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Structure–Activity Relationships of Penem Antibiotics: Crystallographic Structures and Implications for their Antimicrobial Activities

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Abstract—Twelve closely related crystal structures of the penem derivatives revealed a characteristic short contact of the oxygen atom in the C2 side-chains with the S1 atom. The side-chain conformations of the crystal structures showed a good correlation with the antimicrobial activity. In particular, the penems which show high antimicrobial activity have similar torsion angles for S1-C2-C1′-C2′, suggesting that the disposition of the C2′ atom would be important for binding to penicillin-interacting enzymes. Two conformations of the C6 hydroxyethyl group were observed in the crystal structures. Of those two, the conformation with a larger torsion angle (δ = 179.2°) is deduced to be the enzyme-bound conformation in the Michaelis complex. © 1997 Elsevier Science Ltd.

Introduction

Among β-lactam antibiotics, penems show potent activity against a broad spectrum of bacteria, including β-lactamase-producing organisms.[1] Although the penem skeleton was designed as a hybrid of penicillin and cephalosporin,[2] the penem antibiotics show more close structural relation to carbapenem. Thus, C6 alkyl substituents such as ethyl, hydroxymethyl and hydroxyethyl moieties are characteristic substituents in penem antibiotics. Of those three moieties, the hydroxyethyl group with R configuration at C8 is the most effective substituent for the antimicrobial activity. Although sulfur-bearing substituents at C2 are quite effective for antimicrobial activity, cephalosporin-like substituents at C2 have also been shown to be active against various microorganisms.[3] We have previously demonstrated the synthesis and antimicrobial activity of penem antibiotics having chiral carbon in C2 side-chains.[4] In this series of derivatives, we found that penems having cyclic derivatives at C2 were more active than those having acyclic side-chains. It was also interesting that the tetrahydrofuran substituent was more effective for the activity than the corresponding furan ring. Hence, the analysis of the relation of the activity and the conformation of those C2 side-chains should give useful information for elucidation of the enzyme-bound structure of the penem derivatives. Since 3-D structural data of the target enzymes, penicillin-binding proteins, are not available at this moment, we estimated enzyme-bound conformations of those C2 substituents by means of X-ray crystallography and molecular mechanics. In particular, the crystal structures suggested a specific S–O interaction which would contribute to restrict the C2 side-chains in a specific conformation. Examination of the conformation of the C6 side-chain by the same methods has also revealed a plausible conformation for the enzyme binding.

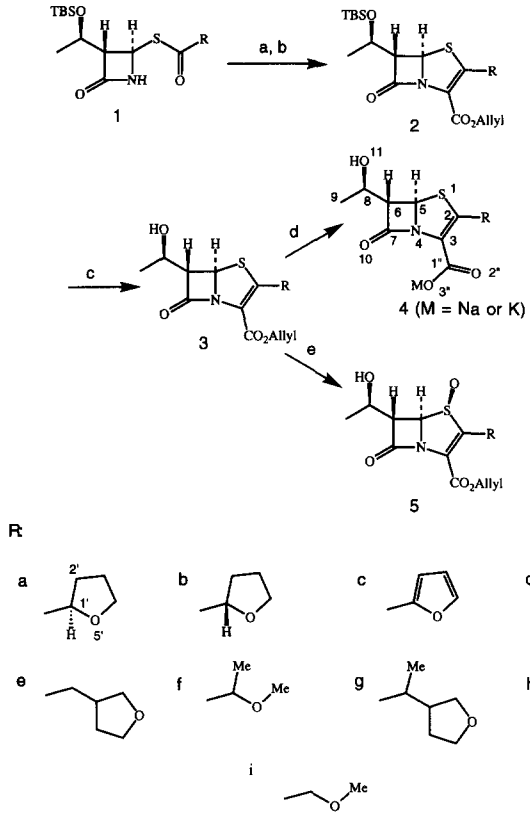
Chemistry

The penem derivatives were prepared by the methods described previously (Scheme 1).[4] The thioesters (**1a–i**) were transformed to the penem structures by means of intramolecular Wittig cyclization reaction. The *t*-butyldimethylsilyl (TBS) group of the penem esters **2a–i** was then removed to afford the alcohol esters **3a–i**. Treatment of the allyl esters **3** with tetrakistriphenylphosphine palladium(0) in the presence of sodium (or potassium) 2-ethylhexanoate gave carboxylate derivatives **4a–i**. A pure diasteromer **4h** (M=H), which was obtained by recrystallization of a diastereomeric mixture of **4e**, was converted to the methyl ester **6** (**4h**, M=Me) by treatment with diazomethane. The ester **6** formed a crystal suitable for crystallographic analysis. The penem β-sulfoxides **5a** and **5b** were prepared by oxidation of the sulfur atom of the allyl esters **3a** and **3b** with *m*-chloroperbenzoic acid in dichloromethane.[5]

The penem derivative **10** was synthesized from the 3*R*,4*S*-azetidinone derivative **7** using the photoisomerization reaction of the 5*S*-penem derivative **9** as a key step (Scheme 2),[6] after removal of the silyl protecting group of **8**. Subsequent deallylation gave the *cis*-penem **11**. The preparation of the 6-dimethyl-substituted penem **12** was described previously.[5]

Crystal structures of penem compounds

Crystal data and experimental details for the penem compounds **3a**, **3c**, **3d**, **4a** (M=Na)[7], **4a** (M=K), **4b** (M=Na), **4i**, **5a**, **5b**, **6** (**4h**, M=Me), **10**,[8] and **12** are shown in Table 1. Three-dimensional structures of those compounds are depicted in Figure 1. Selected torsion angles for the moieties at C-2 [S1-C2-C1′-C2′ (α), S1-C2-C1′-O5′ (β)], the carboxylate at C-3 [C2-C3-



Scheme 1. Reagents: (a) $ClCOCO_2Allyl$, Et_3N; (b) $(EtO)_3P$, reflux in xylene; (c) Bu_4NF, AcOH, THF; (d) $(Ph_3P)_4Pd$, sodium 2-ethylhexanoate; (e) *m*-chloroperbenzoic acid.

C1″-O2″ (γ)], and the hydroxyethyl group at C-6 [C7-C6-C8-O11 (δ)] are listed in Table 2. The atomic distances (d_1) between S1 and O5′ and those (d_2) between the β-lactam carbonyl oxygen (O10) and one of the carboxyl oxygen (O3″) are also shown in Table 2 (for atomic numbering, see compound **4** in Scheme 1).

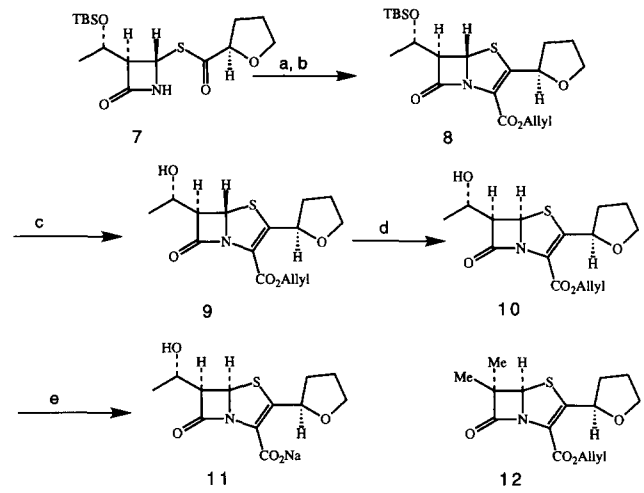
Conformational analysis of penem side-chains at C-2, C-3, and C-6

Conformations of the side-chains at C2 of the crystal structures of **4h** were generated in 10° intervals with respect to the S1-C2-C1′-C2′ (α) and C2-C1′-C2′-C3′ (ε). Energies for those conformations were estimated with MM2 implemented in Macromodel (v. 3.0).[9] Figure 2 shows the energy-contour map for the C2 side-chain. Conformations of the hydroxyethyl side-chain at C6 were also generated in 10° intervals and the conformational energies for the *trans* and *cis* penems **3a** and **10** are plotted in Figure 3.

Results and Discussion

Biological activity of the penem derivatives

Antimicrobial activity of the penem derivatives (**4a–g**, **4i**, and **11**) is shown in Table 3. The activity of five derivatives (**4a–c**, **4f**, and **11**) has been described previously.[4,6] The penem **4a** with the tetrahydrofuran



Scheme 2. Reagents: (a) $ClCOCO_2Allyl$, Et_3N; (b) $(EtO)_3P$, reflux in xylene; (c) Bu_4NF, AcOH, THF; (d) hν; (e) $(Ph_3P)_4Pd$, sodium 2-ethylhexanoate.

ring at C2 is significantly more active than the corresponding acyclic derivative **4f**. On the other hand, the furan-bearing penem **4c** is less active than the tetrahydrofuran derivative **4a**, while the benzene-substituted penem **4d** is more active than the furan derivative **4c**. The penem **4e** with 3-tetrahydrofuranylmethyl at C2 showed an equipotent activity with the 2-tetrahydrofuran-substituted penem **4a**. The compound **4g**, a methylated derivative at C1′ of **4e**, retained the activity. This result contrasts with that of the acyclic derivative **4f**, indicating that the introduction of the methyl group at C1′ is not commonly essential for reduction of the activity as observed in **4f**.

Crystal structures of the penem side-chains at C2

The diastereomeric isomers **4a** (M = Na) and **4b** (M = Na) show distinct torsion angles (β), 28.6° for the *R* isomer **4a** and −39.8° for the *S* isomer **4b**. The O5′ atom of the *R* isomer **4a** has a significantly short atomic distance (2.85 Å) to the S1 atom while the *S* isomer **4b** has a longer distance (3.01 Å) than the *R* isomer. The sodium and potassium salts of **4a** (M = Na and K) showed a slightly longer distance (0.04–0.07 Å) for d_1 than the allyl ester **3a**. The penem sulfoxide **5a**[5] exhibited a closer contact of the O5′ atom to the S1 atom (β = 7.5°, d_1 = 2.78 Å) whereas the side-chain of the isomer **5b** kept a similar conformation to that of **4b** (β = −37.7°, d_1 = 3.00 Å). These short atomic distances are found in all analogs such as 5,6-*cis*-penem **10** and 6,6-dimethyl penem **12** having the 1′*R*-tetrahydrofuran moiety at C2. The methoxymethyl-bearing penem **4i** also showed a short S–O distance (2.85 Å).

Based on the van der Waals radii of sulfur (1.80 Å), oxygen (1.52 Å), and carbon (1.70 Å) atoms we would expect van der Waals contact distances of 3.32 Å for S–O, and 3.50 Å for S–C. However, the present results show that both the S–O and S–C atomic distances of the *S* isomers (**4b** and **5b**) and the carbon analogs (**3d** and **6**) are about 0.3 Å shorter, indicating that those

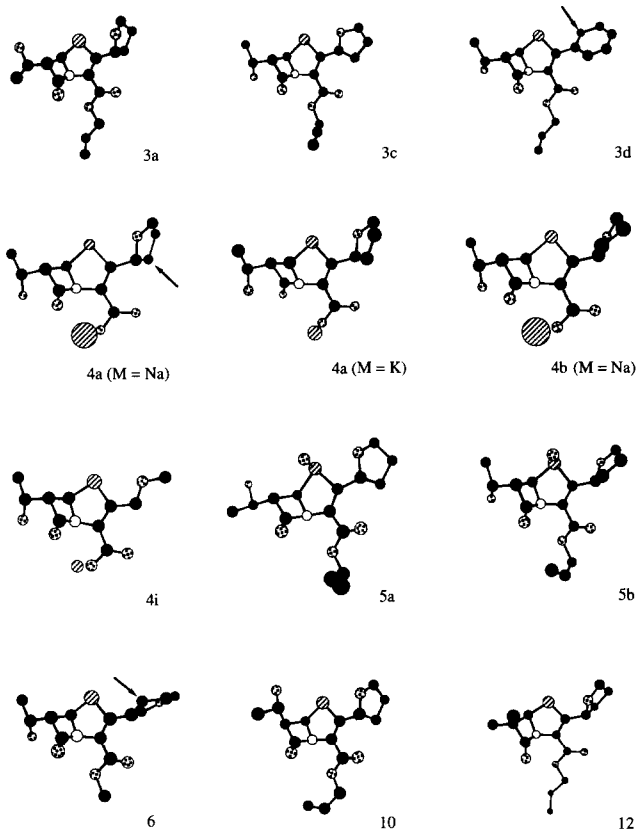


Figure 1. Perspective ball-and-stick view of crystal structures for penem derivatives. Hydrogen atoms are omitted for clarity. Oxygens: large-dotted ball; nitrogens: white ball; carbons: shaded ball; sulfur and metal atoms: hatched ball. The size of the metals (**4a** and **4b**) is dependent on the location. The arrows indicate the carbon atoms corresponding to C2′ or C6′.

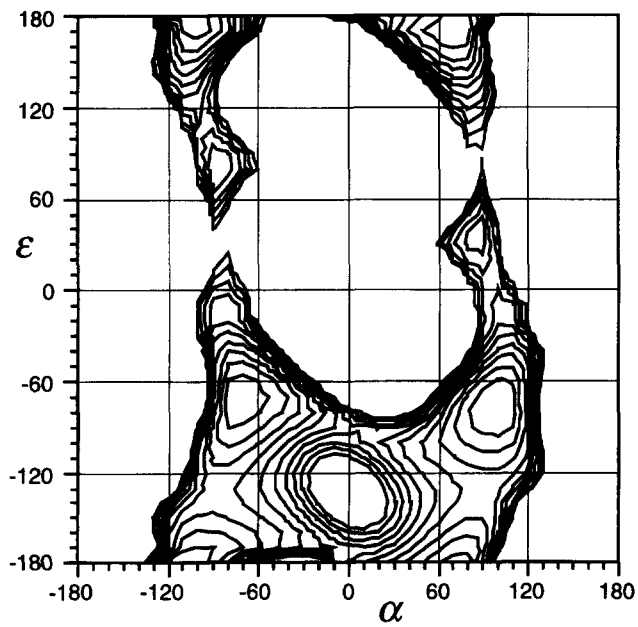


Figure 2. Conformational energy contour for the C2 side-chain of the compound **4h**. α: torsion angle for S1-C2-C1′-C2′; ε: for C2-C1′-C2′-C3′. The contours represent 1 kcal mol^{-1} increments.

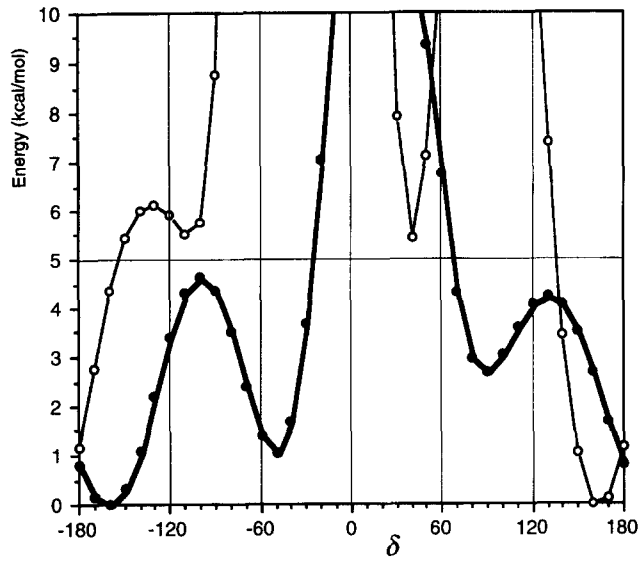


Figure 3. Conformational energy contour for the C6 side-chain of the hydroxyethyl moiety at C6 (δ, C7-C6-C8-O11). The thick and thin lines represent the 5,6-*trans* penem and the 5,6-*cis* penem, respectively.

observed atomic distances are at the van der Waals contacts in these penem derivatives. The O5′ atom of the six *R* isomers was found to be 0.2 Å closer to the S1 atom than that of the *S* isomers. These short atomic distances indicate the presence of a weak but significant interaction between the sulfur and oxygen atoms. The conjugation of the S1 atom to the α,β-unsaturated ester in the penem nucleus could be assumed to be a major factor for this smaller van der Waals radius of the sulfur atom, taking account of a characteristic unusual UV absorption maximum at 305–310 nm for the penem nucleus. A shorter S–O distance in the sulfoxide **5a** (Table 2) suggests that an electron-deficient sulfur atom interacts with an electron-rich oxygen atom. A similar intramolecular short contact has been observed in the crystal of *N*-(2-nitrophenylthio)-*S*,*S*-diphenylsulfoximide[10] in which the sulfur atom of phenylthio group interacts with an oxygen atom of the nitro group. A recent report on 2-thiophenylfuranose derivatives[11] also shows a short contact of the oxygen atom of the tetrahydrofuran with the thiophene sulfur atom at a distance of 3.04 Å. Ab initio computations have suggested that an electrostatic interaction between the positively charged thiophene sulfur and the negatively charged furanose oxygen stabilizes the conformation of the 2-thiophenylfuranose derivatives.[11,12] Hence the S1–O5′ interaction of the penem derivatives stabilizes the conformation of the C2 side-chains in the crystalline state. Furthermore, the shorter distances between the O5′ atom and the S1 atom (2.81–2.88 Å) implies that the C2 side-chain conformation is highly stabilized through the interaction and that this S–O interaction would contribute to the conformation of the C2 side-chain on complex formation with the target enzymes.

The crystal structure of the furan-substituted penem **3c** also shows a close contact of the O5′ with the S1 atom

Table 1. Crystal data and experimental details

Compound	3a	3c	3d	4a (M=Na)	4a (M=K)	4b (M=Na)	4i	5a	5b	6	10	12
Formula	$C_{15}H_{19}O_5NS$	$C_{15}H_{15}O_5NS$	$C_{17}H_{17}O_4NS$	$C_{12}H_{14}O_5NSNa$ 5/2(H_2O)	$C_{12}H_{14}O_5NSK$ 3(H_2O)	$C_{12}H_{14}O_5NSNa$ 5/2(H_2O)	$C_{10}H_{12}O_5NSNa$ 2(H_2O)	$C_{15}H_{19}O_6NS$	$C_{15}H_{19}O_6NS$	$C_{14}H_{19}O_5NS$	$C_{15}H_{19}O_5NS$	$C_{15}H_{19}O_4NS$
Formula weight	325.39	321.35	331.39	352.34	377.46	352.34	317.30	341.38	341.38	313.37	325.39	309.39
Temperature (K)	293	293	293	293	293	293	293	293	293	293	293	293
Radiation	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα	CuKα
Wavelength (Å)	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178	1.54178
Crystal system	Orthorhombic	Monoclinic	Orthorhombic	Orthorhombic	Orthorhombic	Monoclinic	Monoclinic	Monoclinic	Orthorhombic	Monoclinic	Monoclinic	Orthorhombic
Space group	$P2_12_12_1$	$P2_1/a$	$P2_12_12_1$	$P2_12_12$	$P2_12_12$	C2	C2	$P2_1$	$P2_12_12_1$	$P2_1$	$P2_1$	$P2_12_12_1$
Unit-cell dimension												
a (Å)	9.429(1)	21.944(4)	8.651(1)	9.208(1)	9.207(1)	36.183(5)	28.318(3)	9.762(1)	5.017(1)	14.258(1)	10.427(1)	9.026(2)
b (Å)	28.065(3)	4.818(2)	30.551(4)	32.454(2)	33.551(2)	5.729(1)	5.585(2)	13.794(1)	20.279(2)	5.177(1)	17.149(1)	29.723(2)
c (Å)	6.049(1)	14.646(2)	6.166(13)	5.495(3)	5.639(1)	9.244(1)	9.246(1)	6.133(1)	15.861(2)	10.400(1)	4.591(1)	5.944(1)
α (°)												
β (°)		93.93(2)				103.74(1)	90.69(1)	94.11(1)		99.61(1)	101.11(1)	
γ (°)												
Volume (Å^3)	1600.7(4)	1590.9(22)	1629.5(35)	1642.1(10)	1741.8(4)	1861.4(4)	1462.2(7)	823.7(2)	1613.8(4)	756.9(1)	805.6(1)	1594.7(3)
Z	4	4	4	4	4	4	4	2	4	2	2	4
Dx (g cm^{-3})	1.35	1.38	1.35	1.43	1.44	1.25	1.44	1.38	1.41	1.38	1.34	1.29
Absorption coefficient (mm^{-1})	1.957	1.969	1.892	2.307	4.156	2.035	2.504	1.973	2.014	2.048	1.945	1.891
Crystal size (mm)	0.3 × 0.3 × 0.3	0.1 × 0.2 × 0.5	0.1 × 0.1 × 0.3	0.3 × 0.3 × 0.5	0.2 × 0.2 × 0.4	0.2 × 0.05 × 0.5	0.2 × 0.05 × 0.6	0.2 × 0.2 × 0.8	0.1 × 0.1 × 0.5	0.2 × 0.1 × 0.5	0.2 × 0.1 × 0.4	0.5 × 0.3 × 0.2
Scan type	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ	ω/2θ
θ_{max} (°)	63	63	63	63	63	63	63	63	63	63	63	63
No. of refrections collected	1524	2969	1597	1606	1684	1682	1322	1395	1551	1368	1411	1540
No. of refrections observed	1497	2671	1561	1592	1669	1628	1318	1395	1530	1366	1406	1527
Criterion for observed	$F > 3\sigma(F)$	$F > 3\sigma(F)$	$F > 3\sigma(F)$	$F > 3\sigma(F)$					$F > 3\sigma(F)$		$F > 3\sigma(F)$	
No. of refrections used for refinement	1483	2193	1491	1592	1665	1599	1315	1394	1509	1363	1333	1522
R	0.064	0.081	0.08	0.059	0.11	0.169	0.124	0.056	0.064	0.079	0.065	0.084
wR	0.075	0.085	0.083	0.11	0.11	0.192	0.149	0.066	0.076	0.086	0.079	0.086
$(\Delta/\sigma)_{max}$	0.88	3.81	0.47	4.84	0.36	0.26	0.01	0.8	0.29	0.17	0.38	0.27

Method of structure solution: direct methods with the aid of the program MULTAN84 (Main, P.; German, G. and Woolfson, M. M. 1984).
Method of refinement: blocked diagonal matrix methods with the aid of the program RASA5-RP (Rigaku Corporation, Tokyo, Japan).

Table 2. Selected torsion angles and atomic distances in penem derivatives

	α	β	γ	δ	*d*1	*d*2
3a	−102.9	15.7	2.5	179.2	2.81	3.32
4a(M=Na)	−90.5	28.6	−6.3	−42.8	2.85	3.26
4a(M=K)	−91.4	27.0	−6.2	−40.4	2.88	3.30
4b(M=Na)	78.6	−39.8	3.0	−43.7	3.01	3.24
4i	—	−0.6	−10.1	−43.2	2.85	3.25
3c	178.7	−1.8	7.1	−44.5	2.77	3.22
3d	−49.9[a]	128.7[b]	10.7	−63.7	3.17[c]	3.41
5a	−111.8	7.5	11.2	−168.5	2.78	3.26
5b	78.6	−37.7	27.6	−51.9	3.00	3.51
6	−58.8	—	2.1	−47.7	3.26[c]	3.24
10	−110.4	8.2	8.3	159.6	2.81	3.26
12	−95.5	20.7	−0.6	—	2.81	3.48

α: S1-C2-C1′-C2′; β: S1-C2-C1′-O5′; γ: C2-C3-C1″-O2″; δ: C7-C6-C8-O11.
*d*1 (Å): distance between S1 and O5′; *d*2 (Å): distance between O10 and O3″.
[a]S1-C2-C1′-C6′.
[b]S1-C2-C1′-C2′.
[c]Distance between S1 and C2′ (for numbering see compound **4** in Scheme 1).

(the atomic distance, 2.77 Å), leading to a conformation of the furan moiety (β = −1.81°) distinct from the tetrahydrofuran ring found in **3a** and the other analogs (see Table 2). The conformation of the furan ring should be stabilized further through conjugation to the α,β-unsaturated ester moiety in the penem nucleus. The crystal structure of another aromatic ring-substituted penem **3d** showed a twisted conformation for the phenyl group with a torsion angle (α = −49.9°). Since the benzene ring shows a reasonable van der Waals contact (3.17 Å atomic distance) with the S1 atom to C6′ which corresponds to O5′ of furan derivative **3c**, this twist of the benzene ring is a consequence of a steric interaction of the C6′ atom with the S1 atom.

The 3-tetrahydrofurylmethyl-bearing penem **6** (**4h**, M=Me) showed a similar torsion angle for S1-C2-C1′-C2′ (α = −58.8°) with a reasonable van der Waals contact of C2′ to the S1 atom. Thus, the biologically active penem derivatives (**4a**, **4d**, and **4h**) take a similar disposition of C2′ (C6′ for **4d**) whereas the inactive furan derivative (**4c**) shows a quite distinct location of C2′. Hence, it is conceivable that the C2′ atoms of **4a**, **4d**, and **4h** reside at a common binding pocket of the target enzymes. The C2 side-chain of the compound **4h** has four local energy minima with respect to the torsion angles S1-C2-C1′-C2′ (α) and C2-C1′-C2′-C3′ (ε) of **6**, as depicted in a conformational energy-contour map (Figure 2). The two low-energy areas (within 2 kcal mol^{-1} of the lowest energy) at −95° to −35° torsion angle (α) are responsible for the conformation observed in the crystal structure. This indicates that the side-chain exists as a mixture of the four conformations and it is highly probable that the conformation observed in the crystal is close to that formed in the active site of the enzymes.

The acyclic penem derivative **4f** and its epimeric isomer showed a largely reduced potency from the methoxymethyl derivative **4i** which was as highly active as the cyclic derivative **4a**. A plausible factor for the reduction of the activity is a steric effect on the enzyme-bound

Table 3. In vitro antibacterial activity[a] (minimum inhibitory concentration, μg mL^{-1}) of penem derivatives

	Compound number								
Microorganisms	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4i**	**11**
S. aureus 209P JC-1	0.05	0.05	0.39	0.2	0.1	0.78	0.1	0.1	0.78
B. subtilis ATCC 6633	<0.025	0.2	0.78	0.05	0.05	0.39	<0.025	0.39	0.39
E. coli NIHJ JC-2	0.78	6.25	25	3.13	1.56	25	6.25	0.78	0.78
K. pneumoniae PCI 602	0.39	1.56	3.13	3.13[b]	0.1	3.13	0.1	0.78	0.78[b]
S. marcescens IAM 1136	1.56	25	25	12.5[c]	1.56	>50	3.13	3.13	25[c]
E. cloacae 963	0.2	12.5	25	12.5[f]	0.39	>50	3.13[g]	3.13	100[f]
Proteus vulgaris GN 7919	0.78	0.78	3.13	1.56[e]	0.2	25	1.56	0.78	1.56[e]
Pseudomonas aeruginosa No. 12	>50	>50	>50	>100[f]	>50	>50	>50	>50	>100[f]

[a]Minimum inhibitory concentration (MIC) determined in heart infusion agar medium. Inoculum size: 10^6 cells mL^{-1}. Incubation: 24 h at 37 °C.
[b]*K. pneumoniae* ATCC 15380.
[c]*Serratia marcescens* IFO 3736.
[d]*Enterobacter cloacae* NCTC 9394.
[e]*Proteus vulgaris* IFO 3851.
[f]*Pseudomonas aeruginosa* PAO-1.
[g]*Enterobacter cloacae* 91.

conformation of the C2 side-chain. Another possible effect is a change of permeability of the molecule **4f** against the outer-cell membrane. However, **4f** reduced the activity both against Gram-positive and negative bacteria which have quite distinct outer-cell membranes, and the acetoxy derivatives substituted for the methoxy group also lost the activity (data not shown). Furthermore, an epimeric isomer **4g** which has a methyl group at C1′ (methylated analogue of **4e**; configuration not determined) retained the antimicrobial activity. Therefore, the alteration of the permeability is unlikely. Thus, the methyl group of **4f** would have a specific role for the C2′ side-chain conformation to hamper the enzyme-bound conformation. The crystal structure of **4i** also shows the S–O interaction (d_1 = 2.85 Å). Hence, it is conceivable that the introduced Me group at C1′ will cause a repulsive interaction with the methyl of the OMe group and the consequent rotation of the C1′–O2′ bond will eliminate the S1–O2′ interaction. Although a crystal structure of **4f** is not available at this moment, the resulting conformation of the derivative **4f** should be distinct from the favored conformation for the enzyme binding, particularly, the disposition of the introduced methyl group at C1′. Since the retention of the activity of the 1′-methylated analogue **4g** implies that the introduced methyl group does not affect the disposition of the C2′ atom, the alteration of the conformation of the C2 side-chain to the inactive form should be specific for the penem derivatives which have the S–O interaction.

Conformations of the carboxyl group at C3

It is widely accepted that the hydrogen bonding of the carboxylate of β-lactam antibiotics with the hydroxyl group of the particular serine, Ser130, in the active site of β-lactamases and the related enzymes is essential for the binding of the antibiotics.[13,14] Of the 12 crystal structures described herein, the conformation of the carboxyl group is classified into three distinct groups, although the difference is small (within 20° of the torsion angle). A typical conformation can be found in the compound **4a** which has a negative value (−6.26°) for the torsion angle, C2-C3-C1″-O2″ (γ). The second conformation is represented by the compound **3d** having a positive torsion angle (10.7°), and the third one shows a near-zero value (2.45° for **3a**). Although the negative or positive (or near-zero) value should be important for the ligand–enzyme interaction, it would not be possible to determine which is close to the enzyme-bound conformation until the structures of the target enzymes become available.

Conformation of the hydroxyethyl side-chain at C6

The nine crystal structures can be classified into two conformations for the 8*R*-hydroxyethyl group. Of the two conformations, the predominant conformation (δ = −63.7° to −40.4°) was found in the compounds **4a** (M=Na and M=K), **4b**, **3c**, **3d**, **5b**, and **6**, and the other (δ = 159.6° to −168.5°) was in the compounds **3a** and **5a** (Table 2). These conformations are not correlated with a variety of carboxyl substituents at C3, since esters **3c**, **3d**, **5b**, and **6** show torsion angles (−44.5° to −63.7°) similar to the sodium salt **4a**. Thus, the difference of the conformation must be due to independent crystal packing modes. Conformational analysis of the hydroxyethyl side-chain at C6 of the 5*R*,6*S*,8*R* penem derivative **3a** suggested two conformational-energy minima at −160° and −50° (Figure 3).

The crystal structure of the 5,6-*cis* penem **10** had the hydroxyl group disposed in a direction similar to that of the *trans* penem **3a** as seen in Figure 1 (δ = 159.6° for **10** and 179.2° for **3a** in Table 2). Conformational analysis of the hydroxyethyl side-chain of **10** indicated that the side-chain should be strictly localized around the torsion angle observed in the crystal structure (Fig. 3). Since the configuration of the hydroxyl group affects the activity both in 5,6-*cis* and -*trans* penems (more active in 8*R* for *trans* penems, and in 8*S* for *cis*-penems), it is conceivable that the hydroxyl groups play the same role for the binding to the target enzymes and thus the conformation observed in the *trans* penem **3a** is the enzyme-bound conformation.

It has been shown that penem antibiotics acylate β-lactamases as well as penicillin-binding protein.[15–17] A preliminary docking study of the crystal structure of the penem **3a** in the active site of a class A β-lactamase[18] suggested a favored hydrogen-bonding interaction between the hydroxyl group and the side-chain of the Asn132 (Ambler numbering[19]). A site-directed mutagenesis experiment on *Escherichia coli* PBP2 suggested that Asp389 corresponds to Asn132 of β-lactamase from *E. coli*.[20,21] Furthermore, recent crystal-structure analysis of PBP2x of *Streptococcus pneumoniae* has indicated the similarity of the active-site structure to class A enzymes.[22] Hence, we suggest that the C-8 hydroxyl group will interact with the Asp389 residue in PBP2.

Conclusion

Crystallographic analysis of more than 10 closely related structures of the penem derivatives revealed an important interaction of the oxygen atom in the side-chains at C2, which would freeze the active or inactive conformations of the tetrahydrofuran or furan moiety interacting with the S1 atom. It is also conceivable that the side-chain conformations at C2 of the crystal structures correlate well with the activity. In particular, the disposition of the C2′ atom would be important for binding to the enzymes since the active penems have similar torsion angles (α).

Among the two conformations of the C6 hydroxyethyl group observed in the crystal structures, a particular conformation with a larger torsion angle (δ = 179.2°) is selected for the enzyme-bound conformation in the Michaelis complex formation. The interactions of the

side-chains at C2 and C6 with the enzymes would affect the location of the C3 carboxylate group in the interaction with the Ser130 residue to trigger the acylation. Thus, the results of the above analysis of the conformations of the substituents, in particular at C2 and C6 of the penem derivatives, would be useful for understanding structure–activity relationships in penem and related derivatives, and also for design of novel penem-related antibiotics.

Experimental

Melting points (uncorrected) were determined in open capillary tubes with a Buchi 535 apparatus. ^{1}H NMR spectra were recorded on a JEOL JNM-GX270 or Brucker ARX 400 FT NMR spectrometer, and ^{13}C NMR spectra were recorded on a JEOL JNM-GX270, using tetramethylsilane or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TSP) as an internal standard. Positive-ion fast-atom-bombardment mass spectra (FABMS) were obtained on a JEOL JMS-AX5000 instrument, and high-resolution mass spectra (HRMS) were obtained on a JEOL JMS-HX/HX110A instrument. Flash column chromatography was carried out on Merck Kieselgel 60 Art 9385 (230–400 mesh), and preparative TLC of Merck Kieselgel 60 F_{254} Art 13895 (1 mm) was used. Compounds showed satisfactory purity by TLC on Merck Kieselgel 60F_{254} Art 5714 plates (visualized by UV light at 254 nm and/or by 6.3% w/v phosphomolybdic acid in ethanol). The results of elemental combustion analyses, which are indicated only by the elements, are within ± 0.4% of theoretical values and were obtained on a Perkin–Elmer 240B elemental analyzer. IR spectra were determined in KBr, or neat on a Perkin–Elmer 1600 Series FT IR spectrophotometer. UV spectra, CD spectra, and optical rotations were obtained with a Shimazu UV-2000 UV-visible recording spectrophotometer, JASCO J-600 spectropolarimeter and JASCO DIP-181 digital polarimeter, respectively. Water analyses were obtained on a Mitsubishikasei CA-05 moisture meter. For the analysis of carboxylic acids or their salts, reverse-phase HPLC analysis was carried out using TSK ODS-80Tm column (4.6 mm ID × 150 mm) and a set of Shimazu liquid chromatograph 6A system. Acetonitrile/water (including 0.1% v/v trifluoroacetic acid) was employed as eluent (flow rate 1 mL min^{-1}), and a Waters 991J photodiode array detector for UV detection of eluting materials. Purification of the carboxylic acids or their salts was performed on reverse-phase column chromatography with ODS chromatorex (100–200 mesh) of Fuji Davison Chemical Ltd or preparative HPLC separations on a YMC SH-343-5 S-5 120A AM ODS column (20 mm ID × 250 mm), eluting with acetonitrile/water (including 0.1% v/v acetic acid, flow rate 10 mL min^{-1}).

(3*S*,4*R*)-3-((*R*)-1-*tert*-Butyldimethylsilyloxyethyl)-4-(tetrahydro-3-furylacetylthio) azetidin-2-one (1e): general example of substitution at C-4. To a solution of (3*R*,4*R*)-3-((*R*)-1-*tert*-butyldimethylsilyloxyethyl)-4-acetoxyazetidin-2-one (1.21 g, 4.21 mmol) in acetone (1.7 ml) was added water (5.1 ml), tetrahydro-3-furanthioacetic acid (0.67 g, 5.05 mmol), and $NaHCO_3$ (0.42 g, 5.05 mmol) at rt. The reaction mixture was stirred at 60 °C for 4 h. After dilution with AcOEt, the aqueous layer was separated. The organic layer was washed with satd Na_2SO_4 and dried over anhyd $MgSO_4$. After removal of the solvent, the residue was purified by flash chromatography on silica gel (25 g, 3:1 v/v *n*-hexane:AcOEt) to give 1.27 g of the titled compound in 81% yield as a white solid: colorless needles (CH_2Cl_2-*n*-hexane); mp 94–95 °C; IR (cm^{-1}) (KBr) 3094, 1771, 1728, 1694; ^{1}H NMR ($CDCl_3$) δ 0.07 (3H, s) 0.08 (3H, s) 0.88 (9H, s) 1.21 (3H, d, J = 5.9 Hz) 1.50–1.66 (1H, m) 2.01–2.20 (1H, m) 2.58–2.74 (3H, m) 3.16 (1H, dd, J = 2.0 Hz, 4.0 Hz) 3.38–3.47 (1H, m) 3.70–3.97 (3H, m) 4.20–4.31 (1H, m) 5.32 (1H, d, J = 2.0 Hz) 6.27 (1H, brs). Anal. calcd for $C_{17}H_{31}NO_4SSi$: C, 54.66; H, 8.36; N, 3.75. Found: C, 54.72; H, 8.47; N, 3.87.

(5*R*,6*S*)-6-((*R*)-1-*tert*-Butyldimethylsilyloxyethyl)-2-(tetrahydro-3-furylmethyl)penem-3-carboxylic acid allyl ester (2e): general example of cyclization to penem. To a solution of the thioester **1e** (7.68 g, 20.6 mmol) in CH_2Cl_2 (33 ml) was added triethylamine (3.54 ml, 25.4 mmol) at once and then allyl oxalyl chloride (3.37 g, 24.7 mmol) in CH_2Cl_2 (8.2 ml) at −20 °C dropwise for 5 min. The reaction mixture was stirred at the same temp for 10 min. The reaction mixture was poured into water (40 ml) and extracted with CH_2Cl_2 (40 ml). The organic phase was washed with water (40 ml) and satd $NaHCO_3$ (40 ml) and then brine (40 ml), and dried over anhyd $MgSO_4$. The solvent was removed under reduced pressure. The residue was dissolved in xylene (411 ml). To the solution was added triethyl phosphite (17.2 ml) at rt, followed by refluxing for 11 h. After removal of the solvent, the residue was purified by flash chromatography on silica gel (100 g, 5:1 v/v *n*-hexane:AcOEt) to give 5.1 g of the penem ester **2e** in 55% yield based on the azetidinone as a pale yellow solid: colorless needles (CH_2Cl_2-*n*-hexane); mp 51–53 °C; IR (cm^{-1}) (KBr) 1775, 1690, 1605; ^{1}H NMR ($CDCl_3$) δ 0.08 (6H, s) 0.89 (9H, s) 1.25 (3H, d, J = 6 Hz) 1.56–1.71 (1H, m) 2.03–2.15 (1H, m) 2.40–2.54 (1H, m) 2.72–3.14 (2H, m) 3.39–3.51 (1H, m) 3.66 and 3.67 (total 1H, d, J = 5 Hz and 5 Hz) 3.71–3.96 (3H, m) 4.18–4.29 (1H, m) 4.61–4.78 (2H, m) 5.25 (1H, d, J = 10 Hz) 5.40 (1H, d, J = 17 Hz) 5.56 (1H, s) 5.86-6.01 (1H, m). Anal. calcd for $C_{22}H_{35}NO_5SSi$: C, 58.25; H, 7.78; N, 3.09. Found: C, 58.34; H, 7.89; N, 3.17.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-(tetrahydro-3-furylmethyl)penem-3-carboxylic acid allyl ester (3e): general example of deprotection of alcohol. To a solution of the allyl ester **2e** (725 mg, 1.60 mmol) in THF (6.47 ml) was added AcOH (0.40 ml, 7.0 mmol) and then 1.0 M tetra-*n*-butylammonium fluoride in THF (2.44 ml) at rt. The reaction mixture was stirred at 50 °C for 8.5 h. The mixture was poured into water (20 ml) and extracted with AcOEt (20 ml). The organic phase was washed with water (20 ml), satd $NaHCO_3$ (20 ml) twice, satd $KHSO_4$ (20 ml) and brine (20 ml), and dried over anhyd

$MgSO_4$. After concentration under reduced pressure, purification of the residue by flash chromatography on silica gel (15 g, 1:1 v/v *n*-hexane:AcOEt) provided 405 mg of the desired product **3e** as a white solid in 75% yield: colorless needles (CH_2Cl_2–*n*-hexane); mp 94–95 °C; IR (cm^{-1}) (KBr) 3420, 1770, 1700, 1570; ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, J = 6 Hz) 1.97–2.15 (2H, m) 2.39–2.56 (1H, m) 2.76 and 2.84 (total 1H, dd and dd, J = 7 Hz, 14 Hz and J = 7 Hz, 14 Hz) 3.00 and 3.09 (total 1H, dd and dd, J = 7 Hz, 14 Hz and J = 7 Hz, 14 Hz) 3.40–3.51 (1H, m) 3.70 and 3.71 (total 1H, d and d, J = 7 Hz and 7 Hz) 3.74–3.94 (3H, m) 4.18–4.30 (1H, m) 4.66 (1H, dd, J = 5 Hz, 14 Hz) 4.78 (1H, dd, J = 5 Hz, 14 Hz) 5.27 (1H, d, J = 11 Hz) 5.41 (1H, d, J = 17 Hz) 5.60 (1H, d, J = 1 Hz) 5.87–6.14 (1H, m). Anal. calcd for $C_{16}H_{21}NO_5S$: C, 56.62; H, 6.24; N, 4.13. Found: C, 56.61; H, 6.19; N, 4.17.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-(tetrahydro-3-furylmethyl)penem-3-carboxylic acid sodium salt (4e, M=Na): general example of deprotection of carboxylic acid. The ester **3e** (3.39 g, 10 mmol), triphenylphosphine (133 mg, 0.5 mmol), and tetrakis(triphenylphosphine)palladium (120 mg, 0.1 mmol) were dissolved in 5 ml of AcOEt. To this was added a solution of sodium 2-ethylhexanoate (1.99 g, 12 mmol) in AcOEt (5 ml) at rt. The reaction mixture was stirred at the same temp for 1 h, and then poured into water (20 ml). After addition of ether (10 ml), the organic layer was extracted with water (20 ml) twice. The combined aqueous layer was lyophilized. The residue was purified by reverse-phase chromatography on ODS (150 g) with water. The combined fractions for the desired product were lyophilized to give 1.92 g of the desired product **4e** as a pale yellow powder in 60% yield: IR (cm^{-1}) (KBr) 3421, 1766, 1601, 1378; ^1H NMR (D_2O) δ 1.16 and 1.17 (total 3H, d and d, J = 6 Hz and 6 Hz) 1.41–1.62 (1H, m) 1.88–2.04 (1H, m) 2.28–2.46 (1H, m) 2.46–2.58 and 2.58–2.72 (total 1H, m and m) 2.79–3.00 (1H, m) 3.26–3.40 (1H, m) 3.58–3.82 (4H, m) 4.04–4.17 (1H, m) 5.47 (1H, d, J = 2 Hz).

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic acid allyl ester (3a). Yellow prisms (AcOEt–*n*-hexane); mp 97–98 °C; IR (cm^{-1}) (neat) 3471, 1796, 1655, 1493; ^1H NMR ($CDCl_3$) δ 1.35 (3H, d, J = 6.6 Hz) 1.70–2.19 (3H, m) 2.32–2.55 (1H, m) 3.71 (1H, dd, J = 1.3 Hz, 7.9 Hz) 3.78–3.91 (1H, m) 3.91–4.06 (1H, m) 4.15–4.31 (1H, m) 4.64 (1H, dd, J = 5.3 Hz, 13.2 Hz) 4.77 (1H, dd, J = 5.3 Hz, 13.2 Hz) 5.26 (1H, d, J = 9.9 Hz) 5.32–5.49 (2H, m) 5.51 (1H, d, J = 1.3 Hz) 5.86–6.05 (1H, m). Anal. calcd for $C_{15}H_{19}NO_5S$: C, 55.37; H, 5.89; N, 4.30. Found: C, 55.20; H, 5.91; N, 4.27.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*S*)-tetrahydro-2-furyl)penem-3-carboxylic acid allyl ester (3b). Yellow solid ($CHCl_2$–*n*-hexane); mp 58–63 °C; IR (cm^{-1}) (KBr) 3462, 1778, 1706, 1583; ^1H NMR ($CDCl_3$) δ 1.35 (1H, d, J = 6.6 Hz) 1.71–1.87 (1H, m) 1.87–2.02 (2H, m) 2.08 (1H, brd) 2.33–2.50 (1H, m) 3.71 (1H, dd, J = 2.0 Hz, 6.6 Hz) 3.76–3.89 (1H, m) 3.89–4.02 (1H, m) 4.15–4.30 (1H, m) 4.61–4.83 (2H, m) 5.26 (1H, dd, J = 1.3 Hz, 10.6 Hz) 5.40 (1H, t, J = 7.9 Hz) 5.58 (1H, d, J = 2.0 Hz) 5.85–6.03 (1H, m). Anal. calcd for $C_{15}H_{19}NO_5S$: C, 55.37; H, 5.89; N, 4.30. Found: C, 55.08; H, 5.97; N, 4.32.

(5*R,6*S**)-6-((*R**)-1-Hydroxyethyl)-2-(2-furyl)penem-3-carboxylic acid allyl ester (3c)**. The title compound was prepared by the same method described above except for use of a racemic (3*R**,4*R**)-3-((*R**)-1-*tert*-butyldimethylsilyloxyethyl)-4-acetoxyazetidin-2-one: yellow prisms (AcOEt–*n*-hexane); mp 130–135 °C; IR (cm^{-1}) (KBr) 3424, 1772, 1706, 1534; ^1H NMR ($CDCl_3$) δ 1.39 (3H, d, J = 6.3 Hz) 1.84 (1H, d, J = 4.6 Hz) 3.75 (1H, dd, J = 1.3 Hz, 6.8 Hz) 4.21–4.37 (1H, m) 4.70 (1H, dd, J = 5.6 Hz, 13.5 Hz) 4.82 (1H, dd, J = 5.6 Hz, 13.5 Hz) 5.27 (1H, d, J = 9.9 Hz) 5.43 (1H, d, J = 17.2 Hz) 5.61 (1H, d, J = 1.6 Hz) 5.90–6.08 (1H, m) 6.55 (1H, dd, J = 1.7 Hz. 3.6 Hz) 7.53 (1H, d, J = 1.7 Hz) 7.69 (1H, d, J = 3.6 Hz). Anal. calcd for $C_{15}H_{15}NO_5S$: C, 56.06; H, 4.70; N, 4.36. Found: C, 56.08; H, 4.84; N, 4.46.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-phenylpenem-3-carboxylic acid allyl ester (3d). Colorless needles (CH_2Cl_2–*n*-hexane); mp 153–155 °C; IR (cm^{-1}) (KBr) 3464, 1769, 1707, 1560; ^1H NMR ($CDCl_3$) δ 1.39 (3H, d, J = 5.9 Hz) 1.96 (1H, brs) 3.82 (1H, dd, J = 1.3 Hz, 6.6 Hz) 4.21–4.37 (1H, m) 4.48–4.69 (2H, m) 5.13 (1H, d, J = 6.6 Hz) 5.17 (1H, d, J = 13.9 Hz) 5.72 (1H, d, J = 2.0 Hz) 5.68–5.87 (1H, m) 7.29–7.50 (1H, m). Anal. calcd for $C_{17}H_{17}NO_4S$: C, 61.62; H, 5.17; N, 4.23. Found: C, 61.70; H, 5.31; N, 4.28.

(5*R,6*S**)-6-((*R**)-1-Hydroxyethyl)-2-((*S*)-1-methoxyethyl))penem-3-carboxylic acid allyl ester (3f)**. The title compound was prepared by the same method described above except for use of a racemic (3*R**,4*R**)-3-((*R**)-1-*tert*-butyldimethyl-silyloxyethyl)-4-acetoxyazetidin-2-one: IR (cm^{-1}) (neat); ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, J = 5.9 Hz) 1.41 (3H, d, J = 5.9 Hz) 1.84–1.95 (1H, brd) 3.33 (3H, s) 3.74 (1H, dd, J = 2.0 Hz, 7.5 Hz) 4.18–4.31 (1H, m) 4.60–4.73 (1H, m) 4.73–4.90 (1H, m) 5.02 (1H, q, J = 5.9 Hz) 5.27(1H, d, J = 10.7 Hz) 5.42 (1H, d, J = 17.9 Hz) 5.57 (1H, d, J = 2.0 Hz) 5.88–6.06 (1H, m).

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*R* or *S*)-1-(tetrahydro-2-furyl)ethyl)penem-3-carboxylic acid allyl ester (3g). colorless oil; IR (cm^{-1}) (neat) 3350, 1780, 1705, 1565; ^1H NMR ($CDCl_3$) δ 1.12 (3H, d, J = 6.6 Hz) 1.37 (3H, d, J = 5.9 Hz) 1.45–1.68 (1H, m) 1.80 (1H, d, J = 4.6 Hz) 1.93–2.09 (1H, m) 2.14–2.29 (1H, m) 3.43 (1H, t, J = 8.6 Hz) 3.70 (1H, dd, J = 1.3 Hz, 6.6 Hz) 3.72–4.00 (4H, m) 4.19–4.32 (1H, m) 4.60–4.71 (1H, m) 4.71–4.86 (1H, m) 5.27 (1H, d, J = 10.6 Hz) 5.41 (1H, d, J = 17.2 Hz) 5.58 (1H, d, J = 1.3 Hz) 5.86–6.04 (1H, m); HRMS (FAB) m/z ($M+H^+$) calcd for $C_{17}H_{23}NO_5S$: 353.1297. Found: 353.1291.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic acid sodium salt (4a, M=Na). Yellow prisms (acetone–water); mp 144–147

°C (dec); water content 13.07%; UV (H_2O) λ_{max} 306 nm ($\varepsilon = 6.39 \times 10^3$) 256 ($\varepsilon = 3.67 \times 10^3$); CD ($H_2O$) λ_{max} 251 nm ($\theta = +5.59 \times 10^5$) 307 nm ($\theta = -2.44 \times 10^5$); IR ($cm^{-1}$) (KBr) 3330, 1754, 1606, 1378; ^1H NMR (D_2O) δ 1.50 (3H, d, J = 6.5 Hz) 1.99–2.35 (2H, m) 1.49–1.68 (1H, m) 3.94–4.21 (3H, m) 4.35–4.50 (1H, m) 5.73 (1H, t, J = 7.0 Hz) 5.78 (1H, d, J = 1.3 Hz); LC-FABMS m/z 308 ($M+H^+$). Anal. calcd for $C_{12}H_{14}NO_5SNa \cdot 2.5H_2O$: C, 40.90; H, 5.44; N, 3.98. Found: C, 40.75; H, 5.28; N, 3.94.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*S*)-tetrahydro-2-furyl)penem 3-carboxylic acid sodium salt (4b, M=Na). Pale brown needles (acetone–water); mp 144–147 °C (dec); water content 13.08%; UV (H_2O) λ_{max} 304 nm ($\varepsilon = 6.13 \times 10^3$) 255 ($\varepsilon = 4.19 \times 10^3$); CD ($H_2O$) λ_{max} 253 nm ($\theta = +6.27 \times 10^5$) 307 nm ($\theta = -2.18 \times 10^5$); IR ($cm^{-1}$) (KBr) 3300, 1755, 1610, 1580, 1370; ^1H NMR ($CDCl_3$) δ 1.16 (3H, d, J = 6.6 Hz) 1.59–1.77 (1H, m) 1.77–1.94 (2H, m) 2.07–2.28 (1H, m) 3.63–3.85 (2H, m) 4.02–4.17 (1H, m) 5.41 (1H, t, J = 7.3 Hz) 5.48 (1H, s). Anal. calcd for $C_{12}H_{14}NO_5SNa \cdot 2.5H_2O$: C, 40.90; H, 5.44; N, 3.98. Found: C, 40.63; H, 5.21; N, 3.87.

(5*R,6*S**)-6-((*R**)-1-Hydroxyethyl)-2-(2-furyl)penem-3-carboxylic acid potassium salt (4c, M=K).** Brown powder; IR (cm^{-1}) (KBr) 3400, 1778, 1598, 1368; ^1H NMR (D_2O) δ 1.21 (3H, d, J = 6.6 Hz) 3.83 (1H, d, J = 5.9 Hz) 4.09–4.23 (1H, m) 5.58 (1H, s) 6.47 (1H, dd, J = 2.0 Hz, 3.3 Hz) 6.96 (1H, d, J = 3.3 Hz) 7.47 (1H, s).

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-phenylpenem-3-carboxylic acid (4d, M=H). Purification was performed by preparative HPLC: white powder; IR (cm^{-1}) (KBr) 3400, 1782, 1696, 1561; ^1H NMR (MeOH-d_4) δ 1.31 (3H, d, J = 6.6 Hz) 3.76 (1H, dd, J = 1.3 Hz, 7.3 Hz) 4.05–4.19 (1H, m) 5.72 (1H, d, J = 1.3 Hz) 7.30–7.39 (3H, m) 7.42–7.49 (2H, m).

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*R* or *S*)1-(tetrahydro-2-furyl)ethyl)penem-3-carboxylic acid sodium salt (4g, M=Na). White powder; IR (cm^{-1}) (KBr) 3358, 1751, 1600, 1574; ^1H NMR (D_2O) δ 0.91 (3H, d, J = 7.3 Hz) 1.16 (3H, d, J = 6.6 Hz) 1.31–1.49 (1H, m) 1.82–1.99 (1H, m) 2.04–2.23 (1H, m) 3.31 (1H, t, J = 8.6 Hz) 3.39–3.74 (3H, m) 3.80 (1H, t, J = 7.9 Hz) 4.00–4.12 (1H, m) 5.415 (1H, s).

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-3-furylmethyl) penem-3-carboxylic acid methyl ester (6). A solution of the sodium salt **4e** (M=Na, 11 g, 32 mmol) in water (50 ml) was added to 50 ml of AcOEt. This solution was adjusted to pH 2 with 3N HCl. The organic layer was separated. The aqueous layer was saturated with NaCl and extracted with AcOEt (50 ml). The combined organic layer was dried over $MgSO_4$. After removal of solvent, 9.7 g of the carboxylic acid **4e** (M=H) was obtained as a pale yellow solid. Recrystallization from acetone was repeated seven times to afford 0.13 g of **4h** (M=H) as colorless needles (>98% purity).

To a solution of **4h** (M=H) (0.13 g) in AcOEt (10 ml) was added a solution of CH_2N_2 in ether at rt until yellow color remained. After stirring for an additional 10 min at the same temp, AcOH was added to decompose excess CH_2N_2. The resulting solution was washed with satd $NaHCO_3$ and brine, and dried over $MgSO_4$. After removal of the solvent, purification of the residue by flash chromatography on silica gel (5 g, *n*-hexane: AcOEt = 1:1) provided 0.13 g of the desired product **6** as a colorless solid in 96% yield. The ester was recrystallized from MeOH as colorless needles: mp 149–152 °C; IR (cm^{-1}) (KBr) 3406, 1769, 1706, 1579; ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, J = 6.6 Hz) 1.58–1.73 (1H, m) 1.93 (1H, brd) 1.98–2.13 (1H, m) 2.38–2.53 (1H, m) 2.89 (1H, dd, J = 7.3 Hz, 9.9 Hz) 2.99 (1H, dd, J = 7.3 Hz, 9.9 Hz) 3.44 (1H, dd, J = 5.9 Hz, 8.6 Hz) 3.65–3.96 (4H, m) 3.81 (3H, s) 4.18–4.31 (1H, m) 5.59 (1H, d, J = 1.3 Hz). Anal. calcd for $C_{14}H_{19}NO_5S$: C, 53.66; H, 6.11; N, 4.47. Found: C, 53.69; H, 5.87; N, 4.38.

(5*R*,6*S*)-6-((*R*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-2-furylmethyl)penem-3-carboxylic acid sodium salt (4e, M=Na). To the acid **4e** (M=H, 400 mg, 1.43 mmol) was added 1M $NaHCO_3$ (1.34 ml) and stirred at rt. The reaction mixture was purified by reverse-phase chromatography on ODS (60 g) with water as a white powder in 93% yield: IR (cm^{-1}) (KBr) 3370, 1766, 1600, 1578; ^1H NMR (D_2O) δ 1.15 (3H, d, J = 6.6 Hz) 1.43–1.62 (1H, m) 1.87–2.04 (1H, m) 2.28–2.43 (1H, m) 2.66 (1H, dd, J = 7.3 Hz, 13.9 Hz) 2.84 (1H, dd, J = 7.3 Hz, 13.9 Hz) 3.29 (1H, t, J = 8.6 Hz) 3.58–3.83 (4H, m) 4.01–4.17 (1H, m) 5.46 (1H, s).

Allyl-(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylate 1β-oxide (5a). To a solution of penem **3a** (325 mg, 1 mmol) in CH_2Cl_2 (6 ml) was added *m*-chloroperbenzoic acid (215 mg, 80% purity, 1 mmol) at 0 °C. After stirring for 20 min the mixture was poured into satd $NaHCO_3$ and extracted with CH_2Cl_2. The organic phase was dried over anhyd. Na_2SO_4 and concentrated under reduced pressure. Purification of the residue by flash chromatography on silica gel (10 g, *n*-hexane:AcOEt = 3:2) provided 165 mg of the desired product **5a** in 48% yield as a pale yellow solid with 23 mg of 1α-oxide-isomer in 7% yield as a yellow oil: prisms (tetrahydrofuran); UV (CH_3CN) λ_{max} 298 nm ($\varepsilon = 4.59 \times 10^3$); mp 148–149 °C; IR ($cm^{-1}$) (KBr) 3420, 1784, 1725; ^1H NMR ($CDCl_3$) δ 1.38 (3H, d, J = 6.6 Hz) 1.68–1.81 (1H, m) 1.94–2.09 (2H, m) 2.24 (1H, d, J = 4.6 Hz) 2.40–2.57 (1H, m) 3.86 (1H, dd, J = 3.3, 5.3 Hz) 3.92–4.20 (2H, m) 4.38–4.51 (1H, m) 4.70–4.90 (2H, m) 4.84 (1H, d, J = 3.3 Hz) 5.33 (1H, d, J = 10.6 Hz) 5.46 (1H, d, J = 15.8 Hz) 5.49 (1H, t, J = 7.9 Hz) 5.89–6.05 (1H, m); ^{13}C NMR($CDCl_3$) δ 21.58 (CH_3) 26.01 (CH_2) 34.38 (CH_2) 54.34 (CH) 63.90 (CH) 67.12 (CH_2) 68.39 (CH) 69.61 (CH_2) 74.28 (CH) 119.80 (CH_2) 130.62 (CH_2) 133.47 (C) 153.26 (C) 159.48 (C) 166.08 (C); FABMS m/z 342 (M+H). Anal. calcd for $C_{15}H_{19}NO_6S$: C, 52.78; H, 5.61; N, 4.10. Found: C, 52.67; H, 5.61; N, 4.12.

Allyl-(5*R*,6*S*)-6-((*R*)-1-hydroxyethyl)-2-((*S*)-tetrahydro-2-furyl)penem-3-carboxylate-1β-oxide (5b). 1β-Oxide **5b** was also obtained in 25% yield; white solid; mp 110–111 °C; IR (cm^{-1}) (KBr) 3463, 1788, 1718; ^1H NMR ($CDCl_3$) δ 1.38 (3H, d, J = 6.6 Hz) 1.98–2.19 (3H, m) 2.25 (1H, d, J = 5.3 Hz) 2.49–2.53 (1H, m) 3.82 (1H, dd, J = 3.3, 5.3 Hz) 3.85–3.97 (1H, m) 4.07–4.19 (1H, m) 4.38–4.50 (1H, m) 4.70–4.93 (2H, m) 4.90 (1H, d, J = 3.3 Hz) 5.28–5.39 (1H, m) 5.35 (1H, t, J = 7.3 Hz) 5.44 (1H, d J = 17.2 Hz) 5.88–6.05 (1H, m); FABMS FAB *m/z* 342 (M+H).

(3*R*,4*S*)-3-((*S*)-*tert*-Butyldimethylsilyloxyethyl)-4-((*R*)-tetrahydro-2-furoylthio)azetidin-2-one (7). Starting from (3*R*,4*R*)-3-((*S*)-1-*tert*-butyldimethylsilyloxyethyl)-4-acetoxyazetidin-2-one (1.5 g, 5.2 mmol), 1.69 g of the desired product **7** was obtained in a similar way to that described for **1h**, as a colorless solid in 90% yield: mp 75–77 °C; IR (cm^{-1}) (KBr) 3090, 1769, 1693; ^1H NMR ($CDCl_3$) δ 0.08 (6H, s) 0.88 (9H, s) 1.20 (3H, d, J = 5.9 Hz) 1.86–5.16 (3H, m) 2.16 (1H, m) 3.18 (1H, t, J = 2.6 Hz) 3.90–4.12 (2H, m) 4.20–4.34 (1H, m) 4.48 (1H, dd, J = 4.6 Hz, 8.6 Hz) 5.23 (1H, d, J = 2.6 Hz) 6.25 (1H, brs); HRMS (FAB) *m/z* ($M+H^+$). Calcd for $C_{16}H_{30}NO_4SiS$: 360.1665. Found: 360.1652.

(5*S*,6*R*)-6-((*S*)-1-*tert*-Butyldimethylsilyloxyethyl)-2-((*R*)-tetrahydro-2-furyl)penem-3-carboxylic acid allyl ester (8). Starting from the thioester **7** (1.6 g, 4.5 mmol), 1.3 g of the desired product **8** was obtained in a similar way to that described for **3h** as a pale yellow solid in 66% yield: mp 69–70 °C.; IR (cm^{-1}) (KBr) 1769, 1708, 1590; ^1H NMR ($CDCl_3$) δ 0.07 (6H, s) 0.87 (9H, s) 1.25 (3H, d, J = 6.6 Hz) 1.63–1.87 (1H, m) 1.87–2.05 (2H, m) 2.30–2.50 (1H, m) 3.67 (1H, dd, J = 1.3 Hz, 5.3 Hz) 3.73–3.88 (1H, m) 3.88–4.00 (1H, m) 4.14–4.29 (1H, m) 4.59–4.78 (2H, m) 5.24 (1H, d, J = 10.6 Hz) 5.31–5.47 (2H, m) 5.55 (1H, d, J = 1.3 Hz) 5.61–6.02 (1H, m); HRMS (FAB) *m/z* ($M+H^+$). Calcd for $C_{21}H_{33}NO_5SSi$: 439.1848. Found: 439.1840.

(5*S*,6*R*)-6-((*S*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-2-furyl)-penem-3-carboxylic acid allyl ester (9). Starting from the 5*S*,6*R* penem **8** (1.0 g, 2.3 mmol), 0.60 g of the desired product **9** was obtained in a similar way to that described for **3** as a yellow solid in 81% yield: mp 58–61 °C; IR (cm^{-1}) (KBr) 3466, 1778, 1704, 1582; ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, J = 5.9 Hz) 1.68–2.09 (4H, m) 2.33–2.41 (1H, m) 3.71 (1H, d, J = 6.6 Hz) 3.76–3.89 (1H, m) 3.89–4.01 (1H, m) 4.16–4.31 (1H, m) 4.60–4.84 (2H, m) 5.26 (1H, d, J = 10.6 Hz) 5.34–5.47 (1H, m) 5.58 (1H, s) 5.85–6.06 (1H, m); HRMS (FAB) *m/z* ($M+H^+$). Calcd for $C_{15}H_{20}NO_5S$: 326.1062. Found: 326.1062.

(5*R*,6*R*)-6-((*S*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-2-furyl)-penem-3-carboxylic acid allyl ester (10). A solution of the allyl ester **9** (390 mg, 1.2 mmol) in AcOEt (2 mM) was irradiated by means of high-pressure UV lamp (Hanovia) through a Pyrex filter for 50 min. A mixture of the isomerized product and the starting material was obtained in a ratio (2:3). After removal of the solvent, purification of the residue by flash chromatography on silica gel (10 g, *n*-hexane: AcOEt = 1:1) provided 77 mg of the 5*R*,6*R*-*cis*-penem **10** in 20% yield as a white solid: white scaley crystal (recryst from CH_2Cl_2-*n*-hexane); mp 128–131 °C; IR (cm^{-1}) (KBr) 3460, 1777, 1687; ^1H NMR ($CDCl_3$) δ 1.46 (3H, d, J = 5.9 Hz) 1.74 (1H, d, J = 5.3 Hz) 1.73–2.11 (3H, m) 2.40–2.55 (1H, m) 3.80 (1H, dd, J = 4.0, 10.6 Hz) 3.87 (1H, q, J = 6.6 Hz) 4.00 (1H, q, 6.6 Hz) 4.36–4.50 (1H, m) 4.64 (1H, dd, J = 5.9 Hz, 13.2 Hz) 4.77 (1H, dd, J = 5.9 Hz, 13.2 Hz) 5.27 (1H, d, J = 10.6 Hz) 5.38 (1H, t, J = 7.3 Hz) 5.34–5.47 (1H, m) 5.59 (1H, d, J = 4.0 Hz) 5.87–6.02 (1H, m). Anal. calcd for $C_{15}H_{19}NO_5S$: C, 55.37; H, 5.89; N, 4.30. Found: C, 55.43; H, 5.91; N, 4.27.

(5*R*,6*R*)-6-((*S*)-1-Hydroxyethyl)-2-((*R*)-tetrahydro-2-furyl)-penem-3-carboxylic acid sodium salt (11). In a similar way to that described for **4h**, the allyl ester **10** (41 mg, 0.13 mmol), was converted to the salt **11** as a white powder in 25% yield: IR (cm^{-1}) (KBr) 3428, 1773, 1700; ^1H NMR (D_2O) δ 1.26 (3H, d, J = 5.9 Hz) 1.72–2.02 (3H, m) 2.24–2.40 (1H, m) 3.68–3.80 (1H, m) 3.80–3.93 (2H, m) 4.14–4.28 (1H, m) 5.32 (1H, t, J = 7.0 Hz) 5.58 (1H, d, J = 4.0 Hz); FABMS *m/z* (M+H) 286.

(5*R*)-6,6-Dimethyl-2-((*R*)-tetrahydro-2-furyl)-penem-3-carboxylic acid allyl ester (12). In a similar way to that described for **1h**, a diastereomeric mixture of 3,3-dimethyl-4-((*R*)-tetrahydro-2-furoylthio)azetidin-2-one was obtained as a colorless oil in 82% starting from a racemic 3,3-dimethyl-4-acetoxyazetidin-2-one (0.63 g, 5 mmol). Two diastereomers were purified by flash chromatography on silica gel (30 g, 5% AcOEt/*n*-hexane). The 4*R*-isomer was more polar on TLC (AcOEt-*n*-hexane). 4R-isomer: mp 63–65 °C; IR (cm^{-1}) (neat) 3250, 1760, 1700; ^1H NMR ($CDCl_3$) δ 1.29 (3H, s) 1.43 (3H, s) 1.89–2.14 (3H, m) 2.20–2.36 (1H, m) 3.91–4.18 (2H, m) 4.48 (1H, dd, J = 5.3 Hz, 8.6 Hz) 4.98 (1H, s) 6.07, 1H, brs); HRMS (FAB) *m/z* ($M+H^+$). Calcd for $C_{10}H_{16}NO_3S$: 230.0851. Found: 230.0863;

In a similar way to that described for **2h**, starting from a mixture of 4*R*- and 4*S*-isomers of the azetidinone (115 mg, 0.5 mmol), a diastereomeric mixture of the 6,6-dimethylpenem was obtained as a colorless oil in a 37% yield. Further purification by preparative TLC (CH_2Cl_2) gave two diastereomers. The 5*R*-isomer **12** was more polar on TLC (CH_2Cl_2). **12**: mp 82–85 °C; IR (cm^{-1}) (KBr) 1770, 1704, 1566; ^1H NMR ($CDCl_3$) δ 1.48 (3H, s) 1.53 (3H, s) 1.70–1.89 (1H, m) 1.89–2.09 (2H, m) 2.37–2.54 (1H, m) 3.79–3.92 (1H, m) 3.92–4.05 (1H, m) 4.65 (1H, dd, J = 5.3 Hz, 13.2 Hz) 4.78 (1H, dd, J = 5.3 Hz, 13.2 Hz) 5.27 (1H, d, J = 10.6 Hz) 5.31 (1H, s) 5.34–5.49 (1H, m) 5.88–6.06 (1H, m); HRMS (FAB) *m/z* ($M+H^+$). Calcd for $C_{15}H_{20}NO_4S$: 310.113. Found: 310.1088. Anal. calcd for $C_{15}H_{19}NO_4S$.: C, 58.23; H, 6.19; N, 4.53. Found: C, 57.95; H, 6.21; N, 4.52.

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