FORM 25 (12) PATENT granted by (19)

AFRICAN REGIONAL INDUSTRIAL PROPERTY ORGANIZATION (ARIPO)

AP 199 A

(11)

(21) Application Number: AP/P/91/00267

(22) Filing Date: 28.05.91

(24) (45) Date of Grant& Publication 30.06.92

(30) Priority Data:

(33) Country: -

(31) Number: -

(32) Date: -

(84) Designated States:

BW GM MW KE LS

SD SZ UG ZM ZW

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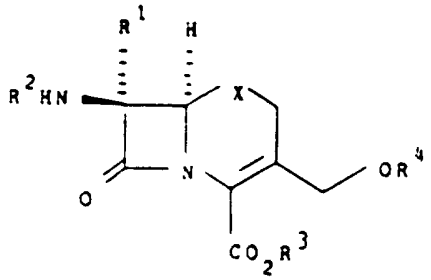
(51) International Patent Classification Int. Cl.[5] C07D 501/34

(54) Title: NOVEL β-LACTAM AND A NOVEL CLASS CEPHALOSPORINS

(57) Abstract: Compound of formula (I) or a salt thereof:

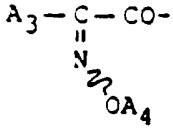


(I)

wherein

R^1 is hydrogen, methoxy or formamido;

R^2 is an acyl group of formula (a)



where A_3 is thiazolyl optionally substituted by an amino group, A_4 is hydrogen or an organic residue; CO_2R^3 is a carboxy group, carboxylate anion, or R^3 is a readily removable carboxy protecting group;

X is S, SO, SO_2, O or CH_2; and R^4 is an unsaturated acyl group novel and useful in the treatment of bacterial infections in humans and animals.

(56) Documents cited: US 4 091 209 US 4 209 616 EP 0 267 733 A2

AP 199

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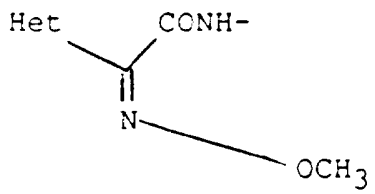
Novel Compounds

This invention relates to novel β-lactam containing compounds, their preparation and their use, and in particular to a novel class of cephalosporins. These compounds have antibacterial properties, and are therefore of use in the treatment of bacterial infections in animals, particularly in mammals including humans, caused by a wide range of organisms.

Compounds of the cephalosporin type are well known as antibacterial antibiotics. They generally comprise 7-β-acylamino ceph-3-em carboxylic acids and their various non-toxic derivatives, e.g. salts, esters, amides, hydrates etc. The ceph-3-em structure may carry various substituents and the nature and position of thse substituents can influence the biological activity of the compounds. GB 1399086 and 1399088 (both Glaxo) disclose generally a broad series of substituted cephalosporin compounds.

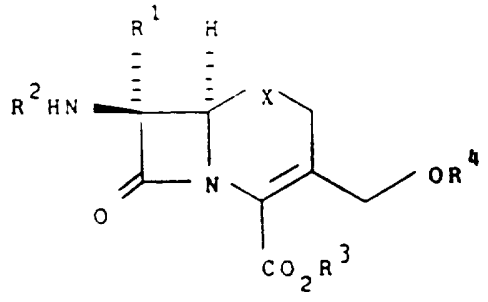
Further compounds of the cephalosporin type are disclosed in DE-OL-2204060 and 2223375, FR-A-2191883 and 2204403, GB 1474520 and US 3912589. These compounds contain a 7-β side chain of the structure:



wherein Het is phenyl, furyl or thienyl.

We have now found a particular class of cephalosporins that possess high levels of antibacterial activity and in addition demonstrate resistance to *in vivo* degradation by esterases.

The present invention provides a compound of formula (I) or a salt thereof:

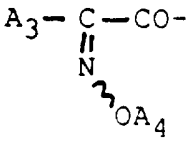


(I)

wherein

R^1 is hydrogen, methoxy or formamido;

R^2 is an acyl group of formula (a)

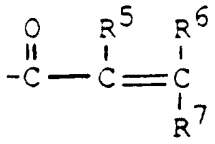
(a) 

where A_3 is thiazolyl optionally substituted by an amino or substituted amino group which may be in protected form and A_4 is hydrogen or an organic residue;

CO_2R^3 is a carboxy group or a carboxylate anion, or R^3 is a readily removable carboxy protecting group;

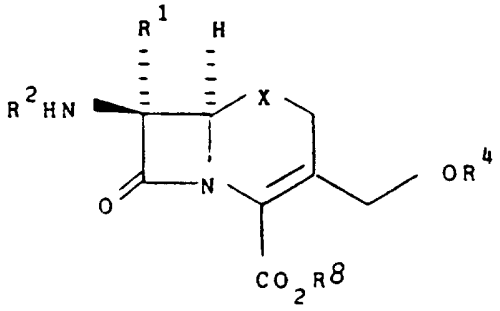
X is S, SO, SO_2, O or CH_2; and

R^4 is a group



where R^5 and R^6 are independently hydrogen or C_{1-6} alkyl, or R^5 and R^6 are together an alkyne bond, and R^7 is hydrogen, C_{1-6} alkyl or C_{2-4} alkenyl.

Since the β-lactam antibiotic compounds of the present invention are intended for use as therapeutic agents in pharmaceutical compositions, it will be readily appreciated that preferred compounds within formula (I) are **pharmaceutically acceptable, i.e. are compounds of formula** (Ia) or pharmaceutically acceptable salts or pharmaceutically acceptable in vivo hydrolysable esters thereof:



(Ia)

wherein R^1, R^2, R^4 and X are as defined with respect to formula (I) and the group CO_2R^8 is a carboxy group or a carboxylate anion.

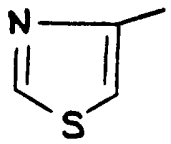
Advantageously R^1 is hydrogen.

In compounds of formula (I) wherein R^1 is formamido, the formamido group can exist in conformations wherein the hydrogen atoms of the -NH-CHO moiety are cis- or trans-; of

these the cis conformation normally predominates.

It will be appreciated that compounds of the invention wherein R^2 is a group of formula (a) can exist as syn and anti isomers or mixtures thereof. Both isomers are encompassed within the scope of this invention. Preferably the compounds of the invention wherein R^2 is a group of formula (a) have the Z configuration (i.e. have the group OA_4 syn to the amide linkage) or are enriched in that isomer.

The thiazolyl system A_3 is preferably a thiazol-4-yl system, i.e.



Suitable values for A_3 within the acyl group R^2 of formula (a) include 2-aminothiazol-4-yl, 5-aminothiazol-4-yl, 2-(2-chloroacetamido)thiazol-4-yl and 2-tritylaminothiazol-4-yl. Preferably A_3 is 2-aminothiazol-4-yl.

Suitable values for the group A_4 include hydrogen, methyl, triphenylmethyl (trityl), ethyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, phenyl, carboxymethyl, carboxypropyl, t-butoxycarbonylmethyl and $CH_nX_{(3-n)}$ where n is 0 - 3 and X is chlorine or fluorine. Particular values for A_4 within the acyl group R^2 of formula (a) include hydrogen, methyl and trityl. Preferably A_4 is hydrogen or methyl.

When used herein in relation to variable A_4, the term organic residue includes any organic residue associated with a 7-position thiazolyloximinoacetamido substituent of an antibacterially active cephalosporin. Suitable values

include inter alia C_{1-6} alkyl, C_{2-6} alkenyl and C_{2-6} alkynyl, each of which may be optionally substituted, C_{3-8} cycloalkyl, aryl and heterocyclyl. Optional substitutents for alkyl, alkenyl and alkynyl include carboxy, C_{1-6} alkoxycarbonyl, hydroxy, C_{1-6} alkoxy, cyano, hydrogen, amino, substituted amino, aryl, heterocyclyl and C_{3-8} cycloalkyl.

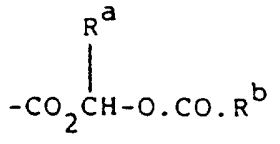
Those compounds of the formula (I) wherein R^3 is a readily removable carboxy protecting group other than a pharmaceutically acceptable in vivo hydrolysable ester or which are in non-pharmaceutically acceptable salt form are primarily useful as intermediates in the preparation of compounds of the formula (Ia) or a pharmaceutically acceptable salt or pharmaceutically acceptable in vivo hydrolysable ester thereof.

Suitable readily removable carboxy protecting groups for the group R^3 include groups forming ester derivatives of the carboxylic acid, including in vivo hydrolysable esters. The derivative is preferably one which may readily be cleaved in vivo.

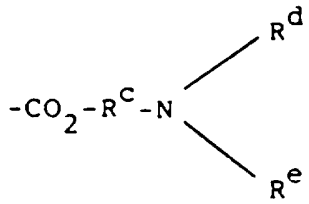
Suitable ester-forming carboxyl-protecting groups are those which may be removed under conventional conditions. Such groups for R^3 include benzyl, p-methoxybenzyl, benzoylmethyl, p-nitrobenzyl, 4-pyridylmethyl, 2,2,2-trichloroethyl, 2,2,2-tribromoethyl, t-butyl, t-amyl, allyl, diphenylmethyl, triphenylmethyl, adamantyl, 2-benzyloxyphenyl, 4-methylthiophenyl, tetrahydrofur-2-yl, tetrahydropyran-2-yl, pentachlorophenyl, acetonyl, p-toluenesulphonylethyl, methoxymethyl, a silyl, stannyl or phosphorus- containing group, an oxime radical of formula $-N=CHR^9$ where R^9 is aryl or heterocyclic, or an in vivo hydrolysable ester radical such as defined below.

A CO_2R^3 carboxyl group may be regenerated from any of the above-mentioned esters by usual methods appropriate to the particular R^3 group, for example, acid- and base- catalysed hydrolysis, or by enzymically-catalysed hydrolysis, or by hydrogenolysis under conditions wherein the remainder of the molecule is substantially unaffected.

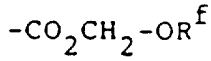
Examples of suitable pharmaceutically acceptable <u>in vivo</u> hydrolysable ester groups include those which break down readily in the human body to leave the parent acid or its salt. Suitable ester groups of this type include those of part formulae (i), (ii), (iii) and (iv):



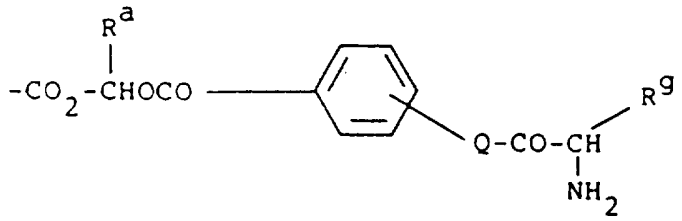
(i)



(ii)



(iii)

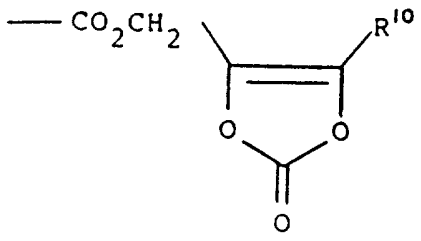


(iv)

wherein R^a is hydrogen, C_{1-6} alkyl, C_{3-7} cycloalkyl, methyl, or phenyl, R^b is C_{1-6} alkyl, C_{1-6} alkoxy, phenyl, benzyl, C_{3-7} cycloalkyl, C_{1-6} alkyl C_{3-7} cycloalkyl, 1-amino C_{1-6} alkyl, or 1-(C_{1-6} alkyl)amino C_{1-6} alkyl; or R^a and R^b together form a 1,2-phenylene group optionally substituted by one or two methoxy phenyl, benzyl, C_{3-7} cycloalkyl, C_{1-6} alkyl C_{3-7} cycloalkyl, 1-amino C_{1-6} alkyl, or 1-(C_{1-6} alkyl)amino C_{1-6} alkyl; or R^a and R^b together form a 1,2-phenylene group optionally substituted by one or two methoxy groups; R^c represents C_{1-6} alkylene optionally substituted with a methyl or ethyl group and R^d and R^e independently represent C_{1-6} alkyl; R^f represents C_{1-6} alkyl; R^g represents hydrogen or phenyl optionally substituted by up to three groups selected from halogen, C_{1-6} alkyl, or C_{1-6} alkoxy; and Q is oxygen or NH.

Examples of suitable in vivo hydrolysable ester groups include, for example, acyloxyalkyl groups such as acetoxymethyl, pivaloyloxymethyl, α-acetoxyethyl, α-pivaloyloxyethyl, 1-(cyclohexylcarbonyloxy)prop-1-yl, and (1-aminoethyl)carbonyloxymethyl; alkoxycarbonyloxyalkyl groups, such as ethoxycarbonyloxymethyl and α-ethoxycarbonyloxyethyl; dialkylaminoalkyl especially di-loweralkylamino alkyl groups such as dimethylaminomethyl, dimethylaminoethyl, diethylaminomethyl or diethylaminoethyl; lactone groups such as phthalidyl and dimethoxyphthalidyl; and esters linked to a second β-lactam antibiotic or to a β-lactamase inhibitor.

A further suitable pharmaceutically acceptable in vivo hydrolysable ester group is that of the formula:



wherein R^{10} is hydrogen, C_{1-6} alkyl or phenyl.

It will be appreciated that also included within the scope of the invention are salts and carboxy-protected derivatives, including in vivo hydrolysable esters, of any carboxy groups that may be present as optional substituents in compounds of formula (I) or (Ia).

Suitable pharmaceutically acceptable salts of the carboxy group of the compound of formula (I) include metal salts, eg aluminium, alkali metal salts such as sodium or potassium, alkaline earth metal salts such as calcium or magnesium, and ammonium or substituted ammonium salts, for example those with lower alkylamines such as triethylamine, hydroxy-lower alkylamines such as 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine or tris-(2-hydroxyethyl)- amine, cycloalkylamines such as dicyclohexylamine, or with procaine, dibenzylamine, N,N-dibenzylethylene- diamine, 1-ephenamine, N-methylmorpholine, N-ethylpiperidine, N-benzyl-β-phenethylamine, dehydroabietylamine, N,N'-bisdehydro-abietylamine, ethylenediamine, or bases of the pyridine type such as pyridine, collidine or quinoline, or other amines which have been used to form salts with known penicillins and cephalosporins. Other useful salts include the lithium salt and silver salt. Salts within

compounds of formula (I), may be prepared by salt exchange in conventional manner.

In compounds of formula (I) or (Ia), the group X may be sulphur or an oxidised sulphur atom, i.e. a sulphoxide (SO) or sulphone (SO_2) group. When X is a sulphoxide group it will be understood that α- and β-isomers may exist; both such isomers are encompassed within the scope of the present invention.

Preferably X is sulphur.

Suitable values for R^4 include 3-methylbut-2-enoyl, 2-methylprop-2-enoyl, but-2-ynoyl, hexa-2,4-dienoyl and 2-methylbut-2-enoyl.

It will be further appreciated that the group R^4 can exist in two isomeric forms which are geometric isomers, depending on the arrangement of the groups attached to the double bond. Both cis and trans isomers are included within the scope of the invention.

Certain compounds of the invention include an amino group which may be protected. Suitable amino protecting groups are those well known in the art which may be removed under conventional conditions without disruption of the remainder of the molecule.

Examples of amino protecting groups include C_{1-6} alkanoyl; benzoyl; benzyl optionally substituted in the phenyl ring by one or two substituents selected from C_1-$_4$ alkyl, C_1-$_4$ alkoxy, trifluoromethyl, halogen, or nitro; C_1-$_4$ alkoxycarbonyl; benzyloxycarbonyl or trityl substituted as for benzyl above; allyloxycarbonyl, trichloroethoxycarbonyl or chloroacetyl.

Also included within the scope of the invention are acid addition salts of any amino group or substituted amino group that may be present as optional substituents in compounds of formula (I) or (Ia), particularly of amino-substituent groups on A^3. Preferred addition salts are the hydrochlorides.

When used herein the term 'aryl' includes phenyl and naphthyl, each optionally substituted with up to five, preferably up to three, groups selected from halogen, mercapto, C_{1-6} alkyl, phenyl, C_{1-6} alkoxy, hydroxy(C_{1-6})alkyl, mercapto(C_{1-6})alkyl, halo(C_{1-6}) alkyl, hydroxy, amino, nitro, carboxy, C_{1-6} alkylcarbonyloxy, **alkoxycarbonyl, formyl, or C_{1-6} alkylcarbonyl groups.**

The terms 'heterocyclyl' and 'heterocyclic' as used herein include aromatic and non-aromatic, single and fused, rings suitably containing up to four hetero-atoms in each ring selected from oxygen, nitrogen and sulphur, which rings may be unsubstituted or substituted by, for example, up to three groups selected from halogen, (C_{1-6})alkyl, (C_{1-6})alkoxy, halo(C_{1-6})alkyl, hydroxy, carboxy, carboxy salts, carboxy esters such as (C_{1-6})alkoxycarbonyl, (C_{1-6})alkoxycarbonyl(C_{1-6})alkyl, aryl, and oxo groups. Each heterocyclic ring suitably has from 4 to 7, preferably 5 or 6, ring atoms. A fused heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring. Compounds within the invention containing a heterocyclyl group may occur in two or more tautometric forms depending on the nature of the heterocyclyl group; all such tautomeric forms are included within the scope of the invention.

When used herein the terms 'alkyl' and 'alkoxy' (or 'lower alkyl' and 'lower alkoxy') include straight and branched chain alkyl groups containing from 1 to 6 carbon atoms, such

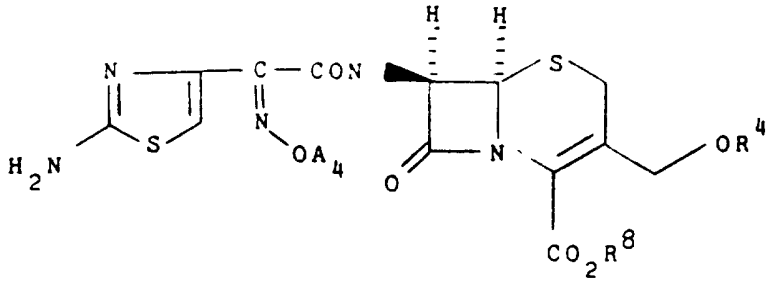
as methyl, ethyl, propyl and butyl. A particular alkyl group is methyl.

When used herein the term 'halogen' refers to fluorine, chlorine, bromine and iodine.

Some of the compounds of this invention may be crystallised or recrystallised from solvents such as organic solvents. In such cases solvates may be formed. This invention includes within its scope stoichiometric solvates including hydrates as well as compounds containing variable amounts of water that may be produced by processes such as lyophilisation.

Since the antibiotic compounds of the invention are intended for use in pharmaceutical compositions it will readily be understood that they are each provided in substantially pure form, for example at least 60% pure, more suitably at least 75% pure and preferably at least 85%, especially at least 95% pure (% are on a weight for weight basis). Impure preparations of the compounds may be used for preparing the more pure forms used in the pharmaceutical compositions; these less pure preparations of the compounds should contain at least 1%, more suitably at least 5% and preferably from 10 to 59% of a compound of the formula (I) or salt thereof.

From the foregoing it will be seen that one preferred subclass of compounds within the invention may be represented by the formula (II) or a pharmaceutically acceptable salt or pharmaceutically acceptable <u>in vivo</u> hydrolysable ester thereof:



(II)

wherein R^4 and R^8 are as hereinbefore defined and A_4 is hydrogen or C_{1-6} alkyl. Particularly preferred compounds of formula (II) have R_4 being 3-methylbut-2-enoyl, 2-methylprop-2-enoyl,but-2-ynoyl, hexa-2,4-dienoyl and 2-methylbut-2-enoyl, A^4 being hydrogen or methyl, with R^8 being hydrogen, or CO_2R^8 being a pharmaceutically acceptable salt or in vivo hydrolysable ester of this acid.

Accordingly, specific compounds within this invention of formula (Ia) which fall within the preferred subclass of compounds of formula (II) include the following, and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof:

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylic acid,

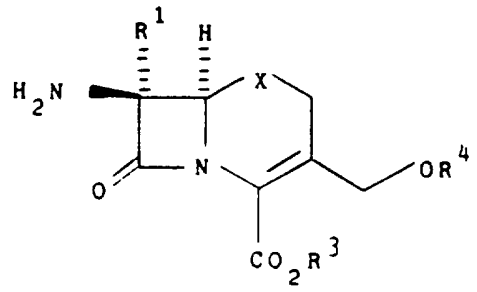
(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(but-2-ynoyloxymethyl)ceph-3-em- 4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid, and

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino=acetamido]-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylic acid.

The present invention further provides a process for the preparation of a compound of formula (I), which process comprises treating a compound of formula (III) or a salt thereof:



(III)

wherein R_1, CO_2R^3, R^4 and X are as hereinbefore defined, wherein any reactive groups may be protected, and wherein the amino group is optionally substituted with a group which permits acylation to take place; with an N-acylating derivative of an acid of formula (IV):

$$R^2OH \qquad (IV)$$

wherein R^2 is as defined with respect to formula (I) and wherein any reactive group may be protected; and thereafter as necessary or desired, carrying out one or more of the following steps:

i) removing any protecting groups;

ii) converting the group CO_2R^3 into a different group CO_2R^3;

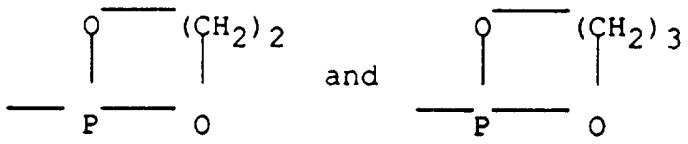
iii) converting the group X into a different group X;

iv) converting the product into a salt.

Suitable compounds of formula (III) include salts and esters, for example an ester in which R^3 is diphenylmethyl.

Acids of formula (IV) may be prepared by methods known in the art, or methods analogous to such processes. Suitable processes include those described, for example, in UK Patent 2 107 307 B, UK Patent Specification No. 1,536,281, and U.K. Patent Specification No. 1,508,064.

Suitable groups which permit acylation to take place and which are optionally present on the amino group of the starting material of the formula (III) include N-silyl, N-stannyl and N-phosphorus groups, for example trialkylsilyl groups such as trimethylsilyl, trialkyltin groups such as tri-n-butyltin, groups of formula $-PR^{11}R^{12}$ wherein R^{11} is an alkyl, haloalkyl, aryl, aralkyl, alkoxy, haloalkyl, aryl, aralkyl, alkoxy, haloalkoxy, aryloxy, aralkyloxy or dialkylamino group, R^{12} is the same as R^{11} or is halogen or R^{11} and R^{12} together form a ring; suitable such phosphorus groups being $-P(OC_2H_5)_2$, $-P(C_2H_5)_2$,



A group which may optionally be introduced onto the amino group in the compound of formula (III) is trimethylsilyl.

Advantageously the silylation reaction may be carried out *in situ*, prior to the acylation reaction, with a silylating agent that does not require concomitant addition of base. Suitable silylating agents include, for example, *N*-(trimethylsilyl)-acetamide, *N*,*O*-bis-(trimethylsilyl)acetamide, *N*,*O*-bis(trimethylsilyl)-trifluoroacetamide, *N*-methyl-*N*-trimethylsilylacetamide, *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide, *N*,*N*'-bis(trimethylsilyl)urea, and *N*,*O*-bis(trimethylsilyl)carbamate. A preferred silylating agent is *N*,*O*-bis(trimethylsilyl)acetamide. The silylation reaction may suitably be carried out in an inert, anhydrous organic solvent such as dichloromethane at room temperature or at an elevated temperature, for example 30 - 60°C, preferably 40 - 50°C.

The above process may optionally be carried out in the presence of a small quantity, for example 0.1 equivalents, of a silyl halide, for example a tri(C_{1-6})alkylsilyl halide, especially trimethylsilyl chloride.

A reactive *N*-acylating derivative of the acid (IV) is employed in the above process. The choice of reactive derivative will of course be influenced by the chemical nature of the substituents of the acid.

Suitable N-acylating derivatives include an acid halide, preferably the acid chloride or bromide. Acylation with an

acid halide may be effected in the presence of an acid binding agent for example, tertiary amine (such as pyridine or dimethylaniline), molecular sieves, an inorganic base (such as calcium carbonate or sodium bicarbonate) or an oxirane, which binds hydrogen halide liberated in the acylation reaction. The oxirane is preferably a (C_{1-6})-1,2-alkylene oxide - such as ethylene oxide or propylene oxide. The acylation reaction using an acid halide may be carried out at a temperature in the range -50°C to +50°C, preferably -20°C to +20°c, in aqueous or non-aqueous media such as water, acetone, tetrahydrofuran, ethyl acetate, dimethylacetamide, dimethylformamide, acetonitrile, dichloromethane, 1,2-dichloroethane, or mixtures thereof. Alternatively, the reaction may be carried out in an unstable emulsion of water-immiscible solvent, especially an aliphatic ester or ketone, such as methyl isobutyl ketone or butyl acetate.

The acid halide may be prepared by reacting the acid (IV) or a salt or a reactive derivative thereof with a halogenating (eg chlorinating or brominating) agent such as phosphorus pentachloride, thionyl chloride, oxalyl chloride or phosgene.

Alternatively, the N-acylating derivative of the acid (IV) may be a symmetrical or mixed anhydride. Suitable mixed anhydrides are anhydrides with, for example, carbonic acid monoesters, trimethyl acetic acid, thioacetic acid, diphenylacetic acid, benzoic acid, phosphorus acids (such as phosphoric, phosphorous, and phosphinic acids) or aromatic or aliphatic sulphonic acids (such as p-toluenesulphonic acid or methanesulphonic acid). When a symmetrical or mixed anhydride is employed, the reaction may be carried out in the presence of a weak base such as pyridine or 2,6-lutidine as catalyst.

Alternative N-acylating derivatives of acid (IV) are the acid azide, or activated esters such as esters with

2-mercaptopyridine, cyanomethanol, p-nitrophenol, 2,4-dinitrophenol, thiophenol, halophenols, including pentachlorophenol, monomethoxyphenol, N-hydroxy succinimide, N-hydroxybenzotriazole, or 8-hydroxyquinoline; or amides such as N-acylsaccharins, N-acylthiazolidin-2-thione or N-acylphthalimides; or an alkylidene iminoester prepared by reaction of the acid (IV) with an oxime.

Other reactive N-acylating derivatives of the acid (IV) include the reactive intermediates formed by reaction in situ with a condensing agent such as a carbodiimide, for example, N,N'-diethyl-, dipropyl- or diisopropylcarbodiimide, N,N'-di-cyclohexyl- carbodiimide, or N-ethyl-N'-[3-(dimethylamino)propyl]- carbodiimide; a suitable carbonyl compound, for example, N,N'-carbonyldiimidazole or N,N'-carbonyldi- triazole; an isoxazolinium salt, for example, N-ethyl-5-phenylisoxazolinium-3-sulphonate or N-t-butyl-5-methylisoxazolinium perchlorate; or an N-alkoxycarbonyl 2-alkoxy-1,2-dihydroquinoline, such as N-ethoxycarbonyl 2-ethoxy-1,2-dihydroquinoline. Other condensing agents include Lewis acids (for example $BBr_3 - C_6H_6$); or a phosphoric acid condensing agent such as diethylphosphorylcyanide. The condensation reaction is preferably carried out in an organic reaction medium, for example, methylene chloride, dimethylformamide, acetonitrile, ethanol, benzene, dioxan or tetrahydrofuran.

A further method of forming the N-acylating derivative of the acid of formula (IV) is to treat the acid of formula (IV) with a solution or suspension preformed by addition of a carbonyl halide, preferably oxalyl chloride, or a phosphoryl halide such as phosphorus oxychloride, to a halogenated hydrocarbon solvent, preferably dichloromethane, containing a lower acyl tertiary amide, preferably N,N-dimethylformamide. The N-acylating derivative of the

acid of formula (IV) so derived may then be caused to react with a compound of formula (III). The acylation reaction may conveniently be carried out at -40° to +30°C, if desired in the presence of an acid binding agent such as pyridine. A catalyst such as 4-dimethylaminopyridine may optionally also be added. A preferred solvent for the above acylation reaction is dichloromethane.

In a preferred reaction, a compound of formula (III) in which the 7-position amino group is unprotected is treated with an N-acylating derivative of the olefinic acid of formula (IV) in the form of a mixed methanesulphonic anhydride in the presence of pyridine. The mixed anhydride is suitably formed **in situ** by reaction of the acid or an ester thereof, optionally in salt form, with methanesulphonyl chloride.

The optional conversion of CO_2R^3 to a different CO_2R^3 and X to a different X, and the optional formation of a salt, may be carried out using methods well known in the art of cephalosporin and penicillin chemistry.

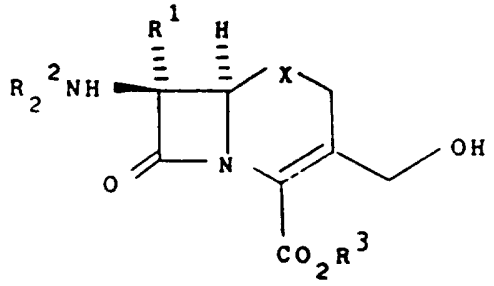
For example, when the group X is S, SO, or SO_2, the group X may be converted into a different group X by methods of oxidation or reduction well known in the art of cephalosporin and penicillin synthesis, as described, for example, in European Patent Application Publication No. 0 114 752. For example, sulphoxides (in which X is SO) may be prepared from the corresponding sulphide (in which X is S) by oxidation with a suitable oxidising agent, for example an organic peracid such as m-chloroperbenzoic acid.

In the process described hereinabove, and in the process described hereinbelow, it may be necessary to remove protecting groups. Deprotection may be carried out by any

convenient method known in the art such that unwanted side reactions are minimised. Separation of unwanted by-products may be carried out using standard methods.

For example, a trityl protecting group at the A_3 or A_4 moiety of the acyl side-chain R^2 may be removed under acid conditions using aqueous hydrochloric acid in formic acid. An acid labile R^3 carboxy protecting group will similarly be removed under the same reaction conditions.

In a further process of the invention, compounds of formula (I) may be prepared by reacting a compound of formula (V):



(V)

wherein R^1, CO_2R^3 and X are as hereinbefore defined, $R_2{}^2$ is R^2 as hereinbefore defined or an acyl group convertible thereto, and any reactive groups are optionally protected; with an acylating olefinic acid of formula (VI) or a derivative thereof:

$$R^4OH \qquad (VI)$$

wherein R^4 is as hereinbefore defined in respect of formula (I); and thereafter, if necessary or desired, carrying out one or more of the following steps:

i) converting the group R_2^2 into a group R^2;

ii) removing any protecting groups;

iii) converting the group CO_2R^3 into a different group CO_2R^3;

iv) converting the group X into a different group X;

v) converting the product into a salt.

A particular group R^3 in compounds of formula (V) is diphenylmethyl.

A particular group R_2^2 in compounds of formula (V) is phenylacetyl.

The compound of formula (VI) is suitably the carboxylic acid, R_4OH.

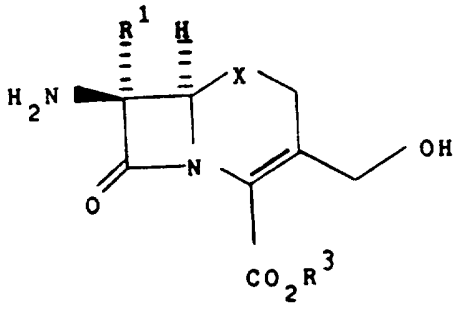
The reaction of a compound of formula (V) with a carboxylic acid of formula (VI) may be carried out under Mitsunobu conditions by treating a mixture of the alcohol of formula (V) and the olefinic acid of formula (VI) in an aprotic solvent with a phosphine derivative, suitably triphenylphosphine, and an azodicarboxylate ester, suitably diethyl azodicarboxylate. The reaction is suitably carried out in tetrahydrofuran as solvent at a temperature of 50-67°C, preferably at reflux temperature.

Where R_2^2 in a compound formula (V) is other than R^2, conversion of R_2^2 to R^2 may be effected via the intermediacy of a compound of formula (III) which has an amino group at the 7-position of the cephalosporin nucleus.

An R_2^2 side-chain may be removed via the Delft procedure commonly used in β-lactam chemistry. Suitable reaction

conditions include treatment with phosphorus pentachloride and N-methylmorpholine in dichloromethane at reduced temperature, for example at -20°C. The product is conveniently isolated initially as a hydrochloride salt which permits any starting materials, isomers or phosphorus-containing materials to be eliminated. The free base may then be isolated by treatment with alkali.

Compounds of formula (V) and salts thereof which are, inter alia intermediates for compounds of the invention as hereinbefore defined may be prepared by selectively acylating the amino function of a compound of formula (VII):



(VII)

wherein R^1, CO_2R^3 and X are as hereinbefore defined with an acylating derivative of an acid of formula (VIII):

$$R_2{}^2OH \qquad \text{(VIII)}$$

wherein $R_2{}^2$ is as hereinbefore defined; and thereafter if necessary carrying out one or more of the following steps:

i) converting the group CO_2R^3 into a different group CO_2R^3;

ii) removing any protecting group;

iii) converting the group X into a different group X;

iv) converting the product into a salt.

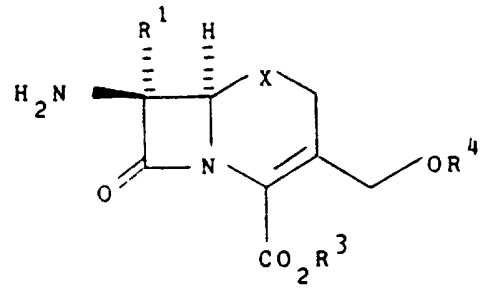
For example, a compound of formula (V) in which R_2^2 is phenylacetyl and R^3 is diphenylmethyl may be prepared by reaction of a compound of formula (VII) in which R^3 is hydrogen with phenylacetyl chloride in aqueous acetone under mildly basic conditions, for example in the presence of sodium bicarbonate, followed by formation of the carboxylic acid and treatment with diphenyldiazomethane.

Compounds of formula (VII) are known compounds or may be prepared from known compounds by methods known in the art of β-lactam chemistry. A convenient starting material for the preparation of compounds of formula (VII) in which X is a sulphur group is the compound 7-amino-cephalosporanic acid (7-ACA) which is readily de-acetylated at the 3-position to the corresponding compound of formula (VII).

Compounds of formulae (IV), (VI), and (VIII) are generally known compounds or are readily prepared from known starting materials using standard methodology.

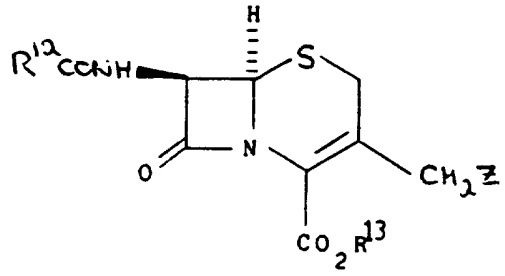
Compounds of formula (III) are believed to be novel compounds and as such form part of the present invention.

Compounds of formula III:



(III)

may be prepared from known compounds such as corresponding N-substituted acetamido derivatives such as 7-phenylacetamido ceph-3-em-4-carboxylates and carboxy derivatives such as esters thereof. Alternatively compounds of formula III may be prepared from known and commercially available compound of formula X:

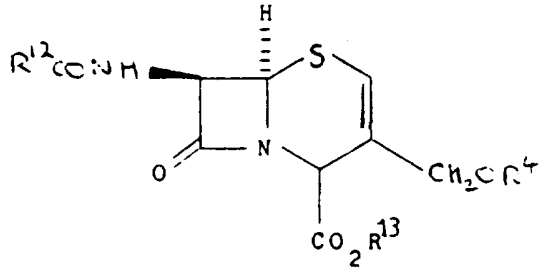


(X)

wherein Z is a halogen, especially chlorine or bromine, $R^{12}CO$ is an acyl group, particularly an amino-protecting acyl group which can be easily removed as understood by those skilled in the art, such as phenylmethyl, and R^{13} is an acyloxy protecting group which can be easily removed as understood by those skilled in the art, such as an ester moiety, especially diphenylmethyl.

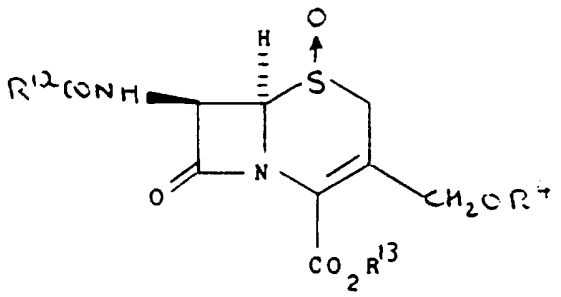
By reaction of compounds of formula X with the acid R^4OH (VI) in the presence of a tertiary ammonium salt of formula

R^x_4NY wherein R^x is alkyl or aryl and Y is the salt anion, especially iodide, for example tetra-n-butyl ammonium iodide, in the presence of sulphite under solvent-aqueous medium phase transfer conditions, compounds of formula XI may be formed:



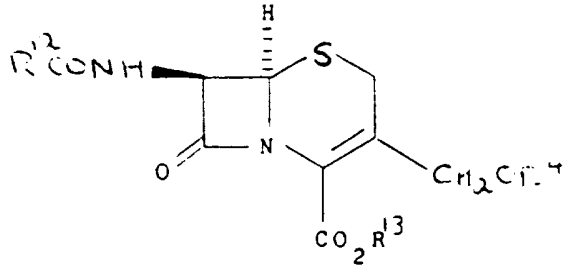
(XI)

Rearrangement of the six-membered ring may be carried out by means of an oxidation-reduction sequence, for example oxidation using a perbenzoic acid, followed by reduction of the resulting sulphoxide (XII):



(XII)

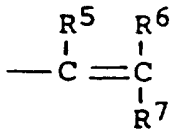
with for example PCl_3, to yield an acetamido derivative of formula (III), i.e. a compound of formula (XIII):



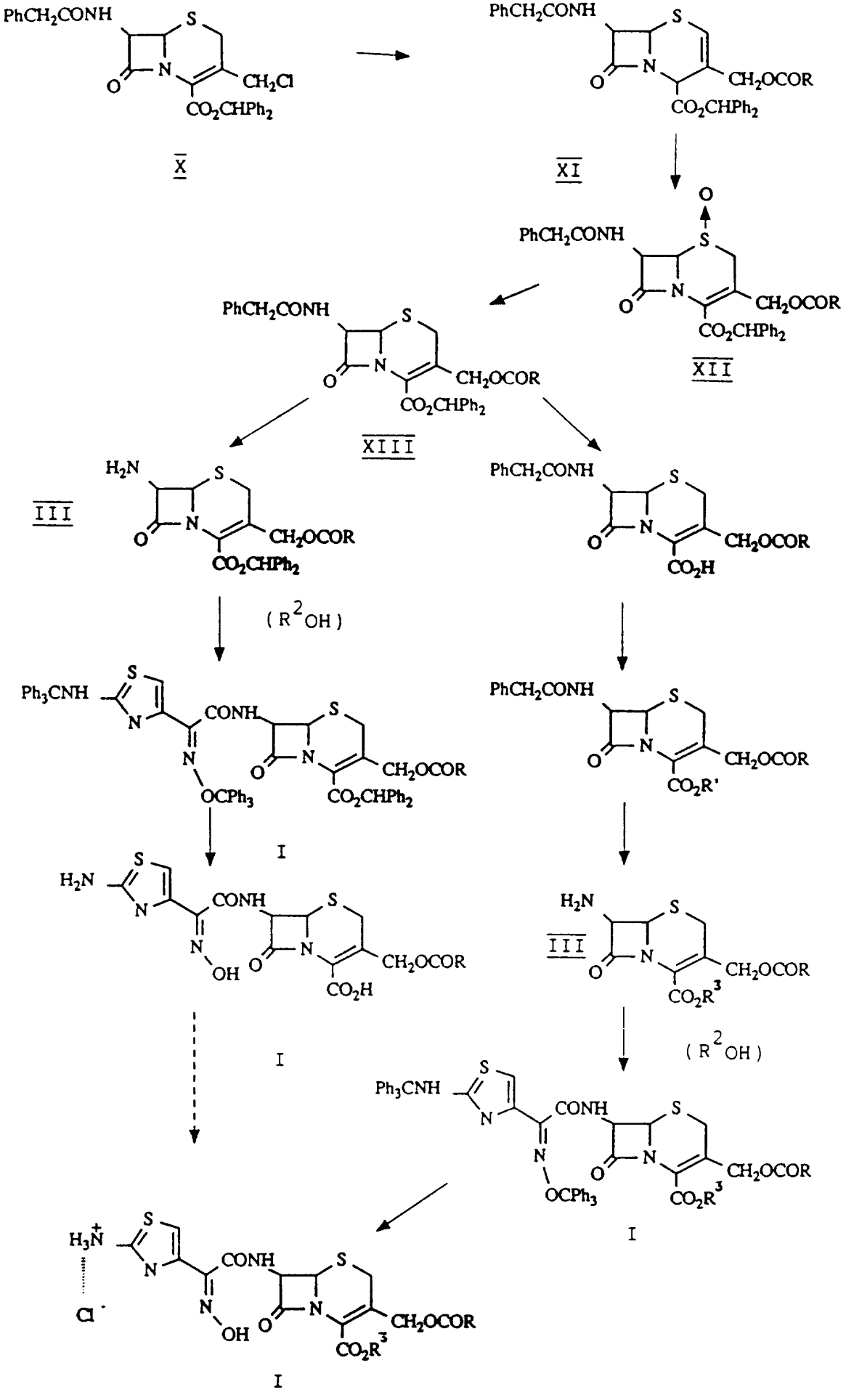
(XIII)

Compound of formula (III) may be obtained from formula (XIII) by removed of the protecting groups $R^{12}CO$ and R^{13}, by for example Delft cleavage when these groups are respectively $PhCH_2CO$ and $CHPh_2$ respectively.

A typical overall reaction scheme to compounds of formula (I) starting from a compound formula (X) in which R^{12} is phenylmethyl, R^{13} is diphenylmethyl and R represents the unsaturated side chain:



is shown below. Although specific substituent groups are shown for the various compounds of formula (I) illustrated (e.g. R^1=hydrogen, R^2=thiazol-4-yl having an amino- or protected amino- substituent or an acid addition salt thereof, A^4=hydrogen or triphenylphosphire) it will be understood by those skilled in the art that the overall synthetic route shown is applicable to all the possibilities for these substituents referred to above:


PhCH2CONH
CH2Cl
CO2CHPh2
X
CH2OCOR
XI
XII
XIII
H2N
III
(R2OH)
Ph3CNH
CONH
OCPh3
I
OH
CO2H
CO2R'
CO2R3
H3N+
Cl-

It should be noted that in processes of this invention ^{2}-cephems may function as intermediates, in the synthetic sequences. Subsequent isomerisation steps by methods well known in cephalosporin chemistry will provide the ^{3}-cephems of the invention.

The present invention also provides a pharmaceutical composition which comprises a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof and a pharmaceutically acceptable carrier. The compositions of the invention include those in a form adapted for oral, topical or parenteral use and may be used for the treatment of bacterial infection in mammals including humans.

The antibiotic compounds according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antibiotics.

The composition may be formulated for administration by any route, such as oral, topical or parenteral. The compositions may be in the form of tablets, capsules, powders, granules, lozenges, creams or liquid preparations, such as oral or sterile parenteral solutions or suspensions.

The topical formulations of the present invention may be presented as, for instance, ointments, creams or lotions, eye ointments and eye or ear drops, impregnated dressings and aerosols, and may contain appropriate conventional additives such as preservatives, solvents to assist drug penetration and emollients in ointments and creams.

The formulations may also contain compatible conventional carriers, such as cream or ointment bases and ethanol or oleyl alcohol for lotions. Such carriers may be present as

from about 1% up to about 98% of the formulation. More usually they will form up to about 80% of the formulation.

Tablets and capsules for oral administration may be in unit dose presentation form, and may contain conventional excipients such as binding agents, for example syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrollidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; or acceptable wetting agents such as sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives, such as suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats, emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters such as glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid, and, if desired, conventional flavouring or colouring agents.

Suppositories will contain conventional suppository bases, e.g. cocoa-butter or other glyceride.

For parenteral administration, fluid unit dosage forms are prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the compound can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing.

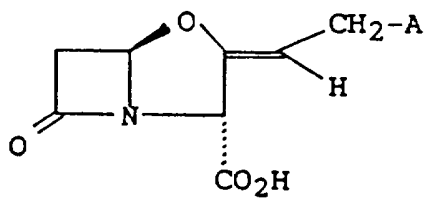
Advantageously, agents such as a local anaesthetic, preservative and buffering agents can be dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilization cannot be accomplished by filtration. The compound can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound.

The compositions may contain from 0.1% by weight, preferably from 10-60% by weight, of the active material, depending on the method of administration. Where the compositions comprise dosage units, each unit will preferably contain from 50-500 mg of the active ingredient. The dosage as employed for adult human treatment will preferably range from 100 to 3000 mg per day, for instance 1500 mg per day depending on the route and frequency of administration. Such a dosage corresponds to 1.5 to 50 mg/kg per day. Suitably the dosage is from 5 to 20 mg/kg per day.

No toxicological effects are indicated when a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof is administered in the above-mentioned dosage range.

The compound of formula (Ia) may be the sole therapeutic agent in the compositions of the invention or a combination with other antibiotics or with a β-lactamase inhibitor may be employed.

Advantageously, the compositions also comprise a compound of formula (XIV) or a pharmaceutically acceptable salt or ester thereof:

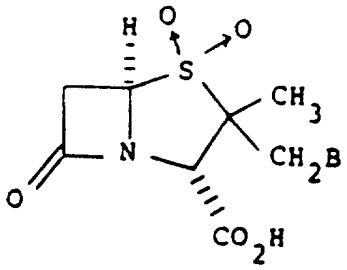


(XIV)

wherein

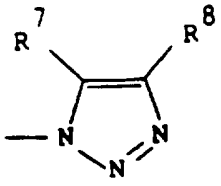
A is hydroxyl, substituted hydroxyl, thiol, substituted thiol, amino, mono- or di-hydrocarbyl-substituted amino, or mono- or di-acylamino; an optionally substituted triazolyl group; or an optionally substituted tetrazolyl group as described in EP 0 053 893.

A further advantageous composition comprises an antibiotic compound according to the invention and a pharmaceutically acceptable carrier or excipienttogether with a β-lactamase inhibitor of formula (XV) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof:



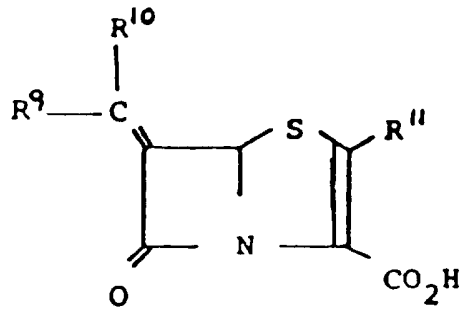
(XV)

wherein B is hydrogen, halogen or a group of formula:



in which R^7 and R^8 are the same or different and each is hydrogen, C_1-$_6$ alkoxycarbonyl, or carboxy or a pharmaceutically acceptable salt thereof.

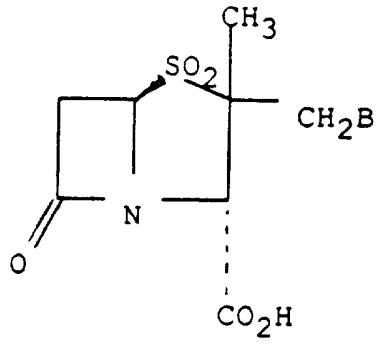
Further suitable β-lactamase inhibitors include 6-alkylidene penem of formula XVI below:



(XVI)

or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, wherein R^9 and R^{10} are the same or different and each represents hydrogen, or a C_{1-10} hydrocarbon or heterocyclic group optionally substituted with a functional group; and R^{11} represents hydrogen or a group of formula R^a or $-SR^a$ where R^a is an optionally substituted C_{1-10} hydrocarbon or heterocyclic group, as described in European Patent Application No. 81301683.9 (Publication Number 0 041 768).

A further advantageous composition comprises a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof together with a compound of formula XVII or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof:



XVII

wherein

B represents hydrogen or chloro.

Further suitable β-lactamase inhibitors include 6β-bromopenicillanic acid and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof and 6β-iodopenicillanic acid and pharmaceutically acceptable salts and in vivo hydrolysable esters thereof described in, for example, EP-A-0 410 768 and EP-A-0 154 132 (both Beecham Group).

Such compositions of this invention which include a β-lactamase inhibitory amount of a β-lactamase inhibitor are formulated in a conventional manner using techniques and procedures per se known in the art.

The present invention provides a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, for use as a therapeutic agent.

The present invention further provides a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo

hydrolysable ester thereof, for use in the treatment of bacterial infections.

The present invention also includes a method of treating bacterial infections in humans and animals which comprises the administration of a therapeutically effective amount of an antibiotic compound of this invention of the formula (Ia) or a pharmaceutically acceptable in vivo hydrolysable ester thereof.

In addition, the present invention includes the use of a compound of formula (Ia) or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, in the manufacture of a medicament for the treatment of bacterial infections.

The antibiotic compounds of the present invention are active against a wide range of organisms including both Gram-negative organisms and Gram-positive organisms.

The following Examples illustrate compounds of the present invention. In vitro biological activity data is presented in the form of a comparative geometric mean MIC (minimum inhibitory concentration) values (μg/ml) for a compound of the present invention and a known compound FK 482.

Example 1

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylate

1(a) Diphenylmethyl (6R,7R)-3-(3-Methylbut-2-enoyloxymethyl)-7-phenylacetamidoceph-3-em-4-carboxylate

To a gently refluxing and vigorously stirred solution of 3-methylbut-2-enoic acid (0.5g) in dry tetrahydrofuran (30ml) under nitrogen were added rapidly and successively triphenylphosphine (1.5g), diphenylmethyl(6R,7R)-3-hydroxymethyl-7-phenylacetamidoceph-3-em-4-carboxylate (2.0g) and diethyl azodicarboxylate (1.0ml). An exothermic reaction ensued; the initial brown colour of the reaction quickly became dark green. After 2 min. the mixture was diluted with toluene (30ml) and evaporated under reduced pressure to low volume. Further toluene was added and the diethyl hydrazodicarboxylate allowed to crystallize. It was filtered off, and the filtrate washed with a little saturated sodium hydrogen carbonate solution, then with saturated brine, dried over anhydrous sodium sulphate, filtered and evaporated to a gum. This was subjected to silica gel chromatography using ethyl acetate-hexane(7:13) as elution solvent. Fractions containing the desired product (tlc analysis)) were combined and evaporated to low volume, whereupon the compound crystallized. It was filtered, washed with a little ether and dried *in vacuo*, to afford the title compound (1.2g). It had ν_{max} (KBr disc) (*inter alia*) 697, 1225, 1647, 1717, 1779 and 3280cm^{-1}; m/z (FAB, MNa^+) 619.

1(b) Diphenylmethyl(6R,7R)-7-amino-3-(3-methylbut-2-enolyloxymethyl)ceph-3-em-4-carboxylate

The product from Example 1(a) (0.6g) was dissolved in dry dichloromethane (20ml) and cooled with stirring under nitrogen to below -20°C. There was added N-methylmorpholine (0.24ml) and a solution of phosphorus pentachloride (resublimed (0.27g) in dichloromethane (7.5ml). The mixture was stirred at -20°C for 1½h. Methanol (2.5ml) was added, stirring continued for ½h, and then water (15ml) was added. After 1½hrs the reaction mixture had reached ambient temperature; ethyl acetate (25ml) was added and the whole mixture was evaporated partially under reduced pressure. A crystalline precipitate appeared. This was collected, washed with ether and with a little acetone, and dried briefly in vacuo. (An infra-red spectrum showed that this material was the hydrochloride of the title compound). The mother liquors were treated with a few ml. of 5M hydrochloric acid and re-evaporated with more ethyl acetate, to yield a further crop of crystals. These were collected, washed with ether and combined with the previous crop. They were then suspended in water (25ml), layered with tetrahydrofuran-toluene- ethyl acetate (1:1:1) and the mixture brought to pH 6 by the cautious additon of 1M NaOH solution. When no crystalline material remained suspended in the solution, the layers were separated, and the resulting non-aqueous phase was washed with water, saturated brine and dried over anhydrous sodium sulphate. The solution was filtered and evaporated to a gum, which partially crystallised on storage at 2-3°C overnight. The remainder solidified on trituration with a little ether; it was filtered off, washed with a little ether and dried in vacuo, to yield the title compound (0.28g) as a colourless crystalline solid, ν_{max} (KBr disc) 697, 1143, 1226, 1654, 1710, 1726, 1765 and 3414cm^{-1}; m/z (FAB, MNa^+) 501.

1(c) Diphenylmethyl (6R,7R)-7-[2-(Z)-Methoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylate

To a solution of 2-(Z)-methoxyimino-2-(2-triphenylmethylaminothiazol-4-yl) acetic acid hydrochloride (0.24g) in dry DMF (4ml) cooled and stirred under nitrogen at -40° was added di-isopropylethylamine (0.26g) and methanesulphonyl chloride (0.12g). The mixture was allowed to stir at -30°C for ½h. To the mixture was then added the product from (b) (0.24g) and di-isopropylamine (0.13g), and it was then stirred at -30° to -10°C for 1h. Toluene (15ml), tetrahydrofuran (10ml), ethyl acetate (10ml) and water (50ml) were added. The layers were separated, and the solvent layer washed with water (50ml) and then with saturated brine. It was dried over anhydrous sodium sulphate, filtered and evaporated, to yield 0.6g of the product as a gum. It was purified by column chromatography on silica gel using ethyl acetate-hexane (2:3) as eluent, to yield the product as a gum (0.32g), together with less pure material (0.04g). It had ν_{max} (KBr disc) 700, 1138, 1223, 1522, 1684, 1718, 1787, 3300 and 3391cm^{-1}.

1(d) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylate

(Two-stage deprotection).

To the product from Example 1(c) (320mg) were added tetrahydrofuran (0.3ml) water (0.3ml) and 98% formic acid (3ml). The mixture was stirred at ambient temperature for 1hr. when most of the starting material had been converted to a new, more polar product (by tlc, 70% ethyl acetate in

hexane). The solvents and acid were co-evaporated with toluene and with tetrahydrofuran, eventually to dryness. Trifluoroacetic acid (3ml) was added and stirred at ice-bath temperature for 10 mins. Toluene and tetrahydrofuran were added and evaporated to dryness. The residue was taken up in toluene-tetrahydrofuran-ethyl acetate (1:1:1), water added and the mixture titrated to pH 7.0 with dilute sodium hydroxide solution. The layers were separated, the aqueous layer acidified to pH 2.0 in the presence of a layer of chloroform. The layers were separated and the aqueous layer extracted with toluene-tetrahydrofuran-ethyl acetate. Water was added to the non-aqueous layer and titrated to pH 7.0 with dilute sodium hydroxide solution. The aqueous phase was separated and evaporated to near dryness with additions of 1-propanol, and then re-evaporated with several portions of acetone. The solid residue was triturated with acetone/ether, filtered off, washed with a little acetone and dried in vacuo, to yield the title compound (72mg). It had ν_{max} (KBr disc) 1145, 1529, 1611, 1680, 1763, 3360 and 3412cm^{-1}.

δ_H (D_2O): 1.70 (3H, d, J 0.8Hz), 2.09 (3H, d, J 0.9Hz) 3.38 and 3.65 (2H, ABq, J 18Hz), 3.97 (3H, s), 4.72 and 4.92 (2H, ABq, J 12.5Hz), 5.00 (1H, s), 5.20 (1H, d, J 5Hz) 5.73-5.74 (1H, irregular t.), 5.80 (1H, d, J 4Hz) and 7.00 (1H, s).

Example 2

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)2-(Z)-hydroxyimino-acetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid

2(a) Diphenylmethyl (6R,7R)-3-(3-Methylbut-2-enoyloxymethyl)-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-triphenylmethoxyiminoacetamido]ceph-3-em-4-carboxylate

To a solution of 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-ylacetic acid, sodium salt (0.52g) in dry N,N-dimethylformamide (6ml), cooled and stirred under nitrogen at -35°C, was added methanesulphonyl chloride (0.18g). After ½h. at -35°C, there was added diphenylmethyl (6R,7R)-7-amino-3-(3-methylbut-2-enoyloxymethyl)-ceph-3-em-4-carboxylate (0.36g, prepared as Example 1b) and pyridine (60mg). The temperature was allowed to rise slowly during 40 min. to ca 0°C. Some of the solvent was removed by evaporation in vacuo, the residue was partitioned between toluene-tetrahydrofuran-ethyl acetate (1:1:1) and water. The aqueous phase was washed with a little toluene, which was combined with the previous solvent phase, which was then washed successively with water and saturated brine, dried over anhydrous sodium sulphate and evaporated to a gum (0.9g). This was subjected to column chromatography on silica gel using ethyl acetate-hexane (3:7) to remove a trace of more polar material; fractions containing the desired compound were combined and evaporated under reduced pressure to a gum, to yield the title compound (065g). ν_{max} (KBr disc) 699, 1218, 1239, 1684, 1715, 1791 and 3389cm^{-1}.

2(b) (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylate acid

To the product from Example 2(a) (650mg) was added 90% formic acid - 10% water (4ml) with stirring. After 40 mins, concentrated hydrochloric acid (0.2ml) was added and stirring continued for a further 40 mins. The insoluble material was filtered off and washed with a little 90% formic acid. The filtrate was evaporated to a gum, which was re-evaporated with toluene to dryness. The residue was treated with ether (10ml) and water (10ml). The insoluble material was removed by filtration (trace only) and the filtrate 'neutralised' in two stages, first to pH 2.5 and then to pH 3.0. Solids crystallised at each stage, they were collected, washed with a few drops of water, then toluene and dried *in vacuo*, to yield the title compound: 1st crop (155mg), 2nd crop (30mg); it had ν_{max} (KBr) 1076, 1144, 1226, 1528, 1637, 1773 and 3321cm^{-1}; *m/z* (FAB, MH^+) 482.

δ_H (D_6-DMSO): 1.89 (3H, d, *J* 0.6Hz), 2.10 (3H, d, *J* 0.7Hz) 3.47 and 3.60 (2H, ABq, *J* 18Hz), 4.69 and 5.02 (2H, ABq, J 13Hz), 5.16 (1H, d, *J* 5Hz), 5.71-5.72 (1H, m), 5.81 (1H, dd, *J* 5 and 8Hz; with D_2O collapses to d, *J* 5Hz), 6.66 (1H, s), 7.15 (s, exch.) and 9.48 (1H, d, *J* 8Hz, exch.).

Example 3

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(E,E)-hexa-2-4-dienoyloxymethyl]ceph-3-em-4-carboxylate

3(a) Diphenylmethyl (6R,7R)-3-[(E,E)-Hexa-2,4-dienoyloxymethyl]-7-phenylacetamidoceph-3-em-4-carboxylate

To a gently refluxing solution of (E,E)-hexa-2,4- dienoic acid (sorbic acid) (0.6g), and triphenylphosphine (1.45g) in dry tetrahydrofuran (30ml) stirred under nitrogen was added diphenylmethyl (6R,7R)-3-hydroxymethyl-7-phenyl-acetamidoceph-3-em-4- carboxylate (2.0g). As soon as it was dissolved, diethyl azodicarboxylate (1.0ml) was added. A vigorous exothermic reaction occurred, the initial dark red- brown colour becoming green. After a few minutes, most of the solvents were removed under reduced pressure, toluene (50ml) added and re-evaporated to a total of ca. 20ml. The insoluble material was filtered off, washed with a little toluene and the filtrate evaporated to a gum, which was subjected to column chromatography on silica gel using ethyl acetate-hexane (7:13) as elution solvent. Fractions containing the desired material (tlc analysis) were combined and evaporated to a gum which crystallised when triturated with ether-hexane. It was filtered off, washed with a little of the same solvent and dried in vacuo, to yield the title product (0.65g), ν_{max} (Nujol mull) 697, 1133, 1655, 1717, 1737, 1785 and 3297cm^{-1}. It had ν_{max} (KBr disc) 699, 1000, 1133, 1185, 1218, 1239, 1615, 1640, 1717, 1780, 3320 and 3402cm^{-1}; m/z (FAB, MNa^+) 513.

3(b) Diphenylmethyl (6R,7R)-7-Amino-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylate

The product from Example 3(a) (0.5g) was dissolved in dry dichloromethane (20ml), cooled and stirred under nitrogen at <-20°C. To it was added N-methylmorpholine (0.20ml), and phosphorus pentachloride (0.22g) in dichloromethane (5.5ml). The reaction was stirred at -20°C for ½h, methanol (2.0ml) added and stirred for ½h while allowing the reaction to warm to near ambient temperture. Water (20ml) was added and vigorous stirring was continued for ½h. Ethyl acetate (50ml) was added and evaporated under reduced pressure. This was followed by the addition of toluene-tetrahydrofuran-ethyl acetate and some water, and titration of the solution to pH 6.0 by the cautious addition of solid potassium carbonate. The layers were separated and the organic layer washed with saturated brine, dried and evaporated to a gum. Tlc on this material showed it to be substantially one product. However, it had a strong odour of phenylacetic acid, so it was taken up in toluene-tetrahydrofuran-ethyl acetate and washed with 5% aqueous sodium hydrogen carbonate solution, then with saturated brine, dried over anhydrous sodium sulphate, filtered and evaporated to a gum. This material was used per se to prepare the methoxime [Example (3c)]. During another experiment to prepare this amino compound the initial hydrochloride crystallized, (cf. 1b) enabling a purer specimen of the free amine to be obtained; this material was used to prepare the hydroxime (Example 4).

3(c) Diphenylmethyl (6R,7R)-7-[2-(Z)-Methoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)-acetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylate

A solution of 2-(Z)-methoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetic acid hydrochloride (0.36g) in N,N-dimethylformamide (6ml) with N,N-di-isopropylethylamine (0.39g) was stirred in an atmosphere of nitrogen and cooled to -40°C. Methanesulphonyl chloride (0.177g) was added and stirred at -30°C to for ½h. The amino compound from Example (3b) (0.36g) in 1:1 tetrahydrofuran-dimethylformamide (2ml) was added. The mixture was stirred and allowed to warm slowly to ambient temperature during 1h. Toluene-tetrahydrofuran-ethyl acetate (1:1:1, 30ml) was added, then water, and the mixture shaken and separated. The organic layer was washed with water and with saturated brine, dried over anhydrous sodium sulphate and evaported to a gum, which was subjected to chromatography on silica gel using ethyl acetate-hexane 2:3) as elution solvent. Fractions containing the desired compound (tlc analysis) were combined and evaporated, to give the title product as a gum (0.22g). It had ν_{max} (film) 700, 1005, 1040, 1137, 1187, 1220, 1242, 1535, 1685, 1725, 1785 and 3295cm^{-1}.

3(d) Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylate

The product from Example (3c) (200mg) in dichloromethane (0.5ml) was treated with trifluoroacetic acid (1ml) at 2-3°C for ½h. It was diluted with tetrahydrofuran and evaporated to a gum and then re- evaporated several times with tetrahydrofuran and finally with a little toluene. The

residue was treated with water (a few ml.) which was discarded. The remaining residue was taken up in toluene-ethyl acetate (1:1) and water and neutralised to pH 7.0 with 0.1M sodium hydroxide solution. The aqueous layer was washed with toluene, and evaporated to near dryness in vacuo. The remaining water was evaporated using 1-propanol and then acetone as co-solvents. The residual syrup was diluted with a little acetone and precipitated with ether, filtered off, washed with ether to give the crude product as a white solid (62mg). This was chromatographed on silica gel using water-ethanol-ethyl acetate (7:23:70) as elution solvent. Fractions containing the desired product (by tlc) were combined, evaporated, re-evaporated with acetone and precipitated with ether, filtered off, washed with ether and dried in vacuo, to afford the title product as a white solid (25mg), ν_{max} ('Nujol'mull) 722, 802, 1002, 1142, 1190, 1215, 1615, 1645, 1765 and 3300 (very broad) cm^{-1}.

δ_H (D_2O): 1.81 (3H, d, J 4.5Hz), 3.40 and 3.69 (2H, ABq, J 18Hz), 3.96 (3H, s), 4.75 and 4.95 (2H, ABq J 12.5Hz) 5.19 (1H, d, J 4.5Hz), 5.79-5.85 (2H, m), 6.26-6.29 (2H, m), 6.99 (1H, s), and 7.26-7.37 (1H, m).

Example 4

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-em-4-carboxylic acid

4(a) Diphenylmethyl (6R,7R)-3-[(E,E)-Hexa-2,4-dienoyloxymethyl]-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-triphenylmethoxyiminoacetamido]ceph-3-em-4-carboxylate

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (0.7g) in dry N,N-dimethylformamide (6ml), stirred at -35°C in an atmosphere of nitrogen, was added methanesulphonyl chloride (0.13g). The mixture was maintained at that temperature for ½h. To this was added the amino nucleus, the compound of Example 3b, (0.5g) dissolved in a small amount of dry tetrahydrofuran, and pyridine (80mg). The temperature was allowed to rise to near ambient during 40 min. Toluene-tetrahydrofuran-ethyl acetate (1:1:1) and water were added, separated, the organic layer was washed with water and saturated brine and dried over anhydrous sodium sulphate. It was evaporated to low volume, and subjected to column chromatography on silica gel using ethyl acetate-hexane(3:7) as eluent; fractions containing the desired compond (by tlc) were combined and evaporated, to yield the title compound (0.65g) and 0.15g of less pure material. It had ν_{max} (KBr disc) 677, 1132, 1184, 1218, 1239, 1641, 1715, 1791 and $3389cm^{-1}$.

4(b) (6R,7R)-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-[(E,E)-hexa-2,4-dienoylyoxymethyl]-ceph-3-em-4-carboxylic acid

The product from Example 4(a) (0.6g) was stirred with a mixture of 98% formic acid (6ml) and 1.0M aqueous hydrochloric acid (0.6ml) at ambient temperature for 1h. Concentrated hydrochloric acid (0.1ml) was added and stirred for a further hour. The insoluble materials were filtered off, washed with a little 90% formic acid and the filtrate evaporated to low volume. Several portions of toluene and/or tetrahydrofuran were added and evaporated, eventually to give a white solid residue. This was partitioned between ether/toluene and water, the pH adjusted to 3.3 by the cautious addition of solid potassium carbonate. After stirring for 1h, this was filtered off, washed with a little ether and with water, and dried in vacuo, to yield the title compound (165mg), ν_{max} (KBr disc) 1001, 1137, 1243, 1641, 1676, 1773 and 3330 (very broad) cm^{-1}.

δ_H (D_6-DMSO): 1.83 (3H, d, J 4.5Hz), 3.50 and 3.64 (2H, ABq, J 18Hz), 4.75 and 5.07 (2H, ABq, J 13Hz), 5.16 (1H, d, J 5Hz) 5.81 (1H, dd, J 5 and 8Hz, collapses to d, J 5Hz with D_2O), 5.88 (1H, d, J 15Hz), 6.28-6.31 (2H, m), 6.67 (1H, s), 7.20-7.39 (m) and 9.51 (1H, d, J 8Hz, exch. with D_2O).

Example 5

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)2-(Z)-hydroxyimino-acetamido]-3-(but-2-ynoyloxymethyl)ceph-3-em-4-carboxylic acid

5(a) Diphenylmethyl(6R,7R)x3-(But-2-ynoyloxymethyl)-7-phenylacetamidoceph-3-em-4-carboxylate

A solution of triphenylphosphine (1.45g) in tetrahydrofuran (30ml) under argon was stirred and heated to gentle reflux. There were added successively and rapidly but-2-ynoic acid (tetrolic acid) (0.5g) diphenylmethyl (6R,7R)-3-hydroxymethyl-7-phenylacetamidoceph-3-em-4-carboxylate (2.0g) and diethyl azodicarboxylate (1.0ml). A vigorous reaction ensued. After stirring for 10 min, an equal volume of toluene was added, and the mixture washed with ice and saturated sodium hydrogen carbonate solution, with water, and saturated brine. The organic solution was dried over anhydrous sodium sulphate, filtered, and evaporated to a gum. Toluene was added and the mixture refrigerated (2-3°C) overnight. The crystalline precipitate of diethyl hydrazodicarboxylate was filtered off and washed with toluene, the filtrate partially evaporated and the residue chromatographed on silica gel using ethyl acetate-hexane (7:13) as elution solvent. A partial purification was effected. Fraction containing the desired product (tlc analysis) were combined and evaporated. The residue was dissolved in ether and allowed to crystallize slowly at 2-3°C, filtered off, washed with ether and was dried in vacuo, to yield the title compound (0.35g) containing a small amount of the ceph-2-em isomer. A further reaction on twice the above scale gave 1.05g of material of this purity. It was found possible to recrystallize the compound from dichloromethane-ethanol by partial evaporation of the dichloromethane and then

refrigeration at 2-3°C; thereby material substantially free from the ceph-2-em was obtained. It had ν_{max} (KBr disc) 697, 1073, 1250, 1522, 1650, 1711, 1725, 1777, 2238 and 3289cm^{-1}, m/z (FAB, MNa^+) 603.

5(b) Diphenylmethyl (6R,7R)-7-Amino-3-(but-2-ynoyloxymethyl)ceph-3-em-4-carboxylate

The product from Example 5a (1.375g) in dry dichloromethane (25ml) was cooled and stirred under nitrogen at -20°C. To it was added N-methylmorpholine (0.57ml), then a solution of phosphorus pentachloride (0.643g) in dichloromethane (16ml). The reaction mixture was stirred at -20°C for ½h. Methanol (6ml) was added and the mixture allowed to warm towards ambient temperature during ½h. Water (25ml) was then added and stirring continued vigorously for ½h. Ethyl acetate (50ml) was added, and the mixture evaporated under reduced pressure to about half volume. This was repeated, then ether was added. A heavy oil separated. The flask and contents were cooled rapidly, whereupon the oil crystallized. It was filtered off, washed with a little 1M hydrochloric acid, then with ether and dried for a short time in air. It was suspended in a mixture of toluene (10ml) tetrahydrofuran (20ml) and water (30ml) and brought to pH 6.5 by the cautious addition of solid potassium carbonate. The layers were separated, and the organic layer washed with water and with saturated brine, dried over sodium sulphate and evaporated to a gum, which eventually crystallized. It was dissolved in dichloromethane and ethanol added to give a slight turbidity. It was seeded and cooled to 2-3°C. After 1h, the crystals were collected by filtration, washed with a little hexane and dried in vacuo, to yield the title product (0.46g). [The mother liquors contained a further quantity (tlc)], ν_{max} (KBr disc) 697,

706, 742, 1077, 1225, 1240, 1709, 1726, 1766, 2235, and 3416cm^{-1}; m/z (FAB, MNa$^+$) 485.

5(c) Diphenylmethyl (6R,7R)-3-(But-2-ynoyloxymethyl)-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-triphenylmethyloxyiminoacetamido]ceph-3-em-4-carboxylate

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (0.7g) in dry N,N-dimethylformamide (6ml), stirred in an atmosphere of nitrogen at -35°, was added methanesulphonyl chloride (0.13g). After ½h, the product from Example 5b (0.45g) was added, followed by pyridine (80mg). The mixture was stirred at -25°C for 45 min Water, toluene, tetrahydrofuran and ethyl acetate was added, stirred and separated. The organic layer was washed with 0.1M hydrochloric acid, dilute sodium hydrogen carbonate solution and saturated brine, dried over anhydrous sodium sulphate, filtered and evaporated to a gum. This was subjected to column chromatography on silica gel using ethyl acetate-hexane (7:13) as eluent. Fractions containing the desired compound (tlc analysis) were combined and collected, to give the title compound (0.63g) and 0.12g of slightly impure material. It had ν_{max} (KBr disc) 699, 751, 1248, 1710, 1791, 2239, and 3384cm^{-1}; m/z (FAB, MNa$^+$) 1138.

5(d) (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(but-2-ynoyloxymethyl)ceph-3-em-4-carboxylic acid

The product from Example 5(c) (0.63g) was moistened with tetrahydrofuran, dissolved in formic acid 98/100% (5ml) containing 1M hydrochloric acid (0.5ml). After ½h, concentrated hydrochloric acid (0.1ml) was added and stirred

for a period >½h. The crystalline precipitate was filtered off and washed with a little 90% formic acid. The filtrate was diluted with toluene and tetrahydrofuran and evaporated to dryness under reduced pressure. The residue was partitioned between ether and water, and adjusted to pH 3.3. The mixture was cooled in ice, filtered off, washed with a little water, then with ether and dried in vacuo, to yield the title product (0.25g), contaminated with some less-polar impurities. It was subjected to silica gel chromatography using water-ethanol-ethyl acetate (2:3:7) as elution solvent, to afford the title product (25mg) as a white solid after evaporation of the solvents and water. It had ν_{max} ('Nujol' mull) 1070, 1255, 1640, 1670, 1710, 1780, 2242 and 3300 (very broad) cm^{-1}.

δ_H (D_6-DMSO): 2.04 (3H, s), 3.48 and 3.63 (2H, ABq, J 18Hz), 4.77 and 5.10 (2H, ABq J 12.5Hz) 5.17 (1H, d, J 5Hz), 5.82 (1H, dd, J 5 and 8Hz, collapses to d, J 5Hz with D_2O), 6.66 (s), 7.15 (s, exch. with D_2O), and 9.49 (1H, d, J 8Hz, exch. with D_2O).

Example 6

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid, its sodium salt and its pivaloyloxymethyl ester

6(a) Diphenylmethyl (6R,7R)-3-(2-Methylprop-2-enoyloxymethyl)-7-phenylacetamidoceph-3-em-4-carboxylate

(i) To a stirred solution of 2-methylprop-2-enoic acid (methacrylic acid) (0.9ml) in gently refluxing tetrahydrofuran (65ml) in an atmosphere of nitrogen were added successively and rapidly triphenylphosphine(3.1g),

diphenylmethyl (6R,7R)-3-hydroxymethyl-7-phenylacetamidoceph-3-em-4-carboxylate (5.14g) and diethyl azodicarboxylate (2.1ml). An exothermic reaction occurred with much darkening. After 2 minutes, it was cooled and diluted with toluene (50ml). The mixture was washed with dilute sodium hydrogen carbonate solution and then with water, and dried over anhydrous sodium sulphate. The solution was filtered off and washed with a little toluene; the filtrate was partially evaporated. The solution was subjected to column chromatography on silica gel using ethyl acetate-hexane (2:3) as eluent. Fractions containing the title product in the purest state were combined and evaporated, dissolved in ether and cooled to 2-3°C overnight. The crystalline product was filtered off, washed with cold ether and dried in vacuo, to yield the title product (1.4g). It was recrystallized by dissolution in dichloromethane (minimum amount), addition of ethanol (10ml) and partial evaporation of the dichloromethane, to yield ^{3}-cephem (1.1g), free from the ^{2}-cephem. A further amount (0.4g) was obtained from less pure column fractions and mother liquors by chromatography and crystallization.

(ii) To a solution of methacrylic acid (1.7ml) in dry pyridine (20ml), cooled and stirred under nitrogen at 0°C, was added methanesulphonyl chloride (1.7ml). After ½h at 0°C, diphenylmethyl(6R,7R)-3-hydroxy-methyl)-7-phenyl-acetamidoceph-3-em-4-carboxylate (5g) was added. The mixture was stirred ½h, then poured onto a mixture of ice and a slight excess of 2.5M sulphuric acid. The product was extracted into ethyl acetate, which was washed with water, dilute sodium hydrogen carbonate solution, and saturated brine, dried over sodium sulphate and evaporated to low volume. The residue was subjected to column chromatography on silica gel using ethyl acetate-hexane (2:3) as elution solvent. Fractions containing the desired product were combined, evaporated and crystallized from ethanol as described in Example 6a(i), to yield the title product (1.0g). It had ν_{max} (KBr disc) 696, 1145, 1224, 1648, 1717, 1781 and 3277cm^{-1}.

6(c) Diphenylmethyl (6R,7R)-3-(2-Methylprop-2-enoyloxymethyl)-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-triphenylmethoxyiminoacetamido]ceph-3-em-4-carboxylate

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (0.87g) in dry N,N-dimethylformamide (10ml), cooled and stirred under nitrogen at -35°C, was added methanesulphonyl chloride (0.3g).

After ½h at <-30°C, there was added the amino compound from Example 6b (0.59g) and dry pyridine (0.1g). The mixture was stirred for 45 min, allowing the temperature to rise to near ambient. A mixture of toluene, tetrahydrofuran, ethyl acetate and water was added, stirred and the layers separated. The solvent layer was washed with dilute hydrochloric acid, dilute sodium hydrogen carbonate solution, water and saturated brine and dried over anhydrous sodium sulphate. The drying agent was filtered off and the filtrate evaporated to low volume, and subjected to chromatography on silica gel using 35% ethyl acetate in hexane as eluent. Fractions containing the desired compound (tlc analysis) were combined and evaporated to a gum (0.9g).

A small quantity (ca 0.1g) of the amino starting material was obtained by washing the column with ethyl acetate.

The title product had (KBr disc) 699, 757, 787, 1150, 1491, 1688, 1720, 1791 and 3386cm^{-1}; m/z (FAB, MNa^+) 1140.

6(d) (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamidol-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid and its sodium salt

The product from Example 6(c) (0.8g) was disolved in 98/100% formic acid (5ml) with stirring. 1.0M hydrochloric acid (0.25ml) and water (0.25ml) were added and the solution was stirred for ½h. Concentrated hydrochloric acid (0.2ml) was added and stirring continued for a further 45min. The insoluble material was filtered off, washed with a little 90% formic acid, and the filtrate diluted with toluene and tetrahydrofuran and evaporated to dryness. The residue was triturated with ether, filtered, washed with ether and dried in vacuo. The solid hydrochloride was moistened with tetrahydrofuran (2-3ml), toluene (20ml) and water (20ml) added and titrated to pH 3.1 by the cautious addition of solid potassium carbonate. The crystalline precipitate was filtered off, washed with toluene/tetrahydrofuran and with water, and dried in vacuo, to yield the title acid (130mg). Further crops (total 150mg) were obtained by evaporation of the mother liquors.

Part (50mg) of the first crop dissolved in water (35ml) and tetrahydrofuran (10ml) was titrated to pH 6.0 with 0.1M sodum hydroxide. The solution was evaporated to near dryness and re-evaporated with ethanol to give a solid, which was then triturated with ether, filtered off, washed with ether, and dried in vacuo overnight, to yield the sodium salt of the title compound (38mg).

The free acid had ν_{max} ('Nujol' mull) 1160, 1634, 1670, 1712, 1775 and 3308cm^{-1}; m/z (FAB, MH^+) 468.

The sodium salt had ν_{max} ('Nujol' mull) 1160, 1535, 1610, 1710, 1765, 3200 ad 3300cm^{-1}.

δ_H (D_6-DMSO): 1.88 (3H, s), 3.52 and 3.66 (2H, ABq, J 18Hz), 4.77 and 5.10 (2, ABq, J 13Hz), 5.16 (1H, d, J 5Hz), 5.71 (1H, s), 5.81 (1H, dd, J 5 and 8Hz, collapses to d, J 5Hz with D_2O), 6.05 (1H, s), 6.65 (1H, s), 7.15 (s, exchanges with D_2O), 9.48 (2H, d, J 8Hz, exchanges with D_2O) and 11.31 (1H, s exchanges with D_2O).

6(e) 2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyimino= acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylate (i.e.) pivaloyloxymethyl ester)

The later crops of free acid from Example 6(d) (150mg) in water (25ml) and tetrahydrofuran (10m) were titrated to pH 6.0 with 0.1M sodium hydroxide solution. The mixture was evaporated under reduced pressure to ca. 10ml, N,N-dimethylformamide (10ml) added and the evaporation continued under high vacuum to ca. 5ml. N,N-Dimethylformamide (10ml) was added and re-evaporated to 5ml, then a further 5ml of solventadded. To this was added a solution in toluene of iodomethyl 2,2-dimethylpropanoate (prepared from the bromomethyl compound (65mg) and sodium iodide (100mg) in acetone (5ml), by stirring at 2-3°C for 10 min. It was filtered, evaporated, taken up in toluene (3ml) and filtered). The mixture was stirred at ambient temperature in an atmosphere of nitrogen for 45min and protected from light. Tetrahydrofuran, toluene and water were added, shaken and separated. The solvent layer was washed with water, dilute sodium hydrogen carbonate solution and saturated brine, dried over sodium sulphate and evaporated to a gum, which was evaporated under high vacuum after the addition of several portions of toluene, and then foamed with a little ether. This residue was triturated with a little ether, filtered, washed with further portions of ether and dried in vacuo overnight to afford the title

product as a buff-coloured solid (60mg). It had ν_{max} (film) 750, 985, 1160, 1295, 1680, 1725, 1755, 1790 and 3300cm^{-1}.

δ_H ($CDCl_3$): 1.23 (9H, s), 1.94 (3H, s), 3.43 and 3.60 (2H, ABq, J 18Hz), 4.91 and 5.26 (2H, ABq, J 14Hz), 5.07 (1H, d, J 5Hz), 5.63 (1H, s), 5.86 and 5.97 (2H, ABq, J 5.5Hz-obscures 7CH), 6.12 (1H, s), 7.03 (1H, s) and 10.45 (1H, bs, exchanges with D_2O).

Example 7

(R,S)-1-Acetoxyethyl(6R,7R)-7-[2-(Aminothiazol-4-yl)-2-(Z)hydroxyimino-acetamido]-3-(2-methylprop-2-enoyloxyoxy=methyl)ceph-3-em4-carboxylate hydrochloride

Example 7a

(R,S)-1-Acetoxyethyl(6R,7R)-3-(2-Methylprop-2-enoyl-oxymethyl)-7-phenylacetamidoceph-3-em-4-carboxylate (via the free acid)

Diphenylmethyl (6R,7R)-3-(2-methylprop-2-en-oyloxy-methyl)-7-phenylacetamidoceph-3-em-4-carboxylate (Example 6(a),3g) was dissolved in 98-100% formic acid (45ml). 5M Hydrochloric acid (1ml) and water (5ml) were added, and the reaction monitored for disappearance of starting material by tlc. Toluene was added and the reaction mixture evaporated to near dryness. Further portions of toluene and tetrahydrofuran were added and evaporated, finally toluene and ethyl acetate. The residue was treated with toluene (10ml) tetrahydrofuran (10ml) ethyl acetate (30ml) and water (20ml) and titrated to pH 8 by the addition of solid potassium hydrogen carbonate. The layers were separated, the aqueous layer was washed with a little ethyl

acetate-toluene, then there were added dichloromethane (20ml), tetrabutylammonium iodide (2g) and 1-bromoethyl acetate (1.0ml). The pH was maintained at 5.5 - 7.5 by the addition of solid potassium hydrogen carbonate in small portions. Further portions (of 0.4ml and 1.1ml) of 1-bromoethyl acetate were added after 1.5 and 3.5h. After 4.5h, the mixture was diluted with ethyl acetate (50ml) and the dichloromethane evaporated under reduced pressure. A little toluene was added, the layers separated, the solvent layer washed with water then brine and dried over anhydrous sodium sulphate. It was filtered, evaporated and subjected to chromatography on silica gel using ethyl acetate and hexane (1:1) as eluent. Fractions containing the desired product were combined and evaporated to a foam (1.33g).

It had ν_{max} (KBr disc): 698, 943, 1073, 1152, 1375, 1528, 1664, 1719, 1760, 1786 and 3289cm^{-1}; m/z (FAB, MNa^+) 525; δ_H ($CDCl_3$): 1.53 (3H, d, J 6Hz), 1.94 (3H, s), 2.08 and 2.09 (3H total, 2 x s), 3.37 and 3.54 (2H, ABq, J 19Hz), 3.60 and 3.69 (2H, ABq, J 16Hz), 4.80-5.25 (3H, m), 5.62 (1H, bs), 5.80-5.90 (1H, m), 6.02 (1H, d, J 12Hz, exch. with D_2O), 6.11 (1H, s), 6.99 and 7.08 (1H total, 2 x q, J 5.5Hz) and 7.20-7.40 (5H, m).

Example 7(b)

(R,S)-1-Acetoxyethyl (6R,7R)-3-(2-Methylprop-2-enoyl-oxymethyl)-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-triphenylmethoxyiminoacetamido]ceph-3-em-4-carboxylate (via) Delft cleavage to the 7-amino-compound)

To a solution of the product from Example 7(a) (1.3g) in dry dichloromethane (15ml) at -28°C was added N-methylmorpholine (0.61ml) and a solution of phosphorus pentachloride (0.66g, 16.5ml of 40mg.ml^{-1} solution in dichloromethane). After

0.5h at <-20°C, methanol (7ml) was added and then after a further 0.5h, during which time the temperature had risen to -9°C, water (40ml). The mixture was stirred vigorously during 1h. Ethyl acetate (50ml) was added and most of the dichloromethane evaporated under reduced pressure. Some diethyl ether was also evaporated from the reaction mixture. Saturated ammonium chloride solution (15ml) ethyl acetate (20ml) tetrahydrofuran (10ml) and toluene (10ml) were added, the mixture stirred vigorously and neutralised to pH 6.5 by the careful addition of solid potassium hydrogen carbonate. The layers were separated, the aqueous layer washed with ethyl acetate, the combined solvent layers washed with water and saturated brine, then dried over anhydrous sodium sulphate and filtered. The solution showed one main polar zone by tlc (silica gel, 1:1 ethyl acetate-hexane) and a faint less polar zone. It was evaporated to an oil, which was immediately redissolved in dry tetrahydrofuran (3ml) and used in the next stage.

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylamino-thiazol-4-yl)acetate (1.5g) in dry N,N-dimethylformamide (15ml) at -35°C under nitrogen was added methanesulphonyl chloride (0.52g). After 0.5h at <-30°C, pyridine (175mg) and the tetrahydrofuran solution of the 7-amino compound prepared in the first stage were added. The mixture was allowed to warm gradually to near ambient temperature during 1h. Toluene, tetrahydrofuran, ethyl acetate and water were added, stirred and separated. The aqueous phase was re-extracted with toluene-ethyl acetate, the solvent layers combined, washed with 1M aqueous hydrochloride acid, potassium hydrogen carbonate solution, water and saturated brine, and dried over anhydrous sodium sulphate. The solution was filtered, the filtrate evaporated to low volume (circa 5ml) under reduced pressure

and subjected to gradient column chromatography on silica gel using ethyl acetate and hexane graded from 1:3 to 2:3 ratio as eluent. Fractions containing the desired product were combined and evaporated, to yield the title product as a foam (1.4g).

It had ν_{max} (KBr disc) 700, 1072, 1153, 1210, 1447, 1491, 1523, 1686, 1720, 1763, 1791, 3056 and 3380cm^{-1}; m/z (FAB, MNa^+) 1060; δ_H 1.5-1.6 (3H, m), 1.95 (3H, s), 2.10 and 2.11 (3H total, 2 x s), 3.22 and 3.49 (2H, ABq, J 19Hz), 4.70-5.30 (3H, m, inc. ABq, J 14Hz), 5.62 (1H, bs), 6.00-6.15 (2H, m, inc. s at 6.12), 6.41 (1H, s), 6.73 (1H, bs, exch. with D_2O), 7.04 and 7.13 (1H total, 2 x q, J 5.5Hz), and 7.15-7.50 (30H, m).

Example 7(c)

(R,S)-1-Acetoxyethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylate hydrochloride (i.e. 'axetil' ester)

To the title product from Example 7(b) (1.38g) was added 98-100% formic acid (14ml) and 5M aqueous hydrochloric acid (0.75ml). The mixture was allowed to stir at ambient temperature during 1.5h. The crystalline precipitate was removed by filtration, and washed with a little 90% formic acid. The filtrate was evaporated to near dryness under reduced pressure, and then re-evaporated to dryness with two successive portions of toluene + tetrahydrofuran, to give a white solid. This was triturated with ether, filtered off, washed with ether and dried in vacuo, to yield the title compound as a colourless solid (0.65g). It had ν_{max} (KBr disc) 946, 1011, 1073, 1154, 1527, 1630, 1676, 1718, 1760,

1786 and 3110cm^{-1}; m/z (FAB, MH^+) 554; δ_H (D_6-DMSO); 1.46 and 1.47 (3H total, 2 x d, J 5.5Hz) 1.89 (3H, s), 2.03 and 2.06 (3H total, 2 x s), 3.61 and 3.74 (2H, ABq, J 18Hz), 4.79, 4.81 and 5.00 (2H, total, 2 x ABq, J 13Hz), 5.22 and 5.24 (1H total, 2 x d, J 5.5Hz), 5.72 (1H, s), 5.80-5.90 (1H, m), 6.05 (1H, s), 6.82 and 6.83 (1H total, 2 x s), 6.90 and 6.99 (1H total, 2 x q, J 5.5Hz), 9.73 (1H, d, J 8Hz, exch. with D_2O) and 12.30 (1H, bs, exch. with D_2O).

Example 8

2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylate hydrochloride

Example 8(a)

2,2-Dimethylpropanoyloxymethyl (6R,7R)-3-(2-Methylprop-2-enoyloxymethyl)-7-phenylacetamidoceph-3-em-4-carboxylate (via the free acid).

The ester (Example 6(a), 2.3g) was deprotected as described in Example 7(a), and then re-esterified using dichloromethane (20ml), tetrabutylammonium iodide (1.5g) and bromomethyl 2,2-dimethylpropanoate (1ml). After stirring for 3h, a further portion (0.8ml) of bromo compound was added and the mixture stirred overnight. Ethyl acetate was added, and the dichloromethane evaporated under reduced pressure. A little toluene was added, the layers separated, the solvent layer washed with water and saturated brine and dried over anhydrous sodium sulphate. It was filtered, evaporated to small volume and subjected to column chromatography using ethyl acetate and hexane (1:1) as eluent. Fractions containing the desired product were

combined and evaporated, to give the title compound as a foam (0.41g). It had δ_H ($CDCl_3$): 1.22 (9H, s), 1.94 (3H, s), 3.35 and 3.54 (2H, ABq, J 19Hz), 3.61 and 3.70 (2H, ABq, J 16Hz), 4.80-5.20 (3H, m), 5.10-5.15 (1H, m), 5.75-5.95 (3H, m), 6.00 (1H, d, J 9Hz, exch. with D_2O), 6.10 (1H, s), and 7.20-7.40 (5H, m).

Example 8(b)

2,2-Dimethylpropanoyloxymethyl (6R,7R)-3-(2-Methyl-prop-2-enoyloxymethyl)-7-[2-(2-triphenylmethylamino-thiazol-4-yl)-2-(Z)-triphenylmethoxyiminoacetamido]-ceph-3-em-4-carboxylate, (via Delft cleavage to the 7-amino compound)

To a solution of the title compound from Example 8(a) (0.41g) in dry dichloromethane (5ml) cooled and stirred at <-25°C in an atmosphere of dry nitrogen were added a solution of phosphorus pentachloride (0.204g) in dichloromethane (5ml) and N-methylmorpholine (0.20ml). After 0.5h methanol (2.5ml) followed at a further interval of 0.5h by water (14ml) were added. The mixture was stirred vigorously during 0.5h, then ethyl acetate (20ml) was added and the dichloromethane evaporated. Saturated ammonium chloride solution (5ml) and toluene (5ml) were added and the pH adjusted to 6.5 by the cautious addition of solid potassium hydrogen carbonate. The mixture was separated, washed with water and saturated brine, dried over anhydrous sodium sulphate, evaporated under reduced pressure to circa 5ml, then tetrahydrofuran (20ml) was added and the solution evaporated to 2-3ml. This was used directly in the next stage (vide infra).

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (0.5g) in dry N,N-dimethylformamide (5ml) at -45°C under nitrogen was added methanesulphonyl chloride (0.16g). After 0.5h at <-30°C, the solution from the first stage was added, followed immediately by pyridine (54mg). The reaction mixture was allowed to warm to ambient temperature during 1h, then there were added toluene (30ml) ethyl acetate (20ml) and water (40ml). The mixture was shaken and separated. The solvent layer was washed with dilute (<1M) sulphuric acid, then with saturated sodium hydrogen carbonate, water and saturated brine, and dried over anhydrous sodium sulphate. The solution was filtered and evaporated to low volume before being subjected to column chromatography on silica gel using ethyl acetate and hexane (1:2) as elution solvent, to afford the product as a foam after evaporation of the solvents. There was obtained 0.29g of the title compound.

It had ν_{max} (KBr disc) 700, 978, 1154, 1290, 1447, 1522, 1685, 1719, 1755, 1792, 2973 and 3325cm^{-1}; m/z (FAB. MNa^+) 1088; δ_H ($CDCl_3$) 1.23 (9H, s), 1.95 (3H, s), 3.21 and 3.49 (2H, ABq, J 19Hz), 4.87 and 5.23 (2H, ABq, J 14Hz), 5.05 (1H, d, J 5Hz), 5.63 (1H, s), 5.88 and 5.96 (2H, ABq, J 5.5Hz), 6.05 (1H, bs, collapses to d, J 5Hz with D_2O), 6.12 (1H, s), 6.46 (1H, s), 6.78 (1H, bs) and 7.20-7.40 (30H, m).

Example 8(c)

2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-[2-(2-Amino-thiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(2-methyl-prop-2-enoyloxymethyl)ceph-3-em-4-carboxylate hydrochloride

The product from Example 8(b) (0.26g) was dissolved in 98-100% formic acid (3ml). 5M aqueous hydrochloric acid (0.14ml) was added and the mixture stirred in an ice-bath, which was not replenished, during 4.5h. The insoluble precipitate was removed by filtration and washed with a little 90% formic acid. The filtrate and washings were evaporated under reduced pressure to dryness with several portions of toluene and tetrahydrofuran, and finally with tetrahydrofuran alone. The gummy residue was triturated with ether, the solid hydrochloride filtered off, washed with ether and dried *in vacuo* to yield the title product (0.10g). It had ν_{max} (Nujol Mull) *inter alia* 1790cm^{-1}; *m/z* (FAB, MH^+ and MNa^+ respectively) 582 and 604; δ_h (D_6-DMSO) 1.15 (9H, s), 1.88 (3H, s), 3.61 and 3.74 (2H, ABq, *J* 18Hz), 4.78 and 5.04 (2H, ABq, *J* 13Hz), 5.24 (1H, d, *J* 5Hz), 5.73 (1H, s), 5.81 and 5.91 (2H, ABq, *J* 6Hz), (obscures 7CH quartet), 6.05 (1H, s), 6.81 (1H, s), and 9.72 (1H, d, *J* 8Hz, exchanges with D_2O).

Example 9

Example 6 (a) via oxidation-reduction sequence:

Example 9(a)

Diphenylmethyl (6R,7R)-3-(2-Methylprop-2-enoyloxy-methyl)-7-phenylacetamidoceph-3-em-1-oxide-4-carboxylate

To a solution of diphenylmethyl (6R,7R)-3-chloro-methyl-7-phenylacetamidoceph-3-em-4-carboxylate ('GClH') (5g) in dichloromethane (30ml) at ambient temperature were added water (15ml) tetrabutylammonium iodide (2g) methacrylic acid (1ml) and sodium metabisulphite (0.2g). The mixture was stirred and neutralised to pH 7.0 with 5M aqueous sodium hydroxide then maintained at pH 6-7 by the occasional addition of 1M sodium hydroxide solution. After 4hr, the layers were separated, the solvent layer was diluted with an equal volume of toluene, then washed with water, sodium metabisulphite solution, sodium hydrogen carbonate solution, saturated brine and dried over anhydrous sodium sulphate. The solution was filtered and evaporated to a gum under reduced pressure (6g).

This material (largely the ^{2} isomer of 6(a)) was redissolved in dichloromethane (100ml), cooled in an ice-bath to 2-3°C and 55% 4-chloroperbenzoic acid (4g) added. After 1.5h, the mixture was washed with water, sodium hydrogen carbonate solution, sodium metabisulphite solution, water and brine, then dried over anhydrous sodium sulphate, filtered and evaporated to near dryness. 2-Propanol was added and re-evaporated to a syrup, which was dissolved in 2-propanol (30ml) and refrigerated overnight. The crystalline mass was broken up, filtered off, washed

with 2-propanol and then ether and dried *in vacuo*, to yield the title product (4.5g).

In a further experiment, 10g of 'GClH' was subjected to an overnight substitution reaction; the oxidant in the second stage was added portion wise (checking by tlc to avoid over-oxidation) to yield 8.7g of the title product. It had ν_{max} (KBr disc) 696, 1034, 1150, 1650, 1717, 1785, and 3285cm^{-1}; *m/z* (FAB, MNa^+ 621; δ_H ($CDCl_3$): 1.90 (3H, s), 3.15 and 3.79 (2H, ABq, *J* 19Hz), 3.59 and 3.66 (2H, ABq, *J* 16Hz), 4.43 (1H, dd, *J* 1.50 and 5Hz), 4.79 and 5.39 (2H, ABq, *J* 14Hz), 5.59 (1H, t, *J* 1.5Hz), 6.05 (1H, s), 6.10 (1H, dd, *J* 5 and 10Hz), 6.73 (1H, d, *J* 10Hz), 6.93 (1H, s), and 7.20-7.50 (*circa*. 15H, m).

Example 9(b)

The sulphoxide from Example 9(a) (8.7g) was dissolved in N,N-dimethylformamide, (70ml) stirred and cooled to -25 to -30°C under an atmosphere of dry nitrogen. Phosphorus trichloride (2.4ml) was added rapidly, the mixture allowed to stir at -25°C for 10 minutes and poured onto ice. The product was filtered off, washed with much water, taken into dichloromethane, washed again with water, dried over anhydrous sodium sulphate, filtered and evaporated to a gum. this was dissolved in a little toluene and subjected to column chromatography on silica gel using ethyl acetate and hexane (1:1) as eluent. Fractions containing the pure compound (by thin layer chromatography) were combined and evaporated. Less pure fractions were combined and rechromatographed using ethyl acetate-hexane (2:3) as elution solvent. Again fractions containing the required compound were combined, evaporated and bulked with the previous material, to obtain a total of 5.6g of the title product after trituration with a little ether, filtration and drying.

Example 10

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid and its sodium salt

Example 10(a)

Diphenylmethyl (6R,7R)-3-(2-Methylprop-2-enoyloxy-methyl)-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]ceph-3-em-4-carboxylate

A solution of 2-(Z)-methoxyimino-2-(triphenylmethyl-aminothiazol-4-yl)acetic acid hydrochloride (0.5g) in dry N,N-dimethylformamide (7ml) was cooled to -35°C under nitrogen. To it was added diisopropylethylamine (0.35ml) and methanesulphonyl chloride (0.08ml) and the mixture stirred for 0.5h. The amino compound from Example 6(b) (0.5g) and dry pyridine (0.08ml) were added, and the mixture allowed to warm slowly to near ambient temperature during 1h. Toluene (6ml), tetrahydrofuran (4ml) and water (20ml) were added, shaken and separated. The aqueous layer was re-extracted with a little toluene, the solvent layers combined, washed several times with water, then with saturated brine, dried over anhydrous sodium sulphate, filtered and evaporated to a gum, which was subjected to chromatography on silica gel using ethyl acetate and hexane (1:1) as elution solvent. Fractions containing the desired compound (by tlc) were combined and evaporated to a foam (0.6g).

The title product had ν_{max} (KBr disc) 700, 754, 1037, 1149, 1220, 1522, 1684, 1719, 1788, 2926, 3303 and 3395cm^{-1}; m/z (FAB, MNa^+) 912; δ_H ($CDCl_3$); 1.92 (3H, s), 3.40 and 3.58

(2H, ABq, J 19Hz), 4.08 (3H, s), 4.09 and 5.17 (2H, ABq, J 14Hz), 5.06 (1H, d, J 3Hz), 5.60 (1H, s), 5.97 (1H, dd, J 5 and 9Hz), 6.08 (1H, s), 6.74, 6.79, 6.95 and 7.00 (4H total) and 7.20-7.50 (circa 25H, m).

Example 10(b)

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid and its sodium salt

To the material from Example 10(a) was added tetrahydrofuran (0.2ml). After an interval of 0.25h, 98-100% formic acid (5ml) was added, the mixture cooled in an ice-bath and after 0.5h, 1M aqueous hydrochloric acid (0.5ml) was added. A gelatinous precipitate appeared which redissolved. After a further 0.5h the mixture was treated with concentrated hydrochloride acid (0.1ml); it was then stirred for 0.75h. The volatile components were removed in vacuo and the residue triturated with ether. It was found to be only partially deprotected, so it was redissolved in formic acid (3ml) at ambient temperature and then treated successively with 1M aqueous hydrochloric acid (0.4ml) then concentrated hydrochloric acid (0.1ml). After 0.75h, the reaction mixture was evaporated under reduced pressure with a mixture of toluene and tetrahyrofuran, to leave a gum which was triturated with ether. The insoluble material was collected by filtration, washed with ether and dried in vacuo. This material was treated with tetrahydrofuran (1ml) and water (3ml) and the pH of the mixture adjusted to 3.3 by the cautious addition of potassium carbonate. The aqueous phase was decanted, and the gum stirred with 1ml of water for 1h. The crystalline free acid was filtered off, washed with a little water and dried in vacuo to yield the title product

(0.1g). It had ν_{max} (KBr disc) 1038, 1156, 1528, 1630, 1675, 1714, 1783, 2939 and 3317cm^{-1}.

It was suspended in a small volume of water and titrated gradually to pH 6.5 with very dilute sodium hydroxide solution. The undissolved material was removed by filtration and the filtrate evaporated to dryness using successively 1-propanol, tetrahydrofuran and acetone as co-solvents. The residue was triturated with acetone-ether, filtered off and dried in vacuo, to yield the title compound as its sodium salt (55mg).

It had ν_{max} ('Nujol' mull) 1038, 1164, 1536, 1614, 1711, 1768 and 3307cm^{-1} and δ_h (D_6-DMSO); 1.88 (3H, s), 3.24 and 3.50 (2H, ABq, J 17Hz), 3.35 (H_2O), 3.83 (3H, s), 4.82 and 5.07 (2H, ABq, J 12Hz9, 5.01 (1H, d, J 5Hz), 5.58 (1H, dd, J 5 and 8Hz), collapses with D_2O to 5.60 (1H, d, J 5Hz), 5.67 (1H, s), 6.02 (1H, s), 6.74 (1H, s), 7.24 (ca 2H, bs, largely exchanges with D_2O) and 9.54 (1H, d, J 8Hz, exchanges with D_2O).

Example 11

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamidol-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylic acid, its sodium salt and 'axetil' ester.

Example 11(a)

Diphenylmethyl (6R,7R)-3-[(Z)-2-Methylbut-2-enoyloxy-methyl]-7-phenylacetamidoceph-3-em-1-oxide-4-carboxylate

A mixture of GClH (see Example 9(a)) (5g), dichloromethane (30ml), water (15ml), tiglic acid [(Z)-2-methylbut-2-enoic

acid, 2.5g] and tetrabutylammonium iodide (1.7g) was stirred and titrated wo pH 7.0 with 10% aqueous sodium hydroxide soution. Sodium hydroxide solution was also used to maintain the solution at neutrality, a little chloroform was added also to replace dichloromethane lost by evaporation, and the reaction mixture stirred overnight. The layers were separated, the solvent layer diluted with toluene (50ml) and washed successively with sodium metabisulphite solution, sodium hydrogen carbonate solution, water and saturated brine, and dried over anhydrous sodium sulphate. The solution was filtered and evaporated under reduced pressure to a gum.

This was redissolved in dichloromethane (100ml) and roughly powdered 55% 3-chloroperbenzoic acid (4g) added. The mixture was stirred during 2h in an ice-bath. Excess sodium metabisulphite solution was added, shaken and separated, then the solvent layer was washed with sodium hydrogen carbonate solution and saturated brine, dried over anhydrous sodium sulphate, filtered and evaporated partially. 2-Propanol (circa 50ml) was added and the evaporation continued. The product began to crystallise; when the volume had been reduced to about 25ml, 2-propanol (50ml) was added, the product collected by filtration, washed with 2-propanol then with ether and dried in vacuo, to yield the title product (3.9g).

A small amount was recrystallised from dichloromethane2-propanol. It had ν_{max} (KBr disc) 697, 1034, 1221, 1249, 1382, 1532, 1648, 1718, 1784, 3031 and 3289cm^{-1}; m/z (FAB, MNa^+) 635; δ_H ($CDCl_3$) 1.79 (6H, s), 3.15 and 3.81 (2H, ABq. J 19Hz), 3.60 and 3.67 (2H, ABq, J 16Hz), 4.44 (1H, d, J 5Hz), 4.78 and 5.38 (2H, ABq, J 14Hz), 6.10 (1H, dd, J 5 and 10Hz), 6.70 (1H, d, J 10Hz), 6.75-6.85 (1H, m), 6.93 (1H, s), and 7.20-7.50 (15H, m).

Example 11(b)

Diphenylmethyl (6R,7R)-3-[(Z)-2-Methylbut-2-enoyloxymethyl]-7-phenylacetamidoceph-3-em-4-carboxylate

The title product from Example 11(a) (3.66g) was dissolved in dry N,N-dimethylformamide under nitrogen and cooled with stirring to -22°C. Phosphorus trichloride (1.0ml) was added all at once. After 8 min at -20°C (external temperature) the mixture was poured onto ice. The solid was collected by filtration, washed with much water and dried in vacuo, to yield 3.4g of the title product.

A small sample recrystallised from tetrahydrofuran-ethanol had ν_{max} (KBr disc) 698, 733, 1259, 1528, 1647, 1705, 1785, 3033 and 3295cm^{-1}; m/z (FAB, MNa^+) 619; δ_H ($CDCl_3$); 1.78 (3H, d, J 6Hz), overlaps with 1.79 (3H, s), 3.34 and 3.52 (2H, ABq, J 19Hz), 3.61 and 3.69 (2H, ABq, J 16Hz), 4.84 and 5.11 (2H, ABq, J 14Hz), 4.95 (1H, d, J 5Hz), 5.87 (1H, q, J 5 and 9Hz), 6.02 (1H, d, J 9Hz), 6.75-6.85 (1H, m), 6.92 (1H, s), and 7.20-7.50 (15H, m).

Example 11(c)

Diphenylmethyl (6R,7R)-7-Amino-3-[(Z)-2-methylbut-2-enoyloxymethyl]-ceph-3-em-4-carboxylate

The product from Example 11(b) (3.3g) dissolved in dichloromethane (40ml) was cooled and stirred at <-20°C under an atmosphere of nitrogen. N-Methylmorpholine (1.34ml) and a solution of phosphorus pentachloride (1.5g) in dichloromethane (34ml) were added. The internal temperature rose from -25°C to -12°C then fell again quickly

to -20°C. The mixture was stirred for 0.5h, methanol (12ml) was added, then after a further 0.5h, water (50ml). During this period the temperature was allowed to rise to near ambient. Ethyl acetate (50ml) was added, and the dichloromethane and much of the ethyl acetate evaporated under reduced pressure. 5M aqueous hydrochloric acid (2ml) was added, and ether (50ml). The ether and aqueous layer were decanted from the heavy oil. This was layered with water, tetrahydrofuran and toluene, and titrated to pH 6.0 with dilute sodium hydroxide solution. The solvent layer was dried over anhydrous sodium sulphate, filtered and evaporated to small volume, then subjected to column chromatography on silica gel using ethyl acetate and hexane (7:3 ratio) as elution solvents. Fractions containing the desired product (by tlc) were combined and evaporated to an oil, which was dissolved in ether (5ml) and refrigerated at -10°C overnight, when it crystallised. It was collected by filtration, washed with a little ether and dried in vacuo, to yield the title compound (1.0g). It had ν_{max} (KBr disc) 703, 1079, 1099, 1220, 1252, 1371, 1630, 1647, 1712, 1779, 1795, 2918, 3345 and 3397cm^{-1}; m/z (FAB, MNa^+) 501; δ_H ($CDCl_3$); 1.79 (3H, d, J 7Hz) overlaps 1.80 (3H, s), 3.40 and 3.57 (2H, ABq, J 19Hz), 4.78 (1H, d, J 5Hz), 4.84 and 5.10 (2H, ABq, J 14Hz), 4.95 (1H, d, J 5Hz), 6.75-6.90 (1H, m). 6.95 (1H, s), and 7.20-7.50 (circa 11H, m, includes exchangeable proton).

Example 11(d)

Diphenylmethyl(6R,7R)-3-[(Z)-2-Methylbut-2-enoyloxymethyl]-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-triphenylmethoxyiminoacetamido]ceph-3-em-4-carboxylate

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (0.75g) in dry

N,N-dimethylformamide (10ml) cooled at -37°C and stirred under nitrogen was added methanesulphonyl chloride (0.18ml). The mixture was stirred 0.5h at between -35°C and -45°C. The product from Example 8(c) (0.5g) and pyridine (85mg) were added, and the mixture allowed to warm to ambient temperature during 45min. There were added toluene (10ml), tetrahydrofuran (5ml) ethyl acetate (5ml) and water (25ml); the mixture was shaken and separated. The solvent layer was washed successively with dilute aqueous hydrochloric acid (circa 0.5M), sodium hydrogen carbonate solution and brine and dried over anhydrous sodium sulphate. The solution was filtered and evaporated to low volume. It was subjected to column chromatography on silica gel using ethyl acetate and hexane (2:3 ratio) as elution solvent. After evaporation of the solvents, the title compound was isolated as a foam (0.89g). It had ν_{max} (KBr disc); 699, 753, 1253, 1447, 1491, 1522, 1690, 1708, 1790, 3057 and 3378cm^{-1}; m/z (FAB, MNa^+ 1154; δ_H ($CDCl_3$); 1.79 (3H, d, J 7Hz), overlaps 1.80 (3H, s), 3.22 and 3.48 (2H, ABq, J 19Hz), 4.86 and 5.16 (2H, ABq, J 14Hz), 5.05 (1H, d, J 5Hz), 6.11 (1H, dd, J 5 and 9Hz, collapses to d, J 5Hz with D_2O), 6.42 (1H, s), 6.75 (1H, s, exch. with D_2O), 6.75-6.90 (1H, m), 6.96 (1H, s), and 7.10-7.60 (35H, m).

Example 11(e)

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylic acid and its sodium salt

The product from Example 11(d) was dissolved in formic acid (98-100%, 7ml) and 1M aqueous hydrochloric acid (0.7ml) and the mixture stirred 0.5h. Concentrated hydrochloric acid (0.1ml) was then added and the mixture stirred during 1h. The precipitate was removed by filtration and washed with a

little 90% formic acid. The filtrate was mixed with toluene and evaporated to near dryness, evaporated again with toluene and tetrahydrofuran, the residue triturated with ether and collected by filtration. The ether-wet material was added to water and titrated to pH 3.5 with dilute aqueous sodium hydroxide solution. The free acid of the title was collected by filtration, washed with water and suspended in water, layered with a little toluene, and titrated to pH 6.5 with dilute aqueous sodium hydroxide solution. The mixture was filtered through a silica filter-aid pad, the aqueous solution diluted with 1-propanol and evaporated to near dryness under reduced pressure. It was re-evaporated with tetrahydrofuran to dryness, the residue triturated with ether, filtered off, washed with ether and dried in vacuo to yield the title sodium salt (175mg). It had ν_{max} (KBr disc); 735, 1264, 1395, 1528, 1675, 1764 and 3324cm^{-1}; (Nujol mull); 1537, 1613, 1767 and 3295cm^{-1}; m/z (FAB, MH^+) 504; δ_H (D_6-DMSO) 1.76 (6H, s), 3.23 and 3.49 (2H, ABq, J 17Hz), 4.80 and 5.05 (2H, ABq, J 12Hz), 5.02 (1H, d, J 5Hz), 5.61 (1H, dd, J 5 and 8 Hz), 6.65 (1H, s), 6.70-6.85 (1H, m), and 9.40 (1H, d, J 8Hz).

Example 11(f)

(R,S)-1-Acetoxyethyl(6R,7R)-7-[2-(2-Aminothiazol-4-yl-2-(Z)-hydroxyiminoacetamido]-3-[(Z)]2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylate hydrochloride

To the product from Example 11(e) (0.97g) was added a solution of 0.1ml 1.0M aqueous hydrochloric acid in formic acid (98-100%, 10ml). When all was in solution, a further 0.9ml of 1M hydrochloric acid was added. After 30min, concentrated aqueous hydrochloric acid (0.1ml) was added. After a further 1.5h at ambient temperature, the insolubles were removed by filtration and washed with a little 90%

formic acid. The filtrate was evaporated to dryness in vacuo, and redissolved in formic acid (4ml), 5M hydrochloric acid (0.15ml) added, and the mixture stirred during 1h. The precipitate was again filtered off, washed with a little 90% formic acid and the filtrate evaporated to dryness in vacuo. The residue was triturated with ether, filtered off, washed with ether and dried, to give the title product as a colourless solid (0.25g). It had ν_{max} (KBr disc) 734, 1073, 1258, 1380, 1527, 1629, 1677, 1708, 1765, 1785 and 3100 (very broad); m/z (FAB, MH^+) 568; δ_H (D_6-DMSO); 1.46 (3H, d, J 5.5Hz), 1.78 (3H, d, J 5.5Hz), 1.79 (3H, s), 2.03 and 2.06 (3H, 2s), 3.60 and 3.73 (2H, ABq, J 18 Hz), 4.76, 4.78 and 4.96 (2H, ABq, with one pair of peaks doubled, J 13Hz), 5.23 and 5.24 (1H, 2d, J 5.5Hz), 5.80-5.95 (1H, m), 6.81 (circa 2H, bs), 6.89 and 6.99 (1H, 2 x q, J 5.5Hz), 9.71 (1H, d, J 8Hz), and 12.20 (1H, bs).

Example 11(q)

(R,S)-1-Acetoxyethyl(6R,7R)-3-[(Z)-2-Methylbut-2-enoyloxymethyl]-7-phenylacetamidoceph-3-em-4-carboxylate (via the free acid)

The product of Example 11(a) (3g) was dissolved in formic acid (98-100%, 50ml) and treated with 1M aqueous hydrochloric acid (5ml). After an interval of 2h (tlc at 1.5h showed little starting material remained), the mixture was evaporated to about ¼ volume, toluene and tetrahydrofuran added and re-evaporated several times to dryness (the residue consisted of an oil and a suspended solid). This was treated with ethyl acetate, toluene, tetrahydrofuran and saturated sodium hydrogen carbonate to give pH 8. The solvent layer was discarded, and to the aqueous layers were added dichloromethane (20ml), chloroform

(10ml), tetrabutylammonium iodide (0.75g), 1-bromoethyl acetate (2.0ml) and solid calcium carbonate (5g). The mixture was stirred vigorously for 2h, then further portions (5g) of calcium carbonate and a further portion (1ml) of 1-bromoethyl acetate were added, and the mixture stirred overnight (covered to prevent loss of solvents). The insolubles were removed by filtration, washed with water, chloroform and much ethyl acetate, and the filtrate partially evaporated to remove chlorinated solvents. Toluene, tetrahydrofuran and ethyl acetate were added, shaken and separated. The solvent layer was washed with water, dried over anhydrous sodium sulphate and filtered. The filtrate was evaporated to a gum, which was subjected to column chromatography on silica gel using ethyl acetate and hexane (1:1) as eluent. The title product was obtained as a foam (0.9g) when fractions containing it (by tlc) were combined and evaporated. It had ν_{max} 733, 944, 1073, 1258, 1380, 1528, 1709, 1785, 3029 and 3303cm^{-1}; δ_H ($CDCl_3$); 1.53 (3H, d, J 5.5Hz), 1.81 (3H, d, J 7Hz), 1.82 (3H, s), 2.09 (3H, s), 3.37 and 3.53 (2H, ABq, J 19Hz), 3.61 and 3.69 (2H, ABq, J 17Hz), 4.80-5.20 (3H, m), 5.80-5.90 (1H, m), 6.00-6.15 (1H, m), 6.80-6.95 (1H, m), 6.99 and 7.09 (1H, 2 x 1, J 5.5Hz), and 7.20- 7.40 (5H, m); m/z (FAB, MNa^+); 5.39.

Example 11(h)

(R,S)-1-Acetoxyethyl (6R,7R)-3-[(Z)-2-Methylbut-2-enoyloxymethyl-7-[2-(2-triphenyl-methylaminothiazol-4-yl)-2-(Z)-triphenylmethoxyimino-acetamido]ceph-3-em-4-carboxylate (via Delft cleavage to the 7-amino compound)

To a solution of the product from Example 11(g) (0.9g) in dry dichloromethane (10ml) cooled and stirred under nitrogen at <-20°C, were added N-methylmorpholine (0.42ml) and a

solution of phosphorus pentachloride (0.46g) in dichloromethane. The mixture was stirred at <-20°C during 0.5h, then added methanol (5ml). After 0.5h, during which time the temperature had risen to -8°C, water (25ml) was added and stirred vigorously for 0.5h. Ethyl acetate was added and the dichloromethane evaporated under reduced pressure. Ethyl acetate (50ml) and saturated ammonium chloride (10ml) were added, and the mixture neutralised at 10°C with solid potassium hydrogen carbonate to pH 6.5. The layers were separated, the solvent layer was washed with saturated brine and dried over anhydrous sodium sulphate, filtered and evaporated to low volume. Tetrahydrofuran (circa 20ml) was added and evaporated to leave a residue of 2-3ml, which was used in the next stage (vide infra). Tlc showed the absence of the starting material, and the presence of a new, more polar zone.

To a solution of sodium 2-(Z)-triphenylmethoxyimino-2-(2-triphenylmethylaminothiazol-4-yl)acetate (1.04g) in dry N,N-dimethylformamide (10ml), cooled and stirred under nitrogen at -40°C, was added methanesulphonyl chloride (0.37g). After 0.5h at -40°C, pyridine (120mg) and the tetrahydrofuran solution, prepared as described above, were added. The reaction mixture was allowed to warm to near ambient temperature during 1h. Toluene, tetrahydrofuran and water were added, stirred and separated. The solvent layer was washed with dilute hydrochloric acid (circa 0.5M), dilute sodium hydrogen carbonate and saturated brine and dried over anhydrous sodium sulphate. The solution was filtered, evaporated to low volume and submitted to column chromatography on silica gel using 35% ethyl acetate in hexane as elution solvent. The title compound was obtained as a foam after evaporation of solvents from the appropriate fractions.

It had ν_{max} (KBr disc); 700, 1072, 1251, 1523, 1690, 1707, 1734, 1764, 1791, 3057 and 3384cm^{-1}; m/z (FAB, MNa^+); 1074, δ_H ($CDCl_3$); 1.55-1.60 (3H, m), 1.81 (3H, d, J 9Hz), 1.83 (3H, s), 2.08, 2.09 (3H, 2 x s), 3.22 and 3.48 (2H, ABq, J 19Hz), 4.88, 4.93 and 5.20 (2H, ABq - one pair of peaks doubled, J 14Hz), 5.04 and 5.05 (1H, 2 x d, J 4.5 Hz), 6.0 - 6.15 (1H, m), 6.40 (1H, s), 6.72 (1H, s, exch. with D_2O), 6.80-6.95 (1H, m), 7.03 and 7.13 (1H, 2 x q, J 5.5Hz), and 7.20-7.40 (30H, m).

Example 12

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylic acid and its sodium salt

Example 12(a)

Diphenylmethyl (6R,7R)-3-[(Z)-2-Methylbut-2-enoyloxy-methyl]-7-[2-(2-triphenylmethylaminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]ceph-3-em-4-carboxylate

To a solution of 2-(Z)-methoxyimino-2-(triphenylmethyl-aminothiazol-4-yl)acetic acid hydrochloride (0.47g) in dry N,N-dimethylformamide (9ml) cooled (under nitrogen) at -38°C and stirred was added N-ethyldiisopropylamine (0.18ml) and methanesulphonyl chloride (80µl). After 0.5h at -35°C, the amine product from Example 8(c) (0.45g) and pyridine (80µl) were added, and the temperature allowed to rise gradually to ambient during 1h. Toluene (10ml) tetrahydrofuran (5ml) ethyl acetate (5ml) and water (25ml) were added, shaken and separated. The aqueous layer was washed with toluene, and the combined solvent layers washed successively with dilute aqueous hydrochloric acid (circa 0.5M), dilute sodium

hydrogen carbonate, water and saturated brine, and dried over anhydrous sodium sulphate. It was evaporated to low volume and subjected to silica gel chromatography using ethyl acetate and hexane (2:3) as eluent. Fractions containing the desired product (by tlc) were combined and evaporated under reduced pressure, to yield the title product as a foam (0.6g).

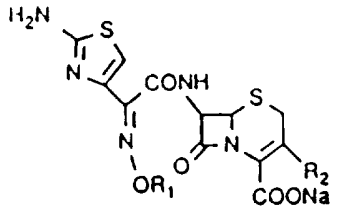
It had ν_{max} (KBr disc); 700, 1037, 1255, 1522, 1685, 1708, 1787, 2935, 3292 and 3390cm^{-1}; m/z (FAB, MNa^+) 926; δ_H ($CDCl_3$); 1.79 (3H, d, J 7Hz), 1.80 (3H, s), 3.40 and 3.57 82H, ABq, J 19Hz), 4.08 (3H, s), 4.89 and 5.15 (2H, Abq, J 14Hz), 5.05 (1H, d, J 5Hz), 5.96 (1H, dd, J 5 and 8Hz), 6.75 (1H, s), 6.80-6.90 (1H, m), 6.95 (1H, s), 7.01 (1H, s), and 7.20-7.50 (25H, m).

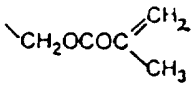
Example 12(b)

(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoximino-acetamido]-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylic acid and its sodium salt

The product from Example 12(a) (0.58g) was dissolved in 98-100% formic acid (5ml). When all was in solution, 1M aqueous hydrochloric acid (0.5ml) was added with vigorous stirring to redissolve the gum which had precipitated. After 0.5h, concentrated hydrochloric acid (0.1ml) was added and the mixture stirred during 45min. The insoluble materials were removed by filtration and washed with a little 90% formic acid, then the filtrate was evaporated to dryness using toluene and tetrahydrofuran as co-solvents. The residue was re-evaporated with tetrahydrofuran, triturated with ether and the hydrochloride filtered off, washed with ether and dried in vacuo, to yield 0.3g. This was suspended in water containing a small amount of toluene,

and titrated to pH 3.5 with very dilute sodium hydroxide solution. Undissolved materials were filtered off, washed with a little water, and with toluene, to give the free acid as a wet solid (circa 0.6g). It was re-suspended in water and titrated to pH 6.5 with very dilute aqueous sodium hydroxide. A small amount of insoluble material was removed by filtration and the filtrate evaporated to near dryness with 1-propanol as co-solvent. The residue was re-evaporated with tetrahydrofuran then acetone, triturated with acetone-ether, filtered off, washed with ether and dried in vacuo, to yield the title product (175mg). It had ν_{max} (KBr disc) 735, 1039, 1262, 1389, 1529, 1609, 1700, 1764, 2939 and 3295cm^{-1}; m/z (FAB, MH^+); 518; δ_H (D_6-DMSO); 1.77 (6H, s), 3.24 and 3.50 (2H, ABq, J 17Hz), 3.84 (3H, s), 4.80 and 5.05 (2H, ABq, J 12Hz), 5.02 (1H, d, J 5Hz), 5.59 (1H, dd, J 5 and 8Hz, collapses to d, J 5Hz with D_2O), 6.73 (1H, s), 6.75-6,85 (1H, m), 7.26 (2H, s, exchanges with D_2O) and 9.55 (1H, d, J 8Hz, exchanges with D_2O).



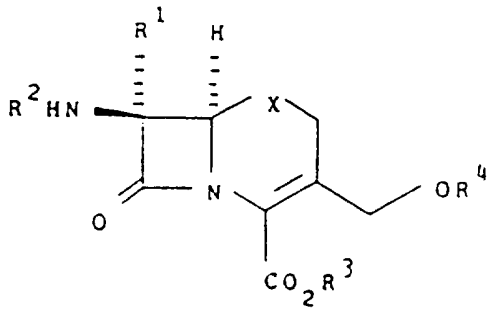
	R_1	R_2
FK482	H	
Ex.6	H	

Geometric Mean MIC's

ORGANISM	No. of strains	FK482	Ex.6
S.aureus Axsens	10	0.23	0.23
S.aureus Axres	10	0.38	0.27
S.epidermidis Axsens	4	0.13	0.18
S.epidermidis Axres	9	0.04	0.13
S.saprophyticus	5	0.11	0.25
H.influenzae Axsens	8	0.30	0.11
H.influenzae Axres	7	0.23	0.08
H.influenzae IR	1	0.50	1.00
B.catarrhalis Axsens	6	0.09	0.13
B.catarrhalis Axres	12	0.22	0.84
E.coli (R-)	5	0.11	0.04
E.coli (R+)	5	0.13	0.07
E.coli (C+)	5	9.19	0.14
K.pneumoniae CFZsens	4	0.07	0.11
K.pneumoniae CFZres	4	0.21	0.25
S.marcescens CTXsens	3	16.00	1.00
S.marcescens CTXres	3	>32	32.00
E.cloacae CTXsens	2	8.00	0.50
E.cloacae CTXres	1	>32	16.00
E.aerogenes	3	12.70	0.79
P.vulgaris	3	0.40	0.20
M.morganii	3	12.70	0.40
P.rettgeri	3	0.03	0.20
P.mirabilis Axsens	3	0.10	0.03
P.mirabilis Axres	5	0.11	0.06
C.freundii	5	0.76	0.14
C.koseri	4	0.06	0.04
P.aeruginosa	3	>32	>32

CLAIMS

1. A compound of formula (I) or a salt thereof:

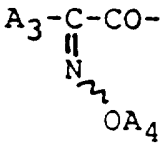


(I)

wherein

R^1 is hydrogen, methoxy or formamido;

R^2 is an acyl group of formula (a)

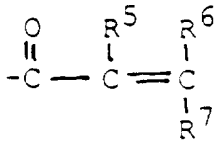
(a) 

where A_3 is thiazolyl optionally substituted by an amino or substituted amino group which may be in protected form and A_4 is hydrogen or an organic residue;

CO_2R^3 is a carboxy group or a carboxylate anion, or R^3 is a readily removable carboxy protecting group;

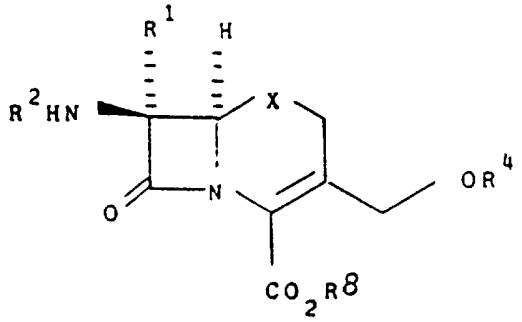
X is S, SO, SO_2, O or CH_2; and

R^4 is a group



where R^5 and R^6 are independently hydrogen or C_{1-6} alkyl, or R^5 and R^6 are together an alkyne bond, and R^7 is hydrogen, C_{1-6} alkyl or C_{2-4} alkenyl.

2. A compound according to claim 1 having a formula IA,



(IA)

wherein R^1, R^2, R^4 and X are as defined in formula (I) in claim 1, and the group CO_2R^8 is CO_2R^3 where CO_2R^3 is a carboxy group or a carboxylate anion, or a pharmaceutically acceptable salt or <u>in vivo</u> hydrolysable ester thereof.

3. A compound according to claim 1 or claim 2 wherein R^1 is hydrogen.

4. A compound according to claim 1, 2 or 3 wherein the group R^2 is a group of formula (a) in a Z configuration.

5. A compound according to any one of the preceding claims wherein the thiazol system A_3 is a thiazol-4-yl system.

6. A compound according to claim 5 wherein A_3 is 2-aminothiazol-4-yl.

7. A compound according to any one of the preceding claims wherein A_4 is selected from hydrogen, methyl, triphenylmethyl, ethyl, cyclopropylmethyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, phenyl, carboxymethyl, carboxypropyl, t-butoxycarbonyl methyl and $CH_nX_{(3-n)}$ where n is 0 - 3 and X is chlorine or fluorine.

8. A compound according to any one of the preceding claims wherein X is sulphur.

9 A compound according to any one of the preceding claims wherein R^4 is selected from 3-methylbut-2-enoyl, 2-methylprop-2-enoyl, but-2-ynoyl, hexa-2,4-dienoyl and 2-methylbut-2-enoyl.

10. A compound acording to claim 2 being:

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(3-methylbut-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-methoxyimino-acetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(but-2-ynoyloxymethyl)ceph-3-em- 4-carboxylic acid,

(6R,7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylic acid,

(6R, 7R)-7-[2-(2-aminothiazol-4-yl)-2-(Z)-hydroxyimino-acetamido]-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylic acid,

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(3-methylbut-2-enoyloxy-methyl)ceph-3-em-4-carboxylate,

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-[(E,E)-hexa-2-4-dienoyloxy-methyl]ceph-3-em-4-carboxylate,

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(2-methylprop-2-enoyloxy-methyl)ceph-3-em-4-carboxylate,

2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyimino=acetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylate,

(R,S)-1-Acetoxyethyl(6R,7R)-7-[2-(Aminothiazol-4-yl)-2-(Z)hydroxyimino-acetamido]-3-(2-methylprop-2-enoyloxyoxy=methyl)ceph-3-em-4-carboxylate hydrochloride,

2,2-Dimethylpropanoyloxymethyl (6R,7R)-7-[2-(2-Amino-thiazol-4-yl)-2-(Z)-hydroxyiminoacetamido]-3-(2-methyl-prop-2-enoyloxymethyl)ceph-3-em-4-carboxylate hydrochloride,

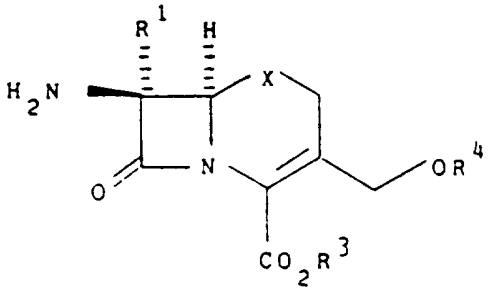
Sodium
(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoxyiminoacetamido]-3-(2-methylprop-2-enoyloxymethyl)ceph-3-em-4-carboxylate,

Sodium
(6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-hydroxyiminoacetamido-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylate,

(R,S)-1-Acetoxyethyl(6R,7R)-7-[2-(2-Aminothiazol-4-yl-2-(Z)-hydroxyiminoacetamido]-3-[(Z)]2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylate hydrochloride, and

Sodium (6R,7R)-7-[2-(2-Aminothiazol-4-yl)-2-(Z)-methoximino-acetamido]-3-[(Z)-2-methylbut-2-enoyloxymethyl]ceph-3-em-4-carboxylate.

11. A process for the preparation of a compound of formula 1 as claimed in claim 1, which process comprises treating a compound of formula (III) or a salt thereof:



(III)

wherein R_1, CO_2R^3, R^4 and X are as hereinbefore defined, wherein any reactive groups may be protected, and wherein the amino group is optionally substituted with a group which permits acylation to take place; with an N-acylating derivative of an acid of formula (IV):

$$R^2OH \qquad (IV)$$

wherein R^2 is as defined with respect to formula (I) and wherein any reactive group may be protected; and thereafter as necessary or desired, carrying out one or more of the following steps:

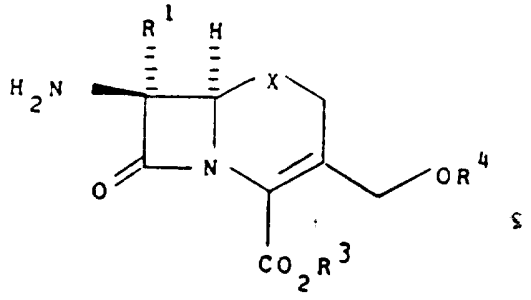
i) removing any protecting groups;

ii) converting the group CO_2R^3 into a different group CO_2R^3 or CO_2R^8;

iii) converting the group X into a different group X;

iv) converting the product into a salt.

12. A compound of formula (III) or a salt thereof:



(III)

wherein R_1, CO_2R^3, R^4 and X are as defined in claim 1, wherein any reactive groups may be protected, and wherein the amino group is optionally substituted with a group which permits acylation to take place.

13. A compound of formula (III) as claimed in claim 12 wherein R^3 is diphenyl methyl.

14. A compound according to claim 13, being;

Diphenylmethyl(6R,7R)-7-amino-3-(3-methylbut-2-enolyloxymethyl)ceph-3-em-4-carboxylate,

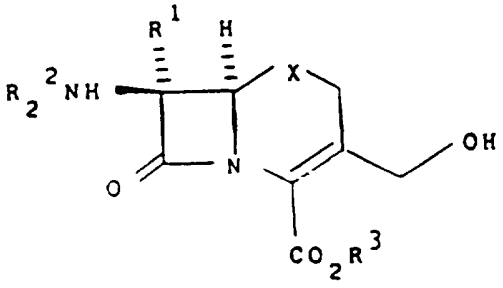
Diphenylmethyl (6R,7R)-7-Amino-3-[(E,E)-hexa-2,4-dienoyloxymethyl]ceph-3-em-4-carboxylate,

Diphenylmethyl (6R,7R)-7-Amino-3-(but-2-ynoyloxymethyl)ceph-3-em-4-carboxylate,

Diphenylmethyl (6R,7R)-7-Amino-3-(2-methylprop-2-enoyloxymethyl)-ceph-3-em-4-carboxylate, or

Diphenylmethyl (6R,7R)-7-Amino-3-[(Z)-2-methylbut-2-enoyloxymethyl]-ceph-3-em-4-carboxylate,

15. A process for the preparation of a compound of formula I as claimed in claim 1, which process comprises reacting a compound of formula (V):



(V)

wherein R^1, CO_2R^3 and X are as defined in formula I, R_2^2 is R^2 as hereinbefore defined or an acyl group convertible thereto, and any reactive groups are optionally protected; with an acylating olefinic acid of formula (VI) or a derivative thereof:

$$R^4OH \qquad (VI)$$

wherein R^4 is as hereinbefore defined in formula (I); and thereafter, if necessary or desired, carrying out one or more of the following steps:

i) converting the group R_2^2 into a group R^2;

ii) removing any protecting groups;

iii) converting the group CO_2R^3 into a different group CO_2R^3 or CO_2R^8;

iv) converting the group X into a different group X;

v) converting the product into a salt.

16. A pharmaceutical composition comprising one or more compounds of formula Ia as claimed in claim 2, or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof; and a pharmaceutically acceptable carrier.

17. A compound of formula Ia as claimed in claim 2, or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, for use as a therapeutic substance.

18. A compound of formula Ia as claimed in claim 2, or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof for use in the treatment of bacterial infections.

19. A method of use of a compound of formula Ia as claimed in claim 2, or a pharmaceutically acceptable salt or in vivo hydrolysable ester thereof, in the manufacture of a medicament for the treatment of bacterial infections.