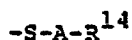


CARBAPENEM PROCESS

BACKGROUND OF THE INVENTION

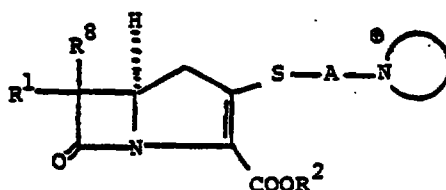
The present invention is directed to a new process for the preparation of carbapenem derivatives having a 2-substituent of the formula



wherein A represents cyclopentylene, cyclohexylene or C₂-C₆ alkylene optionally substituted by one or more C₁-C₄ alkyl groups and R¹⁴ represents a quaternized nitrogen-containing aromatic or non-aromatic heterocycle attached to A through a quaternary nitrogen atom.

The carbapenem derivatives prepared by the process of the present invention are disclosed and claimed by my colleagues Choung U. Kim and Peter F. Misco, Jr., in U.S. Patent Applications Serial Nos. 366,910, 471,379 and 389,652 filed, respectively, April 9, 1982, March 8, 1983 and June 18, 1982; the entire disclosure of each of these applications is incorporated herein by reference.

U.S. Application 366,910 and its continuation-in-part U.S. Application Serial No. 471,379, filed March 8, 1983, which continuation-in-part corresponds to West German Published Patent Application 3,312,533, disclose preparation of carbapenem antibiotics of the formula

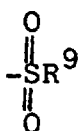
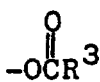
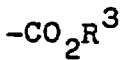
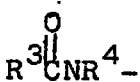
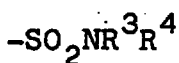
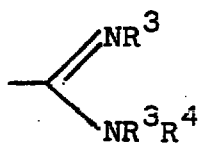
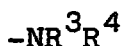


IA

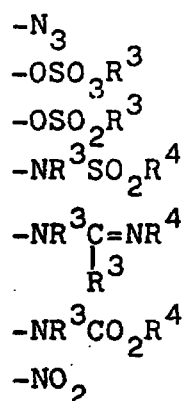
wherein R^8 is hydrogen and R^1 is selected from the group consisting of hydrogen; substituted and unsubstituted: alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl and cycloalkyl-alkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl; and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hereto atom or atoms in the above-named heterocyclic moieties are selected from the group

consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; wherein the substituent or substituents relative to the above-named radicals are selected from the group consisting of

C_1-C_6 alkyl optionally substituted by amino, halo, hydroxy or carboxyl halo



13

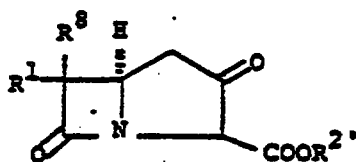


wherein, relative to the above-named substituents, the groups R^3 and R^4 are independently selected from hydrogen; alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl, cycloalkylalkyl and alkylcycloalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; and heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms, or R^3 and R^4 taken together with the nitrogen to which at least one is attached may form a 5- or 6-membered nitrogen-containing heterocyclic ring; R^9 is as defined for R^3 except that it may not be hydrogen; or wherein R^1 and R^8 taken together represent C_2-C_{10} alkylidene or C_2-C_{10} alkylidene substituted by hydroxy; A is cyclopentylene, cyclohexylene or C_2-C_6 alkylene optionally substituted by one or more C_1-C_4 alkyl groups; R^2 is hydrogen, an anionic charge or a conventional

readily removable carboxyl protecting group, providing that when R^2 is hydrogen or a protecting group, there is also present a counter anion; and

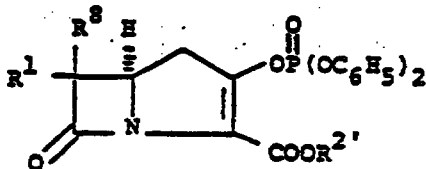


represents a substituted or unsubstituted mono-, bi- or polycyclic aromatic heterocyclic radical containing at least one nitrogen in the ring and attached to A through a ring nitrogen, thereby forming a quaternary ammonium group; and pharmaceutically acceptable salts thereof by the process shown in the following reaction scheme:



III

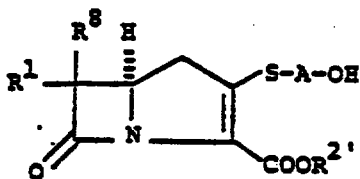
diphenyl chlorophosphate
→



IV

HS-A-OH
→

A = alkylene or
C₅-C₆ cycloalkylene

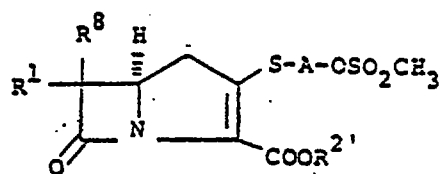


V

methanesulfonyl chloride
→

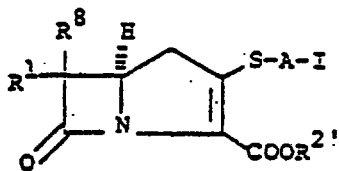
13

- 6 -



VI

I^-



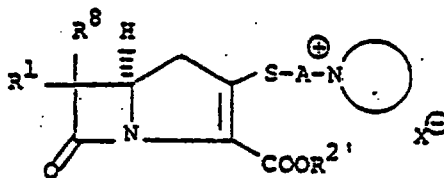
II

N

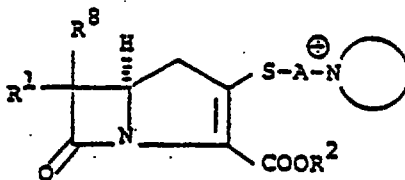
Ag^+

X^-

(X^- = counter anion)



I'A

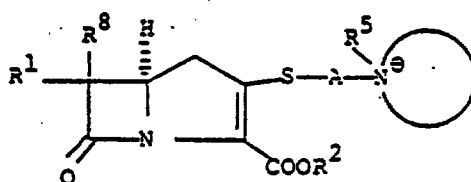


IA

16

- 7 -

Application 389,652 and its continuation-in-part application 499,690 filed June 7, 1983 disclose preparation of carbapenem antibiotics of the formula



IB

wherein R^8 is hydrogen and R^1 is selected from the group consisting of hydrogen; substituted and unsubstituted: alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl and cycloalkyl-alkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; wherein the substituent or substituents relative to the above-named radicals are independently selected from the group consisting of

C₁-C₆ alkyl optionally substituted by
amino, halo, hydroxy or carboxyl

halo

-OR³

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{OCNR}^3\text{R}^4 \end{array}$

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{CNR}^3\text{R}^4 \end{array}$

-NR³R⁴-

$\begin{array}{c} \text{NR}^3 \\ \diagup \\ \text{C} \\ \diagdown \\ \text{NR}^3\text{R}^4 \end{array}$

-SO₂NR³R⁴

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{NHCNR}^3\text{R}^4 \end{array}$

$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}^3\text{CNR}^4 \end{array}$

-CO₂R³

=O

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{OCR}^3 \end{array}$

-SR³

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{SR}^9 \end{array}$

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{SR}^9 \end{array}$

O

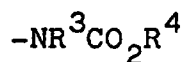
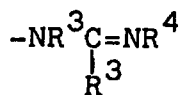
-CN

-N₃

-OSO₃R³

-OSO₂R³

-NR³SO₂R⁴



wherein, relative to the above-named substituents, the groups R^3 and R^4 are independently selected from hydrogen; alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl, cycloalkylalkyl and alkylcycloalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; and heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms, or R^3 and R^4 taken together with the nitrogen to which at least one is attached may form a 5- or 6-membered nitrogen-containing heterocyclic ring; R^9 is as defined for R^3 except that it may not be hydrogen; or wherein R^1 and R^8 taken together represent $\text{C}_2\text{-C}_{10}$ alkylidene or $\text{C}_2\text{-C}_{10}$ alkylidene substituted by hydroxy; R^5 is selected from the group consisting of substituted and unsubstituted: alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl and cycloalkylalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4

oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; wherein the above-named R⁵ radicals are optionally substituted by 1-3 substituents independently selected from:

C₁-C₆ alkyl optionally substituted by amino, fluoro, chloro, carboxyl, hydroxy or carbamoyl; fluoro, chloro or bromo;

-OR³ ;

-OCO₂R³ ;

-OCOR³ ;

-OCONR³R⁴ ;

-OSO₂R³ ;

-OXO ;

-NR³R⁴ ;

R³CONR⁴- ;

-NR³CO₂R⁴ ;

-NR³CONR³R⁴ ;

-NR³SO₂R⁴ ;

-SR³ ;

$\begin{array}{c} \text{O} \\ \parallel \\ \text{S}-\text{R}^9 \end{array}$;

$\begin{array}{c} \text{O} \quad \text{O} \\ \diagdown \quad \diagup \\ \text{S}-\text{R}^9 \end{array}$;

-SO₃R³ ;

-CO₂R³ ;

-CONR³R⁴ ;

-CN; or

phenyl optionally substituted by 1-3 fluoro, chloro, bromo, C₁-C₆ alkyl, -OR³, -NR³R⁴, -SO₃R³, -CO₂R³ or -CONR³R⁴, wherein R³, R⁴ and R⁹ in such R³ substituents are as defined above;

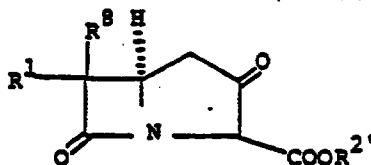
or R^5 may represent a divalent phenylene or C_1-C_4 alkylene group joined to the



ring so as to form a bridged polycyclic group; A is cyclopentylene, or cyclohexylene or C_2-C_6 alkylene optionally substituted by one or more C_1-C_4 alkyl groups; R^2 is hydrogen, an anionic charge or a conventional readily removable carboxyl protecting group, providing that when R^2 is hydrogen or a protecting group, there is also present a counter ion; and



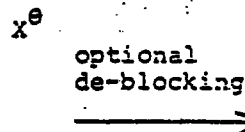
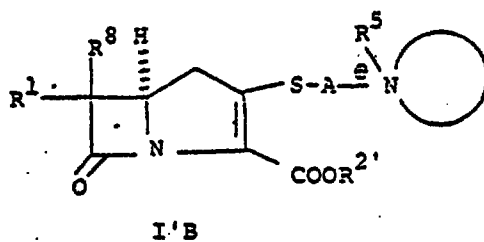
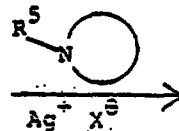
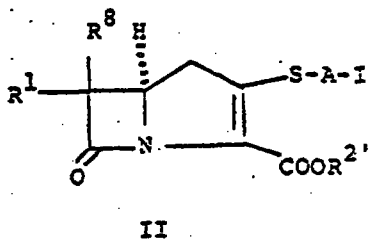
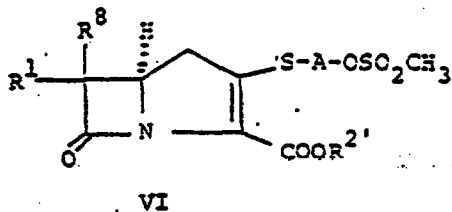
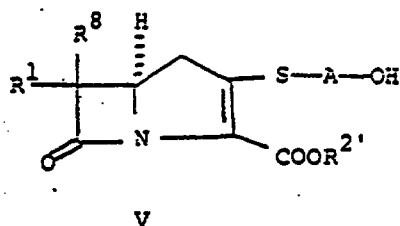
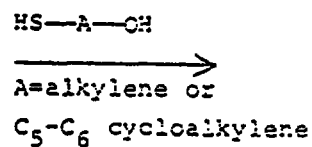
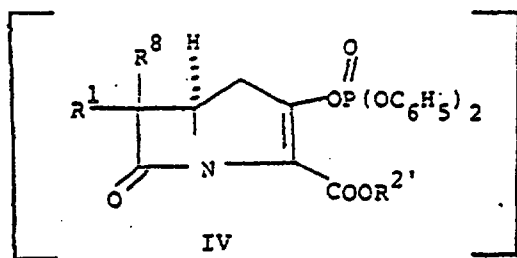
represents a substituted or unsubstituted mono-, bi- or polycyclic non-aromatic heterocyclic radical containing at least one nitrogen in the ring and attached to A through a ring nitrogen, thereby forming a quaternary ammonium group; and pharmaceutically acceptable salts thereof, by the process shown in the following reaction scheme:

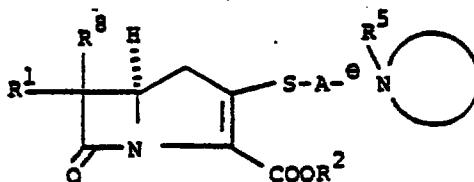


III

diphenyl chlorophosphate





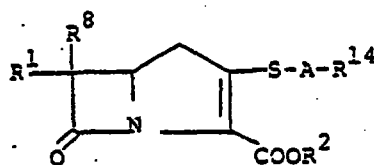


IB

To elaborate on the prior art scheme, starting material III is reacted in an inert organic solvent with diphenyl chlorophosphate in the presence of a base to give intermediate IV. Intermediate IV is then reacted with a mercaptan reagent of the formula HS-A-OH in an inert organic solvent and in the presence of a base to give intermediate V. Intermediate V is then acylated with methanesulfonyl chloride in an inert organic solvent and in the presence of base to give intermediate VI which is reacted with a source of iodide ions in an inert organic solvent to give intermediate II. Intermediate II is reacted with the desired amine in an inert organic solvent and in the presence of silver ion to produce the quaternized product I'A or I'B which may then be de-blocked to give the corresponding de-blocked carbapenem of Formula IA or IB.

The above-described process has several disadvantages. Thus, for example, the process involves several steps which advantageously could be reduced in number. The overall reaction yield is also quite low and the quaternization step is performed on the entire carbapenem compound. It would be desirable to have a new process for producing compounds of Formula IA or IB which (1) involves fewer reaction steps, (2) gives higher yields, (3) allows the quaternized amine to be formed first and then attached to the carbapenem nucleus at a later stage in the synthesis and (4) can be used to more easily form quaternary amine products with a wide variety of amines, i.e. amines with steric hindrance and those with low pK_b values.

The present invention provides a novel process for preparation of carbapenem derivatives of the formula

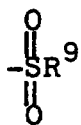
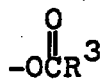
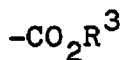
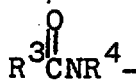
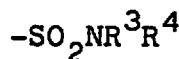
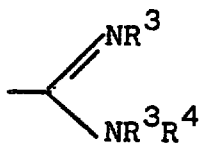
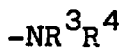
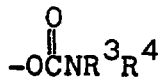


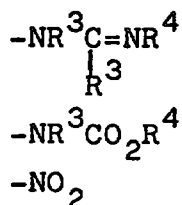
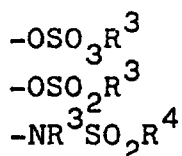
I

wherein R^8 is hydrogen and R^1 is selected from the group consisting of hydrogen; substituted and unsubstituted: alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl and cycloalkylalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; heteroaryl, hetero-aralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; wherein the substituent or substituents relative to the above-named radicals are selected from the group consisting of

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C₁-C₆ alkyl optionally substituted by amino, halo, hydroxy or carboxyl halo

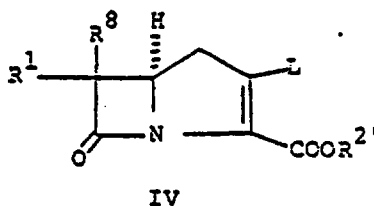




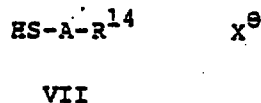
wherein, relative to the above-named substituents, the groups R^3 and R^4 are independently selected from hydrogen; alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl, cycloalkylalkyl and alkylcycloalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; and heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms, or R^3 and R^4 taken together with the nitrogen to which at least one is attached may form a 5- or 6-membered nitrogen-containing heterocyclic ring; R^9 is as defined for R^3 except that it may not be hydrogen; or wherein R^1 and R^8 taken together represent $\text{C}_2\text{-C}_{10}$ alkylidene or $\text{C}_2\text{-C}_{10}$ alkylidene substituted by hydroxy; A is cyclopentylene, cyclohexylene or $\text{C}_2\text{-C}_6$ alkylene optionally substituted by one or more $\text{C}_1\text{-C}_4$ alkyl groups; R^2 is hydrogen, an anionic charge or a conventional readily removable carboxyl protecting group, providing that when R^2 is hydrogen or a protecting group, there is also present a counter

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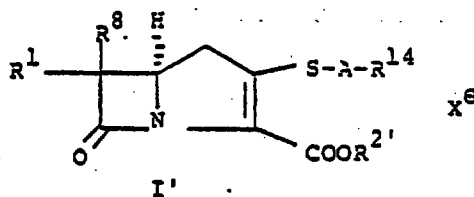
anion; and R^{14} is a quaternized nitrogen-containing aromatic or non-aromatic heterocycle attached to A through a ring nitrogen, thereby forming a quaternary ammonium group, or a pharmaceutically acceptable salt thereof, which process comprises reacting an intermediate of the formula



wherein R^1 and R^8 are as defined above, $R^{2'}$ is a conventional, readily removable carboxyl protecting group and L is a conventional leaving group, with a thiol compound of the formula



wherein A and R^{14} are as defined above and $\text{X}^{\ominus}$ is a counter anion, in an inert solvent and in the presence of base, to produce a carbapenem product of the formula

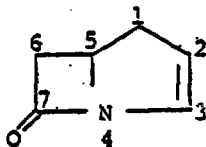


wherein R^1 , R^8 , $R^{2'}$, A, R^{14} and $\text{X}^{\ominus}$ are as defined above and, if desired, removing the carboxyl protecting group $R^{2'}$ to give the corresponding de-blocked compound of formula I, or a pharmaceutically acceptable salt thereof.

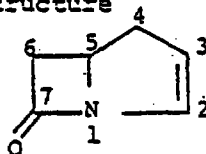
Also provided by the present invention are intermediates of Formula VII and processes for preparing such intermediates.

The carbapenem compounds of Formula I are potent antibacterial agents or intermediates useful in the preparation of such agents.

The compounds of general Formula I above contain the carbapenem nucleus



and may thus be named as 1-carba-2-penam-3-carboxylic acid derivatives. Alternatively, the compounds may be considered to have the basic structure



and named as 7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylic acid derivatives. While the present invention includes compounds wherein the relative stereochemistry of the 5,6-protons is cis as well as trans, the preferred compounds have the 5R,6S (trans) stereochemistry as in the case of thienamycin.

The compounds of Formula I may be unsubstituted in the 6-position or substituted by substituent groups previously disclosed for other carbapenem derivatives. More specifically,

R^8 may be hydrogen and R^1 may be hydrogen or a non-hydrogen substituent disclosed, for example, in European Patent Application 38,869 (see definition of R_6). Alternatively, R^8 and R^1 taken together may be C_2-C_{10} alkylidene or C_2-C_{10} alkylidene substituted, for example, by hydroxy.

To elaborate on the definitions for R^1 and R^8 :

(a) The aliphatic "alkyl", "alkenyl" and "alkynyl" groups may be straight or branched chain having 1-10 carbon atoms; preferred are 1-6, most preferably 1-4, carbon groups; when part of another substituent, e.g. as in cycloalkyl-alkyl, or heteroaralkyl or aralkenyl, the alkyl, alkenyl and alkynyl group preferably contains 1-6, most preferably 1-4, carbon atoms.

(b) "heteroaryl" includes mono-, bi- and polycyclic aromatic heterocyclic groups containing 1-4 O, N or S atoms; preferred are 5- or 6- membered heterocyclic rings such as thienyl, furyl, thiadiazolyl, oxadiazolyl, triazolyl, isothiazolyl, thiazolyl, imidazolyl, isoxazolyl, tetrazolyl, oxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl, pyrazolyl, etc.

(c) "heterocyclyl" includes mono-, bi- and polycyclic saturated or unsaturated non-aromatic heterocyclic groups containing 1-4 O, N or S atoms; preferred are 5- or 6- membered heterocyclic rings such as morpholinyl, piperazinyl, piperidyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, pyrrolinyl, pyrrolidinyl, etc.

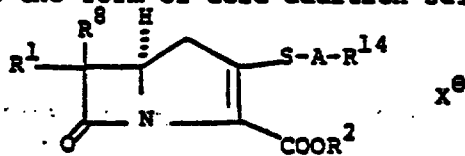
(d) "halo" includes chloro, bromo, fluoro and iodo and is preferably chloro or bromo.

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The term "conventional readily removable carboxyl protecting group" refers to a known ester group which has been employed to block a carboxyl group during the chemical reaction steps described below and which can be removed, if desired, by methods which do not result in any appreciable destruction of the remaining portion of the molecule, e.g. by chemical or enzymatic hydrolysis, treatment with chemical reducing agents under mild conditions, irradiation with ultraviolet light or catalytic hydrogenation. Examples of such ester protecting groups include benzhydryl, p-nitrobenzyl, 2-naphthylmethyl, allyl, benzyl, trichloroethyl, silyl such as trimethylsilyl, phenacyl, p-methoxybenzyl, acetonyl, o-nitrobenzyl, 4-pyridylmethyl and C₁-C₆ alkyl such as methyl, ethyl or t-butyl. Included within such protecting groups are those which are hydrolyzed under physiological conditions such as pivaloyloxymethyl, acetoxymethyl, phthalidyl, indanyl and methoxymethyl. Particularly advantageous carboxyl protecting groups are p-nitrobenzyl which may be readily removed by catalytic hydrogenolysis and allyl which can be removed by Pd(PPh₃)₄-catalyzed reaction.

The pharmaceutically acceptable salts referred to above include the nontoxic acid addition salts, e.g. salts with mineral acids such as hydrochloric, hydrobromic, hydroiodic, phosphoric, sulfuric, etc. and salts with organic acids such as maleic, acetic, citric, succinic, benzoic, tartaric, fumaric, mandelic, ascorbic, lactic, gluconic and malic. Compounds of Formula I in the form of acid addition salts may be written as



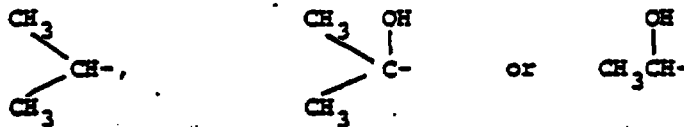
R² = H or protecting group

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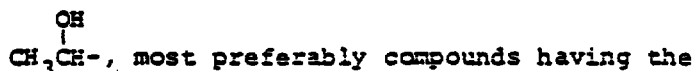
where $X^{\ominus}$ represents the acid anion. The counter anion $X^{\ominus}$ may be selected so as to provide pharmaceutically acceptable salts for therapeutic administration but, in the case of intermediate compounds of Formula I, $X^{\ominus}$ may also be a toxic anion. In such a case the ion can be subsequently removed or substituted by a pharmaceutically acceptable anion to form active end product for therapeutic use. When acidic or basic groups are present in the R^1 group or on the quaternized R^{14} radical, the present invention may also include suitable base or acid salts of these functional groups, e.g. acid addition salts in the case of a basic group and metal salts (e.g. sodium, potassium, calcium and aluminum), the ammonium salt and salts with nontoxic amines (e.g. trialkylamines, procaine, dibenzylamine, 1-phenamine, N-benzyl- β -phenethylamine, N,N'-dibenzylethylenediamine, etc.) in the case of an acidic group.

Compounds of Formula I wherein R^2 is hydrogen, an anionic charge or a physiologically hydrolyzable ester group together with pharmaceutically acceptable salts thereof are useful as antibacterial agents. The remaining compounds of Formula I are valuable intermediates which can be converted into the above-mentioned biologically active compounds.

A preferred embodiment of the present invention comprises compounds of Formula I wherein R^8 is hydrogen and R^1 is hydrogen, CH_3CH_2-

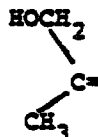


Among this subclass, the preferred compounds are those in which R^1 is



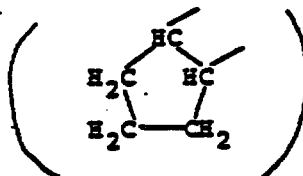
absolute configuration 5R, 6S, 8R.

Another preferred embodiment comprises compounds of Formula I in which R^1 and R^8 taken together form an alkylidene radical of the formula

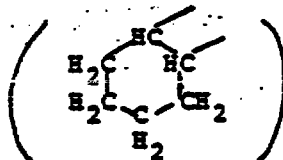


The alkylene or cycloalkylene radical A in the compounds of Formula I may be

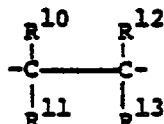
cyclopentylene



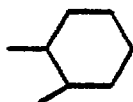
cyclohexylene



or C_2-C_6 alkylene optionally substituted by one or more C_1-C_4 alkyl substituents. Preferred A substituents are cyclopentylene, cyclohexylene or alkylene of the formula



in which R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or C_1-C_4 alkyl. A preferred embodiment comprises those compounds of Formula I in which substituent A is $-CH_2CH_2-$, $-\underset{\text{CH}_3}{\text{CHCH}_2}-$,



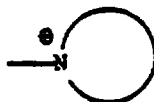
or $-\underset{\text{CH}_3}{\text{CH}_2\text{CH}}-$

In the case of certain compounds of Formula I having a cycloalkylene or branched alkylene A substituent, one or more additional asymmetric carbon atoms may be created which result in formation of diastereoisomers. The present invention includes mixtures of such diastereoisomers as well as the individual purified diastereoisomers.

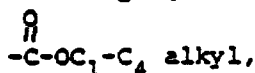
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The quaternized R^{14} substituent may be an optionally substituted mono-, bi- or polycyclic aromatic or non-aromatic heterocyclic radical containing at least one nitrogen in the ring and attached to A through a ring nitrogen, thereby forming a quaternary ammonium group.

One preferred class of R^{14} substituents may be represented by the general formula



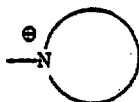
which is meant to define a substituted or unsubstituted mono-, bi- or polycyclic heteroaryl radical containing at least one nitrogen in the ring and attached to a carbon atom of substituent A through a ring nitrogen, thereby forming a quaternary ammonium group. The heteroaryl radical may be optionally substituted by such substituents as C_1-C_4 alkyl, C_1-C_4 alkyl substituted by hydroxy, amino, carboxy or halo, C_3-C_6 cycloalkyl, C_1-C_4 alkoxy, C_1-C_4 alkylthio, amino, C_1-C_4 alkylamino, di(C_1-C_4 alkyl)amino, halo, C_1-C_4 alkanoylamino, C_1-C_4 alkanoyloxy, carboxy,



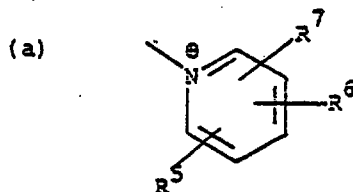
hydroxy, amidino, guanidino, trifluoromethyl, phenyl, phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C_1-C_4 alkyl or C_1-C_4 alkoxy groups, heteroaryl and heteroaryl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 O, N or S atoms and the alkyl moiety associated with heteroaryl has 1-6 carbon atoms.

The heteroaryl radical attached to substituent A is preferably a 5- or 6- membered aromatic heterocyclic radical containing a quaternized nitrogen atom (which is directly bonded to a carbon atom of the alkylene or cycloalkylene radical) and, optionally, one or more additional hetero atoms selected from O, N or S. While, in general, any heteroaryl radical bonded to A

via a quaternized nitrogen atom is found to produce biologically active carbapenem derivatives, a preferred embodiment comprises compounds in which

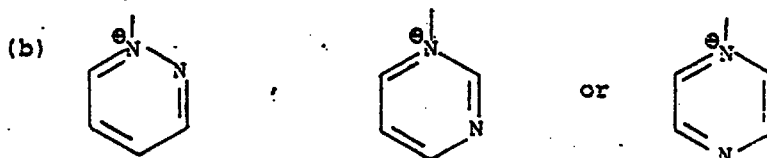


represents a radical selected from the group consisting of



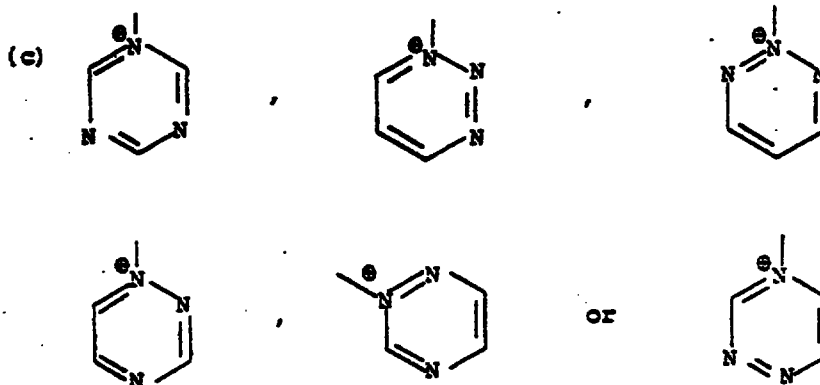
wherein R^5 , R^6 and R^7 are independently selected from hydrogen; C_1-C_4 alkyl; C_1-C_4 alkyl substituted by hydroxy, amino, carboxy or halo; C_3-C_6 cycloalkyl; C_1-C_4 alkoxy; C_1-C_4 alkylthio; amino; C_1-C_4 alkylamino; di(C_1-C_4 alkyl)amino; halo; C_1-C_4 alkanoylamino; C_1-C_4 alkanoyloxy; carboxy;

$-C(=O)OC_1-C_4$ alkyl; hydroxy, amidino; guanidino; trifluoromethyl; phenyl; phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C_1-C_4 alkyl or C_1-C_4 alkoxy groups; and heteroaryl and heteroaralkyl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms; or wherein two of R^5 , R^6 or R^7 taken together may be a fused saturated carbocyclic ring, a fused aromatic carbocyclic ring, a fused saturated heterocyclic ring or a fused heteroaromatic ring,



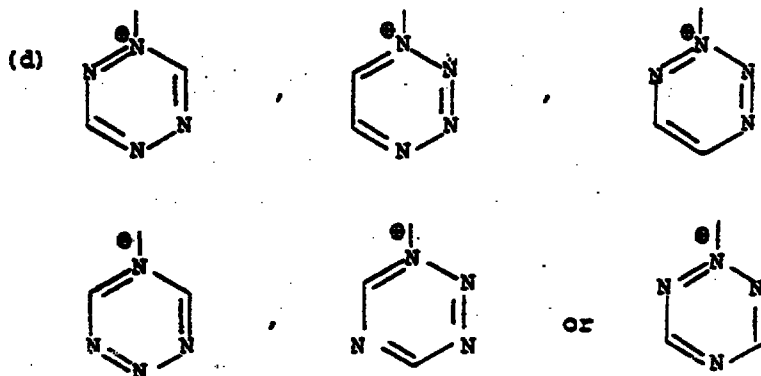
optionally substituted on a carbon atom by one or more substituents independently selected from C₁-C₄ alkyl; C₁-C₄ alkyl substituted by hydroxy, amino, carboxy or halogen; C₃-C₆ cycloalkyl; C₁-C₄ alkoxy; C₁-C₄ alkylthio; amino; C₁-C₄ alkylamino; di(C₁-C₄ alkyl) amino; halo; C₁-C₄ alkanoylamino; C₁-C₄ alkanoyloxy;

carboxy; $\text{-}\overset{\text{O}}{\parallel}\text{C-OC}_1\text{-C}_4$ alkyl; hydroxy; amidino; guanidino; trifluoromethyl; phenyl; phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C₁-C₄ alkyl or C₁-C₄ alkoxy groups; and heteroaryl or heteroaralkyl in which the heteroatom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms, or optionally substituted so as to form a fused carbocyclic or heterocyclic ring;



optionally substituted on a carbon atom by one or more substituents independently selected from C₁-C₄ alkyl; C₁-C₄ alkyl substituted by hydroxy, amino, carboxy or halogen; C₃-C₆ cycloalkyl; C₁-C₄ alkoxy; C₁-C₄ alkylthio; amino; C₁-C₄ alkylamino; di(C₁-C₄ alkyl) amino; halo; C₁-C₄ alkanoylamino; C₁-C₄ alkanoyloxy; carboxy; $\text{-}\overset{\text{O}}{\parallel}\text{C-OC}_1\text{-C}_4$ alkyl; hydroxy; amidino; guanidino; trifluoromethyl; phenyl; phenyl substituted by one, two or three amino, halo,

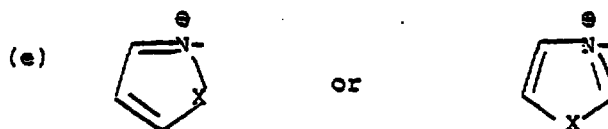
hydroxyl, trifluoromethyl, C_1-C_4 alkyl or C_1-C_4 alkoxy groups; and heteroaryl or heteroaralkyl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms, or optionally substituted so as to form a fused carbocyclic or heterocyclic ring;



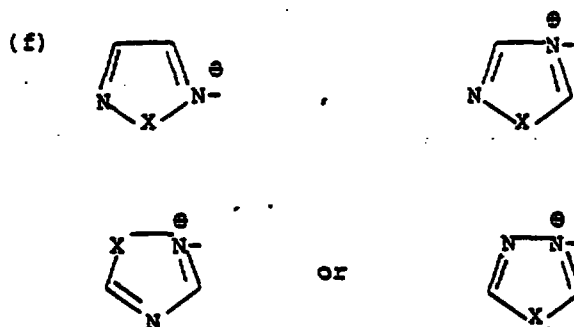
optionally substituted on a carbon atom by one or more substituents independently selected from C_1-C_4 alkyl; C_1-C_4 alkyl substituted by hydroxy, amino, carboxy or halogen; C_3-C_6 cycloalkyl; C_1-C_4 alkoxy; C_1-C_4 alkylthio; amino; C_1-C_4 alkylamino; di(C_1-C_4 alkyl)-amino; halo; C_1-C_4 alkanoylamino; C_1-C_4 alkanoyloxy; carboxy;

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{OC}_1-\text{C}_4 \end{array}$ alkyl; hydroxy; amidino; guanidino; trifluoromethyl; phenyl; phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C_1-C_4 alkyl or C_1-C_4 alkoxy groups; and heteroaryl or heteroaralkyl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms, or optionally substituted so as to form a fused carbocyclic or heterocyclic ring;

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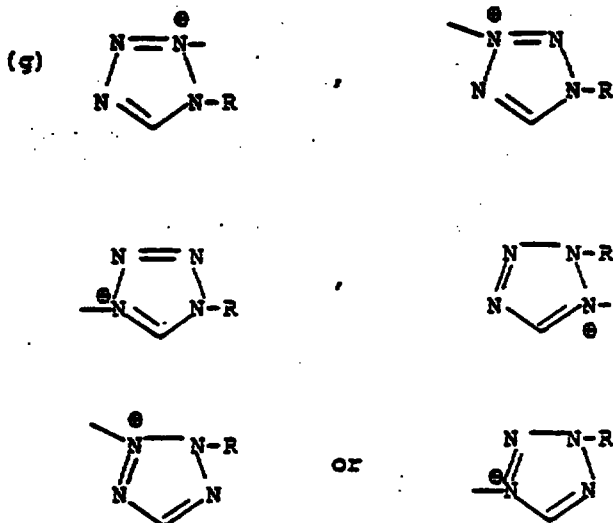


wherein X is O, S or NR in which R is C₁-C₄ alkyl or phenyl, said radical being optionally substituted on a carbon atom by one or more substituents independently selected from C₁-C₄ alkyl; C₁-C₄ alkyl substituted by hydroxy, amino, carboxy or halogen; C₃-C₆ cycloalkyl; C₁-C₄ alkoxy; C₁-C₄ alkylthio; amino; C₁-C₄ alkylamino; di(C₁-C₄ alkyl)amino; halo; C₁-C₄ alkanoylamino; C₁-C₄ alkanoyloxy; carboxy; $\text{-C(=O)-OC}_1\text{-C}_4$ alkyl; hydroxy; amidino; guanidino; trifluoromethyl; phenyl; phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C₁-C₄ alkyl or C₁-C₄ alkoxy groups; and heteroaryl or heteroaralkyl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms, or optionally substituted so as to form a fused carbocyclic or heterocyclic ring;



wherein X is O, S or NR in which R is C₁-C₄ alkyl or phenyl, said radical being optionally substituted on a carbon atom by one or more substituents independently selected from C₁-C₄ alkyl; C₁-C₄ alkyl substituted by hydroxy, amino, carboxy or halogen; C₃-C₆ cycloalkyl; C₁-C₄ alkoxy; C₁-C₄ alkylthio; amino; C₁-C₄ alkylamino; di(C₁-C₄ alkyl)amino; halo; C₁-C₄ alkanoylamino; C₁-C₄ alkanoyloxy; carboxy;

$\text{-}\overset{\text{O}}{\parallel}\text{C-OC}_1\text{-C}_4$ alkyl; hydroxy; amidino; guanidino; trifluoromethyl; phenyl; phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C₁-C₄ alkyl or C₁-C₄ alkoxy groups; and heteroaryl or heteroaralkyl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms; and

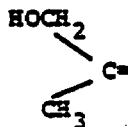


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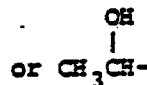
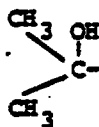
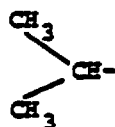
- 29 -

wherein R is C₁-C₄ alkyl or phenyl, said radical being optionally substituted on the carbon atom by a substituent selected from C₁-C₄ alkyl; C₁-C₄ alkyl substituted by hydroxy; amino, carboxy or halogen; C₃-C₆ cycloalkyl; C₁-C₄ alkoxy; C₁-C₄ alkylthio; amino; C₁-C₄ alkylamino; di(C₁-C₄ alkyl)amino; C₁-C₄ alkanoylamino; carboxy; $\begin{array}{c} \text{O} \\ \parallel \\ -\text{C}-\text{OC}_1-\text{C}_4 \end{array}$ alkyl; hydroxy; amidino; guanidino; trifluoromethyl; phenyl, phenyl substituted by one, two or three amino, halo, hydroxyl, trifluoromethyl, C₁-C₄ alkyl or C₁-C₄ alkoxy groups; and heteroaryl or heteroaralkyl in which the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety associated with said heteroaralkyl moiety has 1-6 carbon atoms.

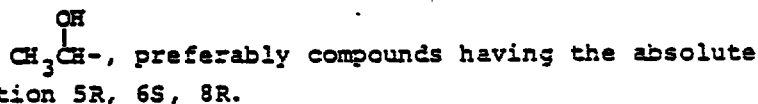
Within the above subclass, the preferred compounds are those in which substituent A is -CH₂CH₂-, $\begin{array}{c} -\text{CHCH}_2- \\ | \\ \text{CH}_3 \end{array}$,  or $\begin{array}{c} -\text{CH}_2\text{CH}- \\ | \\ \text{CH}_3 \end{array}$ and wherein either (a) R¹ and R⁸ taken together represent



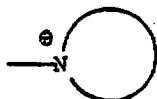
or (b) R⁸ is hydrogen and R¹ represents hydrogen, CH₃CH₂-,



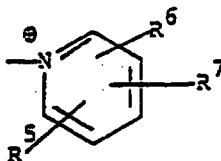
Particularly preferred are the compounds wherein R⁸ is hydrogen and R¹ is

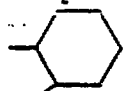


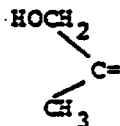
A particularly preferred embodiment of the present invention comprises preparation of compounds wherein



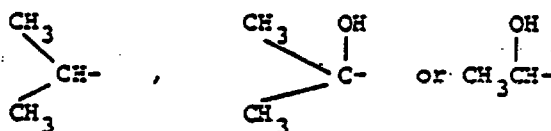
represents a radical of the formula



in which R^5 , R^6 and R^7 are independently selected from the group consisting of hydrogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, C_1 - C_4 alkyl substituted by a hydroxy group, C_1 - C_4 alkylthio, amino, carboxy and carbamoyl. Within this subclass, the preferred compounds are those wherein substituent A is $-CH_2CH_2-$, $-CH(CH_3)CH_2-$,  or $-CH_2CH(CH_3)-$ and wherein either (a) R^1 and R^8 taken together represent



or (b) R^8 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,



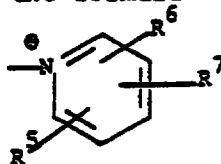
Particularly preferred are the compounds wherein R^8 is hydrogen and R^1 is

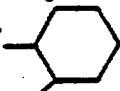
$CH_3CH(OH)-$, preferably compounds having the absolute configuration 5R, 6S, 8R.

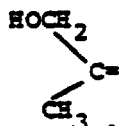
A most preferred embodiment of the present invention comprises preparation of compounds wherein



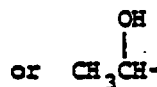
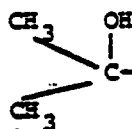
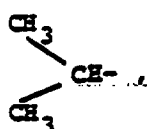
represents a radical of the formula



in which R^5 , R^6 and R^7 are independently selected from the group consisting of hydrogen, C_1 - C_4 alkyl, C_1 - C_4 alkoxy, C_1 - C_4 alkyl substituted by a hydroxy group, C_1 - C_4 alkylthio and amino. Within this subclass, the preferred compounds are those wherein substituent A is $-\text{CH}_2\text{CH}_2-$, $-\text{CH}(\text{CH}_3)\text{CH}_2-$,  or $-\text{CH}_2\text{CH}(\text{CH}_3)-$ and wherein either (a) R^1 and R^8 taken together represent



or (b) R^8 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,

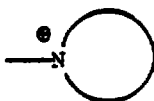


Particularly preferred are the compounds wherein R^8 is hydrogen and R^1 is

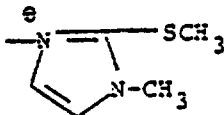


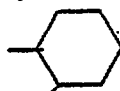
$\text{CH}_3\text{CH}-$, preferably compounds having the absolute configuration 5R, 6S, 8R.

Another preferred embodiment of the present invention comprises preparation of compounds wherein

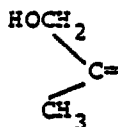


represents a radical of the formula

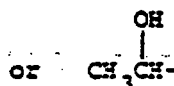
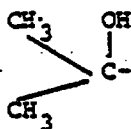
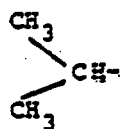


Within this subclass, the preferred compounds are those wherein substituent A is $-\text{CH}(\text{CH}_3)\text{CH}_2-$, $-\text{CH}_2\text{CH}_2-$,  or $-\text{CH}_2\text{CH}(\text{CH}_3)-$

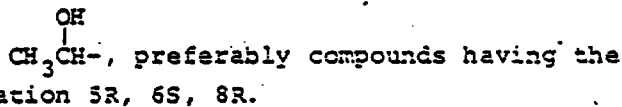
and wherein either (a) R^1 and R^8 taken together represent



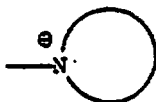
or (b) R^8 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,

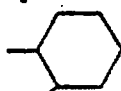


Particularly preferred are the compounds wherein R^8 is hydrogen and R^1 is



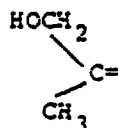
Another preferred embodiment of the present invention comprises preparation of compounds wherein



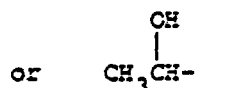
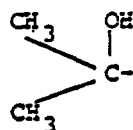
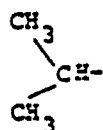
represents a pyridinium radical. Within this subclass, the preferred compounds are those wherein substituent A is $-\text{CH}(\text{CH}_3)\text{CH}_2-$, $-\text{CH}_2\text{CH}_2-$,  or $-\text{CH}_2\text{CH}(\text{CH}_3)-$ and wherein either (a)

R^1 and R^8 taken together represent

4B



or (b) R^8 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,

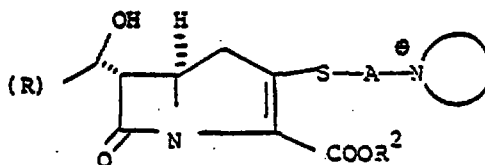


Particularly preferred are the compounds wherein R^8 is hydrogen and R^1 is

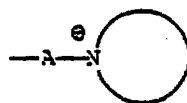


preferably compounds having the absolute configuration 5R, 6S, 8R.

A most preferred embodiment of the present invention comprises the preparation of compounds of the formula

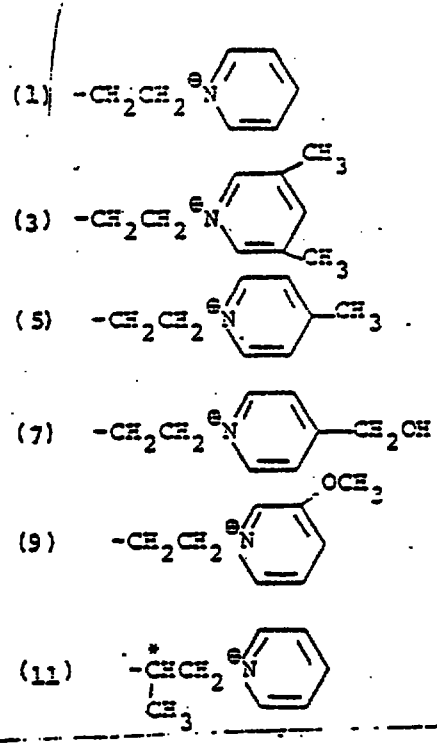


wherein

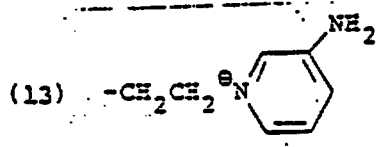


represents

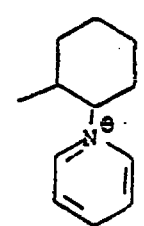
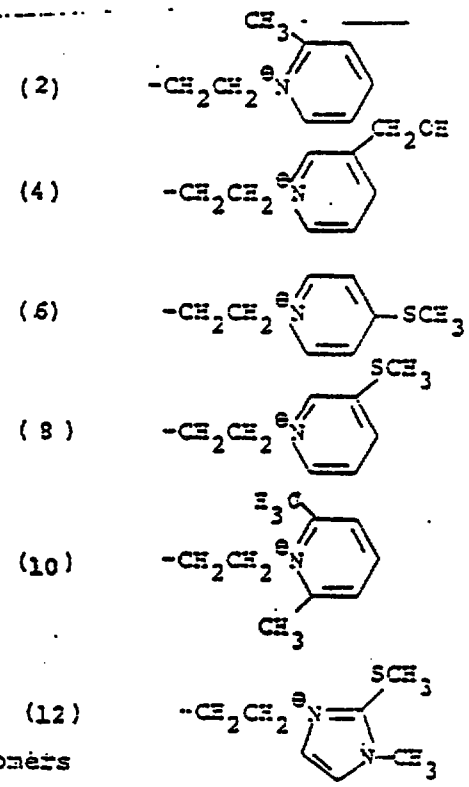
44



R or S diastereoisomers



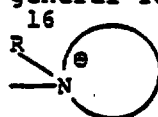
or (14)



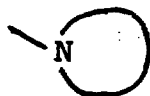
R,R or S,S
diastereoisomers
at two asymmetric
carbons of the
cyclohexyl group

and R^2 is hydrogen, an anionic charge or a conventional readily removable carboxyl protecting group, providing that when R^2 is hydrogen or a protecting group, there is also present a counter anion, and pharmaceutically acceptable acid addition salts thereof.

Another preferred class of quaternized R^{14} substituents may be represented by the general formula



where R^{16} represents either (a) an optionally substituted aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, aryl, araliphatic, heteroaryl, heteroaraliphatic, heterocyclyl or heterocyclyl-aliphatic radical or (b) a divalent phenylene or C_1-C_4 alkylene group joined to the



ring so as to form a bridged polycyclic group. Thus R^{16} may be selected from substituted

and unsubstituted: alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl and cycloalkylalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; heteroaryl, heteroaralkyl, heterocyclyl and heterocyclyl-alkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; wherein the above-named R^{16} radicals are optionally substituted by 1-3 substituents independently selected from:

C₁-C₆ alkyl optionally substituted by amino, fluoro, chloro, carboxyl, hydroxy or carbamoyl; fluoro, chloro or bromo;

-OR³ ;

-OCO₂R³ ;

-OCOR³ ;

-OCONR³R⁴ ;

-OSO₂R³ ;

-oxo ;

-NR³R⁴ ;

R³CONR⁴ - ;

-NR³CO₂R⁴ ;

-NR³CONR³R⁴ ;

-NR³SO₂R⁴ ;

-SR³ ;

$\begin{matrix} \text{O} \\ \parallel \\ -\text{S}-\text{R}^9 \end{matrix}$;

$\begin{matrix} \text{O} & \text{O} \\ \diagdown & / \\ -\text{S}-\text{R}^9 \end{matrix}$;

-SO₃R³ ;

-CO₂R³ ;

-CONR³R⁴ ;

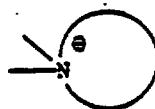
-CN; or

phenyl optionally substituted by 1-3 fluoro,

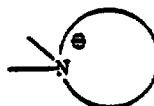
chloro, bromo, C₁-C₆ alkyl, -OR³, -NR³R⁴,

-SO₃R³, -CO₂R³ or -CONR³R⁴, wherein R³, R⁴ and R⁹ in such R¹⁶ substituents are as defined above;

or R^{16} may represent a divalent phenylene or C_1-C_4 alkylene group joined to the

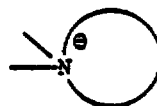


ring so as to form a bridged polycyclic group and



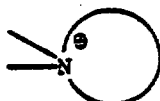
represents a substituted or unsubstituted mono-, bi- or polycyclic non-aromatic (which may be fused to another aromatic or non-aromatic ring) heterocyclic radical containing at least one nitrogen in the ring and attached to A through a ring nitrogen, thereby forming a quaternary ammonium group. The heterocyclic radical may be saturated or unsaturated (with 1-2 double bonds) and may contain up to two additional hetero atoms in addition to the quaternary nitrogen, such additional hetero atoms being selected from O, $S(O)_m$, N, NR^{15} or $NR^{17}R^{18}$ wherein m is 0, 1 or 2, R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl or optionally substituted phenyl and R^{17} and R^{18} are each independently optionally substituted C_1-C_6 alkyl or optionally substituted phenyl.

In a preferred embodiment



represents a non-aromatic 4-7 membered, preferably 5- or 6-membered, N-containing heterocyclic ring containing 0-2 double bonds and 0-2 additional hetero-atoms selected from O, $S(O)_m$, N, NR^{15} or $NR^{17}R^{18}$ wherein m is 0, 1 or 2, R^{15} is hydrogen, C_1-C_6 alkyl optionally substituted by 1-2 substituents independently selected from $-OR^3$, $-NR^3R^4$, $-CO_2R^3$, oxo, phenyl, fluoro, chloro, bromo, $-SO_3R^3$ and $-CONR^3R^4$ or phenyl optionally substituted by 1-3 substituents independently selected from C_1-C_6 alkyl, $-OR^3$, $-NR^3R^4$, fluoro, chloro, bromo, $-SO_3R^3$, $-CO_2R^2$ and $-CONR^3R^4$ and R^{17} and R^{18} are each independently C_1-C_6 alkyl optionally substituted by 1-2 substituents independently selected from $-OR^3$, $-NR^3R^4$, $-CO_2R^3$, oxo, phenyl, fluoro, chloro, bromo, $-SO_3R^3$ and $-CONR^3R^4$ or phenyl

optionally substituted by 1-3 substituents independently selected from C_1-C_6 alkyl, $-OR^3$, $-NR^3R^4$, fluoro, chloro, bromo, $-SO_3R^3$, $-CO_2R^2$ and $-CONR^3R^4$, wherein R^3 and R^4 in such heterocyclic NR^{15} or $NR^{17}R^{18}$ groups are as defined above in connection with the R^{16} substituent. In such preferred embodiment the



ring may be optionally substituted by 1-3 substituents independently selected from

- (a) C_1-C_6 alkyl optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$;
- (b) C_2-C_6 alkenyl optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$;
- (c) C_2-C_6 alkynyl optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$;
- (d) C_3-C_6 cycloalkyl optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$;
- (e) cycloalkylalkyl having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moiety, optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$;

- (f) heteroaryl wherein the hetero atom or atoms are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms, optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$; preferred heteroaryl radicals are 5- or 6-membered aromatic heterocyclic rings;
- (g) heteroaralkyl wherein the hetero atom or atoms are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety has 1-6 carbon atoms, optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$; preferred heteroaralkyl are those in which the heteroaryl radical is a 5- or 6-membered aromatic heterocyclic ring and the alkyl moiety has 1-2 carbon atoms;
- (h) heterocyclyl wherein the hetero atom or atoms are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms, optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$; preferred heterocyclyl are 5- or 6-membered saturated or unsaturated rings;
- (i) heterocyclylalkyl wherein the hetero atom or atoms are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moiety has 1-6 carbon atoms, optionally substituted by 1-2 substituents independently selected from fluoro, chloro, bromo, $-OR^3$, $-OCOR^3$, $-OCONR^3R^4$, oxo, $-NR^3R^4$, $-NR^3COR^4$, $-NR^3CONR^3R^4$, $-NR^3SO_2R^4$, $-SR^3$, $-SO_3R^3$, $-CO_2R^3$ and $-CONR^3R^4$; preferred heterocyclylalkyl are those in which the heterocyclyl moiety is a 5- or 6-membered saturated or unsaturated ring;



(j) fluoro, chloro or bromo;

(k) $-OR^3$;

(l) $-OCO_2R^3$;

(m) $-OCOR^3$;

(n) $-OCONR^3R^4$;

(o) $-OSO_2R^3$;

(p) oxo ;

(q) $-NR^3R^4$;

(r) R^3CONR^4- ;

(s) $-NR^3CO_2R^4$;

(t) $-NR^3CONR^3R^4$;

(u) $-NR^3SO_2R^4$;

(v) $-SR^3$;

(w) $\begin{array}{c} O \\ \nearrow \\ -S-R^9 \end{array}$;

(x) $\begin{array}{c} O \quad O \\ \nearrow \quad \nearrow \\ -S-R^9 \end{array}$;

(y) $-SO_3R^3$;

(z) $-CO_2R^3$;

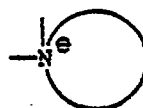
(aa) $-CONR^3R^4$;

(bb) $-CN$; or

(cc) phenyl optionally substituted by 1-3 fluoro, chloro, bromo, C_1-C_6 alkyl, $-OR^3$, $-NR^3R^4$, $-SO_3R^3$, $-CO_2R^3$ or $-CONR^3R^4$.

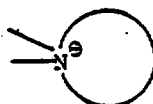
The R^3 , R^4 and R^9 substituents mentioned above are as defined in connection with substituent R^1 .

The



ring as defined above is a non-aromatic heterocycle group. This ring, however, may be fused to another ring which may be a saturated or unsaturated carbocyclic ring, preferably a C_4 - C_7 carbocyclic ring, a phenyl ring, a 4-7 membered heterocyclic (saturated or unsaturated) ring containing 1-3 hetero atoms selected from O, N, S(O)_m, NR^{15} or $NR^{17}R^{18}$ or a 5-6 membered heteroaromatic ring containing 1-3 hetero atoms selected O, S(O)_m, N, NR^{15} or $NR^{17}R^{18}$ in which m, R^{15} , R^{17} and R^{18} are as defined above.

The R^{16} substituent of the non-aromatic R^{14} radical may be either (a) an optionally substituted C_1 - C_6 alkyl, C_2 - C_{10} alkenyl, C_2 - C_{10} alkynyl, C_3 - C_6 cycloalkyl, C_3 - C_6 cycloalkyl- C_1 - C_6 alkyl, phenyl, phenyl- C_1 - C_6 alkyl, phenyl- C_2 - C_6 alkenyl, phenyl- C_2 - C_6 alkynyl, heteroaryl, heteroaralkyl in which the alkyl moiety has 1-6 carbon atoms, heterocyclyl or heterocyclylalkyl in which the alkyl moiety has 1-6 carbon atoms or (b) a divalent phenylene or C_1 - C_4 alkylene group joined to the



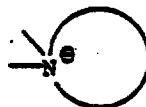
ring so as to form a bridged ring polycyclic group, e.g. a quinuclidine group. The heteroaryl (or heteroaryl portion of heteroaralkyl) substituent may be a mono-, bi- or polycyclic aromatic heterocyclic group containing 1-4 O, N or S atoms; preferred are 5- or 6-membered heterocyclic rings such as thienyl, furyl, thiadiazolyl, oxadiazolyl, triazolyl, isothiazolyl, thiazolyl, imidazolyl, isoxazolyl, tetrazolyl, oxazolyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, pyrrolyl and pyrazolyl. The heterocyclyl (or heterocyclyl portion of heterocyclylalkyl) substituent may be a mono-, bi- or polycyclic saturated or unsaturated non-aromatic heterocyclic group containing 1-4 O, N or S atoms; preferred are 5- or 6-membered heterocyclic rings such as morpholinyl, piperazinyl, piperidyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, pyrrolinyl and pyrrolidinyl.

In the case where the R^{16} substituent is an alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkylalkyl, phenyl, phenylalkyl, phenylalkenyl, phenylalkynyl, heteroaryl, heteroaralkyl, heterocyclyl or heterocyclylalkyl group, such groups may be optionally substituted by 1-3 substituents independently selected from:

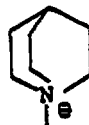
- (a) C_1-C_6 alkyl optionally substituted by, preferably 1-3, amino, fluoro, chloro, carboxyl, hydroxy or carbanoyl groups;
- (b) fluoro, chloro or bromo;
- (c) $-OR^3$;
- (d) $-OCO_2R^3$;
- (e) $-OCOR^3$;
- (f) $-OCONR^3R^4$;
- (g) $-OSO_2R^3$;
- (h) $-oxo$;
- (i) $-NR^3R^4$;
- (j) R^3CONR^4- ;
- (k) $-NR^3CO_2R^4$;
- (l) $-NR^3CONR^3R^4$;

- (m) $-\text{NR}^3\text{SO}_2\text{R}^4$;
 (n) $-\text{SR}^3$;
 (o) $-\text{SOR}^9$;
 (p) $-\text{SO}_2\text{R}^9$;
 (q) $-\text{SO}_3\text{R}^3$;
 (r) $-\text{CO}_2\text{R}^3$;
 (s) $-\text{CONR}^3\text{R}^4$;
 (t) $-\text{CN}$; or
 (u) phenyl optionally substituted by 1-3 substituents independently selected from fluoro, chloro, bromo, $\text{C}_1\text{-C}_6$ alkyl, $-\text{OR}^3$, $-\text{NR}^3\text{R}^4$, $-\text{SO}_3\text{R}^3$, $-\text{CO}_2\text{R}^3$ or $-\text{CONR}^3\text{R}^4$, wherein, relative to the above-named R^{16} substituents, the groups R^3 and R^4 are independently selected from hydrogen; alkyl, alkenyl and alkynyl, having 1-10 carbon atoms; cycloalkyl, cycloalkylalkyl and alkylcycloalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; and heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the heteroaryl and heterocyclyl group or portion of a group is as defined above for R^{16} and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; or R^3 and R^4 taken together with the nitrogen to which at least one is attached may form a 5- or 6-membered nitrogen-containing heterocyclic (as defined above for R^5) ring; and R^9 is as defined above for R^3 except that it may not be hydrogen. A most preferred R^{16} substituent is $\text{C}_1\text{-C}_6$ alkyl, especially methyl.

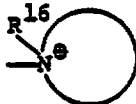
In the case where R^{16} is a divalent phenylene or $\text{C}_1\text{-C}_6$ alkylene group, such group is bonded to another atom of the



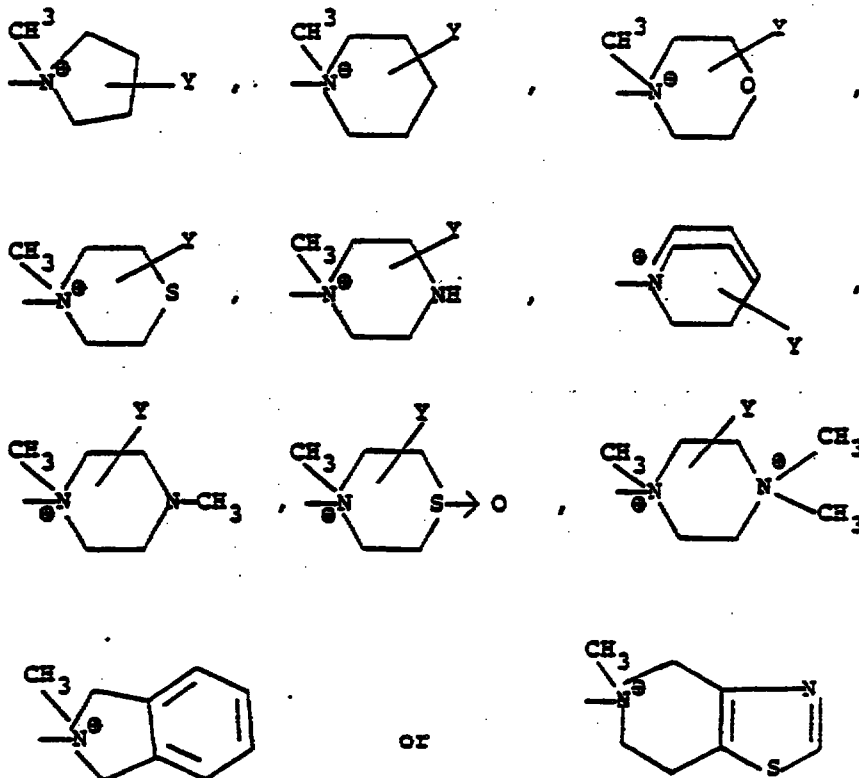
ring so as to form a bridged polycyclic ring, e.g. a quaternized quinuclidine ring of the formula



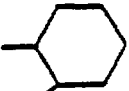
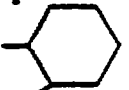
A particularly preferred embodiment of the present invention comprises preparation of compounds of Formula I wherein

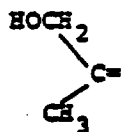


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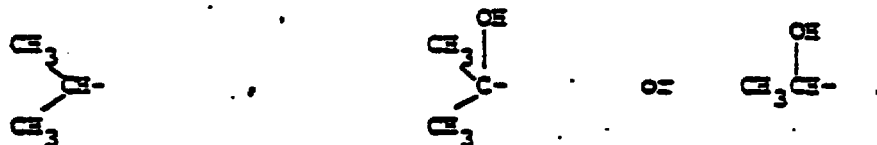


wherein Y is hydrogen, C₁-C₆ alkyl, hydroxy, -SC₁-C₆ alkyl, carboxyl, carbamoyl, chloro, bromo, iodo, fluoro or phenyl. Within this subclass, the preferred compounds are those

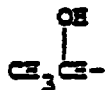
wherein A is $-\text{CH}(\text{CH}_3)\text{CH}_2-$, , $-\text{CH}_2\text{CH}(\text{CH}_3)-$ or $-(\text{CH}_2)_n-$ in which n is 2, 3 or 4, more preferably those in which A is $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{CH}(\text{CH}_3)\text{CH}_2-$,  or $-\text{CH}_2\text{CH}(\text{CH}_3)-$ and most preferably those in which A is $-\text{CH}_2\text{CH}_2-$, and wherein either (a) R^1 and R^8 taken together represent



or (b) R^8 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,

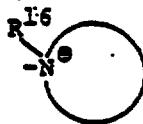


Particularly preferred are the compounds wherein R^8 is hydrogen and R^1 is

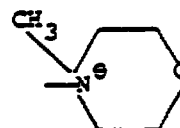
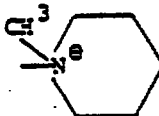
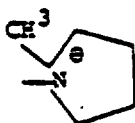


especially compounds having the absolute configuration 5R, 6S, 8R.

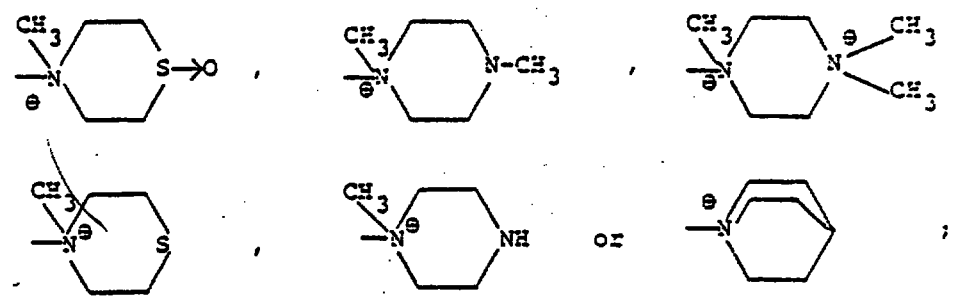
A still more preferred embodiment of the present invention comprises preparation of compounds of Formula I wherein

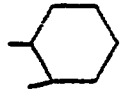


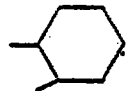
represents



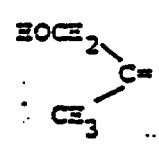
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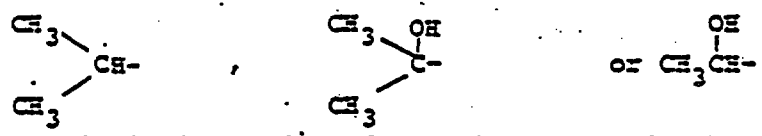
Within this preferred subclass, the preferred compounds are those wherein A is $-\text{CH}(\text{CH}_3)\text{CH}_2-$, , $-\text{CH}_2\text{CH}(\text{CH}_3)-$ or

$-(\text{CH}_2)_n-$ in which A is 2, 3 or 4, more preferably those in which A is $-\text{CH}_2\text{CH}_2-$, $-\text{CH}_2\text{CH}_2\text{CH}_2-$, $-\text{CH}(\text{CH}_3)\text{CH}_2-$, , or $-\text{CH}_2\text{CH}(\text{CH}_3)-$, and

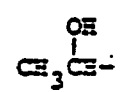
most preferably those in which A is $-\text{CH}_2\text{CH}_2-$, and wherein either (a) R^1 and R^3 taken together represent



or (b) R^3 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,

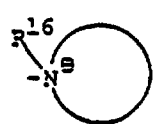


Particularly preferred are the compounds wherein R^6 is hydrogen and R^1 is

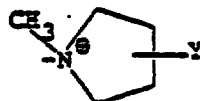


, especially compounds having the absolute configuration 5R, 5S, 8R.

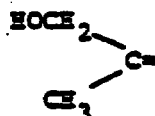
A still more preferred embodiment of the present invention comprises preparation of compounds of Formula I wherein



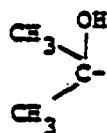
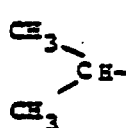
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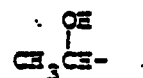
in which Y is hydrogen, C₁-C₆ alkyl, hydroxy, -S-C₁-C₆ alkyl, carboxyl, carbamoyl, chloro, bromo, iodo, fluoro or phenyl. Within this preferred subclass, the preferred compounds are those wherein A is -(CH₂)_n in which n is 2, 3 or 4, most preferably those in which A is -CH₂CH₂- and wherein either (a) R¹ and R⁸ taken together represent



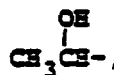
or (b) R⁸ is hydrogen and R¹ represents hydrogen, CH₃CH₂-,



or

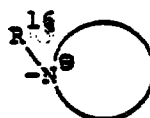


Particularly preferred are the compounds wherein R⁸ is hydrogen and R¹ is



especially compounds having the absolute configuration 5R, 6S, 8R.

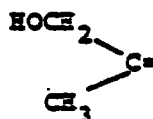
A most preferred embodiment of the present invention comprises preparation of compounds of Formula I wherein



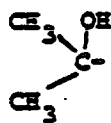
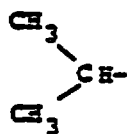
represents



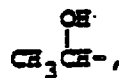
Within this preferred subclass, the preferred compounds are those wherein A is $-(CH_2)_n$ in which n is 2, 3 or 4, most preferably those in which A is $-CH_2CH_2-$ and wherein either (a) R^1 and R^8 taken together represent



or (b) R^8 is hydrogen and R^1 represents hydrogen, CH_3CH_2- ,

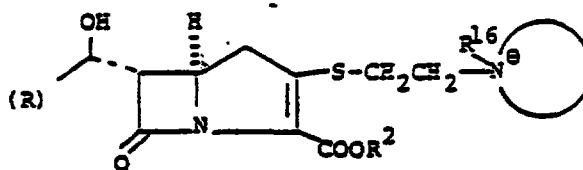


Particularly preferred are the compounds wherein R^8 is hydrogen and R^1 is

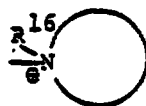


especially compounds having the absolute configuration 5R, 6S, 8R.

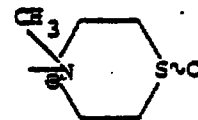
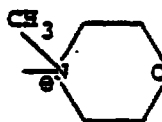
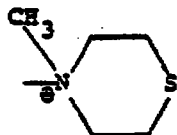
A most preferred embodiment of the present invention comprises preparation of the compounds of the formula



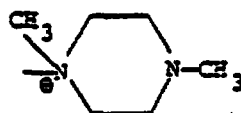
wherein



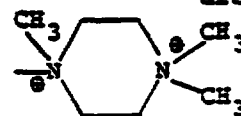
represents



(both c- and s-
diastereoisomers)



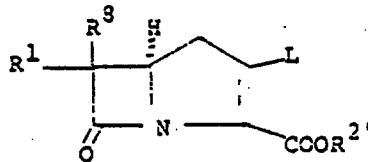
or



and R^2 is hydrogen, an anionic charge or a conventional readily removable carboxyl protecting group, providing that when R^2 is hydrogen or a protecting group, there is also present a counter ion, and pharmaceutically acceptable acid addition salts thereof.

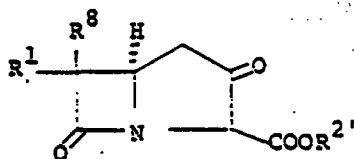
It will be appreciated that certain products within the scope of formula I may be formed as optical isomers as well as epimeric mixtures thereof. It is intended that the present invention include within its scope all such optical isomers and epimeric mixtures. For example, when the 6-substituent is hydroxyethyl, such substituent may be in either the R or S configuration and the resulting isomers as well as epimeric mixtures thereof are encompassed by the present invention.

The process of the present invention utilizes the intermediate of the formula



IV

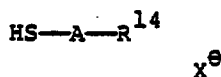
which has been disclosed, for example, in European Patent Application 38,369 and which may be prepared by the general methods described therein. L represents a conventional leaving group (defined as "X" in European Patent Application 38,369) such as chloro, bromo, iodo, benzenesulfonyloxy, p-toluenesulfonyloxy, p-nitrobenzenesulfonyloxy, methanesulfonyloxy, trifluoromethanesulfonyloxy, diphenoxyphosphinyloxy or di(trichloroethoxy)phosphinyloxy. The preferred leaving group is diphenoxyphosphinyloxy. Intermediates of Formula IV are generally formed in situ by reacting an intermediate of the formula



III

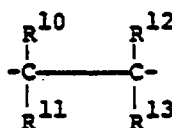
wherein R^1 , R^3 and $R^{2'}$ are as defined above with a suitable acylating agent R^3-L . The preferred intermediate IV where L is diphenoxyphosphinyloxy may be prepared by reacting keto ester III in an inert organic solvent such as methylene chloride, acetonitrile or dimethylformamide with about an equimolar amount of diphenyl chlorophosphate in the presence of a base such as diisopropylethylamine, triethylamine, 4-dimethylaminopyridine or the like at a temperature of from about -20°C to $+40^\circ\text{C}$, most preferably at about 0°C . Intermediate IV may be isolated, if desired, but is conveniently used as the starting material for the process of the present invention without isolation or purification.

In the present process, carbapenem intermediate IV is reacted with a quaternary amine thiol compound of the formula

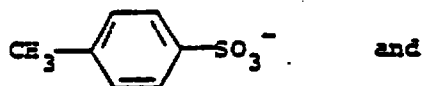


VII

wherein A is cyclopentylene, cyclohexylene or C₂-C₆ alkylene optionally substituted by one or more C₁-C₄ alkyl groups, most preferably cyclopentylene, cyclohexylene or



in which R¹⁰, R¹¹, R¹² and R¹³ are each independently hydrogen or C₁-C₄ alkyl, X[⊖] is a counter anion associated with a strong acid such as Cl[⊖], Br[⊖], CH₃SO₃[⊖], CF₃SO₃[⊖] or



R¹⁴ is a quaternized nitrogen-containing aromatic or non-aromatic heterocycle as defined above. The reaction is carried out in an inert solvent such as acetonitrile, acetonitrile-dimethylformamide, tetrahydrofuran, tetrahydrofuran-H₂O, acetonitrile-H₂O or acetone in the presence of base. The nature of the base is not critical. Best results, however, have been obtained when a non-nucleophilic tertiary amine base such as diisopropylethylamine, 1,3-diazabicyclo[5.4.0]undec-7-ene, 1,5-diazabicyclo[4.3.0]non-5-ene or a tri(C₁-C₄)alkylamine such as triethylamine, tributylamine or tripropylamine is employed. Reaction of intermediate IV with thiol VII may be carried out over a wide temperature range, e.g. -15°C up to room temperature, but is preferably done at a temperature in the range of from about -15°C to +15°C, most preferably at around 0°C.

The carbapenem product produced by reaction of the quaternary amine thiol VII with intermediate IV will have a counter anion associated with it [e.g. (C₆H₅O)₂PO₂[⊖], Cl[⊖] or the anion associated with the quaternary thiol] which may at this stage be substituted by a different counter anion, e.g. one which

is more pharmaceutically acceptable, by conventional procedures. Alternatively, the counter anion may be removed during the subsequent de-blocking step. Where the quaternized carbapenem compound and counter anion form an insoluble product, the product may crystallize out as it is formed and be collected pure by filtration.

Following formation of the desired carbapenem product, the carboxyl protecting group $R^{2'}$ of Compound I' may be optionally removed by conventional procedures such as solvolysis, chemical reduction or hydrogenation. Where a protecting group such as p-nitrobenzyl, benzyl, benzhydryl or 2-naphthylmethyl is used which can be removed by catalytic hydrogenation, intermediate I' in a suitable solvent such as dioxane-water-ethanol, tetrahydrofuran-diethylether-buffer, tetrahydrofuran-aqueous dipotassium hydrogen phosphate-isopropanol or the like may be treated under a hydrogen pressure of from 1 to 4 atmospheres in the presence of a hydrogenation catalyst such as palladium on charcoal, palladium hydroxide, platinum oxide or the like at a temperature of from 0 to 50°C for from about 0.24 to 4 hours. When $R^{2'}$ is a group such as o-nitrobenzyl, photolysis may also be used for deblocking. Protecting groups such as 2,2,2-trichloroethyl may be removed by mild zinc reduction. The allyl protecting group may be removed by using a catalyst comprising a mixture of a palladium compound and triphenyl phosphine in a suitable aprotic solvent such as tetrahydrofuran, methylene chloride or diethyl ether. Similarly, other conventional carboxyl protecting groups may be removed by methods known to those skilled in the art. Finally, as mentioned above, compounds of formula I' where $R^{2'}$ is a physiologically hydrolyzable ester such as acetoxy-methyl, phthalidyl, indanyl, pivaloyloxymethyl, methoxymethyl, etc. may be administered directly to the host without de-blocking since such esters are hydrolyzed in vivo under physiological conditions.

It will be understood that where the R^1 and/or R^8 substituent or the quaternized nucleophile R^{14} attached to substituent A contain a functional group which might interfere with the intended course of reaction, such group may be protected by a conventional blocking group and then subsequently de-blocked to regenerate the desired functional group. Suitable blocking groups and procedures for introducing and removing such groups are well known to those

63

skilled in the art.

As in the case of other β -lactam antibiotics, compounds of general Formula I may be converted by known procedures to pharmaceutically acceptable salts which, for purposes of the present invention, are substantially equivalent to the non-salted compounds. Thus, for example, one may dissolve a compound of Formula I wherein R^2 is an anionic charge in a suitable inert solvent and then add an equivalent of a pharmaceutically acceptable acid. The desired acid addition salt may be recovered by conventional procedures, e.g. solvent precipitation, lyophilization, etc. Where other basic or acidic functional groups are present in the compound of Formula I, pharmaceutically acceptable base addition salts and acid addition salts may be similarly prepared by known methods.

A compound of Formula I where R^2 is hydrogen or an anionic charge, or a pharmaceutically acceptable salt thereof may also be converted by conventional procedures to a corresponding compound where R^2 is a physiologically hydrolyzable ester group, or a compound of Formula I wherein R^2 is a conventional carboxyl protecting group may be converted to the corresponding compound where R^2 is hydrogen, an anionic charge or a physiologically hydrolyzable ester group, or a pharmaceutically acceptable salt thereof.

Certain of the thiol intermediates of Formula VII may be prepared, for example, by reacting a sulfide of the formula

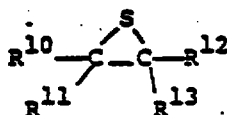


VIIIa



VIIIb

or

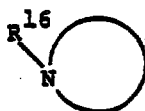


VIIIc

wherein R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or C_1 - C_4 alkyl with a heteroaromatic amine (as defined above) of the formula



or a non-aromatic heterocyclic amine (as defined above) of the formula



and a strong acid. The reaction may be carried out in the presence or absence of an inert organic solvent which is preferably a non-polar organic solvent such as methylene chloride, benzene, xylene, toluene or the like. Where the amine and sulfide reagents are liquids or where a solid amine is soluble in a liquid sulfide reagent, it is preferred to carry out the reaction without use of an additional solvent.

The particular strong acid used in the reaction is not critical and may be, for example, such strong inorganic or organic acids as hydrochloric, hydrobromic, methanesulfonic, p-toluenesulfonic, trifluoromethanesulfonic, etc.

Formation of the quaternary amine thiol intermediate VII may be carried out at a temperature in the range of from about -20°C to about 100°C. Preferred temperatures are generally in the range of about 50-70°C.

The sulfide reagent, aromatic amine and acid are preferably employed so that the sulfide and acid are used in approximately equimolar amounts with the amine being used in excess, e.g. two to three moles of amine per mole of sulfide or acid.

The quaternary amine thiol intermediate will have a counter anion associated with it which will be determined by the particular acid employed. It is, of course, possible to substitute at this point a different counter anion by conventional procedures for use in the subsequent reaction with carbapenem intermediate IV.

The carbapenem derivatives of general Formula I wherein R^2 is hydrogen, an anionic charge or a physiologically hydrolyzable carboxyl protecting group, or the pharmaceutically acceptable salts thereof, are potent antibiotics active against various gram-positive and gram-negative bacteria and they may be used, for example, as animal feed additives for promotion of growth as preservatives in food, as bactericides in industrial applications, for example in waterbased paint and in the white water of paper mills to inhibit the growth of harmful bacteria and as disinfectants for destroying or inhibiting the growth of harmful bacteria on medical and dental equipment. They are especially useful, however, in the treatment of infectious disease in humans and other animals caused by gram-positive or gram-negative bacteria.

The pharmaceutically active compounds provided by the novel process of this invention may be used alone or formulated as pharmaceutical compositions comprising, in addition to the active carbapenem ingredient, a pharmaceutically acceptable carrier or diluent. The compounds may be administered by a variety of means; those of principal interest include: orally, topically or parenterally (intravenous or intramuscular injection). The pharmaceutical compositions may be in solid form such as capsules, tablets, powders, etc., or in liquid form such as solutions, suspensions or emulsions. Compositions for injection, the preferred route of delivery, may be prepared in unit dose form in ampules or in multidose containers and may contain formulatory agents such as suspending, stabilizing and dispersing agents. The compositions may be in ready to use form or in powder form for reconstitution at the time of delivery with a suitable vehicle such as sterile water.

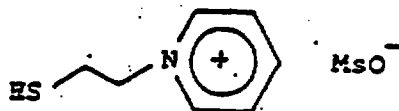
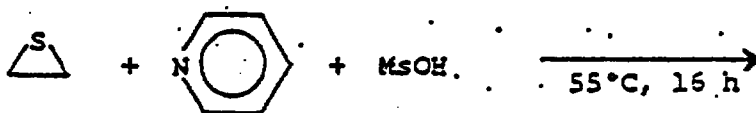
The dosage to be administered depends to a large extent on the particular compound being used, the particular composition formulated, the route of administration, the nature and condition of the host and the particular situs and organism being treated. Selection of the particular preferred dosage and route of application, then, is left to the discretion of the therapist. In general, however, the compounds may be administered parenterally or orally to mammalian hosts in an amount of from about 5 to 200 mg/kg/day. Administration is generally carried out in divided doses, e.g. three to four times a day.

The following examples illustrate but do not limit the scope of the present invention.

Example 1

Preparation of 3-(2-(1-pyridinium)ethylthio)-6α
-(1-(R)-hydroxyethyl)-7-oxo-1-azabicyclo(3.2.0)-hept-2-e
ne-2-carboxylate

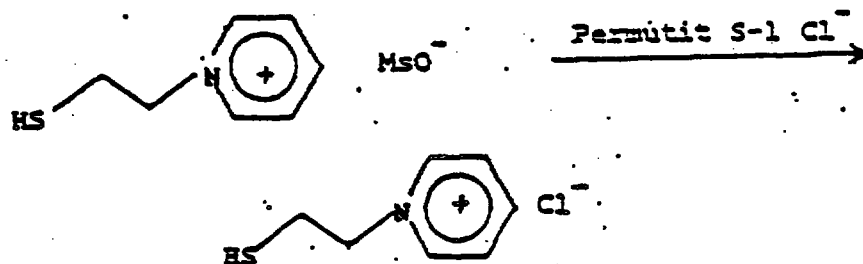
A. 1-(2-mercaptoethyl)pyridinium methanesulfonate



To a suspension of pyridinium methanesulfonate in pyridine prepared by the dropwise addition of methanesulfonic acid (1.95 mL, 0.03 mol) to pyridine (8.0 mL, 0.099 mol) with cooling, was added ethylene sulfide (1.96 mL, 0.033 mol). The resulting mixture was stirred at 55°C for 16 h and concentrated under reduced pressure to a thick syrup which was mixed with few mL of water. The solution was poured on top of a column (40 x 16 cm) of μ -bondapak C-18 which was eluted with water. Lyophilization of the appropriate fractions gave a colorless syrup 6.5 g (91%), ir (film) $\nu_{\max}$: 2300-2600 (br, SH), 1635 (pyridinium), 1490, 1200 (sulfonate), 1068, 1060, 1045, 791, 780 cm^{-1} , ^1Hmr (DMSO- d_6) δ : 2.32 (3H, s, CH_3SO_3^-), 2.61, 2.70, 2.73, 2.82 (1H, B part of A_2B system, SH), 3.07 (2H, m [with D_2O , 3.08 (2H, t, $J=6.5$ Hz)], CH_2S), 4.76 (2H, t, $J=6.5$ Hz, CH_2N^+), 8.19 (2H, m, Hm of pyridinium), 8.6 (1H, m, Ho of pyridinium), 9.08 (2H, dd, $J=6.8$ Hz, $J=1.4$ Hz, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 206 (ϵ 5230), 258 (ϵ 3760) m μ .

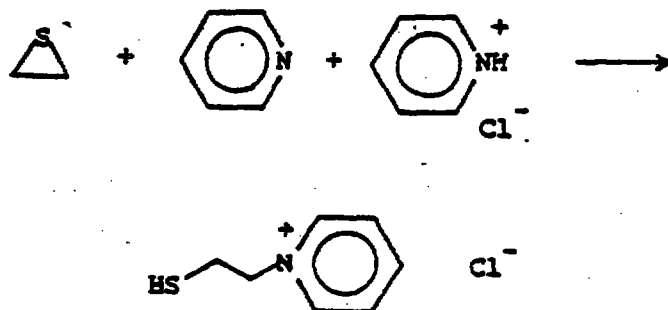
METHOD A

B. 1- (2-mercaptoethyl)pyridinium chloride



An aqueous solution of crude 1-(2-mercaptoethyl)pyridinium methanesulfonate (9.4 g, 0.04 mol) was poured on top of column (2.5 x 41 cm) of permutit* S-1 Cl⁻. The column was eluted with water at a rate of 0.5 mL per min and the appropriate fractions were combined and lyophilized giving a yellowish syrup 7.0 g (100%) which was used as it was for the next step, ¹Hmr (D₂O) δ: 3.22 (sH, m, CH₂S), 4.88 (m, CH₂N⁺), 8.18 (2H, m; Hm of pyridinium), 8.7 (1H, m, Hp of pyridinium), 9.0 ppm (2H, m, Ho of pyridinium).

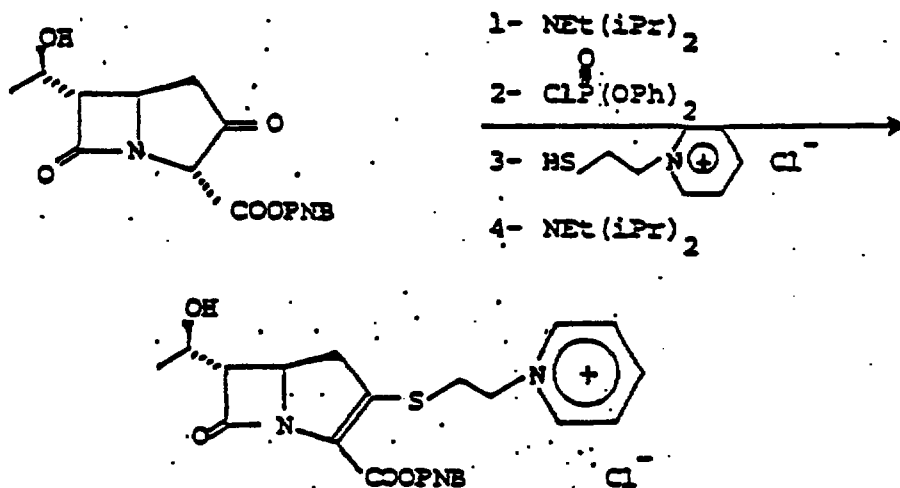
METHOD B



To a precooled (ice bath) pyridine (5.6 mL, 70 mmol) was added pyridine hydrochloride (4.05 g, 35 mmol) and ethylene sulfide (2.1 mL, 35 mmol). The mixture was heated at 65 °C and stirred for 75 min to give a two phases system. The lighter phase was removed. The remaining oil was washed with ether (5 x 10 mL) and pumped under high vacuum to give the title compound (90-100%) which was used as such for the next step.

* Registered Trade Mark

C. Paranitrobenzyl 3- [2-(1-pyridinium)ethylthio]-6 α -
- [1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-
ene-2-carboxylate chloride



A solution of p-nitrobenzyl 6 α -
 [1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0)
 heptane-2-carboxylate (6.09 g, 17.5 mmol) in
 acetonitrile (20 mL) was cooled to +5 °C under a
 nitrogen atmosphere and treated successively with di-
 isopropylethylamine (3.65 mL, 21.0 mmol) and diphenyl
 chlorophosphate (4.34 mL, 21.0 mmol). The resulting
 mixture was stirred for 30 min at 5°C, cooled to -5°C
 and treated successively with a solution of crude
 1-(2-mercaptoethyl)pyridinium chloride (4.3 g, 24
 mmol) in N,N-dimethylformamide (1.0 mL) and dropwise
 with diisopropylethylamine (3.65 mL, 21.0 mmol). The
 reaction mixture was stirred at 0°C for 1 hr, cooled
 to -30°C and stirred for 15 min. more. The solid was
 filtered off and washed with cold (-30°C)
 acetonitrile 5.77 g (65%), ir (nujol) $\nu_{\max}$: 3300
 (OH), 1775 (C=O of β -lactam), 1690 (C=O of PNB
 ester), 1630 (pyridinium), 1605 (phenyl of PNB
 ester), 1515 (NO₂), 1335 cm⁻¹ (NO₂), ¹Hmr (DMSO-d₆)
 δ : 1.17 (3H, d, J=6.1 Hz, CH₃CHOH), 3.2-3.75 (5H, H-4,

* Registered Trade Mark



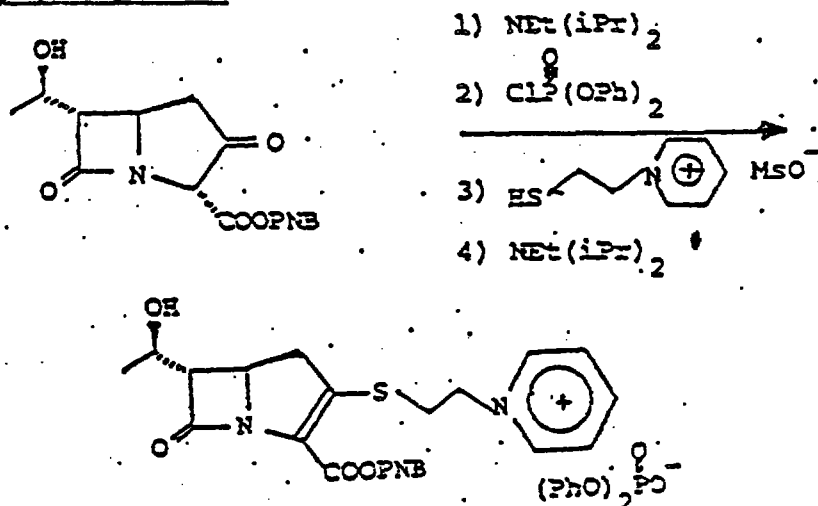
-59a-

H-6, CH₂S), 3.75-4.5 (2H, H-5, CH₃CHOH), 4.92 (2H, brt, $\underline{J}$ =6.5 Hz, CH₂N⁺), 5.18 (1H, d, $\underline{J}$ =4.9 Hz, OH), 5.37 (center of ABq, $\underline{J}_{a,b}$ =14.2 Hz, CH₂ of PNB), 7.69 (2H, d, $\underline{J}$ =8.7 Hz, H_o of PNB), 8.24 (d, $\underline{J}$ =8.7 Hz, H_m of PNB), 8.0-8.4 (4H, H_m of PNB, H_m

of pyridinium), 8.66 (1H, m, Hp of pyridinium), 9.17 (2H, brd, $J=5.5$ Hz, Ho of pyridinium). The filtrate and washing were combined and diluted with ether (150 mL). The supernatant was decanted and the gum was dissolved in water (40 mL) containing enough acetonitrile to have a solution which was poured on top of a column (3 x 10 cm) of μ -bondapak C-18. The column was eluted with 10% acetonitrile - 90% water (150 mL) and 50% acetonitrile - 50% water (100 mL) mixtures. The appropriate fractions were combined and lyophilized after the acetonitrile has been removed under vacuum giving a yellowish powder. An NMR of it showed the presence of the title compound mixed with some p-nitrobenzyl 3-[2-(1-pyridinium)ethylthio]-6c-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (2:1). The powder was dissolved in water (minimum amount) and passed through a column (1.5 x 21 cm) of permutit S-1C1⁻ with water. Lyophilization of the appropriate fractions gave 1.8 g (20%) of the title compound.

D. Paranitrobenzyl

3- [2-(1-pyridinium)ethylthio]-6α- [1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate.



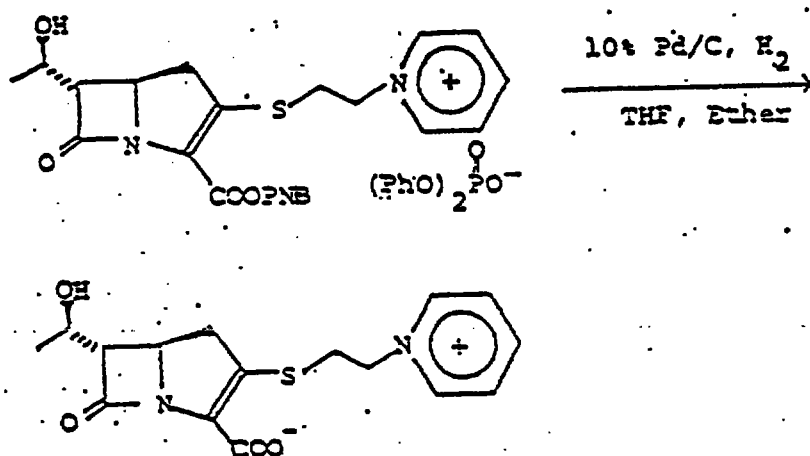
A solution of p-nitrobenzyl 6α - [1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0) heptane-2-carboxylate (0.174 g, 0.50 mmol) in acetonitrile (2 mL) was cooled to 0°C under a nitrogen atmosphere and treated successively with diisopropylethylamine (0.105 mL, 0.60 mmol) and diphenyl chlorophosphate (0.124 mL, 0.60 mmol). The resulting solution was stirred for 30 min at 0°C and treated successively with a solution of 1-(2-mercaptoethyl)pyridinium methanesulfonate (0.170 g, 0.72 mmol) in acetonitrile (0.6 mL) and diisopropylethylamine (0.105 mL, 0.60 mmol). The reaction mixture was stirred at 0°C for 15 min, diluted with cold (0°C) water (7 mL) and poured on top of a column (1.5 x 6.4 cm) of μ -bondapak C-18. The column was eluted with a mixture of acetonitrile (25%-50%) in water (75%-50%). The appropriate fractions were combined and lyophilized after the acetonitrile has been removed under vacuum giving a yellowish powder 0.33 g (92%), ir (KBr)

ν_{max} : 3600-3000 (OH), 1765 (C=O of β -lactam), 1690 (C=O of PNB ester), 1625 (pyridinium), 1585 (phenyl), 1510 (NO_2), 1330 (NO_2), 885 cm^{-1} (NO_2),

¹Hmr (DMSO-d₆) δ: 1.16 (3H, d, J=6.2 Hz, CH₃CHOH), 4.87 (2H, brt, J=6.6 Hz, CH₂S), 5.37 (center of ABq, J_{a,b} = 14.3 Hz, CH₂ of PNB), 6.7-7.5 (phenyl), 7.68 (d, J=8.8 Hz, Ho of PNB), 8.23 (d, J=8.8 Hz, Hm of PNB), 8.0-8.3 (m, Hm of pyridinium), 8.4-8.8 (1H, Hp of pyridinium), 9.09 (2H, dd, J=6.7 Hz, J=1.3 Hz, Ho of pyridinium).

E.3-[2-(1-pyridinium)ethylthio]-6α-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

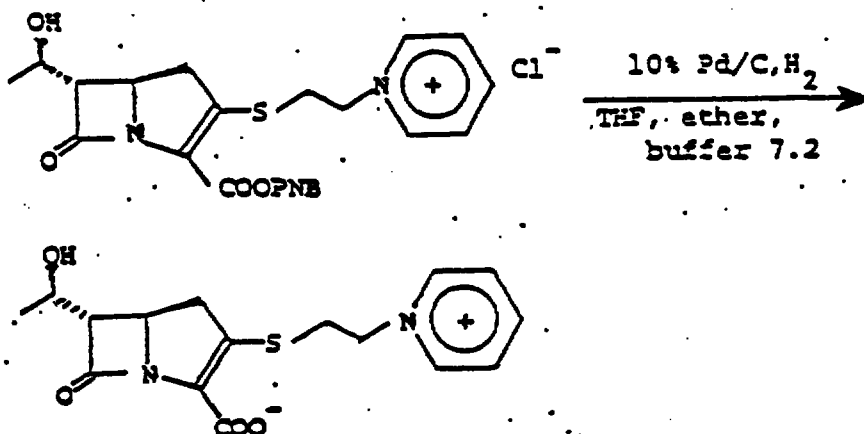
Method A



To a solution of p-nitrobenzyl 3-[2-(1-pyridinium)ethylthio]-6α-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (0.16 g, 0.22 mmol) in wet tetrahydrofuran (10 mL) was added ether (10 mL), potassium phosphate monobasic-sodium hydroxide buffer pH 7.4 (16 mL, 0.05 M) and 10% palladium on charcoal (0.16 g). The resulting mixture was hydrogenated under 40 psi for 1 h at 25°C. The two phases were separated and the organic phase was extracted with water (2 x 3 mL). The aqueous solutions were combined, washed with ether (2 x 10 mL) and poured on top of a column (1.5 x 6.2 cm) of μ-bondpak C-18 after the traces of organic solvents have been removed

under vacuum. Elution of the column with water gave after lyophilization of the appropriate fractions a yellowish powder 0.062 g (84%), ir (KBr) $\nu_{\max}$: 3700-3000 (OH), 1755 (C=O of β -lactam), 1630 (pyridinium), 1590 cm^{-1} (carboxylate), ^1Hmr (D_2O): 1.22 (3H, d, $\underline{J}=6.4$ Hz, CH_3CHOH), 2.92 (d, $\underline{J}=9.1$ Hz, H-4), 2.97 (d, $\underline{J}=9.1$ Hz, H-4), 3.20 (dd, $\underline{J}=2.5$ Hz, $\underline{J}=6.1$ Hz, H-6), 3.44 (t, $\underline{J}=6.0$ Hz, CH_2S), 3.93 (dd, $\underline{J}=9.1$ Hz, $\underline{J}=2.5$ Hz, H-5), 4.82 (t, $\underline{J}=6.0$ Hz, CH_2N^+), 8.04 (m, Hm of pyridinium), 8.5 (m, Hp of pyridinium), 8.82 (dd, $\underline{J}=3.2$ Hz, $\underline{J}=1.1$ Hz, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 259 ($\epsilon 5800$), 296 ($\epsilon 7030$) $\text{m}\mu$, $t_{1/2} = 13.5$ H (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C).

Method B

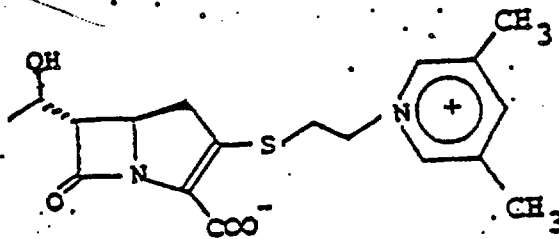


To a solution of p-nitrobenzyl 3-[2-(1-pyridinium) ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo (3.2.0)hept-2-ene-2-carboxylate chloride (5.77 g, 11.4 mmol) in potassium phosphate monobasic-sodium hydroxide buffer (170 mL, 0.2 M, pH 7.22) was added tetrahydrofuran (30 mL), ether (30 mL) and 10% palladium on charcoal (5.7 g). The resulting mixture was hydrogenated at 22°C under 40 psi for 1 h and filtered on a Celite* pad. The pad was washed with water (2 x 15 mL). The filtrate and washings were combined and diluted with ether (100 mL). The aqueous phase was separated, washed with ether (3 x 100 mL) and poured on top of a column (4.5 x 20 cm) of μ -bondapak C-18 after the organic solvents have been removed under vacuum. Elution of the column with water followed by a mixture of 1% acetonitrile in water gave after lyophylization of the appropriate fractions 2.48 g (65%) of the title compound as a yellowish powder. The analytical data were identical to those reported for the compound prepared in the method A.

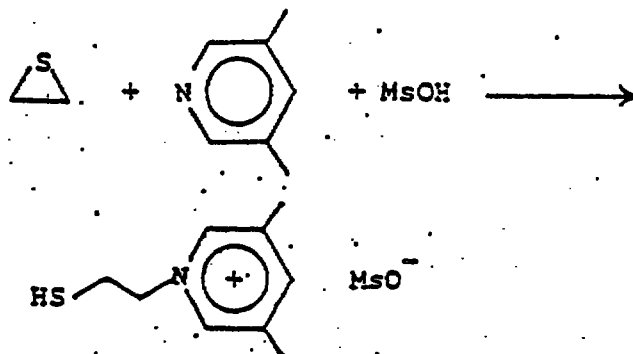
* Registered Trade Mark

Example 2

Preparation of 3-[2-(1-(3,5-dimethylpyridinium)ethylthiol)-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0.)hept-2-ene-2-carboxylate



A. 1-(2-mercaptoethyl)-3,5-dimethylpyridinium methane-sulfonate

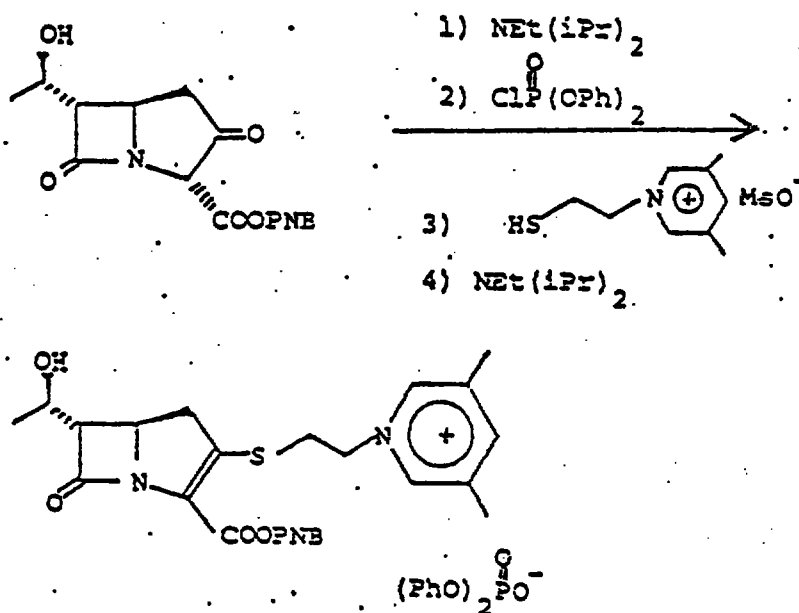


To a suspension of 3,5-lutidinium methanesulfonate in 3,5-lutidine prepared by the addition of methanesulfonic acid (0.65 mL, 0.010 mol) to cold 3,5-lutidine (2.51 mL, 0.022 mol) was added ethylene sulfide (0.655 mL, 0.011 mol). The resulting mixture was stirred under a nitrogen atmosphere at 55°C for 24 h, cooled to 23°C and diluted with water (5 mL) and ether (5 mL). The organic layer was separated and the aqueous solution was washed with ether (6 x 4 mL). The traces of ether were removed under vacuum and the solution was applied on top of a column (2.5 x 6.0 cm) of μ -bondapak C-18. The column was eluted with water and lyophilization of the

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appropriate fractions gave a colourless syrup 2.4 g (91%); ir (film) $\nu_{\max}$: 2520 (SH), 1628 (pyridinium), 1600, 1495, 1325, 1305, 1283, 1200 (sulfonate), 1040, 938, 765, 680 cm^{-1} ; ^1Hmr (DMSO d_6) δ : 2.31 (3H, s, CH_3SO_3^-), 2.47 (6H, s, CH_3 on pyridinium), 2.57, 2.66, 2.69, 2.78 (1H, B part of A_2B system, SH), 3.06 (2H, m [with D_2O added (2H, t, $J=6.5$ Hz)], CH_2S), 4.65 (2H, t, $J=6.5$ Hz, CH_2N^+), 8.34 (1H, s, Hp of pyridinium), 8.79 (2H, s, Ho of pyridinium); uv (H_2O) $\lambda_{\max}$: 271 (ϵ 4860) $\text{m}\mu$. Anal. calcd. for $\text{C}_{10}\text{H}_{17}\text{NO}_3\text{S}_2 \cdot 0.5\text{H}_2\text{O}$: C 44.09, H 6.66, N 5.14, S 23.54; found: C 44.26, H 6.49, N 5.17, S 24.18.

B. Paranitrobenzyl 3-[2-(1-(3,5-dimethylpyridinium)) ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo (3.2.0)hept-2-ene-2-carboxylate diphenylphosphate



To a cold (0°C) solution of p-nitrobenzyl 6 α -(1-(R)-hydroxyethyl)-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2-carboxylate (0.523 g, 1.50 mmol) in acetonitrile (6.0 mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.314 mL, 1.8 mmol) followed by



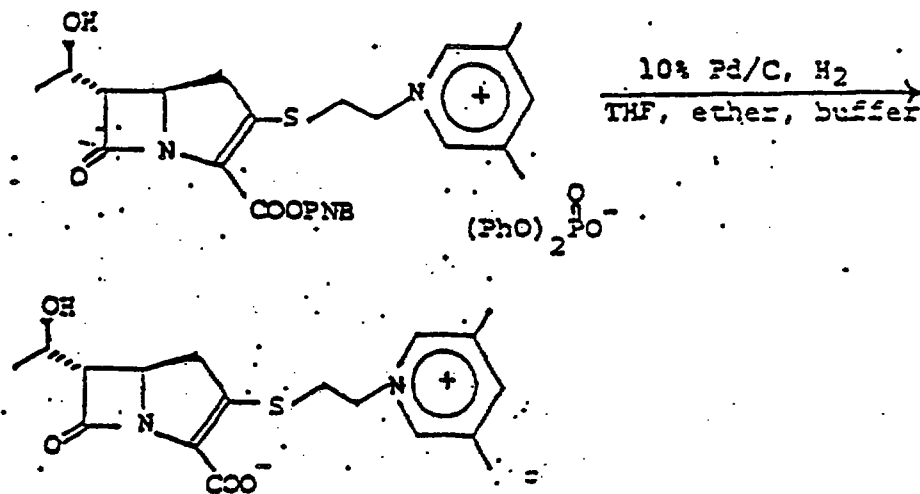
- 66a -

diphenyl chlorophosphate (0.373 mL, 1.8 mmol). The reaction mixture was stirred for 30 min and treated with a solution of 1-(2-mercaptoethyl)-3,5-dimethylpyridinium methanesulfonate (0.493 g, 1.87 mmol) in acetonitrile

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(1.9 mL) followed by diisopropylethylamine (0.314 Ml, 1.8 mmol). The reaction mixture was stirred at 0°C for 1 hr, diluted with cold (0°C) water (26 mL) and poured on top of a column (7.0 x 3.5 cm) of μ -bondapak C-18. Elution of the column with 25-50% acetonitrile - 75-50% water mixture gave after lyophilization of the appropriate fractions 1.01 g (90%) of the title compound as yellowish powder, ir (KBr) $\nu_{\max}$: 3700-3100 (OH), 1778 (C=O of β -lactam), 1700 (C=O of PNB ester), 1635 (pyridinium), 1595 (phenyl), 1521 (NO₂), 1335 (NO₂), 859 cm⁻¹ (NO₂), ¹Hmr (DMSO d₆) δ : 1.16 (3H, d, $J=6.1$ Hz, CH₃CHOH), 2.43 (s, CH₃ on pyridinium), 4.75 (2H, m, CH₂N⁺), 5.38 (center of ABq, $J_{a,b}=14.3$ Hz, CH₂ of PNB), 6.6-7.5 (10H, m, phenyl), 7.70 (2H, d, $J=8.7$ Hz, Ho of PNB), 8.0.-8.5 (3H, m, Hp of pyridinium, Hm of PNB), 8.82 (2H, s, Ho of pyridinium), uv (H₂O) $\lambda_{\max}$: 270 (ϵ 11570), 306 (ϵ 7343) m μ . Anal. calcd. for C₃₇H₃₈N₃O₁₀SP.H₂O: C 58.03, H 5.26, N 5.48, S 4.18; found: C 57.98, H 5.05, N 5.22, S 4.34.

c. 3-[2-(1-(3,5-dimethylpyridinium))ethylthio]-6 α -
[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept
-2-ene-2-carboxylate

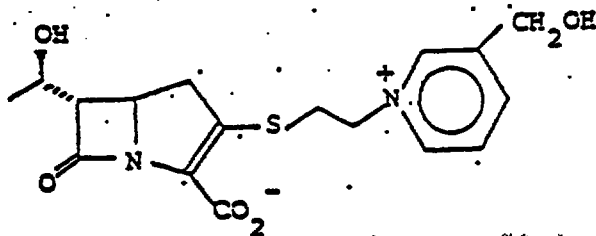


To a solution of p-nitrobenzyl 3-[2-(1-(3,5-dimethylpyridinium)) ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (0.600 g, 0.80 mmol) in wet tetrahydrofuran (36 mL) was added ether (36 mL), potassium phosphate monobasic-sodium hydroxide buffer (0.05M, pH 7.4, 44 mL) and 10% palladium on charcoal (0.60 g). The resulting mixture was hydrogenated under 40 psi at 23°C for 1.25 h. The organic layer was separated and extracted with buffer (2 x 5 mL). Water layers were combined, filtered through a Celite pad, washed with ether (40 mL), pumped to eliminate traces of organic solvents and poured on top of a column (2.5 x 10.0 cm) of μ -bondapak C-18. Elution of the column with water and lyophilization of the appropriate fractions gave the title compound 0.186 g (64%) as a yellowish powder, ir (KBr) ν_{max} : 3700-3100 (OH), 1760 (C=O of β -lactam), 1595 cm⁻¹ (carboxylate), ¹Hmr (D₂O) δ : 1.21 (3H, d, J =6.3 Hz, CH₃CHOH), 2.45 (6H, s, CH₃ on pyridinium), 2.81 (d, J =9.2 Hz, H-4), 2.96 (d, J =9.2 Hz, H-4), 3.22 (dd, J =2.6 Hz, J =6.2 Hz, H-6), 3.40 (t, J =6.2 Hz, CH₂S), 3.84 (dd, J =9.2 Hz, J =2.6 Hz, H-5), 4.15 (m, CH₃CHOH), 4.71

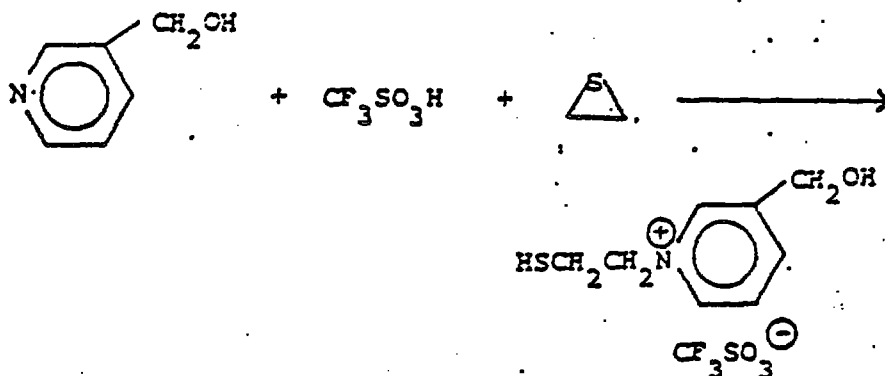
(t, J=6.2 Hz, CH₂N⁺), 8.21(1H, s, Hp of pyridinium), 8.46(2H, s, Ho of pyridinium), uv (H₂O) λ_{max} : 279 (ϵ 8345), 296 (ϵ 7714) m, $[\alpha]_{\text{D}}^{23} + 40.7$ (c 0.53, H₂O), T_{1/2}-16.9 h (measured at a concentration of 10⁻⁴ M in phosphate buffer pH 7.4 at 36.8°C).

Example 3

Preparation of (5R, 6S)-3-[[2-hydroxymethylpyridinio)ethyl]thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

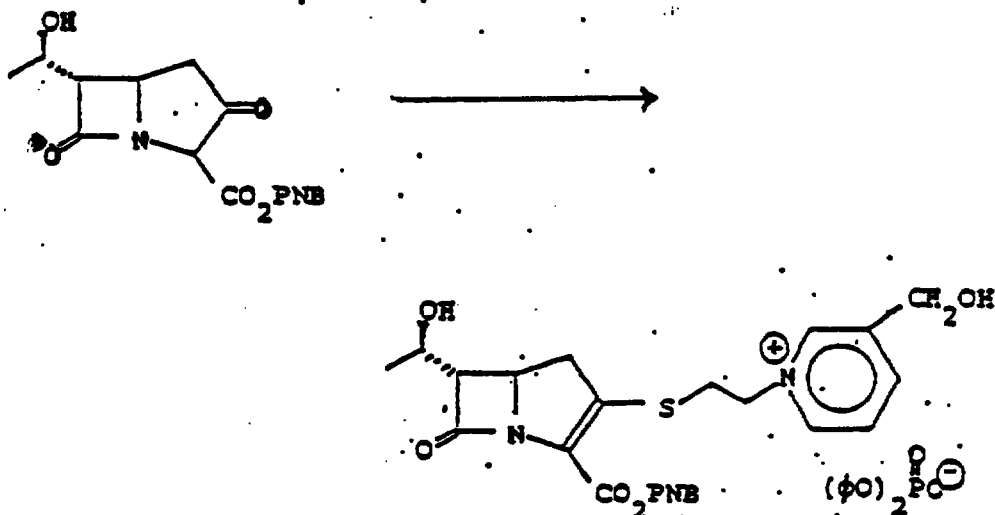


A. 3-Hydroxymethyl-1-(2-mercaptoethyl)pyridinium
trifluoromethanesulfonate



Trifluoromethanesulfonic acid (1.327 mL, 0.015 mol) was added dropwise to 3-pyridinemethanol (2.91 mL, 0.030 mol), followed by ethylene sulfide (0.89 mL, 0.015 mol). The resulting homogeneous mixture was heated (oil bath) at 50-70°C under N₂ for 20 h. The reaction mixture was then taken up in H₂O (15 mL) and extracted with CH₂Cl₂ (5 x 5 mL). The aqueous phase was concentrated in vacuo and then applied to a C₁₈ reverse-phase column. Elution with H₂O followed by evaporation of the relevant fractions gave a pale yellow oil. This material was rechromatographed to give a nearly colourless oil. After drying in vacuo (P₂O₅) this afforded the product (4.50 g, 94%) as a viscous oil. ir (film) ν_{max} : 3450 (s, OH), 2560 (w, SH) cm⁻¹. ¹Hmr (d₆-acetone) δ : 9.10-8.05 (m, 4H, aromatic), 5.01 (t, J =5.5 Hz, 2H, N-CH₂), 4.93 (s, 2H, -CH₂OH), 4.43 (br s, 1H, -OH), 3.43-3.18 (m, 2H, S-CH₂), 2.34-2.10 (m, 1H, SH).

B. p-Nitrobenzyl (5R,6S)-3- [2-(3-hydroxymethyl-pyridinio)ethyl thio]-6- [1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene -2-carboxylate diphenylphosphate

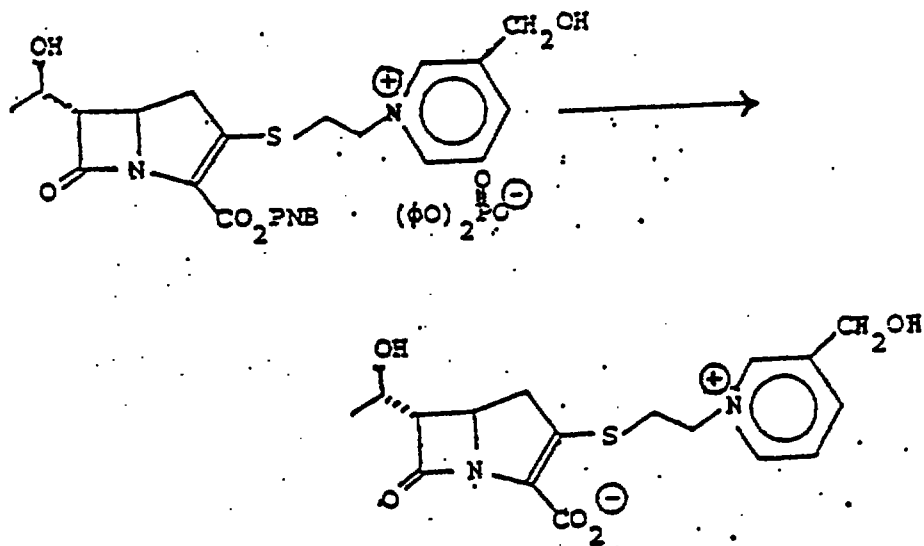


To a solution of p-nitrobenzyl (5R,6S)-6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo [3.2.0]heptane-2-carboxylate (0.174 g, 0.50 mmol) in 2 mL of dry acetonitrile was added diisopropylethylamine (0.096 mL, 0.55 mmol) at 0°C under N₂. Diphenyl chlorophosphate (0.114 mL, 0.55 mmol) was then added dropwise and the reaction mixture was stirred at 0°C for 30 min. A solution 3-hydroxymethyl-1-(2-mercaptoethyl) pyridinium trifluoromethanesulfonate (0.223 g, 0.70 mmol) in 0.50 mL of acetonitrile was then added, followed by diisopropylethylamine (0.122 mL, 0.70 mmol). After being kept at 0°C for 30 min the reaction mixture was concentrated in vacuo and the residual yellow gum was taken up in H₂O (enough acetonitrile was added to aid in dissolving the gum). This solution was applied to a C₁₈ reverse-phase column which was eluted with 15% acetonitrile-H₂O. Lyophilization of the relevant fractions afforded the product (0.305 g, 81%) as a

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beige-coloured solid. ir (KBr) $\nu_{\max}$: 3420 (br, OH), 1775 (β -lactam CO), 1695 ($-\text{CO}_2\text{PNB}$) cm^{-1} ; ^1Hmr (d_6 -acetone) δ : 9.44-7.72 (m, 8H, aromatic), 7.22-6.91 (m, 10H, diphenylphosphate), 5.53, 5.27 (ABq, $\underline{J}$ =14Hz, 2H, benzylic), 5.04 (t, $\underline{J}$ =7.4 Hz, 2H, N-CH₂), 4.75 (s, 2H, CH₂OH), 4.5-3.1 (m, 8H), 1.21 (d, $\underline{J}$ =6.3 Hz, 3H, CHMe).

C. (5R,6S)-3- [2-(3-hydroxymethylpyridinio)ethyl] thio]-6- [1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate



To a solution of p-nitrobenzyl (5R,6S)-3- [2-(3-hydroxymethylpyridinio)ethyl thio]-6- [1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]-hept-2-ene-2-carboxylate diphenylphosphate (0.145 g, 0.194 mmol) in 10 mL of THF containing 5 drops of H₂O, was added 6.0 mL of phosphate buffer (0.05 M, pH 7.4), 0.145 g of 10% palladium-on-charcoal and 10 mL of ether. The mixture was hydrogenated (Parr) at 40 psi for 1 h and then filtered through a pad of Celite. The filter cake was washed with a little H₂O and ether and the aqueous phase was separated and extracted with ether (3x). The aqueous solution was then cooled at 0°C and the pH was adjusted to 7.0 with pH 7.4 buffer. After removing residual volatiles in vacuo the aqueous solution was applied to a C₁₈ reverse-phase column which was eluted with H₂O. Lyophilization of the relevant fractions gave the product (36 mg, 51%) as a light yellow solid. Further purification by reverse-phase hplc gave the pure product (31 mg, 41%) as a solid. ir (KBr)

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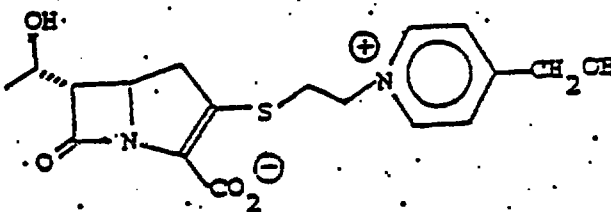
$\nu_{\max}$: 3300(br, OH), 1755 (β -lactam CO), 1590
($-\text{CO}_2^-$) cm^{-1} ; $^1\text{Hnmr}$ (D_2O) δ : 8.78-7.94 (m, 4H aromatic),
4.83 (t, $J=6.0$ Hz, 2H, N- CH_2), 4.83 (s, 2H, CH_2OH),

4.16 (d of q, $J=J'=6.2$ Hz, 1H, H-1'), 3.98 (d, of t, $J=9.1$ Hz, $J'=2.6$ Hz, 1H, H-5), 3.75-3.20 (m, 3H), 3.20-2.65 (m, 2H), 1.22 (d, $J=6.4$ Hz, 3H, CHMe); uv (H₂O) $\lambda_{\max}$: 294 (ϵ 7614), 266 (ϵ 6936) nm; $t_{1/2}$ (pH 7.4, 36.8°C) 14.0 h.

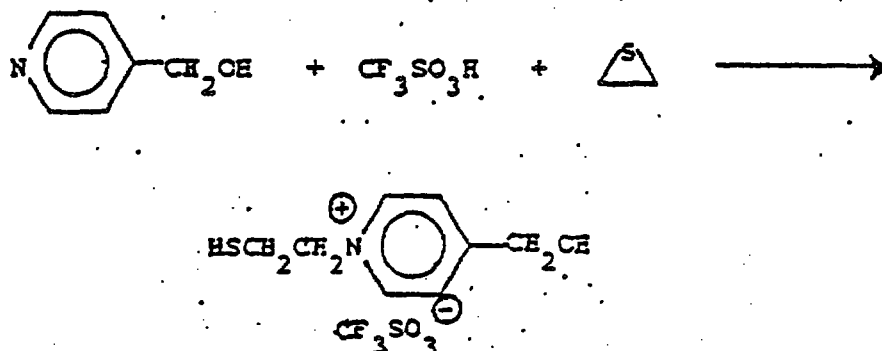
Example 4

Preparation of

(5R,6S)-3- [2-(4-hydroxymethylpyridinio)ethylthio]-6-
[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-
2-ene-2-carboxylate



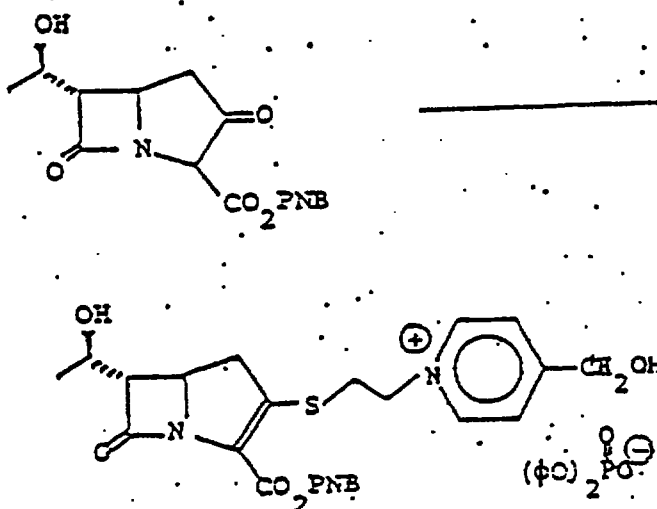
A. 4-Hydroxymethyl-1-(2-mercaptoethyl)pyridinium
trifluoromethanesulfonate



To a solution of 4-pyridinemethanol (1.635 g, 0.015 mol) in 10 mL of CH_2Cl_2 , at 0°C under N_2 , was added dropwise trifluoromethanesulfonic acid (1.327 mL, 0.015 mol). A yellow-brown oil rapidly separated out. An additional equivalent of 4-pyridinemethanol (1.635 g, 0.015 mol) was added to this mixture and the solvent was removed under reduced pressure to give an oil. To this oil was added ethylene sulfide (0.891 mL, 0.015 mol) and the resulting homogeneous mixture was heated (oil bath) at about 60°C for 3 h. The reaction mixture was then taken up in 15 mL of H_2O and the aqueous solution was washed with CH_2Cl_2 (5 x 5 mL). After removing residual organic solvent in vacuo the aqueous solution was applied to a C_{18} reverse-phase column. Elution with H_2O and subsequent evaporation of the relevant fractions afforded an oil which was further dried in vacuo over P_2O_5 to give the product (4.64 g, 97%) as a colourless oil. ir (film)

ν_{max} : 3455 (s, OH), 2565, (w, SH) cm^{-1} ; $^1\text{Hnmr}$ (d_6 -acetone)
 δ : 9.07, 8.18 (ABq, $J=6.8$ Hz, 4H, aromatic), 5.03 (s, 2H, CH_2OH), 4.96 (t, $J=6.5$ Hz, 2H, N- CH_2), 4.09 (br s, 1H, -OH), 3.5-3.1 (m, 2H, S- CH_2), 2.25 (brs; 1H, -SH).

- B. p-Nitrobenzyl (5R,6S)-3-[2-(4-hydroxymethylpyridinio) ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate diphenylphosphate

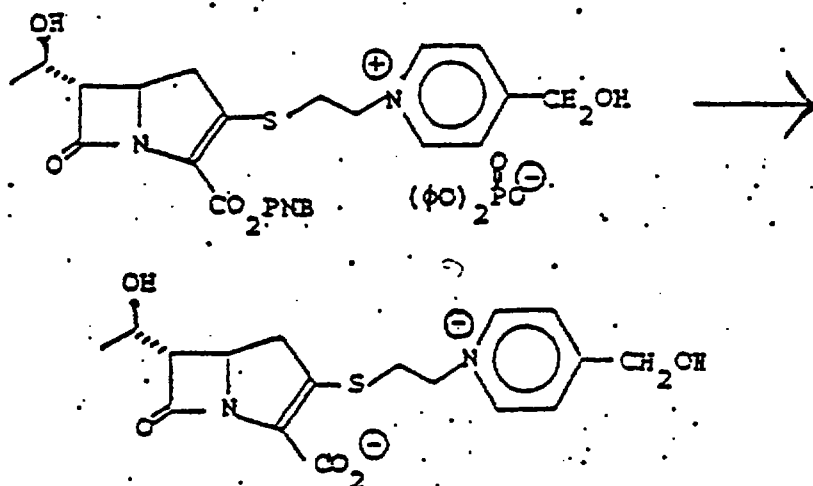


To a solution of p-nitrobenzyl (5R,6S)-6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (0.348 g, 1.0 mmol) in 6 mL of dry acetonitrile, at 0°C under N₂, was added dropwise diisopropylethylamine (0.191 mL, 1.1 mmol) followed by diphenyl chlorophosphate (0.228 mL, 1.1 mmol). The resulting golden-yellow solution was stirred at 0°C for 40 min. To this solution was added a solution of 4-hydroxymethyl-1-(2-mercaptoethyl)pyridinium trifluoromethanesulfonate (0.447 g, 1.4 mmol) in 1 mL of acetonitrile, followed by diisopropylethylamine (0.191 mL, 1.1 mmol). A reddish-black gum separated from the reaction mixture. After 20 min at 0°C the reaction mixture was filtered and concentrated in vacuo. The residue was taken up in a minimum volume of acetonitrile-H₂O (1:1) and applied to a C₁₈ reverse-phase column. Elution with 25% acetonitrile-H₂O and subsequent lyophilization of the relevant fractions

gave the product (0.353 g, 47%) as a cream-coloured solid. ir (KBr) $\nu_{\max}$: 3240 (br, OH), 1775 (β -lactam CO), 1695 ($-\text{CO}_2\text{PNB}$) cm^{-1} ; $^1\text{Hnmr}$ (d_6 -acetone) δ : 9.24-7.84 (m, 8H, aromatic), 7.4-6.9 (m, 10H, diphenylphosphate), 5.52, 5.24 (ABq, $J=14$ Hz, 2H, benzylic), 5.15-4.80 (m, 4H), 4.45-3.05 (m, 7H), 1.35 (d, $J=6.6$ Hz, 3H, CHMe).

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C. (5R,6S)-3-[2-(4-hydroxymethylpyridinio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate



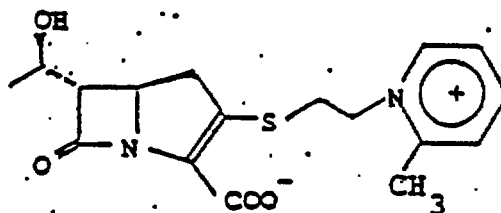
A mixture of p-nitrobenzyl (5R,6S)-3-[2-(4-hydroxymethylpyridinio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate diphenylphosphate (0.348 g, 0.465 mmol) and 10% palladium-on-charcoal (0.35 g) in 11 mL of phosphate buffer (0.05 M, pH 7.4), 5 mL of THF and 10 mL of ether was hydrogenated at 40 psi for 1.25 h. The mixture was then filtered through a Celite pad and the aqueous phase was washed with ether (3 x). The pH of the aqueous solution was then adjusted to 7.0 using additional pH 7.4 buffer. After removing residual volatiles in vacuo the aqueous solution was applied to a C₁₈ reverse-phase column. Elution with 2% acetonitrile-H₂O and subsequent lyophilization gave a yellow-brown solid. This material was re-chromatographed (C₁₈ reverse-phase/H₂O) to give the desired product (0.060 g, 36%) as a light yellow solid. ir (KBr) ν_{max} : 3400 (br, OH), 1755 (β -lactam CO), 1590 (-CO₂⁻) cm⁻¹; ¹Hnmr (D₂O) δ : 8.73, 7.96 (ABq, J=6.8 Hz, 4H, aromatic), 4.93 (s, 2H, CH₂OH), 4.77 (t, J=6.0 Hz,

92
2H, N-CH₂), 4.15 (d of q, $\underline{J}=\underline{J}'=6.3$ Hz, 1H, H-1'), 3.96
(d of t, $\underline{J}=9.2$ Hz, $\underline{J}'=2.6$ Hz, 1H, H-5), 3.65-3.20 (m, 3H),
3.13-2.62 (m, 2H), 1.21 (d, $\underline{J}=6.3$ Hz, 3H, CHMe); uv (H₂O)
 λ_{max} : 295 (ϵ 6880), 256 (ϵ 5595), 224 (ϵ 8111) nm; $t_{1/2}$
(pH 7.4, 36.8°C) 14.5 h

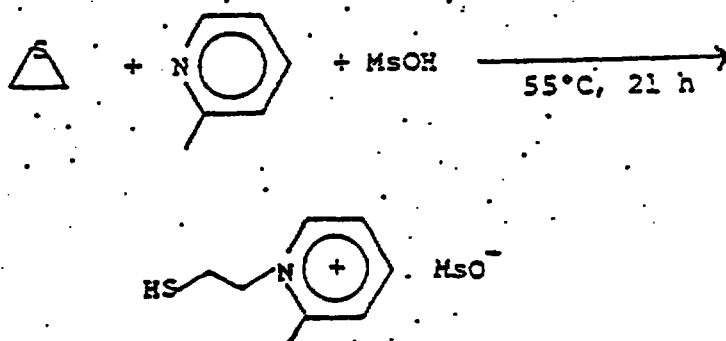
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Example 5

Preparation of 3-[2-(1-(2-methylpyridinium))-ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-carboxylate



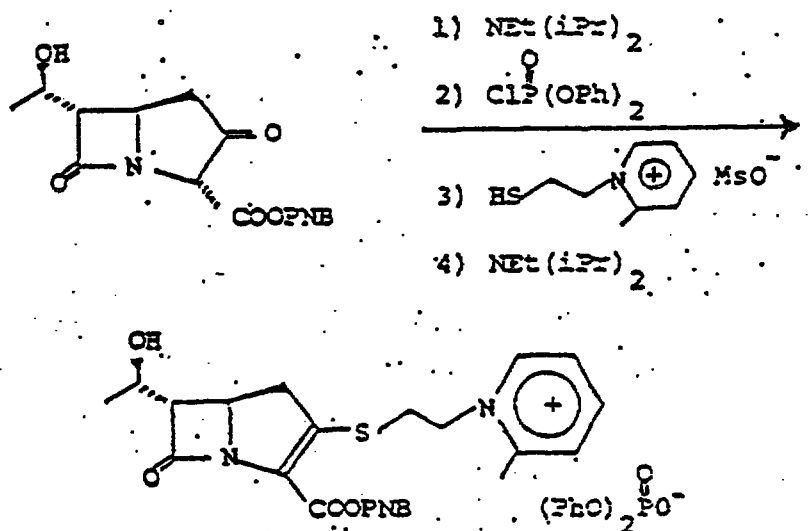
A. 1-(2-mercaptoethyl)-2-methylpyridinium methanesulfonate



To a suspension of 2-methylpyridinium methanesulfonate in 2-methylpyridine prepared by the addition of methanesulfonic acid (0.65 mL, 0.010 mol) to cold 2-methylpyridine (2.17 mL, 0.022 mol) was added ethylene sulfide (0.655 mL, 0.011 mol). The reaction mixture was stirred under a nitrogen atmosphere at 55°C for 21 h, cooled to 23°C and diluted with water (5 mL). The aqueous solution was washed with ether (6 x 4 mL) pumped to remove traces of organic solvents and poured on top of a column (2.5 x 10.0 cm)

of μ -bondapak C-18. The column was eluted with water and lyophilization of the appropriate fractions gave 2.13 g (85%) of the title compound, ir (film) $\nu_{\max}$: 2520 (SH), 1623 (pyridinium) 1574, 1512, 1485, 1412, 1195 (sulfonate), 1038 cm^{-1} , ^1Hmr ($\text{DMSO}-d_6 + \text{D}_2\text{O}$) δ : 2.37 (3H, s, CH_3SO_3^-), 2.83 (3H, s, CH_3 on pyridinium), 3.09 (2H, $J=6.9$ Hz, CH_2S), 4.71 (2H, t, $J=6.9$ Hz, CH_2N^+), 7.93 (2H, m, Hm of pyridinium), 8.44 (1H, m, Hp of pyridinium), 8.89 (1H, m, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 266 ($\epsilon 3550$) $\text{m}\mu$.

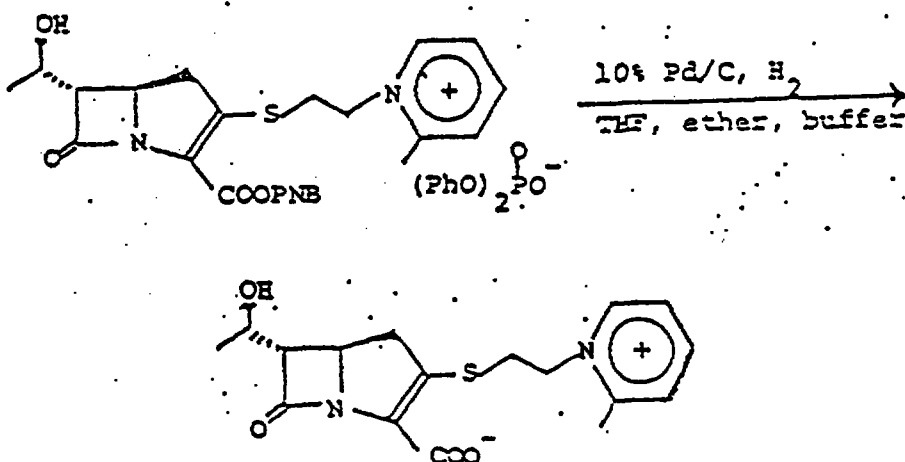
B. Paranitrobenzyl 3-[2-(1-(2-methylpyridinium) ethylthio)]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate



To a cold (0°C) solution of p-nitrobenzyl 6 α -[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2-carboxylate (0.523 g, 1.50 mmol) in acetonitrile (6 mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.314 mL, 1.80 mmol) followed by diphenyl chlorophosphate (0.373 mL, 1.80 mmol). The reaction mixture was stirred for 30 min at 0°C and treated with solution of 1-(2-mercaptoethyl)-2-methylpyridinium methanesulfonate (0.530 g, 2.16 mmol) in acetonitrile (18 mL) followed by diisopropylethylamine (0.314 mL,

1.8 mmol). The reaction mixture was stirred at 0°C for 1 h diluted with cold (0°C) water (26 mL) and poured on top of a column (3.5 x 7.0 cm) of μ -bondapak C-18. Elution of the column with 25% acetonitrile - 75% water and with 50% acetonitrile - 50% water gave after lyophilization of the appropriate fractions 1.06 g, (96%) of the title compound as a yellowish powder, ir (KBr) ν_{max} : 3650-3100 (OH), 1770 (C=O of β -lactam), 1695 and 1690 (C=O of PNB ester), 1630 (pyridinium), 1595 (phenyl), 1518 (NO_2), 1335 (NO_2), 890 cm^{-1} (NO_2), ^1Hmr (DMSO, δ_6) δ : 1.15 (3H, d, $J=6.1$ Hz, CH_3CHOH), 2.87 (s, CH_3 on pyridinium), 3.6-4.4 (2H, m, H-5, CH_3CHOH), 4.75 (2H, m, CH_2N^+), 5.37 (center of ABq, $J=14$ Hz, CH_2 of PNB), 6.5-7.4 (10 H, m, phenyl), 7.70 (2H, d, $J=8.8$ Hz, Ho of PNB), 8.0 (2H, m, Hm of pyridinium), 8.24 (2H, d, $J=8.8$ Hz, Hm of PNB), 8.50 (1H, m, Hp of pyridinium), 8.95 (1H, brd, $J=6.1$ Hz, Ho of pyridinium), uv (H_2O) λ_{max} : 265 ($\epsilon 11990$), 314 ($\epsilon 8020$) m μ

C. 3-[2-(1-(2-methylpyridinium))ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-carboxylate

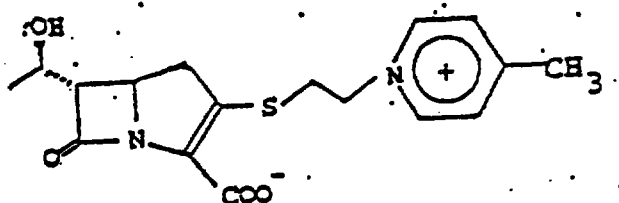


To a solution of p-nitrobenzyl 3-[2-(1-(2-methylpyridinium))ethylthio)-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (0.66 g, 0.90 mmol) in wet tetrahydrofuran (34 mL) was added ether (34 mL), potassium phosphate monobasic-sodium hydroxide buffer (0.15 M, 16.5 mL, pH 7.22) and 10% palladium on charcoal (0.66 g). The resulting mixture was hydrogenated under 40 psi at 23°C for 1.25 h. The organic layer was separated and extracted with buffer (2 x 6 mL). Water layers were combined, filtered through a Celite pad, washed with ether (40 mL), pumped to eliminate traces of organic solvents and poured on top of a column (2.5 x 10 cm) of μ -bondapak C-18. Elution of the column with water and lyophilization of the appropriate fractions gave the title compound 0.098 g (31%) as a yellowish powder, ir (KBr) $\nu_{\max}$: 3650-3100 (OH); 1755 (C=O of β -lactam), 1630 (pyridinium), 1595 cm^{-1} (carboxylate), ^1Hmr (D_2O) δ : 1.20 (3H, d, $J=6.3$ Hz, CH_3CHOH), 2.83 (s, CH_3 on pyridinium), 2.7-3.1 (5H, H-4, CH_3 on pyridinium), 3.1-3.7 (3H, m, CH_2S , H-6), 3.90 (dd, $J=9.1$ Hz, $J=2.6$ Hz, H-5), 3.1 (m, CH_3CHOH), 4.78 (t, $J=6.2$ Hz, CH_2N^+), 7.8 (2H, m, Hm of pyridinium), 8.3 (1H, m, Hp of pyridinium), 8.65 (1H, m, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 268 ($\epsilon 9350$), 296 ($\epsilon 8840$) m μ , $[\alpha]_{\text{D}}^{23} +41^\circ$ (c 0.5, H_2O), $T_{1/2} = 15.0$ h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C).

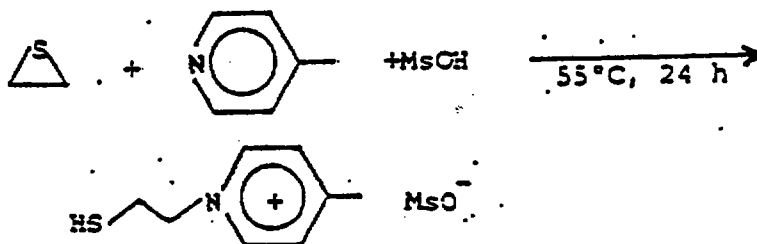
Example 6

Preparation of 3-[2-(1-(4-methylpyridinium)-ethylthio)-6 α -[1(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

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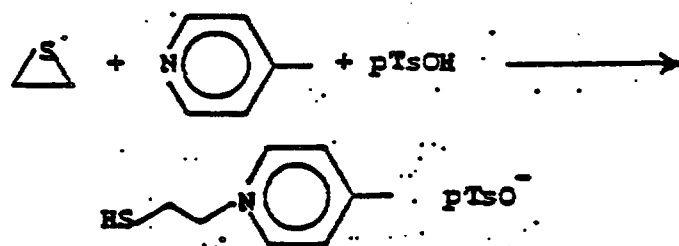
A. 1-(2-mercaptoethyl)-4-methylpyridinium methanesulfonate



To a suspension of 4-picolinium methanesulfonate in 4-picoline prepared by the addition of methanesulfonic acid (0.65 mL, 0.010 mol) to 4-picoline (2.14 mL, 0.022 mol) in cooling was added ethylene sulfide (0.655 mL, 0.011 mol). The reaction mixture was stirred under a nitrogen atmosphere at 55°C for 24 h, cooled to 23°C and diluted with water (5 mL) and ether (10 mL). The organic layer was separated and the aqueous layer was washed with ether (5 x 5 mL) and applied on top of a column (2.5 x 10 cm) of μ -bondapak C-18 after traces of ether have been removed under reduced pressure. Elution of the column with 15% acetonitrile 85% water mixture gave after lyophylization of the appropriate fractions a colorless

syrup 2.66 g (100%), ir (film) $\nu_{\max}$: 2500 (SH), 1640 (pyridinium), 1572, 1520, 1478, 1200 (sulfonate), 1040, 833 and 768 cm^{-1} , ^1Hmr ($\text{DMSO}-d_6$) δ : 2.31 (3H, s, CH_3SO_3^-), 2.62 (s, CH_3 on pyridinium), 2.2-2.9 (4H, SH, CH_3 on pyridinium), 3.04 (2H, m, CH_2s), 4.68 (2H, t, $J=6.4$ Hz, CH_2N^+), 8.01 (2H, d, $J=6.6$ Hz, Hm of pyridinium), 8.89 (2H, d, $J=6.6$ Hz, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 256 ($\epsilon 4100$), 221 ($\epsilon 7544$) $\text{m}\mu$.

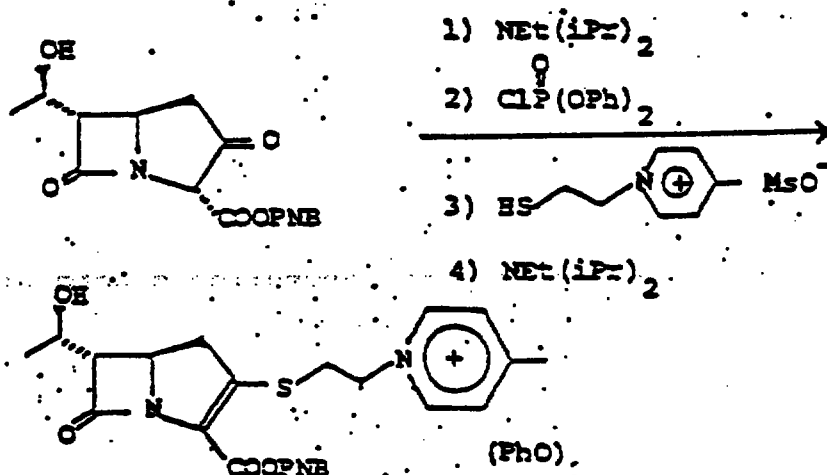
B. 1-(2-mercaptoethyl)-4-methylpyridinium p-toluene-sulfonate



To a suspension of p-toluenesulfonic acid (1.72 g, 0.01 mol) in benzene (6.5 mL) was added 4-picoline (1.17 mL, 0.012 mol). The resulting mixture was stirred under a nitrogen atmosphere at 23°C for 30 min, treated with ethylenesulfide (0.65 mL, 0.011 mol) and stirred at 75° for 24 h. More ethylenesulfide (0.65 mL, 0.011 mol) was added and the stirring was continued at 75°C for 24 h more. The reaction mixture was cooled to 23°C and diluted with water (5 mL) and ether (8 mL). The aqueous layer was separated and washed with ether (3 x 8 mL). The traces of organic solvents were removed under vacuum and the compound was chromatographed on μ -bondapak C-18 with water as eluting solvent to give 2.94 g (90%) of the title compound as a colorless syrup; ir (film) $\nu_{\max}$: 2510 (SH) 1640 (pyridinium), 1595, 1582, 1475, 1200 (sulfonate), 1031, 1010, 818 cm^{-1} , ^1Hmr

(DMSO, d_6) δ : 2.29 (3H, s, CH₃ on pyridinium), 2.61 (s, CH₃ Ph), 2.4-2.8 (4H, SH, CH₃ Ph), 3.03 (2H, m [addition of D₂O gave a t, J = 6.4 Hz, at 3.04], CH₂S), 4.68 (2H, t, J = 6.4 Hz, CH₂N⁺), 7.11, 7.49 (4H, 2d, J = 7.9 Hz, Phenyl), 8.00 (2H, d, J = 6.5 Hz, Hm of pyridinium), 8.89 (2H, d, J = 6.5 Hz, Ho of pyridinium), uv (H₂O) λ_{max} : 256 (ϵ 4315), 222 (ϵ 17045) m μ .

C. Paranitrobenzyl 3-[2-(1-(4-methylpyridinium))ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate

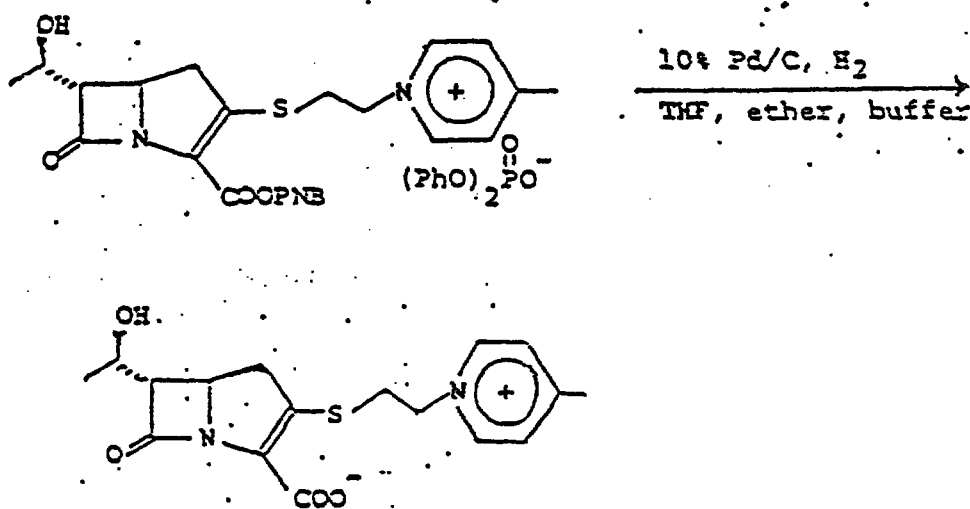


To a cold (0°C) solution of p-nitrobenzyl 6 α -[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2-carboxylate (0.522 g, 1.5 mmol) in acetonitrile (6 mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.314 mL, 1.8 mmol) followed by diphenyl chlorophosphate (0.373 mL, 1.9 mmol). The reaction mixture was stirred for 45 min and treated dropwise with a solution of 1-(2-mercaptoethyl)-4-methylpyridinium methanesulfonate (0.539 g, 2.15 mmol) in acetonitrile (1.8 mL) followed by diisopropylethylamine (0.314 mL, 1.8 mmol). The reaction mixture was stirred at 0°C for 1 h, diluted with cold (0°C) water (24 mL) and poured on top of a column

(2.5 x 8.5 cm) of μ -bondapak C-18. Elution of the column first with 25% acetonitrile -75% water mixture (100 mL) then with 50% acetonitrile 50% water mixture (100 mL) afforded after lyophilization of the

- 5 appropriate fractions 0.91 g (83%) of the title compound as a yellowish powder, ir (KBr) $\nu_{\max}$: 3700-2800 (OH), 1770 (C=O of β -lactam), 1700 (C=O of PNB ester), 1640 (pyridinium), 1595 (phenyl), 1520 (NO₂), 1340 (NO₂), 890 cm⁻¹ (NO₂), ¹Hmr (DMSO, δ) δ : 1.16 (3H, d, $J=6.2$ Hz, CH₃CHOH), 2.61 (s, CH₃ on pyridinium), 3.1-3.7 (3H, m, H-6, CH₂S), 3.7-4.4 (2H, m, H-5, CH₃CHOH), 4.79 (2H, brt, $J=6.3$ Hz, CH₂N⁺), 5.17 (d, $J=4.9$ Hz, OH), 5.37 (center of ABq, $J=14.1$ Hz, CH₂ of PNB), 6.7-7.4 (10 H, m, phenyl), 7.69 (2H, d, $J=8.8$ Hz, Ho of PNB), 8.00 (2H, d, $J=6.5$ Hz, Hm of pyridinium), 8.23 (2H, d, $J=8.8$ Hz, Hm of PNB), 8.92 (2H, d, $J=6.5$ Hz, Ho of pyridinium), uv (H₂O) $\lambda_{\max}$: 262 (ϵ 10835), 311 (ϵ 9670) m μ . Anal. calcd. for C₃₆H₃₆N₃O₁₀SP $\cdot$ 1.5 H₂O: C 56.84, H 5.17, N 5.52, S 4.21; found C56.89, H 5.13, N 5.19, S 4.41.

D. 3-[2-(1-(4-methylpyridinium)ethylthio)-6 α -[1-(B)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

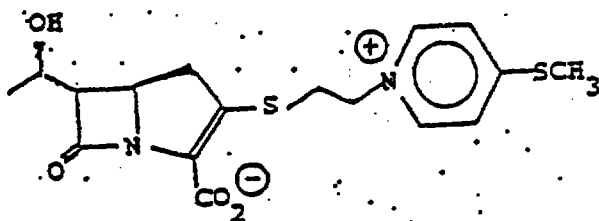


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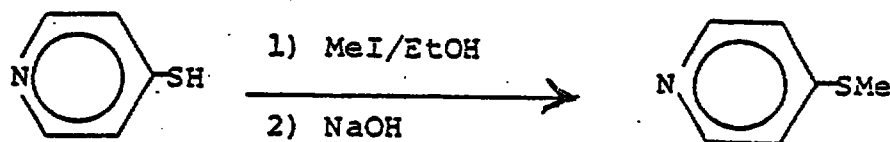
To a solution of p-nitrobenzyl 3-[2-(1-(4-methylpyridinium))-ethylthio]-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (0.587 g, 0.80 mmol) in wet tetrahydrofuran (30 mL) was added ether (30 mL), potassium phosphate mono basic-sodium hydroxide buffer (0.15 M, 14.7 mL, pH 7.22) and 10% palladium on charcoal (0.59 g). The resulting mixture was hydrogenated under 40 psi at 23°C for 1.25 h. The organic layer was separated and extracted with the buffer (2 x 6 mL). The aqueous extracts were combined, filtered through a Celite pad, washed with ether (3 x 20 mL), pumped to remove traces of organic solvents and poured on top of a column (2.5 x 10 cm) of μ -bondapak C-18. Elution of the column with water and lyophilization of the appropriate fractions gave 0.136 g (49%) of the title compound as a yellowish powder, ir (KBr) $\nu_{\max}$: 3700-3000 (OH), 1770 (C=O of β -lactam), 1642 (pyridinium) 1592 cm^{-1} (carboxylate), ^1Hmr (D_2O) δ : 1.19 (3H, t, $J=6.3$ Hz, CH_3CHOH), 2.59 (3H, s, CH_3 on pyridinium), 2.84 (d, $J=9.1$ Hz, H-4), 2.90 (d, $J=9.1$ Hz, H-4), 3.0-3.6 (3H, m, CH_2S , H-6), 3.86 (dd, $J=9.1$ Hz, $J=2.6$ Hz, H-5), 4.12 (m, CH_3CHOH), 4.5-4.9 (CH_2N^+ masked by HOD), 7.80 (2H, d, $J=6.6$ Hz, Hm of pyridinium), 8.58 (2H, d, $J=6.6$ Hz, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 256 (ϵ 5510), 262 (ϵ 5360), 296 (ϵ 7050) m, $[\alpha]_{\text{D}}^{23} + 20.8^\circ$ (C 0.48, H_2O), $T_{1/2}=12.8$ h (measured at a concentration of 10^{-4} M in a phosphate buffer pH 7.4 at 36.8°C).

Example 7

Preparation of (5R) 3- [2-(4-methylthiopyridinio)-ethylthio]-(6S)- [(1R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate



A. 4-Methylthiopyridine



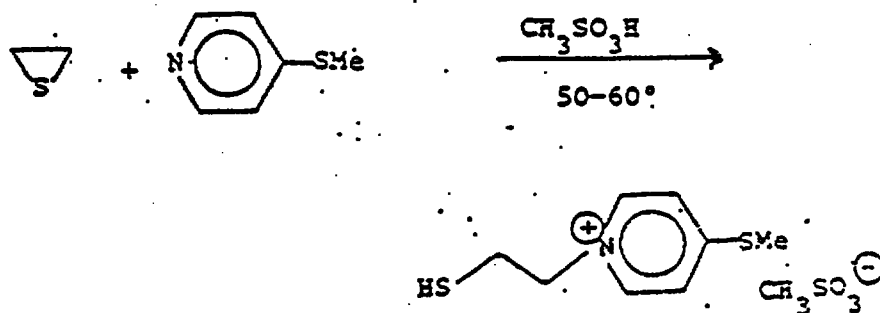
4-Mercaptopyridine (5.55 g, 50.0 mmol; Aldrich) was dissolved in boiling abs. EtOH(50 mL). The insoluble material was removed by filtration over Celite. The filtrate was heated to re-dissolve, and when it cooled to ca. 50°C, methyl iodide (3.17 mL, 51.0 mmol; Aldrich) was added at once. The mixture was cooled to crystallize. Filtration of the solid gave 6.77 g (26.7 mmol, y.53.5%) of the title compound as hydriodide: ^1Hmr (D_2O) δ : 2.70 (3H, s, -SCH₃) and 7.65-7.77-8.35-8.48 ppm (4H, A₂B₂ type, aromatic Hs); ir (Nujol) ν_{max} : 1615, 1585 (aromatic and 780 cm^{-1} ; uv (H_2O)

λ_{max} : 227 ($\epsilon 2.02 \times 10^4$) and 298 nm ($\epsilon 1.64 \times 10^4$).

The hydriodide (6.33 g, 25.0 mmol) was dissolved in H₂O (40 mL) and the insoluble material was removed and washed with H₂O (10 mL). To the filtrate was added at 0-5° NaOH pellet (5 g) and extracted with Et₂O (3 x 25 mL), saturating the aqueous layer with NaCl. The combined organic extracts were washed with brine (x 2), dried (MgSO₄) and evaporated, yielding 2.92 g (23.4 mmol, overall yield 50%) of the title compound as an oil: ¹Hmr (CDCl₃) δ : 2.48 (3H, s, -SCH₃) and 7.03-7.13-8.38-8.48 ppm (4H, A₂B₂ type, aromatic-Hs); ir (film) ν_{max} : 1580 and 800 cm⁻¹.

*Preparation of this compound was reported by King and Ware, J. Chem. Soc., 873 (1939). The procedure, described in this reference was followed.

B. 4-Methylthio-N-(2-mercaptoethyl)pyridinium methanesulfonate

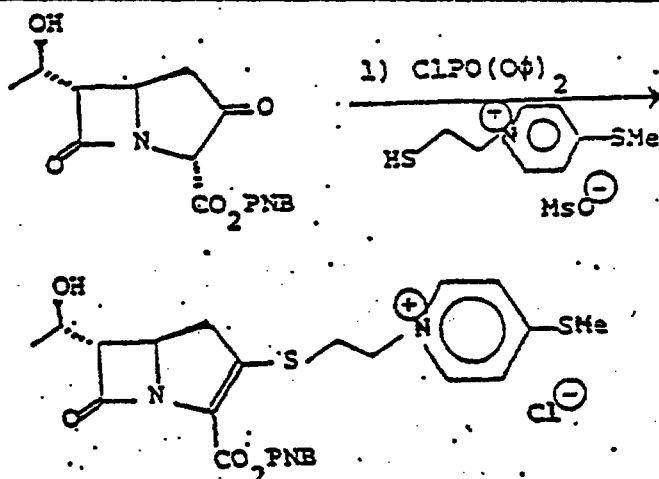


4-Methylthiopyridine (2.75 g, 22.0 mmol) was added slowly to methanesulfonic acid* (0.65 mL, 10.5 mmol) by cooling in an ice-bath. To this solid was added ethylene sulfide* (0.66 mL, 11.0 mmol, Aldrich) and the mixture was heated at 50-60°C for 21 h. As reaction proceeds the solid went to solution. After cooling,

the reaction mixture was dissolved in H₂O (5 mL) and washed with Et₂O (5 x 4 mL). The cloudy aqueous layer was filtered over Celite and the filtrate was purified by reverse phase silica gel column chromatography (C₁₈ micro bondapack 10 g) eluting with H₂O. Each fraction of 10 mL was collected. Fractions 2 and 3 were combined and repurified by the reverse phase column. Fraction 2 gave 1.258 g (4.48 mmol, y. 42.6%) of the title compound as a viscous oil: ¹Hmr (DMSO-d₆, CFT-20) δ: 2.32 (3H, s, MeSO₃[⊖]), 2.72 (3H, s, -SMe), 2.68 (1H, m, SH), 2.9-3.2 (3H, m, -CH₂S-), 4.59 (2H, t, J=6.4 Hz, -CH₂N[⊕]), 7.97 (2H, "d", J=7.2 Hz, aromatic-Hs) and 8.72 ppm (2H, "d", J=7.2 Hz, aromatic-Hs); ir (neat) ν_{max}: 1630, 1200 (br, -SO₃[⊖]), 7.83 and 770 cm⁻¹.

*These reagents were distilled prior to use.

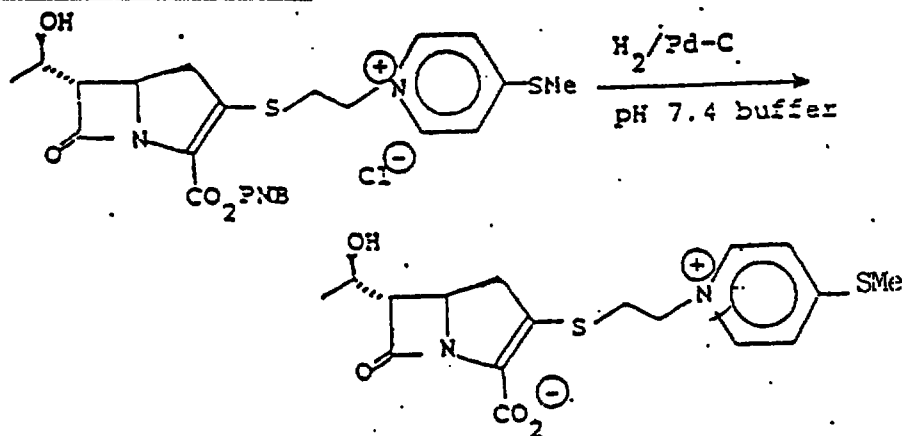
C. (5R) p-Nitrobenzyl 3- [2-(4-Methylthiopyridino) ethylthio]-(6S)- [(1R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate chloride



To a solution of (5R) p-nitrobenzyl 3,7-dioxo-(6S)- [(1R)-hydroxyethyl]-1-azabicyclo [3.2.0] heptane-(2R)-carboxylate (475 mg, 1.36 mmol) and di-isopropylethylamine (0.24 mL, 1.4 mmol) in CH₃CN (5 mL) was added at 0°-5°C under a nitrogen atmosphere diphenyl chloro-

phosphate (0.29 mL, 1.41 mmol). The mixture was stirred at 0°-5°, for 30 min. To this mixture was added an oily suspension of 4-methylthio-N-(2-mercaptoethyl)pyridinium methanesulfonate (678 mg, 1.45 mmol; 60% pure) in CH₃CN (1.5 mL) followed by diisopropylethylamine (0.24 mL, 1.4 mmol). The mixture was stirred at 0°-5°C for 1 h. Immediately after addition of the base, yellowish precipitate formed. The precipitate was filtered and washed with cold CH₃CN (3 mL), yielding 413 mg of yellowish solid. This was triturated from 10% MeOH in H₂O (5 mL) to obtain 341 mg (0.618 mmol, y. 45.4%) of the title compound as white crystals: mp 118°-120°C; ¹Hmr (DMSO-d₆, CFT-20) δ: 1.16 (3H, d, J=6.1 Hz, 1'-CH₃), 2.72 (3H, s, -SCH₃), 3.1-3.7 (5H, m), 3.7-4.3 (2H, m), 4.71 (2H, t, J=6.3 Hz, -CH₂N⁺), 5.15 (1H, d, J=4.9 Hz, OH), 5.20-5.35-5.40-5.55 (2H, ABq, CO₂CH₂-Ar), 7.70 (2H, "d" J=8.8 Hz, nitrophenyl-Hs), 7.97 (2H, "d", J=7.0 Hz, pyridinio-Hs), 8.25 (2H, "d", J=8.8 Hz, nitrophenyl-Hs), and 8.76 ppm (2H, "d", J=7.1 Hz, pyridinio-Hs); ir (Nujol) ν_{max}: 3250 (OH), 1775 (β-lactam), 1700 (ester) and 1625 cm⁻¹ (pyridinio); uv (abs, EtOH) λ_{max}: 308 nm (ε4.47 x 10⁴); [C]_D^{23°} + 24.8 (c 0.5, MeOH); Anal. calcd. for C₂₄H₂₆N₃O₆S₂Cl.H₂O: C 50.56, H 4.95, N 7.37; found: C 50.63, H 4.72, N 6.89.

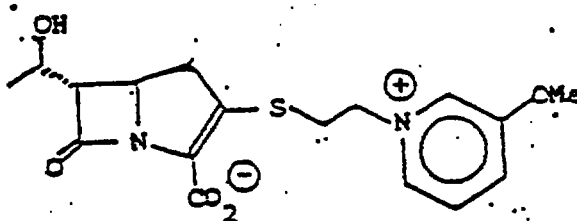
D. (5R) 3-[2-(4-Methylthiopyridinio)ethylthio)-(6S)-[(1R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate



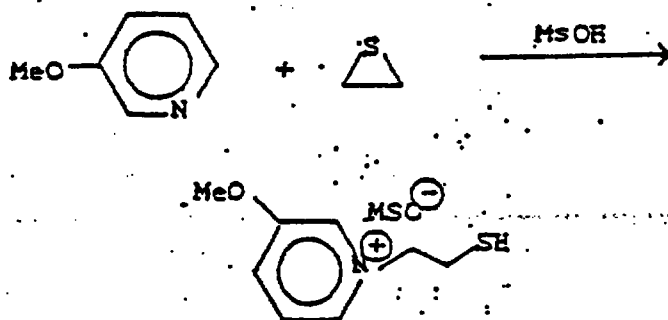
(5R) p-Nitrobenzyl 3-[2-(4-methylthiopyridinio) ethylthio]-(6S)-[(1R)-hydroxyethyl]-7-oxo-1-azabicyclo [3.2.0]hept-2-ene-2-carboxylate chloride (380 mg, 0.688 mmol) was dissolved in THF (31.5 mL) and pH 7.40 phosphate buffer (31.5 mL; 0.05M Fisher) and diluted with Et₂O (31.5 mL). This solution was mixed with 10% Pd-C (380 mg, Engelhard) and hydrogenated at 35 psi on the Parr shaker at room temperature for 1 h. The aqueous layer was filtered over Celite to remove the catalyst and the Celite pad was washed with H₂O (2 x 5 mL). The filtrate and washing were combined and washed with Et₂O (2 x 30 mL). The aqueous layer was pumped off to remove any organic solvents and purified by reverse phase column chromatography (C₁₈ microbondapak, 13 g, Waters Associates) eluting with H₂O. Fractions having a uv absorption at 307 nm were collected (ca. 1 L) and lyophilized to obtain 127 mg (0.334 mmol, y. 48.5%) of the title compound as a yellowish powder: ¹Hmr (D₂O, CFT-20) δ: 1.20 (3H, d, J=6.4 Hz, 1' -CH₃), 2.64 (3H, s, -SCH₃), 2.81 (2H, m, -SCH₂-), 3.19 (1H, dd, J_{6-1'}=6.1 Hz, J₆₋₅=2.6 Hz, 6-H), 3.32 (2H, dd, J=11 Hz, J=5.5 Hz, 4-Hs), 3.92 (1H, dt, J=9.2 Hz, J₅₋₆=2.6 Hz, 5-H), 4.1 (1H, m, 1' -H), 4.61 (2H, t, J=5.9 Hz, -CH₂N⁺), 7.70 (2H, "d", J=7.1 Hz, aromatic-Hs), and 8.40 ppm (2H, "d", J=7.1 Hz, aromatic-Hs); ir (KBr, disc) ν_{max}: 3400 (OH), 1750 (β-lactam), 1630 (pyridinium) and 1590 cm⁻¹ (carboxylate); uv (H₂O) λ_{max}: 231 (ε9800) and 307 nm (ε25000); [α]_D^{23°} + 3.14 (c 0.5, H₂O).

Example 8

Preparation of 3-[2-(3-methoxy-1-pyridinium)ethylthio]-6 α -[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0) - hept-2-ene-2-carboxylate

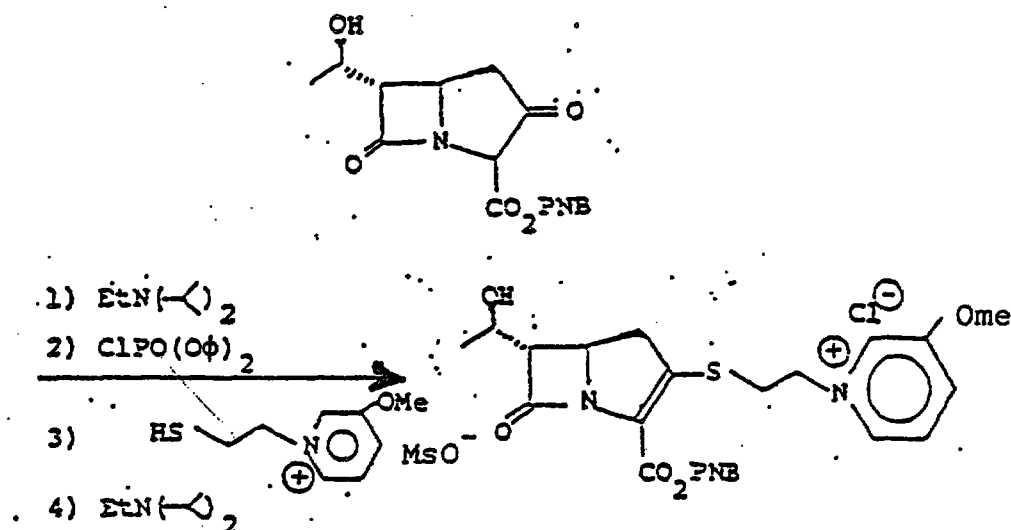


A. 1-(2-mercaptoethyl)-3-methoxypyridinium methanesulfonate



To precooled (5°C) 3-methoxypyridine (698 mg, 6.4 mmol) was added dropwise methanesulfonic acid (0.216 mL, 3.05 mmol) and ethylene sulfide (0.19 mL, 3.2 mmol). The mixture was then heated at 60°C for 18 h, cooled to 20°C, diluted with water (10 mL) and washed with ether (3 x 10 mL). The aqueous phase was pumped under high vacuum for 15 min and poured on a C₁₈ reverse phase column. The title compound was eluted with water. The appropriate fractions were combined and evaporated under high vacuum to give the desired thiol (61.6 mg, yield 76.3%); ir (CH₂Cl₂) ν_{max} : 2550 (w, SH) and 1620, 1600, 1585 cm⁻¹ (m, aromatic); ¹Hmr (DMSO *d*₆) δ : 8.90-7.90 (4H, m, aromatic C-H), 4.72 (2H, t, *J*=6.6 Hz, CH₂N⁺), 4.01 (3H, s, OCH₃), 3.5-3.0 (m, hidden CH₂S), 2.66 (1H, dd, *J*=9.5 Hz, *J*=7.5 Hz, SH) and 2.31 ppm (3H, s, CH₃SO₃⁻)

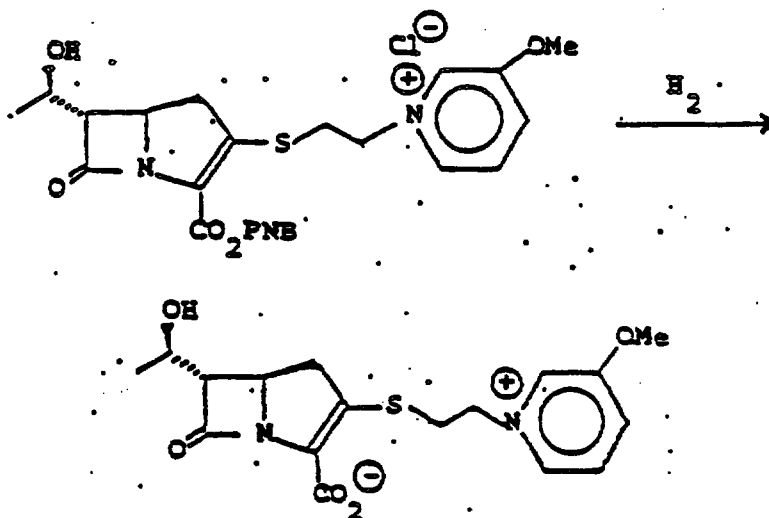
B. para-Nitrobenzyl 3[2-(3-methoxy-1-pyridinium chloride) ethylthio]-6 α -[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo (3.2.0)-hept-2-ene-2-carboxylate



A cold (0°C) solution of p-nitrobenzyl 6 α -[1'-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo-(3.2.0)-heptane-2-carboxylate (1.04 g, 3 mmol) in acetonitrile (12 mL) was treated dropwise with diisopropylethylamine (0.63 mL, 3.6 mmol) and diphenylchlorophosphate (0.75 mL, 3.6 mmol) and stirred at 0°C for 30 min. The resulting enol phosphate was treated with 1-(2-mercaptoethyl)-3-methoxy-pyridinium methanesulfonate (1.14 g, 4.30 mmol) in CH_3CN (7 mL), diisopropylethylamine (0.63 mL, 4.30 mmol), stirred for 30 min. and cooled at -10°C for 30 min. The solid that precipitated out of the mixture was filtered, washed with cold acetonitrile (2 mL) and dried to give the title compound (1.32 g, yield 82%); ir (nujol) ν_{max} 3320 (m, OH), 1780, 1765 (s, β -lactam C=O), 1700, 1695 (m, ester C=O) and 1520 cm^{-1} (s, NO_2); ^1Hmr ($\text{DMSO}-d_6$) δ : 9.01 (1H, bs, H-3 aromatic), 8.75 (1H, bd, $J=5.4\text{ Hz}$, H-6 aromatic), 8.35-7.95 (4H, m, H-aromatic), 7.70 (2H, d, $J=7.7\text{ Hz}$, H-aromatic), 5.37 (2H, center of ABq, $J=13\text{ Hz}$, CH_2PNB), 5.17 (1H, d; $J=4.9\text{ Hz}$, OH), 4.87 (2H, t,

$\underline{J}=6.3$ Hz, $\text{CH}_2\text{-N}^\oplus$), 4.35-3.75 (2H, m, H-5 and H-1'), 4.00 (3H, s, OCH_3) 3.56 (part of a t, $\underline{J}=6.3$ Hz, CH_2S), 3.5-3.20 (3H, m, H-6, H-3) and 1.16 ppm (3H, d, $\underline{J}=6.1$ Hz, CH_3CHO).

C. 3-[2-(3-methoxy-1-pyridinium)ethylthio]-6 α -[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)-hept-2-ene-2-carboxylate



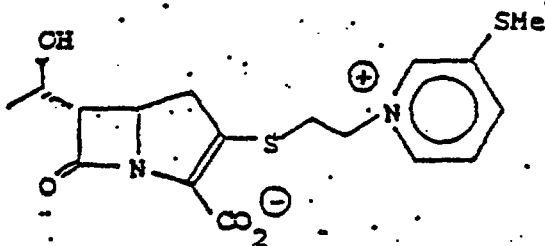
A solution of para-nitrobenzyl 3[2-(3-methoxy-1-pyridinium chloride)ethylthio]-6 α -[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)-hept-2-ene-2-carboxylate (600 mg, 1.12 mmol) in THF (25 mL), ether (25 mL) and pH 7.4 phosphate buffer (0.1M, 25mL) was hydrogenated in a Parr shaker over 10% Pd/C (1.1 g) for 1 h at 40 psi. The mixture was diluted with ether and the aqueous phase was filtered through a #52 hardened filter paper. The aqueous layer was washed with ether (2 x 20 mL), pumped under vacuum and poured on a silica gel reverse phase column. The title compound was eluted with water containing 2 and 5% acetonitrile. The appropriate fractions were combined and lyophilized to give a yellow solid that was repurified by hplc to give the penem carboxylate (150 mg, 38%); ir (nujol) ν_{max} 1750 (s, β -lactam C=O) and 1580 cm^{-1} (s, carboxylate); ^1Hmr (D_2O) δ : 8.55-8.30 (2H, m, H-2, H-6 aromatic); 8.17-7.75 (2H, m, H-3, H-4 aromatic), 4.77 (2H, t, J =5.9 Hz, CH_2N^+), 4.10 (1H, part of 5 lines, J =6.3 Hz, H-1'), 3.97 (3H, s, OCH_3), 3.85, 3.82 (2 lines, part of dt, J =2.6 Hz, part of H-5), 3.42 (2H, t, J =5.9 Hz, $\text{CH}_2\text{-S}$), 3.25

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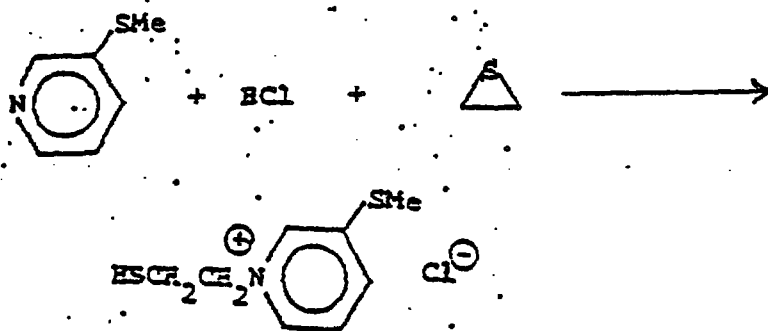
(1H, dd, $J=6.1$ Hz, $J=2.6$ Hz, H-6), 2.99-2.60 (2H, 6 lines, part of H-4) and 1.20 ppm (3H, d, $J=6.4$ Hz, CH₃);
uv (H₂O, c 0.05) λ_{max} : 290 (ϵ 10517), 223 (ϵ 6643); $T_{1/2}$ -
(0.1 M pH 7.4 phosphate buffer, 37°C) 20 h.

Example 9

Preparation of (5R;6S)-3-[2-(3-methylthiopyridinio)
ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo
[3.2.0]hept-2-ene-2-carboxylate



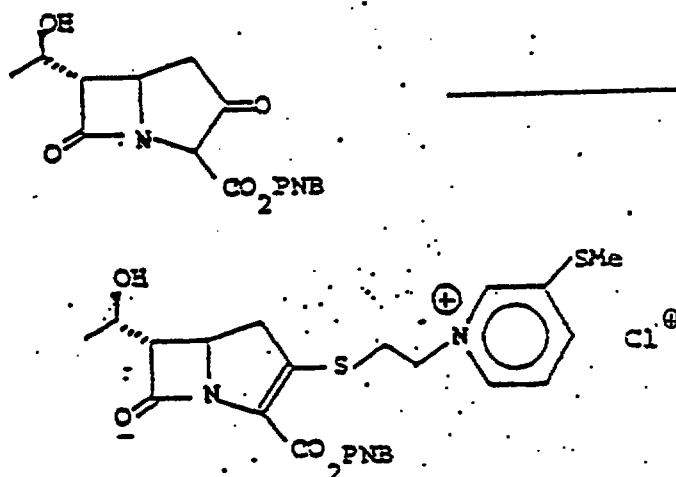
A. 3-Methylthio-1-(2-mercaptoethyl)pyridinium chloride



To a solution of 3-methylthiopyridine¹ (2.00 g, 0.016 mol) in 10 mL of ether was added 15 mL of 1 N HCl and the mixture was well shaken. The aqueous phase was separated, washed with 10 mL of ether and then evaporated. The residual hydrochloride was then dried in vacuo (P₂O₅) to give a white solid. To this solid hydrochloride was added 3-methylthiopyridine (1.88 g, 0.015 mol) and ethylene sulfide (0.89 mL, 0.015 mol) and the resulting mixture was heated (oil bath) at 55-65°C under N₂ for 15 h. This gave a slightly turbid oil which was taken up in 125 mL of H₂O and washed with CH₂Cl₂. The aqueous solution was concentrated to about 25 mL and then a few drops of acetonitrile were added to make the mixture homogeneous. The resulting aqueous solution was applied to a C₁₈ reverse-phase column. Elution with H₂O and subsequent evaporation

of the relevant fractions afforded the product (2.66 g 80%) as a pale yellow, viscous oil. ir (film) $\nu_{\max}$: 2410 (br, -SH) cm^{-1} ; $^1\text{Hnmr}$ (d_6 -DMSO+ D_2O) δ : 8.88-7.88 (m, 4H, aromatic), 4.70 (t, $J=6.5$ Hz, 2H, N- CH_2), 3.08 (skewed t, $J=6.5$ Hz, 2H, S- CH_2), 2.64 (s, 3H, S-Me).
¹Prepared by the method of J.A. Zoltewiez and C. Nisi, J. Org. Chem. 34, 765 (1969).

B. p-Nitrobenzyl(5R,6S)-3-[2-(3-methylthiopyridinio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate chloride



A solution of p-nitrobenzyl (5R,6S)-6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (0.522 g, 1.50 mmol) in 7 mL of dry acetonitrile was cooled at 0°C and then diisopropylethylamine (0.287 mL, 1.65 mmol) was added dropwise. To the resulting yellow-brown solution was added dropwise diphenyl chlorophosphate (0.342 mL, 1.65 mmol) and the reaction mixture was kept at 0°C for 30 min. Diisopropylethylamine (0.313 mL, 1.80 mmol) was then added, followed by a solution of 3-methylthio-1-(2-mercaptoethyl)pyridinium chloride (0.398 g, 1.80 mmol) in 0.70 mL of dry DMF. About a minute after the addition was complete a precipitate separated from the reaction

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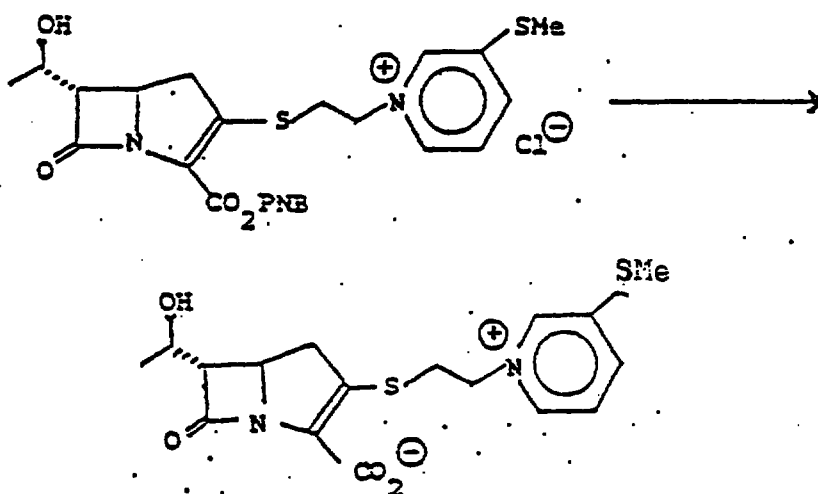
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mixture and further cooling at -10°C for 10 min gave a solid orange-coloured mass. This solid was subsequently triturated with acetonitrile and the residue was collected by filtration. The residue

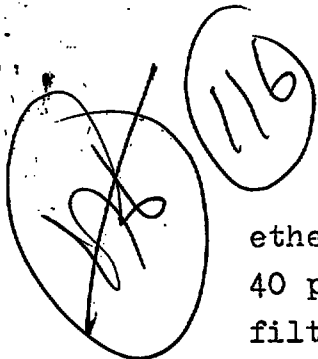
was washed with acetonitrile, then acetone and finally dried in vacuo to give the product (0.455 g, 55%) as a cream-coloured solid. The combined filtrate was evaporated to give a yellow oil which was taken up in a minimum volume of acetonitrile and cooled at 0°C for 30 min. Filtration of this mixture afforded an additional 0.139 g of the product as a light yellow solid. The combined yield was 0.594 g (72%).

ir (KBr) $\nu_{\max}$: 3345 (br, -OH), 1770 (β -lactam CO), 1680 (-CO₂PNB) cm⁻¹; ¹Hnmr (d₆-DMSO) δ : 8.98-7.96 (m, 4H, pyridinium aromatic), 8.20-7.65 (ABq, J=7.0 Hz, 4H, PNB aromatic), 5.53-4.80 (m, 4H), 4.3-3.7 (m, 2H), 3.6-3.25 (m, 6H), 2.66 (s, 3H, S-Me), 1.16 (d, J=6.0 Hz, 3H, CHMe).

15 c. (R,6S)-3-[2-(3-methylthiopyridinio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate



20 To a mixture of p-nitrobenzyl (R,6S)-3-[2-(3-methylthiopyridinio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate chloride (0.551 g, 1.0 mmol) and 10% palladium-on-charcoal (0.55 g) in 25 mL of phosphate buffer
25 (0.05 M, pH 7.4) was added mL of THF and 25 mL of

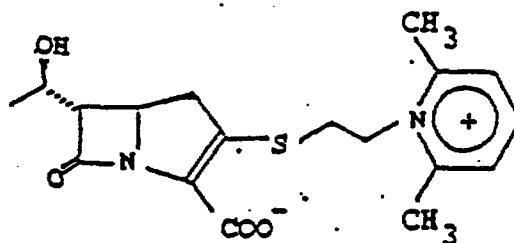
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ether. This mixture was hydrogenated (Parr) at 40 psi for 1 h. The reaction mixture was then filtered through Celite and the filter cake was washed with H_2O and ether. The aqueous phase was separated and washed with additional ether (3 x).

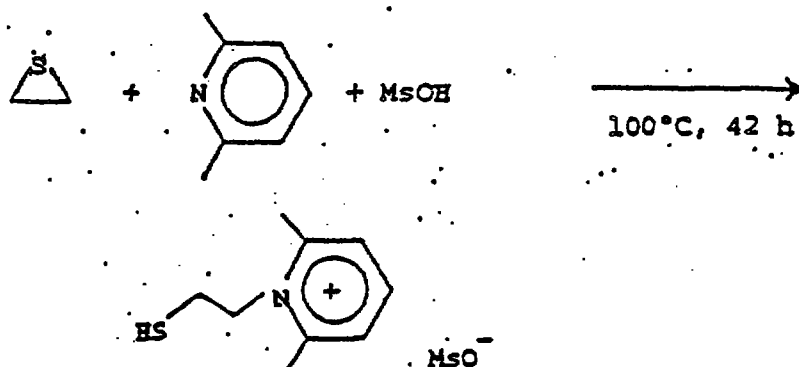
After removing residual organic solvents in vacuo the aqueous solution was cooled at 0°C and the pH was adjusted to 7.0 with saturated aqueous NaHCO₃. This solution was immediately applied to a C₁₈ reverse-phase column. Elution with H₂O and subsequent lyophilization of the relevant fractions afforded 0.25 g of a bright yellow solid. This material was repurified by reverse-phase hplc to give the product (0.210 g, 55%) as a light yellow solid. ir (KBr) ν_{max}: 3400 (br, -OH), 1755 (β-lactam CO), 1590 (-CO₂⁻) cm⁻¹; ¹Hnmr (D₂O) δ: 8.60-7.76 (m, 4H, aromatic), 4.76 (t, J=5.8 Hz, 2H, N-CH₂), 4.13 (d of q, J=J'=6.3 Hz, 1H, H-1'), 3.95 (d of t, J=9.0 Hz, J'=2.8 Hz, 1H, H-5), 3.45-2.75 (m, 5H), 2.59 (s, 3H, S-Me), 1.20 (d, J=6.4 Hz, 3H, CHMe); uv (H₂O) λ_{max}: 296 (ε8509), 273 (ε13005), 231 (ε11576) nm; t_{1/2} (pH 7.4, 36.8°C) 20 h.

Example 10

Preparation of 3-[2-(1-(2,6-dimethylpyridinium)-ethylthio]-6α-[1-(3)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate



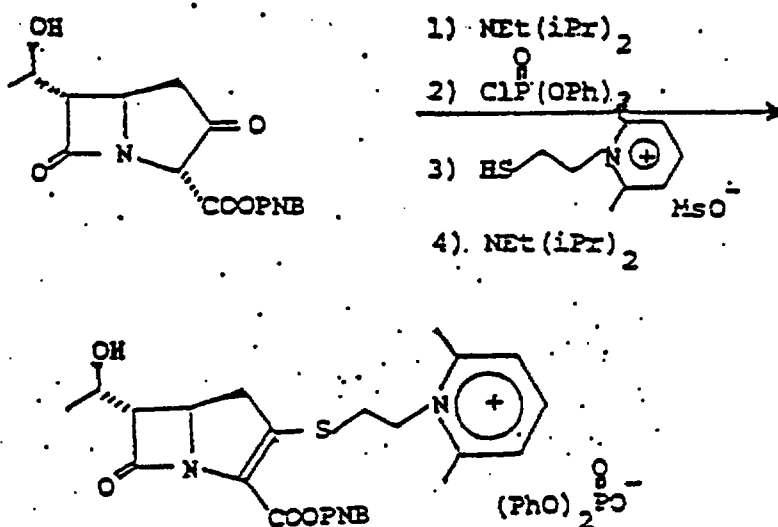
A. 1-(2-mercaptoethyl)-2,6-dimethylpyridinium methane-sulfonate



A mixture of 2,6-dimethylpyridine (19.2 mL, 0.165 mol) and methanesulfonic acid (3.27 mL, 0.050 mol) was stirred for 15 min, treated with ethylene sulfide (4.17 mL, 0.070 mol) and stirred at 100° C for 42 h under a nitrogen atmosphere. After cooling to 25°C, the reaction mixture was diluted with ether (45 mL) and water (30 mL). The two layers were separated and the organic layer was extracted with water (2 x 5 mL). The aqueous layers were combined, filtered through a Celite pad, washed with ether (2 x 15 mL), pumped to remove the traces of organic solvents and poured on top of a column (3.0 x 12 cm) of μ -bondapak C-18. Elution with 3% acetonitrile 97% water mixture gave after lyophilization of the appropriate fractions 2.5 g of the impure title compound as syrup. It was repurified by hplc (μ -bondapak C-18) to give 0.90 g (7%) of the title compound. ir (film) $\nu_{\max}$: 2520 (SH), 1640 and 1625 (pyridinium), 1585, 1490, 1200 cm⁻¹ (sulfonate) ¹Hmr (DMSO-*d*₆ + D₂O) δ : 2.36 (3H, s, CH₃SO₃⁻); 4.62 (2H, m, CH₂N⁺), 7.74 (2H, m, Hm of pyridinium), 8.24 (1H, m, Hp of pyridinium), uv (H₂O) $\lambda_{\max}$: 272 (4080) m μ

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B. Paranitrobenzyl 3-[2-(1-(2,6-dimethylpyridinium))
ethylthio-6 α -[1-(R)-hydroxyethyl]-7-oxo-azabicyclo
(3.2.0)hept-2-ene-carboxylate diphenylphosphate



- 5 To a cold (0°C) solution of p-nitrobenzyl 6 α -[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2-carboxylate (0.658 g, 1.89 mmol) in acetonitrile (6mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.394 mL, 2.26 mmol) and diphenyl
- 10 chlorophosphate (0.468 mL, 2.26 mmol). The reaction mixture was stirred 30 min and treated with a solution of 1-(2-mercaptoethyl)-2,6-dimethylpyridinium methane-sulfonate (0.720 g, 2.73 mmol) in acetonitrile (3 mL) followed by diisopropylethylamine (0.394 mL, 2.26 mmol).
- 15 The reaction mixture was stirred at 0°C for 2 h, diluted with cold (0°C) water (27 mL) and poured on top of a column (2.5 x 9.0 cm) of μ -bondapak C-18. Elution with acetonitrile-water mixtures and lyophilization of the appropriate fractions gave 0.92 g (65%) of the
- 20 title compound, ir (KBr) ν_{max} : 3700-3000 (OH), 1765 (C=O of β -lactam), 1690 (C=O) of PNB ester), 1620 (pyridinium), 1590 (phenyl), 1517 (NO_2), 1330 (NO_2), 880 cm^{-1} (NO_2), ^1Hmr (DMSO, d_6) δ : 1.15 (3H, d, $J=6.2\text{ Hz}$,

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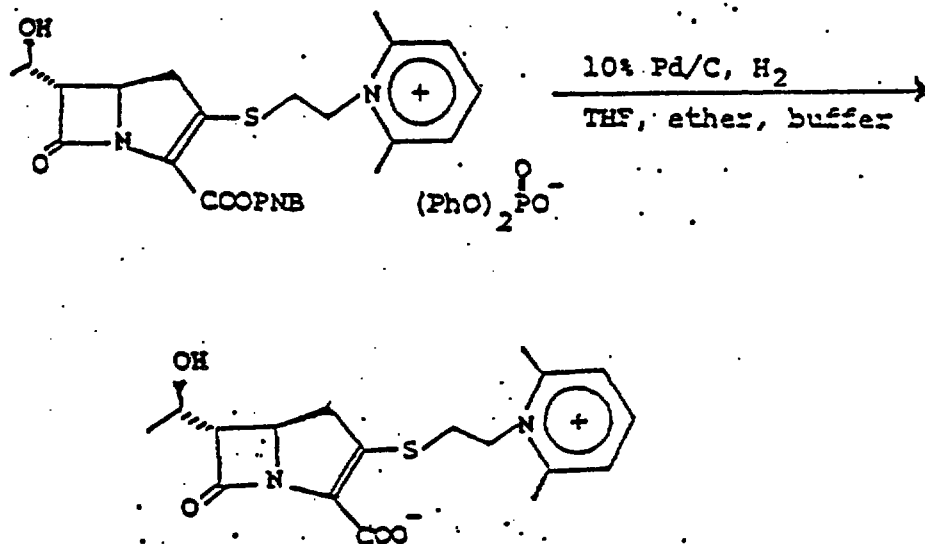
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CH_3CHOH), 2.7-3.7 (11 H, CH_2S , 2- CH_3 on pyridinium, H-4, H-6), 3.7-4.4 (2H, CH_3CHOH , H-5), 4.7 (2H, m, CH_2N^+), 5.14 (1H, d, $J=4.5$ Hz, OH), 5.37 (center of ABq, $J=13.2$ Hz, CH_2 of PNB), 6.7-7.5 (10H, m phenyl), 7.5-8.7 (7H, pyridinium, H's of PNB), uv (H_2O)

5

λ_{max} : 274 ($\epsilon 14150$), 319 ($\epsilon 9445$) m μ

C. 3-[2-(1-(2,6-dimethylpyridinium)ethylthio)-6 α -[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

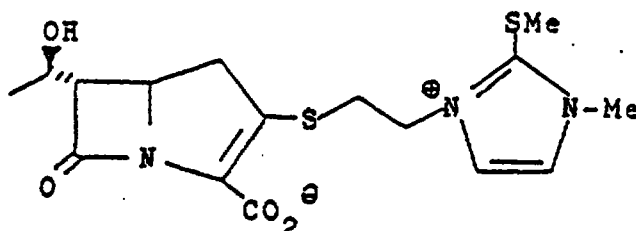


- 5 To a solution of p-nitrobenzyl 3-[2-(1-(2,6-dimethylpyridinium))-ethylthio]-6 α -[1-(R)-hydroxyethyl-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (0.80 g, 1.07 mmol) in wet
- 10 tetrahydrofuran (42 mL) was added ether (42 mL), potassium phosphate monobasic-sodium hydroxide buffer (0.15M, pH 7.22, 21 mL) and 10% palladium on charcoal (0.80 g). The resulting mixture was hydrogenated for 1 h under 40 psi at 23°C and filtered through a Celite
- 15 pad. The two layers were separated and the organic layer was extracted with the buffer (3 x 8 mL). The aqueous phase were combined, washed with ether (50 mL), pumped to remove traces of organic solvent and poured on top of a column (3.0 x 10.2 cm) of μ -bondapak C-18. Elution of the column with 5% acetonitrile - 95% water
- 20 mixture and lyophilization of the appropriate fractions gave the title compound 0.246 g (63%) as a yellowish powder, ir (KBr) ν_{max} : 3700-2800 (OH), 1750 (C=O of

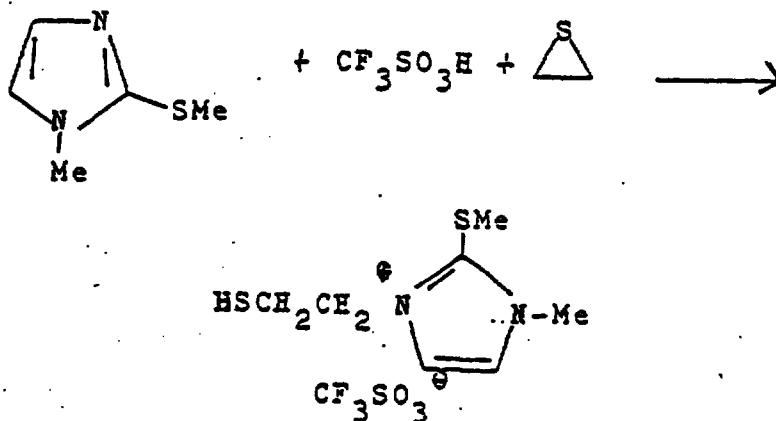
the β -lactam), 1620 (pyridinium), 1585 cm^{-1}
(carboxylate), ^1Hmr (D_2O) δ : 1.23 (3H, d, $J=6.4$ Hz,
 CH_3CHOH), 2.5-3.5 (11H, H-4, H-6, CH_2S , 2CH_3 on
pyridinium), 3.8-4.4 (2H, CH_3CHOH , H-5), 4.5-4.9 (CH_2N^+ ,
5 HOD), 7.64 and 7.74 (2H, A part of A_2B system, Hm of
pyridinium), 8.07, 8.16, 8.18 and 8.27 (1H, B part of
 A_2B system, Hp of pyridinium), uv (H_2O) λ_{max} : 277
($\epsilon 9733$), 300 ($\epsilon 8271$) $\text{m}\mu$, $[\alpha]_{\text{D}}^{23} + 50.7$ (C 0.48, H_2O),
Anal. calcd. for $\text{C}_{18}\text{H}_{22}\text{N}_2\text{O}_4\text{S} \cdot 1.5 \text{H}_2\text{O}$: C 55.51,
10 H 6.47, N 7.19; found: C 55.14, H 6.23, N 6.46.

Example 11

Preparation of (5R, 6S)-3-[2-(2-methylthio-3-methyl-imidazolio)ethyl-thio]-6-[1-(R)-hydroxyethyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate



- 5 A. 2-Methylthio-3-methyl-1-(2-mercaptoethyl)imidazolium trifluoromethanesulfonate



- 10 Trifluoromethanesulfonic acid (1.38 mL, 0.015 mol) was added dropwise to 2-methylthio-1-methylimidazole¹ (4.0 g), 0.03 mol) at 0°C under N₂. Ethylene sulfide (0.9 mL, 0.015 mol) was then added and the mixture was heated at 55°C under N₂ for 24 h. The reaction mixture was triturated with ether (3x) and the residue was taken up in acetone, filtered and evaporated. This
- 15 gave the product (4.2 g, 82%) as a semi-crystalline solid which was used as such without further purification. ir(film) $\nu_{\max}$: 2550 (w, sh) cm⁻¹; ¹Hnmr (d₆-acetone) δ : 7.97 (s, 2H), 4.66 (t, $J=7$ Hz, 2H, methylene), 4.17 (s, 3H, N-Me), 3.20 (d of t, $J=7$ Hz, $J'=9$ Hz, 2H,

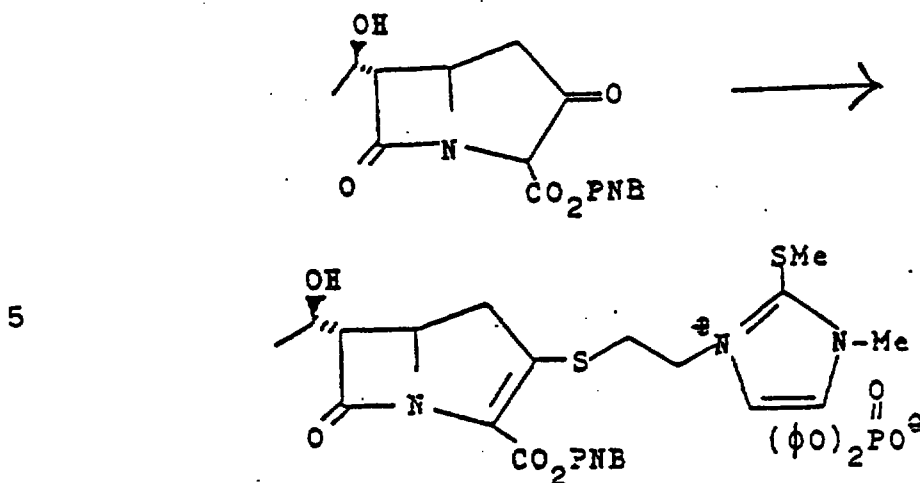
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methylene), 2.72 (s, 3H, S-Me), 2.20 (t, $J=9$ Hz, 1H, -SH).

1. Prepared as per A. Wohl and W. Marckwald, Chem. Ber. 22, 1353 (1889).

B. p-Nitrobenzyl (5R, 6S)-3-[2-(2-methylthio-3-methyl-imidazolio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate diphenyl-phosphate



To a solution of p-nitrobenzyl (5R, 6S)-6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (1.40 g, 4.0 mmol) in 50 mL of dry acetonitrile, at 0°C under N₂, was added dropwise

10 diisopropylethylamine (0.76 mL, 4.4 mmol) followed by diphenyl chlorophosphate (0.91 mL, 4.1 mmol). After stirring the reaction mixture at room temperature for 1h, diisopropylethylamine (0.76 mL, 4.4 mmol) was added and then a solution of 2-methylthio-3-methyl-1-(2-mercapto-

15 ethyl)imidazolium trifluoromethanesulfonate (2.0 g, 5.9 mmol) in 5 mL of acetonitrile was added dropwise. The reaction mixture was kept at room temperature for 1.5 h and was then concentrated in vacuo to give a gum. This gum was taken up in H₂O and applied to a C₁₈ reverse-

20 phase column. Elution with H₂O, then 20% acetonitrile-H₂O and finally 30% acetonitrile-H₂O, followed by lyophilization of the appropriate fractions afforded the product (0.90 g, 30%) as a light yellow solid.

ir(KBr) $\nu_{\max}$: 3380 (br, OH), 1770 (β -lactam CO) cm⁻¹;

25 ¹Hnmr (d₆-acetone) δ : 8.35 (br, s, 1H), 8.24, 7.78



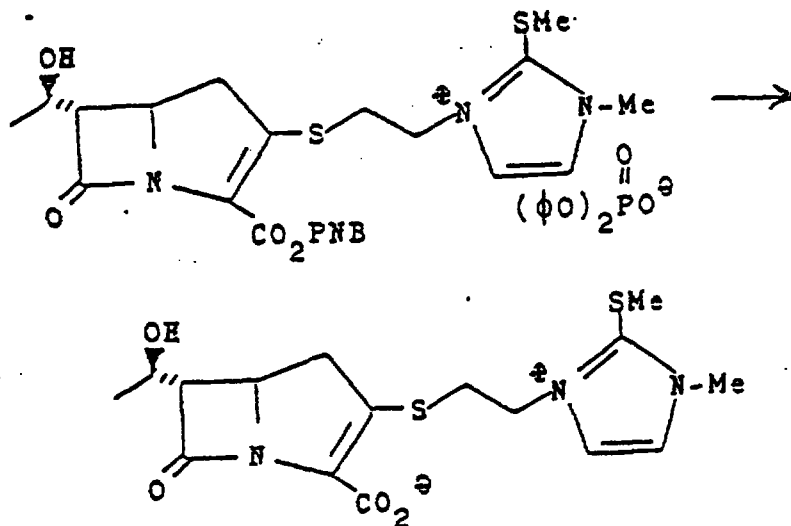
- 102a -

(AB q, $\underline{J}$ =8.8 Hz, 4H, aromatic), 7.89 (br, s, 1H),
7.25-6.91 (m, 10H, diphenylphosphate), 5.50, 5.25
(AB q, $\underline{J}$ =12 Hz, 2H, benzylic), 4.75-4.27 (m, 3H),
4.03 (s, 3H, N-Me), 4.15-2.75 (m, 8H), 2.59 (s, 3H,
S-Me), 1.22 (d, $\underline{J}$ =6.2 Hz, 3H, -CHMe).

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C. (5R, 6S)-3-[2-(2-Methylthio-3-methylimidazolio)ethyl-thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate



5 To a solution of p-nitrobenzyl (5R, 6S)-3-[2-(2-methylthio-3-methylimidazolio)ethyl thio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate diphenylphosphate (1.20 g, 1.56 mmol) in a mixture of 70 mL of THF, 70 ml of ether and 31 mL of
10 phosphate buffer (0.05 M, pH 7.4) was added 1.2 g of 10% palladium-on-charcoal. This mixture was hydrogenated (Parr) at 35 psi for 55 min. The reaction mixture was then filtered through Celite and the filter cake was washed with H₂O and ether. The aqueous phase was
15 separated, cooled at 0°C and the pH was adjusted to 7.0 with saturated aqueous NaHCO₃. After removing residual organic solvents in vacuo the aqueous solution was applied to a C₁₈ reverse-phase column. Elution with H₂O and then 8% acetonitrile-H₂O and subsequent
20 lyophilization of the relevant fractions gave 0.25 g of a solid. This material was repurified by reverse-phase hplc to give the product (0.114 g, 19%) as an off-white solid. ir(KBr) ν_{max} : 3420 (OH), 1750 (β -lactam CO), 1590 (-CO₂⁻) cm⁻¹; ¹Hnmr (D₂O) δ : 7.58

128

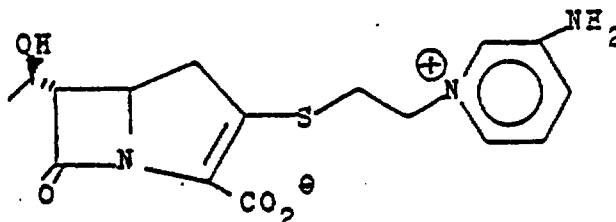
- 103a -

(s, 2H), 4.52 (t, $J=6$ Hz, 2H), 4.28-3.82 (m, 2H), 3.90
(s, 3H, N-Me), 3.40-2.87 (m, 5H), 2.40 (s, 3H, S-Me),
1.20 (d, $J=6.4$ Hz, 3H, -CHMe); uv (H_2O) λ_{max} : 297
(ϵ 7572), 262 (ϵ 6259), 222 (ϵ 7955) nm.

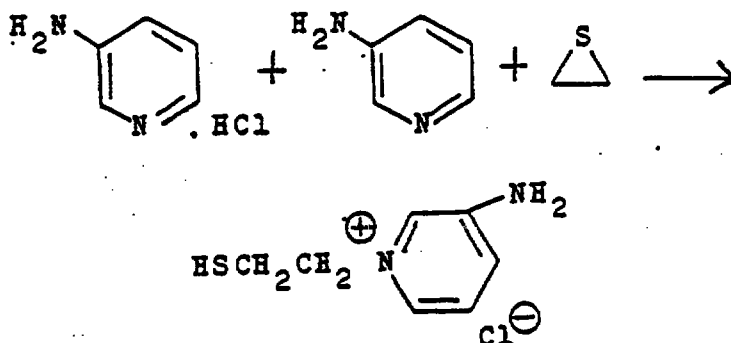
Example 12

Preparation of (5R, 6S)-3-[2-(3-aminopyridinio)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

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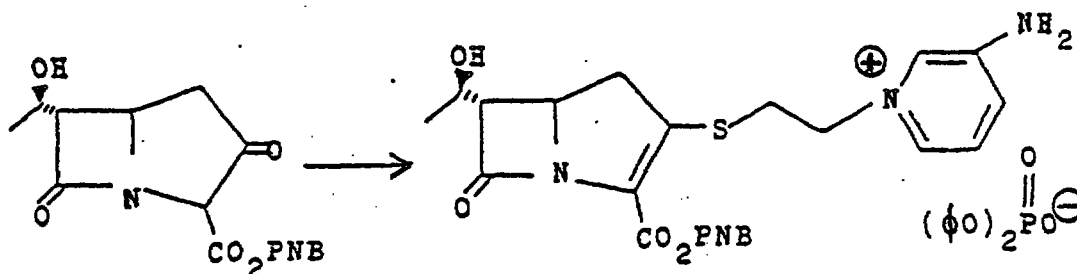


A. 3-Amino-1-(2-mercaptoethyl)pyridinium chloride



3-Aminopyridine (1.50 g, 0.016 mol) was taken up in 15 mL of 1 N methanolic HCl and the resulting solution was evaporated to give the hydrochloride as an oil. To this oil was added 3-aminopyridine (1.32 g, 0.015 mol) and ethylene sulfide (0.89 ml, 0.015 mol) and the resulting mixture was heated (oil bath) at 60-65°C under N₂ for 2 h. Another equivalent of ethylene sulfide (0.89 ml, 0.015 mol) was added and heating was continued at 55-65°C for 65 h. The reaction mixture was washed with CH₂Cl₂ and then taken up in H₂O (25 ml). The aqueous solution was applied to a C₁₈ reverse-phase column which was eluted with H₂O. Evaporation of the relevant fractions gave the product (1.26 g, 44%) as a colorless, viscous oil. ir(film) ν_{max} : 3180 (NH₂) cm⁻¹ ¹Hnmr (d₆-DMSO) δ : 8.19-7.59 (m, 4H, aromatic), 4.59 (t, J=6.2 Hz, 2H, N-CH₂); 3.5 (br s, 2H, -NH₂), 3.20-2.77 (m, 3H).

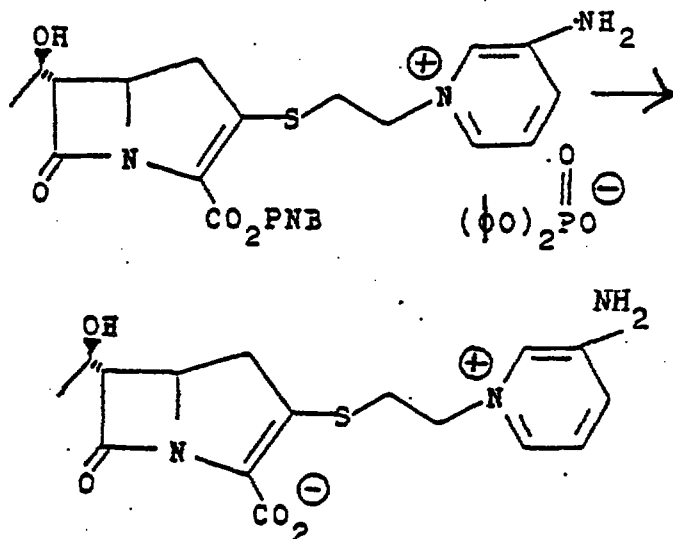
B. p-Nitrobenzyl(5R, 6S)-3-(2-(3-aminopyridinio)ethylthio)-6-(1-(R)-hydroxyethyl)-7-oxo-1-azabicyclo(3.2.0)-hept-2-ene-2-carboxylate diphenylphosphate



- 5 To a solution of p-nitrobenzyl (5R, 6S)-6-(1-(R)-hydroxyethyl)-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2-carboxylate (0.696 g, 2.0 mmol) in 10 mL of dry acetonitrile, at 0°C under N₂, was added dropwise diisopropylethylamine (0.382 mL, 2.2 mmol) followed by
- 10 diphenyl chlorophosphate (0.457 mL, 2.2 mmol). After stirring at 0°C for 30 min. a solution of 3-amino-1-(2-mercaptoethyl)pyridinium chloride (0.475 g, 2.5 mmol) in 1 mL of dry DMF was added, followed by additional diisopropylethylamine (0.435 mL, 2.5 mmol).
- 15 The reaction mixture was kept at 0°C for 1.5 h and was then concentrated in vacuo. The resulting gum was taken up in acetonitrile-H₂O (1:1) and applied to a C₁₈ reverse-phase column. Elution with H₂O, followed by 20% acetonitrile-H₂O and subsequent lyophilization
- 20 of the relevant fractions afforded the product (0.730 g, 50%) as a beige-colored solid. ir(KBr)_{max}: 3330 (br, OH), 3180 (br, NH₂), 1770 (β-lactam CO), 1690 (-CO₂PNB)cm⁻¹; ¹Hnmr (d₆-DMSO) δ: 8.29-7.63 (m, 8 aromatic), 7.2-6.7 (m, 10H, diphenylphosphate), 5.47, 5.18 (AB q, J=14 Hz, 2H, benzylic), 4.73-4.45 (m, 3H), 4.2-3.8 (m, 1H), 3.6-2.6 (m, 8H), 1.15 (d, J=6.2 Hz, 3H, CHMe).
- 25

(31)

C. (5R, 6S)-3-(2-(3-aminopyridinio)ethyl thio)-6-(1-(R)-hydroxyethyl)-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate



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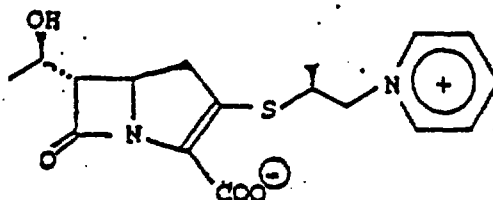
To a mixture of p-nitrobenzyl (5R, 6S)-3-(2-(3-aminopyridinio)ethyl thio)-6-(1-(R)-hydroxyethyl)-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (0.730 g, 1.0 mmol) and 10% palladium-on-charcoal (0.7 g) in 25 mL of a phosphate buffer (0.05 M, pH 7.4) was added 8 mL of THF and 20 mL of ether. This mixture was then hydrogenated (Parr) at 40 psi for 1 h. The resulting mixture was filtered through a pad of Celite and the filter cake was washed with H₂O and ether. The aqueous phase was separated, washed with ether (2 x) and then residual volatiles were removed in vacuo. The aqueous solution was immediately applied to a C₁₈ reverse-phase column which was eluted with H₂O. Lyophilization of the relevant fractions afforded 0.45 g of an off-white solid. This material was repurified by reverse-phase hplc to give the desired product (0.123 g, 35%) as an ivory-colored solid.

ir(KBr) $\nu_{\max}$: 3340 (br), 1750 (br, β -lactam CO),
1580 (br, -CO_2^-) cm^{-1} ; $^1\text{Hnmr}$ (D_2O) δ : 8.07-7.59
(m, 4H, aromatic), 4.61 (t, $\underline{J}$ = 5.8 Hz, 2H, N- CH_2),
4.14 (d of q, $\underline{J}=\underline{J}'$ =6.3 Hz, 1H, H-1'), 3.97 (d of t,
 $\underline{J}$ =9.2 Hz, $\underline{J}'$ =2.6 Hz, 1H, H-5), 3.38 (t, $\underline{J}$ =5.8 Hz,
2H, S- CH_2), 3.24 (d of d, $\underline{J}$ =6.0 Hz, $\underline{J}'$ =
2.6 Hz, 1H, H-6), 3.17-2.57(m, 2H, H-4), 1.21
(d, $\underline{J}$ =6.3 Hz, 3H, CHMe); $\text{uv}(\text{H}_2\text{O})\lambda_{\max}$: 299 (ϵ 7949),
256 (ϵ 8822) nm; $t_{1/2}$ (pH 7.4, 36.8°C) 18.5 h.

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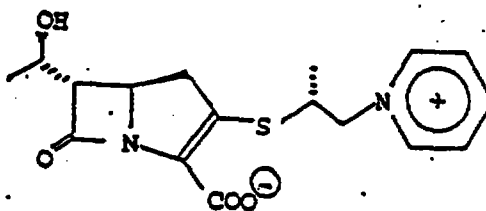
Example 13

PREPARATION OF



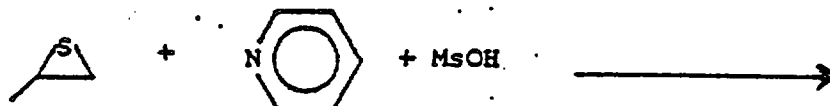
- 5 (5R,6S)3-[1-(S)-methyl-2-(1-pyridinium)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

and



- 10 (5R,6S)3-[1-(R)-methyl-2-(1-pyridinium)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

- A. dl-1-(2-mercapto-2-methylethyl)pyridinium methanesulfonate
dl-1-(2-mercapto-1-methylethyl)
pyridinium methanesulfonate



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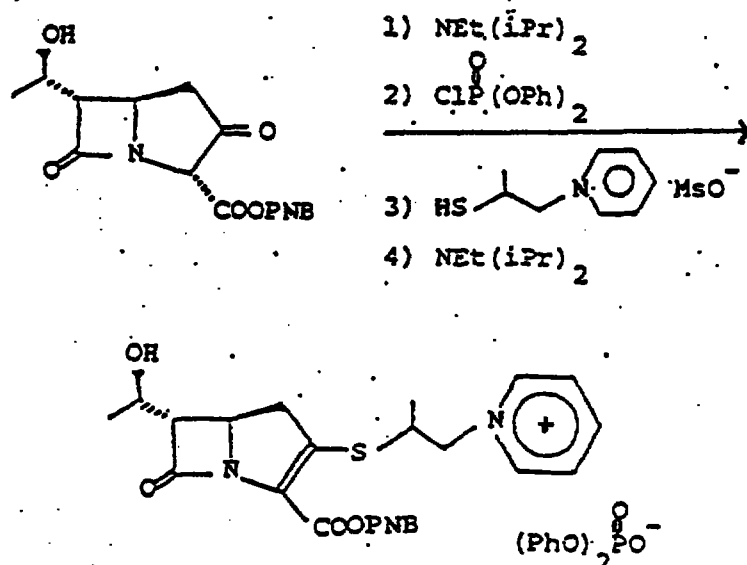
Methanesulfonic acid (1.95 mL, 0.030 mol) was added slowly to cold pyridine (7.33, 0.097 mol) and the resulting mixture was stirred at 40°C for 15 min, treated with dl-propylenesulfide (2.59 mL, 0.033 mol) and stirred at 60°C under a nitrogen atmosphere for 90 h. Pyridine was removed under vacuum; the residue was mixed with water and purified by chromatography (hplc, Prep. Bondapak C-18). The appropriate fractions were combined and lyophilized giving dl-1-(2-mercapto-2-methyl-ethyl)pyridinium methanesulfonate 1.14 g (15%) as a colorless syrup; ir (film) $\nu_{\max}$: 2520 (SH), 1640 (pyridinium), 1180 (s, CH_3SO_3^-), 1040 (CH_3SO_3^-) cm^{-1} , ^1Hmr (DMSO d_6) δ : 1.35 (d, $J=6.8$ Hz, 3H, CH_3CHS), 2.30 (s, 3H, CH_3SO_3^-), 2.90 (d, $J=8.5$ Hz, 1H, SH), 3.2-3.7 (m, CHSH), 4.52 (dd, $J_{\text{gem}}=12.9$ Hz, $J=3.4$ Hz, CHCH_2N^+), 4.87 (dd, $J_{\text{gem}}=12.9$ Hz, $J=6.0$ Hz, CHCH_2N^+), 8.0-8.4 (m, 2H, Hm of pyridinium), 8.5-8.8 (m, 1H, Hp of pyridinium), 9.04 (dd, $J=1.4$ Hz, $J=6.7$ Hz, 2H, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 208 ($\epsilon 5267$), 259 ($\epsilon 3338$),

(135)

Anal. calcd. for $C_9H_{15}NO_3S_2 \cdot 2H_2O$; C 37.88, H 6.71,
N 4.91, S 22.47; found: C 37.49, H 6.85, N 4.86,
S 22.09 and dl-1-(2-mercapto-1-

methylethyl) pyridinium methanesulfonate 0.82 g (11%)
as a colourless syrup; ir (film) $\nu_{\max}$: 2500 (3H), 1628
(pyridinium), 1180 (sulfonate, 1035 (sulfonate) cm^{-1} ,
 ^1Hmr (DMSO d_6) δ : 1.69 (d, $J=6.8$ Hz, 3H, CH_3CHN^+), 2.31
(s, 3H, CH_3SO_3^-), 3.0-3.3 (m, 2H, CH_2S), 4.2-5.2 (m,
1H, CHN^+), 8.0-8.4 (m, 2H, Hm of pyridinium), 8.5-8.8
(m, 1H, Hp of pyridinium), 9.0-9.2 (m, 2H, Ho of
pyridinium), uv (H_2O) $\lambda_{\max}$: 209 ($\epsilon 4987$), 258 ($\epsilon 3838$).
Anal. calcd. for $\text{C}_{15}\text{H}_{15}\text{NO}_3\text{S}_2 \cdot 1.5\text{H}_2\text{O}$: C 39.11, H 6.56,
N 5.07; found: C 39.13, H 5.92, N 5.20.

B. (5R,6S) paranitrobenzyl 3-[1-(R,S)methyl-2-(1-
pyridinium)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-
azabicyclo(3.2.0)hept-2-ene-2-carboxylate
diphenylphosphate



To a cold (0°C) solution of (5R,6S)paranitro-
benzyl 6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo
(3.2.0)heptane-2-carboxylate (0.523 g, 1.5 mmol) in
acetonitrile (6 mL) kept under a nitrogen atmosphere
was added diisopropylethylamine (0.314 mL, 1.8 mmol)

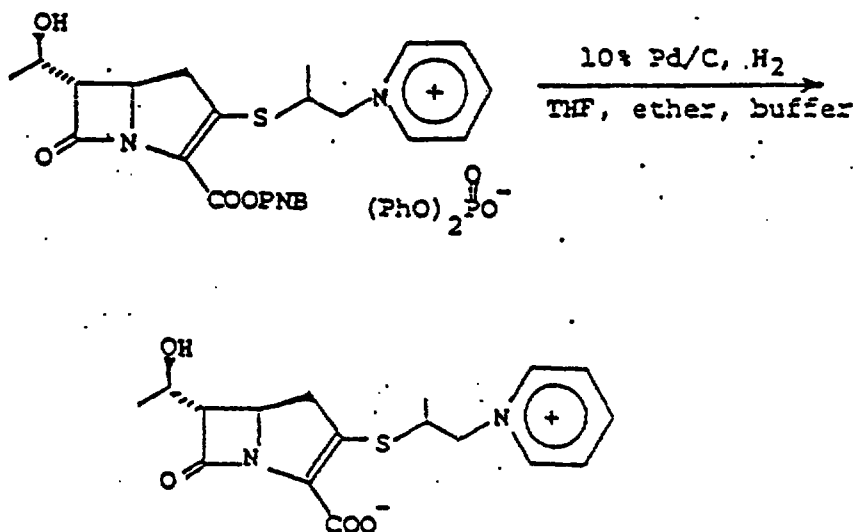
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5 followed by diphenyl chlorophosphate (0.373 mL, 1.8 mmol). The reaction mixture was stirred for 30 min. and treated with a solution of dl-1-(2-mercapto-2-methylethyl) pyridinium methanesulfonate (0.539 g, 2.16 mmol) in acetonitrile (2 mL) and diisopropylethylamine (0.314 mL, 1.8 mmol). The reaction mixture was stirred at 0°C for 1 hr, diluted with cold (0°C)

water (24 mL) and chromatographed over prep bondapak C-18 column (2.5 x 8.5 cm) with 25-50% acetonitrile in water as eluting solvents to give 1.07 g (97%) of the title compound as a yellowish powder after lyophilization;

- 5 ir (KBr) $\nu_{\max}$: 3700-3100 (OH), 1770 (C=O of β -lactam), 1695 (C=O of PNB ester), 1630 (pyridinium), 1590 (phenyl), 1518 (NO_2), 1348 (NO_2), 885 (NO_2) cm^{-1} , ^1Hmr (DMSO d_6) δ : 1.14 (d, $J=6.1$ Hz, 3H, CH_3CHO), 1.33 (d, $J=6.3$ Hz, 3H, CH_3CHS), 4.6-5.0 (m, CH_2N^+), 5.14 (d, $J=5.2$ Hz, 1H, OH), 5.37 (center of ABq, $J=12.4$ Hz, 2H, CH_2 of PNB), 6.6-7.5 (m, 10H, phenyl of phosphate), 7.69 (d, $J=8.7$ Hz, 2H, Ho of PNB), 8.0-8.4 (m, 4H, Hm of PNB, Hm of pyridinium), 8.4-8.8 (m, 1H, Hp of pyridinium), 9.08 (d, $J=5.6$ Hz, 2H, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 263 ($\epsilon 13325$), 308 ($\epsilon 8915$). Anal. calcd. for $\text{C}_{36}\text{H}_{36}\text{N}_3\text{O}_{10}\text{SP.H}_2\text{O}$: C 57.52, H 5.10, N 5.59, S 4.27; found: C 57.76, H 4.96, N 5.36, S 4.35.
- 10
- 15

20 C. (5R,6S)-3-[1-(R and S)-methyl-2-(1-pyridinium) ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo (3.2.0)hept-2-ene-2-carboxylate



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To a solution of (5R,6S)paranitrobenzyl
3-[1-(R,S)methyl-2-(1-pyridinium)ethylthio]-6-[1-(R)-
hydroxyethyl]-7-oxo-1-azabicyclo-(3.2.0)hept-2-ene-2-
5 carboxylate diphenylphosphate (0.60 g, 0.82 mmol) in
wet tetrahydrofuran (33 mL) was added ether (33 mL),
potassium phosphate mono basic-sodium hydroxide
buffer (17 mL, 0.15N, pH 7.22) and 10% palladium on
charcoal (0.60 g). The resulting mixture was
10 hydrogenated for 1 h under 40 psi at 23°C. The two
layers were separated and the

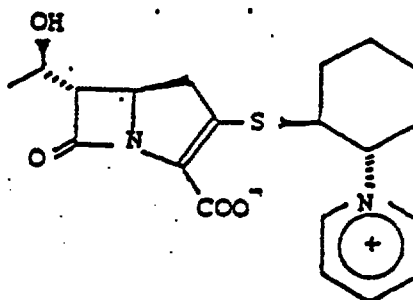
organic layer was extracted with water (3 x 7 mL). The aqueous layers were combined, filtered through a Celite pad, washed with ether (3 x 20 mL) and chromatographed on prep bondapak C-18 column (2.5 x 9.5 cm) with water as eluting solvent to give 0.18 g (63%) of mixture of diastereoisomers. The two diastereoisomers were separated by hplc (prep bondapak C-18) with water as eluting solvent: isomer with lower retention time, 0.068 g (23%) compound "B",

- 10 ir (KBr) $\nu_{\max}$: 1770 (C=O of β -lactam), 1633 (pyridinium), 1593 (carboxylate) cm^{-1} , ^1Hmr (D_2O) δ : 1.20 (d, $J=6.3$ Hz, 3H, CH_3CHO), 1.42 (d, $J=6.9$ Hz, 3H, CH_3CHS), 2.3-3.2 (m, 3H, H-4, H-6), 3.5-3.9 (m, 1H, SCH), 3.9-4.2 (m, 2H, H-5, CH_3CHO), 4.3-5.1 (m, CH_2N^+), 7.8-8.2 (m, 2H, Hm of pyridinium), 8.4-8.7 (m, 1H, Hp of pyridinium), 8.7-9.0 (m, 2H, Ho of pyridinium) uv (H_2O) $\lambda_{\max}$: 260 (ϵ 6727), 300 (ϵ 8245), $[\alpha]_{\text{D}}^{23}$ -39.3° (c, H_2O), $T_{1/2}$ =12.6 h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C); isomer with higher retention time, 0.081 g (28%), compound "A", ir (KBr) $\nu_{\max}$: 1755 (C=O of β -lactam), 1630 (pyridinium), 1590 (carboxylate) cm^{-1} , ^1Hmr (D_2O) δ : 1.18 (d, $J=6.3$ Hz, 3H, CH_3CHO), 1.40 (d, $J=7.0$ Hz, 3H, CH_3CHS), 2.84 (d, $J=9.3$ Hz, 2H, H-4), 3.26 (dd, $J=2.7$ Hz, $J=5.9$ Hz, 1H, H-6), 3.4-4.2 (m, 3H, SCH, CH_3CHO , H-5), 4.2-5.1 (m, CH_2N^+), 7.7-8.1 (m, 2H, Hm of pyridinium), 8.3-8.65 (m, 1H, Hp of pyridinium), 8.65-8.9 (m, 2H, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 259 (ϵ 5694), 296 (ϵ 6936), $[\alpha]_{\text{D}}^{23}$ +96.9° (c 0.56, H_2O), $T_{1/2}$ =15.6 h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C).
- 15
- 20
- 25
- 30

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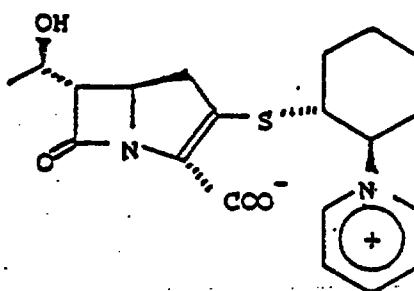
Example 14

PREPARATION OF



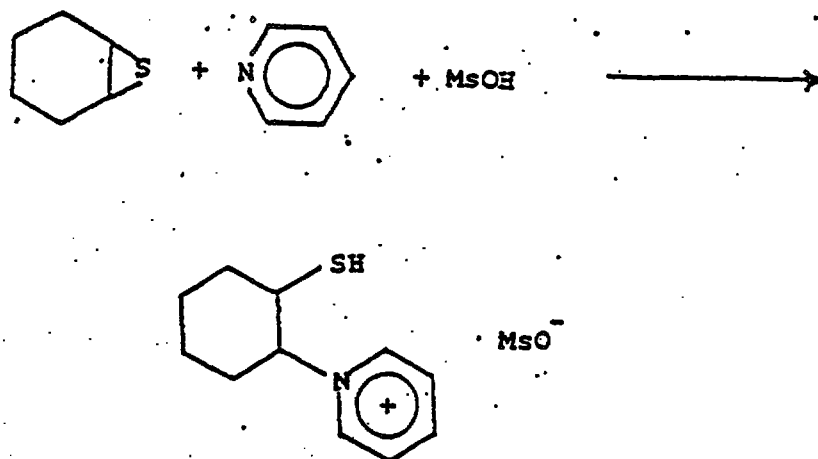
5 (5R,6S)3-[2-(S)-(1-pyridinium)]-1-(S)-cyclohexylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

and



10 (5R,6S)3-[2-[(R)-(1-pyridinium)]-1-(R)-cyclohexylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate.

A. dl-1-(2-mercapto-1-cyclohexyl)pyridinium
methanesulfonate

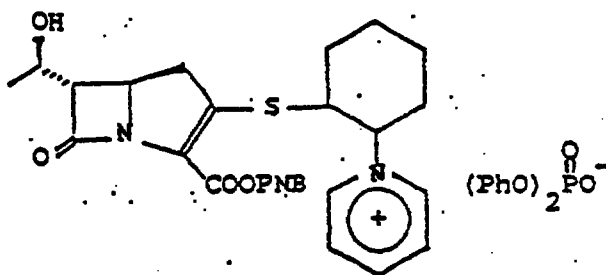
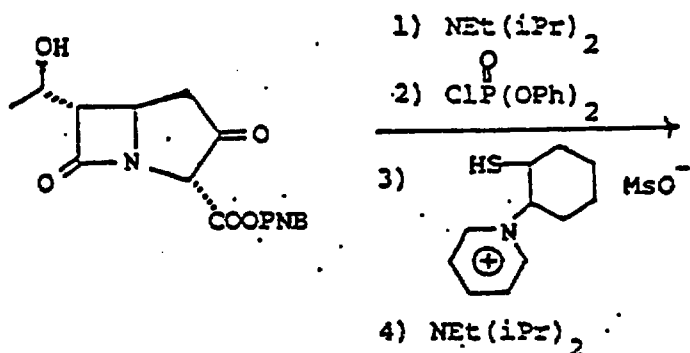


- 5 Methanesulfonic acid (0.65 mL, 0.01 mol) was added dropwise to pyridine (2.42 mL, 0.03 mol) with cooling. The mixture was stirred under a nitrogen atmosphere for 10 min. treated with dl-cyclohexene-sulfide (1.377 g (85 % pure), 0.0102 mol) and stirred
- 10 at 72°C for 25 h. The excess of pyridine was removed under vacuum and the traces were codistilled with water. The residue was mixed with water and chromatographed through prebondapak C-18 column (5 x 13 cm) with 0-2% acetonitrile in water as eluting solvent giving
- 15 after lyophilization a colourless syrup 1.57 g (53%), ir (film) $\nu_{\max}$: 2500 (SH), 1625 (pyridinium), 1190 (SO_3^-), ^1Hmr (DMSO d_6) δ : 1.2-2.5 (m, 8H, cyclohexyl H), 2.32 (s, 3H, CH_3SO_3^-), 2.82 (d, $J=9.8$ Hz, 3H), 3.0-3.5
- 20 γ (m, 1H, CHSH), 4.2-4.9 (m, 1H, CHN^+), 8.0-8.3 (m, 2H, Hm of pyridinium), 8.4-8.8 (m, 1H, Hp of pyridinium), 8.9-9.3 (m, 2H, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 214 ($\epsilon 5365$), 258 ($\epsilon 3500$). Anal. calcd. for $\text{C}_{12}\text{H}_{19}\text{NO}_3\text{S}_2$: C 46.88, H 6.88, N 4.56; found: C 46.61, H 6.46, N 4.65.

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B. (5R,6S)paranitrobenzyl 3-[2- (R or S)-(1-pyridinium)]
-1-(R or S)-cyclohexylthio -6-[1-(R)-hydroxyethyl]-7-oxo-
1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenyl-
phosphate

5

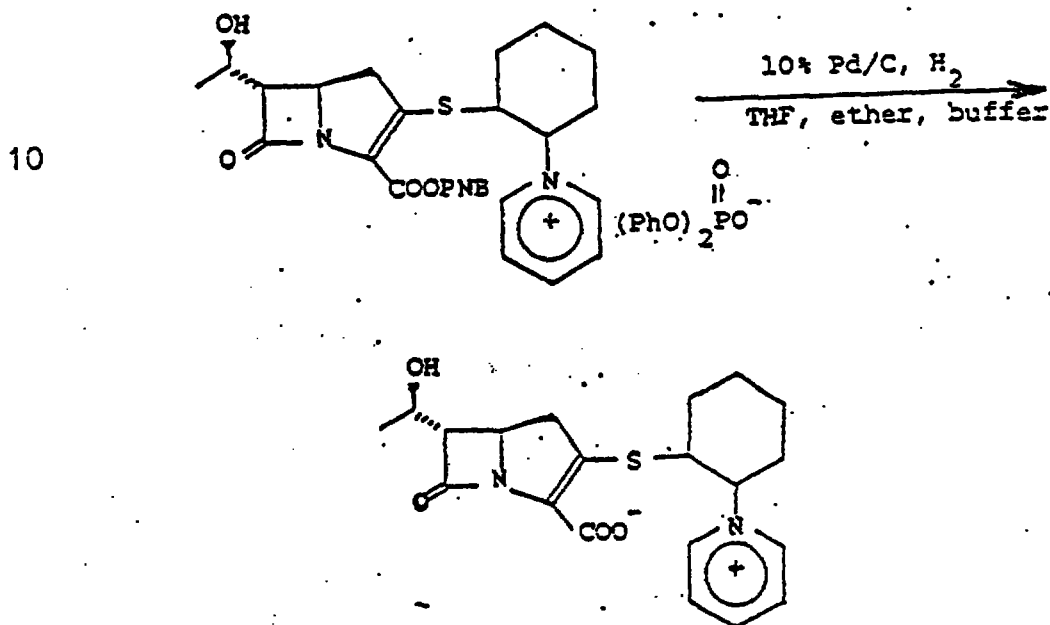


To a cold (0°C) solution of (5R,6S)paranitrobenzyl 6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2-carboxylate (1.37 g, 3.93 mmol) in acetonitrile (15 mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.822 mL, 4.7 mmol) and diphenyl chlorophosphate (0.979 mL, 4.7 mmol). The resulting solution was stirred for 30 min and treated with a solution of dl-1-(2-mercapto-1-cyclohexyl)pyridinium methanesulfonate (1.64 g, 5.66 mmol) in acetonitrile (4.7 mL) followed by diisopropylethylamine (0.822 mL, 4.7 mmol). The reaction mixture was stirred at 0°C for 1 hr, diluted with cold (0°C) water (75 mL) and chromatographed on prebondapak C-18 with 25-50% acetonitrile in water as eluting solvent giving after lyophilization of the appropriate fractions 1.9 g

(53%) of the title compound, ir (KBr) ν_{max} : 3700-3000 (OH), 1770 (C=O of β -lactam), 1700 (C=O of PNB ester), 1628 (pyridinium), 1590 (phenyl), 1515 (NO_2), 1345 (NO_2), 880 (NO_2) cm^{-1} , ^1Hmr (D_2O) δ : 1.13 (d, $\underline{J}=6.1$ Hz, 3H, CH_3CHO), 1.2-2.5 (m, 8H, cyclohexyl H), 2.7-3.5 (m, 4H, H-4, H-6, CHS), 3.5-4.4 (m, 2H, CH_3CHO , H-5), 4.4-5.0 (m, 1H, CHN^+), 5.30 (center of ABq, $\underline{J}=12.8$ Hz, CH_2 of PNB), 6.7-7.4 (m, 10H, phenyl), 7.65 (d, $\underline{J}=8.6$ Hz, 2H,

Ho of PNB), 7.9-8.4 (m, 4H, Hm of PNB, Hm of pyridinium), 8.4-8.8 (m, 1H, Hp of pyridinium), 9.0-9.4 (m, 2H, Ho of pyridinium), uv (H₂O) λ_{max} : 263 (E 9038, 309 (E 6394). Anal. calcd for C₃₉H₄₀N₃O₁₀SP. H₂O: C 59.16, H 5.35, N 5.31; found: C 58.95, H 5.15, N 5.57.

C. (5R,6S) 3-[2-[(R or S)-(1-pyridinium)]-1-(R or S)-cyclohexylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate



15 To a solution of (5R,6S) paranitrobenzyl 3-[2-[(R or S)-(1-pyridinium)]-1-(R or S)-cyclohexylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (1.85 g, 2.34 mmol) in wet tetrahydrofuran (96 mL) was added ether (96 mL), potassium phosphate monobasic-sodium hydroxide buffer (0.15M, pH 7.22, 50 mL) and 10% palladium on charcoal (1.9 g). The resulting mixture

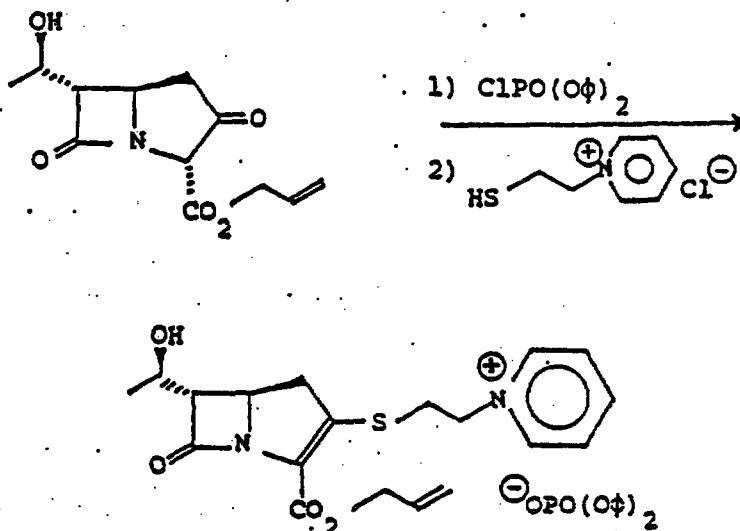
was hydrogenated at 23° under 40 psi for 1.25 h. The organic layer was separated and washed with water (3 x 20 mL). The aqueous solutions were filtered through a Celite pad, washed with ether (2 x 60 mL), pumped to
5 remove the traces of organic solvents and chromatographed on prebondapak C-18 column (4.5 x 9 cm) with 0-5% acetonitrile in water as eluting solvent giving after lyophilization 0.705 g (76%) of a mixture of diastereoisomers. The separation of the diastereo-
10 isomers was done by hplc (prebondapak C-18) with 4% acetonitrile in water as eluting

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- solvent; diastereoisomer with lower retention time, compound "A", (0.29 g, 31%), ir (KBr) $\nu_{\max}$: 1750 (C=O of β -lactam), 1620 (sh, pyridinium), 1685 (carboxylate) cm^{-1} , ^1Hmr (D_2O) δ : 1.21 (d, $J=6.3$ Hz, 3H, CH_3CHO), 1.4-2.5 (m, 8H, cyclohexyl H), 2.5-3.05 (m, 2H, H-4), 3.05-3.25 (m, 1H, H-6), 3.3-3.7 (m, 1H, CHS), 3.9-4.3 (m, 2H, H-5, CH_3CHO), 4.3-4.8 (m, CHN^+), 7.8-8.2 (m, 2H, Hm of pyridinium), 8.3-8.7 (m, 1H, Hp of pyridinium), 8.8-9.1 (m, 2H, Ho of pyridinium),
- 5
- 10 uv (H_2O) $\lambda_{\max}$: 260 ($\epsilon 7123$), 300 ($\epsilon 8685$), $[\alpha]_{\text{D}}^{23} +6.2^\circ$ (c 0.63, H_2O), $T_{1/2} = 16.6$ h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C); Anal. calcd. for $\text{C}_{20}\text{H}_{24}\text{N}_2\text{O}_4\text{S} \cdot 2\text{H}_2\text{O}$: C 56.59, H 6.65, N 6.60, S 7.55; found: C 56.83, H 6.47, N 6.59, S 7.43;
- 15 diastereoisomer with higher retention time, compound "B", (0.35 g, 38%) ir (KBr) $\nu_{\max}$: 1750 (C=O of β -lactam), 1622 (sh, pyridinium), 1588 (carboxylate) cm^{-1} , ^1Hmr (D_2O) δ : 1.19 (d, $J=6.4$ Hz, 3H, CH_3CHO), 1.3-2.5 (m, 8H, cyclohexyl H), 2.5-3.1 (m, 2H, H-4), 3.1-3.3 (m, 1H, H-6), 3.3-3.8 (m, 2H, H-5, CHS), 4.1 (center of m, 1H, CH_3CHO), 4.25-4.7 (m, 1H, CHN^+), 7.8-8.1 (m, 2H, Hm of pyridinium), 8.3-8.7 (m, 1H, Hp of pyridinium), 8.75-9.0 (m, 2H, Ho of pyridinium), uv (H_2O) $\lambda_{\max}$: 259 ($\epsilon 5992$), 296 ($\epsilon 7646$), $[\alpha]_{\text{D}}^{23} +65.3^\circ$ (c 0.43, H_2O),
- 20
- 25 $t_{1/2} = 20.2$ h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C).

Example 15

A. (5R)Allyl 3-[(2-pyridinioethyl)thio]-(6S)-[(1R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate



5

To a solution of (5R) allyl 3,7-dioxo-(6S)-
 [(1R)-hydroxyethyl]-1-azabicyclo(3.2.0)heptane-(2R)-
 carboxylate (473 mg, 1.87 mmol) in CH_3CN (6 mL) was
 added at ca. -10°C under a nitrogen atmosphere
 10 diisopropylethylamine (0.42 mL, 2.4 mmol) followed by
 diphenyl chlorophosphate (0.50 mL, 2.4 mmol). The
 mixture was stirred at 0°C for 30 min, and then cooled
 to -15°C . To this was added an oily suspension of
 N-(2-mercaptoethyl)-pyridinium chloride (527 mg,
 15 3.00 mmol) in CH_3CN (1 mL) containing 5 drops of DMF,
 followed by diisopropylethylamine (0.42 mL, 2.4 mmol).
 The mixture was stirred at -15° for 30 min. and then
 diluted with H_2O (20 mL). This mixture was directly
 purified on a reverse phase silica gel (C_{18} PrepPak,
 20 12 g, Waters Associates) eluting with H_2O (200 mL),
 10% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (100 mL), 20% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (100 mL), 50%
 $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (100 mL) and then 40% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (100 mL).

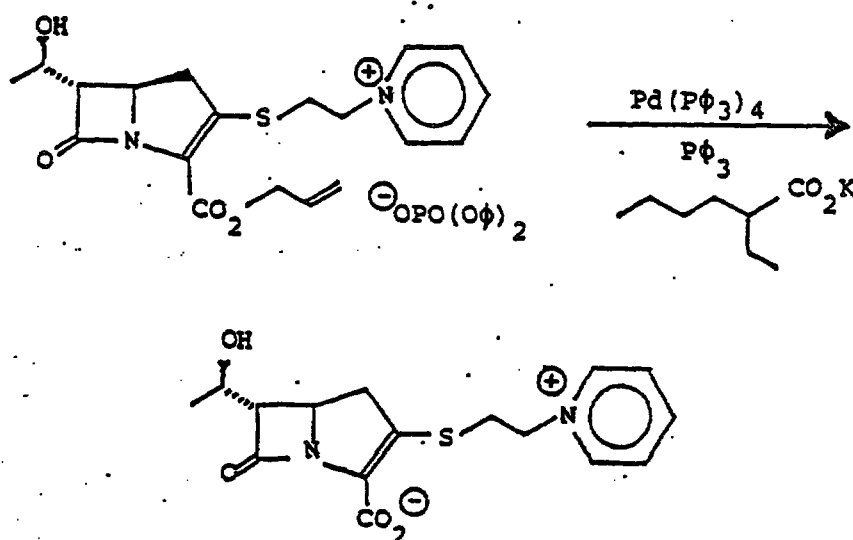
149

Appropriate fractions were collected, the organic solvent removed by a vacuum pump and lyophilized to obtain 786 mg (1.26 mmol, y. 67.3%) of the title compound as brownish powder: ¹Hmr (DMSO-d₆, CFT-20)

5 δ: 1.16 (3H, d, J=6 Hz, 1'-CH₃), 2.6-3.7 (m), 3.75-4.3 (2H, m, 5-H and 1'-H), 4.65 (2H, m, -CO₂CH₂-), 4.87 (2H, t, J=6 Hz, -CH₂-N⁺),

5-6.2 (3H, m, olefinic protons), 6.6-7.4 (m, aromatic protons), 8.15 (2H, "t", $J=7$ Hz, aromatic protons meta to the nitrogen), 8.63 (1H, "t", $J=7$ Hz, aromatic proton para to the nitrogen) and 9.07 ppm (2H, "d", $J=7$ Hz, aromatic protons ortho to the nitrogen); ir (film) ν : 3400 (OH), 1770 (β -lactam), 1690 (ester), 1625 (pyridinio).

10 B. (5R 3-[(2-pyridinioethyl)thio]-(6S)-[(1R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate



To a solution of (5R) allyl 3-[(2-pyridinioethyl)thio]-(6S)-[(1R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate diphenylphosphate (156 mg, 0.25 mmol) in CH_3CN (2 mL) was successively added at ca. 22°C a solution of potassium 2-ethylhexanoate in EtOAc (0.5 M, 0.6 mL; 0.3 mmol), triphenylphosphine (15 mg, 0.057 mmol) and tetrakis(triphenylphosphine) palladium (15 mg, 0.013 mmol). The mixture was stirred at ca. 22°C under a nitrogen atmosphere for 2 h. After addition of anhydrous Et_2O (7 mL), the precipitate was filtered, washed with anhydrous Et_2O (7 mL) and dried in vacuo to yield 101 mg of brownish solid. This was purified

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by reverse phase column chromatography (C₁₈ PrepPAK, 12 g, Waters Associates) eluting with H₂O.

Appropriate fractions (fr. 7-12, each 20 mL) were collected and lyophilized to obtain 53 mg (0.16 mmol, y. 64%) of the title compound as yellowish powder.

5

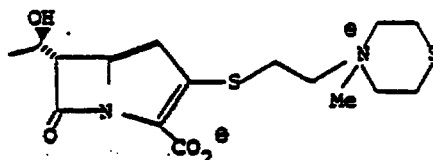
This material was contaminated with potassium diphenylphosphate and potassium 2-ethylhexanoate: ¹Hmr (D₂O, CFT-20) δ: 0.80

5 (t, $J=6.4$ Hz, Me from ethylhexanoate), 1.21 (3H, d, $J=6.3$ Hz, 1'-Me), 2.93 (2H, dd, $J_{1-5}=9$ Hz, $J_{gem}=4$ Hz, 1-Hs), 3.28 (1 H, dd, $J_{6-1}=6.2$ Hz, $J_{6-5}=2.5$ Hz, 6-H), 3.42 (2H, t, $J=6$ Hz, $-CH_2C$), 3.98 (1H, td, $J_{5-1}=9$ Hz, $J_{5-6}=2.5$ Hz, 5-H), 4.15 (1H, q, $J=6.2$ Hz, 1'-H), 4.80 (2H, t, $J=6.0$ Hz, $-CH_2N^+$), 7-7.5 (m, phenyl protons from diphenyl phosphate), 8.03 (2H, m, Hm of pyridinium), 8.56 (1H, m, Hp of pyridinium) and 8.81 ppm (2H, "d", $J=6.5$ Hz, Ho of pyridinium).

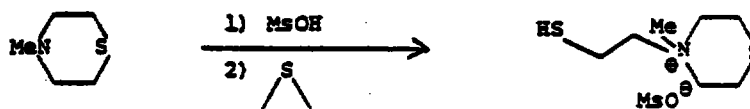
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Example 16

Preparation of 3-[2-(N-Methyl-thiomorpholinium)ethylthio]-6a-[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate



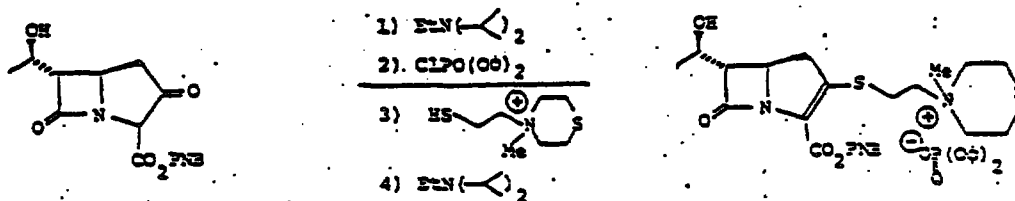
A. N-methyl-N-(2-mercaptoethyl)thiomorpholinium methanesulfonate



To precooled (ice bath) N-methylthiomorpholine* (5.00 g, 42.7 mmol) was added methanesulfonic acid (1.47 mL, 20.5 mmol) and ethylene sulfide (1.30 mL, 21.4 mmol). The mixture was heated at 65°C for 24 h and diluted with water (25 mL). The aqueous solution was washed with diethyl ether (3 x 25 mL), pumped under vacuum and poured over a silica gel reverse phase column; the title compound being eluted with water. The appropriate fractions were combined and evaporated to afford the thiol as an oil (4.80 g, yield 86%); ir (film) ν_{max} : 2550 cm^{-1} (w, SH); ^1Hmr (DMSO d_6) δ : 3.25-2.95 (6H, m, CH_2N^+), 3.32 (3H, s, CH_3N^+), 3.20-2.65 (7H, m, CH_2S , SH) and 2.32 ppm (3H, s, CH_3SO_3).

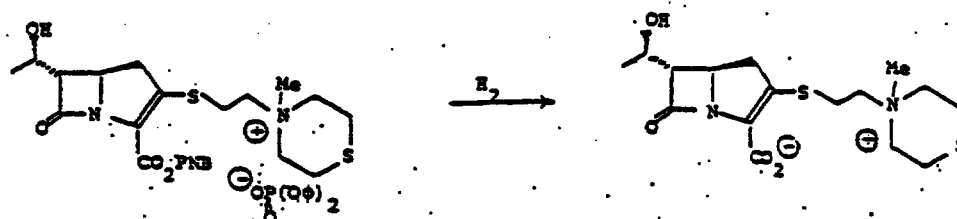
*J.M. Lehn and J. Wagner. Tetrahedron, 25, 4227 (1970)

3. para-Nitrobenzyl 3-[2-(N-methyl-thiomorpholinium diphenyl phosphate) ethylthio]-6c-[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate



A cold (ice bath) solution of para-nitrobenzyl 6c-[1'-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (557 mg, 1.60 mmol) in CH_3CN (8 mL) was treated dropwise with diisopropylethyl amine (0.336 mL, 1.92 mmol) and diphenylchlorophosphate (0.400 mL, 1.92 mmol) and stirred for 30 min. The reaction mixture was treated with N-methyl-N-(2-mercaptoethyl)thiomorpholinium methanesulfonate (893 mg, 2.29 mmol) in CH_3CN (4 mL) and diisopropylethyl amine (0.336 mL, 1.92 mmol) and stirred for 30 min. The solution was diluted with water (20 mL) and poured over a silica gel reversed phase column. The desired compound was eluted with a 50% acetonitrile-water mixture. The appropriate fractions were combined, pumped under vacuum for 2 h. and lyophilized to afford the title compound (1.01 g, yield 85%): ir (nujol) ν_{max} : 1760 (s, β -lactam C=O) and 1510 cm^{-1} (s, NO_2); ^1Hmr ($\text{DMSO}-d_6$) δ : 8.25 (2H, d, $J=8.8$ Hz, H-aromatic), 7.70 (2H, d, $J=8.8$ Hz, H-aromatic), 7.33-6.84 (10 H, m, H-aromatic), 5.37 (2H, center of ABq, $J=14.2$ Hz, CH_2), 5.14 (1H, d, $J=4.5$ Hz, OH), 4.35-3.80 (2H, m, H-1' and H-5), 3.75-3.45 (6H, m, CH_2N^+), 3.31 (3H, s, CH_3N^+), 3.45-2.75 (9H, m, CH_2S , H-6 and H-4) and 1.15 ppm (3H, d, $J=6.2$ Hz, CH_3).

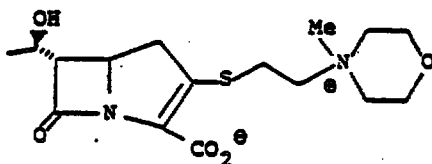
C. 3-[2-(N-methyl-thiomorpholinium)ethylthio]-6c-[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate



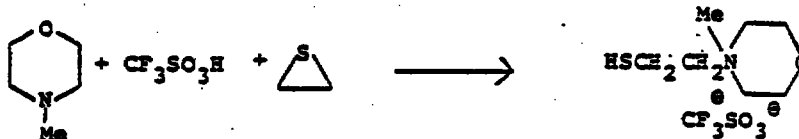
A solution of para-nitrobenzyl 3-[2-(N-methyl-thiomorpholinium diphenylphosphate)ethylthio]-6c-[1'-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (1.31 g, 1.76 mmol) in 0.1M pH 7.4 phosphate buffer (48.8 mL), tetrahydrofuran (20 mL) and diethyl ether (20 mL) was hydrogenated over 10% Pd/C (1.5 g) in a Parr shaker for 1 h at 40 psi. The reaction mixture was diluted with diethyl ether (40 mL) and the phases were separated. The organic phase was washed with water (2 x 5 mL). The aqueous phases were combined, filtered through a #52 hardened filter paper, washed with diethyl ether (2 x 20 mL) and pumped under vacuum. The aqueous solution was poured on a silica gel reverse phase column and the desired carbapenem was eluted with 5% acetonitrile-water. The appropriate fractions were combined, and lyophilized to give title compound as an amorphous solid (205 mg, 31%); *ir* (nujol) ν_{max} : 1750 (s, β -lactam C=O) and 1590 cm^{-1} (s, C=O); ^1Hmr (D_2O) δ : 4.25-3.95 (2H, m, H-1', H-5), 3.70-3.40 (6H, m, CH_2N^+), 3.35 (1H, dd, $J=6.1$ Hz, $J=2.6$ Hz, H-6), 3.08 (3H, s, CH_3N^+), 3.25-2.75 (8H, CH_2S , H-4), and 1.24 ppm (3H, d, $J=6.4$ Hz, CH_3); *uv* (H_2O , c 0.062) λ_{max} : 299 (ϵ 10,962) $T_{1/2}$ 17.7 h (0.1M pH 7 phosphate buffer, 37°C).

Example 17

Preparation of (5R,6S)-3-[2-(1-methylmorpholino)ethylthio]-6-[(R)-1-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate

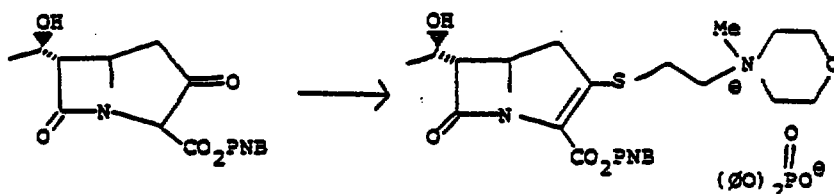


A. 1-Methyl-1-(2-mercaptoethyl)morpholinium trifluoromethanesulfonate



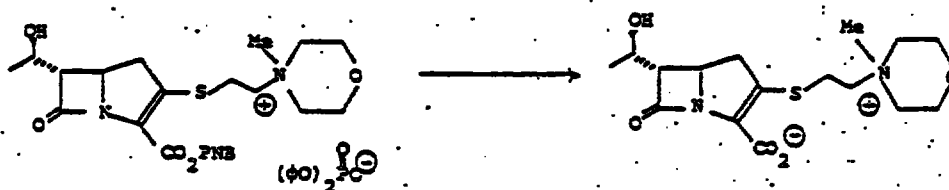
To N-methylmorpholine (3.29 mL, 0.030 mol) was added dropwise trifluoromethanesulfonic acid (1.327 mL, 0.015 mol) at 10°C, followed by ethylene sulfide (0.89 mL, 0.015 mol). The resulting yellow-brown solution was heated (oil bath) at 50-60°C under N₂ for 18 h. Volatile material was then removed in vacuo and the residual oil was taken up in 10 mL of H₂O. The aqueous solution was washed with diethyl ether (3 x 5 mL) and then residual organic solvent was removed in vacuo. The resulting aqueous solution was applied to a C₁₈ reverse-phase column which was eluted with H₂O, then 5% acetonitrile-H₂O and finally 10% acetonitrile-H₂O. Evaporation of the relevant fractions afforded a white solid which was dried in vacuo (P₂O₅) to give the product (1.92 g, 41%). ir (KBr) ν_{max} : 2560 (-SH) cm⁻¹; ¹Hnmr (d₆-acetone) δ : 4.25-3.6 (m, 8H), 3.49 (s, 3H, N-Me), 3.35-2.7 (m, 5H).

B. p-Nitrobenzyl (5R,6S)-3-[2-(1-methylmorpholino)ethylthio]-6-[(R)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate diphenylphosphate



To a solution of p-nitrobenzyl (5R,6S)-6-[(R)-1-hydroxyethyl]-3,7-dioxo-1-azabicyclo[3.2.0]heptane-2-carboxylate (0.348 g, 1.0 mmol) in 25 mL of dry acetonitrile was added dropwise diisopropylethylamine (0.191 mL, 1.1 mmol) and then diphenyl chlorophosphate (0.228 mL, 1.1 mmol) at 0°C under N₂. After stirring at 0°C for 1 h diisopropylethylamine (0.226 mL, 1.3 mmol) was added to the resulting enol phosphate, followed by 1-methyl-1-(2-mercaptoethyl)morpholinium trifluoromethanesulfonate (0.373 g, 1.2 mmol). The reaction mixture was stirred at room temperature for 1.5 h and then concentrated in vacuo. The residual material was taken up in H₂O and applied to a C₁₈ reverse-phase column. Elution with H₂O, then 20% acetonitrile-H₂O and finally 30% acetonitrile-H₂O followed by lyophilization of the relevant fractions gave the product (0.360 g, 40%) as an amorphous solid. ir (film) 3300 (-OH), 1770 (β-lactam CO), 1700 (-CO₂PNB) cm⁻¹; ¹Hnmr (d₆-acetone) δ: 8.25, 7.80 (ABq, J=8.6 Hz, 4H, aromatic), 7.4-6.8 (m, 10H, diphenylphosphate), 5.56, 5.27 (ABq, J=14.2 Hz, 2H, benzylic), 4.42 (d of t, J=9.2 Hz, J'=2.7 Hz, 1H, H-5), 4.1-2.7 (m, 17H), 3.40 (s, 3H, N-Me), 1.22 (d, J=6.2 Hz, 3H, -CHMe).

C. (5R,6S)-3-[2-(1-methylmorpholino)ethylthio]-6-[(R)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

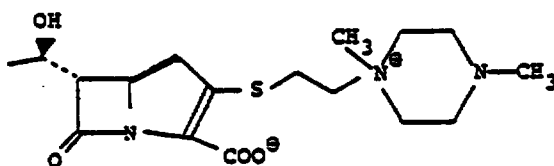


To a solution of p-nitrobenzyl (5R,6S)-3-[2-(1-methylmorpholino)ethylthio]-6-[(R)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate diphenylphosphate (0.360 g, 0.49 mmol) in 13 mL of phosphate buffer (0.05 M pH 7.4) was added 0.36 g of 10% palladium-on-charcoal, 20 mL of tetrahydrofuran and 20 mL of diethyl ether. This mixture was hydrogenated (Parr) at 32 psi for 1 h. The mixture was filtered through Celite and the filter pad was washed with H₂O and diethyl ether. The aqueous phase was separated and the pH was adjusted to 7.0 with additional pH 7.4 phosphate buffer. After removing residual organic solvents in vacuo the aqueous solution was applied to a C₁₈ reverse-phase column. Elution with H₂O and lyophilization of the relevant fractions afforded 0.130 g of an amorphous solid. This material was repurified by reverse-phase hplc to give the pure product (0.058 g, 34%) as an amorphous solid. $\text{ir (KBr)} \nu_{\text{max}}$: 3420 (br, OH), 1750 (β -lactam CO), 1590 ($-\text{CO}_2^-$) cm^{-1} ; $^1\text{Hnmr (D}_2\text{O)}$ δ : 4.35-2.77 (m, 17H), 3.18 (s, 3H, N-Me), 1.23 (d, $J=6.3$ Hz, 3H, CHMe); $\text{uv (H}_2\text{O)} \lambda_{\text{max}}$: 300 (ϵ 6344) nm; $t_{1/2}$ (pH 7.4, 36.8°C) 18.5 h.

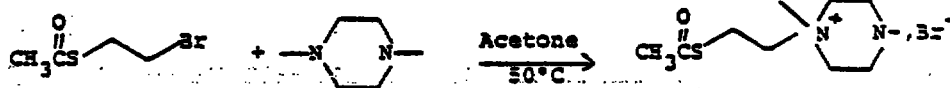
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Example 18

Preparation of (5R,6S) 3-[2-(1,4-dimethyl-1-piperazinium)-ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate



A. 1-(2-acetylthioethyl)-1,4-dimethylpiperazinium bromide

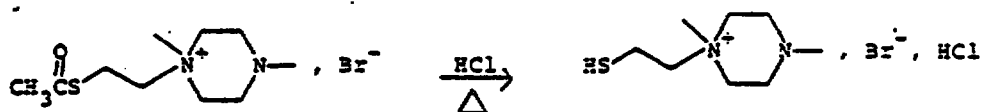


A solution of 2-bromoethyl thiolacetate* (2.20 g, 0.012 mol) and 1,4-dimethylpiperazine (1.95 mL, 0.014 mol) in acetone (4 mL) was stirred at 50°C for 65 h. After cooling to 25°C, the liquid phase was decanted from the gum which was triturated twice in diethyl ether; a hygroscopic yellowish powder, 3.2 g (90%) was obtained; ir (Nujol) ν_{max} : 1685 (C=O of thioester) cm^{-1} ; ^1Hmr (D_2O) δ :

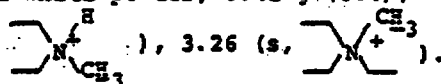
2.37, 2.39 (2s, 6H, CH_3CO , N-CH_3), 3.18 (s, 3H, N-CH_3).

*B. Hansen, Acta Chem. Scand. **11**, 537-40 (1957)

B. 1,4-dimethyl-1-(2-mercaptoethyl)piperazinium bromide hydrochloride

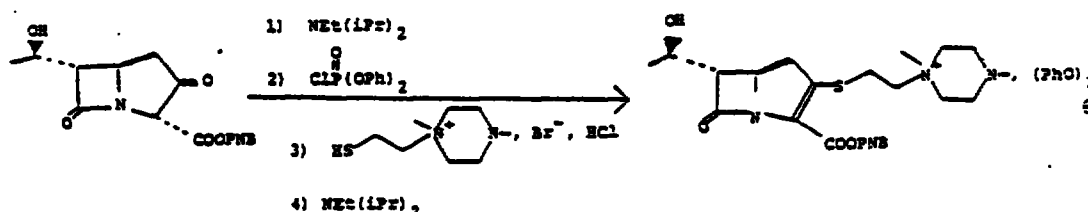


A solution of 1-(2-acetylthioethyl)-1,4-dimethylpiperazinium bromide (1.1 g, 3.7 mmol) in 6N hydrochloric acid (4 mL) was heated at 80°C under a nitrogen atmosphere for 1 h. The solution was concentrated under reduced pressure to give a white powder, 0.41 g (38%), $^1\text{H-NMR}$ (DMSO, d_6) δ : 2.90 (s,



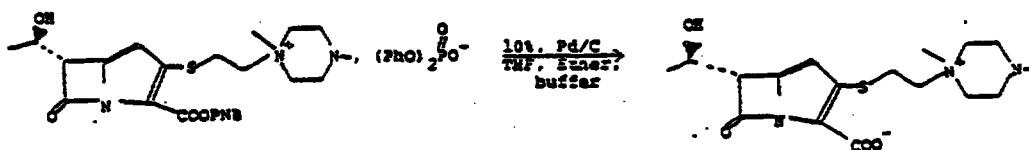
Anal. calcd. for $\text{C}_8\text{H}_{20}\text{N}_2\text{SBr}\cdot\text{H}_2\text{O}$: C 31.03, H 7.16, N 9.05, S 10.35; found: C 31.62, H 7.46, N 9.19, S 10.19.

C. (5R,6S)Paranitrobenzyl 3-[2-(1,4-dimethyl-1-piperazinium)-ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate diphenylphosphate



To a cold (0°C) solution of (5R,6S) paranitrobenzyl 6-[1-(R)-hydroxyethyl]-3,7-dioxo-1-azabicyclo[3.2.0]heptane-2-(R)-carboxylate (0.465 g, 1.33 mmol) in acetonitrile (2 mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.278 mL, 1.59 mmol) and diphenyl chlorophosphate (0.33 mL, 1.59 mmol). The reaction mixture was stirred for 30 min and treated with a suspension of 1,4-dimethyl-1-(2-mercaptoethyl)-piperazinium bromide hydrochloride (0.40 g, 1.37 mmol) in acetonitrile (3 mL)-water (1 mL) mixture and diisopropylethylamine (0.278 mL, 1.59 mmol). After stirring for 18 h at 5°C , cold water (15 mL) was added to the mixture. The resulting solution was chromatographed over PrepPak-500/ C_{18} (Waters Associates) column (2.5 x 7.5 cm) with 25-35% acetonitrile in water as eluting solvents to give a yellowish powder 0.50 g (50%) after lyophilization; ir (KBr) ν_{max} : 1765 ($\text{C}=\text{O}$ of β -lactam), 1690 ($\text{C}=\text{O}$ of PNB ester), 1585 (phenyl), 1512 (NO_2), 875 (NO_2) cm^{-1} , ^1Hmr (DMSO, d_6) δ : 1.16, 1.18 (2d, $J=6.1$ Hz, 3H, CH_3CHOH), 2.44 (s, $\text{N}-\text{CH}_3$), 3.14 (s, $\text{N}-\text{CH}_3$), 5.31 (d, $J=6$ Hz, OH), 5.39 (center of ABq, $J=13$ Hz, CH_2 of PNB), 6.6-7.4 (m, 10H, phenyl of phosphate), 7.71 (d, $J=8.8$ Hz, 2H, Ho of PNB), 8.26 (d, $J=8.8$ Hz, Hm of PNB).

D. (5R,6S) 3-[2-(1,4-dimethyl-1-piperazinium)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate

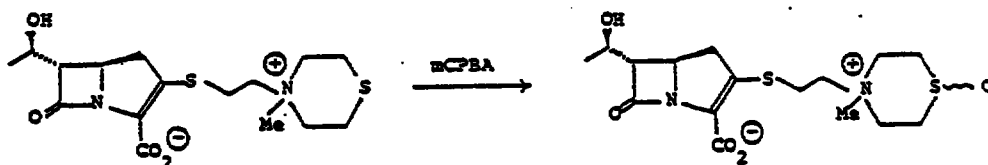
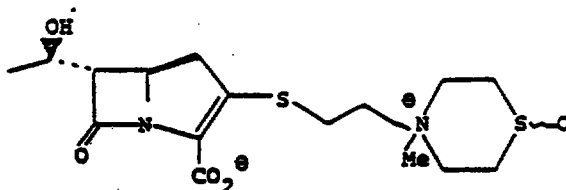


To a solution of (5R,6S) paranitrobenzyl 3-[2-(1,4-dimethyl-1-piperazinium)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate diphenylphosphate (0.47 g, 0.623 mmol) in wet tetrahydrofuran (25 mL) was added diethyl ether (25 mL), potassium phosphate monobasic-sodium hydroxide buffer (13 mL, pH 7.22) and 10% palladium on charcoal (0.47 g). The resulting mixture was hydrogenated at 23°C under 40 psi for 1 h. The two layers were separated and the organic layer was extracted with water (2 x 7 mL). The aqueous layers were combined, filtered through a Celite pad, washed with diethyl ether (2 x 15 mL) and chromatographed on PrepPak-500/C₁₈ (Waters Associates) column (2.5 x 9.5 cm) with water as eluting solvent to give, 0.097 g (43%) after lyophilization; ir (KBr) $\nu_{\max}$: 3000-3700 (OH), 1750 (C=O of β -lactam), 1585 (carboxylate) cm^{-1} , ^1Hmr (D_2O) δ : 1.24 (d, $J=6.4$ Hz, 3H, CH_3CHOH), 2.33 (s, 3H, $\text{N}-\text{CH}_3$), 3.15 (s, $\text{N}-\text{CH}_3$), 4.0-4.5 (m, H-5, CH_3CHOH), uv (H_2O) $\lambda_{\max}$: 296 (e9476), $[\alpha]_{\text{D}}^{23}$ 61.1° (C 0.26, H_2O), $t_{1/2}=12.4$ h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C).

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Example 19

Preparation of (5R,6S)-3-[2-(N-methyl-thiomorpholinium-oxide)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)-hept-2-ene-2-carboxylate



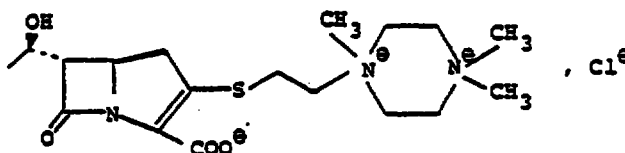
To a cold (-10°C) solution of (5R,6S)-3-[2-(N-methyl-thiomorpholinium)ethylthio]-6-[1-(R)-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)-hept-2-ene-2-carboxylate (608 mg, 1.65 mmol) in a 1:1 mixture of acetonitrile-water (9 mL) was added m-chloroperoxybenzoic acid (334.8 mg, 1.65 mmol) in small portion over a 1 hour period. The mixture was then diluted

with water (15 mL) and washed with diethyl ether (3 x 15 mL). The aqueous phase was pumped under vacuum and passed through a reversed phase silica gel column (H_2O) to give a solid which consisted of a mixture of compounds. This mixture was separated by reversed phase HPLC and afforded fraction A 52.4 mg (yield 12%) and fraction B 23.6 mg (yield 6%) as diastereomers of the title compound; Fraction A: ir (nujol) ν_{max} : 1750 (s, β -lactam C=O) and 1580 cm^{-1} (s, C=O); $^1H_{NMR}$ (D_2O) δ : 4.26-2.91 (20 H, m, H-4, H-5, H-6, H-1', CH_2S , CH_2S-O , CH_3-N^+ and CH_2N^+) and 1.24 ppm (3H, d, $J=6.4$ Hz, CH_3); uv (H_2O , c 0.06) λ_{max} : 302 ($\epsilon 10425$); T 1/2:12 h (0.065 M, pH 7.4 phosphate buffer, 37°C). Fraction B: ir (nujol) ν_{max} : 1750 (s, β -lactam C=O) and 1585 cm^{-1} (s, C=O); $^1H_{NMR}$ (D_2O) δ : 3.86-2.90 (17 H, m, H-4, H-5, H-6, H-1', CH_2S , CH_2S-O , CH_2N^+), 3.25 (3H, s, CH_3N^+) and 1.24 ppm (3H, d, $J=6.4$ Hz, CH_3); uv (H_2O , c 0.05) λ_{max} : 2.99 ($\epsilon 6517$); T 1/2:10.75 h (0.065 M, pH 7.4 buffer solution, 37°C).

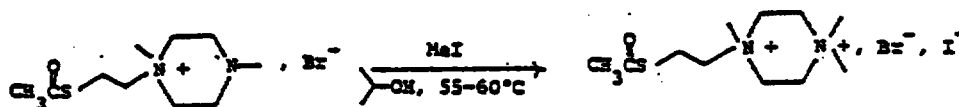
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Example 20

Preparation of (5R,6S)-3-[2-(1,4,4-Trimethyl-1-piperazinium)-ethylthio]-6-[1R-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate chloride

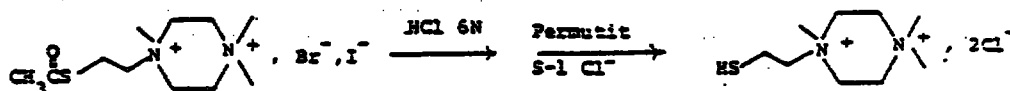


A. 1-(2-acetylthioethyl)-1,4,4-trimethylpiperazinium bromide/iodide



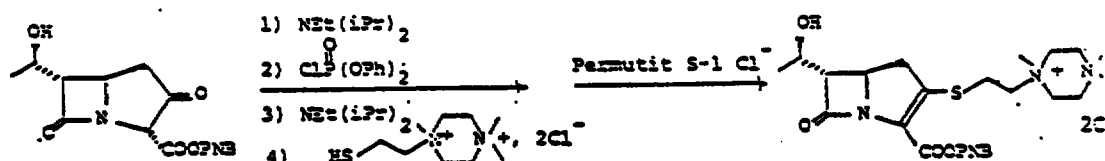
A suspension of 1-(2-acetylthioethyl)-1,4-dimethylpiperazinium bromide (1.48 g, 5.0 mmol) in isopropyl alcohol (10 mL) was treated with methyl iodide (0.373 mL, 6.0 mmol) and heated at 55-60°C for 30 h. The solvents were evaporated under reduced pressure; the residue was triturated in hexane and the solid was filtered, 1.85 g. The solid was dissolved in hot water (8 mL) and the solution was diluted with acetone until turbidity (70-80 mL). Two successive crystallizations gave 1.5 g, mp 220-5°C dec., 68% of the title compound; ir (KBr) $\nu_{\max}$: 1692 cm^{-1} (C=O); ^1Hmr (D_2O) δ : 2.40 (s, 3H, CH_3COO), 3.37 (s, N- CH_3), 3.39 (s, N- CH_3), 3.99 (s); uv (H_2O) $\lambda_{\max}$: 226 (e13144). Anal. calcd for $\text{C}_{11}\text{H}_{24}\text{N}_2\text{OSBrI}$: C 30.08, H 5.51, N 6.38; found: C 30.48, H 5.53, N 6.86.

B. 1-(2-mercaptoethyl)-1,4,4-trimethylpiperazinium bischloride



A mixture of 1-(2-acethylthioethyl)-1,4,4-trimethylpiperazinium bromideiodide (1.84 g, 4.19 mmol) and 6N hydrochloric acid (15 mL) was heated at 57°C under a nitrogen atmosphere for 2.5 h. The solution was concentrated under reduced pressure to dryness. The solid was suspended in water (10 mL) and the well-stirred suspension was treated with permutit S-1 Cl⁻ until a solution was obtained. The solution was poured on a column (1.2 x 60 cm) of permutit S-1 Cl⁻. The column was eluted with water (1.5 mL/min). The appropriate fractions were combined and lyophilized to give a white powder, 0.93 g, mp 190-191°C, 85%; ir (nujol) ν_{max} : 2460 (SH); ^1Hmr (D₂O) δ : 3.4 (s, N-CH₃), 3.45 (s, N-CH₃), 4.07 (s). . Anal. calcd for C₉H₂₂N₂SCl₂·0.75 H₂O: C 39.34, H 8.62, N 10.20, S 11.67; found: C 39.48, H 8.39, N 10.55, S 11.15.

C. (5R,6S) paranitrobenzyl 3-[2-(1,4,4-trimethyl-1-piperazinium)-ethylthio]-6-(1R-hydroxyethyl)-7-oxo-1-azabicyclo(3.2.0)heptane-2-carboxylate bischloride

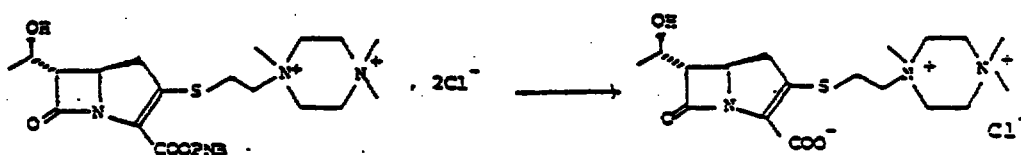


To a cold (5°C) solution of (5R,6S) paranitrobenzyl 6-[1R-hydroxyethyl]-3,7-dioxo-1-azabicyclo(3.2.0)heptane-2R-carboxylate (0.94 g, 2.7 mmol) in acetonitrile (3 mL) kept under a nitrogen atmosphere was added diisopropylethylamine (0.557 mL, 3.2 mmol) and diphenyl chlorophosphate (0.663 mL, 3.2 mmol). The reaction mixture was stirred at 5°C for 30 min and treated with diisopropylethylamine (0.599 mL, 3.44 mmol) and an aqueous solution (4 mL) of 1-(2-mercaptoethyl)-1,4,4-trimethylpiperazinium bischloride (0.90 g, 3.44 mmol). After 1.25 h, diisopropylethylamine (0.1 mL, 0.57 mmol) was added and the stirring was continued for 2 h. A part of the acetonitrile was eliminated under reduced pressure and the resulting red mixture was chromatographed on PrepPak -500/C₁₈ (Water Associates) column with 25-75% acetonitrile in water as eluting solvent to give a yellowish powder (1.4 g) after lyophilization. The powder was solubilized in water and the solution was passed on a column (1.2 x 58 cm) of permutit S-1 Cl⁻ using water as an eluting solvent. Lyophilization of the appropriate fractions gave 1.17 g of a powder that was repurified on a column of PrepPak -500/C₁₈. Lyophilization of the appropriate fractions gave a yellowish powder, 0.80 g (53%);

ir (KBr) ν_{max} : 3400 (br, OH), 1770 (C=O of the β -lactam), 1690 (C=O of PNB ester), 1605 (aromatic), 1515 (NO₂), 1345 (NO₂) cm⁻¹;

¹Hmr (D₂O) δ : 1.26 (d, J=6.3 Hz, 3H, CH₃CHOH), 3.39 (s, NCH₃), 4.00 (s), 5.37 (br, s, CH₂ of PNB), 7.60 (d, J=8.6 Hz, 2H, Ho of -PNB), 8.20 (d, J=9.7 Hz, 2H, Hm of -PNB); uv (H₂O) λ_{max} : 276 (ϵ 12094), 306 (ϵ 10752). Anal. calcd. for C₂₅H₃₆N₄O₆SCl₂·3H₂O: C 46.51, H 6.56, N 8.68, S 4.97, Cl 10.98; found: C 46.31, H 6.18, N 8.57, S 5.36, Cl 11.37.

D. (5R,6S)-3-[2-(1,4,4-trimethyl-1-piperazinium)ethylthio]-6-
[1R-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-
carboxylate chloride

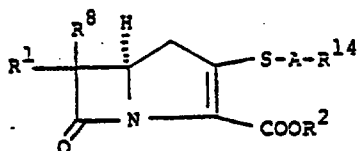


A mixture of (5R,6S) paranitrobenzyl 3-[2-(1,4,4-trimethyl-1-piperazinium)ethylthio]-6-[1R-hydroxyethyl]-7-oxo-1-azabicyclo(3.2.0)hept-2-ene-2-carboxylate bischloride (0.40 g, 0.68 mmol), phosphate buffer (30 mL, 0.05M, pH 7.0), tetrahydrofuran (10 mL), ether (30 mL) and 10% palladium on charcoal (0.40 g) was hydrogenated at 23°C under 35 psi for 1 h. The two phases were separated. The organic phase was extracted with water (10 mL). The aqueous phases were filtered on a Celite pad, washed with ether (10 mL), concentrated under vacuum to 10 mL and chromatographed on PrePak-500/C₁₈ column (2.2 x 11 cm) with water as eluting solvent to give 70 mg (25%) after lyophilization; ir (KBr) $\nu_{\max}$: 3400 (br, OH), 1755 (C=O of the β -lactam), 1585 (carboxylate) cm^{-1} ; ^1Hmr (D₂O) δ : 1.24 (3H, d, $J=6.3$ Hz, CH₃CHOH), 3.36 (s, NCH₃), 3.98 (s); uv (H₂O) $\lambda_{\max}$: 296 (ϵ 7987); $[\alpha]_{\text{D}}^{23}$ 35.9° (c, 0.30, H₂O), $T_{1/2}$ = 9.8 h (measured at a concentration of 10^{-4} M in phosphate buffer pH 7.4 at 36.8°C).

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C L A I M S

1. A process for the preparation of a carbapenem derivative of the formula



I

wherein R^8 is hydrogen and R^1 is selected from the group consisting of hydrogen; substituted and unsubstituted: alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl and cycloalkyl-alkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; heteroaryl, heterocaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms; wherein the substituent or substituents relative to the above-named radicals are selected from the group consisting of



* C L M S *

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C₁-C₆ alkyl optionally substituted by
amino, halo, hydroxy or carboxyl

halo

-OR³

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{OCNR}^3\text{R}^4 \end{array}$

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{CNR}^3\text{R}^4 \end{array}$

-NR³R⁴

$\begin{array}{c} \text{NR}^3 \\ \diagup \\ \text{C} \\ \diagdown \\ \text{NR}^3\text{R}^4 \end{array}$

-SO₂NR³R⁴

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{NHCNR}^3\text{R}^4 \end{array}$

$\begin{array}{c} \text{O} \\ \parallel \\ \text{R}^3\text{CNR}^4 \end{array}$

-CO₂R³

=O

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{OCR}^3 \end{array}$

-SR³

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{SR}^9 \end{array}$

$\begin{array}{c} \text{O} \\ \parallel \\ -\text{SR}^9 \end{array}$

=O

-CN

-N₃

-OSO₃R³

-OSO₂R³

-NR³SO₂R⁴

-NR³C-NR⁴
|
R³

-NR³CO₂R⁴

-NO₂

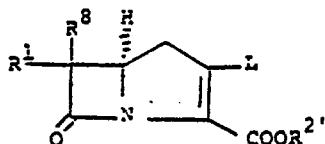


* C L H S *

wherein relative to the above-named substituents, the groups R^3 and R^4 are independently selected from hydrogen; alkyl, alkenyl and alkynyl, having from 1-10 carbon atoms; cycloalkyl, cycloalkylalkyl and alkylcycloalkyl, having 3-6 carbon atoms in the cycloalkyl ring and 1-6 carbon atoms in the alkyl moieties; phenyl; aralkyl, aralkenyl and aralkynyl wherein the aryl moiety is phenyl and the aliphatic portion has 1-6 carbon atoms; and heteroaryl, heteroaralkyl, heterocyclyl and heterocyclylalkyl wherein the hetero atom or atoms in the above-named heterocyclic moieties are selected from the group consisting of 1-4 oxygen, nitrogen or sulfur atoms and the alkyl moieties associated with said heterocyclic moieties have 1-6 carbon atoms, or R^3 and R^4 taken together with the nitrogen to which at least one is attached may form a 5- or 6- membered nitrogen-containing heterocyclic ring; R^9 is as defined for R^3 except that it may not be hydrogen; or wherein R^1 and R^8 taken together represent C_2-C_{10} alkylidene or C_2-C_{10} alkylidene substituted by hydroxy; A is cyclopentylene, cyclohexylene or C_2-C_6 alkylene optionally substituted by one or more C_1-C_4 alkyl groups; R^2 is hydrogen, an anionic charge or a

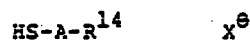
readily removable carboxyl protecting group, providing that when R^2 is hydrogen or a protecting group, there is also present a counter anion; and R^{14} is a quaternized nitrogen-containing aromatic or non-aromatic heterocycle attached to A through a ring nitrogen, thereby forming a quaternary ammonium group, or a pharmaceutically acceptable salt, thereof, which process comprises reacting an intermediate of the formula





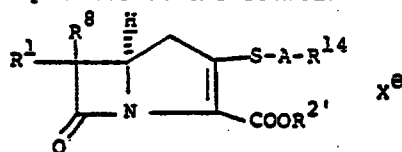
IV

wherein R^1 and R^8 are as defined above, $R^{2'}$ is a readily removable carboxyl protecting group and L is a leaving group, with a thiol compound of the formula



VII

wherein A and R^{14} are as defined above and $\text{X}^{\ominus}$ is a counter anion, in an inert solvent and in the presence of base, to produce a carbapenem product of the formula



I'

wherein R^1 , R^8 , $R^{2'}$, A, R^{14} and $\text{X}^{\ominus}$ are as defined above and, if desired, removing the carboxyl protecting group $R^{2'}$ to give the corresponding de-blocked compound of formula I, or a pharmaceutically acceptable salt thereof.



2. The process according to Claim 1 wherein the base is a non-nucleophilic tertiary amine or a tri(C₁-C₄)alkylamine.

3. The process according to Claim 2 wherein the base is a non-nucleophilic tertiary amine.

4. The process according to Claim 1, 2 or 3 wherein the reaction is carried out at a temperature of -15°C up to room temperature.

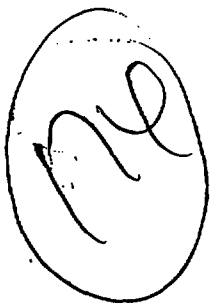
5. The process according to Claim 4 wherein the reaction is carried out at a temperature in the range of from -15°C to +15°C.

6. The process according to Claim 1, 2, 3, 4 or 5 wherein the inert solvent is selected from the group consisting of acetonitrile, a mixture of acetonitrile, dimethylformamide, tetrahydrofuran, a mixture of tetrahydrofuran and water, a mixture of acetonitrile and water and acetone.

7. A process for the preparation of a quaternary amine thiol compound of the formula

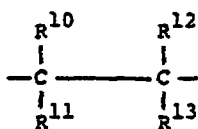


* C L M S *



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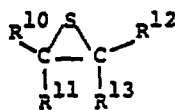
wherein A is cyclopentylene, cyclohexylene or



in which R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or $\text{C}_1\text{-C}_4$ alkyl, $\text{X}^{\ominus}$ is a counter anion and R^{14} represents a substituted or unsubstituted mono, bi- or polycyclic aromatic or non-aromatic heterocyclic radical containing at least one nitrogen in the ring and attached to A through a ring nitrogen, thereby forming a quaternary ammonium group, which process comprises reacting a sulfide of the formula



or



(12)

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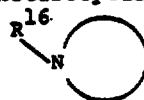
wherein R^{10} , R^{11} , R^{12} and R^{13} are each independently hydrogen or C_1-C_4 alkyl with a strong acid and either (a) a heteroaromatic amine of the formula



wherein



represents a substituted or unsubstituted mono-, bi- or polycyclic aromatic heterocyclic radical containing at least one nitrogen in the ring capable of being quaternized and bonded to a carbon atom of substituent A or (b) a heterocyclic amine of the formula



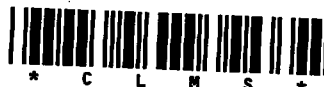
wherein



represents a substituted or unsubstituted mono-, bi- or polycyclic non-aromatic heterocyclic radical containing at least one nitrogen in the ring capable of being quaternized by substituent R^{16} and bonded to a carbon atom of substituent A and R^{16} represents either (a) an optionally substituted aliphatic, cycloaliphatic, cycloaliphatic-aliphatic, aryl, araliphatic, heteroaryl, heteroaraliphatic, heterocyclyl or heterocyclyl-aliphatic radical or (b) a divalent phenylene or C_1-C_4 alkylene group joined to the



ring so as to form a bridged polycyclic group.



8. The process according to Claim 7 wherein the reaction is carried out at a temperature in the range of from -20°C to 100°C.

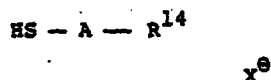
9. The process according to Claim 7 or Claim 8 wherein the strong acid is hydrochloric, hydrobromic, methanesulfonic, p-toluenesulfonic or trifluoromethanesulfonic.

10. The process according to Claim 7, 8 or 9 wherein the reaction is carried out in the presence of a non-polar organic solvent.

11. The process according to claim 10, wherein the non-polar organic solvent is methylene chloride, benzene, xylene or toluene.

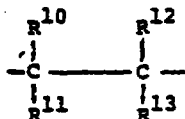
12. The process according to Claim 7, 8, 9, 10 or 11 wherein when the amine and sulfide reagents are liquids or when the amine reagent is a solid, the sulfide reagent is liquid and the solid amine is soluble in the liquid sulfide reagent, the reaction is carried out without additional solvent.

13. A quaternary amine thiol compound of the formula

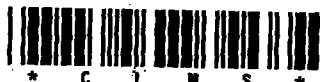


VII

wherein A is cyclopentylene, cyclohexylene or



in which R¹⁰, R¹¹, R¹² and R¹³ are each independently hydrogen or C₁-C₄ alkyl, X[⊖] is a counter anion and R¹⁴ represents a substituted or unsubstituted mono, bi- or polycyclic aromatic or non-aromatic heterocyclic radical containing at least one nitrogen in the ring and attached to A through a ring nitrogen, thereby forming a quaternary ammonium group.



14. A process according to claim 1, substantially as described in any of the applicable Examples in the foregoing description.

15. A process according to claim 7, substantially as described in any of the applicable Examples in the foregoing description.

16. A process according to claim 1, modified by the inclusion of an additional step, in which process R^{14} comprises initially an N-methyl-thiomorpholinium radical, and the initially obtained derivative of formula I is converted into the corresponding compound answering to formula I wherein R^{14} comprises an N-methyl-thiomorpholinium oxide radical, by oxidation with m-chloroperbenzoic acid, the procedure being that of the foregoing Example 19 or an analogous procedure equivalent thereto.

17. A carbapenem derivative prepared by a process according to any of claims 1 to 6 or claim 14.

18. A quaternary ammonium thiol compound prepared by a process according to any of claims 7 to 12 or claim 15.

19. A sulfoxide compound prepared by the process of claim 16.

20. A pharmaceutical composition comprising a carbapenem derivative as claimed in claim 17 and a pharmaceutically acceptable carrier or diluent.

