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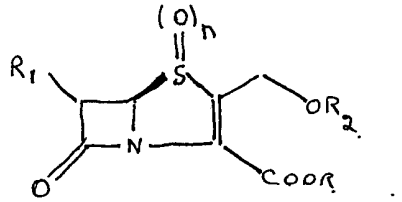
(54) Optically active penems

(57) Preparations of certain 2-penem-3-carboxylic acids, esters and salts of the formula



are described. Reference should be made to the Specification for a full definition of the variables R, R_1, R_2 and n.

The preparations are stereospecific yielding the 5R derivatives, and the introduction of the group R_2 is at a very late stage in the synthesis, enabling a great number of compounds to be prepared.

Particular preparations described lead to sodium salts of compounds in which n=0, R=H, R_1=$CH_3CH(OH)$ and R_2=H, $COCH_3$ or $CONH_2$.

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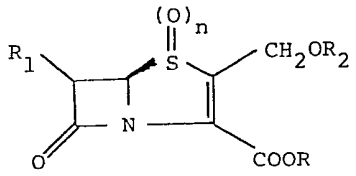
SPECIFICATION

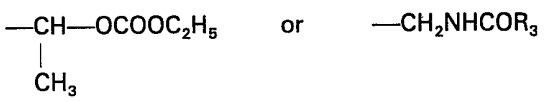
Optically active penems

The invention relates to processes for the preparation of compounds of the general formula I and their pharmaceutically acceptable salts:



I

in which n is 0 or 1, R represents a hydrogen atom, a lower alkyl, 2,2,2-trichloroethyl, acetonyl, allyl, benzyl, *p*-nitrobenzyl, *p*-methoxybenzyl, phenyl, *o*-nitrophenyl, benzhydryl or 1-phenoxyethyl group or a residue known to be hydrolysed "in vivo" and having favourable pharmacokinetic properties such as an acetoxymethyl, pivaloyloxymethyl or phthalidyl group or a group of the formula

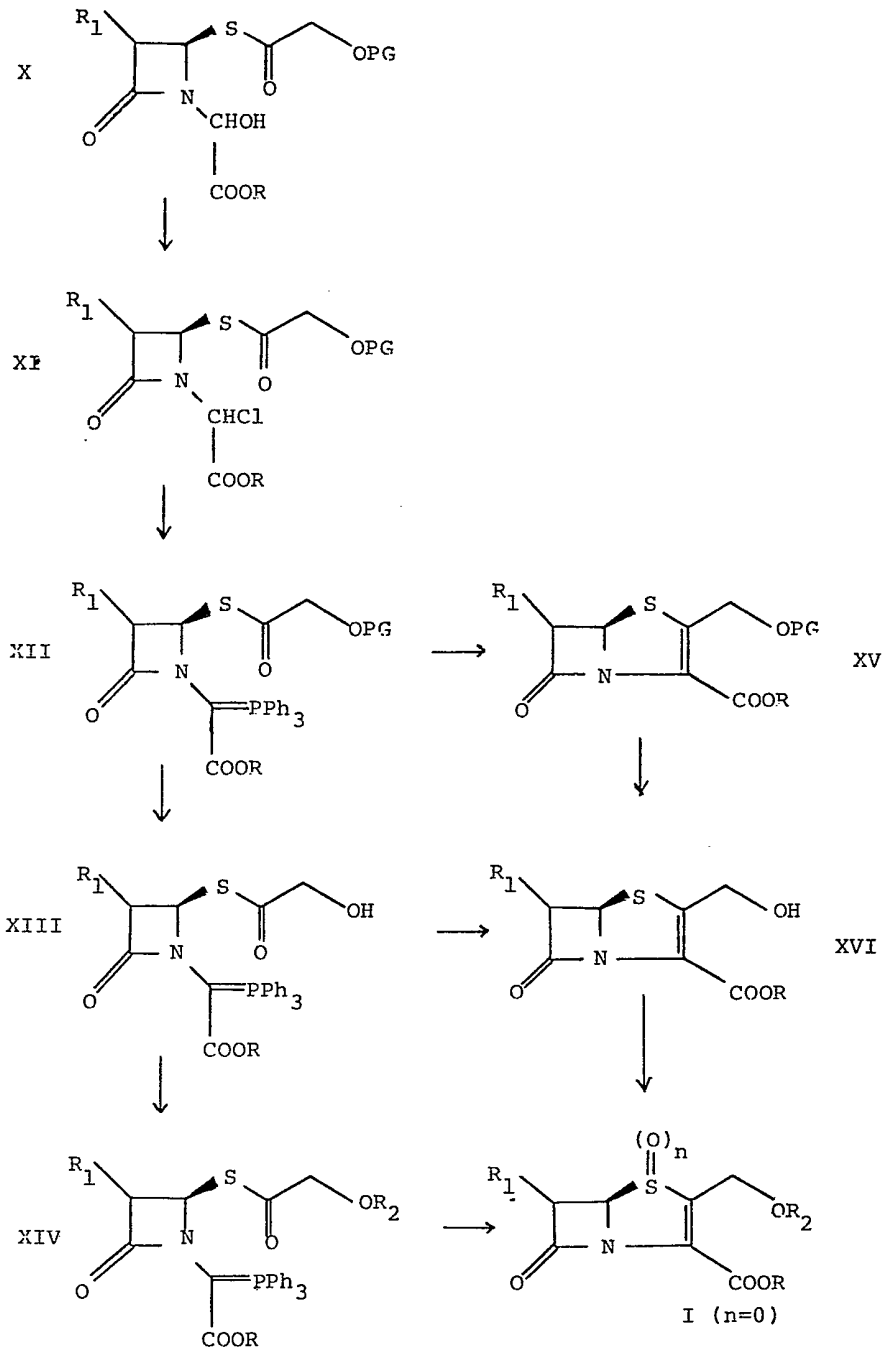


in which R_3 represents an alkyl group having from 1 to 5 carbon atoms or an aryl group, preferably a phenyl or *p*-nitrophenyl group, R_1 represents a hydrogen atom, a lower alkyl, lower alkoxy, cycloalkyl, or hydroxyalkyl group, preferably a hydroxy substituted lower alkyl group such as 1-hydroxyethyl, the alcoholic function of the hydroxyalkyl group being free or protected, the protecting group (if present) preferably being a *p*-nitrobenzyloxycarbonyl, dimethyl-*t*-butylsilyl, diphenyl-*t*-butylsilyl, 2,2,2-trichloroethoxycarbonyl, trimethylsilyl, benzyl, *p*-bromophenacyl, triphenylmethyl or pyranyl group, and R_2 represents a hydrogen atom, an alkyl group having from 1 to 5 carbon atoms, a carbamoyl group, a carbamoyl group substituted by one or two alkyl groups the or each of which has from 1 to 4 carbon atoms, an alkanoyl group having from 2 to 6 carbon atoms optionally substituted by a further alkanoyl group also having from 2 to 6 carbon atoms, a cycloalkylcarbonyl group having from 4 to 7 carbon atoms or an arylcarbonyl or substituted arylcarbonyl group.

The 6-substituent may have either α or β orientation, α-orientation being preferred. When R_1 represents a lower hydroxyalkyl group, the carbon atom bearing the hydroxy group may have either the R or S configuration, but preferably has the R configuration. The carbon atom in position 5 has only the R configuration.

The preferred cycloalkyl groups which R_1 may represent are monocycloalkyl groups having from 4 to 7 carbon atoms, and especially cyclopentyl and cyclohexyl groups. The preferred alkyl groups which R_2 may represent are methyl and ethyl groups. The preferred acrylcarbonyl group which R_2 may represent is benzoyl and, if it is substituted, the preferred substituents are halogen atoms or hydroxy, amino, cyano, nitro, lower alkyl or lower alkoxy groups. The preferred alkanoyl group which R_2 may represent is an acetyl group. If this is substituted, it is preferably by a further acetyl group (acetoacetyl).

The compounds I are prepared according to the invention in accordance with the following reaction scheme, in which Ph represents a phenyl group and PG represents a protecting group, preferably a *p*-nitrobenzyloxycarbonyl, dimethyl-*t*-butyl-silyl, diphenyl-*t*-butyl-silyl, 2,2,2-trichloroethoxycarbonyl, trimethylsilyl, benzyl, *p*-bromophenacyl, triphenylmethyl and pyranyl group.



The invention offers three routes from compound X to compound I. Each route passes through a common sequence of reactions for the conversion of compound X to compound XII.

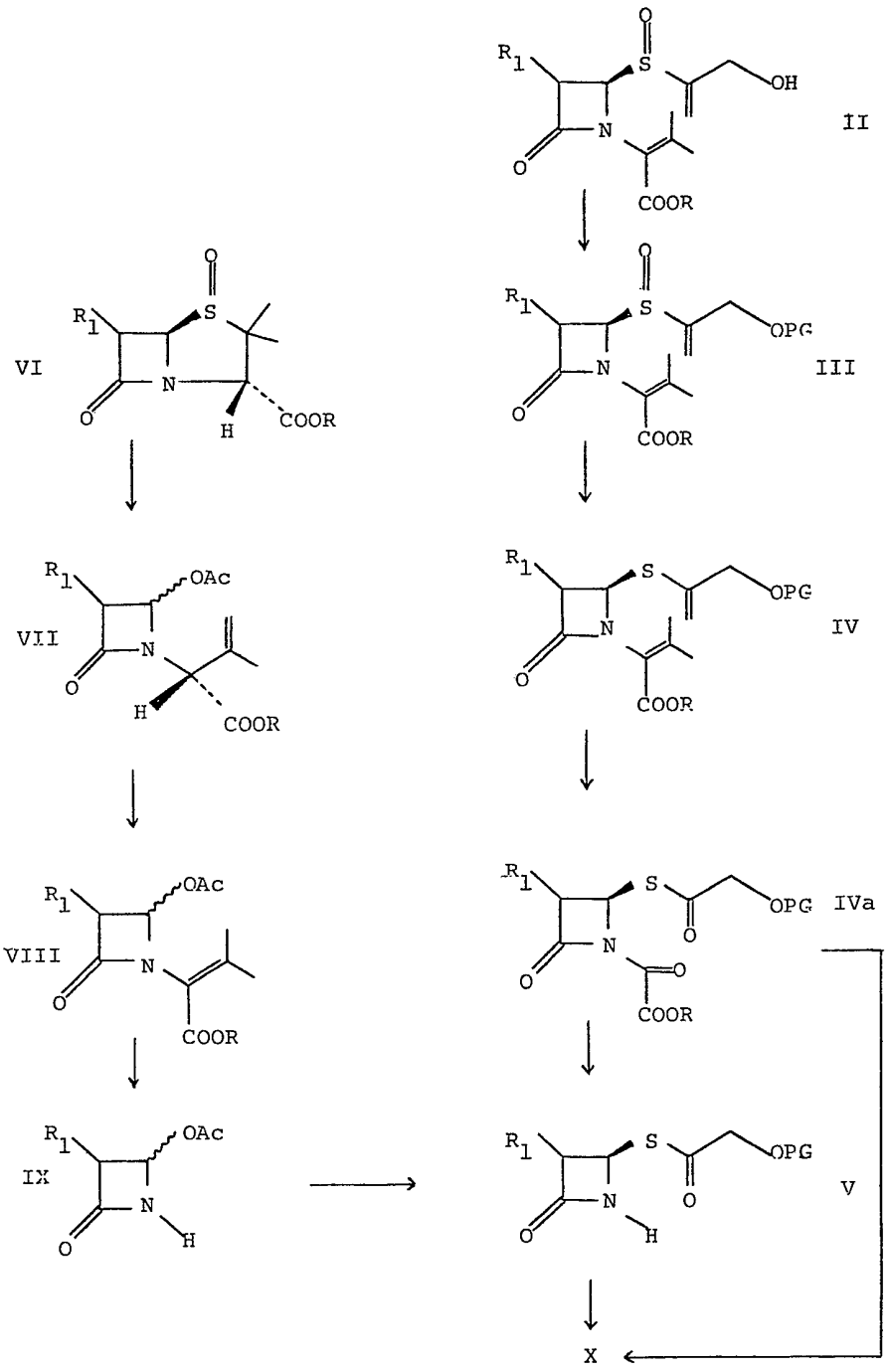
The common sequence of reactions for the conversion of compound X to compound XII comprises chlorination of compound X and reaction of the resultant compound XI with triphenylphosphine. The chlorination may suitably be effected with thionyl chloride at from −20 to 0°C in an inert solvent such as tetrahydrofuran. The reaction with triphenylphosphine may be conducted at from 30 to 60°C, preferably 40°C in an organic solvent such as tetrahydrofuran in the presence of a base such as pyridine or lutidine. Alternatively, it may be conducted in the presence of silica gel at ambient temperature for a few hours.

Each of the three routes from compound XI to compound I comprises three steps: cyclisation, removal of the protecting group PG and introduction of the desired group R_2. Naturally the introduction of the group R_2 follows the removal of the protecting group, and the three routes differ only in whether the cyclisation is carried out as the first, second or third of the three steps. The cyclisation is effected by heating under an inert atmosphere, such as a nitrogen atmosphere, at from 80 to 150°C in an inert solvent such as benzene, toluene or xylene. The conditions for the removal of the protecting group PG depend upon the nature of the protecting group PG. The group R_2 as above defined may be introduced

by reaction with an anhydride or acyl chloride of the general formula $(R_2CO)_2O$ or R_2COCl in which R_2 represents an alkyl group having from 1 to 4 carbon atoms or a cycloalkyl or aryl group; or by reaction with an isocyanate such as trichloroacetyl isocyanate or chlorosulphonyl isocyanate (leading to the compounds I in which R_2 represents a carbamoyl or substituted carbamoyl group); or with a diazoalkane having from 1 to 5 carbon atoms.

If the desired compound I is one in which R_2 represents a hydrogen atom, then the cyclisation is carried out as the first or second step of the three routes from compound XII, and the step of introduction of the group R_2 is omitted. This is because compound XVI is compound I (R_2=H, n=o). If the desired compound I is one in which n is 1, then the sulphur atom of the compound I obtained by one of the methods described may be oxidized in a conventional manner.

The invention also offers three routes to compound X, illustrated by the following reaction scheme in which Ac represents an acetyl group and PG represents a protecting group.



The first route to compound X comprises the protection of the free hydroxy group of compound II (which may be prepared as described in British Patent Specification No. 2043639) with a protecting group, the reduction of the sulphoxide function in the resultant compound III, ozonolysis of both the

carbon-carbon double bonds in the resultant compound IV, methanolysis of the N-substituent in the resultant compound IVa and condensation of the resultant compound V with a glyoxylic ester of the general formula CHOCOOR wherein R is as above defined.

The reduction may be carried out using phosphorus tribromide at a temperature of from −40°C to −20°C in a solvent such as anhydrous dimethylformamide. The ozonolysis may be effected at a temperature of from −80°C to −50°C in a solvent such as diethyl ether, methanol, or preferably, dichloromethane. The methanolysis is preferably conducted in the presence of silica gel or of a catalytic amount of a strong base such as sodium methoxide. The condensation of compound V with the glyoxylic ester is suitably carried out at elevated temperature, from 70 to 100°C in an organic solvent such as benzene or toluene.

The second route to compound X follows the first route from compound II to compound IVa, but then bypasses compound V with a direct reduction of the oxamide function to a carbinolamide function. This can be achieved with zinc and acetic acid.

The third and most preferred route to compound X commences from compound VI, which may be prepared as described in British Patent Specification No. 2043639. This compound may be converted to compound VII by treatment with acetic acid and trimethylphosphite in an inert solvent, such as toluene, under reflux. This reaction has been described by A. Suarato et al (Tet. Lett., 1978, *42* 4059—62). Isomerization of the isopropenyl substituent of compound VII using a base, preferably trimethylamine, in an inert organic solvent such as dichloromethane at from 0 to 20°C, leads to the compound VIII, and this may be converted to compound IX by ozonolysis and methanolysis steps analogous to those described for the conversion of compound IV to compound V. An alternative method of converting compound VIII to compound IX is the procedure described by E. G. Brain et al (J.C.S. Chem. Comm., 1972, 229). Compound IX is condensed with an O-protected hydroxyacetothiol of the general formula $HSCOCH_2OPG$, wherein PG is as above defined, to give compound V. This condensation is preferably conducted in an acetone: water mixture under basic conditions at from 0 to 20°C. Conversion of compound V to compound X is as described for the first route.

Two features of the invention deserve further comment. The carbon atom in position 5, the R configuration of which is "the sole essential stereochemical requirement for antibiotic activity" (H. R. Pfaendler, J. Gosteli and R. B. Woodward, J. A. Chem. Soc., *101*, 1979, 6306), retains its configuration from compound II right through to compound I. The carbon-sulphur bond is not disturbed in any step. In the case of preparation from compound VI, the reaction of compound IX with the O-protected hydroxyacetothiol proceeds stereo-specifically giving only the 3S,4R azetidinone V. Secondly, the group R_2 is introduced at a very late stage in the synthesis, enabling a great number of compounds I to be prepared.

The compounds of the general formula I possess a wide spectrum of antibacterial activity and β-lactamase inhibiting activity and are described and claimed in British Patent Specification No. 2043639.

The invention is illustrated by the following Examples, in which the abbreviation PNB is used for *p*-nitrobenzyl.

Example 1
4-Acetoxy-3R-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)azetidin-2-one
IX: R_1=$CH_3CH(OCOOPNB)$

A solution of 9.1 g (0.02 mole) of methyl 6α-(1R-*p*-nitrobenzyloxycarbonyloxy-ethyl) penicillanate 1-oxide [VI: R=CH_3, R_1=$CH_3CH(OCOOPNB)$] in 100 ml of toluene was treated with 4 ml (0.07 mole) of acetic acid and 13.4 ml of trimethylphosphite. The resulting mixture was refluxed for 3 hours, cooled to room temperature washed with saturated sodium bicarbonate solution (3×50 ml) and with water (50 ml), dried over anhydrous sodium sulphate and evaporated *in vacuo*. The oily residue obtained was purified by column chromatography (cyclohexane:ethyl acetate) to yield 4-acetoxy-3R-(1R-*p*-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-methoxycarbonyl-2-methyl-2-propenyl)-azetidin-2-one [VII: R=CH_3, R_1=$CH_3CH(OCOOPNB)$] as a light yellow oil (7.9 g, 85% yield). The isopropenyl moiety of this compound was isomerized by treatment with triethylamine in dichloromethane at 5°C to yield 4-acetoxy-3R-(1R-*p*-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-methoxycarbonyl-2-methyl-1-propenyl)-azetidin-2-one [VIII: R=CH_3, R_1=$CH_3CH(OCOOPNB)$] in 92% yield. The title compound was prepared as a mixture of cis and trans acetate starting from this material by the following synthetic methods.

Method A

To a solution of 2.46 g (5.29 mmol) of the compound VIII prepared as described immediately above in 200 ml of acetone was added a solution of 4.51 g (21.08 mmol) of sodium metaperiodate in 140 ml of water. 80 ml of 0.1M pH7 phosphate buffer was added, maintaining the temperature at from 10 to 15°C. 65 mg (0.41 mmol) of potassium permanganate was added. The resulting mixture was stirred at room temperature for five hours. The precipitate was filtered off. The filtrate was concentrated to about 200 ml. The aqueous phase was extracted with ethyl acetate. The organic layer was collected, washed with brine, dried over anhydrous sodium sulphate and concentrated in vacuo.

The residue was chromatographed over silica gel eluting with cyclohexane:ethyl acetate mixtures to give the title compound as a foam (1.48 g: 79%).

Method B

To a stirred solution of 7.9 g (17 mmol) of the compound VIII, prepared as previously described in this Example, in 180 ml of acetone, 25 ml of water and 5 ml of 0.1M pH7 phosphate buffer was added portionwise 5.37 g (34 mmol) of potassium permanganate maintaining the temperature at from 15 to 20°C. The mixture was stirred under a nitrogen atmosphere at room temperature for 40 minutes. The organic solvent was eliminated by evaporation in vacuo. The aqueous phase was covered with ethyl acetate. The resulting mixture was stirred and treated with cold aqueous sodium thiosulphate to eliminate the excess potassium permanganate. The organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated under reduced pressure. The resulting residue was purified by column chromatography to give the title compound (4.96 g, 83%).

Method C

The compound VIII, prepared as previously described in this Example, was transformed into the title compound by ozonolysis and subsequent methanolysis (75% overall yield).

IR (neat): 1770—1740 cm^{-1}

PMR ($CDCl_3$) δ: 1.5 and 1.53 (3H, d, J=7Hz); 1.98 and 2.1 (2H, s); 5.3 (1H, m); 5.88 and 5.95 (1H, d, J=1.5 and 4.0Hz); 6.8 (1H, bs); 7.57 (2H, d, J=8Hz); 8.25 (2H, d, J=8Hz).

Example 2

4R-(*t*-butyldiphenylsilyloxyacetylthio)-3S-(1R-*p*-nitrobenzyloxycarbonyloxy-ethyl)-azetidin-2-one V: R_1=CH_3CH(OCOOPNB), PG=*t*-butyldiphenylsilyl

4.2 g of *t*-butyldiphenylsilyloxyacetothiol was dissolved in a solution of 0.56 g of sodium hydroxide in 60 ml of water. After 10 minutes 4.24 g of 4-acetoxy-3S-(1R-*p*-nitrobenzyloxycarbonyloxy-ethyl)-azetidin-2-one, dissolved in dichloromethane, was added. The reaction mixture was vigorously stirred for 1 hour. 70 ml of a dilute solution of citric acid was then added and the organic phase was separated off. The aqueous phase was further extracted with ethyl acetate (3×50 ml). The combined organic extracts were dried over anhydrous sodium sulphate, evaporated and chromatographed on silica gel eluting with cyclohexane: ethyl acetate to obtain the title compound (4.42 g) as a white foam.

IR (neat): 1770—1740, 1690 cm^{-1}.

PMR ($CDCl_3$), δ: 1.13 (9H, l); 1.48 (3H, d, J=7Hz); 3.48 (1, dd, J=2, 6.5Hz); 4.25 (2H, s); 5.2 (1H, m); 5.25 (2H, s); 5.31 (1H, d, J=2Hz); 6.4 (1H, bs); 7.5—7.7 (12H, m); 8.22 (2H, d, J=8Hz).

Example 3

4R-(t-butyldiphenylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-hydroxy-methyl)-azetidin-2-one
X: R=PNB, R_1=CH_3CH(OCOOPNB), PG=t-butyldiphenylsilyl

A solution of 3.11 g (5 mmole) of the compound prepared in Example 2 and 3.20 g (12. 5 mmol) of *p*-nitrobenzyl glyoxylate in 100 ml of benzene was refluxed to remove water by azeotropic distillation until near dryness (5 ml). After refluxing for 2 hours, the reaction mixture was chromatographed on silica gel (ethyl acetate: cyclohexane) giving an epimeric mixture of the title carbinolamide.

Example 4

4R-(t-butyldiphenylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-chloro-methyl)-azetidin-2-one
XI: R=PNB, R_1=CH_3CH(OCOOPNB), PG=*t*-butyldiphenylsilyl

A stirred solution of 3.5 g (4.2 mmol) of the compound prepared in Example 3 in dry tetrahydrofuran at from —5 to 0°C was treated with 0.48 ml (6 mmol) of pyridine and 0.43 ml (6 mmol) of thionyl chloride. After half an hour the reaction mixture was filtered and the filtrate was evaporated *in vacuo* to give an epimeric mixture of the title chloroester as a yellow gum.

Example 5

4R-(t-butyldiphenylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one
XII: R=PNB, R_1=CH_3CH(OCOOPNB), PG=t-butyldiphenylsilyl

A solution in tetrahydrofuran of the compound prepared in Example 4 was treated with 2.2 g (8.5 mmol) of triphenylphosphine and 20 g of silica gel. The mixture was evaporated in vacuo to dryness and the resulting powder was left for two hours at room temperature. The powder was then charged to the top of a column and the phosphorane was eluted with cyclohexane:ethyl acetate mixtures to give the title product (3.2 g) as a light yellow foam.

Example 6

4R-hydroxyacetylthio-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one

XIII: R=PNB, R_1=$CH_3CH(OCOOPNB)$

4 ml of trifluoroacetic acid was added to a stirred solution of 1.07 g (1 mmole) of the compound prepared in Example 5 in 50 ml of ethyl acetate. After fifteen minutes the solvent was removed, 50 ml of toluene was added and the solvent evaporated off again to give 1.3 g of the phosphonium salt. This was dissolved in 50 ml of tetrahydrofuran and treated with 4 equivalents of tetrabutylammonium fluoride. After one hour the mixture was evaporated, dissolved in 50 ml of ethyl acetate and washed with saturated sodium hydrogen carbonate solution (3×25 ml) and water (25 ml). The organic phase was separated off, dried over anhydrous sodium sulphate and evaporated in vacuo. The oily residue was chromatographed on silica gel (cyclohexane:ethyl acetate) to give 0.75 g of the title compound as a foam.

Example 7

p-Nitrobenzyl (5R)-2-hydroxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

XVI: R=PNB, R_1=$CH_3CH(OCOOPNB)$.

0.6 g of the compound prepared in Example 6 was dissolved in 200 ml of toluene and refluxed for 2 hours with a catalytic amount of hydroquinone. The solvent was then evaporated off in vacuo and the residue was purified by column chromatography on silica gel eluting with toluene:ethyl acetate mixtures to give the title product (0.42 g).

UV: λ_{max} (EtOH 95%) 260 nm (ε 19100); 319 nm (ε 8400)

IR: ν_{max} ($CHCl_3$) 3600—3200, 1790, 1745, 1710, 1605, 1580 cm^{-1}

PMR ($CDCl_3$), δ: 1.51 (3H, d, J=7Hz); 3.99 (1H, dd, J=2, 7.5 Hz): 4.69 (2H, bs); 5.15 (1H, m); 5.23 and 5.46 (2H, centres of ABq, J=14Hz); 5.26 (2H, s); 5.64 (1H, d, J=2Hz); 7.51 (2H, d, J=8Hz); 7.61 (2H, d, J=8Hz); 8.20 (4H, d, J=8Hz).

Example 8

p-Nitrobenzyl (5R)-2-(t-butyldiphenylsilyloxymethyl)-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

XV: R=PNB, R_1=$CH_3CH(OCOOPNB)$, PG=*t*-butyl diphenylsilyl

0.3 g of the compound prepared in Example 5 was dissolved in dry toluene and refluxed for 3 hours. The solvent was removed and the mixture was chromatographed on silica gel eluting with cyclohexane:ethyl acetate mixtures to afford the title compound (0.12 g).

Example 9

p-Nitrobenzyl (5R)-2-hydroxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

XVI: R=PNB, R_1=$CH_3CH(OCOOPNB)$

A solution of 0.1 g of the compound prepared in Example 8 in tetrahydrofuran was treated with 3 equivalents of tetrabutylammonium fluoride at −15°C under stirring. The reaction mixture was then poured into 50 ml of ethyl acetate and washed with water (3×30 ml). The dried organic phase was evaporated and chromatographed on silica gel eluting with ethyl acetate:cyclohexane mixtures to give the title compound (20 mg). This material proved to be identical to that obtained in Example 7.

Example 10

Sodium (5R)-2-hydroxymethyl-6S-(1R-hydroxyethyl)-2-penem-3-carboxylate

I: R=H, R_1=$CH_3CH(OH)$, R_2=H, n=O, sodium salt

40 mg of 5% palladium-on-charcoal was added to a solution of 54 mg of the compound prepared in Example 7 (or Example 9) in a mixture of ethyl acetate and water containing 6 mg of sodium bicarbonate. The mixture was hydrogenated at atmospheric pressure for one hour.

A further 20 mg of 5% palladium-on-charcoal was then added and left stirring for half an hour. The mixture was filtered and the aqueous phase was separated off and washed with ethyl acetate. After evaporating off the aqueous phase, the residue was purified on a reverse phase column eluting with water. The title compound (12 mg) was obtained as an amorphous solid.

U.V: λ_{max} (EtOH 95%) 263 nm, 304 nm.

Example 11

p-Nitrobenzyl (5R)-2-acetoxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

I: R=PBM, R_1=$CH_3CH(OCOOPNB)$, R_2=$COCH_3$, n=O

A solution of 350 mg (0.58 mmol) of the compound prepared in Example 7 (or Example 9) in 5 ml of dichloromethane was sequentially treated with 140 mg of pyridine and 80 mg of acetic anhydride, and then stirred at room temperature for six hours. The mixture was washed with sodium hydrogen

carbonate solution (3×5 ml) and water. The dried organic phase was evaporated off and the oily residue was chromatographed on silica gel eluting with cyclohexane:ethyl acetate mixtures to give the title product (200 mg).

UV: λ_{max} (EtOH 95%) 265, 321 nm

IR: ($CHCl_3$), ν_{max} 1795, 1750, 1715, 1610, 1585 cm^{-1}

PMR (CDCl), δ: 1.50 (3H, d, J=7Hz); 2.11 (3H, s), 4.01 (1H, dd, J=1.8, 7.5 Hz); 5.11 and 5.50 (2H, centres of ABq, J=14Hz); 5.15 (1H, m); 5.24 and 5.38 (2H, centres of ABq, J=12Hz); 5.28 (2H, s); 5.70 (1H, d, J=1.8Hz); 7.55 (2H, d, J=8Hz); 7.64 (2H, d, J=8Hz); 8.22 (4H, d, J=8Hz).

Example 12

4R-acetoxyacetylthio-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one

XIV: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$COCH_3$

A stirred solution of 418 mg (0.5 mmol) of the compound prepared in Example 6 in 5 ml of dichloromethane was sequentially treated with 162 mg of pyridine and 90 mg of acetic anhydride, and then stirred at room temperature for six hours. The solution was washed with sodium hydrogen carbonate solution (3×5 ml) and water. The dried organic phase was then evaporated *in vacuo* leaving a gum which was purified by column chromatography to give the title phosphorane (300 mg).

Example 13

p-Nitrobenzyl (5R)-2-acetoxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

I: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$COCH_3$, n=O

300 mg of the compound prepared in Example 12 were dissolved in toluene and the resulting solution was refluxed for 3 hours. The solvent was removed and the mixture chromatographed on silica gel eluting with ethyl acetate:cyclohexane to afford the title penem (140 mg). This product proved to be identical to that obtained in Example 11.

Example 14

Sodium (5R)-2-acetoxymethyl-6S-(1R-hydroxyethyl)-2-penem-3-carboxylate

I; R=H, R_1=$CH_3CH(OH)$, R_2=$COCH_3$, n=O, sodium salt

200 mg of 5% palladium-on-charcoal was added to a solution of 200 mg of the compound prepared in Example 11 (or Example 13) in a mixture of ethyl acetate and water containing 26 mg of sodium bicarbonate. The resulting mixture was hydrogenated at atmospheric pressure for 1 hour. After this time a further 100 mg of 5% palladium-on-charcoal was added until complete absorption of hydrogen. The resulting mixture was filtered and the aqueous phase was separated off and washed with ethyl acetate. The organic phase was discarded and the aqueous phase was evaporated in vacuo. The residue was purified on a reverse phase column eluting with water. Evaporation of the aqueous solution afforded the title product as an amorphous solid (60 mg).

UV: λ_{max} (EtOH 95%) 263 (ε 4630); 305 (ε 5500)

NMR: δ ppm (D_2O): 1.31 (3H, d, J=6.5Hz); 2.19 (3H, s); 3.92 (1H, dd, J=1.5, 7.0Hz); 4.21 (1H, m); 5.10 and 5.44 (2H, centres of ABq, J, 14Hz), 5.67 (1H, d, J=1.5 Hz)

$[\alpha]_D$=+116.9 (c=0.1, EtOH 95%)

Analysis:

$C_{11}H_{12}NO_6SNa \cdot H_2O$ requires	C 40.37; H 4.31; N 4.28
Found:	C 40.41; H 4.26; N 4.29

Example 15

4R-(1-t-butyldimethylsilyloxymethyl-vinylsulphinyl)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-methoxycarbonyl-2-methyl-1-propenyl)-azetidin-2-one

III: R=CH_3, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

1.9 g of 4R-(1-hydroxymethyl-vinylsulphinyl)-3S-(1R-*p*-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-methoxycarbonyl-2-methyl-1-propenyl)-azetidin-2-one were dissolved in 20 ml of dichloromethane. 0.7 ml of triethylamine, 640 mg of *t*-butyldimethylsilyl chloride and 20 mg of dimethylaminopyridine were added under a nitrogen atmosphere. After stirring overnight at room temperature, the solution was washed with water and with ammonium chloride solution, and the solvent was then evaporated off. The residue was chromatographed on silica gel using cyclohexane:ethyl acetate (1:1 by volume) as eluent to afford 0.83 g of the title compound.

PMR ($CDCl_3$), δ (ppm): 0.07 (s, 6H, $Si(CH_3)_2$); 0.88 (s, 9H, $SiC(CH_3)_3$); 1.41 (d, J=6.5Hz, 3H, CH_3CH); 2.14 (s, 3H, $=CH_3$); 2.30 (s, 3H, $=CH_3$); 3.75 (s, 3H, $COOCH_3$), 3.7—3.9 (m, 1H, H-3); 4.48 (bs, 2H, CH_2OSi); 5.25 (s, 2H, CH_2Ph); 5.1—5.2 (m, 2H, *H-4*, CH_3CH); 5.85 (bs, 1H, =H); 5.98 (bs, 1H, =H); and 7.4—8.4 (m, 4H, $PhNO_2$)

I.R. (CH_2Cl_2), ν (cm^{-1}): 1730 (C=O unsat, ester), 1755 (C=O of OCOO), 1780 (C=O, β-lactam)

Mass spectrum (FD): m/e 624

Example 16
4R-(1-t-butyldimethylsilyloxymethyl-vinylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-methoxycarbonyl-2-methyl-1-propenyl-azetidin-2-one
IV: R=CH_3, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

A solution of 0.8 g of the compound prepared in Example 15 in 30 ml of anhydrous dimethylformamide was cooled to −20°C and 0.25 ml of phosphorous tribromide were added. After 15 minutes, the mixture was diluted with ethyl acetate, washed twice with a saturated solution of sodium bicarbonate then with water, and dried over anhydrous sodium sulphate. Evaporation of the solvent afforded 0.7 g of the title compound.

PMR ($CDCl_3$), δ (ppm): 0.05 (s, 6H, $Si(CH_3)_2$); 0.90 (s, 9H, $SiC(CH_3)_3$); 1.48 (d, J=6.5Hz, 3H, CH_3CH); 2.01 (s, 3H, $=CH_3$); 2.24 (s, 3H, $=CH_3$); 3.35 (dd, J=2.5, 7.0Hz, 1H, H-3); 3.73 (s, 3H, $COOCH_3$); 4.08 (t, J=2.0Hz, 2H, CH_2OSi); 5.26 (s, 2H, CH_2Ph); 5.2—5.35 (m, 3H, CH_3CH, H-4, =H); 5.56 (d, J=2.0, 1H, =H); and 7.4—8.4 (m, 4H, $PhNO_2$)

Example 17
4R-(t-butyldimethylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-methoxyoxalyl-azetidin-2-one
IVa: R=CH_3, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

0.7 g of the compound prepared in Example 16 were dissolved in 30 ml of dichloromethane and 10 ml of methanol. The solution was cooled to −78°C and ozone in oxygen was bubbled through the solution until a blue colour appeared. After shaking with an aqueous solution of sodium pyrosulphite and drying over anhydrous sodium sulphate, evaporation of the solvent gave 0.6 g of the title compound.

Example 18
4R-(t-butyldimethylsilyloxyacetylthio)-3S-(1R)-p-nitrobenzyloxycarbonyloxy-ethyl)-azetidin-2-one
V: R_1=$CH_3CH(OCOOPNB)$, PG=*t*-butyldimethylsilyl

0.6 g of the compound prepared in Example 17 was dissolved in 30 ml of methanol and a few grams of silica gel were added. After stirring for 1 hour, the mixture was filtered and the solvent evaporated from the filtrate. The residue was chromatographed on silica gel using cyclohexane:ethyl acetate (3:2 by volume) as eluent to give 0.28 g of the title compound.

PMR ($CDCl_3$), δ (ppm): 0.15 (s, 6H, $Si(CH_3)_2$); 0.95 (s, 9H, $SiC(CH_3)_3$); 1.45 (d, J=6.5Hz, 3H, CH_3CH); 3.42 (dd, J=3.0, 6.0Hz, 1H, H-3); 4.25 (s, 2H, CH_2OSi); 5.26 (s, 2H, CH_2Ph); 5.1—5.3 (m, 2H, $CHCH_3$, *H-4*); 6.70 (bs, 1H, NH); and 7.4—8.4 (m, 4H, $PhNO_2$)

I.R. (CH_2Cl_2), ν (cm^{-1}): 1695 (C=O), 1750 (—OCOO—), 1785 (β-lactam)

Example 19
4R-(t-butyldimethylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-hydroxy-methyl)-azetidin-2-one
X: R=PNB, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

0.34 g of the compound prepared in Example 18 and 0.34 g of *p*-nitrobenzyl glyoxylate in 10 ml of benzene were kept at refluxing temperature for two hours. After evaporating off the solvent, purification of the residue by silica gel column chromatography eluting with cyclohexane:ethyl acetate (3:2 by volume) afforded 0.27 g of the title compound.

PMR ($CDCl_3$), δ (ppm): 0.13 (s, 6H, $Si(CH_3)_2$); 0.95 (s, 9H, $SiC(CH_3)_3$); 1.47 (d, J=6.5Hz, 3H, CH_3CH); 3.52 (m, 1H, H-3); 4.27 (s, 2H, CH_2OSi); 4.0—4.6 (m, 2H, *CH*OH, CH*OH*); 5.25 (s, 4H, two CH_2Ph); 5.1—5.6 (m, 2H, $CHCH_3$, *H-4*); and 7.3—8.3 (m, 8H, two Ph-NO_2)

Example 20
4R-(t-butyldimethylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-chloro-methyl)-azetidin-2-one
XI: R=PNB, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

A solution of 0.27 g of the compound prepared in Example 19 in 3 ml of anhydrous tetrahydrofuran was cooled to 0°C. 0.045 ml of pyridine and 0.03 ml of thionyl chloride were added. After 10 minutes the mixture was filtered. Evaporating off the solvent gave 0.3 g of the title compound, which was used as such for the next step.

Example 21
4R-(t-butyldimethylsilyloxyacetylthio)-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one
XII: R=PNB, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

0.3 g of the compound prepared in Example 20 and 0.45 g of triphenylphosphine were dissolved in 5 ml of dichloromethane and 2 to 3 g of silica gel were added. After evaporating off the solvent, the loaded silica gel was dried, left at room temperature overnight and then washed with cyclohexane to

remove excess triphenylphosphine. The product adsorbed on the silica was chromatographed on silica gel eluting with cyclohexane:ethyl acetate (3:2 by volume). 0.26 g of the title compound were obtained.

PMR ($CDCl_3$), δ (ppm): 0.08, 0.15 (two s, 6H, $Si(CH_3)_2$); 0.89, 0.93 (two s, 9H, $Si(CH_3)_3$); 1.35 (d, J=6.5Hz, 3H, CH_3CH); 4.1—4.2 (m, 2H, CH_2OSi); 4.6—5.0 (m, 1H, $CHCH_3$); 5.20 (bs, 4H, two CH_2—Ph—NO_2); 7.56 (bs, 15H, $(P(Ph)_3)$; and 7.6—8.4 (m, 8H, two Ph—NO_2)

Example 22

4R-hydroxyacetylthio-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one

XIII: R=PNB, R_1=$CH_3CH(OCOOPNB)$

A solution of 0.26 g of the compound prepared in Example 21 and 0.07 ml of acetic acid in 2 ml of anhydrous tetrahydrofuran was treated with a solution of 0.18 g of tetrabutylammonium fluoride in 2 ml of tetrahydrofuran. After stirring at room temperature for 1 hour, the mixture was diluted with ethyl acetate, and washed with water, saturated sodium bicarbonate solution and water again. After drying the mixture and evaporating off the solvent, the residue was purified by silica gel column chromatography eluting with cyclohexane:ethyl acetate (1:3 by volume) giving 0.13 g of the title compound.

PMR ($CDCl_3$), δ (ppm): 1.37 (d, J=6.5Hz, 3H, CH_3CH) 4.2 (m, 2H, CH_2OH); 4.9 (m, 1H, CH_3CH); 5.25 (m, 5H, two CH_2Ph, *H-4*); 7.55 (s, 15H, $P(Ph)_3$); and 7.6—8.4 (m, 8H, two $PhNO_2$)

Example 23

p-Nitrobenzyl (5R)-2-hydroxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

XVI: R=PNB, R_1=$CH_3CH(OCOOPNB)$

A solution of 0.13 g of the compound prepared in Example 22 in 10 ml of xylene was refluxed under a nitrogen atmosphere for 1 hour. Evaporating off the solvent and purification by preparative TLC (silica gel) afforded 50 mg of the title compound.

$[\alpha]_D^{20}$+66° (c=1.3, $CHCl_3$)

PMR ($CDCl_3$), δ (ppm): 1.51 (d, J=6.5, 3H, CH_3CH); 3.55 (bs, 1H, OH); 3.97 (dd, J=2.0, 8.0Hz, 1H, H-6); 4.68 (s, 2H, CH_2OH); 5.19 (dq, J=6.5, 8.0Hz, 1H, $CHCH_3$); 5.25—5.45 (m, 4H, two CH_2Ph); 5.65 (d, J=2.0Hz, 1H, H-5); and 7.4—8.5 (m, 8H, two $PhNO_2$)

Mass spectrum (F.D.) m/e 559

U.V. λ_{max} (CH_2Cl_2): 269 nm (ε 17.000), 323 (6800)

I.R. (CH_2Cl_2) ν (cm^{-1}): 1795, 1755, 1710.

Example 24

p-Nitrobenzyl (5R)-2-(t-butyldimethylsilyloxymethyl)-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

XV: R=PNB, R_1=$CH_3CH(OCOOPNB)$, PG=t-butyldimethylsilyl

A solution of 0.15 g of the compound prepared in Example 21 in 15 ml of xylene was stirred at reflux temperature under a nitrogen atmosphere for 1 hour. The solvent was evaporated off and the residue purified by preparative TLC (silica gel). 70 mg of the title compound were obtained.

Example 25

p-Nitrobenzyl (5R)-2-hydroxymethyl-6S-(1R-p-nitrobenzyl-oxycarbonyl-ethyl)-2-penem-3-carboxylate

XVI: R=PNB, R_1=$CH_3CH(OCOOPNB)$

70 mg of the compound prepared in Example 24 were dissolved in 1 ml of anhydrous tetrahydrofuran. 0.025 ml of acetic acid and a solution of 68 mg of tetrabutylammonium fluoride in 0.5 ml of tetrahydrofuran were added. The mixture was stirred at room temperature for 1 hour, diluted with ethyl acetate, and washed with water, saturated sodium bicarbonate solution and water again. After evaporating off the solvent, the residue was purified by silica gel preparative TLC eluting with cyclohexane:ethyl acetate (3:7 by volume). 30 mg of the title compound were obtained. The material was identical (IR and NMR spectra) to that obtained in Example 23.

Example 26

p-Nitrobenzyl (5R)-2-trichloroacetylcarbamoyloxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate

I: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$CONHCOCCl_3$, n=O

To a solution of 50 mg of the compound prepared in Example 23 (or Example 25) in 1 ml of purified acetone, cooled to 0°C, a solution of 0.06 ml of trichloroacetyl isocyanate in 1 ml of purified acetone was added dropwise. After 20 minutes, the solvent was evaporated off to give 100 mg of the crude title compound.

PMR ($CDCl_3$) δ (ppm): 1.50 (d, J=6.0Hz, 3H, CH_3CH); 4.00 (dd, J=2.0, 8.0Hz, 1H, H-6); 5.1—5.9 (m, 8H, H-5, CHO, two CH_2Ph, CH_2OCO); 7.5—8.4 (m, 8H, two $PhNO_2$); 8.90 (bs, 1H, NH).

Example 27
p-Nitrobenzyl (5R)-2-carbamoyloxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate
I: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$CONH_2$, n=O

100 mg of the crude compound prepared in Example 26 were dissolved in 4 ml of methanol. Silica gel (40 to 63 μm) was added and the mixture was stirred for 3 hours at room temperature and filtered, washing with acetone. The solvent was evaporated from the filrate and the residue was purified by silica gel preparative TLC, with cyclohexane-ethyl acetate (3:7 by volume) as eluent, giving 33 mg of the title compound.

$[\alpha]_D^{20}$+50° (c=2.4, acetone)

PMR ($CDCl_3$), δ (ppm): 1.48 (d, J=6.5Hz, 3H, CH_3CH); 3.95 (dd, J=2.0, 8.0Hz, 1H, H-6): 4.85 (bs, 2H, NH_2); 5.1—5.5 (m, 7H, $CHCH_3$, two CH_2Ph, CH_2OCO); 5.64 (d, J=2.0Hz, 1H, H-5; and 7.4—8.5 (m, 8H, two $PhNO_2$)

I.R. (KBr), ν (cm^{-1}): 1795, 1750, 1710.

Example 28
4R-trichloroacetylcarbamoyloxyacetylthio-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one
XIV: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$CONHCOCCl_3$

120 mg of the compound prepared in Example 22 were dissolved in 2 ml of purified acetone and cooled to 0°C. A solution of 0.1 ml of trichloroacetyl isocyanate in 2 ml of purified acetone was added dropwise and the mixture was stirred for half an hour. Evaporating off the solvent afforded 180 mg of the crude title compound.

Example 29
4R-carbamoyloxyacetylthio-3S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-1-(1-p-nitrobenzyloxycarbonyl-1-triphenylphosphoranylidene-methyl)-azetidin-2-one
XIV: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$CONH_2$

A mixture of 180 mg of the crude compound prepared in Example 28 in 8 ml of methanol and silica gel (40 to 63 μm) was stirred for 4 hours at room temperature. The mixture was filtered, washed with acetone, and the filtrate evaporated. Purification of the residue by silica gel preparative TLC using cyclohexane:ethyl acetate (1:4 by volume) as eluent gave 70 mg of the title compound.

Example 30
p-Nitrobenzyl (5R)-2-carbamoyloxymethyl-6S-(1R-p-nitrobenzyloxycarbonyloxy-ethyl)-2-penem-3-carboxylate
I: R=PNB, R_1=$CH_3CH(OCOOPNB)$, R_2=$CONH_2$ n=O

70 mg of the compound prepared in Example 29 in 8 ml of xylene were heated at reflux temperature for 1 hour under a nitrogen atmosphere. After evaporating off the solvent, purification of the residue by silica gel preparative TLC afforded 30 mg of the title compound, identical (IR and NMR spectra) to that obtained in Example 27.

Example 31
Sodium (5R)-2-carbamoyloxymethyl 6S-(1R-hydroxyethyl)-2-penem-3-carboxylate
I:R=H, R_1=$CH_3CH(OH)$, R_2=$CONH_2$ n=O, sodium salt

30 mg of the compound prepared in Example 27 (or Example 30) were dissolved in 3 ml of ethyl acetate. 2 ml of water, 4.2 mg of sodium bicarbonate and 45 mg of 5% palladium-on-charcoal were added and the mixture underwent hydrogenation at room temperature for 2 hours. After filtration through kieselguhr the aqueous phase was washed with a small amount of cold ethyl acetate, filtered through Waters Sep-Pak C_{18} cartridges and lyophilized. The residue was purified by reverse phase chromatography on Waters Sep-Pak C_{18} cartridges eluting with water. 8 mg of the title compound were obtained.

UV: λ_{max} (H_2O): 259 nm (ε 3600, 308 (5400)

PMR (D_2O), δ (ppm): 1.31 (d, J=6.5Hz, 3H, CH_3CH); 3.91 (dd, J=1.5, 6.0Hz, 1H, H-6); 4.25 (m, 1H, $CHOH$); 5.02, 5.36 (two d, 2H, CH_2OCO); 5.66 (d, J=1.5Hz, 1H, H-5)

$[\alpha]_D^{20}$=+143° (c=O, 97 H_2O).

Claims

1. A method for the preparation of a compound of the general formula I as herein defined or a pharmaceutically acceptable salt of such a compound in which R represents a hydrogen atom, the method comprising chlorinating a compound of the general formula X as herein defined, reacting the resultant compound of the general formula XI as herein defined with triphenylphosphine, and converting the resultant compound of the general formula XII as herein defined into one of the general formula I as herein defined by the steps of

(a) cyclisation by heating under an inert atmosphere in an inert solvent at from 80 to 150°C,
(b) removal of the protecting group PG,
(c) introduction of the group R_2,
(d) oxidation of the 1-S atom by a peracid, and
(e) salifying the free base,

the steps being carried out in that order, or with step (a) following either step (b) or step (c), step (c) being carried out only if in the desired compound R_2 does not represent a hydrogen atom, step (d) being carried out only if in the desired compound *n* is 1, and step (e) being carried out only if the desired compound is a salt of one in which R represents a hydrogen atom.

2. A method according to claim 1 in which the chlorination is effected with thionyl chloride in an inert solvent at from −20 to 0°C.

3. A method according to claim 1 or claim 2 in which the reaction with triphenylphosphine is carried out in an organic solvent at from 30 to 60°C in the presence of a base.

4. A method according to claim 3 in which the base is pyridine or lutidine.

5. A method according to claim 1, the method being substantially as described herein with reference to Examples 4 to 14 or Examples 20 to 31.

6. A method according to claim 1 in which the compound of the general formula X as herein defined is one prepared by reacting a compound of the general formula VI as herein defined with acetic acid and trimethylphosphite in an inert solvent, isomerizing the resultant compound of the general formula VII as herein defined in an inert solvent in the presence of a base at from 0 to 20°C, converting the resultant compound of the general formula VIII as herein defined to one of the general formula IX as herein defined by either ozonolysis and methanolysis or treatment with potassium permanganate in the presence or absence of sodium metaperiodate, reacting the compound of the general formula IX with a compound of the general formula $HSCOCH_2OPG$ wherein PG is as herein defined, and condensing the resultant compound of the general formula V with a glyoxylic ester of the general formula CHOCOOR wherein R is as herein defined.

7. A method according to claim 6 in which the preparation of the compound of the general formula X as herein defined is substantially as described herein with reference to Examples 1 to 3.

8. A method according to claim 1 in which the compound of the general formula X as herein defined is one prepared by protecting the free hydroxy group of a compound of the general formula II as herein defined with a protecting group PG as herein defined, reducing the sulphoxide function in the resultant compound of the general formula III as herein defined with phosphorus tribromide, ozonolysing the resultant compound of the general formula IV as herein defined in a solvent at from −80 to −50°C, methanolysing the N-substituent in the resultant compound of the general formula IVa as herein defined, and condensing the resultant compound of the general formula V with a glyoxylic ester of the general formula CHOCOOR wherein R is as herein defined.

9. A method according to claim 8 in which the preparation of the compound of the general formula X as herein defined is substantially as described herein with reference to Examples 15 to 19.

10. A method according to claim 1 in which the compound of the general formula X as herein defined is one prepared by protecting the free hydroxy group of a compound of the general formula II as herein defined with a protecting group PG as herein defined, reducing the sulphoxide function in the resultant compound of the general formula III as herein defined with phosphorus tribromide, ozonolysing the resultant compound of the general formula IV as herein defined in a solvent at from −80 to −50°C, and reducing the resultant compound of the general formula IVa as herein defined with zinc in acetic acid.

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