A New Chemoenzymatic Approach to the Synthesis of Penems

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A new synthesis of the valuable penem antibiotics FCE 22101 and FCE 22891 which does not require the use of expensive protecting groups is reported. A mixed carbon-silicon protection of two hydroxy groups was used, followed by a selective enzymatic hydrolysis of the resulting 8-*O*-protected-2-carboxylic ester (**18**). Lipase from *Chromobacterium viscosum* and lipoprotein lipase from *Pseudomonas* sp. gave excellent results in both free or immobilized form.

In the last five years, several methods for the synthesis of the valuable penem antibiotics FCE 22101 and FCE 22891 (**1**) have been reported.[1a–d]

These methods can be divided into two major groups: those starting from 4-acyloxyazetidinone (**5**) (path a in Scheme 1),[1a,1c] and those starting from 6-aminopenicillanic acid (**2**) (path b in Scheme 1).[1b,1d] In both classes of synthesis the pivotal intermediate is the diprotected penem (**7**). Two different silyl groups must be used in order to selectively deblock the primary hydroxy group and to transform it into the carbamoyl derivative (**9**). The latter is then deprotected in position 8 and converted into the penem (**1**).

Though this methodology is elegant on a laboratory scale, the use of the t-butyldimethylsilyl (TBDMS) and t-butyldiphenylsilyl (TBDPS) groups as well as the reagent reported for their removal (tetrabutylammonium fluoride) seemed too expensive for industrial application.

On the other hand, the selective carbamoylation of the unprotected 2,8-diol (**10**), failed; a mixture of mono- and di-carbamate being formed even at −78 °C.†

In our attempts to develop an economical and high yielding process for the preparation of FCE 22101 and FCE 22891, we decided to exploit the well documented ability of enzymes to hydrolyse selectively a single ester bond when two or more reactive groups are present in the same substrate.

An 8-*O*-protected 2-carboxylic ester (**18**) (Scheme 2) seemed to be a suitable substrate for this purpose. In this paper we report the synthesis of the latter compound and its selective enzymatic hydrolysis to (**19**) (Scheme 3). The monohydroxy derivative (**19**) was conveniently transformed into FCE 22101.

Results and Discussion

The benzoyloxyazetidinone (**14**) (Scheme 2), synthesized from L-threonine according to Hanessian,[1c] was transformed into its trimethylsilyl (TMS) or tetrahydropyranyl (THP) derivative (**15**) and this then allowed to react with a thiocarboxylate (**13**) (see Experimental section) to give the thioester derivative (**16**). The latter was then treated with allyloxalyl chloride and cyclised with triethyl phosphite[3] to afford (**18**).

Compound (**18a**) was chosen as the preferred substrate and a number of commercial enzyme preparations were tested for the selective hydrolysis of the carboxylic ester in position 2. As shown in Table 1 some lipases were able to catalyse the selective hydrolysis, with, in two cases, a rapid reaction rate (entries 4, 5). In contrast, all the proteases, but one, failed to give the desired product. Moreover, with some proteases we failed to observe any product in the organic extract after 4 h. Although no attempt was made to study the fate of the substrate in such cases, it is likely that an enzyme-catalysed ring cleavage took place. This cleavage may be due either to the action of proteases themselves or to the presence of contaminating β-lactamases in the crude enzyme preparations. Therefore we chose lipases for a more detailed investigation (Table 2).

Some comments on Table 2 should be made. Two bacterial lipases, namely lipase from *Chromobacterium viscosum* (entries 10—12) and lipoprotein lipase from *Pseudomonas* sp. (entry 9) allowed the reaction to occur in high yield, rapidly and with a high substrate/enzyme ratio (up to 200 w/w). Although pancreatin, a much cheaper mammalian enzyme, could be used as a catalyst, because of its lower activity a large amount of protein was required, and this caused problems in stirring and work-up due to the formation of clots and emulsions.

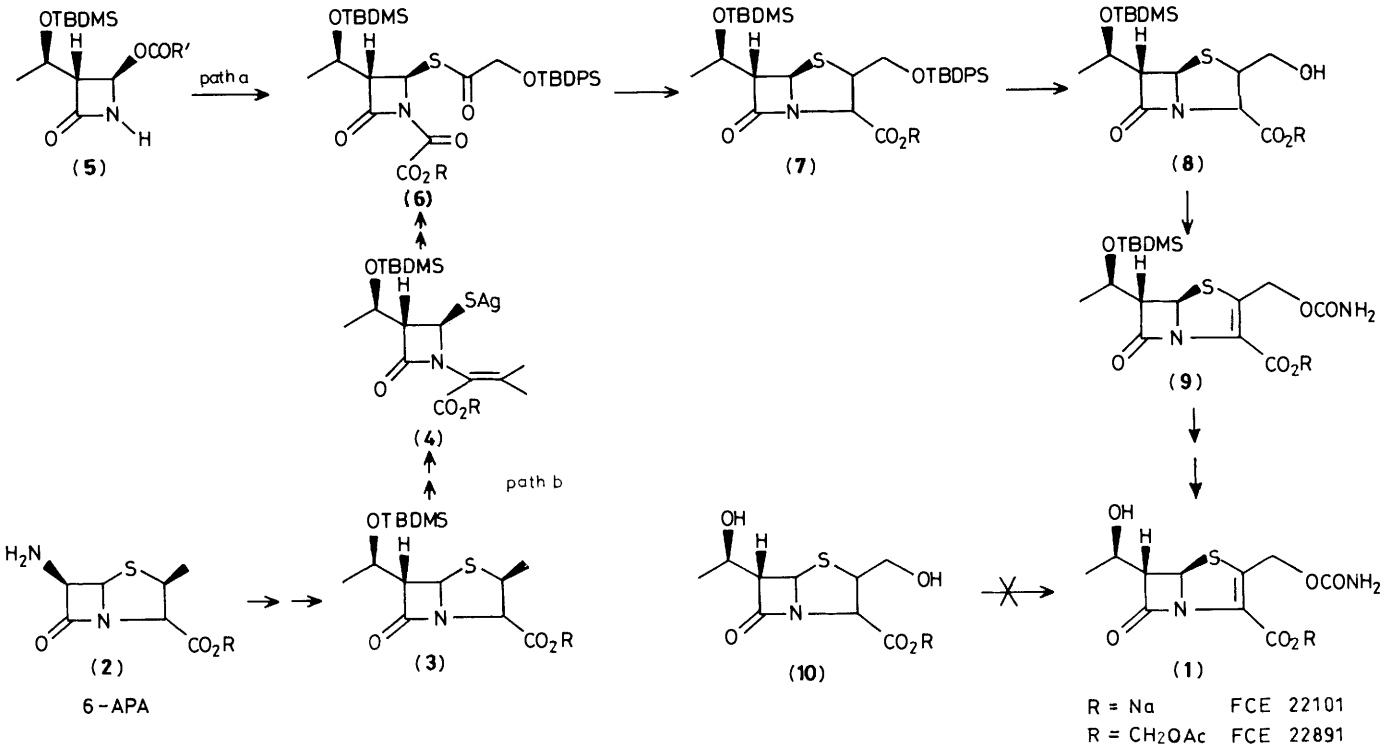
We observed a dramatic decrease of the reaction rate by changing the pH of the buffer (entries 1—3), and therefore chose pH 7.50 in all the successive experiments.

The reaction rate with the other lipases (entries 4—8) was much lower; the reactions were stopped after 20 h and the products isolated by chromatography. Acetic and butyric esters seem roughly equivalent as far as the enzymatic reaction is concerned (entries 10—12). Both the 8-tetrahydropyranyl (**18b**) and the 8-trimethylsilyl (**18a, c**) derivatives can be successfully employed in the enzymatic splitting, but (**18a, c**) proved to be more convenient because of the easier introduction and removal of the trimethylsilyl group. Moreover, yields were lower when tetrahydropyranyl was used as a protecting group.

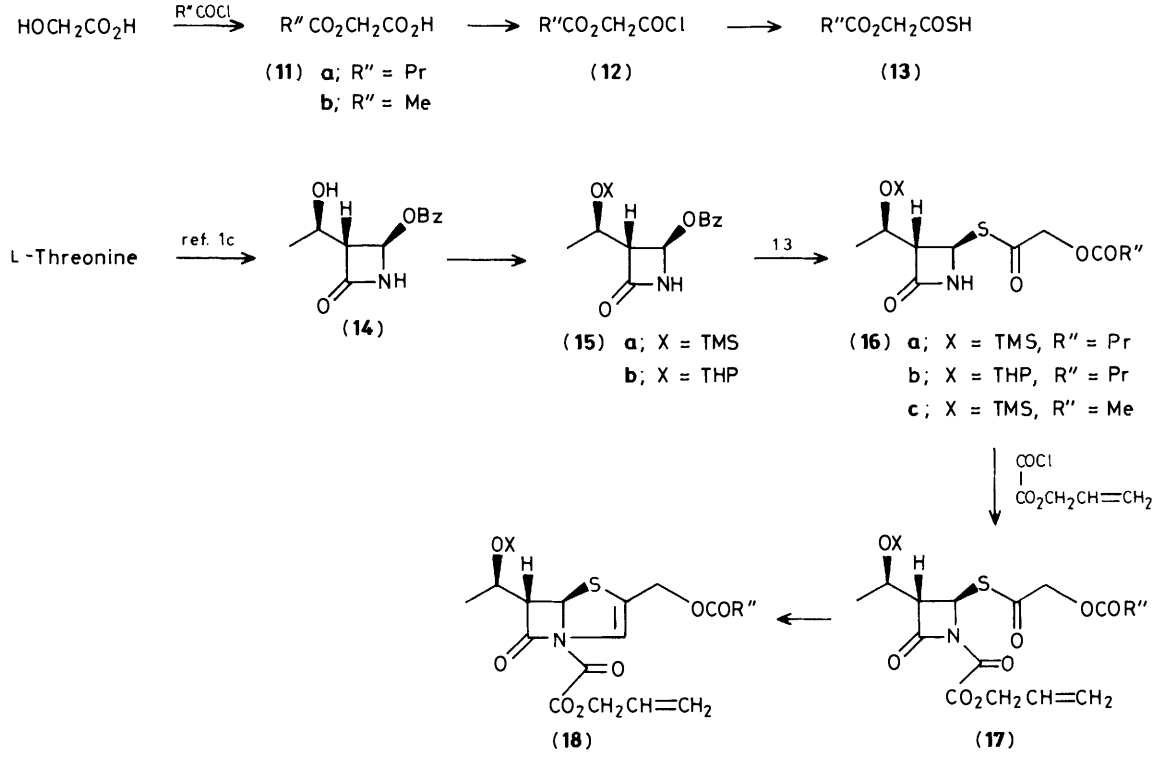
Finally, it should be noted that under our experimental conditions the hydrolytic reaction takes place only at the ester bond in position 2 leaving unaffected both the allyl ester in position 3 and the β-lactam entity. It was also surprising to find that a compound bearing a group as unnatural as the trimethylsilyl was so good a substrate for enzymes.

In order to make this process more attractive from the industrial point of view, we tested the possibility of using the most active enzymes (lipase from *Chromobacterium viscosum* and lipoprotein lipase from *Pseudomonas* sp.) in an immobilized form. Of the immobilization techniques proposed in the literature[4] we decided to use the simplest one, that is adsorption on a suitable carrier. To this end we examined several synthetic adsorbents and found that Amberlite XAD-7 afforded immobilized enzymes with an activity similar to that of the free ones. The adsorbed enzymes can be used several times without appreciable loss of activity. We observed that the reaction product was completely adsorbed onto the polymer, because of the nature of

† This approach was claimed to be successful in the case of a carbapenem.[2]



Scheme 1.



Scheme 2.

the resin itself which has both hydrophobic and hydrophilic sites in its structure.

At the end of the reaction, the product could be recovered by filtration and washing of the resin with an organic solvent. When methylene dichloride was used, no desorption of the enzyme was observed, and more than 90% of its activity remained after six experiments (see Experimental section). The use of this procedure allowed us to increase the substrate/enzyme ratio up to 1 200 w/w.

The monohydroxy derivative (**19**) obtained from the enzy-

Table 1. Enzymatic hydrolysis of (**18a**)[a]

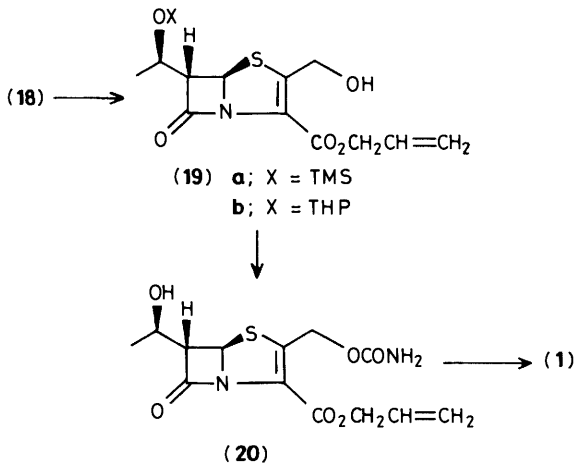
Entry	Enzyme	Manufacturer	Conversion after 4 h[b]
1	PLE	Sigma	*c*
2	Acid phosphatase	Sigma	*c*
3	PPL	Sigma	+
4	Lipase from *Chromobacterium viscosum*	Toyo Jozo	+++
5	Lipoprotein lipase from *Pseudomonas* sp.	Amano	+++
6	Lipase from *Candida cylindricea*	Sigma	+
7	Lipase from wheat germ	Sigma	*c*
8	Lipase SP 225	Novo	*c*
9	Lipase from *Rhizopus delemar*	Sigma	*c*
10	Pancreatin	Unibios	++
11	Alcalase 2.0 T	Novo	*c*
12	Protease from *Streptomyces griseus*	Sigma	*d*
13	Protease from *Streptomyces caespitosus*	Sigma	*c*
14	Protease from *Aspergillus saitoi*	Sigma	*c*
15	Protease from *Aspergillus sojae*	Sigma	*c*
16	Protease from *Rhizopus* sp.	Sigma	+
17	Protease from *Bacillus polymyxa*	Sigma	*d*
18	Papain	Sigma	*c*
19	Acylase I	Sigma	*d*

[a] 30 mg of (**18a**) in 4 ml of phosphate buffer 0.05M pH 7.50; 2—10 mg of enzyme depending on its specific activity: $T = 30$ °C. [b] Conversion estimated by t.l.c. of the organic extract. [c] Only starting material present in the organic extract. [d] No product detected in the organic extract.

Table 2. Enzymatic hydrolysis of (**18a—c**) with lipases[a]

Entry	Substrate	Enzyme (mg)	pH	Time (h)	Conversion (%)[b]	Yield (%)[c]
1	(**18a**)	Pancreatin (750)	7.50	20	100	91
2	(**18a**)	Pancreatin (750)	8.00	20	50	40
3	(**18a**)	Pancreatin (750)	7.00	20	70	58
4	(**18a**)	PPL (60)	7.50	20	35	20
5	(**18a**)	Lipase from *Candida cylindracea* (60)	7.50	20	40	30
6	(**18a**)	Lipase from wheat germ (60)	7.50	20	90	80
7	(**18a**)	Lipase SP 225 (60)	7.50	20	70	50
8	(**18a**)	Lipase from *Rhizopus delemar* (60)	7.50	20	40	20
9	(**18a**)	Lipoprotein lipase from *Pseudomonas* sp. (30)	7.50	1	100	91
10	(**18a**)	Lipase from *Chromobacterium viscosum* (5)	7.50	4	100	92
11	(**18b**)	Lipase from *Chromobacterium viscosum* (20)	7.50	4	100	94
12	(**18c**)	Lipase from *Chromobacterium viscosum* (20)	7.50	5	100	95

[a] All reactions were carried out with 1 g of substrate in 60 ml of phosphate buffer 0.05M at 30 °C. [b] Based on NaOH consumed. [c] Isolated yield.



Scheme 3.

matic reaction was then treated with chlorosulphonyl isocyanate to give the corresponding carbamoyl derivative. It is noteworthy that, when trimethylsilyl was used as protecting group, its removal was accomplished directly during the carbamoylation step. In the case of the tetrahydropyranyl derivative (**19b**), an additional deprotection step was required to afford the carbamate (**20**). FCE 22101 and FCE 22891 were obtained from (**20**) according to published methods.[5]

In conclusion, this new chemoenzymatic approach to the synthesis of penems gave excellent results without using the expensive reagents t-butyldimethylsilyl chloride and t-butyldiphenylsilyl chloride.

Moreover we believe that this mixed carbon-silicon protection of substrates having two or more hydroxy groups could find extensive application in fields other than β-lactam chemistry because of its simplicity and economy.

Experimental

M.p.s were determined on a Tottoli apparatus and are uncorrected. ^{1}H N.m.r. spectra were recorded in $CDCl_3$ solution (TMS internal standard) on a Bruker AM 300 instrument. Optical rotations were measured on $CHCl_3$ solutions with a Perkin-Elmer 241 polarimeter. I.r. spectra were recorded on a Perkin-Elmer 1420 spectrophotometer. Mass spectra were recorded on a Finnigan MAT 8400 instrument at 70 eV. Enzymatic hydrolyses were performed with a Metrohm pH-stat.

For column chromatography, Merck silica gel 60 (70—230 mesh) was used. Amberlite XAD-7 was obtained from Rohm & Haas.

Acylase I (1 020 U/mg), pig liver esterase (PLE, 200 U/mg),

acid phosphatase (4 U/mg), lipase from porcine pancreas (PPL, 11 U/mg), lipase from *Candida cylindracea* (500 U/mg), lipase from wheat germ (7 U/mg), lipase from *Rhizopus delemar* (20 U/mg), protease from *Streptomyces griseus* (5.2 U/mg), protease from *Streptomyces caespitosus* (0.7 U/mg), protease from *Aspergillus sojae* (0.44 U/mg), protease from *Rhizopus* sp. (0.6 U/mg), protease from *Bacillus polymyxa* (1 U/mg) and papain (2.9 U/mg) were purchased from Sigma Chem. Co.; lipase SP 225 and alcalase 2.0 T (2.16 U/g) were obtained from Novo Industri (Denmark); pancreatin (57 U/mg) was purchased from Unibios (Italy); lipoprotein lipase from *Pseudomonas* sp. (1120 U/mg) was purchased from Amano Chem. Co. (Japan) and lipase from *Chromobacterium viscosum* (1200 U/mg) was obtained from Toyo Jozo (Japan).

Synthesis of Acyloxythiocarboxylic Acids (**13a, c**)

(a) *Acyloxycarboxylic Acids* (**11a, c**).—A solution of glycolic acid (53 g, 0.7 mol) and the acid chloride (0.7 mol) in dioxane (350 ml) was refluxed under nitrogen for 5 h. After evaporation of the solvent, the following products were obtained by distillation.

Butyryloxyacetic acid (**11a**) 90% yield, b.p. 90—92 °C (0.1 mmHg); δ 1.0 (3 H, t, *J* 7 Hz, CH_3CH_2), 1.72 (2 H, m, $CH_3CH_2CH_2$), 2.40 (2 H, t, *J* 7 Hz, $CH_3CH_2CH_2$), 4.65 (2 H, s, OCH_2CO_2H), and 10.40 (1 H, br s, CO_2H); $\nu_{max.}$ 1 740 cm^{-1}.

Acetoxyacetic acid (**11c**) 92% yield, b.p. 104—106 °C (4 mmHg); δ 2.15 (3 H, s, CH_3CO), 4.62 (2 H, s, OCH_2CO_2H), and 11.1 (1 H, br s, CO_2H); $\nu_{max.}$ 1 740 cm^{-1}.

(b) *Acyloxycarbonyl Chlorides* (**12a, c**).—To a solution of acyloxycarboxylic acid (**11a, c**) (0.6 mol) in dry benzene (600 ml) containing a few drops of dimethylformamide was added dropwise under nitrogen, oxalyl chloride (103 ml, 1.2 mol) over 0.5 h. The mixture was stirred at room temperature for 2 h; oxalyl chloride (43 ml, 0.5 mol) was added and the solution stirred for an additional 2 h. The solvent was evaporated under reduced pressure and traces of oxalyl chloride were removed by co-distillation with dry benzene to give the crude acid chlorides.

Butyryloxyacetyl chloride (**12a**). δ 1.0 (3 H, t, CH_3CH_2), 1.7 (2 H, m, $CH_3CH_2CH_2$), 2.4 (2 H, t, $CH_3CH_2CH_2$), and 4.9 (2 H, s, OCH_2COCl).

Acetoxyacetyl chloride (**12c**). δ 2.2 (3 H, s, CH_3CO) and 5.0 (2 H, s, OCH_2COCl).

(c) *Acyloxythiocarboxylic Acids* (**13a, c**).—A solution of triethylamine (128 ml, 0.93 mol) in methylene dichloride (1 l) was cooled to 5 °C and hydrogen sulphide (90 g, 2.65 mol) was bubbled through it for 0.5 h. A solution of the crude acyloxycarbonyl chloride (**12a, c**) in methylene dichloride (100 ml) was added dropwise at the same temperature. The reaction mixture was stirred for 2 h at room temperature, and treated with 10% HCl (300 ml). The separated organic phase was washed with 10% HCl (200 ml) and 20% aqueous NaCl, dried (Na_2SO_4), and evaporated. The residue was suspended in water and the pH raised to 6.0 by addition of 10% aqueous NaOH. The mixture was extracted twice with methylene dichloride; 10% HCl was then added to the aqueous layer until the pH reached 1.0 after which it was extracted again with methylene dichloride (3 × 200 ml). The organic extracts were dried (Na_2SO_4) and evaporated to give the pure thiocarboxylic acid.

Butyryloxythioacetic acid (**13a**). An oil, 68% yield from (**11a**); δ 1.0 (3 H, t, *J* 6.9 Hz, CH_3CH_2), 1.72 (2 H, m, $CH_3CH_2CH_2$), 2.42 (2 H, t, *J* 6.9 Hz, $CH_3CH_2CH_2$), 4.5 (1 H, br, SH), and 4.7 (2 H, s, OCH_2COSH); $\nu_{max.}$ 1 750 and 1 705 cm^{-1}.

Acetoxythioacetic acid (**13c**). An oil, 65% yield from (**11c**); δ 2.18 (3 H, s, CH_3), 4.5 (1 H, br, SH), and 4.68 (2 H, s, OCH_2COSH); $\nu_{max.}$ 1 750 and 1 700 cm^{-1}.

(3S,4R)-4-*Acyloxyacetylthio*-3-[(1R)-1-*trimethylsilyloxyethyl*]*azetidin*-2-*one* (**16a, c**).—To a solution of (**14**) (5 g, 21 mmol) in methylene dichloride, at room temperature, under nitrogen, were added in sequence pyridine (5 ml, 63 mmol) and trimethylchlorosilane (5.4 ml, 42 mmol). After being stirred for 15 min at the same temperature, the solution was washed twice with water, dried (Na_2SO_4), and evaporated to afford an oily residue. The crude product was dissolved in acetone (50 ml) and added to a solution of the thioacid (**13**) (30 mmol) and triethylamine (5 ml, 35 mmol) in acetone (35 ml). Water (11.5 ml) was added and the pH adjusted to 9.50 with triethylamine. The mixture was stirred at 25 °C for 1 h and acetone was removed under reduced pressure. The residue was diluted with diethyl ether, washed twice with water, dried (Na_2SO_4), and evaporated. The residue was purified by column chromatography on silica gel (diethyl ether–hexane, 50:50 v/v). The following were prepared in this way.

(3S,4R)-4-*Butyryloxyacetylthio*-3-[(1R)-1-*trimethylsiloxyethyl*]*azetidin*-2-*one* (**16a**). An oil, 68% yield from (**14**); δ 0.15 (9 H, s, TMS), 1.0 (3 H, t, *J* 7.3 Hz, $CH_3CH_2CH_2$), 1.20 (3 H, d, *J* 6.5 Hz, CH_3CH), 1.72 (2 H, m, $CH_3CH_2CH_2$), 2.45 (2 H, t, *J* 7.3 Hz, $CH_3CH_2CH_2$), 3.2 (1 H, m, 3-H), 4.25 (1 H, m, CH_3CH), 4.75 (2 H, s, $SCOCH_2O$), 5.32 (1 H, d, *J* 2 Hz, 4-H), and 6.3 (1 H, br, NH); $\nu_{max.}$ 3 260br, 1 775, 1 755, and 1 700 cm^{-1}; *m/z* 332 ($M^+ - 15$), 288, 218, and 117; $[\alpha]_D^{20}$ +117° (*c* 1.1 in $CHCl_3$).

(3S,4R)-4-*Acetoxyacetylthio*-3-[(1R)-1-*trimethylsiloxyethyl*]*azetidin*-2-*one* (**16c**). An oil, 60% yield from (**14**); δ 0.15 (9 H, s, TMS), 1.20 (3 H, d, *J* 6.5 Hz, CH_3CH), 2.10 (3 H, s, $COCH_3$), 3.20 (1 H, m, 3-H), 4.20 (1 H, m, CH_3CH), 4.80 (2 H, s, $SCOCH_2O$), 5.35 (1 H, d, *J* 2 Hz, 4-H), and 6.1 (1 H, br, NH); $\nu_{max.}$ 3 250br, 1 775, 1 755, and 1 690 cm^{-1}.

(3S,4R)-4-*Butyryloxyacetylthio*-3-[(1R)-1-*tetrahydropyranyloxyethyl*]*azetidin*-2-*one* (**16b**).—A solution of (**14**) (5 g, 21 mmol), dihydropyran (2.9 ml, 32 mmol), and toluene-*p*-sulphonic acid (200 mg) in methylene dichloride (100 ml) was stirred at 25 °C, under nitrogen, for 3 h. The solution was then washed with 5% aqueous $NaHCO_3$ (2 × 50 ml), dried (Na_2SO_4) and evaporated. The crude residue was dissolved in acetone (50 ml) and treated with butyryloxythioacetic acid as described above for (**16a**) to give a yellow oil, which was purified by column chromatography on silica gel (diethyl ether–hexane, 50:50 v/v) to give (**16b**) as an oil, 58% yield from (**14**); δ 1.0 (3 H, t, $CH_3CH_2CH_2$), 1.2—1.32 (3 H, m, CH_3CH), 1.45—1.60 (6 H, m, $CH_2CH_2CH_2$ of THP group), 1.72 (2 H, m, $CH_3CH_2CH_2$), 2.42 (2 H, t, $CH_2CH_2CH_3$), 3.25 (1 H, m, 3-H), 4.2 (1 H, m, CH_3CH), 4.75 (2 H, s, $SCOCH_2O$), 5.32 (1 H, m, 4-H), and 6.5 (1 H, br, NH); $\nu_{max.}$ 3 250br, 1 775, 1 755, and 1 700 cm^{-1}; *m/z* 359 (M^+), 198, 129, and 85.

General Procedure for Ring Closure [*Synthesis of* (**18a—c**)].—To a solution of 4-thioacylazetidinone (**16**) (15 mmol) in dry toluene (50 ml) were added dropwise, at 10 °C, under nitrogen, triethylamine (2.6 ml, 18.6 mmol) and allyloxyoxalyl chloride[6] (2.7 g, 18 mmol). At the end of the addition the mixture was stirred at 10 °C for 10 min. The solution was washed with water (30 ml), 5% aqueous $NaHCO_3$ (30 ml), and water (30 ml), dried (Na_2SO_4), and concentrated to 25 ml. Triethyl phosphite (5.75 ml, 33 mmol) was added and the solution refluxed for 6 h. The reaction mixture was cooled to 20 °C, washed with water (3 × 10 ml), dried (Na_2SO_4), and evaporated to give a crude oil. This was chromatographed on silica gel (diethyl ether–hexane, 30:70 v/v) to afford pure compound (**18**).

Allyl (5R,6S)-2-*butyryloxymethyl*-6-[(1R)-1-*trimethylsilyloxyethyl*]*penem*-3-*carboxylate* (**18a**). As a wax, 50% yield from (**16a**); δ 0.13 (9 H, s, TMS), 0.95 (3 H, t, $CH_2CH_2CH_3$), 1.25 (3 H, d, CH_3CH), 1.7 (2 H, m, $CH_2CH_2CH_3$), 2.3 (2 H, t, $CH_2CH_2CH_3$), 3.7 (1 H, dd, 6-H), 4.2 (1 H, m, 8-H), 4.7 (2

H, m, $CH_2CH{=}CH_2$), 5.2—5.5 (2 H, m, $CH{=}CH_2$), 5.05—5.55 (2 H, m, CH_2OCO), 5.55 (1 H, d, 5-H), and 5.95 (1 H, m, $CH{=}CH_2$); $\nu_{max.}$ 1 770, 1 750, 1 705, and 1 585 cm^{-1}; *m/z* 427 (M^+), 270, 182, and 143; $[\alpha]_D^{20}$ +109.3° (*c* 1.1 in $CHCl_3$).

Allyl (5R,6S)-2-*butyryloxymethyl*-6-[(1R)-1-*tetrahydropyranyloxyethyl*]*penem*-3-*carboxylate* (**18b**). As an oil, 45% yield from (**16b**); δ 0.95 (3 H, t, *J* 6.7 Hz, $CH_2CH_2CH_3$), 1.30—1.37 (3 H, m, CH_3CH), 1.42—1.90 (6 H, m, $CH_2CH_2CH_2$ of THP group), 1.67 (2 H, m, $CH_2CH_2CH_3$), 2.32 (2 H, t, $CH_2CH_2CH_3$), 3.4—3.9 (2 H, m, OCH_2 of THP group), 3.8 (1 H, m, C-6 H), 4.05—4.2 (1 H, m, C-8 H), 4.6—4.85 (3 H, $COOCH_2CH$= and OCH of THP group), 5.05—5.5 (2 H, m, CH_2OCO), 5.2—5.42 (2 H, m, $CH{=}CH_2$), 5.6 (1 H, m, 5-H), and 5.85—6.0 (1 H, m, $CH{=}CH_2$); $\nu_{max.}$ 1 790, 1 745, 1 710, and 1 585 cm^{-1}; *m/z* 439 (M^+), 270, 198, 182, and 85.

Allyl (5R,6S)-2-*acetoxymethyl*-6-[(1R)-1-*trimethylsiloxyethyl*]*penem*-3-*carboxylate* (**18c**). As a wax, 48% yield from (**16c**); δ 0.15 (9 H, s, TMS), 1.28 (3 H, d, CH_3CH), 2.1 (3 H, s, $COCH_3$), 3.72 (1 H, dd, *J* 2 Hz, 6 Hz, 6-H), 4.21 (1 H, m, 8-H), 4.65—4.80 (2 H, m, $COOCH_2CH$=), 5.05—5.44 (2 H, m, CH_2OCO), 5.25—5.50 (2 H, m, $CH{=}CH_2$), 5.55 (1 H, d, *J* 2 Hz, 5 H), and 5.85—5.60 (1 H, m, $CH{=}CH_2$); $\nu_{max.}$ 1 775, 1 745, 1 705, and 1 585 cm^{-1}.

Enzymatic Hydrolysis of (**18a—c**).—(a) *Free enzymes.* The following procedure is representative. To a magnetically stirred suspension of (**18a—c**) in 0.05M phosphate buffer (300 ml) at 30 °C was added the enzyme and the solution maintained at the desired pH with NaOH using a pH-stat. The hydrolysis was allowed to proceed until the NaOH consumption stopped, or was interrupted after 20 h, according to the enzyme used. The reaction mixture was extracted with methylene dichloride and the organic layer was dried (Na_2SO_4) and evaporated to dryness. Chromatography of the residue on silica gel (diethyl ether–hexane, 60:40 v/v) afforded pure (**19a—b**).

Allyl (5R,6S)-2-*hydroxymethyl*-6-[(1R)-1-*trimethylsiloxyethyl*]*penem*-3-*car-boxylate* (**19a**). As an oil; δ 0.15 (9 H, s, TMS), 1.32 (3 H, d, *J* 6.5 Hz, CH_3CH), 3.75 (1 H, dd, *J* 2, 6.5 Hz, 6-H), 3.85 (1 H, br, OH), 4.25 (1 H, m, 8-H), 4.65 (2 H, s, CH_2OH), 4.65—4.95 (2 H, m, CH_2CH=), 5.25—5.50 (2 H, m, $CH{=}CH_2$), 5.60 (1 H, d, *J* 2 Hz, 5-H), and 5.95—6.05 (1 H, m, $CH{=}CH_2$); $\nu_{max.}$ 3 400br, 1 780, 1 700, and 1 575 cm^{-1}; *m/z* 357 (M^+), 272, and 200; $[\alpha]_D^{20}$ 151° (*c* 1.05 in $CHCl_3$).

Allyl (5R,6S)-2-*hydroxymethyl*-6-[(1R)-1-*tetrahydropyranyloxyethyl*]*penem*-3-*carboxylate* (**19b**). As a white solid, m.p. 90—92 °C; δ 1.3—1.4 (3 H, m, CH_3CH), 1.45—1.90 (6 H, m, $CH_2CH_2CH_2$ of THP group), 3.42—3.92 (2 H, m, CH_2O of THP group), 3.6 (1 H, br, OH), 3.80 (1 H, m, 6-H), 4.05—4.22 (1 H, m, 8-H), 4.57—4.82 (5 H, CO_2CH_2CH=, CH_2OH, CH of THP group), 5.2—5.45 (2 H, m, $CH{=}CH_2$), 5.61 (1 H, m, 5-H), and 5.85—6.0 (1 H, m, $CH{=}CH_2$); $\nu_{max.}$ 3 480br, 1 780, 1 690, and 1 575 cm^{-1}; *m/z* 369 (M^+), 200, 158, and 85.

(b) *Immobilized enzymes.* To a solution of 25 mg of either lipase from *Chromobacterium viscosum* or lipoprotein lipase from *Pseudomonas* sp. in 25 ml of 0.05M phosphate buffer (pH 7.50) was added 10 g of Amberlite XAD-7. The mixture was gently stirred overnight at room temperature and then filtered and the resin washed with 25 ml of the same buffer. The immobilized enzyme was added to a magnetically stirred suspension of (**18a**) (5 g) in 0.05M phosphate buffer pH 7.50 (150 ml) at 30 °C and the solution maintained at pH 7.50 with 1M NaOH using a pH-stat. The hydrolysis was allowed to proceed until the NaOH consumption stopped (*ca.* 3 h). The resin containing the enzyme and the reaction product was recovered by filtration and washed with methylene dichloride (3 × 80 ml). The organic solution was dried (Na_2SO_4) and evaporated to dryness to give 4 g of (**19a**) (spectral characteristics as reported above). The resin was washed with phosphate buffer (3 × 50 ml) and used for 6 production cycles, as described above, without appreciable loss of activity.

Allyl (5R,6S)-2-*Carbamoyloxymethyl*-6-[(1R)-1-*hydroxyethyl*]*penem*-3-*carboxylate* (**20**).—(a) *From* (**19a**). A solution of (**19a**) (5 g, 13.3 mmol) in methylene dichloride (50 ml) was cooled to −78 °C; chlorosulphonyl isocyanate (1.4 ml, 16 mmol) was added and the solution stirred for 15 min at the same temperature. Dioxane (40 ml) and water (50 ml) were added, and the temperature was raised to 20 °C over a 2 h period. The mixture was extracted with methylene dichloride (3 × 80 ml). The organic extracts were washed with 5% aqueous $NaHCO_3$ (2 × 200 ml), dried (Na_2SO_4), and evaporated to afford a crude solid. The latter was then crystallized with the aid of hexane, filtered, and washed with hexane to yield a white solid (**20**) (3.3 g, 75%), m.p. 148—149 °C; δ 1.3 (3 H, d, CH_3CH), 3.75 (1 H, dd, 6-H), 4.25 (1 H, m, 8-H), 4.6—4.8 (2 H, m, CO_2CH_2CH=), 5.0 (1 H, br, NH), 5.2—5.5 (2 H, m, $CH{=}CH_2$), 5.3 (2 H, m, CH_2OCONH_2), 5.9—6.0 (1 H, m, $CH{=}CH_2$), and 6.63 (1 H, d, 5-H); $\nu_{max.}$ 1 775, 1 715, 1 705, and 1 580 cm^{-1}; *m/z* 328 (M^+), 243, and 182; $[\alpha]_D^{20}$ +153° (*c* 1.05 in $CHCl_3$).

(b) *From* (**19b**). Compound (**19b**) (5 g, 13.7 mmol) was allowed to react with chlorosulphonyl isocyanate (1.4 ml, 16 mmol) in methylene dichloride at −78 °C, as described before for (**19a**). At the end of the reaction, the crude 8-tetrahydropyranyl-2-carbamoyl derivative was recovered by extraction with methylene dichloride and evaporation of the solvent. The product was dissolved in 80% aqueous ethanol (150 ml), pyridinium toluene-*p*-sulphonate (690 mg, 2.74 mmol) was added, and the resulting mixture stirred for 4 h at 50 °C; toluene-*p*-sulphonic acid (520 mg, 2.74 mmol) was then added and the solution stirred for 2 h. After evaporation of the solvent, the residue was dissolved in ethyl acetate (200 ml), washed with 5% aqueous $NaHCO_3$ (2 × 100 ml), and dried (Na_2SO_4). Evaporation of the solvent gave a crude solid, which was crystallized from hexane, filtered off, and washed with hexane to yield (**20**) (3.0 g, 68%).

References

1 (*a*) G. Franceschi, M. Foglio, M. Alpegiani, C. Battistini, A. Bedeschi, E. Perrone, F. Zarini, F. Arcamone, C. Della Bruna, and A. Sanfilippo, *J. Antiobiotics*, 1983, **36**, 938; (*b*) M. Alpegiani, A. Bedeschi, E. Perrone, and G. Franceschi, *Tetrahedron Lett.*, 1984, **25**, 4171. (*c*) S. Hanessian, A. Bedeschi, C. Battistini, and N. Mongelli, *J. Am. Chem. Soc.*, 1985, **107**, 1438. (*d*) M. Alpegian, A. Bedeschi, F. Giudici, E. Perrone, and G. Franceschi, *ibid.*, p. 6398.

2 S. Uyeo, Belg. Pat. BE 905 784 (1987) (*Chem. Abstr.*, 1988, **108**, 37 494p).

3 C. Battistini, C. Scarafile, M. Foglio, and G. Franceschi, *Tetrahedron Lett.*, 1984, **25**, 2395.

4 I. Chibata, 'Immobilized Enzymes. Research and Development,' Holsted Press, New York, 1978.

5 G. Franceschi, M. Alpegiani, C. Battistini, A. Bedeschi, E. Perrone, and F. Zarini, *Pure Appl. Chem.*, 1987, **59**, 467; see also *Drugs fut.*, 1985, **10**, 119.

6 A. Afonso, F. Hon, J. Weinstein, and A. K. Ganguly, *J. Am. Chem. Soc.*, 1982, **104**, 6138.

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