Inhibitors of Bacterial Signal Peptidase: A Series of 6-(Substituted oxyethyl)penems

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A series of 6-(substituted oxyethyl)penem esters having the (5*S*) stereochemistry which are potent inhibitors of *Escherichia coli* leader peptidase is described. Structure-activity relationships are discussed.

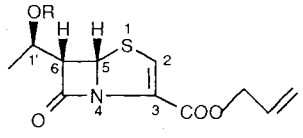
Protein secretion in bacteria is becoming a pathway of widespread interest for the derivation of antibacterial agents[1]. The final destination of considerable quantities of protein (up to 20%) synthesised by bacteria lies outside the cytoplasm. The cytoplasmic membrane represents a hydrophobic barrier which must be crossed by all of these proteins. Signal peptidases ensure release of secreted proteins from the outer surface of the plasma membrane by removal of the hydrophobic signal sequence, or cleavable membrane anchor, from the pre-proteins[2]. These signal peptidases represent attractive targets for novel antibacterial agents.

In *E. coli* there appears to be an essential enzyme, leader peptidase, which fulfils the signal peptidase function[3,4]. It is anchored to the membrane by two hydrophobic domains and the catalytic portion of the molecule is located in the periplasm[5].

We have reported briefly that C-3 esters and amides of penems having the 5*S* stereochemistry are inhibitors of *E. coli* leader peptidase, and that of particular interest were the 6-substituted penems (**1a** and **1b**), with 5*S*, 6*S*, 1′*R* stereochemistry[6]. We now wish to report the synthesis and structure-activity relationships of a series of derivatives of **1a**.

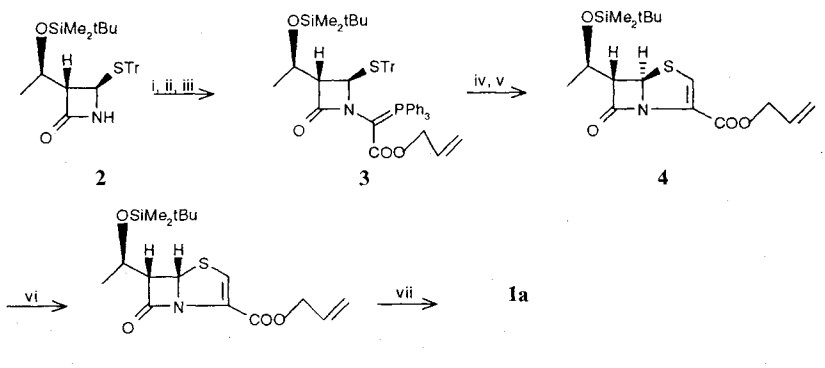
Chemistry

It appeared that an efficient approach to the synthesis of homochiral 5*S* penems could make use of well established methods for synthesis of 5*R* penems followed by a photoisomerisation at C-5[7]. Thus the preparation of **1a** was from the monocyclic β-lactam (**2**)[8]. The 5*R* penem system was built up by the commonly used phosphorane route, and then subjected to UV irradiation from a medium pressure lamp to provide an equilibrium



1a R=H
1b R=Ac

Scheme 1.



Reagents: (i) allyl glyoxylate, (ii) $SOCl_2$, 2,6-lutidine, (iii) PPh_3, 2,6-lutidine (70% for i~iii), (iv) $AgNO_3$, pyridine, MeOH, (v) acetic formic anhydride, DMAP, Et_3NHCl (85% for iv~v), (vi) hν (62%), (vii) Bu_4NF, HOAc (95%).

Table 1. Structures of intermediates.

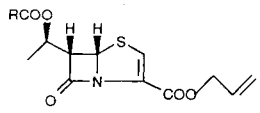
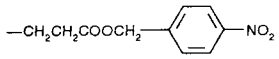
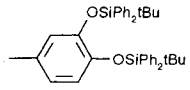
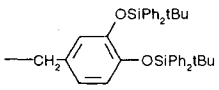
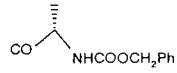
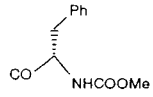
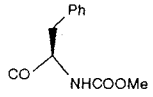
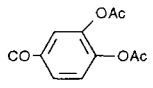
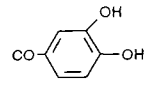
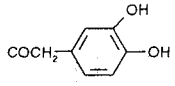
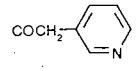
R	RCOOH	
$-CH_2OSiPh_2tBu$	**5**	**6**
	**7**	**8**
	**9**	**10**
	**11**	**12**

Table 2. Inhibition of leader peptidase.

	R	I_{50} μm	% Inhibition at:	
			10 μm	1 μm
1a	H	<1		
1b	COMe	<1		
1c	$CO(CH_2)_{14}Me$		0	0
1d	$COCH_2OH$	<1		
1e	$COCH_2CH_2COO^-Na^+$	12.8		
1f		13.2		
1g			0	0
1h			0	0
1j	COPh	1.65		
1k			21	0
1l			100	76
1m		1.2		
1n		<1		
1p	$CONH_2$		92	28
1q	CONHMe	<1		
1r	CONHPh		24	11
1s	Me	1.4		
1t	Et	<1		
1u	CH_2OMe	1.25		
1v	CH_2COOEt		100	27
1w	$SiMe_2tBu$		0	0
1x	$SO_3^-Na^+$		26	0

mixture of 5*R* and 5*S* penems. The 5*S* penem (**1w**) could be isolated in 62% yield and recovered 5*R* penem recycled. The hydroxyethyl penem (**1a**) was obtained by desilylation (Scheme 1).

In order to probe the requirements for enzyme inhibition, a variety of derivatives of penem (**1a**) were prepared: the secondary hydroxyl was acylated, alkylated and sulfonated. The substituents included two acidic groups, as in **1e** and **1x**, and one weakly basic group as in **1n** as well as a variety of neutral groups. Protected amino acid substituents (**1f**, **1g**, **1h**) or carbamate substituents (**1p**, **1q**, **1r**), might mimic a peptide substrate.

Acetic anhydride gave **1b** and palmitoyl and benzoyl chlorides **1c** and **1j**. Appropriate isocyanates provided **1p**, **1q**, **1r**. Other acyl derivatives were prepared using carboxylic acids (sometimes suitably protected), carbodiimide and DMAP (4-dimethylamino pyridine)[9]. The protected carboxylic acids (**5**, **7**, **9** and **11**) which were used, and penem intermediates (**6**, **8**, **10** and **12**) prepared from them, are shown in Table 1.

Ethers were prepared by standard methods. The sulfate (**1x**) was prepared using TMS chlorosulfate in pyridine.

Biology

The enzyme assay used for these compounds has been described[6]. The synthetic nonapeptide Phe-Ser-Ala-Ser-Ala-Leu-Ala-Lys-Ile-NH_2 was used as substrate in the assay. Peptide concentration was 500 μM and enzyme concentration 2 μM. Table 2 shows I_{50} results for the compounds, or if this was not determined, the % inhibition at compound concentrations of 10 μM and 1 μM. The limit of detection of the assay was such that I_{50} figures of below 1 μM cannot be quoted accurately.

Amongst acyl derivatives, larger hydrophobic groups reduce activity from that of acetyl (**1b**): palmitoyl (**1c**) has no activity, benzoyl (**1j**) and diacetoxybenzoyl (**1k**) reduced activity. However, the two catechols (**1l**, **1m**) show quite good activity. The inactivity of the two phenylalanine derivatives (**1g**, **1h**) probably indicates intolerance of two large groups on the α carbon of the substituent, since the alanine derivative (**1f**) is active. The weakly basic pyridine (**1n**) is well tolerated but the ionised carboxylate (**1e**) much less so (the ionised sulfate (**1x**) is also poorly active). The glycolate (**1d**) has good activity.

Of the carbamates, the *N*-methyl (**1q**) is far superior to unsubstituted (**1p**) or *N*-phenyl (**1r**). Again, in the ether series, a large hydrophobic group (TBDMS, **1w**) destroys activity, The ethyl ether (**1t**) is preferred, but other small alkyl groups are also active.

In summary, a few compounds appear in this assay to have a similar degree of activity to the initial leads (**1a** and **1b**). These have substituents which are unionised and either small (some ethers, **1d**, **1q**, can be hydrophilic or hydrophobic) or a little larger but hydrophilic (**1l**, **1m**, **1n**). However, the lack of any significantly better activity than that of (**1a** or **1b**) suggests that none of the other substituents offers considerably increased binding to the enzyme.

None of these compounds showed antibacterial activity at a useful level against a range of organisms in a standard MIC test. However, by means of a whole cell pulse-chase assay it has been demonstrated that compounds (**1a**, **1b** and **1q**) do inhibit processing of preproteins in the cytoplasmic membranes of certain strains of *E. coli* and *Staphylococcus aureus*. It has also been shown by biophotometer studies that the presence of **1a** and **1b** results in lowered growth rates of certain microorganisms. These results will be reported in detail elsewhere.

Experimental

All compounds were chromatographically pure as shown by TLC on Merck silica gel 60 F_{254} plates. Chromatography was carried out using Merck Silica gel 60, eluting with EtOAc-hexane mixtures unless otherwise stated. Optical rotations were measured on an AA-1000 polarimeter (Optical Activity Ltd). IR spectra were recorded on a Perkin-Elmer 1605 FTIR, NMR spectra at 250 MHz on a Bruker AC-F 250 machine and UV spectra on a Beckman DU-68 spectrophotometer. Mass spectra: EI and CI spectra were obtained on a VG TRIO-2, FAB spectra on a VG ZAB using Xe gas, and electrospray spectra on a Finnigan MAT TSQ700 instrument. MPs were determined on a Kofler hot stage apparatus and are uncorrected. All new compounds gave satisfactory IR, NMR, UV and MS and/or microanalysis. Compound **5** was prepared as previously[10].

Phosphorane (3)

A solution of azetidinone (**2**) (1 g, 2 mmol) and allyl glyoxylate (340 mg, 3 mmol) in benzene (10 ml) was refluxed under a Dean-Stark apparatus for 1 hour, cooled and treated with Et_3N (0.1 ml). After 90 minutes the solution was evaporated and the residue redissolved in THF (18 ml), cooled to -20°C and treated with 2,6-lutidine (0.47 ml) and thionyl chloride (0.23 ml). The mixture was warmed to room temperature, filtered and evaporated. The residue was taken up in benzene (20 ml) and evaporated, then taken up in dioxan (1.5 ml) and treated with 2,6-lutidine (0.47 ml) and triphenylphosphine (4 g). After stirring at 40°C for 18 hours, the mixture was diluted with EtOAc, washed with 0.5 N HCl and water, dried and evaporated. Chromatography

provided **3** as a foam (1.2 g, 70%): IR ν_{max} ($CHCl_3$) cm^{-1} 1750, 1615, 1260, 1105

Allyl (5*R*,6*S*,1′*R*)-6-(1′-*t*-Butyldimethylsilyloxyethyl)-penem-3-carboxylate (**4**)

A solution of phosphorane (**3**) (1.2 g, 1.39 mmol) in CH_2Cl_2 (1.5 ml)-MeOH (10 ml) was treated with pyridine (0.146 ml, 1.81 mmol) and a 0.15 M solution of $AgNO_3$ in MeOH (12.1 ml). After 30 minutes the solution was evaporated and remaining MeOH chased off with $CHCl_3$. The residue was dissolved in CH_2Cl_2 (18 ml), ice-cooled and treated with acetic formic anhydride (1.12 ml), DMAP (0.17 g) and Et_3NHCl (1.93 g). After stirring for 30 minutes the solid was filtered off, the filtrate diluted with EtOAc (100 ml), washed with 0.5 N HCl, water and $NaHCO_3$ solution, dried and allowed to stand for 1.5 hours to complete the cyclisation to penem. Chromatography provided the 5*R*-penem (**4**) (437 mg, 85%). A sample crystallised from hexane/pentane as colourless needles: MP 91~93°C; $[\alpha]_D^{22}$ +142° (*c* 0.9; $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1771, 1705, 1557; ^1H NMR ($CDCl_3$) δ 0.10 (6H, s, Me_2Si), 0.90 (9H, s, *t*Bu), 1.27 (3H, d, J=6 Hz, CH_3CH–), 3.6~3.7 (1H, m, 6-H), 4.25 (1H, quintet, J=6 Hz, CH_3CH–), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (2H, m, CH_2=CH–), 5.72 (1H, d, J=1.7 Hz, 5-H), 5.8~6.1 (1H, m, CH_2=CH–), 7.23 (1H, d, J=0.9 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 260 (2970), 317 (6830); FAB-MS (matrix 3-NOBA-Na) *m/z* 392 (100%, M+Na).

Anal Calcd for $C_{17}H_{27}NO_4SSi$:
C 55.3, H 7.4, N 3.8, S 8.7.
Found:
C 55.3, H 7.6, N 3.8, S 8.7.

Allyl (5*S*,6*S*,1′*R*)-6-(1′-*t*-Butyldimethylsilyloxyethyl)-penem-3-carboxylate (**1v**)

A solution of penem (**4**) (247 mg) in EtOAc (100 ml) in the vessel of a photochemical reactor was purged with argon for 2 hours and then irradiated through pyrex by a Hanovia medium pressure UV lamp for 30 minutes. The solution was evaporated and chromatographed; the first-eluted compound was the required 5*S*-penem (112 mg, 45%) as a white solid: $[\alpha]_D^{21}$ −214° (*c* 0.7, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1796, 1712, 1650, 1557; ^1H NMR ($CDCl_3$) δ 0.12 (6H, s, Me_2Si), 0.88 (9H, s, *t*Bu), 1.43 (3H, d, J=6 Hz, CH_3CH–), 3.92 (1H, ddd, J=10.5, 4.3 and 0.7 Hz, 6-H), 4.37 (1H, dq, J=10.1 and 6 Hz, CH_3CH–), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (2H, m, CH_2=CH–), 5.81 (1H, d, J=4.2 Hz, 5-H), 5.9~6.1 (1H, m, CH_2=CH–), 7.33 (1H, d, J=0.7 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 255 (2950), 314 (7570); FAB-MS (matrix 3-NOBA-Na) *m/z* 392 (100%, M+Na).

Anal Calcd for $C_{17}H_{27}NO_4SSi$: C 55.3, H 7.4, N 3.8.
Found: C 55.3, H 7.6, N 3.9.

The second-eluted compound was recovered (**4**) (89 mg, 36%).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Hydroxyethyl)penem-3-carboxylate (**1a**)

Silylated penem (**1v**) (1.94 g, 5.25 mmol) was dissolved in AcOH (3.6 ml, 63 mmol) and treated with a 1 M solution of TBAF in THF (21 ml). After 7 hours, the solution was diluted with EtOAc (150 ml), washed with water (twice) and $NaHCO_3$ solution, dried and evaporated. Chromatography gave (**1a**) as a pale yellow gum (1.27 g, 95%): $[\alpha]_D^{24}$ −249° (*c* 1.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3600, 3510 (br), 1796, 1710, 1650, 1560; ^1H NMR ($CDCl_3$) δ 1.47 (3H, d, J=6 Hz, CH_3CH–), 2.0 (1H, br s, OH), 3.89 (1H, dd, J=10.2 and 3.7 Hz, 6-H), 4.34 (1H, dq, J=10.2 and 6.1 Hz, CH_3CH–), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (2H, m, CH_2=CH–), 5.84 (1H, d, J=3.0 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.31 (1H, s, 2-H); UV λ_{max}^{EtoH} nm (ε) 253 (2650), 313 (6780); MS *m/z* 255.0567 (M^+, calcd for $C_{11}H_{13}NO_4S$ 255.0565).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Acetoxyethyl)penem-3-carboxylate (**1b**)

A solution of penem (**1a**) (480 mg, 1.88 mmol) in CH_2Cl_2 (10 ml) was ice-cooled and treated with Et_3N (0.52 ml), Ac_2O (0.44 ml) and a catalytic quantity of DMAP. After 30 minutes, the solution was diluted with EtOAc, washed with 0.5 N H_2SO_4, water and $NaHCO_3$ solution, dried and evaporated. Chromatography gave **1b** (543 mg, 97%). Crystallisation from EtOAc-hexane gave colourless needles: MP 45~46°C; $[\alpha]_D^{20}$ −264° (*c* 0.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1730, 1715, 1655, 1560; ^1H NMR ($CDCl_3$) δ 1.52 (3H, d, J=6.3 Hz, CH_3CH–), 2.05 (3H, s, OAc), 4.06 (1H, ddd, J=10.2, 4 and 0.8 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (3H, m, CH_2=CH– and CH_3CH–), 5.83 (1H, d, J=4.2 Hz, 5-H), 5.8~6.1 (1H, m, CH_2=CH–), 7.31 (1H, d, J=0.8 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 255 (1830), 313 (5870).

Anal Calcd for $C_{13}H_{15}NO_5S$: C 52.5, H 5.1, N 4.7.
Found: C 52.7, H 5.1, N 4.8.

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Palmitoyloxyethyl)penem-3-carboxylate (**1c**)

A solution of penem (**1a**) (51 mg, 0.2 mmol) in CH_2Cl_2 (2 ml) was treated with Et_3N (0.14 ml), palmitoyl chloride (275 mg) and a catalytic quantity of DMAP. After 1 hour, the solution was diluted with EtOAc, washed with 0.5 N H_2SO_4, water and $NaHCO_3$ solution, dried and evaporated. Chromatography gave (**1c**) as a gum (70 mg, 72%); $[\alpha]_D^{21}$ −172° (*c* 0.7, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1720, 1650, 1565; ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, $-CH_2CH_3$), 1.28 (24H, s, palmitoyl), 1.51 (3H, d, J=6.3 Hz, CH_3CH–), 1.60 (2H, m, $-CH_2CH_2CO$–), 2.27 (2H, t, $-CH_2CO$–), 4.06 (1H, ddd, J=10.2, 3.9 and 0.7 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.4 (3H, m, CH_2=CH– and CH_3CH–), 5.82 (1H, d, J=4.0 Hz, 5-H), 5.8~6.1 (1H, m, CH_2=CH–), 7.31 (1H, d, J=0.7 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 256 (2930), 311 (7500); FAB-MS (matrix 3-NOBA-Na) *m/z* 516 (70%, M+Na), 170 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Benzoyloxyethyl)penem-3-carboxylate (**1j**)

Analogous preparation to that for **1c** gave **1j** as colourless needles (59%); MP 146~148°C; $[\alpha]_D^{22}$ −283° (*c* 0.5, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 1791, 1702, 1648, 1555; ^1H NMR ($CDCl_3$) δ 1.68 (3H, d, J=6.3 Hz, CH_3CH–), 4.22 (1H, dd, J=9.8 and 4.3 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (2H, m, CH_2=CH–), 5.5~5.7 (1H, m, CH_3CH–), 5.91 (1H, d, J=4.0 Hz, 5-H), 5.9~6.1 (1H, m, CH_2=CH–), 7.33 (1H, s, 2-H), 7.4~7.5 (2H, m, Ar-H), 7.55~7.7 (1H, m, Ar-H), 7.9~8.1 (2H, m, Ar-H); UV λ_{max}^{EtOH} nm (ε) 229 (14975), 313 (7150).

Anal Calcd for $C_{18}H_{17}NO_5S$: C 60.2, H 4.8, N 3.9, S 8.9.
Found: C 60.2, H 4.7, N 4.0, S 8.7.

3,4-(Bis-*t*-butyldiphenylsilyloxy)benzoic Acid (**9**)

A solution of 3,4-dihydroxybenzoic acid (308 mg, 2 mmol) in DMF (4 ml) was treated with imidazole (560 mg) and *t*-butyldiphenylsilyl chloride (2.08 ml). After stirring for 3 days, the mixture was diluted with EtOAc, washed with 0.5 N H_2SO_4 and water, dried and evaporated. Chromatography gave *t*-butyldiphenylsilyl 3,4-(bis-*t*-butyldiphenylsilyloxy)benzoate as a white foam (1.5 g, 86%): IR ν_{max} ($CHCl_3$) cm^{-1} 1696; ^1H NMR ($CDCl_3$) δ 0.79 (9H, s, *t*Bu), 1.16 (18H, s, *t*Bu), 6.46 (1H, d, J=8.5 Hz, Ar-H), 7.1~7.9 (32H, m, Ar-H). A solution of this ester in MeOH (12 ml) +CH_2Cl_2 (8 ml) was treated with a solution of KF (110 mg) in MeOH (3 ml). After 1 hour the solution was diluted with EtOAc, washed with 0.5 N H_2SO_4 and water, dried and evaporated. Crystallisation from EtOAc-hexane gave the acid (**9**) as white microcrystals (823 mg, 75%): MP 237~239°C; IR ν_{max} (KBr) cm^{-1} 1682, 1596, 1577, 1515; ^1H NMR ($CDCl_3$) δ 1.12 (9H, s, *t*Bu), 1.19 (9H, s, *t*Bu), 6.40 (1H, d, J=8.4 Hz, Ar-H), 7.09 (1H, dd, J=6.4 and 2.0 Hz, Ar-H), 7.19 (1H, d, J=2.0 Hz, Ar-H), 7.3~7.9 (20H, m, Ph); UV λ_{max}^{EtOH} nm (ε) 252 (12170), 291 (3580).

Anal Calcd for $C_{39}H_{42}NO_4Si_2$: C 74.2, H 6.7.
Found: C 74.2, H 6.9.

3,4-(Bis-*t*-butyldiphenylsilyloxy)phenylacetic Acid (**11**)

Analogous preparation to that for **9** and isolation by chromatography gave **11** as a white foam (89%): IR ν_{max} ($CHCl_3$) cm^{-1} 1711, 1605, 1580, 1510; ^1H NMR ($CDCl_3$) δ 1.14 (9H, s, *t*Bu), 1.17 (9H, s, *t*Bu), 3.06 (2H, s, $-CH_2COOH$), 6.2~6.4 (3H, m, Ar-H), 7.2~7.5 (12H, m, Ar-H), 7.7~7.9 (8H, m, Ar-H); Electrospray-MS *m/z* 662 (100%, M+NH_4).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(*t*-Butyldiphenylsilyloxy)-acetoxyethyl]penem-3-carboxylate (**6**): General Method for Carbodiimide/DMAP-mediated Coupling

A solution of penem (**1a**) (51 mg, 0.2 mmol) and **5** (250 mg, 0.8 mmol) in CH_2Cl_2 (1 ml) was cooled to −15°C and treated with DMAP (25 mg, 0.2 mmol) and 1-dimethylaminopropyl-3-ethylcarbodiimide hydrochloride (300 mg). The reaction was stirred for 20 minutes at −15°C and 30 minutes at 0°C, diluted with EtOAc, washed with 0.5 N H_2SO_4, water and $NaHCO_3$ solution, dried and evaporated. Chromatography gave **6** as a gum (98 mg, 89%): $[\alpha]_D^{19}$ −138° (*c* 1.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1765, 1715, 1650, 1560; ^1H NMR ($CDCl_3$) δ 1.10 (9H, s, *t*Bu), 1.46 (3H, d, J=6.3 Hz, CH_3CH–), 3.92 (1H, ddd, J=10.5, 4.0 and 0.7 Hz, 6-H), 4.23 (2H, s, $-COCH_2O$–), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (3H, m, CH_2=CH– and CH_3CH–), 5.68 (1H, d, J=4.0 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.29 (1H, d, J=0.7 Hz, 2-H), 7.3~7.5 (6H, m, Ph-H), 7.6~7.7 (4H, m, Ph-H); UV λ_{max}^{EtOH} nm (ε) 258 (3930), 312 (8310); FAB-MS (matrix 3-NOBA-Na) *m/z* 574 (30%, M+Na), 359 (80%), 170 (100%).

In a similar way were made the following:

Allyl (5*S*,6*S*,1′*R*)-6-(1′-*p*-Nitrobenzylsuccinyloxyethyl]penem-3-carboxylate (**8**)

Gum (90%): IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1735, 1670, 1615, 1560, 1520; ^1H NMR ($CDCl_3$) δ 1.51 (3H, d, J=6.3 Hz, CH_3CH–), 2.5~2.9 (4H, m, $-CH_2CH_2$–), 4.08 (1H, ddd, J=10.0, 4.0 and 0.7 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (5H, m, $-CH_2Ar$, CH_2=CH– and CH_3CH–), 5.79 (1H, d, J=4.0 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.32 (1H, d, J=0.8 Hz, 2-H), 7.52 (2H, d, Ar-H), 8.22 (2H, d, Ar-H); Thermospray-MS *m/z* 508 (100%, M+NH_4).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(3,4-Bis-*t*-butyldiphenylsilyloxybenzoyloxy)ethyl]penem-3-carboxylate (**10**)

Gum (20%): $[\alpha]_D^{20}$ −98° (*c* 0.8, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1794, 1715, 1660, 1600, 1585, 1570, 1515; ^1H NMR ($CDCl_3$) δ 1.14 (9H, s, *t*Bu), 1.18 (9H, s, *t*Bu), 1.33 (3H, d, J=6.2 Hz, CH_3CH–), 3.73 (1H, dd, J=10.2 and 4.1 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.16 (1H, dq, J=10.3 and 6.2 Hz, CH_3CH–), 5.2~5.4 (3H, m, 5-H and CH_2=CH–), 5.8~6.0 (1H, m, CH_2=CH–), 6.44 (1H, d, J=9.0 Hz, Ar-H), 7.0~7.1 (2H, m, Ar-H), 7.21 (1H, d, J=0.6 Hz, 2-H), 7.3~7.5 (12H, m, Ph-H), 7.7~7.8 (8H, m, Ph-H); UV λ_{max}^{EtOH} nm (ε) 261 (18140), 302 (13100); FAB-MS (matrix 3-NOBA-Na) *m/z* 890 (35%, M+Na), 613 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(3,4-Bis-*t*-butyldiphenylsilyloxyphenylacetyloxy)ethyl]penem-3-carboxylate (**12**)

White foam (100%): IR ν_{max} ($CHCl_3$) cm^{-1} 1792, 1718, 1650, 1604, 1575, 1561, 1514; ^1H NMR ($CDCl_3$) δ 1.14 (9H, s, *t*Bu), 1.17 (9H, s, *t*Bu), 1.27 (3H, d, J=6.3 Hz, CH_3CH–), 2.99 (2H, s, $-CH_2Ar$), 3.59 (1H, dd, J=10.0 and 3.9 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 4.98 (1H, dq, J=10.4 and 6.2 Hz, CH_3CH–), 5.19 (1H, d, J=4.2 Hz, 5-H), 5.2~5.5 (2H, m, CH_2=CH–), 5.9~6.1 (1H, m, CH_2=CH–), 6.24 (1H, dd, J=8.2 and 2.1 Hz, Ar-H), 6.3-6.4 (2H, m, Ar-H), 7.11 (1H, d, J=0.8 Hz, 2-H), 7.3~7.5 (12H, m, Ph-H), 7.7~7.9 (8H, m, Ph-H); UV λ_{max}^{EtOH} nm (ε) 290 (5310), 313 (6990); FAB-MS (matrix 3-NOBA-Na) *m/z* 904 (25%, M+Na), 197 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(Benzyloxycarbonyl-L-alanyloxy)ethyl]penem-3-carboxylate (**1f**)

Gum (60%): $[\alpha]_D^{21}$ −250° (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3420, 1795, 1715, 1660, 1565, 1510; ^1H NMR ($CDCl_3$) δ 1.40 (3H, d, J=7.2 Hz, CH_3CH–), 1.51 (3H, d, J=6.2 Hz, CH_3CH–), 4.06 (1H, dd, J=10.1 and 3.8 Hz, 6-H), 4.28 (1H, quintet, J=7.2 Hz, alanyl CH), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.11 (2H, s, $-CH_2Ph$), 5.1~5.5 (4H, m, NH, CH_2=CH– and CH_3CH–), 5.78 (1H, d, J=3.9 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.2~7.4 (6H, m, Ph-H and 2-H); UV λ_{max}^{EtOH} nm (ε) 252 (3400), 312 (7890); FAB-MS (matrix 3-NOBA-Na) *m/z* 483 (60%, M+Na), 176 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(Methoxycarbonyl-L-phenylalanyloxy)ethyl]penem-3-carboxylate (**1g**)

Gum (83%): $[\alpha]_D^{20}$ −200° (*c* 0.8, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3440, 1795, 1715, 1660, 1565, 1510; ^1H NMR ($CDCl_3$) δ 1.42 (3H, d, J=6.3 Hz, CH_3CH–), 3.04 (2H, d, J=6.5 Hz, $-CH_2Ph$), 3.68 (3H, s, OMe), 4.01 (1H, dd, J=10.1 and 4.2 Hz, 6-H), 4.51 (1H, q, J=7.3 Hz, –COCHN–), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.13 (1H, d, J=7.8 Hz, NH), 5.2~5.5 (3H, m, CH_2=CH– and CH_3CH–), 5.77 (1H, d, J=4.0 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.1~7.4 (6H, m, Ph-H and 2-H); UV λ_{max}^{EtOH} nm (ε) 254 (2750), 314 (6720); FAB-MS (matrix 3-NOBA-Na) *m/z* 483 (55%, M+Na), 176 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(Methoxycarbonyl-D-phenylalanyloxy)ethyl]penem-3-carboxylate (**1h**)

Gum (82%): $[\alpha]_D^{21}$ −201° (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3450, 1795, 1715, 1660, 1565, 1510; ^1H NMR ($CDCl_3$) δ 1.50 (3H, d, J=6.3 Hz, CH_3CH–), 3.04 (2H, d, J=6.5 Hz, $-CH_2Ph$), 3.64 (3H, s, OMe), 3.91 (1H, dd, J=10.0 and 4.0 Hz, 6-H), 4.5~4.8 (3H, m, –COCHN– and $-CH_2CH$=), 5.11 (1H, d, J=7.8 Hz, NH), 5.2~5.5 (3H, m, CH_2=CH– and CH_3CH–), 5.63 (1H, d, J=4.0 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.1~7.4 (6H, m, Ph-H and 2-H); UV λ_{max}^{EtOH} nm (ε) 254 (3780), 312 (8630); FAB-MS (matrix 3-NOBA-Na) *m/z* 483 (35%, M+Na), 176 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(3,4-Diacetoxybenzoyloxy)-ethyl]penem-3-carboxylate (**1k**)

Gum (15%): $[\alpha]_D^{18}$ −170° (*c* 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1790, 1720, 1650, 1605, 1585, 1560; ^1H NMR ($CDCl_3$) δ 1.63 (3H, d, J=6.3 Hz, CH_3CH–), 2.33 (6H, s, $COCH_3$), 4.20 (1H, dd, J=10.0 and 4.0 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (2H, m, CH_2=CH–), 5.54 (1H, dq, J=10.0 and 6.3 Hz, CH_3CH–), 5.88 (1H, d, J=4.0 Hz, 5-H), 5.9~6.1 (1H, m, CH_2=CH–), 7.29 (1H, d, J=8.5 Hz, Ar-H), 7.32 (1H, d, J=0.8 Hz, 2-H), 7.82 (1H, d, J=2.0 Hz, Ar-H), 7.91 (1H, dd, J=8.5 and 2.0 Hz, Ar-H); UV λ_{max}^{EtOH} nm (ε) 236 (5880), 313 (2680); FAB-MS (matrix 3-NOBA-Na) *m/z* 498 (30%, M+Na), 176 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(3-Pyridylacetoxy)ethyl]-penem-3-carboxylate (**1n**)

Gum (53%): $[\alpha]_D^{18}$ −209° (*c* 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1792, 1717, 1650, 1563; ^1H NMR ($CDCl_3$) δ 1.52 (3H, d, J=6.3 Hz, CH_3CH–), 3.62 (2H, s, $-CH_2Ar$), 4.06 (1H, dd, J=10.3 and 4.0 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.4 (3H, m, CH_2=CH– and CH_3CH–), 5.72 (1H, d, J=4.0 Hz, 5-H), 5.8~6.0 (1H, m, CH_2=CH–), 7.25 (1H, d, J=0.7 Hz, 2-H), 7.25~7.35 (1H, m, Ar-H), 7.66 (1H, d, J=7.9 Hz, Ar-H), 8.54 (2H, br s, Ar-H); UV λ_{max}^{EtOH} nm (ε) 256 (5420), 260 (5510), 312 (7400); FAB-MS (matrix thioglycerol) *m/z* 375 (100%, M+H).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Hydroxyacetoxyethyl)penem-3-carboxylate (**1d**)

A solution of the silylated penem (**6**) (95 mg, 0.17 mmol) in THF (1 ml) was treated with AcOH (0.06 ml) and a THF solution of 1 M TBAF (0.35 ml). After 1 hour the solution was diluted with EtOAc, washed with water, $NaHCO_3$ solution and water, dried and evaporated. Chromatography gave **1d** as a gum (40 mg, 74%): $[\alpha]_D^{19}$ −248° (*c* 0.3, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3580, 1794, 1745, 1717, 1650, 1560; ^1H NMR ($CDCl_3$) δ 1.57 (3H, d, J=6.3 Hz, CH_3CH–), 2.49 (1H, br s, OH), 4.0~4.2 (3H, m, 6-H and $-CH_2OH$), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.5 (3H, m, CH_2=CH– and CH_3CH–), 5.85 (1H, d, J=4.0 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 7.33 (1H, d, J=0.7 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 256 (3380), 311 (7610); ionspray-MS *m/z* 644 (10%, 2M+NH_4), 331 (100%, M+NH_4).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Sodiosuccinyloxyethyl)penem-3-carboxylate (**1e**)

A solution of penem (**8**) (170 mg, 0.35 mmol) in THF (15 ml) was ice-cooled, stirred vigorously and treated with 1 M NH_4Cl solution (12 ml) and iron powder (1.5 g). After 1.25 hours the mixture was diluted with EtOAc and water, acidified with dil. H_2SO_4 to pH 2 and filtered. The layers were separated and the organic washed with water and evaporated. The residue was taken up in THF (5 ml), 0.1 N $NaHCO_3$ (5 ml) added and stirred 3 hours. After washing with ether, remaining organic solvent was evaporated and the aqueous solution applied to a column of HP20SS resin. Elution with water, then 20% acetone-water, followed by partial evaporation and freeze-drying of the compound-containing eluate gave **1e** as a hygroscopic solid (35 mg, 27%): $[\alpha]_D^{21}$ −241° (*c* 0.2, H_2O); IR ν_{max} (KBr) cm^{-1} 1791, 1718, 1575, 1560; ^1H NMR (D_2O) δ 1.44 (3H, d, J=6.3 Hz, CH_3CH–), 2.3~2.7 (4H, m, $-CH_2CH_2$–), 4.48 (1H, dd, J=9.8 and 3.8 Hz, 6-H), 4.6~4.8 (2H, m, $-CH_2CH$=), 5.2~5.4 (3H, m, CH_2=CH– and CH_3CH–), 5.9~6.1 (2H, m including 1H, d, J=4.0 Hz at δ 5.94, 5-H and CH_2=CH–), 7.69 (1H, s, 2-H); UV $\lambda_{max}^{H_2O}$ nm (ε) 252 (3120), 316 (6380); FAB-MS (matrix thioglycerol) *m/z* 378 (20%, M+H).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(3,4-Dihydroxybenzoyloxy)-ethyl]penem-3-carboxylate (**1l**)

Desilylation of (**10**) was carried out analogously to that of (**6**) to give **1l** as a foam (72%): $[\alpha]_D^{20}$ −208° (*c* 1.3, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3538, 3368, 1795, 1714, 1649, 1608, 1562, 1524; ^1H NMR ($CDCl_3$) δ 1.63 (3H, d, J=6.4 Hz, CH_3CH–), 4.19 (1H, ddd, J=9.7, 4.0 and 0.7 Hz, 6-H), 4.6~4.8 (2H, m, –CH_2CH=), 5.2~5.6 (3H, m, CH_2=CH– and CH_3CH–), 5.85~6.05 (2H, m including 1H, d, J=4.0 Hz at 5.88 δ, 5-H and CH_2=CH–), 6.44 (2H, br s, OH), 6.91 (1H, d, J=8.3 Hz, Ar-H), 7.35 (1H, d, J=0.7 Hz, 2-H), 7.4~7.6 (2H, m, Ar-H); UV λ_{max}^{EtOH} nm (ε) 221 (19440), 264 (11970), 305 (12420); FAB-MS (matrix 3-NOBA-Na) *m/z* 414 (7%, M+Na), 176 (100%), (matrix thioglycerol) *m/z* 392 (8%, M+H), 149 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-[1′-(3,4-Dihydroxyphenyl-acetoxy)ethyl]penem-3-carboxylate (**1m**)

Desilylation of (**12**) was carried out analogously to that of (**6**) to give **1m** as a gum (80%): $[\alpha]_D^{20}$ −185° (*c* 0.4, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3550, 3410, 1795, 1717, 1649, 1614, 1562, 1520; ^1H NMR ($CDCl_3$) δ 1.49 (3H, d, J=6.3 Hz, CH_3CH–), 3.47 (2H, s, –CH_2Ar), 4.04 (1H, dd, J=10.2 and 4.0 Hz, 6-H), 4.6~4.8 (2H, m, –CH_2CH=), 5.2~5.4 (3H, m, CH_2=CH– and CH_3CH–), 5.69 (1H, d, J=4.0 Hz, 5-H), 5.8~6.1 (3H, m, CH_2=CH– and OH, reduces to 1H, m, on D_2O exchange), 6.63 (1H, dd, J=8.0 and 2.0 Hz, Ar-H), 6.7~6.8 (2H, m, 2H and Ar-H), 7.25 (1H, d, J=3.6 Hz, Ar-H); UV λ_{max}^{EtOH} nm (ε) 291 (6070), 313 (7100); electrospray-MS *m/z* 428 (100%, M+Na).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Carbamoyloxyethyl)penem-3-carboxylate (**1p**)

A solution of penem (**1a**) (100 mg, 0.39 mmol) in EtOAc (0.3 ml) was treated with trichloracetyl isocyanate (0.3 ml) and left for 5 hours. The mixture was diluted with EtOAc, washed with $NaHCO_3$ solution, dried and evaporated. Chromatography caused decomposition of the trichloracetyl carbamate and gave **1p** as a solid (95 mg, 81%). Crystallisation (EtOAc-hexane) provided colourless needles: MP 161~163°C; $[\alpha]_D^{21}$ −290° (*c* 0.7, $CHCl_3$); IR ν_{max} (KBr) cm^{-1} 3439, 3357, 3291, 3211, 1776, 1717, 1661, 1620, 1553; ^1H NMR ($CDCl_3$) δ 1.53 (3H, d, J=6.3 Hz, CH_3CH–), 4.06 (1H, dd, J=9.9 and 3.6 Hz, 6-H), 4.60 (2H, br s NH_2), 4.6~4.8 (2H, m, –CH_2CH=), 5.1~5.4 (3H, m, CH_2=CH– and CH_3CH–), 5.85 (1H, d, J=4.0 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 7.32 (1H, d, J=0.8 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 254 (2860), 312 (7790); FAB-MS (matrix 3-NOBA-Na) *m/z* 321 (70%, M+Na), 176 (100%).

Anal Calcd for $C_{12}H_{14}N_2O_5S$: C 48.3, H 4.7, N 9.4, S 10.8.
Found: C 48.2, H 4.6, N 9.4, S 10.8.

Allyl (5*S*,6*S*,1′*R*)-6-(1′-*N*-Methylcarbamoyloxy-ethyl)penem-3-carboxylate (**1q**)

A solution of penem (**1a**) (300 mg, 1.18 mmol) in methyl isocyanate (2.5 ml) was treated with a catalytic quantity of DMAP and left for 3 days. The solvent was evaporated and the residue dissolved in toluene, washed with 0.5 N H_2SO_4 and water, dried and evaporated. Chromatography and crystallisation (EtOAc-hexane) gave **1q** (200 mg, 54%) as colourless needles: MP 127~129°C; $[\alpha]_D^{20}$ −256° (*c* 0.4, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3466, 1793, 1717, 1650, 1561, 1520; ^1H NMR ($CDCl_3$) δ 1.52 (3H, d, J=6.3 Hz, CH_3CH–), 2.80 (3H, d, J=4.9 Hz, CH_3NH), 4.03 (1H, dd, J=9.9 and 4.0 Hz, 6-H), 4.60 (1H, br s NH), 4.6~4.8 (2H, m, –CH_2CH=), 5.1~5.4 (3H, m, CH_2=CH– and CH_3CH–), 5.84 (1H, d, J=4.0 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 7.34 (1H, d, J=0.8 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 252 (2940), 312 (8130); MS *m/z* 312.0782 (M^+, calcd for $C_{13}H_{16}N_2O_5S$ 312.0780).

Anal Calcd for $C_{13}H_{16}N_2O_5S$: C 50.0, H 5.2, N 9.0.
Found: C 50.0, H 5.0, N 8.8.

Allyl (5*S*,6*S*,1′*R*)-6-(1′-*N*-Phenylcarbamoyloxy-ethyl)penem-3-carboxylate (**1r**)

A solution of penem (**1a**) (24 mg, 0.094 mmol) in phenyl isocyanate (0.5 ml) was treated with a catalytic quantity of DMAP. After 1 hour the mixture was dissolved in dichloromethane, washed with 1 N HCl and brine, dried and evaporated. Chromatography followed by preparative HPLC (C18 column, eluted with MeCN-H_2O 2:1) gave **1r** as a gum (5 mg, 17%); IR ν_{max} ($CHCl_3$) cm^{-1} 3430, 3300(br), 1795, 1715, 1650, 1560, 1525; ^1H NMR ($CDCl_3$) δ 1.60 (3H, d, J=6.3 Hz, CH_3CH–), 4.10 (1H, ddd, J=10.2, 4.0 and 0.8 Hz, 6-H), 4.6~4.8 (2H, m, –CH_2CH=), 5.2~5.5 (3H, m, CH_2=CH– and CH_3CH–), 5.88 (1H, d, J=4.0 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 6.58 (1H, br s, NH), 7.0~7.1 (1H, m, Ph-H), 7.3~7.5 (5H, m, Ph-H and 2-H); MS (ammonia chemical ionisation) *m/z* 392 (5%, M+NH_4), 94 (100%).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Methoxyethyl)penem-3-carboxylate (**1s**)

A solution of penem (**1a**) (50 mg, 0.2 mmol) in nitromethane (2 ml) was ice-cooled and treated with powdered Na_2CO_3 (400 mg) and trimethyloxonium fluoroborate (250 mg). After stirring for 4 hours with ice cooling and a further 3 hours at room temperature, the mixture was filtered through celite, diluted with CH_2Cl_2, washed with water, dried and evaporated. Chromatography gave **1s** as a gum (9 mg, 17%): $[\alpha]_D^{21}$ −210° (*c* 1, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1715, 1650, 1560; ^1H NMR ($CDCl_3$) δ 1.40 (3H, d, J=5.6 Hz, CH_3CH–), 3.33 (3H, s, OMe), 3.8~4.0 (2H, m, 6-H and CH_3CH–), 4.6~4.8 (2H, m, –CH_2CH=), 5.2~5.5 (2H, m, CH_2=CH–), 5.81 (1H, d, J=3.8 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 7.31 (1H, s, 2-H); UV λ_{max}^{EtOH} nm (ε) 253 (2690), 314 (6520); MS *m/z* 269.0726 (M^+, calcd for $C_{12}H_{15}NO_4S$ 269.0722).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Ethoxyethyl)penem-3-carboxylate (**1t**)

Penem (**1t**) was prepared analogously to (**1s**), apart from the use of CH_2Cl_2 as solvent, to give a gum (12%): $[\alpha]_D^{19}$ −214° (*c* 0.6, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1795, 1715, 1650, 1560; ^1H NMR ($CDCl_3$) δ 1.18 (3H, t, *J*= 7.0 Hz, CH_3CH_2–), 1.40 (3H, d, *J*=5.5 Hz, CH_3CH–), 3.35 and 3.68 (2H, 2dq, *J*=8.8 and 7.0 Hz, CH_3CH_2–), 3.8~4.0 (2H, m, 6-H and CH_3CH–), 4.6~4.8 (2H, m, –CH_2CH=), 5.2~5.5 (2H, m, CH_2=CH–), 5.82 (1H, d, *J*=3.7 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 7.33 (1H, s, 2-H); UV λ_{max}^{EtOH} nm (ε) 253 (3230), 313 (7370); MS *m/z* 283.0882 (M^+, calcd for $C_{13}H_{17}NO_4S$ 283.0878).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Methoxymethoxyethyl)penem-3-carboxylate (**1u**)

A solution of penem (**1a**) (54 mg, 0.21 mmol) in CH_2Cl_2 (0.2 ml) was treated with diisopropylethylamine (0.14 ml) and chloromethylmethyl ether (0.06 ml) and stirred for 18 hours. The solution was diluted with EtOAc, washed with 1 N HCl and $NaHCO_3$ solution, dried and evaporated. Chromatography gave **1u** as a gum (54 mg, 85%): $[\alpha]_D^{20}$ −235° (*c* 0.9, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1791, 1715, 1650, 1563; ^1H NMR ($CDCl_3$) δ 1.48 (3H, d, *J*=6.2 Hz, CH_3CH–), 3.38 (3H, s, OMe), 3.98 (1H, ddd, *J*=10.3, 4.0 and 0.8 Hz, 6-H), 4.1~4.25 (1H, m, CH_3CH–), 4.6~4.8 (4H, m, –CH_2CH= and –OCH_2O–), 5.2~5.5 (2H, m, CH_2=CH–), 5.84 (1H, d, *J*=4.0 Hz, 5-H), 5.85~6.05 (1H, m, CH_2=CH–), 7.32 (1H, d, *J*=0.8 Hz, 2-H); UV λ_{max}^{EtOH} nm (ε) 252 (3130), 314 (7700); MS *m/z* 299.0833 (M^+, calcd for $C_{13}H_{17}NO_5S$ 299.0827).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Ethoxycarbonylmethoxyethyl)penem-3-carboxylate (**1v**)

A solution of penem (**1a**) (25 mg, 0.1 mmol) in CH_2Cl_2 (0.5 ml) at 0°C was treated with boron trifluoride etherate (3 mg) and ethyl diazoacetate (0.02 ml) and stored at 4°C for 3 days. Chromatography gave **1v** as a gum (5 mg, 15%): $[\alpha]_D^{20}$ −228° (*c* 0.1, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1790, 1752, 1716, 1650, 1560; ^1H NMR ($CDCl_3$) δ 1.30 (3H, t, *J*=7.2 Hz, CH_3CH_2–), 1.45 (3H, d, *J*= 5.4 Hz, CH_3CH–), 4.0~4.1 (3H, m, –OCHHCO–, CH_3CH– and 6-H), 4.13 (1H, d, *J*=15.8 Hz, –OCHH-CO–), 4.23 (2H, q, *J*=7.2 Hz, –OCH_2CH_3), 4.6~4.8 (2H, m, –CH_2CH=), 5.2~5.5 (2H, m, CH_2=CH–), 5.89 (1H, d, *J*=3.7 Hz, 5-H), 5.9~6.0 (1H, m, CH_2=CH–), 7.31 (1H, s, 2-H); UV λ_{max}^{EtOH} nm (ε) 314 (7500); MS *m/z* 341.0937 (M^+, calcd for $C_{15}H_{19}NO_6S$ 341.0933).

Allyl (5*S*,6*S*,1′*R*)-6-(1′-Sodiosulfatoethyl)penem-3-carboxylate (**1x**)

A solution of penem (**1a**) (51 mg, 0.2 mmol) in pyridine (1 ml) was ice-cooled and treated with trimethylsilyl chlorosulfate (0.1 ml, 0.6 mmol). After 4 hours, the solvent was evaporated and the residue chromatographed on silica: non-polar material was eluted with EtOAc and the required compound with *n*-butanol - ethanol - water (4 : 1 : 1). After evaporation of butanol and ethanol, the remaining solution was adjusted to pH 7 with dil. NaOH and passed through a column of Amberlite IR120(Na). The solution of sodium salt was finally chromatographed on HP20SS resin, eluting with water and then 20% acetone-water. An aqueous solution was freeze-dried to provide **1x** as a white solid (12.5 mg, 17.5%): $[\alpha]_D^{20}$ −268° (*c* 0.1; H_2O); IR ν_{max} (KBr) cm^{-1} 1786, 1713, 1645, 1560, 1268, 1220; ^1H NMR (D_2O) δ 1.58 (3H, d, *J*=6.3 Hz, CH_3CH–), 4.39 (1H, dd, *J*=10.0 and 3.8 Hz, 6-H), 4.76 (2H, dd, *J*=5.4 and 1.2 Hz, –CH_2CH=), 4.9~5.1 (1H, m, CH_3CH–), 5.3~5.5 (2H, m, CH_2=CH–), 5.9~6.1 (2H, m including 1H, d, *J*=4.0 Hz at δ 6.0, 5-H and CH_2=CH–), 7.75 (1H, d, *J*=0.8 Hz, 2-H); UV $\lambda_{max}^{H_2O}$ nm (ε) 250 (2930), 314 (7560); electrospray-MS *m/z* 737 (100%, 2M+Na), 358 (75%, M+H).

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