N,N-diethylaniline or the like. Most preferably, the base is ammonium hydroxide, triethylamine or N-methylmorpholine.

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The acylation is advantageously carried out at a pH of about 5 to about 7.5 and, preferably, at a pH of about 6.2 to 6.8. The process of the present invention may be carried out at a temperature of about -50°C to about room temperature and, preferably, at about -10°C to -40°C. After the acylation is completed, the reaction mixture is acidified with an appropriate acid and, preferably, with sulfuric acid to a pH of about 1.8 to 2.6 to produce the sulfate salt of the desired cefepime antibiotic which is substantially free of the anti-isomer and the Δ^2 isomer. If desired, the sulfate salt of cefepime may be converted to other cefepime salts such as the dihydrochloride hydrate as described by Kaplan, et al, in U.S. Patent No. 4,910,301.

In one aspect of the present invention, there is provided an acylation process for the preparation of the broad-spectrum antibiotic cefepime which is substantially free of the anti-isomer and the Δ^2 -isomer which comprises reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate salt which is substantially free of the Δ^2 isomer with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in an aqueous or, preferably, mixed aqueous-organic solvent under carefully controlled pH conditions of about 5.0 to 7.5.

In another aspect of the present invention, there is provided a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer having the formula



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In still another aspect of the present invention, there is provided a process for the preparation of a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer which comprises reacting the anhydrous crystalline acid hydrochloride salt of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid with a mixture containing at least one molar equivalent of oxalyl chloride and at least one molar equivalent to a slight excess of

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dimethylformamide to an amount of said oxalyl chloride in an inert organic solvent at a temperature of less than -10°C to produce a stable, crystalline syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer.

When the N-acylation of the compound of Formula IV is complete as ascertained by known detection methods available in the art, for example, thin-layer chromatography, high pressure liquid chromatography and spectroscopic methods. According to the preferred process of the present invention, a sufficient amount of an acid such as hydrochloric acid, sulfuric acid or the like is added to the completed reaction to ensure crystallization of the desired cefepime salt and then diluted with an appropriate water-miscible organic solvent such as methyl ethyl ketone, acetone, isopropanol, butanol or the like to induce or complete the crystallization. Preferably, the aqueous-organic reaction mixture is treated with a sufficient amount of sulfuric acid to crystallize the sulfate salt of cefepime which is substantially free of the anti-isomer and the Δ^2 isomer. The cefepime sulfate may then be converted to the preferred crystalline cefepime dihydrochloride monohydrate by the method described by Kaplan et al., in U.S. Patent No. 4,910,301. The cefepime sulfate which is produced in the present process may be neutralized with a base and, preferably, with a weakly basic ion-exchange resin known in the art and, preferably, commercially available such as Amberlite LA-2, Dowex WGR, Bio-Rad AG3-X4A, Amberlite IRA 93, Amberlite IRA 35 or the like to produce an aqueous or aqueous-organic solution containing the zwitterion

form of cefepime. The solution is then treated with a sufficient amount of hydrochloric acid and, optionally, with a water-miscible organic solvent to induce the crystallization of the preferred
5 crystalline cefepime dihydrochloride hydrate. Most preferably, the aqueous-organic reaction mixture obtained from the aqueous acylation of the present process is treated with a sufficient amount of hydrochloric acid to induce and ensure crystallization
10 of said antibiotic, cefepime dihydrochloride hydrate when a water-miscible organic solvent such as acetone is added. The amount of water-miscible organic solvent to be added should be sufficient to produce complete crystallization of said antibiotic and,
15 advantageously, in an amount of about 2 to about 9 volumes of the aqueous amount of the aqueous-organic reaction mixture to produce the temperature stable crystalline cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the
20 Δ^2 isomer.

When it is desired to prepare only the cefepime dihydrochloride monohydrate, the aqueous-organic reaction mixture from the aqueous acylation is
25 advantageously treated with a sufficient amount of hydrochloric acid and diluted with an appropriate amount of a water-miscible organic solvent as described herein to ensure the crystallization of the desired monohydrate form. Alternatively, when it is
30 desired to prepare the stable cefepime dihydrochloride dihydrate, the aqueous-organic reaction mixture may be advantageously treated with a greater equivalent concentration of hydrochloric acid and an amount of water-miscible organic solvent to hold the
35 crystallization at the cloud point before additional

organic solvent is added to complete the crystallization. However, it should be appreciated by those skilled in the art that if the isolation step from the aqueous-organic reaction mixture of the process is not carefully controlled, it is possible that a mixture of crystalline cefepime dihydrochloride monohydrate and dihydrate forms may be produced. In any event, the preparation of only one of the desired hydrates can be prepared from either hydrate or a mixture of hydrates by following the recrystallization procedures described herein.

The crystalline cefepime dihydrochloride monohydrate prepared by the present process may be used to prepare a stable crystalline cefepime dihydrochloride dihydrate by recrystallization under controlled concentrations of solvent and hydrochloric acid, and the amount of time held at the cloud point (initial crystallization) as described herein. Alternatively, the crystalline dihydrochloride dihydrate prepared by the present process may also be used to prepare a stable crystalline cefepime dihydrochloride monohydrate by recrystallization under different controlled conditions as described herein. Thus the process of the present invention may be used to produce either the desired monohydrate or dihydrate of said antibiotic.

In contrast to the labile cefepime dihydrochloride dihydrate described in U.S. Patent No. 4,910,301 which readily loses the second mole of water, the crystalline cefepime dihydrochloride dihydrate which
5 may be produced by the present process has been found to have a well-defined crystalline structure which retains the second mole of water. The new crystalline dihydrate form (needle-like crystals) has been found to be remarkably stable and its crystal morphology
10 does not change under various conditions, for example, in air at a temperature of 70°C for more than two months, under vacuum with P₂O₅ at 50°C for 48 hours, under oven drying at 70°C for 96 hours and under high or low relative humidity conditions. The crystalline
15 dihydrate exhibits characteristic infrared absorption peaks at 3574 cm⁻¹ and 3432 cm⁻¹ as indicated by FT-IR diffuse reflectance spectroscopy with KBr and a 13 mm sample cup using a Nicolet 20SX spectrometer. This temperature and moisture stable crystalline dihydrate
20 form of cefepime is also characterized by an x-ray powder diffraction pattern as shown in Table 1 wherein "d" refers to interplanar spacings and "I/I₀" refers to relative percent intensities. The X-ray pattern was collected with a Rigaku Geigerflex X-Ray
25 Diffractometer and a nickel filtered copper (K_α) radiation wavelength of 1.5425 Å;

TABLE 1

Stable Cefepime Dihydrochloride Dihydrate

5	d	I/I ₀ (%)
	13.14	15
10	12.78	13
	8.82	24
	6.62	18
	6.41	100
	4.94	17
15	4.79	10
	4.74	12
	4.52	13
	4.41	36
	4.1	63
20	3.75	50
	3.6	11
	3.53	16
	3.41	36
	3.31	9
25	3.19	22
	2.84	30
	2.67	16
	2.61	6
	2.57	14
30	2.5	4
	2.48	9
	2.27	15

35

Thus, an embodiment of the present invention provides a process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ^2 isomer which comprises reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate which is substantially free of the Δ^2 isomer with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in a mixed aqueous-organic solvent at a pH of about 5.0 to 7.5.

A preferred embodiment of the present invention further comprises the preparation of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer by
5 reacting the anhydrous acid hydrochloride salt of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid with a mixture containing at least one molar equivalent of oxalyl chloride and at least one molar equivalent to a slight excess of
10 dimethylformamide to an amount of said oxalyl chloride in an inert organic solvent at a temperature of less than -10°C .

A more preferred embodiment of the present
15 invention provides a process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ^2 isomer which comprises reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate which is
20 substantially free of the Δ^2 isomer with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of the anti-isomer in an aqueous-organic solvent and further comprises the addition of a sufficient amount of
25 hydrochloric acid and a water-miscible organic solvent to the completed reaction mixture.

The most preferred embodiment of the present invention provides a process for the preparation of
30 the antibiotics, cefepime dihydrochloride monohydrate and cefepime dihydrochloride dihydrate directly from the aqueous-organic reaction mixture of the present process.

The utility of cefepime (Compound V) is shown in Abruaki et al, U.S. Patent No. 4,406,899. The stable dihydrate form of cefepime produced by the present process exhibits the antibiotic properties of the
5 above-mentioned cefepime of U.S. Patent No. 4,406,899 and finds utility as an antibiotic in like manner.

It is understood that the specification and the examples are illustrative and are not to be construed
10 as limiting the scope of the invention.

DESCRIPTION OF SPECIFIC EMBODIMENTSExample 1

5 Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

A suspension of (25 g, 124.25 mmol) 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid in toluene (250 ml) was gassed with HCl at 20°-28°C. The HCl was introduced subsurface in two aliquots of 8.1 g (222.2 mmol) and 4.8 g (131.7 mmol) with 30 minutes agitation between aliquots. After 1 hour at 20°C, the product was collected by filtration under a nitrogen atmosphere, washed with toluene (50 ml) and hexane (250 ml), and dried at 20°-25°C in vacuo to yield 28.68 g (97%) of the title compound.

Example 2

20 Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of (0.77 ml, 10 mmol) dimethylformamide in dichloromethane (40 ml) at 5°C was added (0.89 ml, 10 mmol) 98% oxalyl chloride in dichloromethane (4.1 ml). Dropwise addition kept the temperature at 4°-5°C. To the resulting suspension, cooled to -27°C, was added (2.37 g, 10 mmol) 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride prepared in Example 1. The suspension was stirred for 2.5 hours at -25°C. Filtration under a nitrogen atmosphere and washing with dichloromethane (50 ml) and hexane (100 ml) gave 1.78 g (69.5%) of white, crystalline title compound after drying at 20°C in vacuo.

The title acid chloride acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester hydrochloride in pyridine solution to give a single zone (TLC) product coincident with and inseparable from an authentic sample of the desired desacetoxy cephalosporin ester.

Example 3

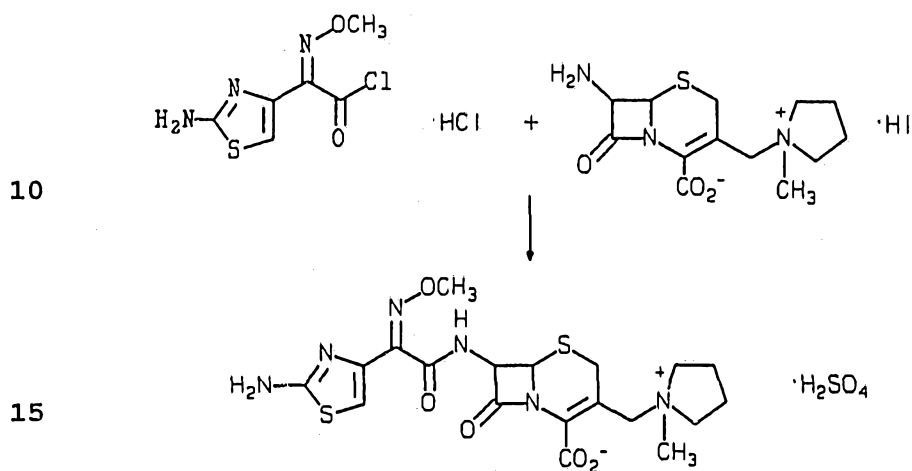
10 Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

To a solution of (1.55 ml, 20 mmol) dimethylformamide in dichloromethane (80 ml) at 5°C was added
15 (1.78 ml, 20 mmol) of 98% pure oxalyl chloride in dichloromethane (8.2 ml). Addition time was 5 minutes at 5°-8°C. The resulting suspension was agitated 10 minutes at 5°C and then cooled to -30°C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetic acid
20 hydrochloride (4.75 g, 20 mmol) from Example 1 was added. The suspension was stirred for 2.5 hours at -25° to -30°C. Filtration under a nitrogen atmosphere and washing with dichloromethane (75 ml) and hexane (100 ml) gave 3.57 g (69.7%) of crystalline title
25 compound after drying at 20°C in vacuo.

An aliquot of the solid acid chloride hydrochloride product acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester hydro-
30 chloride in pyridine solution to give essentially single zone (TLC) product which was coincident with and inseparable from an authentic sample of the desired desacetoxy cephalosporin ester.

Example 4

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate (cefepime)



7-Amino-3-[(1-methyl-1-pyrrolidinio)methyl] cep-3-em-4-carboxylate monohydroiodide (0.85 g, 2.0 mmol) [prepared according to the procedures described by S. P. Brundidge, *et al.*, in U.S. Patent No. 4,714,760] was dissolved in 9 ml of acetone-water (2:1) with triethylamine at pH 6.5 and 20°C. Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (0.56 g, 2.2 mmol) [prepared in Example 3] was added using triethylamine to control the pH in the 5-7 range. Assay of the resulting solution by high pressure liquid chromatography showed 58% yield of the desired cephalosporin (cefepime). Acidification with sulfuric acid to pH 2.2 gave 0.63 g of the title antibiotic as its sulfate salt (51% activity yield), as described by Aburaki, *et al.*, in U.S. Patent No. 4,406,899, issued September 27, 1983, and by Kaplan, *et al.*, in U.S. Patent No. 4,910,301, issued March 20, 1990.

Example 5Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl
chloride hydrochloride

5

To a solution of (9.75 ml, 125.9 mmol) dimethylformamide in dichloromethane (450 ml) at 5°C was added dropwise a solution of (11.21 ml, 125.9 mmol) oxalyl chloride (98%) in dichloromethane (15 ml). Addition was completed in 10 minutes at 5°-7°C. To the resulting slurry, cooled to -25°C, was added (28.5 g, 119.9 mmol) syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt in one aliquot. The slurry was stirred for 3.5 hours at -25°
15 to -30°C, filtered under a nitrogen atmosphere, washed with dichloromethane (100 ml) and hexane (400 ml), and dried at 20°-25°C in vacuo. Yield of crystalline title compound was 30.7 g (72.5%).

20 The title acid chloride acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester hydrochloride in pyridine solution to give essentially one zone (TLC) of the desired desacetoxy cephalosporin ester by reference to an authentic sample.

25

The title acid chloride (200 mg, 0.8 mmol) was hydrolyzed in water. ¹H NMR of the isolated product was identical to the starting syn acid.

Example 6Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl
chloride hydrochloride

5

To a solution of (8.13 ml, 105 mmol) dimethylformamide in dichloromethane (350 ml) at 5°C was added dropwise (9.34 ml, 105 mmol) oxalyl chloride (98% pure) in dichloromethane (5 ml). Maximum
10 temperature reached during the addition was 7°C. The resulting suspension was stirred for 10 minutes at 5°C and then cooled to -27°C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (23.8 g, 100
15 mmol) was added in one aliquot. The suspension was stirred for 2.5 hours at -25° to -30°C, filtered under a nitrogen atmosphere, washed with dichloromethane (25 ml) and hexane (125 ml), and dried at 20°C in vacuo. Yield was 21.39 g (83.5%) of crystalline acid chloride hydrochloride.

20

Anal. Calcd. for $C_6H_7N_3O_2SCl_2$:

C, 28.14; H, 2.76; N, 16.41; S, 12.52;

Found: C, 28.25; H, 2.93; N, 16.32; S, 12.67.

25

1H NMR (DMSO- d_6) δ : 3.93 (CH₃), 7.04 (H5).

Example 7Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

5

A suspension of (87 g, 432.4 mmol) syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid in toluene (870 ml) at 22°C was gassed with two aliquots of HCl; 17.5 g, 480 mmol in 30 minutes and 15.0 g, 410
10 mmol in 20 minutes with 20 minutes agitation between aliquots. The slurry was agitated 1.5 hours at 25°C, filtered under a nitrogen atmosphere, washed with toluene (100 ml) and hexane (400 ml), and dried at 20°-25°C in vacuo. Yield of the title compound was
15 100.2 g (97.5%).

Anal. Calcd. for $C_6H_8N_3O_3SCl$:

C, 30.32; H, 3.39; N, 17.68; S, 13.49;
Cl, 14.92;

20 Found: C, 30.51; H, 3.39; N, 17.54; S, 13.37;
Cl, 14.90.

Example 8Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl
chloride hydrochloride

5

To a solution of (32.4 ml, 419.7 mmol) dimethylformamide in dichloromethane (400 ml) at 5°C was added (37.4 ml, 419.7 mmol) 98% oxalyl chloride dropwise. The resulting suspension was cooled to
10 -25°C and added to a -25°C suspension of (95 g, 399.7 mmol) syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride from Example 7. The suspension was stirred for 2.5 hours at -25° to -28°C, filtered under a nitrogen atmosphere, washed with
15 dichloromethane (100 ml) and hexane (500 ml), and dried at 20°-25°C in vacuo. The yield of crystalline title compound was 84.3 g (82.3%).

Anal. Calcd. for $C_6H_7N_3O_2SCl_2$:

20 C, 28.14; H, 2.76; N, 16.41; S, 12.52;

Found: C, 27.90; H, 3.10; N, 16.14; S, 12.27.

1H NMR (DMSO- d_6) δ : 3.95 (CH₃), 7.04 (H5).

25

Example 9

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate (cefepime)

To a solution of 240 ml acetone and 80 ml water was added 20.0 g of 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]-ceph-3-em-4-carboxylate
10 hydriodide (0.047 mol) and set stirring. Using a Radiometer ABU80 auto titrator with end point set at pH 6.5 and filled with N-methylmorpholine, syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (20.0 g, 0.0785 mol) [prepared in
15 Example 5] was added in 4 portions at 5 minute intervals keeping the pH at 6.5. After the addition was completed, the thin slurry was stirred for an additional 20 minutes at room temperature. The pH of the reaction mixture was lowered to pH 2.65 with 21 ml
20 of 6N H₂SO₄. Precipitation of the title compound occurred. The slurry was seeded and stirred at room temperature for 20 minutes. The pH of the slurry was readjusted to 1.8 with 16 ml of 6N H₂SO₄, and stirring was continued for another 60 minutes. The slurry was
25 filtered in vacuo and washed with 70 ml water-acetone (1:1) and then 70 ml acetone to give 24.09 g (88.5% stoich. weight yield) of the title compound, which is identical to the compound of Example 4 and to the cefepime described by Aburaki, et al., in U.S. Patent
30 No. 4,406,899, issued September 27, 1983, and by Kaplan, et al., in U.S. Patent No. 4,910,301, issued March 20, 1990.

Example 10Preparation of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

5

To a solution of dimethylformamide (8.76 ml, 0.113 mole) in dichloromethane (375 ml) at 5°C, add oxalyl chloride (9.64 ml, 0.111 mole) dropwise keeping the temperature at 5°-6°C. Agitate the suspension for 10 minutes and then cool to -25°C. Add syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt (25.0 g) in aliquots over 11 minutes under a dry nitrogen atmosphere. Agitate the slurry for 2.5 hours at -25°C. Filter the product in a dry nitrogen atmosphere, and wash the cake with dichloromethane (80 ml). Dry the product at 20°-25°C in vacuo over P₂O₅ to yield 23.88 g (88.6%) of the title compound as a pale-yellow, crystalline solid.

20

Anal. Calcd. for C₆H₇N₃O₂SCl₂:

C, 28.14; H, 2.76; N, 16.41; S, 12.52;

Cl, 27.68;

Found: C, 28.06; H, 2.71; N, 16.26; S, 12.30;

Cl, 27.23.

25

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d₄ (¹H NMR), as shown in Figure 1.

30

¹H NMR (CD₄CO₂D) δ: 4.14 (CH₃), 7.10 (H5). Residual acid hydrochloride level from CH₃ (4.11) integral is 5.1%. A trace level of isomeric H5 is seen at 7.67 ppm.

35

Example 11Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl
5 chloride hydrochloride

To a solution of dimethylformamide (17.92 ml, 231.9 mmol) in dichloromethane (375 ml) at 5°C was added oxalyl chloride (19.76 ml, 220.8 mmol).
10 Addition time was 15 minutes at 5°-6°C. The resulting suspension was agitated 10 minutes at 5°-6°C and then cooled to -25°C. 2-(2-Aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (25.0 g, 105.2 mmol) was added. The resulting solution was seeded
15 with the title compound to produce the product slurry. The suspension was agitated for 3.5 hours at -25°C, filtered in a dry nitrogen atmosphere, washed with dichloromethane (150 ml), and dried at 20°-25°C in vacuo. Yield was 9.61 g (35.7%) of crystalline title
20 compound.

An aliquot of the solid acid chloride hydrochloride product acylated 7-amino desacetoxy cephalosporanic acid diphenylmethyl ester
25 hydrochloride in pyridine solution to give essentially single zone (TLC) product which was coincident with and inseparable from an authentic sample of the desired desacetoxy cephalosporin ester.

Example 12Preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

5

The experimental procedure of Example 1 in Czechoslovakian Patent No. 238,950 was repeated as follows:

10 A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (4.0 g) having a KF of 0.06% was slurried in 30 ml of benzene at 21°C. One drop of dimethylformamide was added, followed by 5.0 grams of powdered phosphorous pentachloride
15 in one aliquot. The temperature rose to 34°C in approximately 2 minutes and was then raised to 40°C in 1 minute to provide a complete solution. The solution was allowed to cool, and at 36°C, a precipitate formed. After stirring for 30
20 minutes, the temperature was 22°C. The light-yellow solid was collected by filtration under a dry nitrogen atmosphere and washed with 30 ml of benzene and 20 ml of heptane. Yield was 2.88 g after drying in vacuo over P₂O₅ at 20°-25°C for 18
25 hours.

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d₄ (¹H NMR) as shown in
30 Figure 2 which shows the H5 at 7.56 ppm and CH₃ at 4.34 ppm. This spectrum is consistent with a product of the title compound having the anti-isomer configuration and not the syn-isomer as disclosed by said Czechoslovakian patent.

35

Example 13Preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

5

The experimental procedure of Example 2 in Czechoslovakian Patent No. 238,950 was repeated as follows:

10 A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (4.0 g) having a KF of 0.06% was slurried in 20 ml of acetonitrile which had been adjusted to have a KF of 0.22%. A drop of dimethylformamide was added, and the

15 temperature was 20°C. On addition of 6.0 grams of powdered phosphorus pentachloride, the temperature rose to 40°C and complete solution was obtained. The solution was cooled to 20°C, and a precipitate formed at 33°C. After stirring for 30 minutes,

20 the product was collected under a dry nitrogen atmosphere and washed with 30 ml of benzene and 20 ml of heptane. Yield was 1.86 g after drying for 18 hours at 20°-25°C in vacuo over P₂O₅.

25 The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d₄ (¹H NMR) as shown in Figure 3 which shows the H5 at 7.56 ppm and CH₃ at 4.31 ppm. The ¹H NMR spectrum is consistent with a

30 product of the title compound having the anti-isomer configuration and not the syn-isomer as disclosed by said Czechoslovakian patent.

Example 14Preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino
acetyl chloride hydrochloride

5

The experimental procedure of Example 3 in Czechoslovakian Patent No. 238,950 was repeated as follows:

10 Concentrated hydrochloric acid (0.16 ml) was added to 30 ml of dichloromethane. After cooling to -10°C, 6.5 g of phosphorus pentachloride was added in increments. After warming to 0°C, 4.0 g of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino
15 acetic acid having a KF of 1.06% was added in one aliquot. The temperature rose to 2°C. A complete solution was obtained after 9 minutes at 0°C. After 40 minutes, a precipitate started to form. The product slurry was agitated for 2.8 hours at
20 2°-3°C, filtered under a dry nitrogen atmosphere, washed with 30 ml of benzene and 20 ml of heptane, and dried in vacuo at 20°-25°C over P₂O₅ for 18 hours. The yield was 3.42 g of light-yellow powder.

25

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance spectrum in acetic acid-d₄ (¹H NMR) as shown in Figure 4 which shows the H5 at 7.56 ppm and CH₃ at
30 4.31 ppm. The ¹H NMR spectrum is consistent with a product of the title compound having the anti-isomer configuration and not the syn-isomer as disclosed by said Czechoslovakian patent.

35

Example 15Attempted preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

5

The general procedure described in Example 7 of U.S. Patent No. 4,203,899 for the conversion of protected aminothiazolacetic acid to the corresponding acid chloride was applied to the unprotected
10 aminothiazolacetic acid described herein as follows:

A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt (2.38 g, 0.01 mole) was suspended in 30.5 ml of
15 benzene and cooled to 20°C. Oxalyl chloride (2.09 ml, 0.024 mole) was added, followed by dimethylformamide (0.50 ml, 0.0065 mole). The temperature rose to 22°C with vigorous gas evolution. Within 20 minutes at 20°C, the gassing
20 had subsided and the slurry was agitated at 20°C ± 2°C for 2 hours. The slurry was concentrated in vacuo to remove the solvent, and the resulting yellow product was dried in vacuo over P₂O₅ at 20°-25°C for 16 hours. Yield was 2.59 g.

25

The product of the foregoing experiment was characterized by proton nuclear magnetic resonance (¹H NMR) spectrum in acetic acid-d₄ which shows the H5 at 7.60 ppm and CH₃ at 4.37 ppm. The spectrum of the
30 product is consistent with the title compound having the anti-isomer configuration.

Example 16Attempted preparation of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

5

The general procedure described in Example 59 of U.S. Patent No. 4,203,899 for the conversion of protected aminothiazolacetic acid to the corresponding acid chloride was applied to the unprotected
10 aminothiazolacetic acid described herein as follows:

A sample of syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt (2.38 g, 0.01 mole) was suspended in 25 ml of
15 dichloromethane. After cooling to 4°C, 2.08 g (0.01 mole) of phosphorous pentachloride was added. With ice cooling, the temperature rose to 6°C, and after cooling back to 4°C, the slurry was stirred for 1 hour. The precipitate was collected
20 by filtration under a dry nitrogen atmosphere, washed with dichloromethane (10 ml), and dried in vacuo at 20°-25°C to give 1.4 g of pale-yellow solid.

25 The product of the foregoing experiment was characterized by proton nuclear magnetic resonance (¹H NMR) spectrum in acetic acid-d₄ which shows the H5 at 7.61 ppm and the CH₃ at 4.34 ppm. The spectrum of the product is consistent with the title compound having
30 the anti-isomer configuration. Additionally, the product is contaminated with unconverted acid (¹H NMR having H5 at 7.07 ppm and CH₃ at 4.06 ppm) which was further confirmed by spiking with starting acid.

Example 17

Acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]-ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (anti-form from Example 14)

To a precooled solution of 9 ml acetone and 3.4 ml water at 10°C was added 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl] ceph-3-em-4-carboxylate HI salt (1.13 g, 2.66 mmol). 2-(2-Aminothiazol-4-yl)-2-methoxyiminoacetyl chloride hydrochloride (1.09 g, 4.21 mmol) [prepared in Example 14] was added in 5 portions at 0°C along with triethylamine (0.37 ml, 2.66 mmol) to keep the pH at 6.0-7.0. The reaction mixture was stirred at ambient temperature for 15 minutes. Assay of the resulting solution by high pressure liquid chromatography (gradient C₁₈ column, 2% to 25% acetonitrile in 0.005M NH₄H₂PO₄) showed 72.4 area percent of anti-cefepime at 13.08 minutes and no detectable syn-cefepime which was expected at about 8.5 minutes retention time. Acidification with sulfuric acid to pH 1.9 gave 1.48 g of anti-cefepime as the sulfate salt. The identification of the product was confirmed by ¹H-NMR spectroscopy (DMSO-d₆) and was shown to contain 0.58 mole of triethylamine salt.

Example 18

Acylation of 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate HI salt using 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (anti-isomer from Example 14)

To a precooled solution of 108 ml of acetone and 40.5 ml of water at 10°C was added 7-amino- [(1-
10 methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate HI salt (13.5 g, 0.0317 mol). The pH of the slurry was adjusted to 7.0 using 2.7 ml of 14% NH₄OH. With the temperature at 10°C, 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (13.05 g,
15 0.015 mol) [prepared by the procedure of Example 14] was added portionwise over 60 minutes while using 14% NH₄OH (27 ml) to maintain a pH of 6.3-7.0 during the first half of the addition and at pH 6.1-6.6 during the second half of the addition. The reaction mixture
20 was stirred at ambient temperature for 30 minutes. The reaction mixture was polished filtered and washed with 6 ml of 2:1 acetone/water then 6N H₂SO₄ (15 ml) was slowly added to the filtrate to adjust the pH to 1.87-1.90. After stirring for 1 hour, the insolubles
25 were filtered off and the cake washed with 21 ml of 2:1 acetone/water and then with 30 ml of acetone. To the filtrate was added 1 liter of acetone over 30 minutes and the mixture was stirred at 5-8°C for 40 minutes. The product was collected by filtration,
30 washed twice with 24 ml of 4:1 acetone/water, 60 ml of acetone and dried under vacuum to yield 20.64 g (116% stoich weight) of anti-cefepime as the sulfate salt (95.4% pure by HPLC). The ¹H-NMR spectra was consistent with the structure of anti-cefepime
35 containing about 3 moles of ammonium salts.

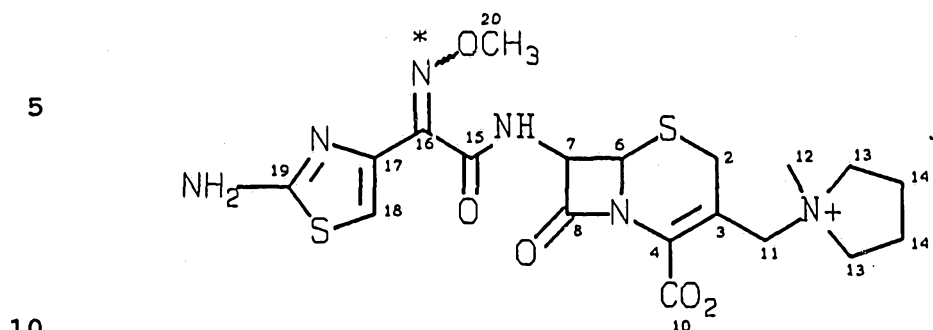
Example 19

Comparison of a product of Example 9 (cefepime syn-isomer) and a product of Example 17 (cefepime anti-isomer) show the following differences in physical characteristics.

High pressure liquid chromatography of the cefepime isomers was carried out on a Waters μ Bondapak C₁₈ column (3.9 x 300 mm) using a solvent system mixture of 1000 ml water containing 2.88 g (0.013 mol) heptane sulfonic acid sodium salt and pH adjusted to 4.0 with acetic acid and 100 ml acetonitrile at a flow rate of 2.0 ml/minute. The products were visualized with a Water Model 450 variable wavelength detector set at 254 λ to give the following results.

	<u>Retention Time</u> (min.)
<u>Syn</u> -isomer of cefepime (Example 9)	10.5
<u>Anti</u> -isomer of cefepime (Example 17)	37.8

The proton NMR spectra of the syn and anti-methoxime isomers of cefepime as the dihydrochloride salts was carried out on a Bruker AMX-400 FT NMR spectrometer using deuterated dimethyl sulfoxide as the solvent. The reported chemical shifts are referenced to DMSO at 2.49 ppm. The numbering system shown below in the Formula and Table are for convenience only.

Syn- and anti-cefepime

* Wavy bond denotes syn- and anti-methoxyimino isomers.

Comparison Table of Proton NMR Chemical Shifts (ppm)

15

<u>Assignment</u>	<u>Syn-Cefepime</u>	<u>Anti-Cefepime</u>
C2-H2	4.04, 3.65	4.02, 3.65
C6-H	5.33	5.31
C7-H	5.88	5.85
20 C11-H2	4.60, 4.31	4.59, 4.30
C12-H3	2.93	2.93
C13-H4	3.7, 3.4	3.6, 3.3
C14-H4	2.10	2.10
C18-H4	6.88	7.57
25 C20-H3	3.92	4.05
NH	9.83	9.56
NH2	8.60	8.70

30

The ¹H NMR spectra of the two methoxime isomers of cefepime, as shown above, are significantly different. The thiazole ring CH(18) of the syn-(Z) methoxime isomer, at 6.88 ppm, falls upfield of the anti-(E) methoxime isomer CH(18), at 7.57.

35

Example 20Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

5

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (85.3 g, 424 mmol) in dichloromethane (570 ml) was milled under nitrogen for 15 minutes in a blender. The resulting fine suspension was diluted with
10 dichloromethane (100 ml), and transferred under nitrogen to a 1L Buchi jacketed reactor. The reactor was pressurized with nitrogen (5 psi) and the mixture stirred at 375 rpm and cooled to -2°C. Hydrogen chloride (15.4 g, 424 mmol) was introduced into the
15 headspace of the reactor at 0.2 g per minute. There was a temperature rise of 2°C. The mixture was stirred for a further 30 minutes at 0°C, filtered, and washed with dichloromethane (350 ml) under nitrogen. The solid was dried in vacuo at 45°C for 18 hours.
20 The title compound was an off-white powder (110.9 g, 111% uncorrected yield).

Anal. Calcd. for $C_6H_8N_3O_3S\text{Cl}$:

25 C, 30.32; H, 3.39; N, 17.68;
S, 13.49; Cl, 14.91;
Found: C, 29.37; H, 3.17; N, 16.34;
S, 12.70; Cl, 16.99.

^1H NMR (DMSO- d_6) δ : 4.05 (s, 3H, CH_3), 5.9 (s, 15
30 mole % residual CH_2Cl_2), 7.1 (s, 1H, C-5 H). Signals were also seen at 4.18 (s, 3H, CH_3) and 7.7 (s, 1H, C-5 H) corresponding to ca. 2% of anti-isomer.

Example 21Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride salt

5

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (25 g, 124 mmol) in acetonitrile (125 ml) under nitrogen was titrated with a 1.39M solution of HCl in acetonitrile (89.2 ml, 123.9 mmol) and was maintained at 10° to 15°C. The mixture was stirred for a further 30 minutes at 10° to 15°C, filtered, and washed with acetonitrile (200 ml) under nitrogen. The solid was dried in vacuo at 45°C for 3 hours. The title compound was an off-white powder (29.5 g, 97.4% uncorrected yield).

¹H NMR (CD₃OD) δ: 2.05 (s, 13 % wt/wt, residual acetonitrile), 4.1 (s, 3H, CH₃), 7.1 (s, 1H, C-5 H). Signals were also seen at 4.2 (s, 3H, CH₃) and 7.8 (s, 1H, C-5 H) corresponding to ca. 0.5% of anti-isomer.

Example 22

25

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid hydrochloride (56.24 g, 210 mmol), containing approximately 11% wt/wt residual acetonitrile) in dichloromethane (450 ml) was milled under nitrogen for 3 minutes in a blender, then cooled to -35°C and transferred under nitrogen over 5 minutes to a well stirred slurry of Vilsmeier reagent, also at -35°C.

The slurry of Vilsmeier reagent was prepared by adding oxalyl chloride (28.2 g, 221 mmol) portionwise to a solution of dimethylformamide (16.89 g, 231 mmol) in dichloromethane (300 ml) at 0°C, followed by cooling to -35°C. During the addition, the reaction temperature rose to -28°C. After the addition, the reaction mixture was seeded with the product. After a further 2.5 hours at -28°C to -35°C the mixture was filtered and the filter cake washed with dichloromethane (200 ml) under nitrogen. Nitrogen was passed through the cake for 30 minutes, then the solid was dried in vacuo at room temperature for 12 hours. The title compound was obtained as an off-white powder (42.9 g, 72% yield).

15

¹H NMR (CD₃OD) δ: 4.06 (s, 3H, CH₃), 7.12 (s, 1H, C-5 H). Signals were seen at 7.18 corresponding to ca. 5% of the acid hydrochloride salt, and at 7.80 (s, C-5 H) corresponding to ca. 0.5% of the anti-isomer. After derivatization with diethylamine in acetonitrile, HPLC analysis showed the title compound (syn-isomer, as its diethylamide derivative) with a retention time of 9.6 minutes, the acid hydrochloride salt with a retention time of 2.8 minutes, and the anti-isomer (as its diethylamine derivative) with a retention time of 16.4 minutes. The ratio of the syn-isomer:acid hydrochloride salt:anti-isomer was 90:5:<1.

Example 23Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl
chloride hydrochloride

5

Syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid (84.7 g, 421 mmol) in dichloromethane (570 ml) was milled under nitrogen for 20 minutes in a blender. The resulting fine suspension was diluted with
10 dichloromethane (100 ml), and transferred under nitrogen to a 1L Buchi jacketed reactor. The reactor was pressurized with nitrogen (5 psi) and the mixture stirred at 375 rpm and cooled to -2°C. Hydrogen chloride (15.3 g, 421 mmol) was introduced into the
15 headspace of the reactor at 0.2 g per minute. There was a temperature rise of 2°C. The mixture was stirred for a further 30 minutes at 0°C, milled for 3 minutes in the blender, then cooled to -35°C and transferred under nitrogen over 5 minutes to a well
20 stirred slurry of Vilsmeier reagent, also at -35°C. The slurry of Vilsmeier reagent was prepared by adding oxalyl chloride (56.1 g, 439 mmol) portionwise to a solution of dimethylformamide (33.8 g, 462 mmol) in dichloromethane (880 ml) at 0°C, followed by cooling
25 to -35°C. During the addition, the reaction temperature rose to -28°C. After the addition, the reaction mixture was seeded with product. After a further 2.5 hours at -28°C to -35°C the mixture was filtered and the filter cake washed with
30 dichloromethane (350 ml) under nitrogen. Nitrogen was passed through the cake for 30 minutes, then the solid was dried in vacuo at room temperature for 12 hours. The title compound was obtained as an off-white powder (95.2 g, 89% uncorrected yield).

35

Anal. Calcd. for $C_6H_7N_3O_2SCl_2$:
C, 28.14; H, 2.76; N, 16.41;
S, 12.52; Cl, 27.68;
Found: C, 28.11; H, 2.62; N, 16.20;
S, 12.22; Cl, 26.74.

1H NMR (CD_3OD) δ : 4.06 (s, 3H, CH_3), 7.12 (s, 1H, C-5 H). Signals were also seen at 7.18 (s, C-5 H) corresponding to ca. 4% of acid hydrochloride salt, and at 7.80 (s, C-5 H) corresponding to ca. 2% of the anti-isomer. After derivatization with diethylamine in acetonitrile, HPLC analysis showed the title compound (as its diethylamide derivative) with a retention time of 9.6 minutes, the starting acid with a retention time of 2.8 minutes, and the anti-isomer (as its diethylamide derivative) with a retention time of 16.4 minutes. The ratio of syn-isomer:starting acid:anti-isomer was 90:4:2.

20

EXAMPLE 24

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate dihydrochloride hydrates

7-Amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate hydriodide (15.01 g; 35.29 mmol) was dissolved gradually in 60 ml of water while keeping the pH below 6.5 using triethylamine. Acetone (120 ml) was added, and the resulting aqueous acetone solution was cooled to between $-15^\circ C$ and $-20^\circ C$. While maintaining the pH below 7.5 and above 5 using a Radiometer ABU80 autotitrator filled with

triethylamine set at a pH 6.5 endpoint, syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (14.6 g active; 45.4 mmol) was added over a period of 2 hours and 7 minutes. The reaction
5 mixture was allowed to warm gradually from -12° to 5°C until the acylation reached its completion (as indicated by HPLC); then the reaction mixture was polish filtered.

10 The filtrate was acidified with 12N hydrochloric acid (17.6 ml; 0.212 mol), then acetone (210 ml) was added to initiate crystallization. The slurry was stirred for 1.25 hours then diluted further with acetone (195 ml). The slurry was cooled to 0 - 5°C,
15 stirred for 0.75 hours then filtered. After vacuum filtration, the wet cake was washed with acetone and dried in vacuo at 45°C overnight. The dihydrochloride hydrate salt form of the title compound was isolated with 95.5% purity (12.8 g; 63.8% stoichiometric weight
20 yield).

EXAMPLE 25

5 Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-
methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-
methyl]ceph-3-em-4-carboxylate dihydrochloride
10 hydrates

To a cold (-30°C) solution of acetone (120 ml) and water (40 ml), 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate hydriodide (15.0 g; 10 35.0 mmol) and syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (10.49 g active; 40.9 mmol) were added simultaneously and separately as solids over 30 minutes. The reaction pH was maintained between 5.5 and 7.0 using a Radiometer 15 ABU80 autotitrator filled with triethylamine set at a pH 6.5 endpoint, and the temperature was maintained between -20°C and -40°C. Once the addition of reactant was finished, the resulting thin, opaque slurry was warmed to 0 - 5°C and stirred until the 20 solids dissolved (20 minutes) at which point high performance liquid chromatography indicated the acylation was complete. The reaction mixture was then polish filtered.

25 The filtrate was acidified with 12N hydrochloric acid (17.6 ml; 0.212 mol), then acetone (150 ml) was added to initiate crystallization. The slurry was stirred for 1 hour then diluted further with acetone (315 ml) over 30 minutes, stirred for 30 minutes at 30 room temperature and cooled to 0 - 5°C for 1 hour. After vacuum filtration, the product was washed with acetone (250 ml) and dried in vacuo at 45°C. The title compound was isolated as its dihydrochloride hydrate salt with 94.9% purity (17.68 g; 87.7% 35 stoichiometric weight yield). Water content by Karl Fischer method was 4.45%.

EXAMPLE 26

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-
methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-
5 methyl]ceph-3-em-4-carboxylate dihydrochloride
hydrates

7-Amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate hydriodide (14.75 g; 34.7 mmol) was
10 dissolved gradually in 60 ml of water at room
temperature while keeping the pH below 6.5 using
triethylamine. The red orange solution was cooled to
0°C - 5°C and was decolorized using activated carbon
(3 g). The carbon was filtered, and the resulting
15 light amber solution was stored at 0° - 5°C. The
carbon cake was washed with 22.5 ml of water. The
wash was combined with 68 ml of acetone, and the
resulting aqueous acetone solution was cooled to
-30°C. Maintaining the temperature between -20°C and
20 -30°C and maintaining the pH between 5.5 and 6.5 using
a Radiometer ABU80 autotitrator filled with
triethylamine set at a pH 6.5 endpoint, the solution
of decolorized 7-amino-3[(1-methyl-1-
pyrrolidinio)methyl]ceph-3-em-4-carboxylate was
25 dripped steadily into the cold aqueous acetone
solution from the carbon cake wash while
simultaneously adding syn 2-(2-aminothiazol-4-yl)-2-
methoxyimino acetyl chloride hydrochloride (9.06 g
active; 35.4 mmol) and 180 ml acetone (steady stream).
30 Once the reactant addition was complete, the reaction
mixture was warmed to 0 - 5°C and was allowed to stir
until the solids dissolved. When the acylation was
complete (as indicated by HPLC), the reaction mixture
was polish filtered.

The filtrate was acidified with 12N hydrochloric acid (17.6 ml; 0.212 mol) then diluted at room temperature with acetone (690 ml) over one hour and held at room temperature for one hour. The product
5 was vacuum filtered, washed with acetone (250 ml), then dried in vacuo at approximately 45°C for 15 hours. The title compound was isolated with a 89.6% purity (15.44 g; 76.4% stoichiometric weight yield). Water content by Karl Fischer method was 4.07%.

10

EXAMPLE 27

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-
15 methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-
methyl]ceph-3-em-4-carboxylate dihydrochloride
hydrates

To a cold (-30°C) solution of acetone (120 ml) and
20 water (40 ml), 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate hydrochloride (14.34 g; 43.0 mmol) and syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (11.01 g active; 43.0 mmol) were added
25 simultaneously and separately as solids over 30 minutes. The reaction pH was maintained between 5.5 and 7.0 using a Radiometer ABU80 autotitrator filled with triethylamine set at a pH 6.5 endpoint, and the temperature was maintained between -20°C and -40°C.
30 Once the reactant addition was finished, the resulting thin, opaque slurry was warmed to 0° - 5°C and stirred until the solids dissolved (20 minutes) at which point high performance liquid chromatography indicated the acylation was complete. The reaction mixture was
35 polish filtered.

The filtrate was acidified with 12N hydrochloric acid (21.4 ml; 0.257 mol), then acetone (75 ml) was added to initiate crystallization. The crystallization slurry was stirred for 1 hour then
5 diluted with acetone (505 ml) over 30 minutes. After stirring for 30 minutes at room temperature, the slurry was cooled to 0 - 5°C for 1 hour and filtered. The product was washed with acetone (250 ml) and dried
10 in vacuo at 45°C. The title compound was isolated as its dihydrochloride hydrate salt with 93.5% purity (18.81 g; 85.1% stoichiometric weight yield). Water content by Karl Fischer method was 4.2%.

15

EXAMPLE 28Conversion of Cefepime Dihydrochloride Monohydrate to Cefepime Dihydrochloride Dihydrate

20 Cefepime dihydrochloride monohydrate (300 g, purity by HPLC 99.9%, KF 3.8%) was dissolved in deionized water (1200 ml). Hydrochloric acid 6N (132 ml, 1.5 equivalent) was added. The solution was filtered and washed with deionized water (300 ml).

25

Acetone (1500 ml) was added to the filtered solution. Additional acetone (4000 ml) was added dropwise over 20 minutes. The solution was held at cloud point until heavy dihydrate crystals formed
30 (needles by microscopic analysis; seeding optional at cloud point). Additional acetone (8000 ml) was added over a 25 minute period. The thick slurry was stirred at 25°C for one hour.

The crystal form was confirmed to be dihydrate (needles) by microscopic analysis comparing the crystals to an authentic sample. The slurry was filtered and washed with acetone (2 x 1500 ml). The
5 cake was dried at 40°C in vacuo for 15 hours. The yield of cefepime dihydrochloride dihydrate was 305.10 g (98.6%), purity by HPLC 99.0%, KF 6.5%.

EXAMPLE 29

10

Conversion of Cefepime Dihydrochloride Dihydrate to Cefepime Dihydrochloride Monohydrate

Cefepime dihydrochloride dihydrate (15.0 g, purity
15 by HPLC 99.2%, KF 6.4%) was dissolved in deionized water (75 ml). Hydrochloric acid 6N (0.9 ml, 0.2 equivalent) was added. The solution was filtered through a .45 micron filter.

20 Acetone (200 ml) was added dropwise to the filtered solution over 20 minutes to give a cloudy solution (optional seeding at this point). Without holding at this point more acetone (400 ml) was added dropwise over 40 minutes. The slurry was cooled in a
25 ice bath at 0° to 5°C for one hour.

The crystal form was confirmed to be monohydrate by microscopic analysis comparing the crystals to an authentic sample. The slurry was filtered and washed
30 with acetone (2 x 60 ml). The cake was dried at 40°C in vacuo for 15 hours. The yield of cefepime dihydrochloride monohydrate was 13.28 g (91.8%), and the crystal structure was confirmed to be the same as described by Kaplan, et al in U.S. Patent
35 No. 4,910,301.

EXAMPLE 30

5 Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-
methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-
methyl]ceph-3-em-4-carboxylate dihydrochloride
hydrates

To a cold (-22°C) solution of acetone (120 ml) and water (40 ml), 7-amino-3-[(1-methyl-1-pyrrolidinio)-
10 methyl]ceph-3-em-4-carboxylate hydriodide (14.67 g active; 0.0345 mol) and syn-2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (9.93 g active; 0.0388 mol) were added simultaneously and separately as solids over 25 minutes. The reaction pH
15 was maintained between 5.0 and 7.0 using a Radiometer ABU80 autotitrator filled with triethylamine set at a pH 6.5 endpoint, and the temperature was maintained between -20°C and -30°C. Once the reactant addition was finished, the resulting opaque slurry was warmed
20 to 0 - 5°C and stirred until the solids dissolved at which point high performance liquid chromatography indicated the acylation was complete. The reaction mixture was polish filtered, and the filtrate was divided into two equal portions.

25

Method A

One portion of the above filtrate was acidified with 12N hydrochloric acid (8.8 ml; 0.106 mol) and diluted with acetone (114 ml) until the filtrate
30 clouded. The filtrate was seeded with cefepime dihydrochloride dihydrate crystals (0.5 g) and the slurry was heated at 40°C for approximately 3 hours. The mixture was cooled at 0 - 5°C for 1 hour and filtered. The product was washed with acetone and
35 dried in vacuo at room temperature. The

dihydrochloride salt of the title compound was crystallized with 93.3% purity (7.34 g; 67.3% stoichiometric weight yield). Water content by Karl Fischer analysis was 4.1% and FT-IR (Diffuse Reflectance with KBr) analysis with absorbance peaks at 3574 cm^{-1} and 3432 cm^{-1} indicated the product to be a mixture of monohydrate (granular-like crystals) and dihydrate (needle-like crystals) forms of the title compound.

10

Method B

The second filtrate portion was acidified with 12N hydrochloric acid (8.8 ml; 0.106 mol) and diluted with acetone (206 ml) over 1 hour. The slurry was held until crystallization was observed, then it was cooled to 0 - 5°C and held 1 hour. The slurry was filtered and the product was washed with acetone then dried in vacuo at 45°C. The dihydrochloride salt of the title compound was crystallized with 95.3% purity (8.6 g; 85.3% stoichiometric weight yield). Water content by Karl Fischer analysis was 4.6% and FT-IR (Diffuse Reflectance with KBr) analysis indicated that the product was the monohydrate form of the title compound with less than about 0.4% of the dihydrate form.

25

EXAMPLE 31

Preparation of 7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate dihydrochloride dihydrate

To a cold (-22°C) solution of acetone (120 ml) and water (40 ml), 7-amino-3-[(1-methyl-1-pyrrolidinio)-methyl]ceph-3-em-4-carboxylate hydriodide (14.61 g active; 0.0344 mol) and syn 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride (9.94 g active; 0.0388 mol) were added simultaneously and separately as solids over 25 minutes. The reaction pH was maintained between 5.0 and 7.5 using a Radiometer ABU80 autotitrator filled with triethylamine set at a pH 6.5 endpoint, and the temperature was maintained between -20°C and -30°C. Once the reactant addition was finished, the resulting opaque slurry was warmed to 0-5°C and stirred until the solids dissolved at which point high performance liquid chromatography indicated the acylation was complete. The reaction mixture was polish filtered, and the filtrate was divided into two equal portions.

25

Method A

One portion of the above filtrate was acidified with 12N hydrochloric acid (11.7 ml; 0.1404 mol) and acetone was added to the filtrate with stirring until it clouded. The filtrate was seeded with cefepime dihydrochloride dihydrate crystals (0.3 g) and the slurry was heated at 50°C for approximately 1 hour. The mixture was cooled to room temperature, diluted with acetone and stirred for 15 hours. The slurry was again heated at 40°C for 1 hour and diluted with

35

acetone. A total of 280 ml of acetone was used to crystallize the product. After cooling the mixture gradually to 0-5°C for 1 hour, the slurry was filtered, washed with acetone (125 ml) and the product
5 dried in vacuo at 45°C. The title compound, cefepime dihydrochloride dihydrate, was isolated with a 97.8% purity (8.19 g; 80.9% stoichiometric weight yield). Water content by Karl Fischer analysis was 6.5%, and FT-IR (Diffuse Reflectance with KBr) analysis showed
10 absorbance peaks at 3574 cm⁻¹ and 3432 cm⁻¹ which confirmed the product to be the dihydrate (needle-like crystals) form.

Method B

15 The second filtrate portion was acidified with 12N hydrochloric acid (14.6 ml; 0.1752 mol) and acetone was added to the filtrate with stirring until it clouded. The filtrate was seeded with cefepime dihydrochloride dihydrate crystals (0.3 g) and the
20 slurry was stirred for approximately 1.5 hours at room temperature. The mixture was diluted further with acetone, stirred at room temperature for 15 hours then heated at 40°C for 1 hour. Additional acetone was added to dilute the mixture to provide a total of
25 231 ml of acetone. After cooling gradually to 0-5°C for 1 hour, the product was collected by filtration, washed with 125 ml of acetone and dried in vacuo at 45°C. The title compound, cefepime dihydrochloride dihydrate, was isolated with 96.3% purity (8.68 g;
30 85.7% stoichiometric weight yield). Water content by Karl Fischer analysis was 6.7%, and FT-IR (Diffuse Reflectance with KBr) analysis showed absorbance peaks at 3574 cm⁻¹ and 3432 cm⁻¹ which confirmed the product to be the dihydrate (needle-like crystals) form.

~~WHAT IS CLAIMED IS:~~

The claims defining the invention are as follows:

1. A process for the preparation of an antibiotic, cefepime dihydrochloride hydrate which is
5 substantially free of the anti-isomer and the Δ^2 isomer which ~~comprises~~ ^{includes} reacting 7-amino-3-[(1-methyl-1-pyrrolidinio)methyl]ceph-3-em-4-carboxylate with the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride substantially free of
10 the anti-isomer in an aqueous-organic solvent at a pH of about 5.0 to 7.5.
2. A process of Claim 1 further ~~comprising~~ ^{including} the preparation of said syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetyl chloride hydrochloride
15 substantially free of the anti-isomer by reacting the anhydrous acid hydrochloride salt of the syn-isomer of 2-(2-aminothiazol-4-yl)-2-methoxyimino acetic acid with a mixture containing at least one molar
20 equivalent of oxalyl chloride and at least one molar equivalent to a slight excess of dimethylformamide to an amount of said oxalyl chloride in an inert organic solvent at a temperature of less than -10°C .
- 25 3. A process of Claim 1 further ~~comprising~~ ^{including} the addition of a sufficient amount of an acid and a water-miscible organic solvent to the reaction mixture to produce said antibiotic.
- 30 4. A process of Claim 1 wherein the pH is about 6.2 to 6.8.
5. A process of Claim 1 wherein the aqueous-organic solvent is aqueous-acetone.



6. A process of Claim 2 wherein the amount of oxalyl chloride is 1.0 to about 2.0 molar equivalents and the amount of dimethylformamide is a slight equimolar excess of said oxalyl chloride.
- 5
7. A process of Claim 6 wherein the amount of oxalyl chloride is 1.05 molar equivalents and the amount of dimethylformamide is 1.075 equivalents to said anhydrous acid hydrochloride salt.
- 10
8. A process of Claim 2 wherein the temperature is about -15°C to -40°C .
9. A process of Claim 2 where the inert organic
15 solvent is dichloromethane or acetonitrile.
10. A process of Claim 3 wherein the acid is sulfuric acid.
- 20 11. A process of Claim 10 further ^{including} ~~comprising~~ the neutralization of the resulting sulfate salt with base followed by the addition of a sufficient amount of hydrochloric acid to produce said antibiotic.
- 25 12. A process of Claim 1 wherein said antibiotic contains from 2.5% to 7.0% by weight of water.
13. A process of Claim 1 wherein said antibiotic is cefepime dihydrochloride monohydrate.
- 30 14. A process of Claim 1 wherein said antibiotic is cefepime dihydrochloride dihydrate.

15. A process for the preparation of antibiotic, cefepime dihydrochloride hydrate which is substantially free of the anti-isomer and the Δ^2 isomer, substantially as hereinbefore described with reference to any one of the examples.

5

16. An antibiotic, cefepime dihydrochloride hydrate prepared by the process of any one of claims 1 to 15.

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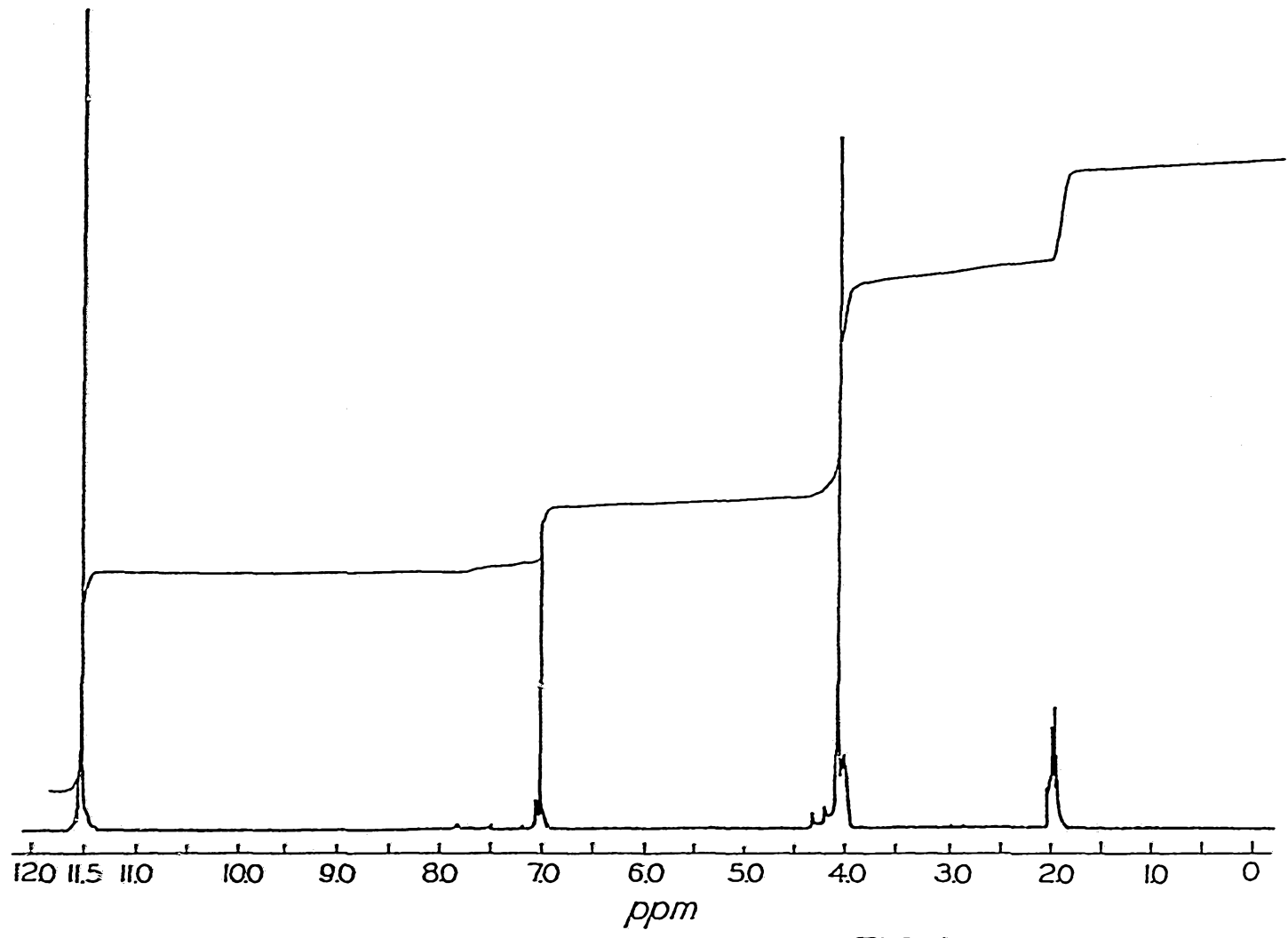


FIG. I

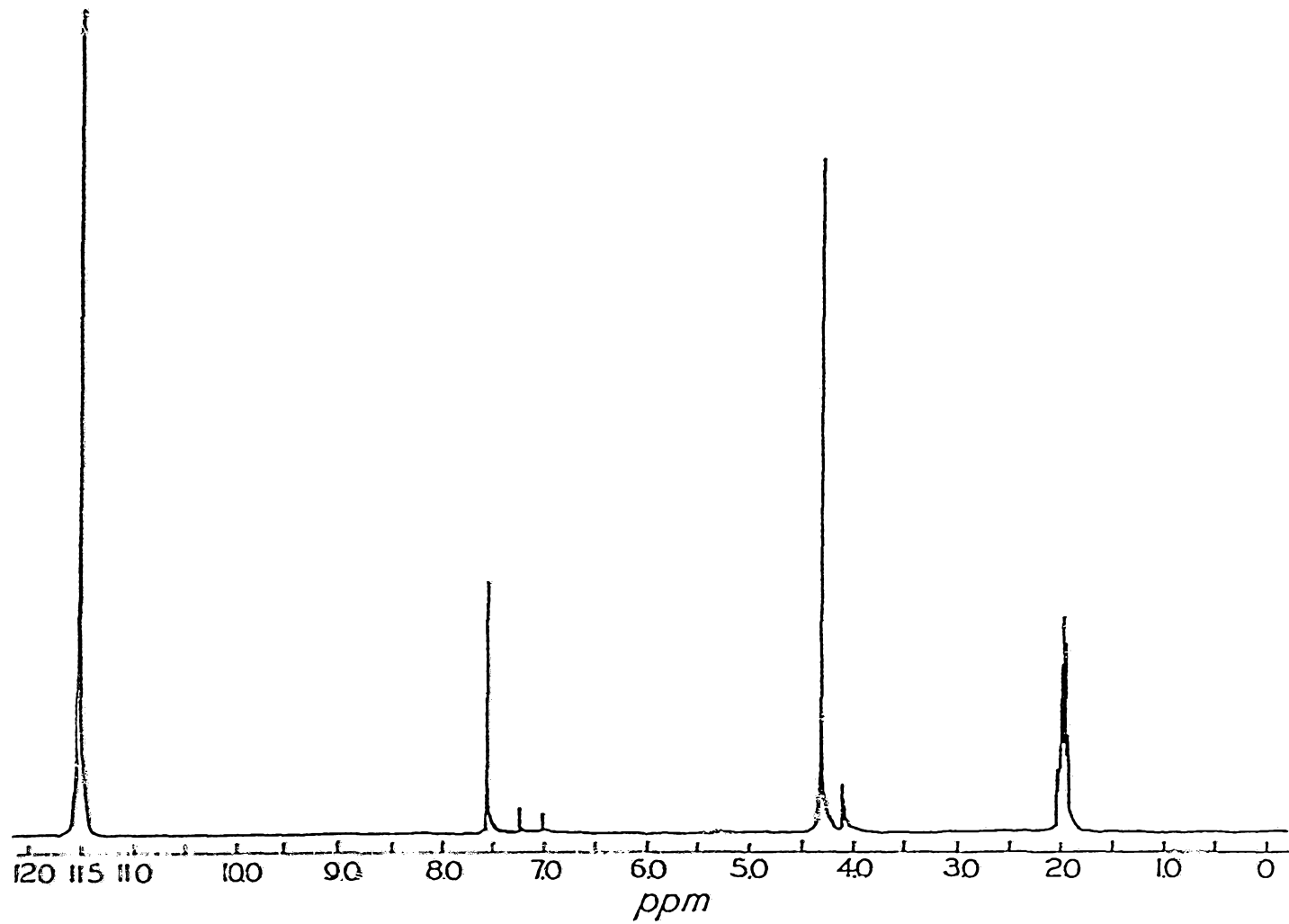


FIG. 2

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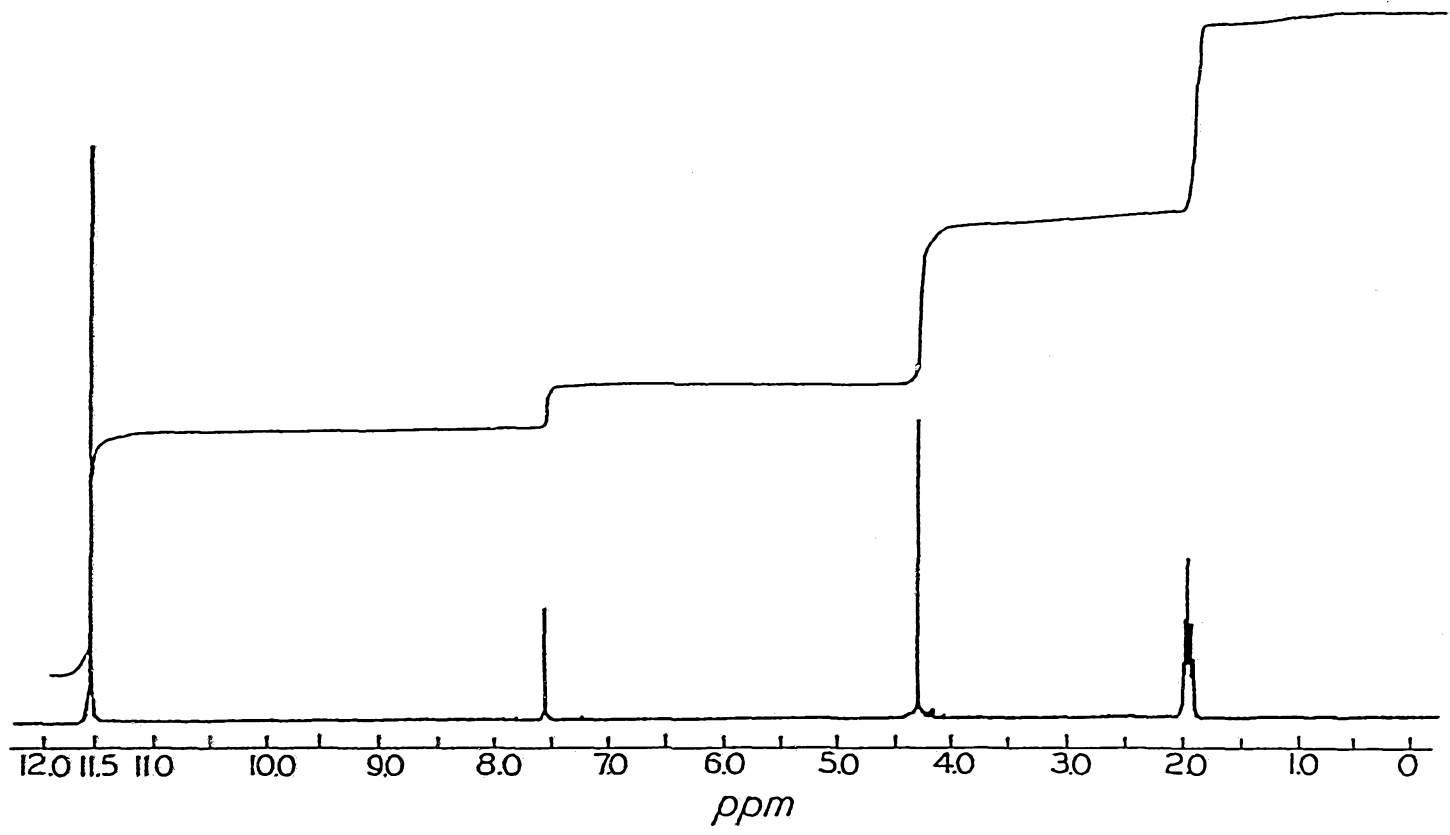


FIG. 3

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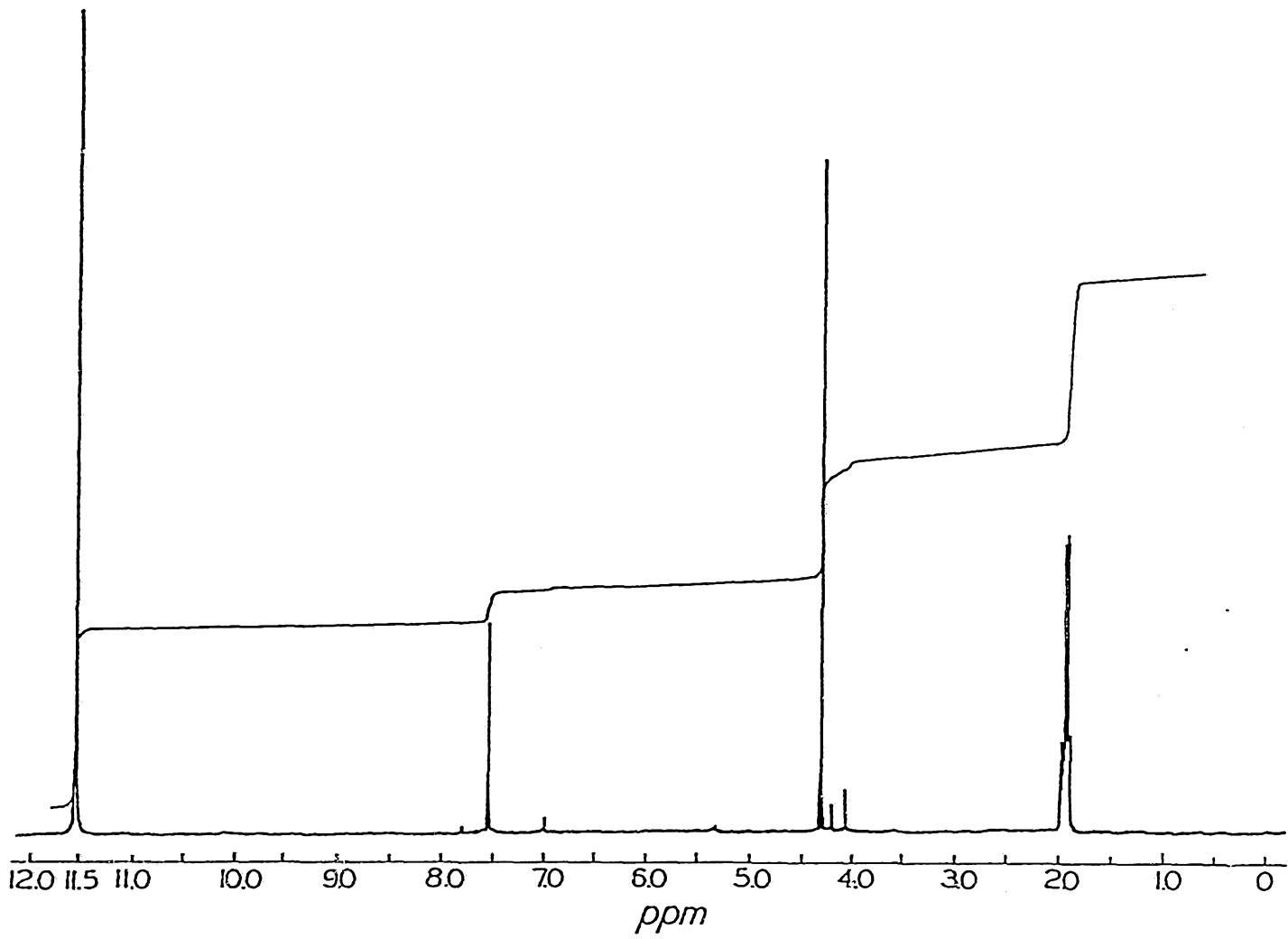


FIG. 4