SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONS IN THE CLASS OF 2-(PYRIDYL)PENEMS

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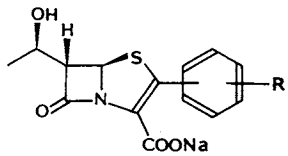
The isosteric CH→N substitution in the class of 2-arylpenems results in improved antibacterial activity, with retention of the favorable characteristic of stability towards renal dehydropeptidase. High therapeutic efficacy was demonstrated in experimental mice septicemias with the 2-(3-pyridyl) derivative **2b** and its orally absorbed acetoxymethyl ester prodrug **4n**.

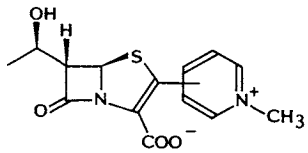
Recently we described a series of penems differing from our previous 2-CH_2X series in the interposition of a *p*-phenylene spacer between the nucleus and the CH_2X group[1]. Originally, we synthesized some simple 2-arylpenems (**1a** and **1b**) and found that they are characterized by excellent renal dehydropeptidase (DHP-I) stability and remarkable oral bioavailability (Table 2). These favorable properties had to be set against poor activity against most Gram-negative bacteria and exceedingly high serum protein binding. To overcome these drawbacks, which in the first instance could be related to the lipophilicity of compounds, either the introduction of a charged substituent on the phenyl ring[1], or an isosteric CH→N substitution within the ring was contrived. Using the latter approach, this paper presents the preparation and bioactivity of penems carrying at C-2 2-, 3-, or 4-pyridyl moieties as in **2a**～**2c**, and where the substitution on the pyridine ring was varied as in **2d**～**2k**.

Two quaternary ammonium derivatives (**2l** and **2m**) were also prepared. The po bioavailability was achieved through the preparation of an acetoxymethyl ester prodrug (**4n**; Fig. 2).

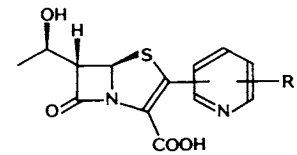
Fig. 1. Structure of penems **1a**, **1b** and **2a**～**2m**.



1a R=H
1b R=4-CH_3

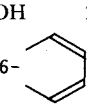


3-Pyridyl (**2l**)
4-Pyridyl (**2m**)



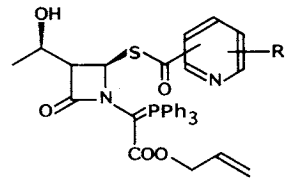
2-Pyridyl (**2a***)	R=H	3-Pyridyl (**2g***)	R=6-CH_3
3-Pyridyl (**2b**)	R=H	3-Pyridyl (**2h***)	R=6-OCH_3
4-Pyridyl (**2c***)	R=H	3-Pyridyl (**2i**)	R=6-$CONH_2$
3-Pyridyl (**2d***)	R=6-Cl	3-Pyridyl (**2j**)	R=6-CH_2OH
3-Pyridyl (**2e**)	R=6-OH	2-Pyridyl (**2k**)	R=4,5-OH

3-Pyridyl (**2f***) R=5,6-

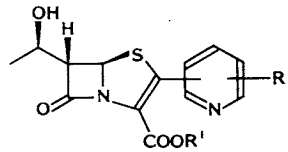


* Isolated as the sodium salt.

Fig. 2. Structure of phosphoranes **3a**~**3k** and penems **4a**~**4k** and **4n**.



2-Pyridyl (**3a**) R=H
3-Pyridyl (**3b**) R=H
4-Pyridyl (**3c**) R=H
3-Pyridyl (**3d**) R=6-Cl
3-Pyridyl (**3e**) R=6-O
3-Pyridyl (**3f**) R=5,6-
3-Pyridyl (**3g**) R=6-CH_3
3-Pyridyl (**3h**) R=6-OCH_3
3-Pyridyl (**3i**) R=6-$CONH_2$
3-Pyridyl (**3j**) R=6-CH_2O-Si(Ph)(Ph)-
2-Pyridyl (**3k**) R=4,5-O



2-Pyridyl (**4a**) R=H R′=
3-Pyridyl (**4b**) R=H R′=
4-Pyridyl (**4c**) R=H R′=
3-Pyridyl (**4d**) R=6-Cl R′=
3-Pyridyl (**4e**) R=6-O R′=
3-Pyridyl (**4f**) R=5,6- R′=
3-Pyridyl (**4g**) R=6-CH_3 R′=
3-Pyridyl (**4h**) R=6-OCH_3 R′=
3-Pyridyl (**4i**) R=6-$CONH_2$ R′=
3-Pyridyl (**4j**) R=6-CH_2O-Si(Ph)(Ph)- R′=
2-Pyridyl (**4k**) R=4,5-O R′=
3-Pyridyl (**4n**) R=H R′=CH_2OAc

Chemistry

Silver (3*S*,4*R*)-1-[[allyloxy)carbonyl](triphenylphosphoranylidene)methyl]-3-[(1*R*)-hydroxyethyl]azetidin-2-one-4-thiolate, prepared by modification of a published procedure[2], was converted to the phosphorane thioesters **3a**~**3k** (Fig. 2) by reaction with the appropriate pyridyl carboxylic chloride (2 mol equiv, CH_2Cl_2, 0°C to room temperature), in turn obtained from the corresponding potassium or sodium carboxylate ($(COCl)_2$, benzene, room temperature)[3].

Use of pyridines **5a**~**7a** required a protection-deprotection sequence (Fig. 3). Silver carbonate mediated reaction[4] of **5a** and **6a**[5] with allyl bromide (benzene, room temperature, 24 hours) yielded the fully protected derivatives **5b** and **6b**. Selective *O*-alkylation was demonstrated by ^{1}H NMR chemical shift of the pyridine α-protons when compared with that of the tautomeric pyridone form†. Ester hydrolysis (KOH 1 equiv, EtOH reflux, 2 hours) led to the potassium salts **5c** and **6c**. *tert*-Butyldiphenylsilyl *O*-protection

† Compare, for instance, the chemical shifts of the H_α in **6c** (8.50 ppm as free acid) and in **5c** (8.50 ppm) vs the H_α chemical shifts when the tautomeric pyridone forms are present (7.40 ppm in **6a** and 8.05 ppm in **5a** sodium salt respectively).

Fig. 3. Pyridines **5a**~**5c**, **6a**~**6c** and **7a**~**7c**.

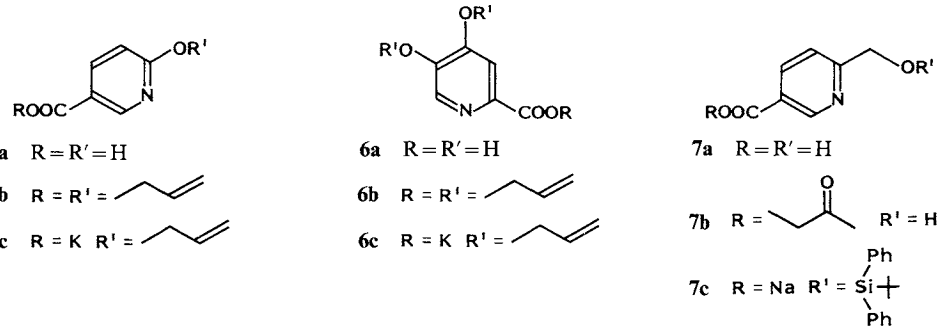


Table 1. *In vitro* antibacterial activity[a] of penems.

Strain No.	**1a**	**1b**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**
Staphylococcus aureus Smith	0.19	0.04	0.19	0.09	0.09	0.02	0.02	0.01
S. aureus 39/2 (Pen^+)	0.19	0.04	0.19	0.09	0.09	0.02	0.04	0.02
Streptococcus pyogenes ATCC 12384	0.04	0.02	0.19	0.02	0.01	0.02	0.04	0.001
S. faecalis ATCC 6057	3.1	3.1	6.2	1.5	3.1	0.78	1.5	0.78
Escherichia coli K-12	12.5	12.5	0.78	0.78	3.1	1.5	0.78	6.2
E. coli TEM	12.5	25	0.78	0.78	0.78	1.5	0.39	6.2
E. coli B	0.78	0.39	0.78	0.78	1.5	0.39	0.78	0.02
Salmonella typhi ATCC 14028	3.1	6.2	0.78	0.39	1.5	0.78	0.39	6.2
Klebsiella aerogenes 1082 E	3.1	3.1	0.78	0.39	1.5	1.5	0.78	6.2
Enterobacter cloacae P 99	12.5	25	0.78	0.78	1.5	1.5	0.39	25
Citrobacter freundii ATCC 8090	3.1	3.1	0.78	0.39	1.5	0.78	0.78	6.2

Strain No.	**2g**	**2h**	**2i**	**2j**	**2k**	**2l**	**2m**
Staphylococcus aureus Smith	0.02	0.02	0.09	0.04	0.09	0.09	>50
S. aureus 39/2 (Pen^+)	0.02	0.02	0.09	0.04	0.09	0.09	>50
Streptococcus pyogenes ATCC 12384	0.01	0.09	0.09	0.01	0.01	0.02	>50
S. faecalis ATCC 6057	1.5	1.5	1.5	1.5	1.5	25	>50
Escherichia coli K-12	0.39	1.5	0.78	0.39	1.5	12.5	>50
E. coli TEM	0.39	0.78	0.39	0.19	3.1	12.5	>50
E. coli B	0.19	0.19	0.39	0.19	0.78	12.5	>50
Salmonella typhi ATCC 14028	0.39	1.5	0.39	0.19	1.5	25	>50
Klebsiella aerogenes 1082 E	0.19	0.39	0.39	0.19	1.5	12.5	>50
Enterobacter cloacae P 99	0.78	1.5	0.78	0.78	1.5	6.2	>50
Citrobacter freundii ATCC 8090	0.19	0.39	0.39	0.39	1.5	6.2	>50

[a] MICs (μg/ml) were determined by the standard 2-fold agar dilution method in Mueller-Hinton Agar (Difco). Spots of 10^4 bacteria were automatically applied to the surface of the agar using a multipoint inoculator.

was used in the synthesis of **2j**. Selective silylation of the benzylic hydroxyl required preventive esterification of **7a** (chloroacetone, *N*,*N*-dimethylformamide, triethylamine, overnight). Reaction of **7b** with *tert*-butyldiphenylsilyl chloride (3 mol equiv, imidazole 10 mol equiv, CH_2Cl_2, few minutes) and mild alkaline hydrolysis (KOH 1.2 mol equiv, CH_3CN-H_2O, 10°C, 2 hours) led to the desired *O*-protected reagent **7c**.

Heating of phosphoranes thioesters **3a**~**3k** (benzene, reflux, 8~12 hours) uneventfully afforded penem allyl esters **4a**~**4k**, which were deblocked with tetrakis(triphenylphosphine)palladium to **2a**~**2k** as sodium salts or free acids, depending upon conditions (see Experimental part). The quaternary ammonium derivatives **2l** and **2m** were prepared simply by mixing the phosphoranes **3b**, **3c** with MeI (MeCN, room

Table 2. Pharmacokinetics parameters[a] and DHP-I stability[b] of penems.

	1a	1b	2a	2b	2c	2d	2e	2f	2g	2h	2i	2l	4n
$t_{1/2}\beta$ (minutes)	13	8	>3	5.5	4	>3	3	14	3	8	7.5	—	14
AUC (μg/minute/ml)	2,209	748	286	268	692	286	146	928	—	667	251	—	180
po absorption[c] (%)	13	72	<1	<1	1.3	—	—	—	—	—	—	—	67
DHP-I stability[d]	95.5	—	—	91.5[e]	—	—	89.5	—	73	—	—	98.7	—

[a] After iv administration at 10 mg/kg in mice, with the exception of **4n**, which was administered orally.
[b] % of unreacted starting material after 1 hour incubation of 30 μg/ml of penem and 1 μg/ml of purified porcine renal enzyme, at 37°C, pH 7.1 MOPS buffer.
[c] $([AUC]_{po}/[AUC]_{iv}) \times 100$.
[d] Corresponding data for imipenem, taken as a reference compound, was 14.
[e] At 100 μg/ml.

Table 3. Therapeutic efficacy in mice of isosteric 2-phenyl and 2-pyridyl penems.

Organism	ED_{50}[a] (mg/kg)				
	1a	1b	2b	2g	4n
Staphylococcus aureus Smith[b]	≫1	≫1	<0.16	0.32 (0.08~1.21)	0.47 (0.17~1.3)
Streptococcus pyogenes 3 ATCC 12384[b]	—	—	—	0.3 (0.02~3.6)	0.55 (0.21~1.44)
Escherichia coli G[c]	—	—	7.6 (5.5~10.4)	6.4 (4.97~5.36)	16.33 (12.24~21.8)

[a] Confidence limits for $P=0.95$.
[b] Treatment: 2 hours after challenge.
[c] Treatments: 0.5, 1.5 and 6 hours after challenge.

temperature, overnight). Quaternarization at the pyridine nitrogen resulted, in fact, in thioester carbonyl activation sufficient for spontaneous Wittig reaction, which, after palladium mediated deallylation, gave zwitterions **2l** and **2m**. As the compounds synthesized exhibited no significant po absorption as such, the ester prodrug **4n** was prepared by reaction of **2b** (sodium carboxylate) with acetoxymethyl bromide (0.9 mol equiv, *N*,*N*-dimethylformamide, −20°C, overnight).

Biological Results and Discussion

According to our expectations, replacement of the 2-phenyl ring of **1a** with pyridine indeed increased activity against Gram-negative organisms, while retaining a remarkable potency against Gram-positive strains.

Among the unsubstituted pyridyl penems **2a**~**2c** the *m*-isomer proved the most active. In spite of major differences in electronic and polarity parameters, ring substitution in **2d**~**2k** did not significantly affect intrinsic activity. Hammet σ values of the ring substituent fairly correlated with chemical reactivity[6] (data not shown) but not with antimicrobial activity; on the contrary the most reactive compounds (**2l** and **2m**), where the pyridine is quaternarized, proved poorly or not active. Low outer membrane permeation accounts for the narrow spectrum of the quinoline derivative **2f**. Compound **2f**, in fact, in spite of its excellent activity on Gram-positive bacteria and *Escherichia coli* B (a Gram-negative strain characterized by reduced permeability barriers), resulted poorly active on *E. coli* K-12 and most Gram-negative organisms. Pharmacokinetic parameters of 2-pyridyl penems in mice are reported in Table 2.

The compounds showed plasma half-lives equal or superior to that displayed by our clinical candidate FCE 22101, and variable AUC values (Table 2). More interestingly, they retained most of the DHP-I

stability characteristic of the 2-phenyl derivative **1a**, which stands as one of the most stable penems in our *in vitro* hydrolysis experiments utilizing the purified porcine renal enzyme[7]. The *in vivo* activity in mice of compounds **2b** and **2g** is reported in Table 3; against *Staphylococcus aureus* and *E. coli* infections, **2b** and **2g** showed strong therapeutic efficacy, in contrast with the isosteric compounds of the phenyl series, **1a** and **1b**, which were probably affected by an exceedingly high protein binding. The acetoxymethyl ester **4n**, characterized by a high po bioavailability (67%), associated with good therapeutic efficacy, should deserve further evaluation.

Experimental

IR spectra were recorded on a Perkin-Elmer 457 or 1420 IR spectrophotometer. FAB-MS were recorded on a Varianmat 311/A mass spectrometer equipped with a combined EI/FI/FD ion source. ^{1}H NMR were recorded at 200 MHz on a Varian XL-200 spectrometer. UV spectra were recorded on a Farmitalia-Carlo Erba Strumentazione UV Spectracomp 301 spectrophotometer.

In Vitro Antibacterial Activity

MICs were determined by the standard 2-fold serial dilution method in Mueller-Hinton agar (Difco). Plates were inoculated with 10^4 cfu/spot using a multipoint inoculator (Denley Instruments Ltd., Bolney, Sussex). MICs were read after 18 hours of incubation at 37°C as the lowest concentration that inhibited visible growth.

Therapeutic Activity in Experimental Infections in Mice

Protection tests were done in female albino CD-1 COBS mice, weighting 21~23 g, experimentally infected with *S. aureus*, *Streptococcus pyogenes* and *E. coli*. Mice were challenged by the ip route. Seven animals were used at each of the dose concentrations of antibiotic tested, which were given subcutaneously (compounds **1a**, **1b**, **2b** and **2g**) or orally (compound **4n**) at 0.1 mg/10 g weight concentration. The mortality was recorded daily and the number of animal surviving on day 5 was used to calculate the median ED_{50}.

Phosphoranes **3a**~**3k**

General Preparation Method *e.g.* **3b**: 3-Pyridyl carboxylic chloride (2.73 g) in abs CH_2Cl_2 (50 ml) was added dropwise to a stirred solution of silver (3*S*,4*R*)-1-[[(allyloxy)carbonyl](triphenylphosphoranylidene)methyl]-3-[(1*R*)-hydroxyethyl]azetidin-2-one-4-thiolate (5.97 g) in abs CH_2Cl_2 (200 ml) containing pyridine (100 μl) at 5°C. After 10 minutes the cooling bath was removed and the reaction mixture was stirred for additional 15 minutes. Ethyl acetate (150 ml) was added and the suspension was filtered through a filter-aid bed. The organic solution was washed with aq $NaHCO_3$ and brine, then dried (Na_2SO_4) and evaporated *in vacuo* to give after SiO_2 chromatography with *n*-hexane - EtOAc (from 50 : 50 to 0 : 100) followed by EtOAc - acetone (90 : 10) the phosphorane thioester **3b** (3.2 g): IR ($CHCl_3$) cm^{-1} 1750, 1680, 1610.

Potassium 4,5-Diallyloxypyridine-2-carboxylate **6c**: 4,5-Dihydroxypyridine-2-carboxylic acid (**6a**) (1.85 g) and Ag_2CO_3 (11 g) were suspended in benzene (40 ml) at room temperature and allyl bromide (15 ml) was added. The reaction mixture was stirred for 30 hours in the dark. The suspension was diluted with benzene (40 ml) and filtered through a Celite bed. The solution was evaporated *in vacuo* to give, after silica gel column chromatography (*n*-hexane - EtOAc, 60 : 40) allyl 4,5-diallyloxypyridine-2-carboxylate **6b** (1.67 g). To the ester dissolved in EtOH 95% (80 ml), KOH (420 mg) was added and the solution was refluxed for 2 hours. EtOH was concentrated *in vacuo* to 20 ml, Et_2O (100 ml) was added and the insoluble potassium salt **6c** (1.53 g) was obtained after filtration and drying *in vacuo*: ^{1}H NMR as free acid (DMSO-d_6) δ 4.8 (2H, m, CH_2), 5.27~5.47 (2H, m, CH_2), 5.95~6.13 (1H, m, CH), 7.67 (1H, s, aromatic CH), 8.27 (1H, s, aromatic CH). In the same way potassium 6-allyloxypyridine-3-carboxylate **5c** was obtained: ^{1}H NMR (DMSO-d_6) δ 4.78 (2H, m, CH_2), 5.2~5.4 (2H, m, CH_2), 5.95~6.15 (1H, m, CH), 6.63 (1H, d, aromatic CH), 7.98 (1H, dd, aromatic CH), 8.48 (1H, d, aromatic CH).

Sodium 6-*tert*-Butyldiphenylsilyloxymethylpyridine-3-carboxylate **7c**: To a solution of potassium

6-hydroxymethylpyridine-3-carboxylate **7a**, prepared by a modification of a published procedure[8], 2.4 g in DMF (20 ml) chloroacetone (1 ml) was added at room temperature. The solution was stirred for 28 hours and then concentrated *in vacuo*. The residue was taken up in EtOAc - H_2O, the organic layer was dried (Na_2SO_4) and evaporated *in vacuo* to give crude **7b**. The crude oil was dissolved in CH_2Cl_2 (70 ml) and imidazole (1.6 g) and *tert*-butyldiphenylsilyl chloride were successively added at 0°C while stirring. After 15 minutes, the suspension was poured in water. The organic phase was separated, washed with brine, dried (Na_2SO_4), concentrated *in vacuo*, and purified by SiO_2 chromatography eluting with *n*-hexane - EtOAc mixtures to give acetonyl 6-*tert*-butyldiphenylsilyloxymethylpyridine-3-carboxylate (2.6 g). The diprotected pyridine was dissolved in MeCN (20 ml) and water (6 ml) and NaOH 0.4 M (15 ml) was added dropwise during 1 hour (TLC monitoring). The solution was concentrated *in vacuo* to small volume, toluene was added (2 × 100 ml) and the solution evaporated twice *in vacuo*. The waxy solid was taken up in acetone (20 ml) and *n*-hexane (70 ml). The acetonic layer was evaporated *in vacuo* to dryness to give **7c** as a foam (2.3 g): ^1H NMR (DMSO-d_6) δ 1.03 (9H, s, CH_3), 4.76 (2H, s, CH_2), 7.3 ~ 7.7 (11H, m, aromatic CH), 8.13 (1H, dd, aromatic CH), 8.8 (1H, d, aromatic CH).

Penems Esters **4a** ~ **4k**: General Preparation Method *e.g.* **4b**

A stirred solution of phosphorane **3b** (3.2 g) in benzene (20 ml) was refluxed for 8 hours. The solvent was removed *in vacuo* and the residue chromatographed with *n*-hexane - EtOAc (30 : 70) to yield the penem **4b** (1.63 g), contaminated with some Ph_3PO, as an oil: IR ($CHCl_3$) cm^{-1} 1780, 1720; ^1H NMR (90 MHz, $CDCl_3$) δ 1.33 (3H, d, CH_3), 3.75 (1H, dd, CH), 4.5 (1H, m, CH), 4.85 (2H, m, CH_2), 5.2 ~ 5.6 (2H, m, CH_2), 5.9 (1H, m, CH), 6.45 (1H, d, CH), ~7.4 (1H, m, aromatic CH), 8.05 (1H, m, aromatic CH), 8.8 ~ 8.9 (1H, m, aromatic CH), 9.1 (1H, d, aromatic CH).

Penem Acids **2b**, **2e**, **2i** ~ **2j**: General Deprotection Procedure

Penem **2b**: To a stirred solution of penem ester **4b** (650 mg) in CH_2Cl_2 - THF (1 : 1) (80 ml) glacial acetic acid (0.8 ml), Ph_3P (280 mg), and $(Ph_3P)_4Pd$ (280 mg) were added. The reaction mixture was stirred at room temperature for 30 minutes, then Et_2O (40 ml) was added. The precipitate was collected by centrifugation and purified by reverse phase chromatography (LiChroprep RP-18) eluting with water - acetone (95 : 5). The UV active fractions were collected and concentrated *in vacuo* to small volume. The white precipitate was collected by filtration and dried *in vacuo* to yield **2b** as an amorphous solid (330 mg), mp 205 ~ 208°C (dec). Spectral data are collected in Table 4.

Penems Sodium Salts **2a**, **2c**, **2d**, **2f** ~ **2h**: General Deprotection Procedure

Penem **2a**: To a stirred solution of penem ester **4a** (1.03 g) in THF - CH_2Cl_2 (1 : 1) (70 ml) Ph_3P (200 mg), sodium 2-ethylhexanoate (410 mg) and $(Ph_3P)_4Pd$ (200 mg) were sequentially added. The mixture was stirred at room temperature for 30 minutes and, after dilution with Et_2O (200 ml), the precipitated salt was collected by centrifugation, dissolved in water and chromatographed (LiChroprep RP-18) eluting with water. The appropriate fractions were collected and freeze-dried to give the title compound (453 mg). Spectral data are collected in Table 4.

Penem **2k**: To a stirred solution of the penem **4k** (200 mg) in THF - CH_2Cl_2 (1 : 1) (20 ml) Ph_3P (35 mg), sodium 2-ethylhexanoate (31 mg), glacial acetic acid (0.3 ml), and $(Ph_3P)_4Pd$ (200 mg) were added successively. The mixture was stirred at room temperature for 30 minutes and, after dilution with Et_2O (200 ml), the precipitated salt was collected by centrifugation, dissolved in water and chromatographed on reverse phase (LiChroprep RP-18) eluting with water - acetone (9 : 1) to give 125 mg of the title compound.

Acetoxymethyl (5*R*,6*S*)-6-[1(*R*)-Hydroxyethyl]-2-(3-pyridyl)penem-3-carboxylate **4n**

A solution of acetoxymethyl bromide (125 mg) in DMF (1 ml) was added to a solution of **2b** (320 mg) in DMF (8 ml) at −20°C under stirring. After 2.5 hours stirring another portion of acetoxymethyl bromide (75 mg) in DMF (5 ml) was added. Stirring was continued overnight. The reaction mixture was poured in EtOAc - water and the organic layer washed twice with brine, dried over anhydrous Na_2SO_4, filtered and evaporated *in vacuo*. The oily residue was chromatographed on SiO_2 eluting with *n*-hexane - EtOAc mixtures

Table 4. Spectroscopic data of penem compounds **1a**, **1b** and **2a**~**2k**.

Compound	^{1}H NMR (200 MHz, D_2O[a]) δ (ppm)	IR (KBr) cm^{-1}		UV $\lambda_{max}^{H_2O}$ nm
		β-Lactam	COO^-	
1a	1.34 (3H, d, CH_3), 3.98 (1H, dd, CH), 4.28 (1H, dq, CH), 5.80 (1H, d, CH), 7.45 (5H, m, aromatic CH)	1770	1600	322
1b	1.17 (3H, d, CH_3), 2.30 (3H, s, CH_3), 3.75 (1H, dd, CH), 3.97 (1H, dq, CH), 5.66 (1H, d, CH), 7.33, 7.16 (4H, ABq, aromatic CH)	1770	1600	324
2a	1.36 (3H, d, CH_3), 4.13 (1H, dd, CH), 4.33 (1H, dq, CH), 5.81 (1H, d, CH), 7.86 (2H, m, aromatic CH), 8.33 (1H, m, aromatic CH), 8.73 (1H, m, aromatic CH)	1780	1590	378
2b	1.17 (3H, d, CH_3), 3.86 (1H, dd, CH), 4.00 (1H, dq, CH), 5.77 (1H, d, CH), 7.41 (1H, dd, aromatic CH), 7.83 (1H, ddd, aromatic CH), 8.55 (1H, dd, aromatic CH), 8.59 (1H, d, aromatic CH)	1760	1600	332
2c	1.31 (3H, d, CH_3), 4.07 (1H, dd, CH), 4.27 (1H, dq, CH), 5.85 (1H, d, CH), 7.57 (2H, m, aromatic CH), 8.54 (2H, m, aromatic CH)	1780	1630	339[b]
2d	1.32 (3H, d, CH_3), 4.02 (1H, dd, CH), 4.28 (1H, dq, CH), 5.81 (1H, d, CH), 7.49 (1H, d, aromatic CH), 7.87 (dd, aromatic CH), 8.38 (1H, d, aromatic CH)	1765	1605	332
2e	1.33 (3H, d, CH_3), 3.94 (1H, dd, CH), 4.28 (1H, dq, CH), 5.76 (1H, d, CH), 6.61 (1H, d, aromatic CH), 7.7~7.8 (2H, m, aromatic CH)	1760	1610	310
2f	1.26 (3H, d, CH_3), 3.76 (1H, dd, CH), 4.18 (1H, dq, CH), 5.78 (1H, d, CH), 7.73, 7.57 (2H, m, aromatic CH), 7.97 (2H, m, aromatic CH), 8.35 (1H, d, aromatic CH), 9.01 (1H, d, aromatic CH)	1750	1605	344[b]
2g	1.32 (3H, d, CH_3), 2.51 (3H, s, CH_3), 4.00 (1H, dd, CH), 4.27 (1H, dq, CH), 5.79 (1H, d, CH), 7.30 (1H, d, aromatic CH), 7.76 (1H, dd, aromatic CH), 8.41 (1H, d, aromatic CH)	1755	1600	328
2h	1.33 (3H, d, CH_3), 3.93 (3H, s, CH_3), 3.98 (1H, dd, CH), 4.28 (1H, dq, CH), 5.76 (1H, d, CH), 6.88 (1H, d, aromatic CH), 7.81 (1H, dd, aromatic CH), 8.18 (1H, d, aromatic CH)	1765	1600	324
2i	1.35 (3H, d, CH_3), 4.08 (1H, dd, CH), 4.31 (1H, dq, CH), 5.88 (1H, d, CH), 8.05 (2H, m, aromatic CH), 8.67 (1H, m, aromatic CH)	1755	1600[c]	348
2j	—	—	—	332
2k	1.16 (3H, d, CH_3), 3.71 (1H, dd, CH), 3.96 (1H, dq, CH), 5.54 (1H, d, CH), 6.67 (1H, s, aromatic CH), 7.78 (1H, s, aromatic CH)	—	—	338

[a] Except for **2b** and **2k**, analyzed in DMSO-d_6, and **2f**, analyzed in Me_2CO-d_6.
[b] Phosphate buffer, pH 7.4.
[c] $-CONH_2$: Carbonyl stretching at 1675 cm^{-1}.

to yield the title product as a foam (220 mg): IR ($CHCl_3$) cm^{-1} 1785, 1760 (sh), 1720; UV $\lambda_{max}^{H_2O}$ nm 340; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.38 (3H, d, CH_3), 2.06 (3H, s, CH_3), 3.85 (1H, dd, CH), 4.29 (1H, dq, CH), 5.69, 5.74 (2H, ABq, CH_2), 5.76 (1H, d, CH), 7.33 (1H, m, aromatic CH), 7.81 (1H, m, aromatic CH), 8.6~8.7 (2H, m, aromatic CH).

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-2-(1-methyl-3-pyridinium)penem-3-carboxylate (**2l**):

To a solution of **3b** (500 mg) in MeCN (10 ml) an excess of MeI (1 ml) was added and the mixture was let stand overnight. The solution was then evaporated *in vacuo* and the crude product was purified by SiO_2 column chromatography eluting with EtOAc-hexane mixtures, to give 300 mg of the allyl ester

of the title compound (as iodide). This compound was deprotected as described above for penem **2b**. After purification by reverse phase column the UV active fractions were collected and lyophilized to yield zwitterion **2l**. IR (KBr) cm^{-1} 1770, 1595; ^1H NMR (200 MHz, D_2O) δ 1.33 (3H, d, CH_3), 4.11 (1H, dd, CH), 4.30 (1H, dq, CH), 4.39 (3H, s, CH_3), 5.91 (1H, d, CH), 8.01 (1H, dd, aromatic CH), 8.54 (1H, d, aromatic CH), 8.73 (1H, d, aromatic CH), 8.96 (1H, s, aromatic CH); UV $\lambda_{max}^{H_2O}$ nm 338, mass spectrum (FAB): *m/z* 307 (100, MH^+), 263 (20, $MH^+ - CO_2$).

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